PHYSIOLOGICAL PERFORMANCE CURVES ACROSS PHYLOGENETIC AND FUNCTIONAL BOUNDARIES: WHEN ARE THEY USEFUL?

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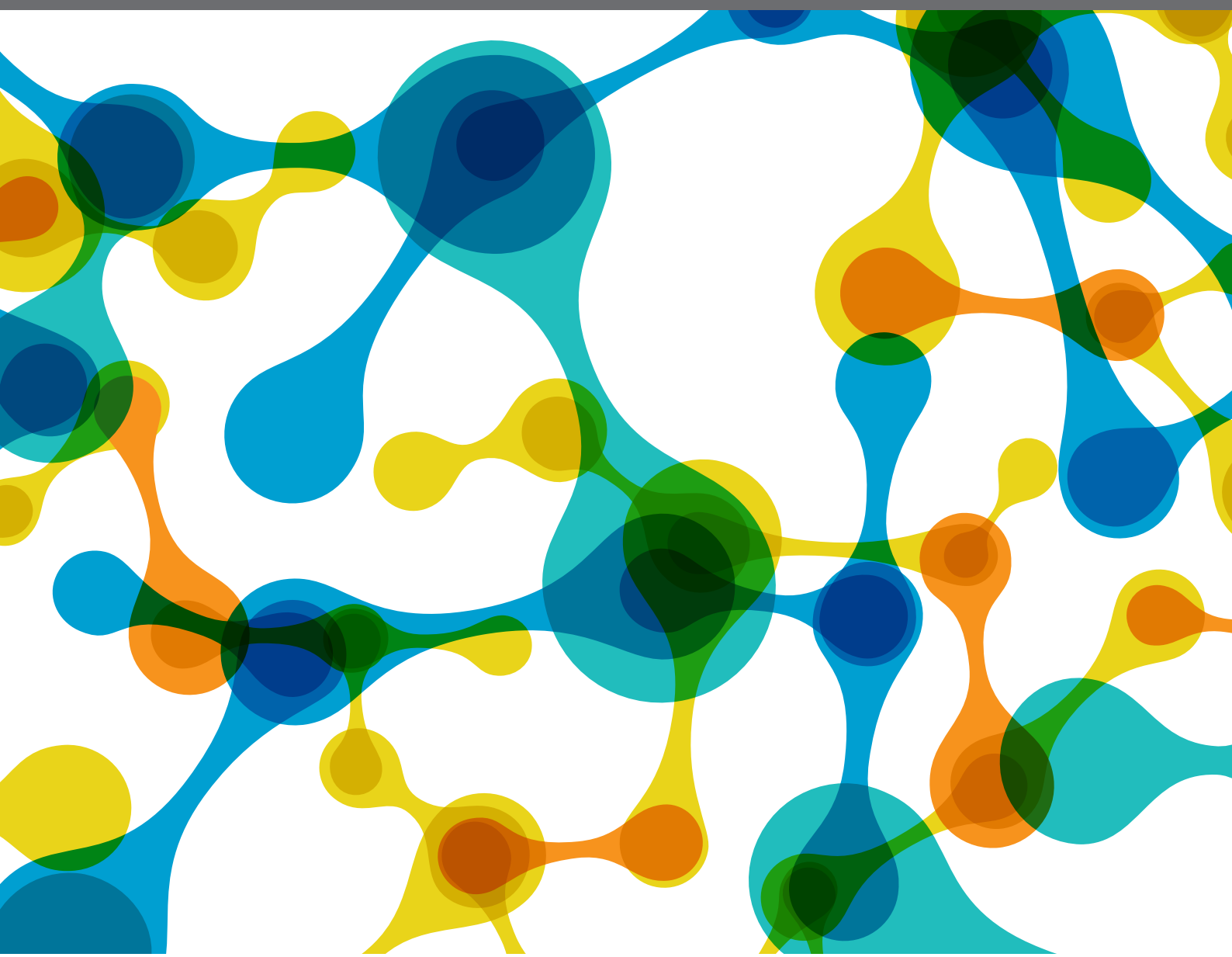

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PHYSIOLOGICAL PERFORMANCE CURVES ACROSS PHYLOGENETIC AND FUNCTIONAL BOUNDARIES: WHEN ARE THEY USEFUL?

Topic Editors:
Frank Seebacher, The University of Sydney, Australia
Alexander G. Little, Queen's University, Canada

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Plate-Based Respirometry to Assess Thermal Sensitivity of Zebrafish Embryo Bioenergetics *in situ*

*Erik Rollwitz and Martin Jastroch**

Department of Molecular Biosciences, The Wenner-Gren Institute, Stockholm University, Stockholm, Sweden

Oxygen consumption allows measuring the metabolic activity of organisms. Here, we adopted the multi-well plate-based respirometry of the extracellular flux analyzer (Seahorse XF96) to investigate the effect of temperature on the bioenergetics of zebrafish embryos (*Danio rerio*) *in situ*. We show that the removal of the embryonic chorion is beneficial for oxygen consumption rates (OCR) and penetration of various mitochondrial inhibitors, and confirm that sedation reduces the variability of OCR. At 48 h post-fertilization, embryos (maintained at a routine temperature of 28°C) were exposed to different medium temperatures ranging from 18°C to 37°C for 20 h prior OCR measurement. Measurement temperatures from 18°C to 45°C in the XF96 were achieved by lowering the room temperature and active in-built heating. At 18°C assay temperature, basal OCR was low due to decreased ATP-linked respiration, which was not limited by mitochondrial power, as seen in substantial spare respiratory capacity. Basal OCR of the embryos increased with assay temperature and were stable up to 37°C assay temperature, with pre-exposure of 37°C resulting in more thermo-resistant basal OCR measured at 41°C. Adverse effects of the mitochondrial inhibitor oligomycin were seen at 37°C and chemical uncouplers disrupted substrate oxidation gradually with increasing assay temperature. Proton leak respiration increased at assay temperatures above 28°C and compromised the efficiency of ATP production, calculated as coupling efficiency. Thus, temperature impacts mitochondrial respiration by reduced cellular ATP turnover at lower temperatures and by increased proton leak at higher temperatures. This conclusion is coherent with the assessment of heart rate, an independent indicator of systemic metabolic rate, which increased with exposure temperature, peaking at 28°C, and decreased at higher temperatures. Collectively, plate-based respirometry allows assessing distinct parts of mitochondrial energy transduction in zebrafish embryos and investigating the effect of temperature and temperature acclimation on mitochondrial bioenergetics *in situ*.

Keywords: extracellular flux, zebrafish, embryo, oxygen consumption, temperature, proton leak, mitochondria

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Frank Seebacher,
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Paul Craig,
University of Waterloo, Canada
Christopher Souders,
University of Florida, United States

****Correspondence:***
Martin Jastroch
martin.jastroch@su.se

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INTRODUCTION

All species require temperature adaptation of their bioenergetics for maintenance of metabolism and life. Low temperatures decrease cellular metabolism of ectothermic species as they mainly lack thermogenic capabilities for maintaining constant body temperatures. Hence, ectotherms must balance temperature-sensitive cellular energy production and consumption to ensure physiological functions, such as growth, activity, and reproduction. While water temperatures fluctuate naturally due to weather and seasons, temperature sensitivity becomes increasingly important in the long-term scope of global warming, where aquatic ecosystems and thus ectotherms are challenged. The global mean ocean surface temperature is expected to increase by 1.8–4°C by the end of this century (Rhein et al., 2013), which will presumably elevate ocean warming, acidification, and hypoxia (Hoegh-Guldberg and Bruno, 2010). The effect of warming will depend on the thermal niche of the respective species. Species living below their thermal optimum will benefit from warm conditions, while others close to their thermal limit will suffer (Shephard et al., 2010; Eymann et al., 2020).

Many factors, such as physiological limitations, macromolecules, and genetic traits, need to be considered when looking at the plasticity of ectothermic (and endothermic) species in their response to changing environmental temperatures. An important physiological factor is aerobic scope; it represents the absolute difference between the maximum and standard rates of organismal aerobic metabolism (Gleeson, 1981; Halsey et al., 2018). In aquatic ectotherms, a decrease in aerobic scope is considered as the beginning of physiological thermal limitation on both ends of the thermal window, caused by a reduced capacity of the cardiovascular and pulmonary system to meet the increasing oxygen demand (Portner and Knust, 2007). The slowing of ventilation and circulation in the cold and the insufficient increase in the warm cause a mismatch between oxygen delivery and demand, which results in a limitation of thermal tolerance (Pörtner, 2001). Additional molecular constraints impact optimal function of organisms across a wide range of temperatures. Ectotherms in cold environments may increase the production of enzymes to compensate for decreased catalytic activity (Guderley, 2004). These modifications, also referred to as extrinsic factors, can occur relatively fast in response to environmental changes. Changes in temperature may also affect the structure and stability of proteins and enzymes, such as the loss of substrate binding affinity in teleost fish with increasing assay temperatures (Fields and Somero, 1998). Processes that aim to stabilize proteins, for example, by altering amino acid composition and secondary structures of proteins (Tattersall et al., 2012), occur at a slower pace during development or over generations, also referred to as intrinsic modifications (Travis et al., 1999; Fangue et al., 2009). Some genetic traits may shift expression to genes that provide better protection against the prevailing environmental conditions to alter the optimal thermal window of ectotherms. Further, behavioral changes and selection of microhabitats need to be considered when looking at ectothermic model organisms (Angilletta et al., 2009). Taken together, the response to thermal fluctuations is complex due to many factors. A general molecular mechanism or genetical program that enables aquatic ectotherms to maintain basic physiological function in a relative wide thermal spectrum remains elusive.

To measure thermal viability in aquatic ectotherms, several methodological approaches and model organisms with various thermal tolerance have been used, such as annelids (*Arenicola marina*) sipunculids (*Sipunculus nudus*), bivalves (*Ostrea edulis*), cephalopods (*Lolliguncula brevis*), mollusks (*Laternula elliptica*), crustaceans (*Maja squinado*), and teleost fish (*Fundulus heteroclitus*; Zielinski and Po, 1996; Pörtner and Zielinski, 1998; Urban and Silva, 1998; Sommer and Pörtner, 1999; Frederich et al., 2000; Schulte, 2015; Eymann et al., 2020). The range of parameters to judge thermal plasticity includes gene expression, enzyme activities, motility (e.g., swimming speed), and heart and metabolic rates (e.g., indirectly *via* oxygen consumption; Lahnsteiner and Mansour, 2012; Little et al., 2013; Ferreira et al., 2014; Veilleux et al., 2015; Pichaud et al., 2019).

From the experimental point of view, a good model organism has a sequenced genome and can be genetically manipulated, thereby offering the possibility to establish causality of molecular mechanisms.

The zebrafish (*Danio rerio*) is a vertebrate model used broadly in many disciplines ranging from developmental biology to drug discovery, offering an array of established tools ranging from genetic modification to standardized behavioral analysis. Thermal adjustments of its metabolism can be investigated over a large thermal window, as the zebrafish naturally inhabits freshwater with a wide temperature range of 16.5 to 34°C (Engeszer et al., 2007) in the tropics of South Asia. Previous studies measured metabolic rates as oxygen consumption of living zebrafish in response to different temperatures, using metabolic chambers/tanks (Little et al., 2013). To further understand the underlying mitochondrial mechanisms, it would be advantageous to isolate mitochondria and determine mitochondrial activities with Clark-type oxygen electrodes, as has been done in mollusks, for example, Kurochkin et al.(2009). The quantity and purity of isolated mitochondria, however, are limited for a small organism, such as the zebrafish. With new technologies, such as plate-based respirometry of the Seahorse extracellular flux analyzer, however, it may be possible to get insights into mitochondrial mechanisms and adaptations in response to thermal challenges by subjecting living organisms to the assay and measure oxygen consumption *in situ* (Divakaruni et al., 2014). A few studies demonstrated how to adapt the Seahorse system to the zebrafish and how to measure respiration of embryos. Stackley et al. investigated the change of embryonic bioenergetics over the course of early development at a standard temperature of 28.5°C, showing the increase of oxygen consumption rates (OCR) from 3 to 48 h post-fertilization (hpf) in all respiratory parameters except proton leak (Stackley et al., 2011). The high individual variability of OCR (Souders et al., 2018) could be mitigated by sedation using tricaine (MS-222) pre-exposure (Raftery et al., 2017). Spheroid capture plates of the 24-well Seahorse system were used to determine the impact of the chorion on the respiratory responses to chemical uncoupler FCCP (Carbonyl cyanide-p-trifluoromethoxyphenylhydrazone)

during development (Souders et al., 2018). Lee et al. (2019) curated Seahorse studies using whole zebrafish embryos, providing a list until 2019, where experimental conditions have been summarized to optimize the assay for testing toxins and pollutants (Lee et al., 2019). Plate-based respirometry is found in a few studies for testing the impact of toxins during early embryo development. For example, Shim and colleagues used 96-well Seahorse technology, enabling higher n-values to test the bioenergetic effects of triclosan, a synthetic antimicrobial agent commonly used in consumer goods (Shim et al., 2016).

In this paper, we applied the XF96 Seahorse extracellular flux analyzer platform to investigate the bioenergetic effects of various assay temperatures (from 18°C to 45°C) and the effect of pre-exposing the zebrafish embryos to temperatures ranging from 18°C to 37°C.

MATERIALS AND METHODS

Animals

Maintenance

Fertilized eggs of wild-type (AB-strain) zebrafish (*Danio rerio*) were obtained from the Zebrafish core facility at Karolinska Institute after crossing under controlled conditions using breeding traps. Dividers were pulled at 6 AM and eggs were collected for transport to Stockholm University. The eggs were kept in E3 medium (5 mM NaCl, 0.17 mM KCl, 0.33 mM CaCl2, 0.4 mM MgCl2, and 10^{-5}% Methylene Blue, pH 7.2). The freshly laid eggs were kept at 28.5°C and picked up within 3 h. During transport, the embryos were briefly exposed to approximately 22°C before returning to 28.5°C. Up to 200 embryos were maintained in one large petri dish (150 mm × 20 mm, P5606, Sarstedt, Germany). Checking for dead embryos and medium replacement was done twice during the first experimental day and once in the morning of the second day (see **Supplementary Figure** for comprehensive description of the study design). The studies were approved by the Stockholm North Ethical Committee with the permit number 14049–2019.

Dechorionation

At 24 h post-fertilization (24 hpf), the chorion was removed by a combination of enzymatic digestion and mechanical force using thin forceps. Embryos were transferred with a plastic Pasteur pipette from their large petri dishes to a beaker containing E3 medium. Pronase from *Streptomyces griseus* (SKU: 10165921001, Sigma-Aldrich, United States) was added at a concentration of 2 mg/ml. The medium was swirled in a continuous movement of the beaker and the digestion states were checked microscopically for approximately 5 min, or until the first embryos separated from their chorion. Additionally, the chorion integrity was checked by gently nudging the chorionated embryos with a pipet tip. The Pronase treatment was considered complete when the choria appeared soft. Embryo survival rates of nearly 100% after enzymatic digestion were achievable with careful treatment and immediate deactivation of Pronase activity, by shortly washing the embryos five times in Pronase-free E3 medium. Notably, solely the washing steps separated a fair amount of choria and embryos, while the residual individuals had to be separated with forceps. For this, the chorion of remaining embryos was removed by tearing the chorion with two forceps (Dumont no. 5) after transfer to a smaller petri dish (100 mm x 20 mm, P5606, Sarstedt, Germany). We improved the duration of chorion removal of ~350 embryos from 60 to 30 min during the course of our studies. Chorion removal is also possible without Pronase treatment, but results in a significant number of crushed embryos (~15% in our hands), due to the mechanical force imposed on the embryos while tearing the rigid chorion with forceps. Furthermore, the time of chorion removal will increase about 3–5 times.

Temperature Exposure

The embryos were checked for viability before exposing them to either 18°C, 23°C, 28°C, 33°C, or 37°C in sealed 50 ml falcon tubes (62.547.254, Sarstedt, Germany) in a temperature-controlled water bath. Up to 70 embryos were transferred into the falcon tubes containing 50 ml of E3 medium. The embryos were exposed to the respective temperature for 20 h (see **Supplementary Figure**). We did not term temperature exposure "acclimation", as we did not evaluate acclimation steady states. At 48 hpf, the zebrafish embryos were transferred to respirometric analysis.

Microscopical Phenotyping

Embryos were transferred from the temperature incubation tubes into a petri dish (100 mm multi 20 mm, P5606, Sarstedt, Germany) for microscopy. The embryos were categorized into the phenotypes of normal, curved, and "other" (collection of very minor diverse phenotypes, e.g., showing edema) by visual inspection. Images were taken with the EVOS XL core microscope (Invitrogen, United States) at 4x magnification. Scale bars were added during post-processing with the aid of a Bürker counting chamber and the software AxioVision (release 4.8, Carl Zeiss, Germany).

Protein and DNA Quantification

Embryo lysis, protein, and DNA quantification were performed to evaluate differences in biomass of the embryos in response to temperature pre-exposure.

Lysis of Zebrafish Embryos

A petri dish (100 mm x 20 mm, P5606, Sarstedt, Germany) was filled with RIPA buffer (SDS 0.1%, NaCl 150 mM, IGEPAL CA-630 1%, deoxycholic acid 0.5%, and TRIS 50 mM), and one embryo at a time was transferred with as little E3 medium as possible. Individual embryos were transferred into a 2 ml safe lock reaction tube using a pipet set to 10 µl and a pipet tip which was cut to avoid shearing. The embryos (a pool of five) were either stored at 20°C or processed directly. For lysis, a 3 mm carbide bead and 50 µl of RIPA buffer were added to the tubes and the five embryos were lysed

with the TissueLyser LT (QIAGEN, Netherlands) for 5 min at a frequency of 40 Hz. Afterward, the samples were placed on ice for 30 min and the lysate was diluted 1:3 by adding distilled water. The dilution was centrifuged at 4°C for 30 min at full speed with a table top centrifuge (Centrifuge 5,427 R, Eppendorf, Germany), and the supernatant (about 130 µl) was removed and transferred to a fresh reaction tube for storage at 20°C.

Protein Quantification

Protein concentration was determined using Bradford reagent. The protein samples were diluted 1:3 to reduce measurement interference with the RIPA buffer. Dilutions of 2 mg/ml non-free fatty acid-free BSA (A7906, Sigma-Aldrich, United States) stock were used as standard. In a black 96-well microplate (655,096, Greiner Bio-One), 5 µl of sample were added to 250 µl Bradford reagent (B6916, Sigma), mixed and incubated for 5 min at room temperature, and shielded from light. The absorbance was detected using an EnSpire Multimode Plate Reader (PerkinElmer, United States) at 595 nm. All samples were measured in triplicates.

DNA Quantification

DNA concentration was determined using the Quant-iT™ PicoGreen® dsDNA Kits (P7589, Invitrogen, United States), following the manufacturer's protocol (high range standard curve, 1 µg – 0.01 µg). For the DNA standard curve, 2 µg/ml of stock dsDNA solution was diluted in 1:3 RIPA buffer. The fluorescent signal was detected with a CLARIOstar plate reader (BMG Labtech, Germany) adjusted to the PicoGreen pre-set (top optic, excitation: 483–15, dichroic: auto 502.8, and emission: 530–30). All samples were measured in triplicates.

Seahorse (XF96) Temperature

To adjust the XF96 Seahorse extracellular flux analyzer (Agilent, United States) to various temperatures, the desired environmental and tray temperature were set in the menu instrument/administration/temperature control. To achieve 18°C measurement temperature, the analyzer was placed in a temperature-controlled 5°C room for passive cooling. Notably, the analyzer was moved back to room temperature after the measurement to avoid condensation in the machine. For 23°C and 28°C assay temperatures, cooling of the Seahorse was supported by desk fans circulating cool room air into the vents. The analyzer maintained assay temperatures between 33°C and 45°C without any external support, while a thermal fuse prevented the use of the machine beyond 45°C by disabling the in-built heaters.

Seahorse Measurements

Cartridge Preparation

The Seahorse cartridges (102,416, Agilent, United States) were hydrated by adding 200 µl of XF calibrant solution (100,840, Agilent, United States) to the utility plate and incubated overnight at 37°C.

Sedation and Transfer to the Seahorse Well Plate

Tricaine (E10521, Merck, United States) was used to reduce embryo movement in the Seahorse well and to stabilize OCR, as previously shown by Raftery et al. (2017). The embryos were incubated in a petri dish containing 125 mg/ml tricaine in E3 medium and then transferred into the Seahorse XF96 cell culture plates (101,085, Agilent, United States) with a 100 µl cut pipet tip before adding 170 µl of XF base medium with minimal DMEM (103,334, Agilent, United States) to each well. The central position of the embryos was checked with a microscope and corrected with a shortened Microloader™ pipet tip (EP5242956003, Eppendorf, Germany), if necessary. For analyzing the effect of sedation on the variability of OCR, tricaine was injected *via* port A of the XF96 cartridge.

Measurement Protocol

The baseline of embryonic respiration was measured in 15 cycles, with one cycle consisting of 1 min mixing, 1 min waiting, and 2 min measuring. The last three cycle values before oligomycin addition were averaged to determine embryonic embryonal respiration. Oligomycin (O4876, Sigma-Aldrich, United States), injected *via* port A at a final concentration of 25 µm to inhibit ATP synthase, served to determine respiration linked to ATP synthesis and to proton leak. Thirty cycles were required to establish steady-state rates at low temperatures, and the average of three lowest consecutive points was taken as value for proton leak. Subsequently, 8 µm carbonyl cyanide-p-trifluoromethoxyphenylhydrazone (FCCP; C2920, Sigma-Aldrich, United States) was added (port B) to uncouple respiration and maximize substrate oxidation for eight cycles. The average of the three highest points determined maximal respiration. Finally, 1.5 µm rotenone (R8875, Sigma-Aldrich, United States) and 1.5 µm antimycin A (A8674, Sigma-Aldrich, United States) served to block mitochondrial respiration and determine non-mitochondrial respiration as the average of the three lowest consecutive points. This value was subtracted to receive mitochondrial respiration rates. ATP-linked respiration was calculated by subtracting proton leak respiration from basal respiration. Spare respiratory capacity was calculated by subtracting basal mitochondrial from maximal respiration. Coupling efficiency (CE) reports the fraction of mitochondrial respiration dedicated to ATP synthesis and is the quotient of ATP-linked/basal mitochondrial respiration. In cases, where proton leak respiration is negligibly low, or slightly negative values were received by subtraction of non-mitochondrial respiration, CE was set to 1. **Figure 1** depicts a schematic measurement example of embryos exposed and measured at 28°C. Mitochondrial inhibitors and FCCP were dissolved in dimethyl sulfoxide and diluted with XF base medium (103,334, Agilent, United States) with minimal DMEM. The mitochondrial stress assay is a fatal experiment for the embryo. The embryos are considered alive during the measurement of basal respiration and may die during oligomycin treatment (~70 min after start), since ATP synthase inhibition *via* oligomycin is irreversible (Wyatt and Buckler, 2004). Furthermore, FCCP and finally rotenone/antimycin A treatment are deleterious for the organism.

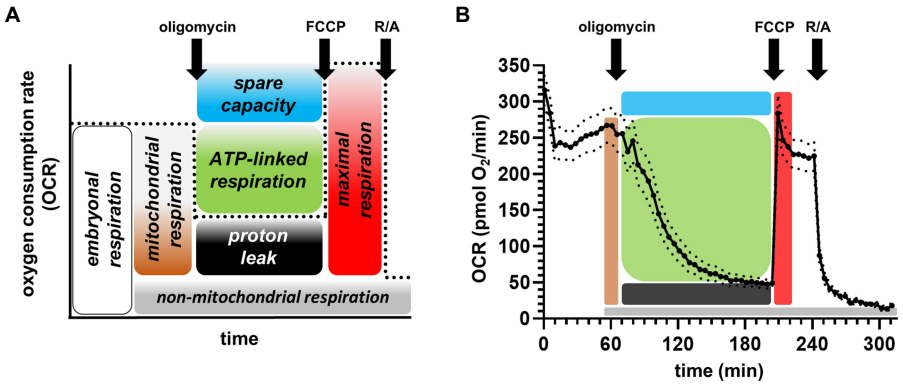

FIGURE 1 | Partitioning embryonic respiration into bioenergetic modules. **(A)** General scheme depicting the analysis of the mitochondrial stress assay, showing the different respiratory modules. FCCP: carbonyl cyanide-p-trifluoromethoxyphenylhydrazone; R/A: rotenone and antimycin A. **(B)** Exemplary averaged trace measuring embryos at 28°C. The solid trace represents the mean, while the dotted lines depict the standard error of the mean.

Analysis of Embryo Heart Rate

Heartbeats per second of the transparent embryos were counted visually using the microscope. All embryos were removed from the water bath incubation simultaneously and placed in fresh 100 mm x 20 mm petri dishes (P5606, Sarstedt, Germany) filled with E3 medium at RT. The embryos were anesthetized with five drops of 4 g l^{-1} tricaine solution to reduce movement, since Raftery et al. (2017) demonstrated no impact of 75 mg/l – 175 mg/l tricaine treatment on heart rate within 2 hours. Then, the embryos were aligned vertically and the heart rate was recorded for 15 s with a four-digit hand-held tally counter (ENM, United States). The final heart rate was determined by counting temperature pre-exposed embryos on two different experimental days.

