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RESEARCH ARTICLE

Breaking free from thermodynamic constraints: thermal acclimation and metabolic compensation in a freshwater zooplankton species

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ABSTRACT

Respiration rates of ectothermic organisms are affected by environmental temperatures, and sustainable metabolism at high temperatures sometimes limits heat tolerance. Organisms are hypothesized to exhibit acclimatory metabolic compensation effects, decelerating their metabolic processes below Arrhenius expectations based on temperature alone. We tested the hypothesis that either heritable or plastic heat tolerance differences can be explained by metabolic compensation in the eurythermal freshwater zooplankton crustacean *Daphnia magna*. We measured respiration rates in a ramp-up experiment over a range of assay temperatures (5–37°C) in eight genotypes of *D. magna* representing a range of previously reported acute heat tolerances and, at a narrower range of temperatures (10–35°C), in *D. magna* with different acclimation history (either 10 or 25°C). We discovered no difference in temperature-specific respiration rates between heat-tolerant and heat-sensitive genotypes. In contrast, we observed acclimation-specific compensatory differences in respiration rates at both extremes of the temperature range studied. Notably, there was a deceleration of oxygen consumption at higher temperature in 25°C-acclimated *D. magna* relative to their 10°C-acclimated counterparts, observed in active animals, a pattern corroborated by similar changes in filtering rate and, partly, by changes in mitochondrial membrane potential. A recovery experiment indicated that the reduction of respiration was not caused by irreversible damage during exposure to a sublethal temperature. Response time necessary to acquire the respiratory adjustment to high temperature was lower than for low temperature, indicating that metabolic compensation at lower temperatures requires slower, possibly structural changes.

KEY WORDS: Acclimation, *Daphnia*, Plasticity, Respiration, Temperature

INTRODUCTION

Few aspects of ecological physiology have been studied more thoroughly than temperature dependence of respiration rate in poikilothermic organisms, particularly in relation to upper temperature tolerance limit (Fry, 1971; Hochachka and Somero, 2002; Angilletta, 2009; Schulte, 2015). It has been shown in numerous model systems that respiration rate increases with temperature in a manner well described by the Arrhenius equation over a range of temperatures, up to a peak or plateau, followed by a rapid decrease as temperatures become too high to sustain normal metabolism (Schulte et al., 2011). Numerous attempts have been made to interpret the shape of this respiratory temperature performance curve with respect to the physiological response to temperature, width of temperature tolerance range, and where temperature optima may fall (Huey and Stevenson, 1979; Paladino et al., 1980; Schoolfield et al., 1981; Huey and Kingsolver, 1989, 1993; Pörtner, 2002, 2006; Frazier et al., 2006; Neves et al., 2015).

The Arrhenius phase can be thought of as a constraint imposed by fundamental principles of thermodynamics; the plateau phase therefore reflects either some new constraint not relevant at lower temperatures or an adaptive compensatory effort of the organism to limit respiration to a sustainable level (Hochachka, 1967; Gadgil and Bossert, 1970; Padfield et al., 2016). The temperature at which the transition from the Arrhenius-prescribed respiration rates to the plateau occurs is termed the Arrhenius breakpoint temperature (ABT; Schulte, 2015) and whether it is indicative of organisms' ability to sustain maximal respiration rate and/or maintain active metabolism at elevated temperatures is subject to debate. It has been repeatedly proposed that, for aquatic organisms, the constraint limiting the maximum temperature tolerance limit is the availability of oxygen, as respiratory demands increase with temperature while oxygen availability (and possibly transport efficiency) drops (Pörtner, 2001, 2010; Verberk et al., 2016; but see Jutfelt et al., 2018 for criticism of this view). Constraints such as oxygen availability reduce the range of the so-called aerobic scope, the difference between basal and maximum respiration, limiting the amount of energy available for growth and reproduction. The process just described is known as the oxygen- and capacity-limited thermal tolerance hypothesis (Fry and Hart, 1948; Pörtner, 2001; Pörtner and Knust, 2007; Pörtner and Farrell, 2008; see Schulte, 2015 and Verberk et al., 2016 for recent reviews).

Despite oxygen availability constraints, there is considerable variation in the ABT and in the plateau height among different species (Martinez et al., 2016a,b), individuals (Salin et al., 2016), populations from different geographic and latitudinal regions (Angilletta et al., 2002; Sommer and Pörtner, 2004; Morley et al., 2009; Harada et al., 2019) and, importantly, with different acclimation histories (Noguchi et al., 2015; Pascal and Chong, 2016; Sandersfeld et al., 2017). This variability suggests that the transition from Arrhenius respiration rates to the plateau is not fully constrained by thermodynamics and oxygen availability, and the deceleration of oxygen consumption may reflect an adaptive compensatory adjustment in metabolic demands at extreme temperatures to circumvent oxygen limitation, a plastic response termed metabolic compensation (Hochachka, 1967). The exact mechanisms of such compensation, as well as its role in extending

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the upper temperature tolerance limit, remain unclear (Schulte, 2015).

Freshwater zooplankton crustaceans of the genus *Daphnia* and their responses to temperature have been of interest to ecological physiologists for nearly a century (Brown, 1929; MacArthur and Baille, 1929; Stier and Wolf, 1932) and ample data on thermal adaptation have been accumulated (Armitage and Lei, 1979; Lamkemeyer et al., 2003; Yampolsky et al., 2014a; Jansen et al., 2017). In this paper, we investigate the role of thermal acclimation in plastic response in *Daphnia* that allows longer survival at high temperatures, and we test the hypothesis that such plasticity exists in the form of metabolic compensation resulting in reduced oxygen consumption at sublethal temperatures. We follow the Angilletta (2009) definition of acclimation as both the process and the outcome of adjustment of organisms' physiology and biochemistry during an exposure to a novel environment that results in better performance and, potentially, in higher fitness in that environment. Specifically, thermal acclimation results from an exposure to elevated temperatures and results in better performance at these or even higher temperatures (e.g. longer survival at a constant sublethal temperature or higher temperature tolerance limit). Beneficial thermal acclimation has been convincingly demonstrated in a variety of ectotherms (Fry, 1971; Angilletta, 2009), including *Daphnia* (Armitage and Lei, 1979; McKee, 1995; Lamkemeyer et al., 2003; Zeis et al., 2004; Williams et al., 2012; Yampolsky et al., 2014a,b; Coggins et al., 2017; Martin-Creuzburg et al., 2019; Burton et al., 2020), but details of the physiological mechanisms are largely unknown. Two lines of evidence suggest that this mechanism may be tightly linked to changes in respiration and/or metabolic demands for oxygen. Firstly, perhaps the most obvious plastic response to elevated temperatures is the upregulation of hemoglobins (Wiggins and Frappell, 2000; Gerke et al., 2011; Williams et al., 2012; Yampolsky et al., 2014a; Cuenca Cambronero et al., 2017), which significantly correlates with heat tolerance measured in both short- and long-term survival experiments. This includes correlated heat-tolerance response caused by exposure to mild hypoxia (Zeis et al., 2013; Coggins et al., 2017). Secondly, gene expression studies (Yampolsky et al., 2014b; Becker et al., 2018) indicate that acclimation to higher temperatures is accompanied by downregulation of a broad spectrum of gene expression-related proteins, consistent with the idea of adjustments aiming to decrease metabolic energy expenditures. These observations lead us to the hypothesis that acclimation to high temperature in *Daphnia* may be accomplished by means of metabolic compensation.

Metabolic compensation is likely to be accompanied by changes in mitochondrial function (Sommer and Pörtner, 2004; Martinez et al., 2016a,b; Harada et al., 2019), most importantly by uncoupling the electron transport chain from ATP production (Mölich et al., 2012; Martinez et al., 2016a,b; Bryant et al., 2018), which also ameliorates reactive oxygen species production (Brand, 2000). Therefore, we also test the hypothesis that the thermal response in oxygen consumption rate is mirrored by changes in mitochondrial membrane potential. Finally, in *Daphnia*, as well as in a variety of filter-feeding organisms, ventilation and feeding is accomplished by movement of the same filtering appendages (Pirow and Buchen, 2004) and thus we expect that any adjustment in oxygen consumption will be either mirrored, or, potentially, constrained by changes in filtering activity.

In summary, past studies provide evidence that acclimation to elevated temperatures in *Daphnia* may be accomplished through metabolic compensation that allows organisms to achieve lower metabolic oxygen demand. In the current study, we test this hypothesis using several genotypes of *Daphnia magna* from geographically distinct populations (Table S1) that are acclimated to either 10 or 25°C and whose respiration rate is measured over both the Arrhenius range (10–20°C) and plateau range (22.5–35°C) and demonstrate that the rates of oxygen and food consumption as well as, to some extent, changes in mitochondrial potential are consistent with this hypothesis.

MATERIALS AND METHODS

Clone origin, maintenance and thermal acclimation

Lineages of *Daphnia magna* Straus 1820 from eight different genotypes, representing arbitrary defined high and low acute temperature tolerance levels (Yampolsky et al., 2014a,b; Coggins et al., 2017; Martin-Creuzburg et al., 2019; see Table S1), were maintained at 18°C with a 12 h:12 h light:dark cycle in 100 ml flasks of oxygen-saturated COMBO medium (Kilham et al., 1998) with 6–10 individuals per flask. All cultures were fed the green algae *Scenedesmus obliquus* to a final concentration of 50,000 cells per individual per flask every 2 days to ensure that food limitation never occurred. COMBO medium and flasks were replaced every 4 days. Not all eight clones were used in all experiments, but both heat-tolerant and heat-sensitive clones were represented in each experiment (Table S1). Acclimation to either 10 or 25°C was achieved by transferring adult *D. magna* from 18°C-maintained stocks to each of these temperatures, at which their offspring were reared for two generations before experimental work was conducted. Wet mass of all individuals was measured at the conclusion of each respiration or feeding experiment.

Respirometry

Oxygen consumption was measured using two 4-channel FireSting O_2 Fiber Optic Oxygen Meters (PyroScience, Aechen, Germany; sensor software: Pyro Oxygen Logger, https://www.pyroscience.com/en/downloads/laboratory-devices) in ramp-up and temperature acclimation experiments (see below) and, additionally, by a Loligo respirometer (Loligo Systems, Viborg, Denmark; sensor software: MicroResp, https://www.loligosystems.com/microresp-version-1-automated-microplate-respirometry-software) with a SDR SensorDish sensor (PreSens Precision Sensing, Regensburg, Germany). Sensors were placed inside an incubator set to assay temperatures ranging from 5 to 37°C with experimental animals allowed to ramp up or down from their rearing (acclimation) temperature to the assay temperature over the course of 1 h. In the experiments in which the FireSting O_2 oxygen meter was used, each replicate measurement consisted of three adult female *D. magna* from the same clone–acclimation treatment combination (two for the larger 10°C-reared ones) placed in an airtight 4 ml vial (4 ml Ox Vial, PyroScience) filled with 0.2 µm-filtered COMBO water without air bubbles equilibrated at each assay temperature, each of which was randomly assigned to measurement channels with uniform distribution of clones and acclimation histories among runs. Additionally, in the Loligo measurements, contrasting treatments were paired and placed into adjacent wells and clones were distributed uniformly across the respirometry microplate. Measurements were carried out at 5 s intervals for a duration long enough to result in at least a 0.5 mg l^{-1} drop in oxygen concentration (typically a 1–1.5 mg l^{-1} drop and never below 5 mg l^{-1} or 75% of saturation at the end of the measurement), which was 3 h at 5 and 10°C, and 90 min for all other assay temperatures. In each run using both respirometers, oxygen levels were also measured in one blank vial containing only sterile COMBO medium.

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To ensure circulation across the measurement area of the vials, their lids were modified with an additional airtight space for a magnetic stir rod bringing the total volume of the chambers to 5 ml. To prevent damage to test organisms, the space with the stir rod was covered with 59% open nylon mesh. Measurement vials were placed in a 4-liter dry bath filled with Lab Armor beads (Lab Armor LLC, Cornelius, OR, USA) to help maintain temperature stability, and the bath was positioned on a multi-position magnetic stirrer with the same stirring intensity for each vial. An external temperature probe for each FireSting system was placed at each end of the bath to ensure homogenous temperature between channels.

In experiments in which the Loligo oxygen meter was used, each replicate consisted of a single individual placed into a 1.7 ml (active animals) or 0.2 ml (anesthetized animals) well of a 24-well Loligo microplate sealed with PCR film and equilibrated to the assay temperature. COMBO water was treated in the same manner as described above. Measurements were carried out at 15 s intervals for a duration long enough to result in at least a 1 mg l^{-1} drop in oxygen concentration, which was typically 2 h at 10°C or 90 min for 25 and 35°C assay temperatures. Each plate contained at least two blank wells with sterile COMBO water. In all experiments, measurements of oxygen concentration started after a 15 min break-in period and lasted for 90 min at 10°C and 60 min at higher temperatures. In each experiment the first two runs were used to estimate the effect size, and sample size (number of runs) necessary for achieving statistical significance (raw P-value<0.01) was determined assuming normal distribution of residual variance within run and treatment combination. No additional analysis was done until the set number of runs was achieved. If the required number of runs was unrealistically high, no attempt was made to achieve statistical significance and the effect at such conditions was deemed non-detectable. Individual observations were excluded from the analysis if the *D. magna* died during measurement (showed no heartbeat at the end of the measurement; see below) or was damaged before the measurement completion. This was a pre-established exclusion criterion.

Ramp-up experiment

The purpose of this experiment was to evaluate the influence of increasing temperature on *D. magna* oxygen consumption in animals with heritable differences in acute temperature tolerance but a shared thermal history. *Daphnia magna* adults raised at 18°C were transferred to 5°C, maintained at that temperature overnight, after which their oxygen consumption was measured at incrementally increasing temperatures up to 37°C, in 5°C increments (with the exception of the 7°C increment in the last measurement), with *D. magna* maintained at each next temperature overnight prior to the measurement. In this and all other experiments, *D. magna* were weighed to the nearest 0.1 mg after the last respiration measurement. Total number of individuals used in this experiment was 91, with the number of replicates per assay temperature varying between nine and 32 (lower at the highest temperatures due to loss of some animals; if one individual in a replicate was lost, this whole replicate was not re-used in further measurements for consistency).

Temperature acclimation experiments

The role of acclimation to a high or low temperature on oxygen consumption in newly experienced temperatures was investigated by measuring oxygen consumption with *D. magna* maintained for at least two generations at either 10 or 25°C and transferred directly to each of the assay temperatures with a 1 h ramp to reach the assay temperature. Two such experiments were conducted. In acclimation experiment 1, the goal was to assess the effect of thermal acclimation in a broader set of clones (8) and with more detailed assay temperature increments (from 10 to 25°C in 5°C increments, and from 27.5 to 35°C in 2.5°C increments). Three replicates of each genotype from each acclimation temperature (T_{acc}) were measured at each assay temperature. In acclimation experiment 2, a lower number of clones (4) were assayed at three assay temperatures (10, 25 and 35°C), but a higher number of replicates per clone and treatment was used (10–30) and both active and basal respiration were measured (see below). FireSting O_2 and Loligo respirometers (see above) were used in acclimation experiments 1 and 2, respectively. Two to six runs were conducted at each assay temperature in each experiment, with a total of 46 replicates (135–138 individuals) per assay temperature (total of 1091 individuals) in experiment 1 and 43–108 replicates per assay temperature (total 217 individuals) in experiment 2 (see Tables S2 and S3 for details).