Statistics

All data are presented either individually or as means ± SEM. Students *t*-test was applied to test for the difference of OCR of chorionated vs. dechorionated embryos. Ordinary one-way ANOVA followed by Tukey's multiple comparisons test was applied to investigate the impact of sedation on OCR and the impact of temperature on protein/DNA content. Two-way ANOVA followed by Tukey's multiple comparisons test was performed to test for differences in OCR in response to temperature pre-exposure and different assay temperatures. For differences in heart rate, ordinary one-way ANOVA was applied and followed by Dunnett's multiple comparisons test, with the standard temperature of 28°C as control value. Values of $p < 0.05$ were considered statistically significant. All statistical tests were performed using GraphPad Prism version 8.0.0 for Windows, GraphPad Software, United States.

RESULTS

Analysis of Cellular and Mitochondrial Respiration

The well-established mitochondrial stress assay for extracellular flux analyzers was applied to measure the bioenergetics of the zebrafish embryos. **Figure 1A** depicts the scheme of the assay and how to calculate the values for the different modules of mitochondrial energy transduction upon the injections of compounds. ATP synthase inhibitor oligomycin served to partition basal mitochondrial respiration into ATP-linked and proton leak respiration, chemical uncoupler (FCCP) stimulates respiration to determine maximal respiration/substrate oxidation and spare respiratory capacity of the embryo. Finally, the respiratory chain inhibitors rotenone and antimycin A (R/A) enabled correction for non-mitochondrial respiration. A typical averaged respiratory trace of embryos maintained and measured at 28°C is shown in **Figure 1B**, and the criteria to average values for embryonic, proton leak, FCCP, and non-mitochondrial respiration are described in Material and Methods.

Impact of the Chorion on Respiration

Next, we assessed the impact of the chorion on respiration one day after chorion removal. The microscopic images (**Figure 2A**) depict the embryos with and without choria, which were positioned centrally in the well to reduce variability between the respiratory traces. Furthermore, embryos with chorion appeared to be crushed after the experimental run, e.g., leaking of the yolk sack, while straightened embryos without the chorion appeared to be more intact (see supplemental images). In our hands, the chorion removal 24 h prior measurement resulted in increased basal respiration and improved the response to FCCP (**Figure 2B**). Thus, chorion removal was used for all following experiments.

Impact of Sedation on Respiration

Tricaine has been used previously (Raftery et al., 2017) to reduce the noise of the respiratory traces. We confirmed the positive effect of tricaine in our experiments, showing that the variability of the respiratory readouts was reduced (**Figure 2C**) and importantly, did not significantly change the average OCR value.

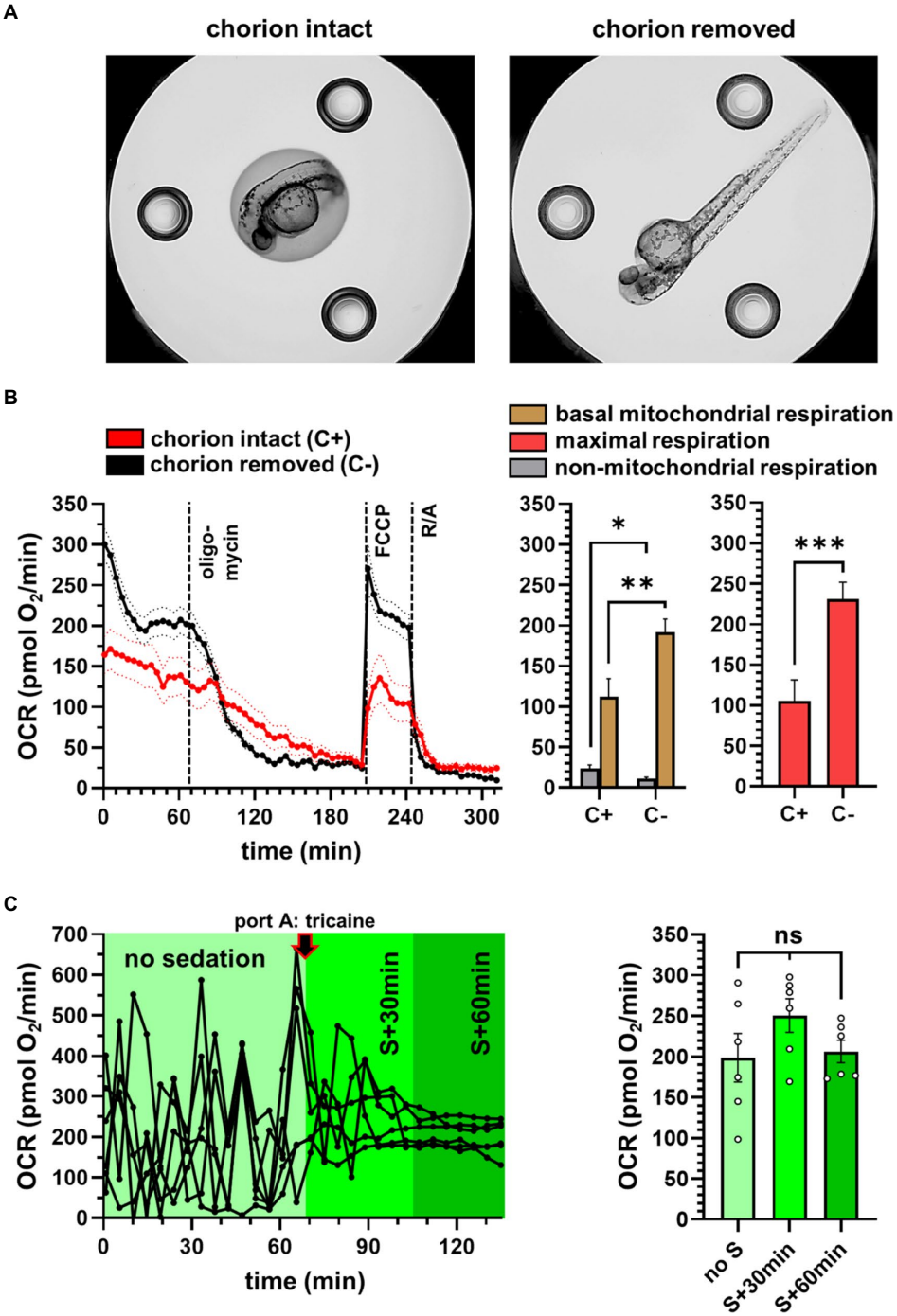

FIGURE 2 | Experimental conditions to measure zebrafish embryos in the XF96 Seahorse. **(A)** Microscopic image of the zebrafish embryos in the Seahorse well, without (left) and with (right) chorion removal. **(B)** Effect of the chorion on OCR, showing reduced OCR with an intact chorion. Mean traces represent 36 wells for each group. **(C)** Effect of sedation on the respiratory traces. Respiratory variability was evaluated on individual traces using six individual embryos. S: sedation. $^{*}p<0.05$; $^{**}p<0.005$; $^{***}p<0.0005$.

Temperature Exposure and Morphological Consequences

Next, we exposed the embryos, previously maintained at 28°C, to medium temperatures of 18°C, 23°C, 28°C, 33°C, and 37°C for 20 h, before moving them to the measurement well plate (**Figure 3A** and **Supplementary Figure**). The embryos showed pronounced changes in morphology toward a curved phenotype with exposure to 37°C (**Figure 3B**), prompting us to investigate changes in biomass in response to temperature exposure. We found that neither protein nor DNA content was significantly

different between experimental groups (**Figure 3C**). Thus, we did not correct the OCR for biomass.

Measurements of Embryonic Respiration at Different Temperatures

First, we set up the XF96 in stable thermal environments to achieve stable measurement temperatures as described in Material and Methods. Temperatures registered in the tray, which reflect closest the measurement temperature, and the interior temperature of the instrument can be retrieved. Exemplified for one plate (**Figure 4A**), the temperature appeared to be quite stable over the measurement time. Overall, the OCR traces of differentially pre-exposed embryos at different assay temperatures reveal the increase of respiration

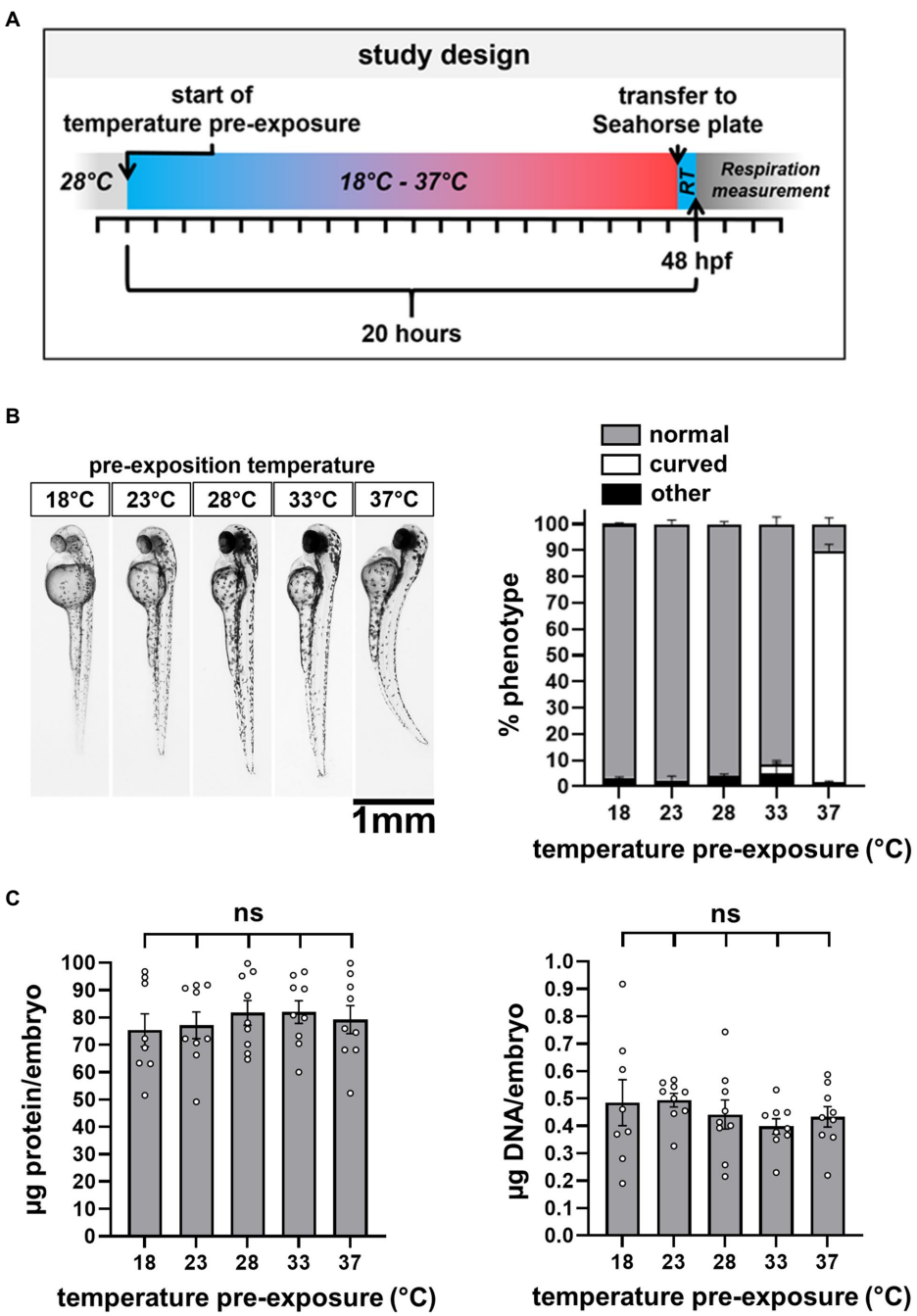

FIGURE 3 | Study design and temperature-dependent changes in embryo morphology. **(A)** Short outline of the study design. A comprehensive outline can be found in the **Supplementary Figure**. RT: room temperature of 22 ± 1°C. **(B)** Light microscopy to evaluate shape changes, categorized in normal, curved, and other. **(C)** Quantification of protein and DNA content per embryo.

rates with increasing temperatures and respiratory failure at higher assay temperatures (**Figure 4B**).

Analysis of Respiratory Traces at Different Temperatures

Next, we analyzed the respiration traces of the assay temperatures from 18°C to 37°C, partitioning respiration into functional modules as described above (**Figure 1**). Basal mitochondrial respiration increased with temperature up to 28°C and remained stable up to 37°C (**Figure 5A**). Non-mitochondrial respiration was low throughout the different measurement conditions. Proton leak rates were negligible up to 23°C but then increased with increasing assay temperature (**Figure 5B**), while ATP-linked respiration remained stable between 28°C and 37°C. Maximal respiration between 18°C and 28°C was stable, while spare respiratory capacity decreased simultaneously (**Figure 5C**). Given the decreasing effects of FCCP with increasing temperatures, the maximal respiration rates at 33°C and 37°C remained below basal respiration, presumably due to damage over time and thus, were highly confounded and not used for further analysis. Increasing proton leak and stable ATP-linked respiration should decrease the efficiency to convert nutrient energy to ATP, and this is seen as decreasing coupling efficiency (CE) with higher temperatures (**Figure 5D**). Notably, CEs were calculated for each fish individually and are therefore a powerful internally standardized parameter for individual mitochondrial efficiency.

Mitochondrial Factors Potentially Limiting Metabolic Performance

To get insights into the mitochondrial limitation for systemic metabolism, we plotted selected mitochondrial respiration parameters against assay temperatures. Mitochondrial activity is low at 18°C assay temperature, but this is not caused by the limitation of substrate oxidation, as maximal respiration is not reduced and substantial spare respiratory capacity is available (**Figure 5C**). Therefore, low mitochondrial activity must be controlled by low ATP synthase activity, or more likely, by low cellular ATP turnover. Respiration linked to ATP turnover increases steadily with assay temperature, depicted in the temperature performance curve of ATP-linked respiration (**Figure 6A**). Coupling efficiency is stable up to 28°C assay temperature and then drops by about 0.15 = 15%, meaning that 15% more energy is lost as heat due to increased proton leak at temperatures above 28°C (**Figure 6B**). The lack of trustable FCCP rates at 33–37°C prevented us from analyzing limitations of substrate oxidation. Collectively, we would expect a decrease of metabolic performance at lower and higher temperatures. Using heart rate as an independent indicator of systemic metabolic rate (**Figure 6C**), we found peak rates for embryos pre-exposed to 28°C, while the heart rate was significantly decreased in embryos pre-exposed to 18°C and 37°C.

Resilience of Embryonic Respiration to High Temperatures by Pre-exposure to Warm Conditions

Finally, we analyzed whether temperature pre-exposure improves temperature resilience of embryonic respiration at higher temperatures. Therefore, we plotted basal mitochondrial respiration measured between 18°C and 45°C of the cold (18°), normal (28°), and warm (37°C) pre-exposed embryos (**Figure 6D**). In 18°C pre-exposed embryos, oxygen consumption was generally lower. Respiration of embryos at 18°C and 28°C, the latter resembling the routine maintenance temperature, withstands assay temperatures up to 37°C. Embryos which were pre-exposed to 37°C, however, maintained higher basal respiration rates up to 41°C. At 45°C assay temperatures, respiration of all embryos collapsed immediately.

DISCUSSION

The life cycle bottlenecks, which represent the most temperature-sensitive life stages, are often not clearly defined (Dahlke et al., 2020). We decided to investigate the developing embryo as vulnerable bioindicator for temperature sensitivity and climate change, as we can apply Seahorse technology on whole embryos. The assessment of respiration rates in zebrafish embryos with the XF96 extracellular flux analyzer allows measuring systemic oxidative metabolism in a multi-well format, determining with various mitochondrial effector injections, which modules of mitochondrial energy transduction are changed in response to different treatments. These changes in oxygen consumption enable us to address distinct bioenergetic mechanisms. We investigated the effects of temperature pre-exposure and assay temperature on the mitochondrial performance of zebrafish embryos to receive mechanistic insights underlying systemic metabolism and its limits. We show that slow respiration in the cold is caused by low ATP turnover and is not limited by mitochondrial oxidative power, suggesting slow cellular metabolism. In the warm, basal mitochondrial respiration is stable before dropping above 37°C, paralleled by an increasing proton leak which becomes more impactful for the consumption of proton motive force and therefore, limits mitochondrial efficiency. Our experiments also show that the thermal window of stable embryonic respiration rates can be extended toward higher temperatures by pre-exposing the embryos to the warmth.

The plate-based respirometry of the XF96 analyzer can measure up to 92 individuals in parallel and imposes less shearing stress on the embryos as compared to chamber-based respirometry, which requires constant stirring. XF analyzers have been used for zebrafish bioenergetics in previous studies (Stackley et al., 2011; Shim et al., 2016; Raftery et al., 2017; Souders et al., 2018; Lee et al., 2019). In contrast to previous observations showing reduced respiration after chorion removal in islet capture plates of the XF24 system (Souders et al., 2018), we found that dechorionation of embryos enhanced respiration and responses to mitochondrial inhibitors and uncouplers in the XF96 system. Notably, our embryos were dechorionated at a later developmental stage of post-fertilization, which could

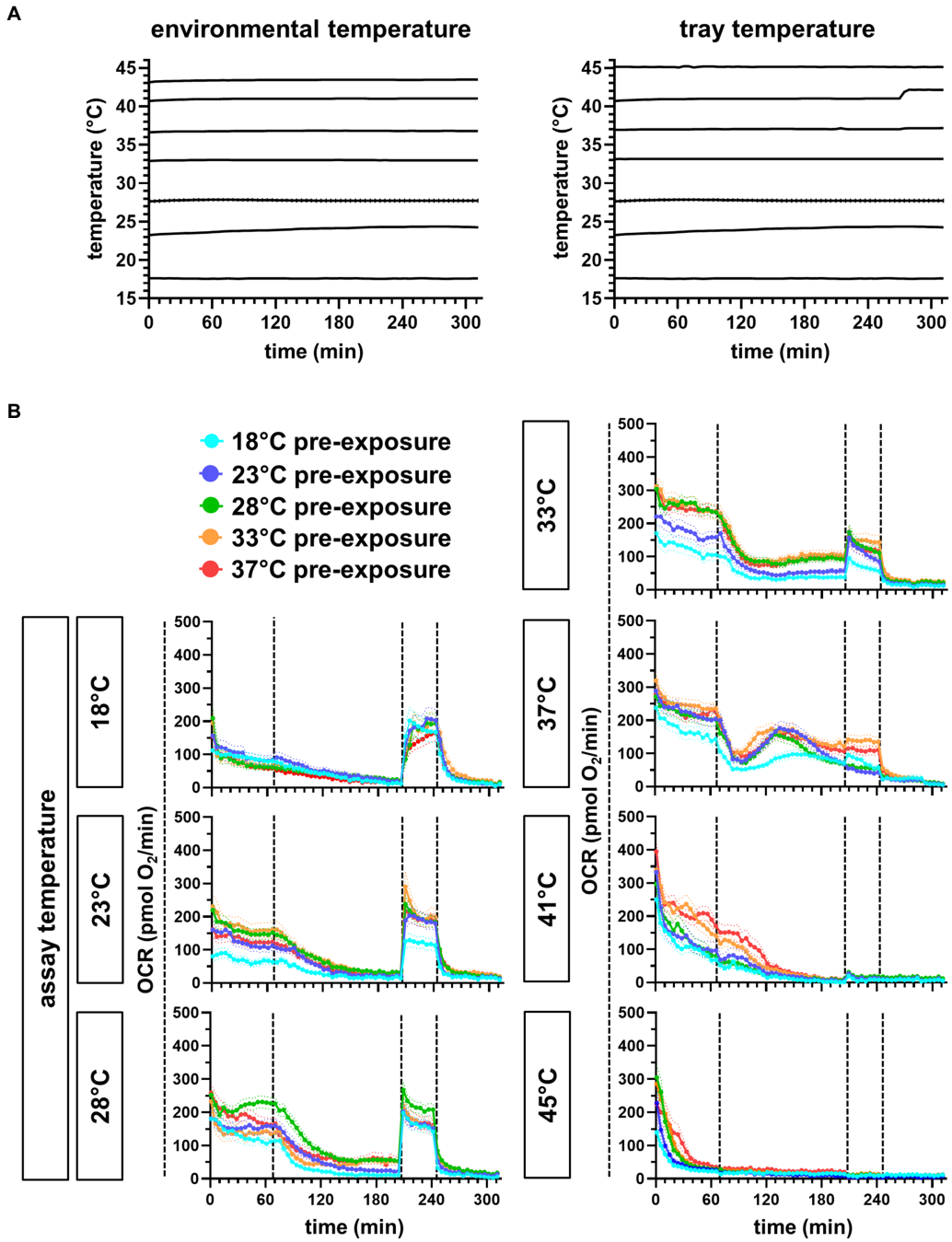

FIGURE 4 | Continuous temperature registration of the XF96 Seahorse and respiration traces of differentially exposed embryos at different assay temperatures. **(A)** Instrument temperatures of the interior (environmental temperature) and of the tray, where the plate wells are located. **(B)** Pre-exposure temperatures are depicted in different trace colors. The mean trace is represented by n individual measurements ($n = 16$ for 18°C, 41°C, and 45°C; $n = 32$ for 23°C, 28°C, 33°C, and 37°C, measured on two experimental days).

explain discrepant results. However, physical damage on the embryo in the small respiratory chamber of about 2–3 µm clearance also appeared to be visually absent when the chorion had been removed prior measurement. We confirmed that sedation with tricaine reduces variability of oxygen traces that may be caused by spontaneous activity of the embryos. The mitochondrial inhibitor concentrations were deduced from previous publications, where effective inhibition of the ATP synthase in 50 hpf embryos was shown with 25 µm oligomycin (Gibert et al., 2013), maximal stimulation of substrate oxidation in 48 hpf zebrafish with 8 µm FCCP, and full inhibition of complex I and III with 1.5 µm rotenone/antimycin A

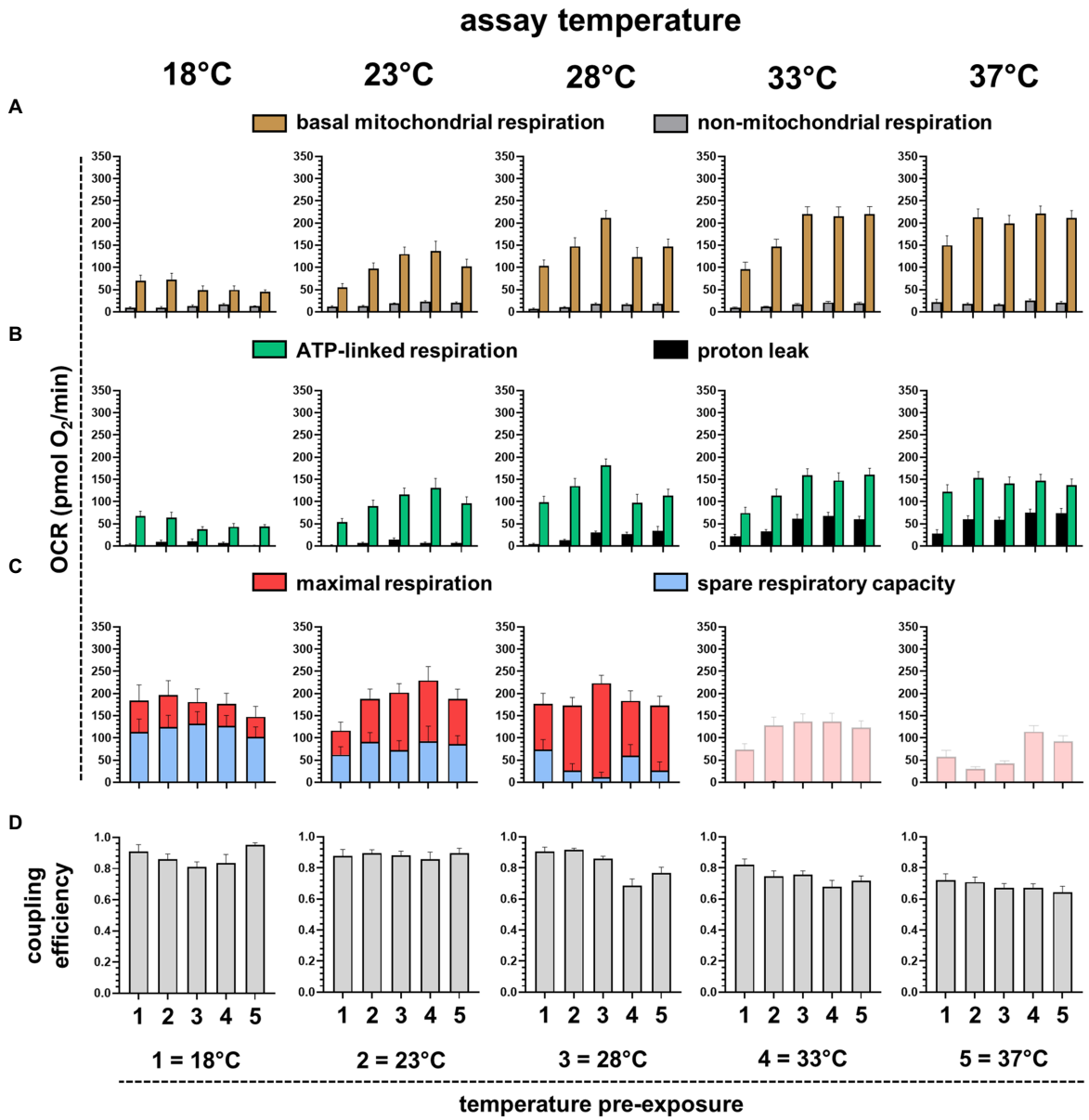

FIGURE 5 | Analysis of different respiratory parameters of embryos pre-exposed to different temperatures and measured at different assay temperatures. **(A)** Mitochondrial (brown) and non-mitochondrial (gray) respiration determined with rotenone and antimycin A. **(B)** ATP-linked (green) and proton leak (black) respiration determined using oligomycin. **(C)** Maximal respiration (red) induced with FCCP and spare respiratory capacity (blue), determined by subtracting basal respiration. At 33°C and 37°C, FCCP did not induce respiration above basal respiration and thus, could not be further analyzed. **(D)** Coupling efficiency (CE) calculated as fraction of respiration dedicated to ATP synthesis (ATP-linked respiration) of basal mitochondrial respiration. Averaged values represent 16–32 individuals.