In acclimation experiment 2, respiration rate was measured in both active *D. magna* and in *D. magna* anesthetized with 1% urethane (Ross and Ross, 2008), which blocks swimming and filtering activity (but does not inhibit heartbeat). To achieve this, after each active metabolism measurement the plate was unsealed and the animals were moved to a 0.2 ml plate with a 1% urethane solution in COMBO medium equilibrated to the assay temperature. The plates were sealed again and an additional measurement was conducted, starting 15 min after transfer into urethane solution, to achieve at least a 0.5 mg l^{-1} drop in oxygen concentration. Because the duration of both measurements at 35°C assay temperature is close to survival time of 10°C-acclimated *D. magna* at this temperature, the presence of heartbeat at the end of measurement was recorded and measurements of respiration rate were discarded if no heartbeat was detected. As urethane immobilizes filtering appendages that are also the main site of oxygen exchange with the environment (Pirow and Buchen, 2004), these measurements yield the basal respiration rate that reflects absolute minimum in oxygen consumption: both the demand and the intake are minimized, the former due to incapacitation of all muscles except the heart and the latter due to oxygen exchanged limited to diffusion (which in turn is limited due to low gradient of O_2 concentration). Thus the respiration rates measured this way are lower-bound estimates of the true basal rates.

Feeding and swimming rate measurements

In order to assess the amount of metabolic deceleration shown by 10°C- and 25°C-acclimated *D. magna* during a sublethal 35°C exposure, we assayed filtration rate and swimming rate in four clones of *D. magna* acclimated to these temperatures as described above and assayed at either 10, 25 or 35°C, with ramping times similar to those used in the respiration experiments. To measure swimming activity, five adult females from each clone and each T_{acc} were placed into a 250 ml culture flask with 200 ml of COMBO water of the assay temperature, which were then placed into a uniformly lit chamber and videotaped using a Canon PowerShot camera for 1 min. The videos were then traced semi-automatically using Tracker software (https://physlets.org/tracker) with the human tracer blinded with respect to clone and treatment. Average velocity while swimming and percentage of time spent active was recorded for each individual.

To measure feeding (filtering) rate, two or three replicate females per clone per acclimation temperature (total of 20 or 21 replicates per assay temperature, 61 total) were placed individually into 50 ml culture flasks containing 20 ml of COMBO water with 200,000

cells ml^{-1} *Scenedesmus* algae (2× the standard food concentration) at each of the assay temperatures and allowed to feed for the amount of time sufficient to decrease algae concentration 2- to 3-fold, with flasks kept in the darkness on a 60 rpm shaker. These times were 24, 8 and 2.5 h at the assay temperatures of 10, 25 and 35°C, respectively. Algae fluorescence was then measured in two replicate 3 ml samples placed into 24-well plates and measured using a SDR SensorDish sensor (see above). Flasks with algae but no *D. magna* served as blanks. The fluorescence values of blanks were averaged across each plate and subtracted from each non-blank observation.

Recovery experiment

To assess whether the respiration deceleration observed in the 25°C-acclimated *D. magna* during exposure to 35°C (see below) was reversible, *D. magna* from the 25°C-acclimated cohort (12 replicates, 34 individuals) were exposed to 35°C for 1 h. After the 1 h exposure, this sample was split into two equal portions: one placed back in 25°C and the other in 20°C. After 24 h of recovery, both replicates were placed in 25°C and oxygen consumption was measured at that temperature. As a control for this experiment, oxygen consumption was simultaneously measured in 25°C-acclimated *D. magna* never exposed to 35°C (16 replicates, 48 individuals).

Acclimation reversal time experiment

The time required to exhibit respiratory responses characteristic of acclimation to 10 or 25°C after experiencing a lifetime acclimation at the opposite temperature was evaluated using *D. magna* from 18 and 25°C acclimation that were exposed to 10°C between 0 and 72 h before measurement at 10°C (24 replicates per acclimation temperature, 139 individuals total) and those from the 10°C acclimation that were exposed to 25°C between 0 and 24 h (12 replicates, 24 individuals) before measurement at 35°C. Exposure times were based on observational data from short-term reciprocal acclimation temperature switches described in an earlier paper (Coggins et al., 2017).

Mitochondrial membrane potential measurements

In order to complement respiration rate measurements with estimates of mitochondrial membrane potential ($\Delta\psi$), we exposed 10°C- and 25°C-acclimated *D. magna* to rhodamine-123 (Emaus et al., 1986; Hasan, 2019) for 2 h at each of the three assay temperatures: 10, 25 or 35°C. Individual adult females from four clones, 25–29 replicates per assay temperature, were incubated in 0.5 ml of 10 µmol l^{-1} solution of rhodamine-123 in COMBO water in the dark. After the 2 h exposure, *D. magna* were rinsed three times with COMBO water and photographed under a Leica fluorescent microscope with 450/490 nm excitation filter, 510 nm longpass dichromatic mirror, and 515 nm suppression filter LP. Four regions of interest (RoIs) were measured using ImageJ software: second epipodite (left or right chosen randomly), heart, brain, and head without neural tissue with the median fluorescence intensity in the RoI used as the measurement and the median in a background region used as the blank. Images were blinded prior to measurent. Four to five individuals per each of four clones (FI-FSP1-16-2, GB-EL75-69, IL-MI-8 and HU-K-6; see Table S1) per acclimation temperature per assay temperature were used in this experiment. Only live *D. magna* were measured (detected by heartbeat), so the final number of observations was 2–5 per genotype per treatment.

Statistical analysis

Data were analysed using JMP statistical software (www.jmp.com). Respiration rates were estimated by linear regression of oxygen concentration over the range of measurements where linear decline of oxygen concentration was observed. Blank oxygen consumption estimates in each replicate run were subtracted from each respiration rate estimate obtained in this run. Wet mass was used as a covariable in all analyses, except for fitting Arrhenius and logistic curves and *post hoc* pairwise Tukey's tests. In these analyses, mass-specific respiration rates obtained by normalization to wet mass were used.

The Arrhenius equation:

$$R = Ae^{-\frac{E_a}{k_B}T} \quad (1)$$

(where A is a pre-exponent parameter, E_a is the activation energy, k_B is Boltzmann's constant and T is absolute temperature in K), was fitted to respiration rates over the range of assay temperatures in which the log-transformed mass-specific respiration rate was showing a monotonous and linear relationship with inverse temperature, i.e. between 5 and 25°C.

Because groups of *D. magna* with different genetic (ramp-up experiment) or thermal acclimation (temperature acclimation experiments) showed differences in range of assay temperatures where the data no longer followed the Arrhenius equation, three different approaches were used to test the differences between these groups. The first approach aimed to detect differences in ABT between clonal or T_{acc} groups. To achieve this, experiment-wide approximate breakpoint temperature was determined visually and then linear regression in Arrhenius coordinates (log R versus $1/T$) was conducted for each of the compared groups separately, first for Arrhenius range and then for the plateau range (Fig. S1). The ABT was then determined as the temperature at which the two regression lines intersect, i.e. as:

$$\mathrm{ABT} = \frac{I_P - I_A}{S_A - S_P}, \quad (2)$$

where I_A and I_P are the intercepts and S_A and S_P are the slopes of the linear regressions over the Arrhenius and plateau ranges, respectively. Fig. S1 presents a graphic explanation of this analysis. The standard error of ABT was calculated as the standard error of a ratio of two differences, namely:

$$\mathrm{SE(ABT)} = \mathrm{ABT}\left(\frac{\mathrm{SE}(I_P) + \mathrm{SE}(I_A)}{I_P - I_A} + \frac{\mathrm{SE}(S_A) + \mathrm{SE}(S_P)}{S_A - S_P}\right). \quad (3)$$

For presentation purposes, both ABTs and their standard errors were converted to °C units.