(Lee et al., 2019). Some compound concentrations, however, may have to be systematically re-assessed in future studies, as, for example, temperature may alter the sensitivity to uncoupler reagents. FCCP treatment at high assay temperatures (33°C and 37°C) did not induce higher respiration rates above basal levels, indicating vulnerability of the embryos. Furthermore, some cellular functions of the embryos may decay during the experiments, e.g., due to ATP depletion after oligomycin injection, which may also confound uncoupler-induced respiration rates. These caveats can be overcome by either changing the uncoupling compounds and/or concentrations depending on temperature, by changing measurement times, or by directly injecting various compounds in the first port to split the mitochondrial stress assay into separate experiments. Similarly, further experimentation could be applied to exclude any deleterious effects of the sedative tricaine at different assay temperatures or in differently temperature-exposed embryos. We applied concentrations in our experimental set up that were either used in many publications

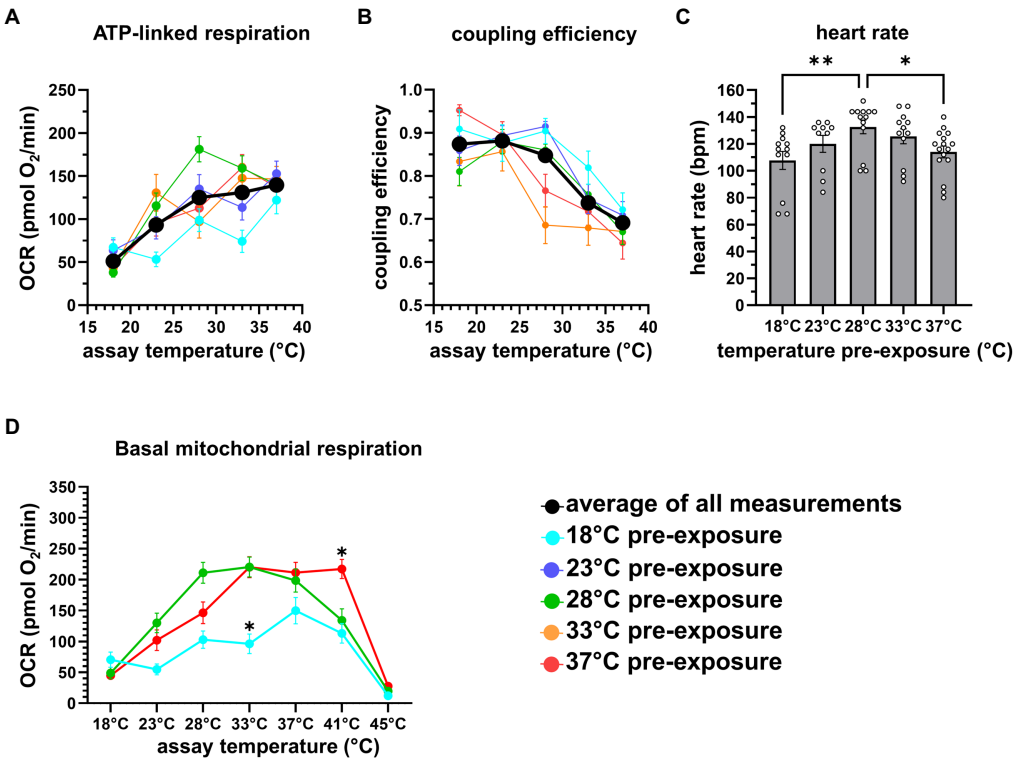

FIGURE 6 | Temperature sensitivity of respiration. **(A)** Colored traces depict the averages of ATP-linked respiration of different temperature pre-exposures, the black solid trace represents the average of all measurements. **(B)** Colored traces indicate coupling efficiency of different pre-exposures, the black solid trace represents the average of all measurements. **(C)** Bar chart depicting the average of the heart rate ($n = 12–14$) in response to temperature pre-exposure, measured at room temperature. **(D)** Basal mitochondrial respiration of 18°C, 28°C, and 37°C pre-exposed embryos is plotted vs. assay temperature, revealing lower respiratory activity of 18°C pre-exposed embryos, and the resilient respiration rates of 37°C vs. 28°C pre-exposed embryos at higher assay temperature. *$p < 0.05$; **$p < 0.005$.

by others, briefly checked for sensitivity, or which did not show obvious adverse effects (e.g., tricaine over time) during the conventional mitochondrial stress assay.

We chose to start exposure to different temperatures not before 24 hpf despite reducing the exposure time to 20 h, as direct exposure of fertilized eggs to 33°C and 37°C would result in low survival rates, which are likely due to the impairment of gastrulation, a critical developmental step that is marked by blastoderm epiboly occurring at approximately 5.25 hpf (Kimmel et al., 1995). Maintaining the embryos on the standard temperature of 28.5°C ensured undisturbed onset of development and increased survival in the later temperature exposure experiments.

In the oxygen traces at 37°C assay temperature, we found reproducible increases of proton leak respiration 30 min after oligomycin injection. The molecular nature of this increase remains unknown but could reside in apoptotic processes, which increase mitochondrial membrane permeability, such as permeability transition.

From all the OCR parameters, it transpires that embryonic metabolism is reduced at 18°C, reflected by decreased ATP-linked respiration. Notably, ATP-linked respiration did not further increase at higher temperatures, thus increasing the impact of the mitochondrial proton leak. We quantified the energetic efficiency to produce ATP by calculating coupling efficiency CE (Kabra et al., 2021). CE is the fraction of mitochondrial respiration linked to ATP synthesis (CE = ATP-linked respiration/basal mitochondrial respiration) and decreases from >0.85 to about 0.7 above 33°C assay temperature. Thus, the efficiency to convert nutrient energy to ATP decreases by about 15% and could negatively impact energy metabolism. These observations are in accordance with data of isolated mitochondria in ectotherms. For example, substrate oxidation in isolated fish muscle mitochondria increases with assay temperature (Guderley and Johnston, 1996), and proton permeability of isolated liver mitochondria increases with acclimation temperatures, as shown for the common carp (Jastroch et al., 2007) or the cane toad (Trzcionka et al., 2008).

Which mitochondrial parameters appear most important for metabolic performance in response to temperature? Using embryo heart rates as indicator of systemic metabolism, we found a bell-shape distribution over exposure temperatures, peaking around 28°C. Slower metabolism below 28°C is best reflected in the reduced ATP-linked respiration. At higher temperature above 28°C, CE is impacted by the proton leak, possibly limiting metabolic performance. Thus, these two parameters could be used to explain temperature phenomena on metabolic performance. Importantly, CE as internally standardized parameter can also be used in cross-study

comparisons, as experimental differences of absolute values are eliminated.

We found that 37°C pre-exposed individuals expand their thermal window for stable mitochondrial respiration rates up to 41°C, demonstrating some capacity to expand tolerance of higher temperatures. This observation could be instrumental for judging the impact of shifting environmental temperatures, e.g., during global warming. At least during early development, pre-exposure to higher temperatures is beneficial.

This project explored the impact of temperature on mitochondrial bioenergetics. With these assays, we aim to investigate effects of genetically modified zebrafish to identify mechanisms that are causally linked to thermo-tolerance. Furthermore, the experimental protocol can be used to understand how environmental pollutants (e.g., heavy metal ions) interfere with the temperature-bioenergetics axis of an aquatic organism, also using other species.

Collectively, we show that the Seahorse extracellular flux analyzer platform can robustly assess mitochondrial function *in situ* and that the analysis of respiratory parameters could be integrated in modeling of temperature sensitivity of metabolic performance in small organisms.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, and further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

The animal study was reviewed and approved by the Stockholm North Ethical Committee with the permit number 14049–2019.

AUTHOR CONTRIBUTIONS

ER performed all experiments and analyzed the data. ER and MJ wrote the manuscript. MJ conceptualized the study. All authors have edited and proof-read the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphys.2021.746367/full#supplementary-material

REFERENCES

Angilletta, M. J., Sears, M. W., and Pringle, R. M. (2009). Spatial dynamics of nesting behavior: lizards shift microhabitats to construct nests with beneficial thermal properties. *Ecology* 90, 2933–2939. doi: 10.1890/08-2224.1

Dahlke, F. T., Wohlrab, S., Butzin, M., and Pörtner, H.-O. (2020). Thermal bottlenecks in the life cycle define climate vulnerability of fish. *Science* 369, 65–70. doi: 10.1126/science.aaz3658

Divakaruni, A. S., Paradyse, A., Ferrick, D. A., Murphy, A. N., and Jastroch, M. (2014). Analysis and interpretation of microplate-based oxygen consumption and pH data. *Methods Enzymol.* 547, 309–354. doi: 10.1016/B978-0-12-801415-8.00016-3

Engeszer, R. E., Patterson, L. B., Rao, A. A., and Parichy, D. M. (2007). Zebrafish in the wild: a review of natural history and new notes from the field. *Zebrafish* 4, 21–40. doi: 10.1089/zeb.2006.9997

Eymann, C., Götze, S., Bock, C., Guderley, H., Knoll, A. H., Lannig, G., et al. (2020). Thermal performance of the European flat oyster, Ostrea edulis (Linnaeus, 1758)—explaining ecological findings under climate change. *Mar. Biol.* 167:17. doi: 10.1007/s00227-019-3620-3

Fangue, N. A., Richards, J. G., and Schulte, P. M. (2009). Do mitochondrial properties explain intraspecific variation in thermal tolerance? *J. Exp. Biol.* 212, 514–522. doi: 10.1242/jeb.024034

Ferreira, E. O., Anttila, K., and Farrell, A. P. (2014). Thermal optima and tolerance in the Eurythermic goldfish (*Carassius auratus*): relationships between whole-animal aerobic capacity and maximum heart rate. *Physiol. Biochem. Zool.* 87, 599–611. doi: 10.1086/677317

Fields, P. A., and Somero, G. N. (1998). Hot spots in cold adaptation: localized increases in conformational flexibility in lactate dehydrogenase A4 orthologs of Antarctic notothenioid fishes. *Proc. Natl. Acad. Sci.* 95, 11476–11481. doi: 10.1073/pnas.95.19.11476

Frederich, M., DeWachter, B., Sartoris, F. J., and Pörtner, H. O. (2000). Cold tolerance and the regulation of cardiac performance and Hemolymph distribution in *Maja squinado* (Crustacea: Decapoda). *Physiol. Biochem. Zool.* 73, 406–415. doi: 10.1086/317735

Gibert, Y., McGee, S. L., and Ward, A. C. (2013). Metabolic profile analysis of zebrafish embryos. *J. Visualized. Exp: JoVE.* 71:e4300. doi: 10.3791/4300

Gleeson, T. T. (1981). Preferred body temperature, aerobic scope, and activity capacity in the monitor lizard, Varanus salvator. *Physiol. Zool.* 54, 423–429. doi: 10.1086/physzool.54.4.30155835

Guderley, H. (2004). Metabolic responses to low temperature in fish muscle. *Biol. Rev.* 79, 409–427. doi: 10.1017/S1464793103006328

Guderley, H., and Johnston, I. I. (1996). Plasticity of fish muscle mitochondria with thermal acclimation. *J. Exp. Biol.* 199, 1311–1317.

Halsey, L. G., Killen, S. S., Clark, T. D., and Norin, T. (2018). Exploring key issues of aerobic scope interpretation in ectotherms: absolute versus factorial. *Rev. Fish Biol. Fish.* 28, 405–415. doi: 10.1007/s11160-018-9516-3

Hoegh-Guldberg, O., and Bruno, J. F. (2010). The impact of climate change on the world's marine ecosystems. *Science* 328, 1523–1528. doi: 10.1126/science.1189930

Jastroch, M., Buckingham, J. A., Helwig, M., Klingenspor, M., and Brand, M. D. (2007). Functional characterisation of UCP1 in the common carp: uncoupling activity in liver mitochondria and cold-induced expression in the brain. *J. Comp. Physiol. B.* 177, 743–752. doi: 10.1007/s00360-007-0171-6

Kabra, U. D., Affourtit, C., and Jastroch, M. (2021). Respiratory parameters for the classification of dysfunctional insulin secretion by pancreatic islets. *Meta* 11:405. doi: 10.3390/metabo11060405

Kimmel, C. B., Ballard, W. W., Kimmel, S. R., Ullmann, B., and Schilling, T. F. (1995). Stages of embryonic development of the zebrafish. *Dev. Dyn.* 203, 253–310. doi: 10.1002/aja.1002030302

Kurochkin, I. O., Ivanina, A. V., Eilers, S., Downs, C. A., May, L. A., and Sokolova, I. M. (2009). Cadmium affects metabolic responses to prolonged anoxia and reoxygenation in eastern oysters (*Crassostrea virginica*). *Am. J. Phys. Regul. Integr. Comp. Phys.* 297, R1262–R1272. doi: 10.1152/ajpregu.00324.2009

Lahnsteiner, F., and Mansour, N. (2012). The effect of temperature on sperm motility and enzymatic activity in brown trout Salmo trutta, burbot Lota lota and grayling Thymallus thymallus. *J. Fish Biol.* 81, 197–209. doi: 10.1111/j.1095-8649.2012.03323.x

Lee, S., Lee, H., and Kim, K.-T. (2019). Optimization of experimental conditions and measurement of oxygen consumption rate (OCR) in zebrafish embryos exposed to organophosphate flame retardants (OPFRs). *Ecotoxicol. Environ. Saf.* 182:109377. doi: 10.1016/j.ecoenv.2019.109377

Little, A. G., Kunisue, T., Kannan, K., and Seebacher, F. (2013). Thyroid hormone actions are temperature-specific and regulate thermal acclimation in zebrafish (Danio rerio). *BMC Biol.* 11, 1–15. doi: 10.1186/1741-7007-11-26

Pichaud, N., Ekström, A., Breton, S., Sundström, F., Rowinski, P., Blier, P. U., et al. (2019). Cardiac mitochondrial plasticity and thermal sensitivity in a fish inhabiting an artificially heated ecosystem. *Sci. Rep.* 9, 1–11. doi: 10.1038/s41598-019-54165-3

Pörtner, H. (2001). Climate change and temperature-dependent biogeography: oxygen limitation of thermal tolerance in animals. *Naturwissenschaften* 88, 137–146. doi: 10.1007/s001140100216

Portner, H. O., and Knust, R. (2007). Climate change affects marine fishes Through the oxygen limitation of thermal tolerance. *Science* 315, 95–97. doi: 10.1126/science.1135471

Pörtner, H.-O., and Zielinski, S. (1998). Environmental constraints and the physiology of performance in squids. *S. Afr. J. Mar. Sci.* 20, 207–221. doi: 10.2989/025776198784126421

Raftery, T. D., Jayasundara, N., and Di Giulio, R. T. (2017). A bioenergetics assay for studying the effects of environmental stressors on mitochondrial function in vivo in zebrafish larvae. *Comp. Biochem. Physiol. Part C: Toxicol. Pharmacol.* 192, 23–32. doi: 10.1016/j.cbpc.2016.12.001

Rhein, M., Rintoul, S. R., and Aoki, S. (2013). "Observations: ocean," in *Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change*. eds. T. F. Stocker, D. Qin, G.-K. Plattner, M. Tignor, S. K. Allen, J. Boschung, A. Nauels, Y. Xia, V. Bex and P. M. Midgley (Cambridge: Cambridge University Press), 255–316.

Schulte, P. M. (2015). The effects of temperature on aerobic metabolism: towards a mechanistic understanding of the responses of ectotherms to a changing environment. *J. Exp. Biol.* 218, 1856–1866. doi: 10.1242/jeb.118851

Shephard, S., Beukers-Stewart, B., Hiddink, J. G., Brand, A. R., and Kaiser, M. J. (2010). Strengthening recruitment of exploited scallops Pecten maximus with ocean warming. *Mar. Biol.* 157, 91–97. doi: 10.1007/s00227-009-1298-7

Shim, J., Weatherly, L. M., Luc, R. H., Dorman, M. T., Neilson, A., Ng, R., et al. (2016). Triclosan is a mitochondrial uncoupler in live zebrafish. *J. Appl. Toxicol.* 36, 1662–1667. doi: 10.1002/jat.3311

Sommer, A., and Pörtner, H. (1999). Exposure of Arenicola marina to extreme temperatures:adaptive flexibility of a boreal and a subpolar population. *Mar. Ecol. Prog. Ser.* 181, 215–226. doi: 10.3354/meps181215

Souders, C. L., Liang, X., Wang, X., Ector, N., Zhao, Y. H., and Martyniuk, C. J. (2018). High-throughput assessment of oxidative respiration in fish embryos: advancing adverse outcome pathways for mitochondrial dysfunction. *Aquat. Toxicol.* 199, 162–173. doi: 10.1016/j.aquatox.2018.03.031

Stackley, K. D., Beeson, C. C., Rahn, J. J., and Chan, S. S. L. (2011). Bioenergetic profiling of zebrafish embryonic development. *PLoS One* 6:e25652. doi: 10.1371/journal.pone.0025652

Tattersall, G. J., Sinclair, B. J., Withers, P. C., Fields, P. A., Seebacher, F., Cooper, C. E., et al. (2012). Coping with thermal challenges: physiological adaptations to environmental temperatures. *Compr. Physiol.* 2, 2151–2202. doi: 10.1002/cphy.c110055

Travis, J., McManus, M. G., and Baer, C. F. (1999). Sources of variation in physiological phenotypes and their evolutionary significance. *Am. Zool.* 39, 422–433. doi: 10.1093/icb/39.2.422

Trzcionka, M., Withers, K. W., Klingenspor, M., and Jastroch, M. (2008). The effects of fasting and cold exposure on metabolic rate and mitochondrial proton leak in liver and skeletal muscle of an amphibian, the cane toad Bufo marinus. *J. Exp. Biol.* 211, 1911–1918. doi: 10.1242/jeb.016519

Urban, H.-J., and Silva, P. (1998). Upper temperature tolerance of two Antarctic Mollusks (Laternula elliptica and Nacella concinna) from potter cove, King George Island, Antarctic peninsula. *Reports. Polar Res. Alfred. Wegener. Institut. Polar. Mar. Res.* 299, 230–236.

Veilleux, H. D., Ryu, T., Donelson, J. M., van Herwerden, L., Seridi, L., Ghosheh, Y., et al. (2015). Molecular processes of transgenerational acclimation to a warming ocean. *Nat. Clim. Chang.* 5, 1074–1078. doi: 10.1038/nclimate2724

Wyatt, C. N., and Buckler, K. J. (2004). The effect of mitochondrial inhibitors on membrane currents in isolated neonatal rat carotid body type I cells. *J. Physiol.* 556, 175–191. doi: 10.1113/jphysiol.2003.058131

Zielinski, S., and Po, H. O. (1996). Energy metabolism and ATP free-energy change of the intertidal worm Sipunculus nudus below a critical temperature. *J. Comp. Physiol. B.* 166, 492–500. doi: 10.1007/BF02338292

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Plasticity of Performance Curves in Ectotherms: Individual Variation Modulates Population Responses to Environmental Change

Frank Seebacher[1]** *and Alexander G. Little*[2]

[1]School of Life and Environmental Sciences, University of Sydney, Sydney, NSW, Australia, [2]Department of Biology, Biosciences Complex, Queen's University, Kingston, ON, Canada

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Jörn Rittweger,
Helmholtz Association of German Research Centers (HZ), Germany

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Jonas Böcker,
Helmholtz Association of German Research Centers (HZ), Germany
Franck Brocherie,
Institut national du sport, de l'expertise et de la performance (INSEP), France

***Correspondence:**
Frank Seebacher
frank.seebacher@sydney.edu.au

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Many ectothermic animals can respond to changes in their environment by altering the sensitivities of physiological rates, given sufficient time to do so. In other words, thermal acclimation and developmental plasticity can shift thermal performance curves so that performance may be completely or partially buffered against the effects of environmental temperature changes. Plastic responses can thereby increase the resilience to temperature change. However, there may be pronounced differences between individuals in their capacity for plasticity, and these differences are not necessarily reflected in population means. In a bet-hedging strategy, only a subsection of the population may persist under environmental conditions that favour either plasticity or fixed phenotypes. Thus, experimental approaches that measure means across individuals can not necessarily predict population responses to temperature change. Here, we collated published data of 608 mosquitofish (*Gambusia holbrooki*) each acclimated twice, to a cool and a warm temperature in random order, to model how diversity in individual capacity for plasticity can affect populations under different temperature regimes. The persistence of both plastic and fixed phenotypes indicates that on average, neither phenotype is selectively more advantageous. Fish with low acclimation capacity had greater maximal swimming performance in warm conditions, but their performance decreased to a greater extent with decreasing temperature in variable environments. In contrast, the performance of fish with high acclimation capacity decreased to a lesser extent with a decrease in temperature. Hence, even though fish with low acclimation capacity had greater maximal performance, high acclimation capacity may be advantageous when ecologically relevant behaviour requires submaximal locomotor performance. Trade-offs, developmental effects and the advantages of plastic phenotypes together are likely to explain the observed population variation.

Keywords: bet-hedging, plasticity, swimming performance, acclimation, environmental variation

INTRODUCTION

Temperature is one of the most relevant physical state variables in biology because physiological rates and hence fitness are influenced by the thermal environment (Tattersall et al., 2012). High temperatures, in particular, cause damage to proteins and membranes and can thereby disrupt fundamental processes such as movement, growth and reproduction (Tattersall et al., 2012). Variation in the thermal environment can be a strong predictor of individual fitness and population persistence (Kingsolver and Buckley, 2017). In the current era of global warming, understanding thermal effects on organisms has assumed a new urgency because of their potential role in determining the success and biogeography of populations and species (Sinclair et al., 2016; Woods et al., 2018).

At a reductionist level, living organisms are comprised of networks of interacting biochemical pathways (Costanzo et al., 2021). Thermodynamics dictates that the rate of biochemical reactions depends on the temperature of the system. The thermal sensitivity of higher organismal traits such as locomotor performance or metabolic rate is then determined by the thermodynamics of flux through underlying biochemical pathways. Hence, each physiological rate has a characteristic temperature response, which is captured by 'thermal performance curves' (TPC; Huey and Kingsolver, 1989).

A TPC describes the change in a physiological rate across a range of acutely changing temperatures. The shape of TPCs is characteristically in the form of an inverted 'U', where rates increase with an increase in temperature until a maximum is reached beyond which rates decline with further temperature increases (Huey and Kingsolver, 1989; McKenzie et al., 2021; **Figure 1**). The decrease in rates at lower temperatures is caused by thermodynamic constraints in Gibb's free energy, while the decline in rates at high temperatures results from a loss of the quaternary or tertiary structures of enzymes and damage to membranes (DeLong et al., 2017).

However, TPCs are not fixed within individuals over time (Sinclair et al., 2016) or consistent among individuals within populations or species (Careau et al., 2014; Seebacher et al., 2015). Long-term exposure to different temperature regimes within or across generations can shift the TPC of individuals. Hence, transgenerational, developmental or reversible plasticity can result in changes in the maximum, mode and breadth of TPCs (Schulte et al., 2011; LeRoy et al., 2017). These epigenetic effects are at least partly regulated by DNA methylation and histone acetylation (Simmonds and Seebacher, 2017; Loughland et al., 2021). Plasticity can be beneficial if performance is optimised at the acute thermal environment experienced by individuals. However, there is a potential cost to plasticity if the temperature range at which performance maxima occur mismatches the prevailing thermal conditions in the environment (Bateson et al., 2014).

Importantly, TPCs and their plasticity also vary between individuals within populations (Seebacher et al., 2015). A potential ramification of this individual variation is that means of thermal performance across samples of individuals do not necessarily represent the population as a whole. Instead, population responses may be determined by a bet-hedging strategy (Haaland et al., 2020). In a bet-hedging scenario, populations comprise individuals with high capacity for acclimation that can fully compensate for an environmental change given sufficient time to acclimate and individuals with low acclimation capacity that cannot compensate physiological rates at all. We found this to be the case in mosquitofish (*Gambusia holbrooki*; Seebacher et al., 2015; Loughland and Seebacher, 2020). It is possible that variation in the plasticity of TPCs may disadvantage some individuals under particular conditions, but promote population resilience as a result of the increased diversity of phenotypes (Schindler et al., 2015). Our aim was to explore these relationships to document variation in the plasticity of TPCs between individuals and to test how this variation may affect population responses. In mosquitofish, we also observed a trade-off between the capacity for acclimation and performance under warm conditions (Seebacher et al., 2015). Hence, here, we model how differences in the plasticity of TPCs together with this trade-off affect population performance in different thermal environments.

We collated a large data set from previously published studies on swimming performance of mosquitofish (608 individuals), in which each individual was acclimated twice to determine the capacity for reversible plasticity (Seebacher et al., 2015; Loughland and Seebacher, 2020). We subsampled this data set to characterise the effects of variation in individual phenotypes on population responses. This data set is unique because data from double-acclimated animals that permit calculation of individual plasticity are rare and because the large number of samples available makes our modelling approach possible; to the best of our knowledge, similar data sets do not exist for other species. However, we acknowledge that data from a single species may lack generality, and our results represent a proof of concept but do not necessarily apply to all ectotherms. Our aims were (a) to characterise the variation in plasticity of TPCs between individuals within populations and (b) to determine the extent to which sample means drawn from population mask individual variation. Finally, (c) we modelled the extent to which variation in capacity for plasticity between individuals influences population responses to environmental temperature variation.

MATERIALS AND METHODS

Data

We re-analysed three different data sets on thermal acclimation of swimming performance (critical sustained swimming speed, U_{crit}) in mosquitofish (*Gambusia holbrooki*; Seebacher et al., 2015; Loughland and Seebacher, 2020). In each data set, individual fish were acclimated twice, to a cool (18 or 20°C) and to a warm (28°C) temperature in random order; acclimation temperatures corresponded to spring and summer temperatures at the capture site (Seebacher et al., 2014). All fish were sourced from the same wild population (Manly Dam, Australia 33°78′S; 151°26′E) and were acclimated for 3–4 weeks to the different

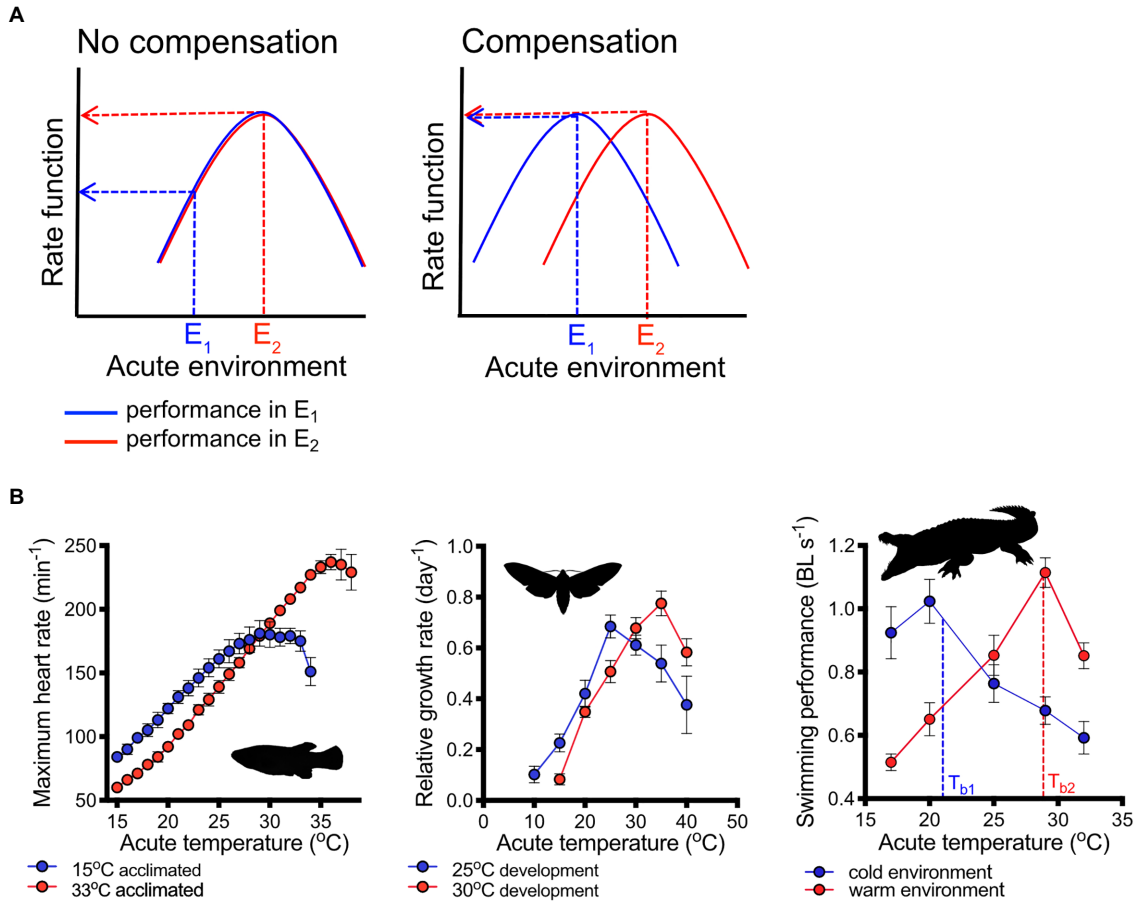

FIGURE 1 | Plasticity of thermal performance curves. Ideally, plasticity can shift performance curves so that physiological rate functions are perfectly buffered from changes in the thermal environment. The case of perfect compensation is shown (**A**, right panel) schematically as a right shift of the thermal performance curve (blue line = performance in cool environment, E1; red line = performance in warm environment, E2). Lack of compensation (left panel) would result in a substantial decrement in performance as temperatures decrease. Examples **(B)** of shifts in performance curves demonstrating different degrees of compensation: heart rate in killifish [*Fundulus heteroclitus*; redrawn from Safi et al. (2019), left panel] and growth rates in moth (*Maduca sexta*) larvae [central panel, redrawn from Kingsolver et al. (2020)] compensate partially for different acclimation or developmental temperatures, respectively. Thermal performance curves of swimming in crocodiles (*Crocodylus porosus*, right panel) thermoregulating in simulated winter (cool) and summer (warm) environments shifted horizontally so that swimming performance remained nearly constant at the different regulated body temperatures [T_{b1} and T_{b2} in cool and warm environments, respectively; redrawn from Glanville and Seebacher (2006)]. Means ± s.e. are shown in **(B)**, and images are from PhyloPic (http://phylopic.org/).

temperatures. After acclimation, U_{crit} of each fish was measured at different acute test temperatures. Experimental fish were of mixed sex (184 females, 424 males) and were sexually immature at the start of acclimation treatments. Fish were separated from each other during acclimation treatments to ensure that females were not pregnant at the time of U_{crit} measurements.

In the first data set (data set 1), $n = 48$ wild-caught fish were each acclimated to 20 and 28°C in random order, and after each acclimation treatment, U_{crit} was measured at 20, 28 and 32°C acute test temperatures in each fish (Seebacher et al., 2015). In the second data set (data set 2), U_{crit} of 416 double-acclimated (to 18 and 28°C), wild-caught fish was measured only at the acute test temperature that coincided with acclimation temperatures (Loughland and Seebacher, 2020). The third data set (data set 3) from the same publication (Loughland and Seebacher, 2020) was collected from third- or fourth-generation offspring (total $n = 144$ fish) bred in outdoor mesocosms from parents collected at the study site. Each of these fish was acclimated to 20 and 28°C, and U_{crit} of each fish was measured at 20 and 28°C acute test temperatures after each acclimation treatment.

Double acclimation permitted calculation of an index of acclimation capacity for each fish (Seebacher et al., 2015):

$$\text{Acclimation capacity} = 1 - \frac{(P_{28} - P_{20})}{[(P_{28} + P_{20})/2]},$$

where P_{28} is the U_{crit} of a fish that is acclimated to 28°C and measured at 28°C acute test temperature, while P_{20} is the equivalent measure at 20°C (or 18°C in data set 2). The acclimation capacity index indicates relative thermal compensation

(i.e. the ability to maintain relatively constant performance across thermal conditions) by contrasting the difference between P_{20} and P_{28}. Acclimation capacity approaches 1 as P_{20} approaches P_{28} and decreases as P_{20} decreases. If a fish overcompensated for low temperatures and $P_{20} > P_{28}$, the index will be >1. The index is based on the difference between P_{20} and P_{28} and is a dimensionless number that is independent from the absolute values of P_{20} and P_{28}. More details of experimental procedures are given in the original publications (Seebacher et al., 2015; Loughland and Seebacher, 2020).

Consequences of Variation on Interpretation of Samples

Our purpose here was to mimic typical approaches in the literature to assess acclimation of populations to test whether subsampling of populations can reflect true variation and responses of the population. Hence, we randomly subsampled the combined data sets 1 and 3 ($n = 192$ fish, see **Supplementary Material** for R code) to draw 10 samples of eight replicates each for warm- and cold-acclimated fish. Each of these 10 data sets mimics a fairly typical experiment in the literature, for example as in our study on mosquitofish where we compared acclimation in spring- and summer-caught fish (Seebacher et al., 2014).

We analysed each data set with a permutational analysis [in the R package lmPerm (Wheeler and Torchiano, 2016)] with acclimation temperature and acute test temperature as independent factors. Permutational analyses do not make assumptions about underlying data distributions but use the data *per se* to infer significant differences (Drummond and Vowler, 2012). Hence, values of p in permutational analyses are not associated with any particular distribution, and there are no test statistics (such as F or t; Ludbrook and Dudley, 1998). The value of p in permutational tests has the practical meaning of denoting the number of randomised permuted data sets for which the treatment effects were as or more extreme than the observed experimental data divided by the total number of permutations.

Consequences of Individual Variation for Population Responses

Our aim here was to model how populations comprised of individuals with different acclimation capacities respond to different environmental conditions. We modelled different phenotypic compositions of populations by selecting subpopulations from the complete data set (data sets 1, 2 and 3 combined; $n = 608$ fish), which were the top 10% of fish with the highest acclimation capacity (high), the bottom 10% with the lowest acclimation capacity (low) and the central 10% (centre); each of these subpopulations was comprised of 61 fish.

From the P_{20} and P_{28} values of each fish, we determined the slope of change in U_{crit} between these temperatures to estimate U_{crit} at intermediate temperatures assuming that acclimation has taken place. We simulated environmental conditions within the measured range (20–28°C) by either assuming constant conditions of 20 or 28°C or letting temperatures vary between 20 and 28°C. To model variable temperatures, we assumed sinusoidal temperature variation with a mean of 24°C and an amplitude of 4°C. We randomised the phase of the sinusoidal temperature fluctuation 100 times for each fish and recalculated U_{crit} (from the slope between P_{20} and P_{28}) each time. We thereby 'exposed' each fish to the complete temperature variation. From the simulated data set, we calculated mean U_{crit} and 95% confidence intervals for each subpopulation (using $n = 61$ for CI calculations to represent the number of fish in the simulation rather than the number of simulated values).

Mean values for each subpopulation may mask the underlying distributions of U_{crit}, which may be important for ecological responses. For each subpopulation (low, centre, high acclimation capacity), we therefore modelled U_{crit} distributions in the variable environment (as determined above) as the per cent of U_{crit} values that fell above a given fraction of maximum speed. The low acclimation capacity subpopulation had the highest maximum U_{crit}, which we used as maximum U_{crit} in all simulations. However, rather than using the single highest U_{crit} value, which is not representative of most fish, we defined the 90th percentile of the low acclimation capacity subpopulation as the maximum U_{crit}. We then determined the percentage of U_{crit} values that fell above a given fraction of this maximum U_{crit} for each subpopulation, which we defined as 'achievable' U_{crit}. See **Supplementary Material** for R code.

RESULTS AND DISCUSSION

Variation in Plasticity of Thermal Performance Curves Between Individuals

Thermal performance curves varied considerably between individuals (**Figure 2**). In both cold- and warm-acclimated fish from data set 1 (**Figure 2A**), U_{crit} tended to increase from 20 to 28°C and decrease at 32°C. However, this pattern was not consistent among individuals, and frequently U_{crit} increased between 28 and 32°C. In fish from data sets 1 and 3 ($n = 192$; **Figure 2B**), U_{crit} mostly increased between 20 and 28°C at both acclimation temperatures, but there was considerable variation in thermal sensitivity (i.e. Q_{10} values). Similarly, responses to acclimation differed considerably between individuals (**Figure 2C**). At both 20 and 28°C test temperatures, acclimation to 28°C led to either increased or decreased performance in different individuals (data from data sets 1 and 3).

Similar to the patterns of thermal sensitivity described above, there was pronounced variation in acclimation capacity within the total population (data sets 1–3, $n = 608$ fish; **Figure 3**). Fish phenotypes ranged from having the capacity to fully compensate (and even overcompensate) for the 8–10°C temperature difference following acclimation, to having no capacity for acclimation at all so that U_{crit} changed passively (thermodynamically) with an acute change in temperature. Interestingly, there was a trade-off in capacity for acclimation

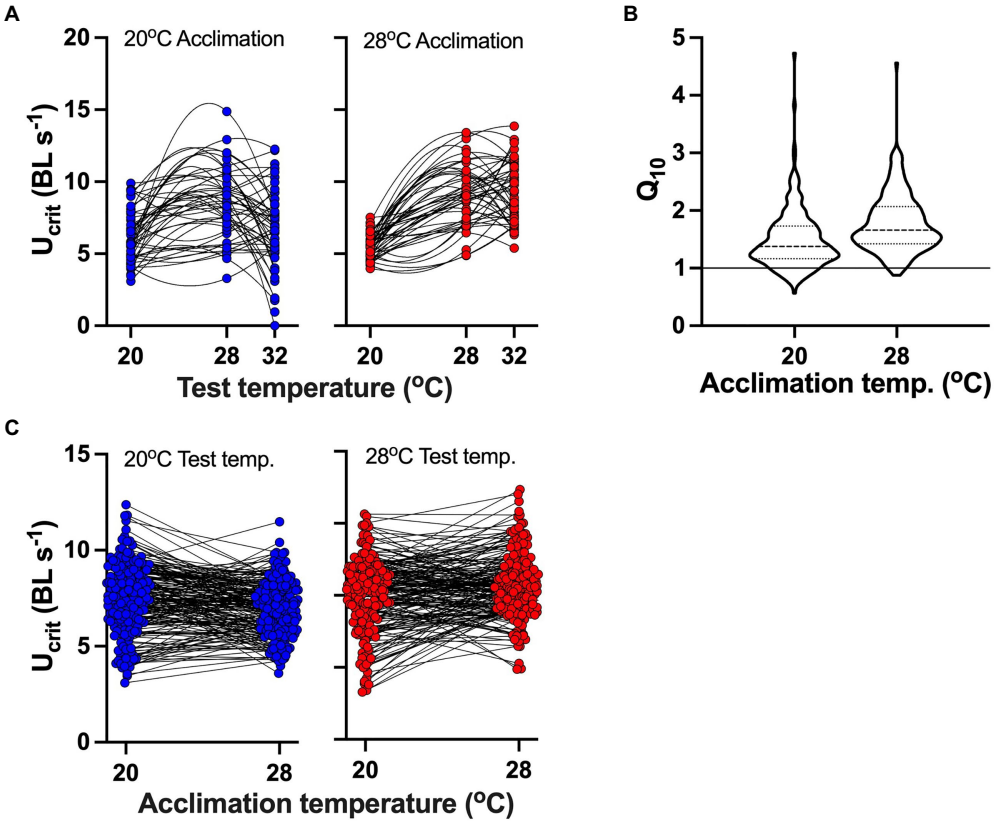

FIGURE 2 | Variation in individual responses to different acclimation and test temperatures. **(A)** Nonlinear performance (quadratic fit) curves for a subset ($N = 48$; data set 1) of fish for which we recorded swimming performance at three test temperatures; blue symbols indicate acclimation to 20°C, and red symbols indicate acclimation to 28°C. **(B)** Violin plot of thermal sensitivity (Q_{10} values between 20 and 28°C acute test temperatures) of individuals (from data sets 1 and 3; $n = 192$ fish) shows considerable variation among individuals acclimated to 20 and 28°C. Thick broken line in violin plots shows mean, and thin broken lines show 95% confidence intervals; the solid line in the plot indicated $Q_{10} = 1$, below which U_{crit} decreased with increasing test temperature. **(C)** Change in performance across acute test temperatures following acclimation to different temperatures. Thin black lines connect datapoints taken from the same individual. Note the pronounced differences in the directions of change in response to different acute test and acclimation temperatures. **(B,C)** are plots of the same data.

with performance at warm conditions (P_{28}): P_{28} decreased significantly (regression: $Y = 12.15 - 3.39x$; $R^2 = 0.22$, $p < 0.0001$) as P_{20} increased ($Y = 3.97 + 4.67x$; $R^2 = 0.54$, $p < 0.0001$) with increasing acclimation capacity. Across both acclimation treatments, mean performance of fish increased slightly but significantly with increasing acclimation capacity ($Y = 8.06 + 0.68x$, $R^2 = 0.014$, $p = 0.0032$; **Figure 3**). However, the persistence of both plastic and fixed phenotypes in the population indicates that on average, plasticity is not necessarily advantageous over fixed phenotypes, but also that plasticity does not carry a cost that would select against it.

Increased variation of phenotypes can render the populations as a whole more resilient to change if different phenotypes are advantageous under different environmental conditions (Schindler et al., 2015; Blondel et al., 2021). Plasticity is advantageous by buffering performance under cooler conditions, such as in winter, while fixed phenotypes perform better at high temperatures during summer. This evolutionary bet-hedging can increase population persistence (Schindler et al., 2015). Mechanistically, it is an interesting question of what mediates the observed variation between individuals. Reactive oxygen species (ROS) and heat shock proteins can be induced by acute heat or cold exposure (Liu et al., 2018). Cold acclimation also increased ROS production in mosquitofish (Loughland and Seebacher, 2020) and grass snakes (Bury et al., 2018), and salmon acclimated to 20°C had greater rates of oxidative phosphorylation but reduced ROS production compared to 12°C acclimated fish (Gerber et al., 2020). Mosquitofish with high acclimation capacity also have greater antioxidant capacities (Loughland and Seebacher, 2020). Increased ROS as a result of reduced antioxidant capacity can decrease swimming performance (Ghanizadeh-Kazerouni et al., 2016) and may at least partly explain the observed patterns in our study. Alternatively, the dynamics of signalling pathways, from endocrine (e.g. thyroid hormone) to epigenetic (e.g. histone acetylation and DNA methylation), may differ between individuals and thereby cause individual variation (Simmonds and Seebacher, 2017; Little, 2021; Loughland et al., 2021).

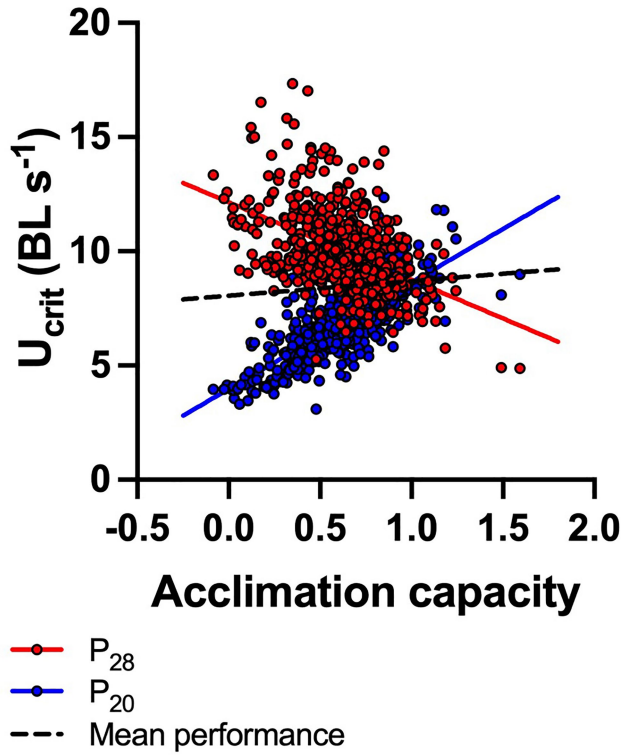

FIGURE 3 | Trade-off between capacity for acclimation and swimming performance (U_{crit}) under warm conditions. Acclimation capacity is shown as a dimensionless index (see main text) that indicates the capacity for reversible acclimation (1 = perfect compensation; >1 = cold-acclimated fish perform better; <1 = warm-acclimated fish perform better). Performance at 28°C test temperature of fish acclimated to 28°C (P_{28}, red circles) decreased as acclimation capacity increased, while P_{20} (U_{crit} of 20°C acclimated fish measured at 20°C test temperature) increased. Across both acclimation treatments, mean performance increased slightly with increasing acclimation capacity (broken black line). $N = 608$ fish (both P_{20} and P_{28} are plotted for each fish), and significant regression lines are shown (P_{20}: $Y = 3.97 + 4.67x$; $R^2 = 0.54$, $p < 0.0001$; P_{28}: $Y = 12.15 - 3.39x$; $R^2 = 0.22$, $p < 0.0001$; mean performance: $Y = 8.06 + 0.68x$, $R^2 = 0.014$, $p = 0.0032$).

Consequences of Individual Variation on Interpretation of Samples

Acclimation did not have a significant main effect in any of the ten random samples (all $p > 0.3$), but test temperature was significant in all samples, and on average, U_{crit} increased with increasing test temperature (all $p < 0.003$; **Figure 4A**). There was a significant interaction between test and acclimation temperatures in samples 1 and 7 ($p < 0.05$) and at a one-tailed significance level in sample 3 ($p = 0.083$); the interaction was not significant in any of the other samples (all $p > 0.24$). Knowing the true acclimation capacity of individual fish showed that means mask the variation in acclimation capacity between individuals, and in many samples, individual acclimation capacity ranged from close to no capacity for acclimation (values → 0) to perfect compensation for the acclimation temperature difference between treatments (values → 1; **Figure 4B**).

The relatively small sample size simulated here can lead to fundamentally different conclusions about the population as a whole: in most cases, the data showed that there was no acclimation response, but three samples indicated that there was. These results serve as a cautionary note to avoid undersampling of populations and presenting means in the absence of individual values. However, even if larger samples were collected, sample means would mask the underlying variation and obscure the bet-hedging dynamics discussed above. Experimental approaches that compare individuals from different acclimation treatments may not be sufficient to test for costs or trade-offs associated with plasticity, which would require knowledge of within-individual acclimation capacities. Understanding individual variation in acclimation capacity would be necessary to predict how populations can respond to climate variability, where diversity of phenotypes may be important to increase resilience. Sample means can show population trends across time or contexts.

Consequences of Variation for Population Responses

Our simulations showed that in the fluctuating environment, the average U_{crit} was similar in all three subpopulations, indicating that capacity for acclimation did not affect mean performance under these circumstances (**Figure 5**). However, performance in the stable cool environment decreased in the centre and low acclimation subpopulations, but it increased in the stable warm environment in those subpopulations. In contrast, U_{crit} of fish with high acclimation capacity did not vary significantly (95% CI) between either the stable or the fluctuating environments.

The achievable U_{crit} in a variable environment was defined as the per cent of U_{crit} (= achievable U_{crit}) that was greater than a given fraction of the maximal (90th percentile) U_{crit}. In other words, the achievable U_{crit} is synonymous with the per cent of time fish could swim faster than a given fraction of maximum in an environment that varied over time (**Figure 6**). Fish with low acclimation capacity had the highest maximum U_{crit} (90th percentile = 11.7 body lengths s^{-1}), but in a variable environment, their achievable U_{crit} declined rapidly. This result is not surprising because in fish with low acclimation capacity, U_{crit} changes thermodynamically in proportion with the acute temperature change. In contrast, fish with central or high acclimation capacity compensated at least partially (centre) for the decline in environmental temperatures. Hence, even though these groups had lower maximum U_{crit} (90th percentile: 10.3 and 10.4 BL s^{-1}, respectively), their achievable U_{crit} was higher than in the low acclimation subpopulation below approximately 0.8 of maximal U_{crit}. The high acclimation capacity subpopulation had the highest achievable U_{crit} (**Figure 6**).