The second approach to reveal differences in deviations from Arrhenius expectations was to fit a logistic equation to specific respiration rates measured in each group of *D. magna* separately:

$$R = \frac{R_{max}}{1 + e^{-k(T - t_0)}}, \quad (4)$$

where R is the specific respiration rate (µg O_2 mg^{-1} wet mass min^{-1}), T is the assay temperature (°C) and the three fitted parameters R_{max}, k and T_0 are the maximal respiration rate, strength of temperature effect and inflection temperature, respectively. While lacking a mechanistic basis in determining the thermal respiration curve, a logistic curve fits the exponential increase followed by a plateau well and can quantify the degree of curvilinearity in its shape if compared with a linear fit. Maximum likelihood estimates of standard errors around these parameters were used to evaluate the differences between groups of *D. magna*. Logistic versus linear fits were compared using Akaike's information criterion (AIC) (Akaike, 1974) and a log-ratio test. As an

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alternative to using specific respiration rates in this analysis, we could correct the raw respiration rates by the residuals from their linear regression on body masses. The results of this version of the analysis were similar to those reported below and are not presented.

Finally, analysis of covariance (ANCOVA) models were evaluated with *D. magna* wet mass and assay temperature as continuous covariables, and T_{acc} (e.g. 10 versus 25°C) treated as a fixed categorical variable. Clones and experimental blocks were treated as random effects with the assumption of no interactions with the main effects. When temperature tolerance level was included in the model (i.e. in the ramp-up experiment analysis), clones were nested within the temperature tolerance groups (Table S1). To assess non-linearity of the effect of assay temperature on respiration, this covariable was included in each model as both a linear and a quadratic effect, and the model was implemented for each T_{acc} separately. In order for a *post hoc* Tukey's test to be conducted across assay temperature values, this analysis was repeated with the assay temperature treated as a categorical factor and the quadratic term dropped from the model. Likewise, in the membrane potential experiment and in acclimation experiment 2, where the assay temperature had only three levels, this variable was treated as a fixed categorical factor.

RESULTS

Ramp-up experiment

For both the low and high thermal tolerance groups, the respiration rate followed the Arrhenius equation until approximately 25°C, after which it decelerated (resulting in an S-shape response) and eventually dropped above 30°C (Fig. 1A; Table S2). Estimates of ABT were virtually identical (Fig. S1A): 18.8±0.46 and 18.7±0.46°C for the high and low heat tolerance groups of clones. The logistic curve was a better fit than a linear one (Table S2) both overall and for each of the heat tolerance groups with no indication of difference between the two groups. The presence of a significant non-linearity in the respiration rate response to assay temperature is confirmed by the significant quadratic term in the ANCOVA (F=20.41, P<1.5×10^{-5}). While there was an overall significant difference in specific respiration rate among clones (d.f.=5, F=3.208, P<9.5×10^{-3}), it showed no correlation with previously measured high versus low acute temperature tolerance in each clone (Fig. 1A) with non-significant heat tolerance group effect when tested against clones nested within each group (d.f.=1, F=0.009, P>0.9). Likewise, there were no clone×assay temperature interactions (d.f.=5, F=0.488, P>0.77).

Temperature acclimation experiments

Daphnia magna acclimated to 25°C and transferred directly into the assay temperatures without an 8 h ramp also showed a logistic-shaped response curve to the increasing assay temperature (Fig. 1B, red symbols; Table 1). The leveling off occurred at approximately the same temperature as for the ramp-up experiment, but at a slightly lower asymptote (0.012 versus 0.015 µg O_2 mg^{-1} wet mass min^{-1}). Daphnids from the 10°C acclimation had significantly higher respiration rate at both the lowest and the highest temperatures than their 25°C-acclimated counterparts (Fig. 1B), but not at intermediate temperatures, showing little deceleration of respiration rate with increasing temperature (Fig. 1B, blue symbols). This resulted in a significantly lower ABT (Fig. S1B): 19.3±1.38 and 23.7±0.56°C for the 10°C- and 25°C-acclimated *D. magna*, respectively. Moreover, the linear regression was a better fit than logistic curve by AIC (Table S2). In contrast, the 25°C-acclimated animals showed a clear deceleration of respiration rate above 25°C. The normalized AIC for the logistic versus linear fit was 0.997, suggesting that the logistic fit was over 300 times more likely to be the best fit rather than the linear one. *Post hoc* Tukey's tests for pairwise differences indicated that the difference between 10°C- and 25°C-acclimated *D. magna* was significant (P<0.05) and that the respiration rate of 25°C-acclimated animals measured at 35°C was not significantly different from that at 22.5 or 20°C, while the same comparisons for the 10°C-acclimated animals were individually significant (Fig. 2B). The quadratic term for assay temperature in the ANCOVA for respiration rate with wet mass as a covariable was significant for the 25°C-acclimated *D. magna* (F=9.43, P<0.002), but not for 10°C-acclimated *D. magna* (F=2.88, P>0.09).

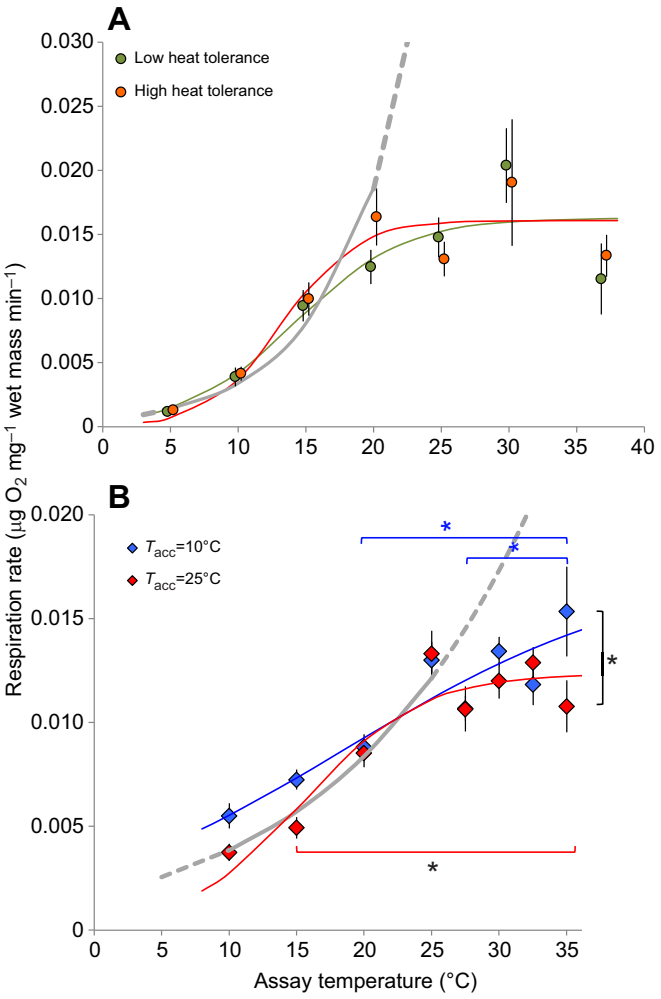

Fig. 1. Temperature ramp-up and acclimation experiments. (A) Specific respiration rates (means and s.e.m.) in *Daphnia magna* in a ramp-up experiment with clones from high (orange) and low (green) temperature tolerance groups. Sample sizes were N=9 to 32 per assay temperature (see Yampolsky et al., 2020, tab 'Ramp-UP' for details). (B) Specific respiration rates in *D. magna* acclimated to either 10°C (blue) or 25°C (red) and transferred directly into the assay temperature. Logistic functions fitted with parameters are listed in Table 1. Grey lines denote Arrhenius equations fitted for assay temperatures <30°C (solid lines: fitted; dashed line: extrapolated). Horizontal brackets show Tukey's test differences between respiration rate at 35°C and the closest lower assay temperatures at which the *post hoc* pairwise comparison is significant, within each acclimation temperature (T_{acc}) group. Vertical bracket shows the results of Tukey's test between acclimation temperature groups within each assay temperature. *P<0.05. Sample size was N=46 per assay temperature (see Yampolsky et al., 2020, tab 'Acclimation-1' for details).