These nonlinear distributions of achievable U_{crit} could present a selective advantage for plastic phenotypes if the ecological outcomes of movement are maximised at a lower than maximal speed. Animals rarely move at their maximum speed, and the functional outcomes of responses such as escaping a predator may be optimised at fractions of maximal

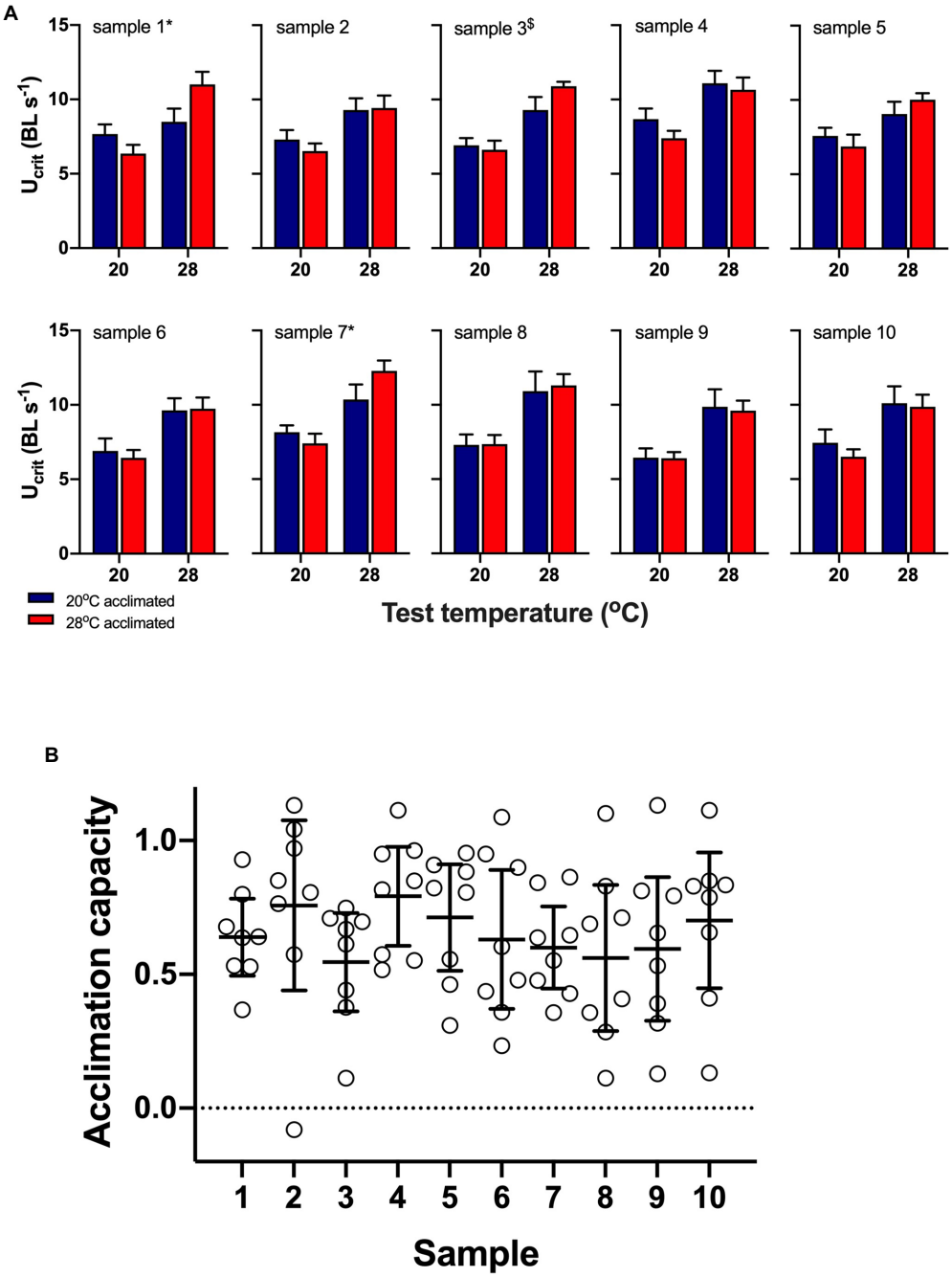

FIGURE 4 | Data from ten random samples of eight fish from the pool of 192 fish (data sets 1 and 3). **(A)** mean (± s.e.) data from the ten samples showing swimming performance (U_{crit}) of 20°C (blue bars) and 28°C (red bars) acclimated fish measured at 20 and 28°C acute test temperatures. Two-factor permutational analyses showed that acclimation did not have a significant main effect in any sample, but test temperature was significant in all samples. There was a significant interaction between test and acclimation temperatures in samples 1 and 7 ($p = 0.013$ and $p = 0.044$, respectively, indicated by *; sample 3: $p = 0.083$ indicated by $). **(B)** Means (± s.e.) mask the variation in acclimation capacity between individuals, and in most samples, individual acclimation capacity ranged from close to no capacity for acclimation (values → 0) to perfect compensation for the acclimation temperature difference between treatments (values → 1).

speed (Wilson et al., 2015). Success in escaping from a predator may be highest at a submaximal speed because at maximal speeds, precision of movement, information processing and endurance decline, while energetic costs increase (Wilson et al., 2015; Wynn et al., 2015). Conversely, speeds below a given threshold may simply be too slow for the prey to escape (Husak and Fox, 2006). Hypothetically, if it is assumed that a fraction of 0.7 maximal U_{crit} is

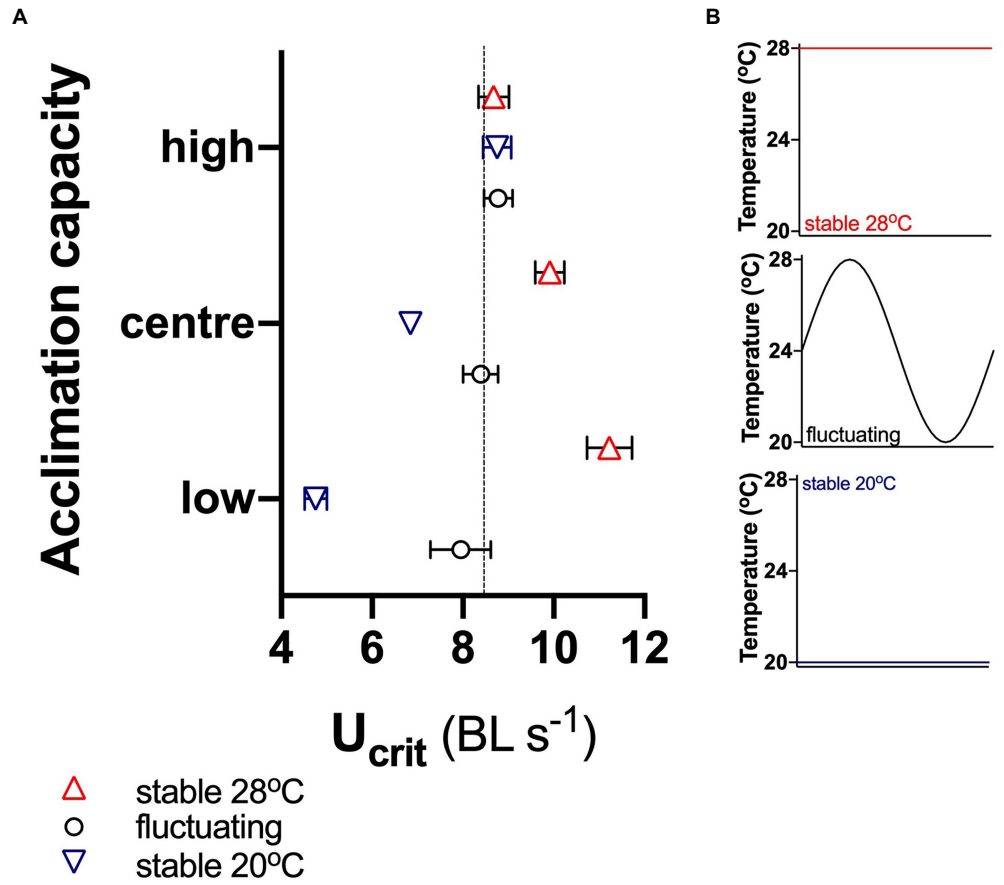

FIGURE 5 | Simulated responses to environmental change. **(A)** U_{crit} of fish with high acclimation capacity (top 10% of the total population of 608 fish), with low acclimation capacity (bottom 10%) and of the central 10% of the total population in different environments: stable at 20 and 28°C and fluctuating between 20 and 28°C **(B)**. Means ± 95% confidence intervals are shown, and the dotted horizontal line in **(A)** indicates the mean of the total population of 608 fish in the fluctuating environment.

necessary for escape from predators (Husak and Fox, 2006), the different distributions of achievable U_{crit} in our subpopulations will influence the success of escaping. Only 46% of U_{crit} values in the low acclimation capacity subpopulation fall above 0.7 maximal speed, while 71% of U_{crit} values were above 0.7 of maximal speed in the high acclimation capacity subpopulation. Hence, fish with high acclimation capacity would be vulnerable around 30% of the time, while those with low acclimation capacity would be vulnerable more than half the time. This hypothetical example demonstrates that the buffering of U_{crit} for low temperatures may translate to a selective advantage across acclimation conditions.

CONCLUSION

Predictably variable environments are often thought to produce plastic phenotypes. Conversely, plasticity is thought to be selected against in stable environments, which implies that there is a cost of plasticity (DeWitt and Scheiner, 2004; Angilletta, 2009; Auld et al., 2010). Hence, the expectation is that depending on environmental context, populations – or even species – are comprised of either plastic or fixed phenotypes. We show that this is not the case, at least for mosquitofish. The trade-off between plasticity and maximal performance could be interpreted as a cost of plasticity because highly plastic individuals have lower maximal performance. However, animals rarely perform at maximal capacities under natural circumstances, and we show that plastic individuals have an advantage if the outcomes of fitness-related activities such as predator escape are optimised at submaximal performance levels.

Nonetheless, fixed phenotypes persist in the population, so that the advantages of plasticity are not sufficient to replace fixed phenotypes. It is possible that the greater performance of fixed individuals at warm temperatures may be advantageous during summer, when mean lake temperatures increase substantially and there are frequent heat waves. The capacity for plasticity may be outstripped by the degree of temperature rise during these times so

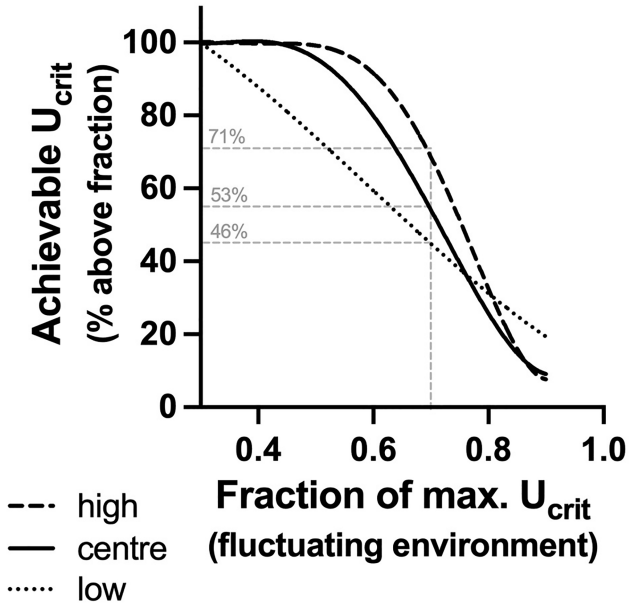

FIGURE 6 | Achievable U_{crit} in a variable environment. Per cent of U_{crit} (= achievable U_{crit}) in each subpopulation that was greater than a given fraction of the maximal (90th percentile) U_{crit} of the low acclimation capacity subpopulation. Fish with low acclimation capacity (low, fine broken line) had the highest maximum U_{crit} (90th percentile = 11.7 BL s^{-1}), but in a variable environment (20–28°C fluctuations), the achievable U_{crit} declined rapidly. In contrast, fish with central or high acclimation capacity [centre (solid line) and high (broken line), respectively] had lower maximum U_{crit} (90th percentile: 10.3 and 10.4 BL s^{-1}, respectively), but their achievable U_{crit} was higher below approximately 0.8 of maximal U_{crit}. The thin grey lines indicate a hypothetical scenario, where escape from a predator requires performance that is at least 0.7 of maximal U_{crit}. Fish with high acclimation capacity would achieve this 71% of the time, while fish with low acclimation capacity would achieve this only relatively rarely (46%) with centre fish in between (53%).

that there is higher mortality of plastic phenotypes. Additionally, developmental temperatures can influence phenotypes, and cold conditions during early development produce more plastic individuals, and warm conditions produce individuals that perform better at warm temperatures (Seebacher et al., 2014; Loughland et al., 2021). In a short-lived species like mosquitofish, births at different times of year – and therefore at different temperatures – may suffice to balance population phenotypes. Trade-offs, developmental effects and the advantages of plastic phenotypes together are likely to explain the observed population variation. Note also that different traits within organisms can differ in the plasticity of their performance curves, which adds an additional layer of complexity and trade-offs (Wilson et al., 2002; Bozinovic et al., 2020). The contention that variable environments produce plasticity is likely to be too simplistic, because it does not capture the dynamics of natural populations.

DATA AVAILABILITY STATEMENT

Publicly available data sets were analysed in this study. These data can be found here: Electronic supplementary material for: Seebacher et al., 2015. Dryad Digital Repository: https://doi.org/10.5061/dryad.v6wwpzgs2

AUTHOR CONTRIBUTIONS

FS and AL conceived the ideas and edited the manuscript. FS analysed the data and wrote the manuscript. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphys.2021.733305/full#supplementary-material

REFERENCES

Angilletta, M. J. Jr. (2009). *Thermal Adaptation*. UK: Oxford University Press.

Auld, J. R., Agrawal, A. A., and Relyea, R. A. (2010). Re-evaluating the costs and limits of adaptive phenotypic plasticity. *Proc. R. Soc. B* 277, 503–511. doi: 10.1098/rspb.2009.1355

Bateson, P., Gluckman, P., and Hanson, M. (2014). The biology of developmental plasticity and the predictive adaptive response hypothesis. *J. Physiol.* 592, 2357–2368. doi: 10.1113/jphysiol.2014.271460

Blondel, L., Paterson, I. G., Bentzen, P., and Hendry, A. P. (2021). Resistance and resilience of genetic and phenotypic diversity to "black swan" flood events: A retrospective analysis with historical samples of guppies. *Mol. Ecol.* 30, 1017–1028. doi: 10.1111/mec.15782

Bozinovic, F., Cavieres, G., Martel, S. I., Alruiz, J. M., Molina, A. N., Roschzttardtz, H., et al. (2020). Thermal effects vary predictably across levels of organization: empirical results and theoretical basis. *Proc. R. Soc. B* 287:20202508. doi: 10.1098/rspb.2020.2508

Bury, S., Cichoń, M., Bauchinger, U., and Sadowska, E. T. (2018). High oxidative stress despite low energy metabolism and vice versa: Insights through temperature acclimation in an ectotherm. *J. Therm. Biol.* 78, 36–41. doi: 10.1016/j.jtherbio.2018.08.003

Careau, V., Biro, P. A., Bonneaud, C., Fokam, E. B., and Herrel, A. (2014). Individual variation in thermal performance curves: swimming burst speed and jumping endurance in wild-caught tropical clawed frogs. *Oecologia* 175, 471–480. doi: 10.1007/s00442-014-2925-7

Costanzo, M., Hou, J., Messier, V., Nelson, J., Rahman, M., VanderSluis, B., et al. (2021). Environmental robustness of the global yeast genetic

interaction network. *Science* 372:eabf8424. doi: 10.1126/science.abf8424

DeLong, J. P., Gibert, J. P., Luhring, T. M., Bachman, G., Reed, B., Neyer, A., et al. (2017). The combined effects of reactant kinetics and enzyme stability explain the temperature dependence of metabolic rates. *Ecol. Evol.* 7, 3940–3950. doi: 10.1002/ece3.2955

DeWitt, T., and Scheiner, S. (2004). *Phenotypic Plasticity: Functional and Conceptual Approaches*. UK: Oxford University Press.

Drummond, G. B., and Vowler, S. L. (2012). Different tests for a difference: how do we do research? *J. Physiol.* 590, 235–238. doi: 10.1113/jphysiol.2011.225235

Gerber, L., Clow, K. A., Mark, F. C., and Gamperl, A. K. (2020). Improved mitochondrial function in salmon (*Salmo salar*) following high temperature acclimation suggests that there are cracks in the proverbial 'ceiling'. *Sci. Rep.* 10:21636. doi: 10.1038/s41598-020-78519-4

Ghanizadeh-Kazerouni, E. G., Franklin, C. E., and Seebacher, F. (2016). UV-B exposure reduces locomotor performance by impairing muscle function but not mitochondrial ATP production. *J. Exp. Biol.* 219, 96–102. doi: 10.1242/jeb.131615

Glanville, E. J., and Seebacher, F. (2006). Compensation for environmental change by complementary shifts of thermal sensitivity and thermoregulatory behaviour in an ectotherm. *J. Exp. Biol.* 209, 4869–4877.

Haaland, T. R., Wright, J., and Ratikainen, I. I. (2020). Generalists versus specialists in fluctuating environments: a bet-hedging perspective. *Oikos* 129, 879–890. doi: 10.1111/oik.07109

Huey, R. B., and Kingsolver, J. G. (1989). Evolution of thermal sensitivity of ectotherm performance. *Trends Ecol. Evol.* 4, 131–135. doi: 10.1016/0169-5347(89)90211-5

Husak, J. F., and Fox, S. F. (2006). Field use of maximal sprint speed by collared lizards (*Crotaphytus collaris*): compensation and sexual selection. *Evolution* 60, 1888–1895. doi: 10.1111/j.0014-3820.2006.tb00532.x

Kingsolver, J. G., and Buckley, L. B. (2017). Quantifying thermal extremes and biological variation to predict evolutionary responses to changing climate. *Philos. Trans. R. Soc. B* 372:20160147. doi: 10.1098/rstb.2016.0147

Kingsolver, J. G., Moore, M. E., Hill, C. A., and Augustine, K. E. (2020). Growth, stress, and acclimation responses to fluctuating temperatures in field and domesticated populations of Manduca sexta. *Ecol. Evol.* 10, 13980–13989.

LeRoy, A., Loughland, I., and Seebacher, F. (2017). Differential effects of developmental thermal plasticity across three generations of guppies (*Poecilia reticulata*): canalization and anticipatory matching. *Sci. Rep.* 7:4313. doi: 10.1038/s41598-017-03300-z

Little, A. G. (2021). Thyroid hormone regulation of thermal acclimation in ectotherms: Physiological mechanisms and ecoevolutionary implications. *Mol. Cell. Endocrinol.* 530:111285. doi: 10.1016/j.mce.2021.111285

Liu, Z.-P., Gu, W.-B., Tu, D.-D., Zhu, Q.-H., Zhou, Y.-L., Wang, C., et al. (2018). Effects of both cold and heat stress on the liver of the giant spiny frog (*Quasipaa spinosa*): stress response and histological changes. *J. Exp. Biol.* 221:jeb186379. doi: 10.1242/jeb.186379

Loughland, I., Little, A. G., and Seebacher, F. (2021). DNA methyltransferase 3a mediates developmental thermal plasticity. *BMC Biol.* 19:11. doi: 10.1186/s12915-020-00942-w

Loughland, I., and Seebacher, F. (2020). Differences in oxidative status explain variation in thermal acclimation capacity between individual mosquitofish (*Gambusia holbrooki*). *Funct. Ecol.* 34, 1380–1390. doi: 10.1111/1365-2435.13563

Ludbrook, J., and Dudley, H. (1998). Why permutation tests are superior to t and F tests in biomedical research. *Am. Stat.* 52, 127–132.

McKenzie, D. J., Zhang, Y., Eliason, E. J., Schulte, P. M., Claireaux, G., Blasco, F. R., et al. (2021). Intraspecific variation in tolerance of warming in fishes. *J. Fish Biol.* 98, 1536–1555. doi: 10.1111/jfb.14620

Safi, H., Zhang, Y., Schulte, P. M., and Farrell, A. P. (2019). GThe effect of cute warming and thermal acclimation on maximum heart rates of the common killifish Fundulus heteroclitus. *J. Fish Biol.* 95, 1441–1446.

Schindler, D. E., Armstrong, J. B., and Reed, T. E. (2015). The portfolio concept in ecology and evolution. *Front. Ecol. Environ.* 13, 257–263. doi: 10.1890/140275

Schulte, P. M., Healy, T. M., and Fangue, N. A. (2011). Thermal performance curves, phenotypic plasticity, and the time scales of temperature exposure. *Integr. Comp. Biol.* 51, 691–702. doi: 10.1093/icb/icr097

Seebacher, F., Beaman, J. E., and Little, A. G. (2014). Regulation of thermal acclimation varies between generations of the short-lived mosquitofish that developed in different environmental conditions. *Funct. Ecol.* 28, 137–148. doi: 10.1111/1365-2435.12156

Seebacher, F., Ducret, V., Little, A. G., and Adriaenssens, B. (2015). Generalist–specialist trade-off during thermal acclimation. *R. Soc. Open Sci.* 2:140251. doi: 10.1098/rsos.140251

Simmonds, A. I. M., and Seebacher, F. (2017). Histone deacetylase activity modulates exercise-induced skeletal muscle plasticity in zebrafish (*Danio rerio*). *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 313, R35–R43. doi: 10.1152/ajpregu.00378.2016

Sinclair, B. J., Vasseur, D., Marshall, K. E., Sewell, M. A., Levesque, D. L., Willett, C. S., et al. (2016). Can we predict ectotherm responses to climate change using thermal performance curves and body temperatures? *Ecol. Lett.* 19, 1372–1385. doi: 10.1111/ele.12686

Tattersall, G. J., Sinclair, B. J., Withers, P. C., FIields, P. A., Seebacher, F., Cooper, C. E., et al. (2012). Coping with thermal challenges: physiological adaptations to environmental temperatures. *Compr. Physiol.* 2, 2151–2202. doi: 10.1002/cphy.c110055

Wheeler, R. E., and Torchiano, M. (2016). Permutation tests for linear models in R. R Package Version 2.1.0.

Wilson, R. S., Husak, J. F., Halsey, L. G., and Clemente, C. J. (2015). Predicting the movement speeds of animals in natural environments. *Integr. Comp. Biol.* 55, 1125–1141. doi: 10.1093/icb/icv106

Wilson, R., James, R. S., and Damme, R. V. (2002). Trade-offs between speed and endurance in the frog *Xenopus laevis*: a multi-level approach. *J. Exp. Biol.* 205, 1145–1152. doi: 10.1242/jeb.205.8.1145

Woods, H. A., Kingsolver, J. G., Fey, S. B., and Vasseur, D. A. (2018). Uncertainty in geographical estimates of performance and fitness. *Methods Ecol. Evol.* 9, 1996–2008. doi: 10.1111/2041-210X.13035

Wynn, M. L., Clemente, C., Nasir, A. F. A. A., and Wilson, R. S. (2015). Running faster causes disaster: trade-offs between speed, manoeuvrability and motor control when running around corners in northern quolls (*Dasyurus hallucatus*). *J. Exp. Biol.* 218, 433–439. doi: 10.1242/jeb.111682

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Thermal Acclimation to the Highest Natural Ambient Temperature Compromises Physiological Performance in Tadpoles of a Stream-Breeding Savanna Tree Frog

Leonardo S. Longhini[1], Lucas A. Zena[2], Elias T. Polymeropoulos[3], Aline C. G. Rocha[1], Gabriela da Silva Leandro[1], Cynthia P. A. Prado[1], Kênia C. Bícego[1] and Luciane H. Gargaglioni[1]**

[1] Departamento de Morfologia e Fisiologia Animal, Universidade Estadual Paulista Júlio de Mesquita Filho, Jaboticabal, Brazil, [2] Department of Physiology, Institute of Biosciences, University of São Paulo, São Paulo, Brazil, [3] Institute for Marine and Antarctic Studies, University of Tasmania, Hobart, TAS, Australia

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***Correspondence:**
Luciane H. Gargaglioni
luciane.gargaglioni@unesp.br
Lucas A. Zena
lucas.zena@bioenv.gu.se

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Amphibians may be more vulnerable to climate-driven habitat modification because of their complex life cycle dependence on land and water. Considering the current rate of global warming, it is critical to identify the vulnerability of a species by assessing its potential to acclimate to warming temperatures. In many species, thermal acclimation provides a reversible physiological adjustment in response to temperature changes, conferring resilience in a changing climate. Here, we investigate the effects of temperature acclimation on the physiological performance of tadpoles of a stream-breeding savanna tree frog (*Bokermannohyla ibitiguara*) in relation to the thermal conditions naturally experienced in their microhabitat (range: 18.8–24.6°C). We quantified performance measures such as routine and maximum metabolic rate at different test (15, 20, 25, 30, and 34°C) and acclimation temperatures (18 and 25°C). We also measured heart rate before and after autonomic blockade with atropine and sotalol at the respective acclimation temperatures. Further, we determined the critical thermal maximum and warming tolerance (critical thermal maximum minus maximum microhabitat temperature), which were not affected by acclimation. Mass-specific routine and mass-specific maximum metabolic rate, as well as heart rate, increased with increasing test temperatures; however, acclimation elevated mass-specific routine metabolic rate while not affecting mass-specific maximum metabolic rate. Heart rate before and after the pharmacological blockade was also unaffected by acclimation. Aerobic scope in animals acclimated to 25°C was substantially reduced, suggesting that physiological performance at the highest temperatures experienced in

their natural habitat is compromised. In conclusion, the data suggest that the tadpoles of *B. ibitiguara,* living in a thermally stable environment, have a limited capacity to physiologically adjust to the highest temperatures found in their micro-habitat, making the species more vulnerable to future climate change.

Keywords: aerobic scope, critical thermal maximum, heart rate, autonomic blockade, climate change, aclimatation, amphibian, oxygen consumption

INTRODUCTION

Global warming affects the behavior, distribution, and physiology of many animal species (Parmesan and Yohe, 2003; Parmesan, 2006; Charmantier et al., 2008; Chen et al., 2009; Clusella-Trullas and Chown, 2013; Foden et al., 2013; Settele et al., 2014; Seebacher et al., 2015; Sandblom et al., 2016a; Pacifici et al., 2017). Since the pre-industrial times, the global average temperature has increased by 1.0°C, and during the past decade, record-breaking storms, forest fires, droughts, heat waves, and floods around the world have been documented (IPCC, 2021). It is predicted that extreme weather events and elevated temperature peaks will become more regular in the future (Schär et al., 2004; Diffenbaugh and Ashfaq, 2010) and are likely to influence the performance and survival of a wide range of species globally.

Ectotherms, for instance, are likely to be affected by global warming since many physiological rates such as heart rate and metabolism are strongly influenced by environmental temperature (T_a). The respiratory and cardiovascular systems are tightly coupled to maintain suitable oxygen delivery to metabolically active tissues, and cardiorespiratory adjustments are generally required whenever metabolic demands change (Overgaard et al., 2012; Hillman and Hedrick, 2015). The effect of T_a on metabolic rate typically follows an exponential curve in many ectotherms, roughly doubling for every 10°C increase in T_a (i.e., Q_{10} = ∼2, Rocha and Branco, 1998; Overgaard et al., 2012), which is generally accompanied by similar increases in heart rate (f_H) (Bícego-Nahas and Branco, 1999; Hedrick et al., 1999; Seebacher and Franklin, 2011; Overgaard et al., 2012; Zena et al., 2015, 2016). However, many ectotherms remodel their physiology to reduce the extent to which physiological reaction rates change in response to changes in temperature, i.e., thermal acclimation, which is essential for the maintenance of individual performance over a wide range of temperatures (Pough et al., 1992; Rome et al., 1992; Angilletta, 2009; Seebacher et al., 2015). Acclimation may manifest as a reversible change of an organism's thermal sensitivity when exposed to a new thermal condition, where a physiological rate remains relatively constant despite variations in ambient temperature (Seebacher et al., 2015). For instance, cardiorespiratory functions such as heart rate reset, so that the initially elevated values progressively decrease upon prolonged exposure to moderately high temperatures (Overgaard et al., 2012; Sandblom et al., 2014; Seebacher et al., 2015; Ekström et al., 2016). Such a phenomenon can occur via two mechanisms: (1) reduction of the intrinsic f_H; (2) increase in cholinergic tone and thus reduction of f_H, or even a combination of both. This plasticity of cardiovascular control after prolonged exposure to high T_a has already been observed in fish (Ekström et al., 2016; Sandblom et al., 2016b).