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Table 1. ANCOVA of the effects of replicate temperature-acclimation experiments, assay temperature (T), acclimation temperature (T_{acc}) and their interaction on *Daphnia magna* respiration rate

Source	d.f.	MS	F	P
Wet mass (mg)	1	0.0406	67.16	1×10^{-14}
Experiment	1	0.0078	12.88	0.0004
T	2	0.0349	57.66	1.5×10^{-21}
Experiment×T	2	0.0022	3.71	0.026
T_{acc}	1	0.0045	7.47	0.007
Experiment×T_{acc}	1	0.0002	0.37	0.55
$T\times T_{acc}$	2	0.0026	4.24	0.015
Experiment×$T\times T_{acc}$	2	0.0003	0.42	0.66
Clone	4	0.0002	0.4	0.81
Error	268			

Wet mass of animals was used as a covariable; clones were treated as a random effect.

In order to confirm the observed differences in respiration rates between 10°C- and 25°C-acclimated *D. magna* measured at the opposite ends of the assay temperature range, we measured respiration rate of *D. magna* acclimated to two temperatures at assay temperatures of 10, 25 and 35°C (acclimation experiment 2). These results are presented in Fig. 2A, Table 1 and Table S3. Consistent with the results shown in Fig. 1B, active respiration was significantly higher in 10°C-acclimated than in 25°C-acclimated *D. magna* assayed at 10°C (P<0.0001; Table S3), but also when assayed at 35°C (P<0.01; Fig. 2A), due to no further increase in respiration rate beyond 25°C assay temperature in 25°C-acclimated *D. magna* (Fig. 2A, continuous lines). In contrast, basal respiration rate measured in urethane-anesthetized animals (Fig. 2A, dashed lines) showed no difference between 10°C- and 25°C-acclimated *D. magna* at any assay temperature (Table S3). The 25°C-acclimated *D. magna* showed somewhat higher respiration rate than 10°C-acclimated *D. magna*, but this difference was not significant once the data were body mass- and block effect-corrected. This observed deceleration of oxygen consumption rate in 25°C-acclimated *D. magna* is largely caused by the reduction of active metabolism rather than in the lower-bound estimates of basal metabolism in immobilized animals.

Joint analysis of both acclimation experiments (with experiment as a block effect) is presented in Table 1. There was a significant difference between experiments, possibly owing to different equipment used, different volumes of chambers and different stirring regimes. A modestly significant assay temperature×acclimation temperature term (P<0.015) indicated differential respiratory responses to current temperatures. More specifically, these differences were largely due to the differences between the two acclimation groups at 37°C (*post hoc* Tukey's tests; Fig. 2A,B).

The deceleration of specific respiration rate observed in 25°C-acclimated *D. magna* was not caused by irreversible damage, because a full recovery occurred after 24 h at either 25 or 20°C (Fig. 3).

Feeding rates

In a pattern similar to that of active respiration results (Fig. 2A,B), feeding rate continued to increase with temperature beyond 25°C in 10°C-acclimated *D. magna*, but not in their 25°C-acclimated counterparts (Fig. 2C), although the difference between feeding rate estimates of the two acclimation groups obtained at 35°C did not reach statistical significance (Table S4). Feeding rate measured at 10°C was significantly higher in *D. magna* acclimated to this temperature than in those acclimated to 25°C.

Swimming activity

In contrast, swimming activity did not follow the pattern of respiration and feeding rate and was not consistent with the hypothesis of metabolic compensation. Portion of time spent actively swimming decreased with assay temperature in both acclimation groups equally (Fig. S2A). Average velocity while active exhibited the opposite pattern relative to the prediction of metabolic compensation in 25°C-acclimated *D. magna*: consistent

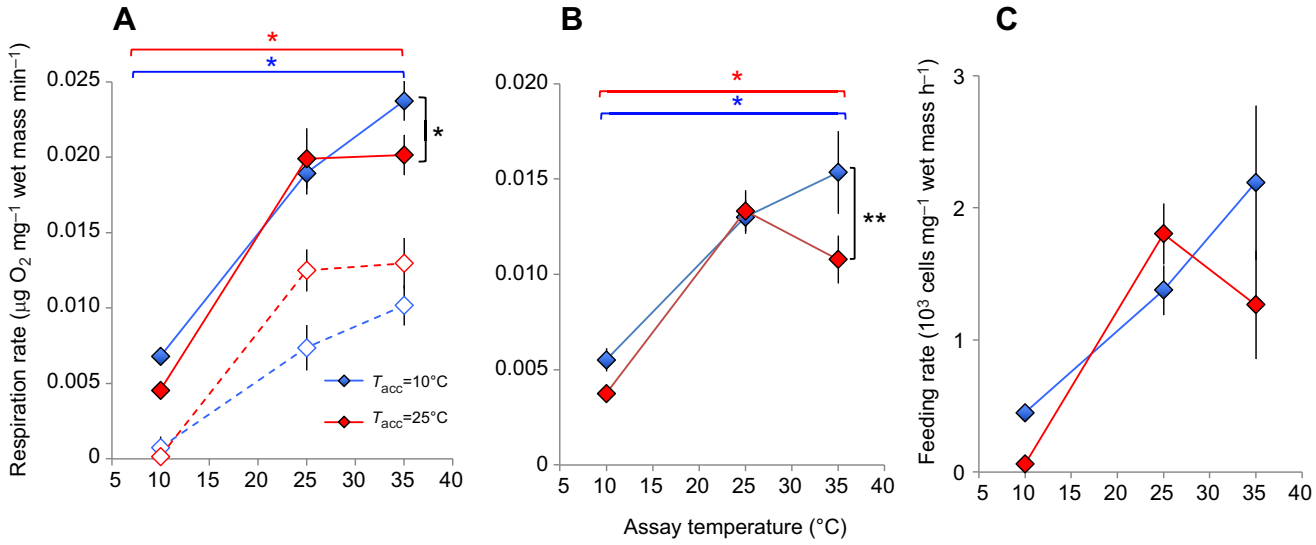

Fig. 2. Acclimation experiment 2. (A) Results of acclimation experiment 2 with active (continuous lines, filled symbols) and basal (dashed lines, open symbols) specific respiration rate (means and s.e.m.) measured in *D. magna* acclimated to either 10°C (blue) or 25°C (red) at three assay temperatures. Sample size was N=43 to 108 per assay temperature (see Yampolsky et al., 2020, tab 'Acclimation-1' for details). (B) Same data as in Fig. 1B; for comparison purposes, data points for the same three assay temperatures are shown. (C) Feeding rate (10^3 cells mg^{-1} wet mass h^{-1} measured in the same two acclimation temperature (T_{acc}) groups at the same three assay temperatures. Horizontal and vertical brackets show the results of Tukey's test, as in Fig. 1B. *P<0.05; **P<0.01. Tukey's tests for respiration rates were conducted for each experiment separately; the same tests conducted for both experiments together (see Table 1) yield identical results. Sample size: 66, 43 and 108 individuals for acclimation temperatures 10, 25 and 35°C, respectively.

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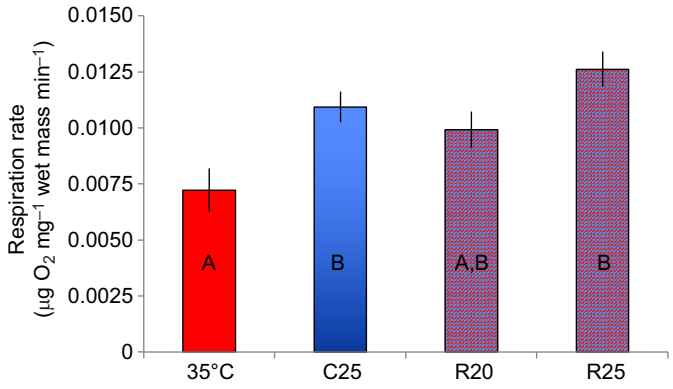

Fig. 3. Recovery experiment. Recovery of specific respiration rate (means and s.e.m.) in 25°C- acclimated *D. magna* after 1 h at 35°C followed by 24 h at either 20°C (R20) or 25°C (R25). *Daphnia magna* with no exposure to the higher temperature (control) are labelled C25. The difference among the four treatments is significant (one-way ANCOVA with *D. magna* wet mass as a covariable, d.f.=3, F=6.03, P<0.0018). Uppercase letters indicate levels different from each other by Tukey's test (levels sharing a letter are not significantly different, P=0.05). Sample sizes: exposure+recovery treatment, N=6 in each recovery treatment; controls, N=16.

with previous results of Zeis et al. (2004) it decreased with assay temperature in 10°C-acclimated *D. magna*, but not in their 25°C-acclimated counterparts (Fig. S2B). None of the within-assay temperature comparisons was significant owing to strong T_{acc}×clone interactions (data not reported).

Acclimation time experiment

The time necessary for the daphnids from the different lifetime acclimation temperatures to exhibit differences in respiration rate appeared to be asymmetric. In the down-acclimation experiment, *D. magna* previously reared at 18°C reached the elevated respiration rate of cold-acclimated *D. magna* within 72 h (Fig. 4A), while *D. magna* previously reared at 25°C failed to reach an elevated respiration rate at 10°C. However, the difference between 10°C-reared *D. magna* and 25°C-reared *D. magna* in respiration rate at 35°C was eliminated in as little as 1 h of exposure of 10°C-reared *D. magna* to 25°C (Fig. 4B).

Mitochondrial membrane potential

Mitochondrial membrane potential ($\Delta\psi$) increased with the assay temperature and, for the RoIs in the head, did so in a T_{acc}-specific manner (Fig. 5, Table 2). In all RoIs, the 10°C-acclimated *D. magna* had a higher $\Delta\psi$ measured at 10°C than their 25°C-acclimated counterparts (Fig. 5). This difference disappeared or even, in the brain RoI, became inverted (Fig. 5A) at higher assay temperatures. In the non-neural head tissues, $\Delta\psi$ increased with the assay temperature more rapidly in the 10°C-acclimated *D. magna* (Fig. 5B, Table 2). In the heart and the epipodite there was no significant interaction between acclimation and assay temperatures.

DISCUSSION

Of the two aspects of *Daphnia* biology affecting heat tolerance – geographic origin and recent acclimation history – only the latter had a strong effect on the thermal respiratory response. Despite strong evidence of local thermal adaptation in space (Yampolsky et al., 2014a; Seefeldt and Ebert, 2019) and in paleogeographic history (Geerts et al., 2015; Jansen et al., 2017; Shaw et al., 2018) of *Daphnia* populations, we saw no difference between geographically distinct heat-tolerant and heat-sensitive clones of *D. magna* with respect to the shape of their respiratory temperature performance curve. Whatever the plastic responses are that determine heat tolerance differences among genotypes, they are not likely to be related to changes in respiratory metabolism.

We show that the same genotypes acclimated to different rearing temperatures (10 versus 25°C) differ in their temperature performance curve shape (Fig. 1B) in a manner consistent with beneficial metabolic plasticity resulting in thermal compensation. Individuals acclimated to contrasting temperatures show evidence of metabolic compensation at both extremes of the studied temperature range. At 10–15°C, the cold-acclimated *D. magna* have higher specific respiration rates than warm-acclimated ones, indicating physiological and biochemical adjustments to compensate for temperature-limited low metabolic rate and consistent with the classic 'shift to the left' (Armitage and Lei, 1979). A similar pattern, consistent with McKee (1995) and Müller et al. (2018) data, is observed for feeding rate, which is not surprising, considering that oxygen intake and filtering rate are both proportional to the activity of filtering appendages (Pirow and Buchen, 2004) and therefore these two responses are difficult to untangle. At the temperature optimum (20–25°C), the two acclimation histories result in identical respiration and feeding rates. Cold-acclimated *D. magna* showed a lower ABT (i.e. organisms initiated deceleration of respiration rate at lower temperature), but this deceleration was slower than in their warm-acclimated counterparts, resulting in higher respiration rate at the sublethal temperature than in the warm-acclimated ones. These findings corroborate previous thermal acclimation studies establishing that *D. magna* thermal tolerance curves and breakpoints can shift (Armitage and Lei, 1979; Lamkemeyer et al., 2003; Zeis et al., 2004). This result is also consistent with data for killifish indicating that 25°C-acclimated animals have a higher oxygen consumption rate at assay temperatures above 35°C than their 15°C-acclimated counterparts (Schulte et al., 2011). Based on these observations, we hypothesize that a more significant reduction of respiration rate at 35°C in the warm-acclimated *D. magna* (Figs 1B and 2A,B) reflects beneficial metabolic compensation, consistent with the increased ability of warm-acclimated *D. magna* to survive longer at this temperature.

Compensatory changes in respiration rates are visible in total metabolism, but not in the lower-bound estimates of basal metabolism measurements obtained in urethane-anesthetized *D. magna* (Fig. 2A), in which striated muscles are inactive, including those in moving antennae and filtering appendages. This can be interpreted in two different ways. Firstly, one may hypothesize that basal metabolic demands are constrained and thus not subject to acclimatory changes. In this case, it is possible that most of respiratory acclimation occurs through reduction of muscular activity. This is consistent with the swimming velocity data (Fig. S2B) at 10°C, but not at 35°C, at which temperature the 25°C-acclimated *D. magna* showed higher swimming velocity than their 10°C-acclimated counterparts. However, owing to immobilization of filtering appendages in urethane-anesthetized *D. magna*, oxygen intake is limited to diffusion and the lack of response may simply reflect constraints imposed by existing gradients of oxygen concentration (Pirow and Buchen, 2004). Secondly, it is possible that basal respiration rate at 10°C is too close to resolution of our respirometry set-up and we simply failed to detect differences even if they were present.