Thermal acclimation of metabolic rate and cardiorespiratory functions seem to be crucial for many ectotherms, favoring plastic phenotypes by conferring resilience against predictable (e.g., seasons) and unpredictable changes in T_a (Seebacher et al., 2015; Sandblom et al., 2016a). Nevertheless, tropical ectotherms usually experience smaller annual/seasonal changes in environmental temperature, and therefore may be more vulnerable to the impacts of global warming, which bring them closer to their thermal tolerance limits (i.e., difference between minimum [CT_{min}] and maximum [CT_{max}] critical temperatures) (Somero and DeVries, 1967; Ghalambor et al., 2006; Deutsch et al., 2008; Nilsson et al., 2009; Huey et al., 2012). A lack of comprehensive analyses of the capacity for physiological plasticity across taxonomic groups and geographic regions precludes generalizations regarding thermal plasticity and hence predictions of the impacts of climate change on ectotherms (Simon et al., 2015). According to the International Union for Conservation of Nature (IUCN), more than 50% of amphibian species are susceptible to climate change, and such vulnerability is exacerbated for this particular group of vertebrates since it exhibits several life stages in which normal development requires a contrasting habitat or microhabitat (e.g., water-dependent larval-development with limited dispersal capability) (Foden et al., 2008; Lawler et al., 2010).

Tadpoles are an ideal organism to study thermal physiological adaptations. For instance, their relatively small size and the high heat capacity and thermal conductivity of water make tadpoles virtually isothermal with the environment (Lutterschmidt and Hutchison, 1997). Thus, in consideration of taxonomic as well as geographic diversity, we chose to investigate the thermal acclimation in tadpoles of *Bokermannohyla ibitiguara* (Cardoso, 1983), an endemic anuran amphibian from the Cerrado, a threatened savanna-like morphoclimatic domain in central Brazil (Nali and Prado, 2012). Adults of *B. ibitiguara* are associated with gallery forests, while the tadpoles develop in permanent streams (Haddad et al., 1988; Nali and Prado, 2012; Nali et al., 2020). The significance of the species under consideration is highlighted as "data deficient" by the IUCN (Caramaschi and Eterovick, 2004), and its vulnerability to environmental changes, such as temperature, remains unknown.

We investigated the interacting effects of thermal acclimation (18 vs 25°C) (as a form of phenotypic plasticity) on thermal tolerance and physiological mechanisms of tadpoles of *B. ibitiguara* in relation to recorded T_a experienced in the natural habitat. For this purpose, we determined the CT_{max}

during acute gradual temperature increases and calculated the warming tolerance (WT, the difference between CT_{max} and maximum temperature found in the micro-habitat). We also evaluated the aerobic scope by measuring routine and maximum metabolic rate at different test temperatures. Additionally, the body characteristics of tadpoles of both acclimation groups were evaluated, and routine f_H was measured before and after pharmacological autonomic blockade in both groups. Given that some anuran species display mechanisms of thermal compensation (e.g., reset of resting f_H, changes in oxygen consumption or increases of CT_{max}), we predicted *B. ibitiguara* tadpoles to display a shift in their thermal tolerance after at least 3 weeks of warm acclimation. Further, warm acclimation and its consequential increase in temperature-induced oxygen demand will result in a chronically altered rate of oxygen consumption and increased capacity for oxygen delivery through modifications in the cardiorespiratory activity, represented by changes in f_H.

MATERIALS AND METHODS

Animal Collection and Maintenance

The anuran species *B. ibitiguara* (Hylidae) is endemic to the Serra da Canastra mountain range in the state of Minas Gerais, southeastern Brazil. Premetamorphic tadpoles (between stages 26 and 30, according to Gosner, 1960; see **Table 1** for biometrics) were collected in one semi-permanent stream (**Figure 1**) located in a rural area, in the municipality of Sacramento (20°16′21.9″S, 47°04′24.5″W; 677 m elevation; **Supplementary Figure 1**), Minas Gerais state. Using an aquarium fishing net, we collected approximately 25 tadpoles during both day and nighttime on each of the three fieldtrips in February, April and December of 2019. The tadpoles used in the present study originate from different clutches since several adults reproduce in the same stream (Nali and Prado, 2012). Animals were transported in plastic bags to our laboratory at the Department of Animal Morphology and Physiology, UNESP, Jaboticabal, Brazil (approximately 21°14′S and 48°17′W), where they were maintained in two glass aquariums (90 L) under natural photoperiod and temperature set for each acclimation group – 18 and 25°C). Tadpoles did not undergo metamorphoses during any of the experimental protocols. Although the larval period length of *B. ibitiguara* is unknown, stream-breeding species in the genus *Bokermannohyla* are known to exhibit a prolonged larval development phase that may last around 4–5 months (Leite and Eterovick, 2010; Eterovick et al., 2020).

After two days of habituation to the laboratory environment, tadpoles obtained during the first fieldtrip were divided into two acclimation groups, 18 and 28°C. We choose to acclimate tadpoles initially to 28°C in order to test their capacity to tolerate temperatures above the warmest temperature found in their habitat (i.e., 24.6°C); however, all tadpoles exhibited signs of reduced food intake and showed poor body condition. Tadpoles obtained during the second and third fieldtrip were divided into two acclimation groups: 18 and 25°C (hereafter T_{acc18} and T_{acc25}, respectively). Therefore, 18°C was chosen because it is coldest temperature that tadpoles may develop in, while 25°C closely represents the warmest condition for *B. ibitiguara* since the maximum temperature found in their habitat was 24.6°C (see section "Microhabitat Temperature").

For acclimating tadpoles to 18 ± 0.01°C, a stainless-steel coil was positioned inside the aquarium and connected to an external circulation bath via plastic tubes (PolyScience 9112A11B Programmable, Model 9112 Refrigerated Circulator). For acclimating tadpoles to 25 ± 0.02°C, we used a heater controlled by a thermostat (Roxin Ht-1300, 100w) maintained inside the aquarium. Each acclimation temperature was achieved by increasing or decreasing water temperatures by 2°C per day until it reached the desired temperature. All individuals were acclimated at their final treatment temperatures for at least 3 weeks, which is considered a typical acclimation time for small aquatic organisms (Barrionuevo and Fernandes, 1998). Animals were fed daily with herbivore fish food (Maramar, maxi green, 75% vegetable origin). To ensure good water quality, an external filtration system (mechanical, chemical and biological filtration - model HF-0400, Atman, Santo André, São Paulo, Brazil) was used in each aquarium along with an external air pump to maintain water oxygen saturation. Furthermore, twice a week, 20–30% of the aquarium water was removed with animal waste (via a siphon) and replaced with clean water from an artesian well. Thermal gradients inside the aquaria were avoided by creating water motion by the filtration system and the air pumps, and the thermal environment was tested regularly. Animal collection was approved by the Brazilian environmental agency (SISBIO-ICMBio, #621361), and all experimental protocols were approved by the local Animal Care and Use Committee (CEUA-FCAV-UNESP; #02205/18).

Microhabitat Temperature and Environmental Data

The stream temperature and dissolved oxygen from which tadpoles were collected was recorded for every field trip (four in total: February, April and December of 2019, and July of 2020) at three different sites along the stream. For this, we used a portable dissolved oxygen and temperature polarographic meter (YSI, Model 550A). Additionally, one temperature logger (iButton; Maxim Integrated, San Jose, CA, United States), previously coated in a biologically inert wax mixture (20% Elvax; DuPont, NC, United States; 80% histological paraffin wax), was positioned in the water close to the bottom of the stream, where the tadpoles were found, to record water temperature fluctuations every hour for a year (between April 24th of 2019 and July 25th of 2020). We obtained the mean daily minimum (T_{min}), maximum (T_{max}) and average (T_{mean}) temperatures of the stream water. However, for our final analyses, we considered temperatures recorded only between October and May of 2019, which corresponds to the months of greatest rainfall, consequently with water in the stream, and during the reproductive phase of the species (October–June, Nali and Prado, 2012).

Environmental data were acquired from a weather station located at the Sacramento city, MG, (19°52′48″S, 47°25′48″ W;

TABLE 1 | Comparisons of body characteristics of tadpoles in different laboratory acclimation groups (T_{acc18} and T_{acc25}°C).

Groups	TL (mm)	PL (mm)	BW (mm)	Body mass (g)
T_{acc18} (N = 9)	54.7 ± 1.5	17.9 ± 0.6	9.9 ± 0.5	1.3 ± 0.1
T_{acc25} (N = 10)	46.7 ± 1.3	14.8 ± 0.3	7.6 ± 0.3	0.7 ± 0.07
T_{acc18} *vs* T_{acc25}	$t_{(17)}$ = 3.96; P < 0.001	$t_{(17)}$ = 4.13; P < 0.001	$t_{(17)}$ = 3.66; P < 0.001	$t_{(17)}$ = 4.76; P < 0.001

Values shown are means ± s.e.m. for total body length (TL), partial length (PL), body width (BW) in millimeter (mm) and body mass in grams (g). We tested differences with unpaired t test at a 0.05 significance level. T values and the corresponding degrees of freedom are also shown, all parameters are significantly different between acclimation groups.

altitude: 913.12 m) at 87.2 km distance from the stream. The data included daily values for precipitation (mm) and mean ambient temperature (T_{mean}; °C) recorded for 2019.

Body Characteristics

After the acclimation phase, the tadpoles of each group (T_{acc18}: N = 9; T_{acc25}: N = 10) were individually weighed on a digital scale (0.01g, Model LW 303i, Bel Engineering, Italy) and measured using calipers (0.01 mm) to obtain the average body mass (BM), total body length (TL, from snout to the end of the tail), partial length (PL, from snout to the insertion of the tail) and body width (BW).

Upper Thermal Limits

Critical thermal maximum (CT_{max}), defined as the thermal point at which activity becomes disoriented, and an animal loses its ability to escape from conditions that lead to death (Cowles and Bogert, 1944), was determined using the dynamic method previously performed in tadpoles (Lutterschmidt and Hutchison, 1997; Duarte et al., 2012; Kern et al., 2015; Agudelo-Cantero and Navas, 2019). The experiment started at the acclimation temperature of each group, then animals were exposed to a constant heating rate of 0.1°C min^{-1} (**Supplementary Figure 2**) inside a water bath, until we observed immobility after five consecutive taps on the tail using a glass stick (Simon et al., 2015; Badr et al., 2016; Moyano et al., 2017; Agudelo-Cantero and Navas, 2019). The ramp increases in temperature experienced by the tadpoles were continuously measured (sample rate: 1 kHz) using a temperature sensor (MLT415/M Thermistor temperature sensor, ADInstruments®, Sydney, Australia). Once an individual reached its CT_{max}, we quickly transferred it into a plastic container with water at ∼25°C to allow recovery. Only animals that survived after 24 hours were included in the analysis (T_{acc18}: N = 7; T_{acc25}: N = 8 – of the 16 animals tested, only one died within 24 h; the tadpoles for each acclimation groups originated from different collection events).

We also estimated the warming tolerance (WT), which provides a measure of the relative severity of warming that each species can withstand before reaching critical performance levels (Deutsch et al., 2008). This metric was calculated as the difference between the organism's CT_{max} and the maximum microhabitat temperature (T_{max}), i.e., WT = CT_{max} – T_{max}) (Duarte et al., 2012). We considered T_{max} to be the mean daily maximum temperature recorded at the stream between October and May of 2019.

Measuring Oxygen Consumption in Tadpoles

A different sub-sample of tadpoles was used to study the metabolic rates in each acclimation group. The rate of oxygen consumption (≅ metabolic rate = $\dot{M}O_2$) was measured in resting tadpoles (T_{acc18}: N = 8; T_{acc25}: N = 8) and after forced activity at five test temperatures (15, 20, 25, 30, and 34°C) using fluorescence-based intermittent-flow respirometry (Steffensen, 1989; Clark et al., 2013; Rosewarne et al., 2016; Svendsen et al., 2016). Since it was not possible to keep the tadpoles immobile during respirometry trials, $\dot{M}O_2$ measurements represent routine metabolic rates ($r\dot{M}O_2$), indicating the rate of oxygen consumed during low levels of voluntary activity (Fry, 1971; Seebacher and Grigaltchik, 2014).

Each animal was placed in a cylindrical, acrylic respirometer (total volume of 43 mL), submerged in an experimental tank filled with aerated water (PO_2 = 21 kPa). Through a hole in the upper part of the respirometer, we placed an oxygen sensor (PSt3, PreSens, Regensburg, Germany) and the partial pressure of O_2 was recorded as per cent of saturation and with a sampling rate of 0.2 Hz using customized software for the O_2 analyzer (FIBOX3, PreSens, Germany). Inside the experimental tank surrounding the respirometer, an additional aerator was placed to ensure adequate oxygenation of the surrounding water. A submerged recirculation aquarium mini-pump (mini pump A, Sarlobetter, Brazil) was placed within the tank in order to flush the water inside the respirometry chamber. A separate pump (ECEEN, 43GPH), also located within the tank, was used to recirculate water inside the sealed respirometer, and therefore ensure proper mixing for measuring $\dot{M}O_2$. Adjustment and maintenance of each test temperature was performed using an external water bath with a coil connected to the experimental tank (PolyScience 9112A11B Programmable, Model 9112 Refrigerated Circulator). The O_2 sensor was calibrated daily at the test temperatures using 100% aerated distilled water and 0% oxygen by dipping the O_2 sensor in 100 mL distilled water with 1 g dissolved Na_2SO_3 (1% sodium sulphite solution, which acted as an O_2 scavenger).

Tadpoles were placed into the respirometer for habituation at the first test temperature (15°C) for at least one hour, which is sufficiently long for recovery from handling stress (Kern et al., 2014; Seebacher and Grigaltchik, 2014; Longhini et al., 2017). After one hour, the respirometer was sealed and $\dot{M}O_2$ was determined in duplicates at each test temperature (15, 20, 25, 30, and 34°C), always ensuring that O_2 saturation was kept above 80% (Jensen et al., 2013) during each cycle. At the end of the experimental protocol for measurements of $\dot{M}O_2$,

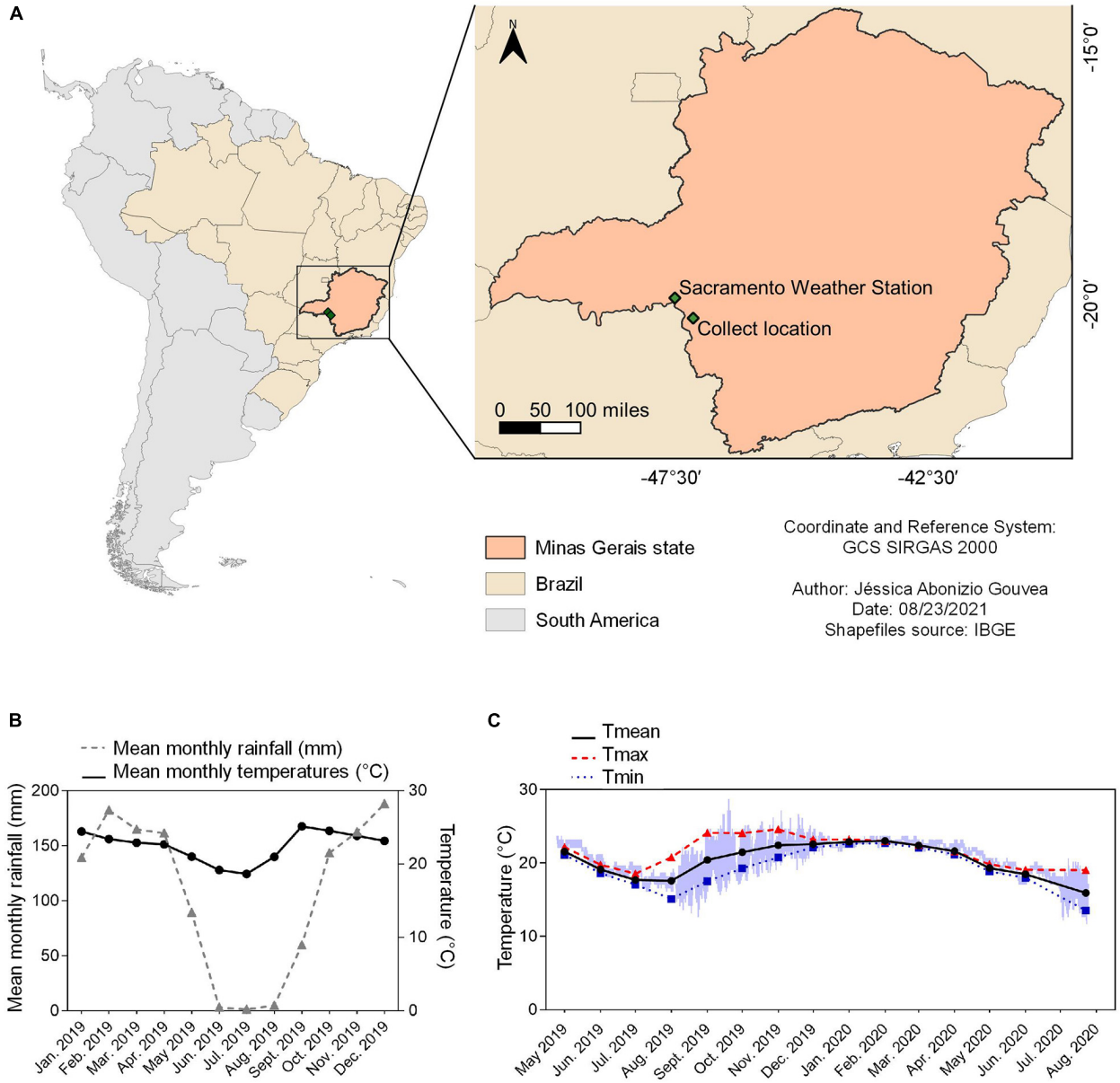

FIGURE 1 | Summary of environmental data for the location that B. ibitiguara inhabits, compared to the weather station. **(A)** Minas Gerais state highlighted on the map of Brazil, showing the capture site of tadpoles of *B. ibitiguara* **(B)** and the nearest weather station **(C)**, at Sacramento city (at a distant of 87.2 km). **(B)** Mean monthly temperatures (black line) and rainfall (blue line) for the year 2019 obtained from the Sacramento weather station. **(C)** Minimum (blue line), maximum (red line) and mean (black line) monthly records of water temperatures in the permanent stream where tadpoles were captured. The data logger collected temperature data every hour from April 24th of 2019 to July 25th of 2020. The time interval estimated for the dried season is from May to September [see graph in panel **(B)**], when, at some point (the total duration is impossible to be determined) the stream displayed decreased water levels and thus and thus, the temperature sensor recorded air temperature.

tadpoles were removed from the respirometer, and their wet body mass was recorded using digital scales (±0.01 g). Then, animals were transferred to plastic containers with water at ∼25°C. All tadpoles survived the experiments performed for measuring routine metabolic rate.

For measuring maximum metabolic rate ($m\dot{M}O_2$), we used the manual chasing method immediately before tadpoles were introduced into the respirometer (Clark et al., 2013). This method was chosen because *B. ibitiguara* tadpoles are bottom dwellers, found mostly resting on rocky or silty substrates (Leite and Eterovick, 2010), under or above submerged leaves in the stream. This method makes it possible to achieve $m\dot{M}O_2$ levels due to excess post-exercise oxygen consumption (Reidy et al., 1995; Briceño et al., 2020). For the chasing protocols, a different group of animals (T_{acc18}: $N = 8$; T_{acc25}: $N = 8$) were placed in a 500 mL beaker inside the same experimental box used for measurements

of $r\dot{M}O_2$. Using a glass stick, we chased the individual for 5 min continuously or until exhaustion occurred (no response after 5 consecutive taps on the tail). After the chasing protocol, tadpoles were immediately placed inside the respirometer that was sealed for measurement of $m\dot{M}O_2$. Tadpoles were exposed to the same test temperature (15, 20, 25, 30, and 34°C) and randomly for both acclimation groups). All tadpoles survived the experiments performed for measuring $m\dot{M}O_2$, except animals initially tested at 34°C from T_{acc25} (N = 2), which represents 11% of total individuals. The respirometry system (acrylic chamber, tubes and pumps) was cleaned daily at the end of each experimental protocol using chlorine to avoid any microbial/algal growth. The background $\dot{M}O_2$ was measured in the respirometer without tadpoles as controls, and we subtracted O_2 consumption of the controls from the experimental values.

The $\dot{M}O_2$ (μmol g^{-1} h^{-1}) during each measurement phase was derived from the slope of the linear regression of O_2 content (μmol L^{-1}) over time (h) according to the equation:

$$\dot{M}O_2 = V_{RE} W_o^{-1} \frac{dCO_2}{d\tau}$$

where V_{RE} is the effective volume of water in the respirometer, calculated as the total respirometer volume minus the organism volume, W_o is the organism mass (we assumed a density of 1 kg L^{-1}) and $dCO_2/d\tau$ is the slope of the linear decrease in O_2 content during the time the chamber was sealed (Svendsen et al., 2016). For final $\dot{M}O_2$ calculations, we only considered slopes with $r^2 \geq 0.95$.

Drugs

To study the autonomic control of heart rate (f_H), atropine (cholinergic muscarinic antagonist; 3.0 mg kg^{-1}) and sotalol (β-adrenergic antagonist; 3.0 mg kg^{-1}) were purchased from Sigma-Aldrich (St Louis, MO, United States) and dissolved in amphibian Ringer solution (composition in mmol l^{-1}: 46.9 NaCl; 21.0 KCl; 2.40 CaCl; 1.29 MgCl; 3.14 $NaHCO_3$; according to Zena et al., 2016; Longhini et al., 2017). Drugs and doses were chosen based on previous studies performed on both tadpole and adult anuran amphibians (Zena et al., 2016; Longhini et al., 2017).

Heart Rate Measurement and Pharmacological Autonomic Blockade of Heart Rate (f_H)

A different sub-sample of tadpoles was used to study the autonomic control for each acclimation group (T_{acc18}: N = 8; T_{acc25}: N = 8). Heart rate was measured using a non-invasive methodology as previously described (Longhini et al., 2017). Briefly, we coupled two parallel electrodes, made from hypodermic needles (40 mm × 1.20 mm, 18G), to a 20 mL plastic syringe positioned inside the experimental tank and connected to a recirculation pump to ensure adequate water exchange between the outside and the inside of the syringe. The electrodes were wired and connected to a signal amplifier (A-M Systems, model 1700, Sequim, WA, United States), allowing the collection of electrical signals from the tadpole's heart by a direct contact between the electrodes and the animal's ventral surface. Biological signals were recorded at a sampling rate of 1 kHz by an acquisition system (PowerLab System, ADInstruments®, Sydney, Australia) and further analyzed offline (Chart Software, version 7.3, ADinstruments®, Sydney, Australia) using the software's built-in filters (low-pass: 50 Hz) over the raw signals. The online signals were amplified (10.000× gain) and filtered (bandpass: 0.1–5 KHz). The f_H averages were obtained from 5 minutes of a visibly stable recording that did not contain any obvious artefact resulting from tadpole movements by using the LabChart software's signal detection tools (version 7.3, Sydney, Australia). In addition, the water system was grounded to attenuate the noise by using a ground wire connected to the amplifier.

The experimental protocol for the blockade of sympathetic and parasympathetic modulation on the heart was initiated after one hour of the tadpoles' habituation to the experimental apparatus, which was followed by recordings of baseline f_H measurements for an additional hour. After baseline recordings, tadpoles were gently removed from the experimental apparatus and handled to receive an intraperitoneal injection of atropine. Recording of f_H occurred for one hour after the muscarinic blockade. Subsequently, sotalol hydrochloride injection was performed to achieve a full autonomic blockade, and f_H was recorded for an additional hour. Intraperitoneal injections were performed using a dental needle (Mizzy, 200 μm outside diameter) connected by a polyethylene tube (PE-10, Clay Adams, Parsippany, NJ, United States) to a Hamilton syringe (5 μL). Injections were standardized so that the volume injected into the peritoneal cavity was 0.46 μL g^{-1}. The autonomic blockade protocol was performed twice in each individual, following an interval of 7 days between the first and the second experiment. At first, the blockage was induced in each individual in their respective acclimation group (T_{acc18} and T_{acc25}), that is, at their respective acclimation temperatures, 18 and 25°C. After 7 days, each tadpole was again subjected to the autonomic blockade, but in this case in the form of an acute exposure to the opposite temperature of acclimation, i.e., T_{acc18} was exposed to 25°C for 1 h and T_{acc25} was exposed to 18°C for 1 h before the pharmacological blockade. At the end of the experiments, tadpoles were euthanized by placing them in a solution of benzocaine hydrochloride (250 mg L^{-1}) buffered to pH 7.7 with sodium bicarbonate (Longhini et al., 2017). All tadpoles survived to experiments performed for the autonomic blockade, excepted one animal (5%) from T_{acc25}, which died during the habituation to the experimental apparatus when acutely exposed to 18°C.

Statistical Analyses

For comparing the thermal tolerance parameters (CT_{max} and WT) and body characteristics of tadpoles between the two acclimation groups, we used an unpaired t-test. To verify the effect of acclimation (T_{acc18} *vs.* T_{acc25}), test temperatures (18 *vs.* 25°C), selective autonomic blockade, and their interaction on f_H (response variable), we fitted linear mixed models by using the R package nlme (Pinheiro et al., 2021). We also fitted linear mixed models for comparing the effects of acclimation (T_{acc18} *vs.* T_{acc25}), test temperatures (15, 20, 25, 30, and 34°C) and their interaction on mass-specific $r\dot{M}O_2$ and $m\dot{M}O_2$. In all cases, individuals were included as random effects (intercept) to

account for the repeatability of the data throughout the study. Absolute aerobic scope (AAS) was calculated as the difference between mean values of $m\dot{M}O_2$ and $r\dot{M}O_2$, while the factorial aerobic scope (FAS) was obtained as the ratio of the mean values for $m\dot{M}O_2$ to $r\dot{M}O_2$. Factorial and absolute scope were fitted using a Gaussian curve using the Graphpad software, version 8.0.[1] We also constructed stream temperature frequency histograms of daily values recorded every hour by the data logger, which were bin centered at 0.5 degree interval.

All statistical analyses were performed using R software v. 3.6.3 (R Core Team, 2020). For all analyses, statistical significance was accepted when $P \leq 0.05$. When significant effects were found in linear models, these were further explored by Tukey's test for pairwise comparisons within each acclimation treatment. Normality of the residuals were visually inspected by using histograms. Homogeneity of variance for each model was visually inspected and tested using a Levene's test. When necessary, appropriate data transformations were performed (log transformation).

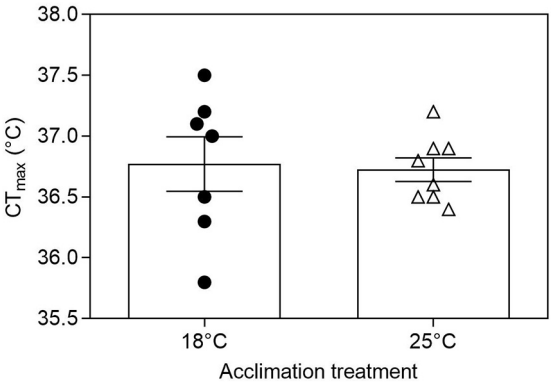

FIGURE 2 | Critical thermal maxima (CT_{max}) for tadpoles of *Bokermannohyla ibitiguara*. Acclimation treatment (18 and 25°C) did not affect CT_{max} of premetamorphic tadpoles ($P = 0.84$). Data are presented as mean ± s.e.m. (18°C, $N = 7$; 25°C, $N = 8$).

RESULTS

Microhabitat Temperature

Data logger recordings for seasonal temperature changes in the stream where tadpoles of *B. ibitiguara* were collected (sampled between April 24th of 2019 and July 25th of 2020) is shown in **Figure 1**. During the dry season, we observed that the stream's flow ceased completely, leaving only non-adjacent pools of stagnant water, which explains the high daily temperature variations between August and November of 2019 (see **Figure 1**). During our last field trip (July 25th of 2020), we found that the temperature logger was completely emerged from the dried stream bed. By only considering the months in which the stream bed was filled (October–May) according to field observations, T_{max} was 24.6 ± 0.6; T_{min} was 18.8 ± 0.7, while T_{mean} was 21.9 ± 0.8.

For each field trip, we also measured stream water temperature manually at the points where we collected tadpoles, either during daylight or nighttime: February 2019: 24.2°C (15h50); 23°C (16h00) and 22.6°C (8h30); April 2019: 22.5°C (19h30); 22.3°C (20h07) and 22.2°C (10h10); December 2019: 23°C (16h33); 22.7°C (19h48); and July 2020: 21.1°C (12h05); resulting in a T_{mean} of 22.1 ± 0.6. We also measured the dissolved O_2 in the same location points of collection: 5.6 ± 1.7 mg L^{-1} (range: 4.1–7.3 mg L^{-1}; February 2019); 7.7 ± 0.1 mg L^{-1} (range: 7.3–7.8 mg L^{-1}; April 2019); 5.7 ± 0.6 mg L^{-1} (range: 4.6–6.7 mg L^{-1}; December 2019) and 4.6 ± 0.3 mg L^{-1} (range: 4.1–5.1 mg L^{-1}; July 2020).

Body Characteristics of Acclimation Groups

After the acclimation treatment, all morphological traits were significantly different between T_{acc18} and T_{acc25} (see **Table 1**). However, none of the acclimation regimes affected the allometric relationships obtained from the residuals of the regressions between total length *vs.* body mass (T_{acc18}: 0.0004 ± 0.3 *vs.* T_{acc25}: −0.02 ± 0.3; $t_{(17)} = 0.05$; $P = 0.96$), total length *vs.* partial length (T_{acc18}: 0.009 ± 0.3 *vs.* T_{acc25}: 0.008 ± 0.3; $t_{(17)} = 0.001$; $P = 0.99$), and total length *vs.* body width (T_{acc18}: 0.02 ± 0.3 *vs.* T_{acc25}: 0.05 ± 0.3; $t_{(17)} = 0.04$; $P = 0.96$).

Thermal Tolerance

Tadpoles of *B. ibitiguara* of both acclimation groups exhibited similar CT_{max} (T_{acc18}: 36.8 ± 0.2°C *vs.* T_{acc25}: 36.7 ± 0.09°C; $t_{(13)} = 0.19$; $P = 0.84$; **Figure 2**). The heating rate did not differ between the two groups (slope for T_{acc18}: 0.089°C min^{-1} *vs.* slope for T_{acc25}: 0.091°C min^{-1}; $F_{(1,107)} = 0.61$, $P = 0.43$; see **Supplementary Figure 2**). WT was also the same for both acclimated groups (T_{acc18}: 12.1 ± 0.6°C *vs.* T_{acc25}: 12.1 ± 0.2°C; $t_{(13)} = 0.21$; $P = 0.84$).

Effects of Temperature on Aerobic Metabolism

The body mass was significantly different for $r\dot{M}O_2$ ($F_{(1,13)} = 14.43$, $P < 0.001$) and $m\dot{M}O_2$ ($F_{(1,13)} = 37.68$, $P = 0.003$). In the subsequent analysis, body mass was considered as a possible factor of influence in a covariance analysis. The results for total $\dot{M}O_2$ are described in detail in the supplementary material (**Supplementary Figure 3**). Mass-specific values for $r\dot{M}O_2$ and $m\dot{M}O_2$ are shown in **Figures 3A,B**, respectively. Routine metabolic rate increased with increasing temperature in both acclimation groups (T_{acc18} and T_{acc25}) (Test temperature effect: $F_{(1,62)} = 302.5$, $P < 0.0001$; **Figure 3A**). Acclimation significantly affected $r\dot{M}O_2$ ($F_{(1,14)} = 68.2$, $P < 0.0001$) in tadpoles acclimated to 25°C showing a higher $r\dot{M}O_2$, with values increasing up to 30°C and showing no further increase when tadpoles were exposed to 34°C (30.06 ± 0.1°C: 15.95 ± 1.2 μmol O_2 g^{-1} h^{-1} *vs.* 34 ± 0.07°C: 16.01 ± 1.9 μmol O_2 g^{-1} h^{-1}; $t_{(56)} = 0.221$, $P = 1.0$). In contrast, mass-specific $r\dot{M}O_2$ continues to increase up to 34°C for T_{acc18} (30.4 ± 0.12°C: 5.37 ± 0.5 μmol O_2 g^{-1} h^{-1} *vs.* 34.4 ± 0.06°C: 7.44 ± 0.7

[1] www.graphpad.com

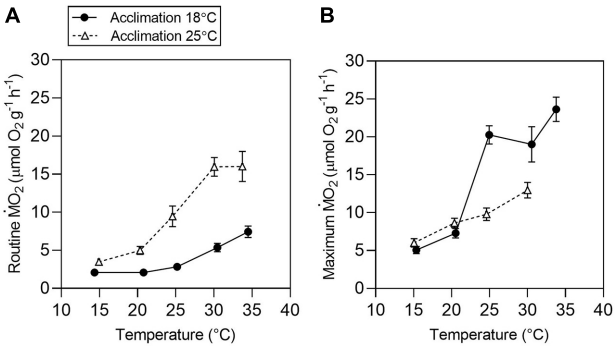

FIGURE 3 | Temperature effects on aerobic metabolism in tadpoles of *Bokermannohyla ibitiguara*. Mass-specific routine [**(A)** $r\dot{M}O_2$] and maximum [**(B)** $m\dot{M}O_2$] metabolic rates for tadpoles acclimated to 18°C (N = 8) and 25°C (N = 8) and exposed to different test temperatures (15, 20, 25, 30, and 34°C). Data are presented as mean ± s.e.m.

μmol O_2 g^{-1} h^{-1}; interaction effect: $F_{(1,62)}$ = 4.58, P = 0.03). Maximum metabolic rate also increased with increasing test temperature for both T_{acc18} and T_{acc25}. The $m\dot{M}O_2$ of T_{acc18} increased up to 24.9 ± 0.04°C after which no further increase was detected until the temperature reaches 33.7 ± 0.01°C (24.9 ± 0.04°C: 20.2 ± 1.2 μmol O_2 g^{-1} h^{-1} *vs.* 33.7 ± 0.01°C: 23.6 ± 1.6 μmol O_2 g^{-1} h^{-1}; $t_{(49)}$ = −2.267, P = 0.43). In contrast, despite a continuous increase in $m\dot{M}O_2$ for T_{acc25} up to 30 ± 0.05°C, it never reached values similar to T_{acc18} with increasing temperature (interaction effect: $F_{(1,54)}$ = 9.3, P < 0.0001; **Figure 3B**).

Aerobic scope over a range of water temperatures is presented as the absolute difference between mean values of $r\dot{M}O_2$ and $m\dot{M}O_2$ (**Figure 4B**), and as a factorial term calculated as the ratio of the mean values for $m\dot{M}O_2$ to $r\dot{M}O_2$ (**Figure 4A**) with water temperature histograms from the micro-habitat of *B. ibitiguara* measured every hour from the October to May period (with water flow in the stream). For both ways of obtaining the scope, T_{acc25} visually exhibited a smaller amplitude in relation to T_{acc18}. In addition to an apparent reduction in aerobic scope for T_{acc25} relative to T_{acc18}, the former exhibits maximum values around 20°C, while the latter around 30°C. Furthermore, in T_{acc18} the maximum performance is above the average stream temperature, while the performance is shifted to the left at lower temperatures in T_{acc25}.

Temperature Effects on Heart Rate

Acclimation temperatures did not affect f_H responses to acute changes in temperature (Acclimation effect: $F_{(1,12)}$ = 0.014; P = 0.90; **Figure 5**), while test temperature significantly affected f_H (Test temperature effect: $F_{(1,62)}$ = 635.997, P < 0.0001). Regardless of the acclimation group, routine heart rate (f_H) increased significantly when acutely exposed from 18 to 25°C (18°C: 52.07 ± 1.6 beats min^{-1} *vs.* 25°C: 80.3 ± 1.6 beats min^{-1}; $t_{(62)}$ = 12.609, P < 0.0001; **Figure 5**) with a Q_{10} of 1.9.

Pharmacological treatment with atropine and sotalol significantly affected f_H (treatment effect: $F_{(2,62)}$ = 33.372; P < 0.0001). Atropine increased the f_H of tadpoles at the test temperature of 18°C relative to routine values (atropine: 59.5 ± 0.8 beats min^{-1} *vs.* routine: 52.07 ± 1.6 beats min^{-1}; $t_{(62)}$ = 3.318, P = 0.0182, respectively). Sotalol evoked a slight reduction in f_H, although not significantly different from atropine values (double blockade: 54.4 ± 1.1 beats min^{-1} *vs.* atropine: 59.5 ± 0.8 beats min^{-1}; $t_{(62)}$ = 2.261, P = 0.2257; respectively). When tadpoles were acutely exposed to 25°C, atropine also increased f_H relative to routine values (atropine: 98.1 ± 1.9 beats min^{-1} *vs.* routine: 80.3 ± 1.6 beats min^{-1}; $t_{(62)}$ = 7.933, P < 0.0001, respectively), although with a larger amplitude effect (interaction effect: $F_{(2,62)}$ = 5.707; P = 0.005). Conversely, sotalol evoked a significant reduction in f_H relative to atropine values (double blockade: 85.5 ± 2.2 beats min^{-1} *vs.* atropine: 98.1 ± 1.9 beats min^{-1}; $t_{(62)}$ = 5.639, P < 0.0001; respectively).

DISCUSSION

Tadpoles of the anuran *B. ibitiguara* have limited phenotypic plasticity when acclimated to the warmest temperature (∼25°C for at least 3 weeks) found in their micro-habitat. We found that thermal tolerance (i.e., CT_{max}) did not differ between acclimation groups (18 and 25°C) and that cardiorespiratory parameters such as routine f_H and $r\dot{M}O_2$ increased significantly with high acclimation temperature (i.e., 25°C). Conversely, $m\dot{M}O_2$ showed a mild increase with acute changes in temperature in tadpoles acclimated to 25°C, thereby remaining low relative to $m\dot{M}O_2$ values from tadpoles acclimated to 18°C. Therefore, tadpoles exhibited a reduced aerobic metabolic scope when acclimated to 25°C. Our results indicate that *B. ibitiguara* tadpoles are highly susceptible to future events of global warming, in which an average increase of 3°C in the stream temperature that tadpoles inhabit can impact species survival success mainly owing to limited phenotypic plasticity of cardiorespiratory functions.

Thermal Tolerance of Tadpoles

It is generally expected that species with restricted geographical distributions are exposed to low seasonal temperature variations and therefore show a narrower range of thermal tolerance limits, which may include low capacity for physiological plasticity, such as thermal acclimation (Brattstrom, 1968; Huey and Kingsolver, 1993; Bernardo and Spotila, 2006; Gifford and Kozak, 2012). Chronic acclimation to a high T_a of 25°C, did not result in changes to the upper thermal tolerance levels in tadpoles of *B. ibitiguara* from the Cerrado (18°C: 36.8 ± 0.2°C *vs.* 25°C: 36.7 ± 0.09°C). Thus, thermal acclimation appears to be absent. Although a general pattern of increased CT_{max} at relatively high acclimation temperatures has previously been suggested in anurans (Brattstrom, 1968; Navas et al., 2008), some anuran amphibians show a limited scope for or absence of acclimation capacity (Rome et al., 1992; Bovo et al., 2020). Brazilian anuran tadpoles found in contrasting morphoclimatic domains, such as *Rhinella ornata* in the Atlantic forest and *Rhinella granulosa* in drier habitats in the Caatinga exhibit CT_{max} of 42.5 and 44.4°C, respectively (Simon et al., 2015). Tadpoles that develop in ephemeral tropical ponds experience large daily

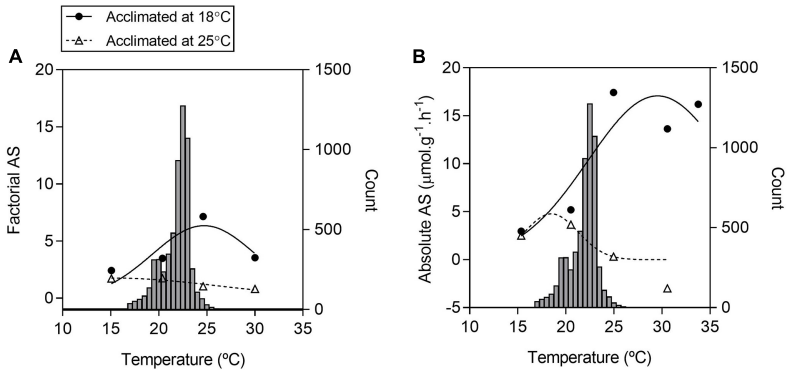

FIGURE 4 | Frequency of water temperature and aerobic scopes of tadpoles of *Bokermannohyla ibitiguara* over a range of test temperatures. Factorial aerobic scope [**(A)** FAS] is calculated from the ratio between mean values obtained for mass-specific maximum metabolic rate ($m\dot{M}O_2$) and routine metabolic rate ($r\dot{M}O_2$). The absolute aerobic scope [**(B)** AAS] is calculated from the difference between mean values for $m\dot{M}O_2$ and mean values for $r\dot{M}O_2$ over a range of different temperatures (15 ± 0.06°C to 30 ± 0.05°C). Histograms of the frequency of stream temperature are repeated in panels **(A,B)**, representing the records collected by the data logger every hour between October and May. The right axis indicates the count of records of each water temperature, and the left axis corresponds to the calculated aerobic scope.

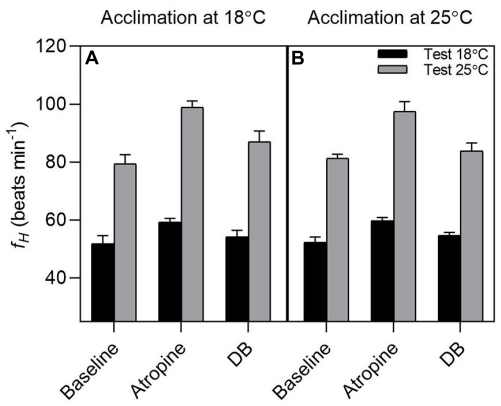

FIGURE 5 | Effect of pharmacological blockade on heart rate in tadpoles of *Bokermannohyla ibitiguara*. The effects of pharmacological blockade (atropine alone and double blockade = atropine + sotalol) on heart rate (f_H) in tadpoles acclimated at 18°C (N = 8) and tadpoles acclimated at 25°C (N = 7) at different experimental test temperatures (18 and 25°C). Regardless of the treatment treatment used, the f_H was significantly altered by temperature of 25°C ($P < 0.001$). Data are shown as means ± s.e.m.

temperature fluctuations and can exhibit CT_{max} above 40°C (Abe and Neto, 1991). In contrast, tadpoles from *B. ibitiguara* exhibit low CT_{max} values, which may result from an adaptation to their micro-habitat that seems to keep low T_a oscillations for most part of the year (**Figure 1C**). *B. ibitiguara* is known to inhabit streams surrounded by gallery forests in a topographically complex landscape at altitudes up to 1.500 m (Nali et al., 2020). In the present study, we sampled tadpoles from a stream at 670 m altitude, a micro-habitat in which there are no large daily or seasonal temperature fluctuations, likely to be related to the presence of gallery forests alongside the streams that the tadpoles inhabit (**Supplementary Figure 1**).

In order to evaluate the heat-shock risk that tadpoles of *B. ibitiguara* may experience, that is, how fast the tadpole's performance would decline when approaching the upper thermal limit, we estimated their warming tolerance (Duarte et al., 2012). Since CT_{max} was virtually the same between both acclimation groups, values estimated for warming tolerance were similar and relatively high (T_{acc18}: 12.44 ± 0.5°C and T_{acc25}:12.42 ± 0.2°C) compared to other tadpole species (Duarte et al., 2012; Simon et al., 2015). This suggests that tadpoles of *B. ibitiguara* tolerate warming before temperatures become deleterious and ultimately lethal, meaning that these tadpoles are in some way resistant to rapid episodes of thermal stress (Duarte et al., 2012; Gutiérrez-Pesquera et al., 2016). Such elevated WT values are in between those recorded for tadpoles living in cool ponds and streams of the subtropical Atlantic Forest in northern Argentina (i.e., WT = 13.2°C; Duarte et al., 2012), and in the Atlantic Forest in southeastern Brazil (i.e., WT = 9.0°C; Simon et al., 2015). In the case of *B. ibitiguara*, adults only reproduce in cool streams that are thermally insulated by gallery forests (Nali and Prado, 2012). Thus the likelihood of long-term thermal heat stress resulting from anthropogenic land-use changes such as deforestation would expose streams to higher daily and seasonal variation in temperature, which may impact the survival of tadpoles. As such, Pintanel et al. (2019) found strong variation in the maximum temperatures in habitats between forests and open environments inhabited by tropical Andean frogs. Their results suggest that environmental thermal variability differences could lead, through local adaptations, to different thermal tolerances. Thus, species tended to be thermal specialists in the less variable thermal environments, similar to what we describe for *B. ibitiguara*.

Effect of Temperature on Aerobic Metabolism

Previous studies have shown that small aquatic ectotherms may be able to acclimate within a relatively short timeframe (Brown et al., 2004; Rohr et al., 2018). In fact, it is clear that the increase in the stream temperature by 3.1°C relative to the average value (T_{mean}: 21.9°C) would considerably impact *B. ibitiguara* tadpoles'

survival success, as tadpoles acclimated at 25°C (T_{acc25}) exhibited a relatively high $r\dot{M}O_2$ compared to tadpoles acclimated at 18°C (T_{acc18}). This suggests that *B. ibitiguara* is unable to show thermal compensation of cardiorespiratory functions at 3°C above their habitat's average temperature (i.e., 21.9°C). In fact, although $r\dot{M}O_2$ measurements were possible at 34°C in acclimated tadpoles to 25°C, $m\dot{M}O_2$ measurements at the same test temperature were unsuccessful, as tadpoles did not withstand the chase protocol and some ($N = 2$) died during the initial phase of the subsequent respirometry measurements. Although CT_{max} in T_{acc25} tadpoles was relatively higher (36.7 ± 0.09°C) than the temperature at which tadpoles died (∼34°C), we must consider that tadpoles were warmed relatively fast (i.e., 0.1°C min^{-1}). Therefore, we must recognize that the chosen warming protocol to obtain the CT_{max} may have overestimated CT_{max} values, since a slower heating rate could have returned lower CT_{max} values as previously suggested (Chown et al., 2009; Rezende et al., 2011; Ribeiro et al., 2012; Simon et al., 2015).

Noteworthily, in addition to the acclimation temperature at 25°C, we also tested a higher temperature (28°C – tested in tadpoles collected on our first fieldtrip) in which tadpoles were maintained for up to 3 weeks. However, animals exhibited signs of reduced food intake and showed poor body condition, which was also observed in T_{acc25} (visual observation, see **Supplementary Figure 4**). Other studies have also observed such deleterious effects of high acclimation temperatures in different taxa, such as arthropods, urchins, zooplankton and salmon (Rall et al., 2010; Lemoine and Burkepile, 2012; Alcaraz et al., 2014; Hvas et al., 2017). For instance, Healy and Schulte (2012), studying the fish *Fundulus heteroclitus,* found that at temperatures where both $r\dot{M}O_2$ and $m\dot{M}O_2$ were still increasing exponentially with temperature and aerobic scope was maximal, the fish had difficulty maintaining body mass during long-term acclimation. This suggests that there are limitations to the ability to take up, process or assimilate enough nutrients to support the high metabolic rates at high acclimation temperatures (Edwards, 1971; Schulte, 2015).

Effects of Temperature on Body Size and Developmental Implications

We found significant differences in body measurements between acclimation groups, with T_{acc25} overall, exhibiting smaller body size characteristics compared to T_{acc18} after 3 weeks of acclimation (**Table 1**). Our data corroborate the decrease in growth observed at the highest acclimation temperature (27°C) in weatherfish larvae of *Misgurnus fossilis* (Schreiber et al., 2017). We recognize our limitations in drawing conclusions about the effect of acclimation temperature on body characteristics due to the lack of data preceding the experiments. However, after the completion of the experimental protocols on aerobic metabolism, tadpoles were returned to their acclimation temperatures, and their further development was observed. Interestingly, the tadpoles from T_{acc25} did not metamorphose, in contrast to individuals of T_{acc18}, of which many developed as expected. Normally, environmental stressors such as temperature, prolonged droughts and hypoxic environments would accelerate metamorphosis by increasing the hypothalamus-pituitary-interrenal axis activity (Kikuyama et al., 1993; Owerkowicz et al., 2009; Heinrich et al., 2011; Rollins-Smith, 2017). The putatively reduced growth and the prevention of metamorphosis in tadpoles of T_{acc25} may indicate changes in energy allocation, with most of it being diverted to maintain a high $r\dot{M}O_2$ (Ruthsatz et al., 2018; Weerathunga and Rajapaksa, 2020). Both thyroid and glucocorticoid hormones are known to trigger metamorphosis in amphibians, and elevated temperatures may activate the hypothalamus-pituitary-interrenal axis and accelerate metamorphosis (Duellman and Trueb, 1994; Crespi and Denver, 2004; Ruthsatz et al., 2018). However, the release of hormones for metamorphosis may demand a high metabolic cost, which could have been disrupted in *B. ibitiguara* tadpoles at 25°C due to the high temperature-driven routine metabolic demand, leading to a trade-off between maintaining body condition or metamorphosis. Interestingly, during a field trip in the middle of the dry season (July 2020), *B. ibitiguara* tadpoles could still be found in what seemed to be permanent water ponds, despite the flow of the stream having ceased. We confirmed that these ponds exhibited a temperature of 21.1°C (time of the day 12h05, similar to the manual measurements obtained in other months) and O_2 concentration (4.7 mg/L) did not differ from values when the stream had a running flow (see microhabitat values in the results section). Therefore, it seems that *B. ibitiguara* tadpoles can survive through the dry season in suitable thermal conditions by potentially delaying metamorphosis until the following rainy season.