Similar interpretational uncertainty applies to the evidence of metabolic compensation observed in filtering rates at temperature extremes. On the one hand, it may be fully constrained by respiration rates due to the effect of filtering apparatus movement intensity on both. On the other hand, it may reflect reduced demand for food, commensurate with reduced overall respiration. Finally, it

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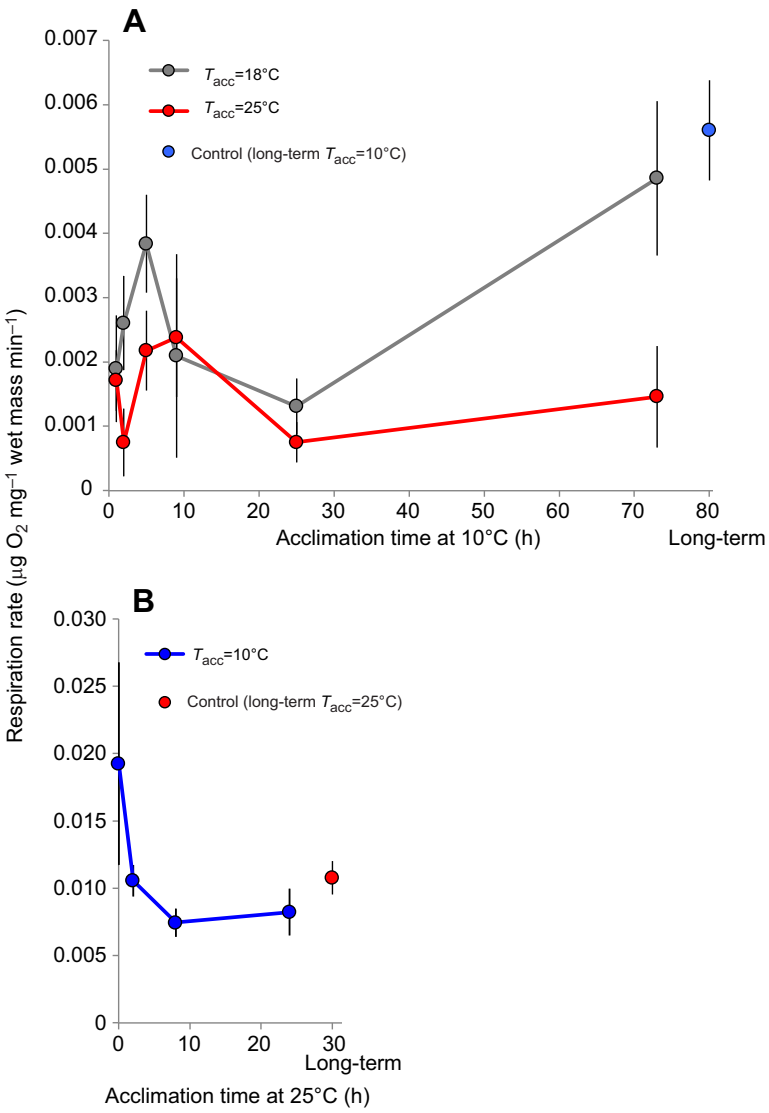

Fig. 4. Time necessary to achieve acclimation. Time course of specific respiration rate (means and s.e.m.) in daphnids from different acclimation temperatures (T_{acc}) when held at 10°C. (A) *Daphnia magna* previously acclimated to either 18 or 25°C exposed to 10°C for 0–72 h prior to respiration measurement at this temperature; control: respiration rate measured in *D. magna* after long-term acclimation to 10°C. Sample size: *N*=24 replicates. (B) *Daphnia magna* previously acclimated to 10°C exposed to 25°C for 0–24 h prior to respiration measurement at 37°C; control: respiration rate measured in *D. magna* after long-term acclimation to 25°C. Sample size: *N*=12 replicates.

may indirectly reflect reduced intensity of filtering apparatus movement, resulting in lower respiratory costs. The latter two possibilities are fully consistent with our previous finding that warm-acclimated *Daphnia* show across-the-board downregulation of gene expression pathways (Yampolsky et al., 2014a,b).

If the reduction of food consumption is indeed due to reduced activity of the filtering apparatus and not to increased rejection of food particles *Daphnia* are known to be capable of (Hartmann and Kunkel, 1991), then this reduction also translates as reduced respiration rate, as the same appendages that filter food also enable water flow through the filtering chamber where gas exchange occurs (Smirnov, 2017). This is consistent with the reduced oxygen consumption at sublethal temperatures observed in active, but not in anesthetized animals. Whether the observed differences between filtering rates are due to reduced movement of filtering appendages or to reduced ingestion is not known, and can be further investigated by direct observations of filtering appendages movement rate.

The reduction of swimming activity in both cold- and warm-acclimated *D. magna* observed at sublethal temperatures may be either a manifestation of reduction of metabolic costs, or, perhaps, a beneficial plastic response aimed at achieving passive sinking to lower, colder layers of water, or both. Either way, these responses are acclimation independent, as the proportion of time spent actively swimming decreased equally in both acclimation groups (Fig. S2A) and average velocity decreased in cold-acclimated, but increased in warm-acclimated, *D. magna* (Fig. S2B), contrary to the hypothesis that warm-acclimated *D. magna* compensate more than their cold-acclimated counterparts.

The mitochondrial membrane measurements partly corroborate the conclusions about higher reduction of respiratory metabolism in warm-acclimated *D. magna*. As expected, mitochondrial membrane potential increased with the assay temperature in all tissues (Fig. 5). Higher $\Delta\psi$ observed at 10°C in all tissues in *D. magna* acclimated to that temperature than in those acclimated to 25°C is consistent with difference in respiration rate (Figs 1B and 2B). If the deceleration of respiration rate at the highest assay temperatures in the 25°C-acclimated *D. magna* was caused by a deceleration in $\Delta\psi$, we would expect to see lower $\Delta\psi$ in 25°C-acclimated than in 10°C-acclimated *D. magna*. We observed this only in the non-neural head tissue (Fig. 5B), where membrane potential continues to increase beyond the 25°C assay temperature in cold-acclimated *D. magna*, but not in warm-acclimated ones, matching the pattern observed in the respiration rate experiments. This is consistent with the hypothesis

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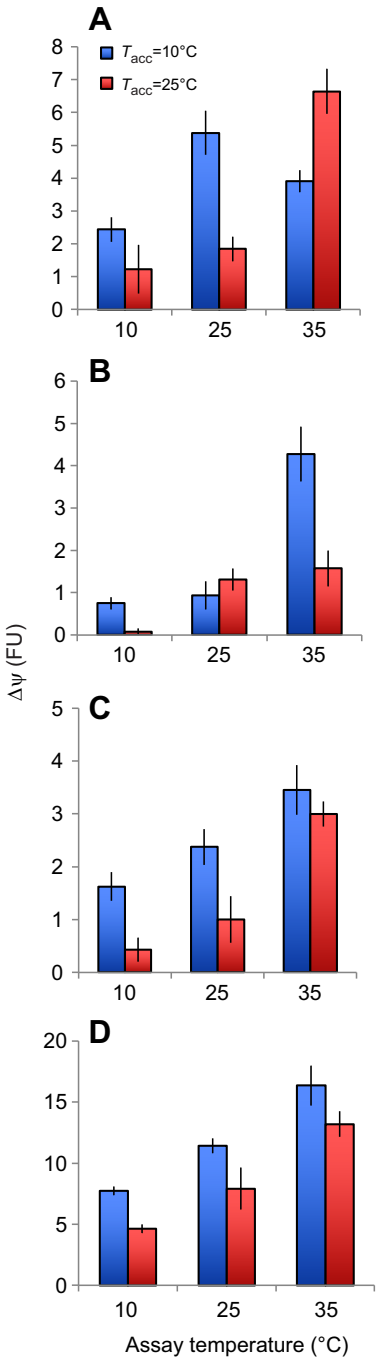

Fig. 5. Mitochondrial membrane potential measured as rhodamine-123 fluorescence (arbitrary fluorescence units, FU) at three assay temperatures in *D. magna* acclimated to either 10 or 25°C. (A) Brain; (B) non-neural head tissue; (C) heart; (D) epipodite. Sample size: *N*=25 to 29 per assay temperature (see Yampolsky et al., 2020, tab 'mtPotential' for details).

Table 2. ANCOVA of the effects of acclimation temperature (T_{acc}), assay temperature (T), clones and their interactions on mitochondrial membrane potential measured as rhodamine-123 fluorescence in four regions of interest (brain, head, heart and epipodite)

Source	d.f.	MS	*F* ratio	*P*>*F*
Brain				
T_{acc}	1	3.3	0.17	0.7
T	2	89.7	19.94	**0.0018**
$T\times T_{acc}$	2	60.3	44.79	**7.35×10^{-5}**
Clone	3	11.5	0.51	0.7
T_{acc}×clone	3	19.4	14.92	**0.0024**
T×clone	6	4.5	3.6	0.07
$T\times T_{acc}$×clone	6	1.3	0.34	0.91
Error	59	3.7		
Head				
T_{acc}	1	19.4	36.56	**0.005**
T	2	43.6	105.9	**7.15×10^{-5}**
$T\times T_{acc}$	2	13.8	44.6	**9.75×10^{-6}**
Clone	3	0.4	0.7	0.61
T_{acc}×clone	3	0.5	1.8	0.23
T×clone	6	0.4	1.43	0.34
$T\times T_{acc}$×clone	6	0.2	0.13	0.99
Error	59	2		
Heart				
T_{acc}	1	10.8	1.65	0.29
T	2	33.3	15.6	**0.0037**
$T\times T_{acc}$	2	1.1	0.55	0.6
Clone	3	1.8	0.26	0.85
T_{acc}×clone	3	6.6	3.35	0.1
T×clone	6	2.2	1.08	0.46
$T\times T_{acc}$×clone	6	2	1.42	0.2213
Error	60	1.4		
Epipodite				
T_{acc}	1	70.5	0.55	0.51
T	2	539.4	10.08	0.012
$T\times T_{acc}$	2	21.8	0.43	0.67
Clone	3	34.2	0.25	0.86
T_{acc}×clone	3	132.4	2.58	0.15
T×clone	6	55.3	1.06	0.47
$T\times T_{acc}$×clone	6	52	8.26	**7.04×10^{-7}**
Error	60	6.3		

P-values below 0.01 are given in bold.

that warm-acclimated *D. magna* achieve metabolic compensation by reducing mitochondrial membrane potential. One might hypothesize that such reduction is achieved by upstream regulatory mechanisms rather than through the expression of uncoupling proteins, as uncoupling would generate extra heat, further damaging rather than protecting mitochondria or other cellular components (Martinez et al., 2016a,b; Bryant et al., 2018). Of course, this conclusion only makes sense under the assumption that there is a direct link between tissue-specific patterns of membrane potential as measured by rhodamine assay and whole-organism respiration rates.

In other tissues, however, the predicted pattern was not observed (Fig. 5A,C,D). Notably, in the brain (Fig. 5A), the compensation effects occur in cold- rather than warm-acclimated *D. magna*, indicating that any possible thermal effects on neural function are not related to heat tolerance. No evidence of metabolic compensation occurs in the heart, or in mitochondria-rich epipodite tissues.

If the observed deceleration of respiration below the Arrhenius curve is indeed beneficial (i.e. not caused by direct heat damage), then it should be fully reversible. The opposite is not true: reversibility of changes does not prove their adaptive nature. For example, if deceleration of respiration rate is caused by oxygen availability or transport limitation, it may be fully reversible, and yet fully constrained. Yet, reversible phenotypic plasticity, termed 'phenotypic elasticity' (Wiesenthal et al., 2018) is thought to be indicative of adaptive value, particularly when such reversible changes carry an energetic cost. In this study the 24 h recovery at either 20 or 25°C after a sublethal temperature exposure resulted in reversal of respiration back to values measured in untreated controls (Fig. 3). While this does not prove the adaptive nature of such changes, it at least indicates that the plateau on the temperature performance curve is not caused by direct heat damage. Other recent

work further corroborates the ability of *Daphnia* to recover to optimal performance after acclimation to a suboptimum temperature, showing recovery of filtration capacity following acclimation to potentially lethal temperatures (Müller et al., 2018). The partial recovery found by Müller et al. is consistent with the idea that different physiological systems of an organism will have different thermal optima and tolerance ranges (Schulte, 2015).

Fast restoration of respiration after exposure to sublethal temperature (Fig. 3) and fast acquisition of the plastic response (Fig. 4B) indicate that the observed heat-induced metabolic rate plasticity is unlikely to be caused by any long-term structural changes in respiratory systems. The opposite direction of acclimation was much slower: it took 18°C-acclimated *D. magna* 72 h to reach a respiration rate at 10°C that was comparable to respiration of daphnids acclimated to this colder temperature and the 25°C-acclimated individuals did not reach the 10°C respiration rate at the end of the 3 day experiment (Fig. 4A). This is a very well documented effect: upward thermal acclimation is faster than downward acclimation (Fry, 1971). While this difference can be fully explained by a purely thermodynamic argument (Fry, 1971), we wish to speculate that acclimation to cold is different in nature in the sense that respiratory compensation requires structural changes, for example changes in membrane (Kraffe et al., 2007; Grim et al., 2014; Holmbeck and Rand, 2015) or proteome (Schwerin et al., 2009; Dietrich et al., 2018) composition. Our previous work demonstrated that reciprocal transfers from high and low acclimation temperatures on a scale of several days had a marked effect on lipid composition (Coggins et al., 2017) and that whole-body fatty acid composition can influence thermal tolerance when diet is enriched with a fluidity-increasing polyunsaturated fatty acid (Martin-Creuzburg et al., 2019). This is consistent with the idea of slower, structural changes necessary for acclimation to cold.

In conclusion, we provide evidence of metabolic compensation following acclimation to moderately low (10°C) versus moderately high (25°C) temperatures. This at least partially elastic, rapidly achievable response is consistent with sublethal (35°C) temperature tolerance of the two acclimation groups, and is observed in total, but not basal, respiratory metabolism. It is also not correlated with geographic origin or heritable heat tolerance level of genotypes tested. The lack of differences among genotypes indicates that the plastic compensation mechanism is conserved and therefore other mechanisms have to be invoked to explain local thermal adaptation.

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Competing interests
The authors declare no competing or financial interests.

Author contributions
Conceptualization: B.L.C., J.R.B., L.Y.Y.; Methodology: J.R.B., L.Y.Y.; Validation: R.H., L.Y.Y.; Formal analysis: B.L.C., L.Y.Y.; Investigation: B.L.C., C.E.A., A.C.P., R.H., M.N.E., L.Y.Y.; Resources: J.R.B.; Data curation: B.L.C., C.E.A., L.Y.Y.; Writing - original draft: L.Y.Y.; Writing - review & editing: B.L.C., C.E.A., A.C.P., L.Y.Y.; Visualization: C.E.A., L.Y.Y.; Supervision: J.R.B., L.Y.Y.; Project administration: J.R.B., L.Y.Y.

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Data availability
Measurements of respiration rates and mitochondrial potential are available from the Dryad Digital Repository (Yampolsky et al., 2020): https://doi.org/10.5061/dryad.c2fqz616k

Supplementary information
Supplementary information available online at
https://jeb.biologists.org/lookup/doi/10.1242/jeb.237727.supplemental

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