The major weakness in our study stems from the fact that the effects of acclimation on $r\dot{M}O_2$ and subsequently AAS/FAS cannot be confidently discerned from body size and developmental effects. In particular, the observed increase in $r\dot{M}O_2$ in T_{acc25} after acclimation could be a result of accelerated development at a higher T_a, as $\dot{M}O_2$ generally increases throughout development (Szdzuy et al., 2008; Sartori et al., 2017). Although we cannot exclude the possibility that the tadpoles did not develop faster (although smaller) than tadpoles in the T_{acc18} group, the results are more supportive of stunted, rather than accelerated growth. Given the lack of information on developmental characteristics in this species and the fact that metamorphosis did not occur in this group, we are confident that the increase in $r\dot{M}O_2$ is a genuine effect of acclimation, resetting metabolism to an intrinsically higher level and negatively impacting physiological performance and possibly survival. Furthermore, considering global warming will affect most species for many generations, it is important to investigate whether transgenerational and developmental plasticity may allow this species to compensate for climate change, since parental history and egg development may be relevant to the offspring's thermosensitivity (Seebacher and Grigaltchik, 2014; Donelson et al., 2018).

Effect of Temperature on Maximum Metabolic Rate

Tadpoles of *B. ibitiguara* are mostly sedentary, unless feeding or escaping from predators, where high levels of $\dot{M}O_2$ are

required. In regards to the temperature dependency of active oxygen consumption, the $m\dot{M}O_2$ did not increase much beyond 30°C in T_{acc18} (**Figure 3B**). Conversely, $r\dot{M}O_2$ continued its exponential increase in T_{acc18}, until the temperature approached a lethal level (34°C), while $r\dot{M}O_2$ in T_{acc25} reached a plateau at 30°C. This same response was observed in weatherfish larvae (Schreiber et al., 2017) and by Fry (Fry, 1947; Fry and Hart, 1948) when exercising goldfish (*Carassius auratus*), predicting that the optimal temperature for aerobic scope is created by the failure of $m\dot{M}O_2$ to continue increasing with temperature (Farrell, 2009). Tadpoles of *Limnodynastes peroni* also show an exponential increase in $r\dot{M}O_2$ (Seebacher and Grigaltchik, 2014). Animals acclimated to the cold (15°C), showed significantly higher O_2 consumption rates at higher experimental temperatures (20 and 25°C) compared to the group acclimated at higher temperature (i.e., 25°C). In addition, tadpoles of *L. peroni* acclimated to 15°C were more active than animals acclimated to 25°C, which suggests that more oxygen was used by tadpoles acclimated to 15°C for a given level of activity. An alternative explanation is that low temperature activity requires more ATP per unit of muscle power than at high temperature. In our case, both $r\dot{M}O_2$ and $m\dot{M}O_2$ increased exponentially in parallel, up to temperatures close to 25°C before the critical maximum temperature that could be tolerated by the tadpoles was reached. Our data corroborates the notion that in more stable environments, such as the stream that the tadpoles of *B. ibitiguara* inhabit, optimal physiological processes may be constrained by a limited range of environmental temperatures (Gabriel, 2005; Gabriel et al., 2005). In addition, as global water temperatures rise, O_2 solubility in the water is reduced (Dejours, 1981) and therefore animals will face additional challenges to meet the higher oxygen demand of increased metabolic rates (Pörtner et al., 2006).

Effect of Temperature and Autonomic Blockade on Routine Heart Rate

In this study, the effect of prolonged exposure to elevated temperature, i.e., thermal acclimation, did not cause any compensatory response in the autonomic control of f_H. Thermal acclimation may reset resting f_H so that the initially elevated f_H progressively reduces over time upon exposure to the elevated temperature. Such a response is primarily achieved by reducing intrinsic f_H and/or increasing the inhibitory cholinergic tone on the heart (Haverinen and Vornanen, 2007; Ekström et al., 2016; Sandblom et al., 2016b). The treatment with atropine increased the tadpole's heart rate at both test temperatures, although the magnitude of the response was temperature dependent, with a more pronounced tachycardia at the higher experimental temperature. In addition, sotalol treatment following atropine reduced f_H to near baseline values, suggesting routine f_H and intrinsic f_H are very similar. This suggests that both cholinergic and adrenergic tone exhibit virtually equal influences on routine f_H. In fact, the lack of acclimation response in the autonomic control of f_H and intrinsic f_H in *B. ibitiguara* may be explained by the fact that tadpoles inhabit temperature stable environments. This contrasts with eurythermal species that exhibit thermal acclimation of autonomic control of f_H with consequent improvements in cardiac function (Seibert, 1979; Sureau et al., 1989; Ekström et al., 2016; Sandblom et al., 2016b).

It is interesting to note that changes in f_H with acute warming (from 18 to 25°C in T_{acc18}) and acute cooling (from 25 to 18°C in the T_{acc25}) are equal. However, as previously discussed, routine values for metabolic rate for acclimation group T_{acc25} are considerably elevated relative to acclimation group T_{acc18} (for approximately the same temperature interval, that is, from 20 to 25°C (see **Supplementary Figure 5**). Since routine f_H did not differ between acclimation groups, the maintenance of a high routine metabolic rate for T_{acc25} tadpoles can only be explained by increases in cardiac output due to adjustments in stroke volume, and/or increases in arteriovenous extraction. Indeed, increases in stroke volume was previously observed in tadpoles of *Xenopus leavis*, in which significant adjustments in cardiac output after exposure to acute hypoxia occurred by increasing both f_H and stroke volume (Francis Pan and Burggren, 2013). Yet, this hypothesis remains untested in tadpoles and requires further studies.

CONCLUSION AND PERSPECTIVES

Our study demonstrates that tadpoles of *B. ibitiguara* have a limited phenotypic plasticity in response to acclimation to high temperatures, since the thermal tolerance was not different between acclimation groups (18 and 25°C), and cardiorespiratory functions (i.e., routine f_H and $r\dot{M}O_2$) increased substantially with high temperature acclimation. On the other hand, $m\dot{M}O_2$ remained low in relation to the lower temperature of acclimation. Consequently, the tadpoles presented a reduced aerobic metabolic scope when acclimated to a higher temperature (25°C) and therefore an increased vulnerability to climate-driven increases in temperature. In addition, our hypothesis that there would be f_H compensation due to elevation in cholinergic tone or reductions of intrinsic f_H was not confirmed, since cholinergic and adrenergic tone exhibit virtually equal influences on resting f_H independent of acclimation group. These findings may be related to the fact that *B. ibitiguara* tadpoles develop in a stable micro-habitat in which daily and seasonal changes in water temperature are narrow. Such traits may reflect the characteristics of the gallery forests alongside streams that the tadpoles inhabit. Further, tadpoles may find favorable conditions throughout their habitat to allow a prolonged larval phase and possibly adjust the time of metamorphosis to the beginning of the next rainy season.

This lack of plasticity during the larval phase of *B. ibitiguara* has important conservation implications, because adults of this anuran amphibian are habitat specialists, always associated to a topographically complex landscape that has endured anthropogenic modification (Nali et al., 2020). Moreover, in recent years the region where the study was conducted has experienced prolonged droughts and streams have been used to capture water, contributing to more frequent drying periods. Also, the Brazilian Cerrado is one of the most threatened tropical savannas in the world, with nearly a 100 endemic amphibians' species, including *B. ibitiguara* (Nali and Prado, 2012; Valdujo et al., 2012;

CEPF: Critical Ecosystem Partnership Fund, 2017). Therefore, in a scenario with prolonged droughts, gradual increases in ambient temperatures and degradation of remaining gallery forests in non-protected areas of the Brazilian Cerrado, the survival of this species will likely be affected. Thus, even if CT_{max} values found here are above the temperatures the species usually experience, the probability of experiencing high temperatures above their optimal temperatures would increase in the future. In addition, there are very few studies on this topic, despite the enormous diversity of anuran species in Brazil and in the Neotropical region.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The animal study was reviewed and approved by animal collection was approved by the Brazilian Environmental Agency (SISBIO-ICMBio, #621361), and all experimental protocols were approved by the local Animal Care and Use Committee (CEUA-FCAV-UNESP; #02205/18).

AUTHOR CONTRIBUTIONS

LSL, LAZ, ETP, and LHG designed the research. LSL, LAZ, ACGR, and GSL performed the experiments and LSL, LAZ, and ETP analyzed the data. LAZ and LHG supervised the project. All authors interpreted the data and provided critical and intellectual input during the preparation of the manuscript and approved the final version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphys.2021.726440/full#supplementary-material

REFERENCES

Abe, A. S., and Neto, J. R. (1991). Tolerance to high temperatures in tadpoles of *Leptodactylus fuscus* and *Hyla fuscovaria* in temporary ponds (Amphibia, Leptodactylidae, Hylidae). *Zool. Anz.* 226, 280–284.

Agudelo-Cantero, G. A., and Navas, C. A. (2019). Interactive effects of experimental heating rates, ontogeny and body mass on the upper thermal limits of anuran larvae. *J. Therm. Biol.* 82, 43–51. doi: 10.1016/j.jtherbio.2019.03.010

Alcaraz, M., Felipe, J., Grote, U., Arashkevich, E., and Nikishina, A. (2014). Life in a warming ocean: thermal thresholds and metabolic balance of arctic zooplankton. *J. Plankton Res.* 36, 3–10. doi: 10.1093/plankt/fbt111

Angilletta, M. J. J. (2009). *Thermal Adaptation: A Theoretical and Empirical Synthesis*. Oxford: Oxford University Press. doi: 10.1093/acprof:oso/9780198570875.001.1

Badr, A., El-Sayed, M. F., and Vornanen, M. (2016). Effects of seasonal acclimatization on temperature dependence of cardiac excitability in the roach, *Rutilus rutilus*. *J Exp Biol.* 219, 1495–1504. doi: 10.1242/jeb.138347

Barrionuevo, W. R., and Fernandes, M. N. (1998). Time-course of respiratory metabolic adjustments of a South American fish, *Prochilodus scrofa*, exposed to low and high temperatures. *J. Appl. Ichthyol.* 14, 37–41. doi: 10.1111/j.1439-0426.1998.tb00611.x

Bernardo, J., and Spotila, J. R. (2006). Physiological constraints on organismal response to global warming: Mechanistic insights from clinally varying populations and implications for assessing endangerment. *Biol. Lett.* 2, 135–139. doi: 10.1098/rsbl.2005.0417

Bícego-Nahas, K. C., and Branco, L. G. (1999). Seasonal changes in the cardiorespiratory responses to hypercarbia and temperature in the bullfrog, *Rana catesbeiana*. *Com. Biochem. Physiol. A Mol. Integr. Physiol.* 124, 221–229. doi: 10.1016/S1095-6433(99)00119-1

Bovo, R. P., Kohlsdorf, T., and de Andreade, D. O. V. (2020). "Fisiologia térmica em anfíbios," in *Fisiologia Térmica de Vertebrados. [recurso eletrônico]*, eds K. C. Bícego and L. H. Gargaglioni (São Paulo: Cultura Acadêmica), 147–175.

Brattstrom, B. H. (1968). Thermal acclimation in anuran amphibians as a function of latitude and altitude. *Comp. Biochem. Physiol.* 24, 93–111. doi: 10.1016/0010-406X(68)90961-4

Briceño, F. A., Fitzgibbon, Q. P., Polymeropoulos, E. T., Hinojosa, I. A., and Pecl, G. T. (2020). Temperature alters the physiological response of spiny lobsters under predation risk. *Conserv. Physiol.* 8, 1–16. doi: 10.1093/conphys/coaa065

Brown, J. H., Gillooly, J. F., Allen, A. P., Savage, V. M., and West, G. B. (2004). Toward a metabolic theory of ecology. *Ecology* 85, 1771–1789. doi: 10.1890/03-9000

Caramaschi, U., and Eterovick, P. C. (2004). *Bokermannohyla ibitiguara*. *IUCN Red List Threatened Species* 2004:e.T55509A11320909.

Cardoso, A. J. (1983). Descrição e biologia de uma nova espécie de Hyla Laurenti, 1768 (Amphibia, Anura, Hylidae). *Iheringia Série Zoologia* 62, 37–45.

CEPF: Critical Ecosystem Partnership Fund (2017). *Perfil do Ecossistema Hotspot de Biodiversidade do Cerrado*. Brasilia: ISPN & CI, 495.

Charmantier, A., McCleery, R. H., Cole, L. R., Perrins, C., Kruuk, L. E. B., and Sheldon, B. C. (2008). Adaptive phenotypic plasticity in response to climate change in a wild bird population. *Science* 320, 800–803. doi: 10.1126/science.1157174

Chen, I. C., Shiu, H. J., Benedick, S., Holloway, J. D., Chey, V. K., Barlow, H. S., et al. (2009). Elevation increases in moth assemblages over 42 years on a tropical mountain. *Proc. Natl. Acad. Sci. U.S.A.* 106, 1479–1483. doi: 10.1073/pnas.0809320106

Chown, S. L., Jumbam, K. R., Sørensen, J. G., and Terblanche, J. S. (2009). Phenotypic variance, plasticity and heritability estimates of critical thermal limits depend on methodological context. *Funct. Ecol.* 23, 133–140. doi: 10.1111/j.1365-2435.2008.01481.x

Clark, T. D., Sandblom, E., and Jutfelt, F. (2013). Aerobic scope measurements of fishes in an era of climate change: respirometry, relevance and recommendations. *J. Exp. Biol.* 216, 2771–2782. doi: 10.1242/jeb.084251

Clusella-Trullas, S., and Chown, S. L. (2013). Lizard thermal trait variation at multiple scales: a review. *J. Comp Physiol. B* 183, 323–332.

Cowles, R. B., and Bogert, C. M. (1944). A preliminary study of the thermal requirements of desert reptiles. *Bull. Am. Museum Nat. Hist.* 83, 261–296.

Crespi, E. J., and Denver, R. J. (2004). Roles of corticotropin-releasing factor, neuropeptide-y, and corticosterone in the regulation food intake in Xenopus laevis. *J. Neuroendocrinol.* 16, 279–288. doi: 10.1111/j.0953-8194.2004.01168.x

Dejours, P. (1981). *Principles of Comparative Respiratory Physiology. 2nd revised edn*. Amsterdam: Elsevier/North-Holland Biomedical Press.

Deutsch, C. A., Tewksbury, J. J., Huey, R. B., Sheldon, K. S., Ghalambor, C. K., Haak, D. C., et al. (2008). Impacts of climate warming on terrestrial ectotherms across latitude. *Proc. Natl. Acad. Sci. U.S.A.* 105, 6668–6672. doi: 10.1073/pnas.0709472105

Diffenbaugh, N. S., and Ashfaq, M. (2010). Intensification of hot extremes in the United States. *Geophys. Res. Lett.* 37:L15701. doi: 10.1029/2010GL043888

Donelson, J. M., Wong, M., Booth, D. J., and Munday, P. L. (2018). Transgenerational plasticity of reproduction depends on rate of warming across generations. *Evol. Appl.* 9, 1072–1081. doi: 10.1111/eva.12386

Duarte, H., Tejedo, M., Katzenberger, M., Marangoni, F., Baldo, D., Beltrán, J. F., et al. (2012). Can amphibians take the heat? Vulnerability to climate warming in subtropical and temperate larval amphibian communities. *Glob. Chang. Biol.* 18, 412–421. doi: 10.1111/j.1365-2486.2011.02518.x

Duellman, W. E., and Trueb, L. (1994). *Biology of Amphibians*. Baltimore, MA: The Johns Hopkins University Press.

Edwards, D. J. (1971). Effect of temperature on rate of passage of food through the alimentar canal of the plaice *Pleuronectes platessa* L. *J. Fish. Biol.* 3, 433–439. doi: 10.1111/j.1095-8649.1971.tb05915.x

Ekström, A., Hellgren, K., Grans, A., Pichaud, N., and Sandblom, E. (2016). Dynamic changes in scope for heart rate and cardiac autonomic control during warm acclimation in rainbow trout. *J. Exp. Biol.* 219, 1106–1109. doi: 10.1242/jeb.134312

Eterovick, P. C., Souza, A. M., and Sazima, I. (2020). *Anfíbios Anuros da Serra do Cipó–Anuran Amphibians of the Serra do Cipó*. Belo Horizonte: Editora Grafion, 294.

Farrell, A. P. (2009). Environment, antecedents and climate change: lessons from the study of temperature physiology and river migration of salmonids. *J. Exp. Biol.* 212, 3771–3780. doi: 10.1242/jeb.023671

Foden, W., Mace, G., Vié, J.-C., Angulo, A., Butchart, S., DeVantier, L., et al. (2008). "Species susceptibility to climate change impacts," in *The 2008 Review of The IUCN Red List of Threatened Species*, eds J.-C. Vié, C. Hilton-Taylor, and S. N. Stuart (Gland: IUCN).

Foden, W. B., Butchart, S. H. M., Stuart, S. N., Vié, J.-C., Akçakaya, H. R., Angulo, A., et al. (2013). Identifying the world's most climate change vulnerable species: a systematic trait-based assessment of all birds, amphibians and corals. *PLoS One* 8:e65427. doi: 10.1371/journal.pone.0065427

Francis Pan, T. C., and Burggren, W. W. (2013). Ontogeny of hypoxic modulation of cardiac performance and its allometry in the African clawed frog *Xenopus laevis*. *J. Comp. Physiol. B* 183, 123–133. doi: 10.1007/s00360-012-0686-3

Fry, F. E. J. (1947). Effects of the environment on animal activity. *Publ. Ontario Fish. Res. Lab.* 68, 1–52.

Fry, F. E. J. (1971). "The effect of environmental factors on the physiology of fish," in *Fish Physiology*, eds W. S. Hoar and D. J. Randall (San Diego, CA: Academic Press), 1–99. doi: 10.1016/S1546-5098(08)60146-6

Fry, F. E. J., and Hart, J. S. (1948). Cruising speed of goldfish in relation to water temperature. *J. Fish. Res. Board. Can.* 7, 169–175. doi: 10.1139/f47-018

Gabriel, W. (2005). How stress selects for reversible phenotypic plasticity. *J. Evol. Biol.* 18, 873–883. doi: 10.1111/j.1420-9101.2005.00959.x

Gabriel, W., Luttbeg, B., Sih, A., and Tollrian, R. (2005). Environmental tolerance, heterogeneity, and the evolution of reversible plastic responses. *Am. Nat.* 166, 339–353. doi: 10.1086/432558

Ghalambor, C. K., Huey, R. B., Martin, P. R., Tewksbury, J. J., and Wang, G. (2006). Are mountain passes higher in the tropics? Janzen's hypothesis revisited. *Integr. Comp. Biol.* 46, 5–17. doi: 10.1093/icb/icj003

Gifford, M. E., and Kozak, K. H. (2012). Islands in the sky or squeezed at the top? Ecological causes of elevational range limits in montane salamanders. *Ecography* 35, 193–203. doi: 10.1111/j.1600-0587.2011.06866.x

Gosner, K. L. (1960). A simplified table for staging anuran embryos and larvae with notes on identification. *Herpetologica* 16, 183–190.

Gutiérrez-Pesquera, L. M., Tejedo, M., Olalla-Tárraga, M. Á, Duarte, H., Nicieza, A., and Solé, M. (2016). Testing the climate variability hypothesis in thermal tolerance limits of tropical and temperate tadpoles. *J. Biogeogr.* 43, 1116–1178. doi: 10.1111/jbi.12700

Haddad, C. F. B., Andrade, G. V., and Cardoso, A. J. (1988). Anfíbios anuros do parque nacional da Serra da Canastra, Estado de Minas Gerais. *Brasil Florestal* 64, 9–20.

Haverinen, J., and Vornanen, M. (2007). Temperature acclimation modifies sinoatrial pacemaker mechanism of the rainbow trout heart. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 292, 1023–1032. doi: 10.1152/ajpregu.00432.2006

Healy, T. M., and Schulte, P. M. (2012). Factors affecting plasticity in whole-organism thermal tolerance in common killifish (*Fundulus heteroclitus*). *J. Comp. Physiol. B* 182, 49–62. doi: 10.1007/s00360-011-0595-x

Hedrick, M. S., Palioca, W. B., and Hillman, S. S. (1999). Effects of temperature and physical activity on blood flow shunts and intracardiac mixing in the toad Bufo marinus. *Physiol. Biochem. Zool.* 72, 509–519. doi: 10.1086/316693

Heinrich, E. C., Farzin, M., Klok, C. J., and Harrison, J. F. (2011). The effect of developmental stage on the sensitivity of cell and body size to hypoxia in *Drosophila melanogaster*. *J. Exp. Biol.* 214, 1419–1427. doi: 10.1242/jeb.051904

Hillman, S. S., and Hedrick, M. S. (2015). A meta-analysis of in vivo vertebrate cardiac performance: implications for cardiovascular support in the evolution of endothermy. *J. Exp. Biol.* 218, 1143–1150. doi: 10.1242/jeb.118372

Huey, R. B., Kearney, M. R., Krockenberger, A., Holtum, J. A. M., Jess, M., and Williams, S. E. (2012). Predicting organismal vulnerability to climate warming: roles of behaviour, physiology, and adaptation. *Philos. Trans. R. Soc. B* 367, 1665–1679. doi: 10.1098/rstb.2012.0005

Huey, R. B., and Kingsolver, J. G. (1993). Evolution of resistance to high temperature in ectotherms. *Am. Nat.* 142, 21–46. doi: 10.1086/285521

Hvas, M., Folkedal, O., Imsland, A., and Oppedall, F. (2017). The effect of thermal acclimation on aerobic scope and critical swimming speed in Atlantic salmon, Salmo salar. *J. Exp. Biol.* 220, 2757–2764. doi: 10.1242/jeb.154021

IPCC (2021). *Climate Change 2021: The Physical Science Basis. Contribution of Working Group I to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change*, eds V. Masson-Delmotte, P. Zhai, A. Pirani, S. L. Connors, C. Péan, S. Berger, et al. Cambridge: Cambridge University Press.

Jensen, M. A., Fitzgibbon, Q. P., Carter, C. G., and Adams, L. R. (2013). Recovery periods of cultured spiny lobster, *Sagmariasus verreauxi* juveniles: effects of handling, force feeding, exercising to exhaustion and anaesthesia on oxygen consumption and ammonia-N excretion rates. *Aquaculture* 410–411, 114–121. doi: 10.1016/j.aquaculture.2013.06.020

Kern, P., Cramp, R. L., and Franklin, C. E. (2014). Temperature and UV-B-insensitive performance in tadpoles of the ornate burrowing frog: an ephemeral pond specialist. *J. Exp. Biol.* 217, 1246–1252. doi: 10.1242/jeb.097006

Kern, P., Cramp, R. L., and Franklin, C. E. (2015). Physiological responses of ectotherms to daily temperature variation. *J. Exp. Biol.* 218, 3068–3076. doi: 10.1242/jeb.123166

Kikuyama, S., Kawamura, K., Tanaka, S., and Yamamoto, K. (1993). Aspects of amphibian metamorphosis: hormonal control. *Int. Rev. Cytol.* 145, 105–148. doi: 10.1016/S0074-7696(08)60426-X

Lawler, J. J., Shafer, S. L., and Blaustein, A. R. (2010). Projected climate impacts for the amphibians of the western hemisphere. *Conserv. Biol.* 24, 38–50. doi: 10.1111/j.1523-1739.2009.01403.x

Leite, F. S. F., and Eterovick, P. C. (2010). Description of the tadpole of *Bokermannohyla martinsi* (Anura: Hylidae), morphological and ecological comparison with related Bokermannohyla tadpoles. *J. Herpetol.* 44, 431–440. doi: 10.1670/09-079.1

Lemoine, N. P., and Burkepile, D. E. (2012). Temperature−induced mismatches between consumption and metabolism reduce consumer fitness. *Ecology* 93, 2483–2489. doi: 10.1890/12-0375.1

Longhini, L. S., Zena, L. A., da Silva, G. S. F., Bícego, K. C., and Gargaglioni, L. H. (2017). Temperature effects on the cardiorespiratory control of American bullfrog tadpoles based on a non-invasive methodology. *J. Exp. Biol.* 220, 3763–3770. doi: 10.1242/jeb.160911

Lutterschmidt, W. I., and Hutchison, V. H. (1997). The critical thermal maximum: history and critique. *Can. J. Zool.* 75, 1561–1574. doi: 10.1139/z97-783

Moyano, M., Candebat, C., Ruhbaum, Y., Álvarez-Fernández, S., Claireaux, G., Zambonino-Infante, J. L., et al. (2017). Effects of warming rate, acclimation temperature and ontogeny on the critical thermal maximum of temperate marine fish larvae. *PLoS One* 12:e0179928. doi: 10.1371/journal.pone.0179928

Nali, R. C., Becker, C. G., Zamudio, K. R., and Prado, C. P. A. (2020). Topography, more than land cover, explains genetic diversity in a Neotropical savanna tree frog. *Divers. Distrib.* 26, 1798–1812. doi: 10.1111/ddi.13154

Nali, R. C., and Prado, C. P. A. (2012). Habitat use, reproductive traits and social interactions in a stream-dweller treefrog endemic to the Brazilian Cerrado. *Amphib. Reptil.* 33, 337–347. doi: 10.1163/15685381-00002836

Navas, C. A., Otani, L., and Carvalho, J. E. (2008). Thermal relationships and exercise physiology in anuran amphibians: integration and evolutionary implications. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* 151, 344–362. doi: 10.1016/j.cbpa.2007.07.003

Nilsson, G. E., Crawley, N., Lunde, I. G., and Munday, P. L. (2009). Elevated temperatures reduces the respiratory scope of coral reef fishes. *Glob. Chang. Biol.* 15, 1405–1412. doi: 10.1111/j.1365-2486.2008.01767.x

Overgaard, J., Andersen, J. L., Findsen, A., Pedersen, P. B. M., Hansen, K., Ozolina, K., et al. (2012). Aerobic scope and cardiovascular oxygen transport is not compromised at high temperatures in the toad *Rhinella marina*. *J. Exp. Biol.* 215, 3519–3526. doi: 10.1242/jeb.070110

Owerkowicz, T., Elsey, R. M., and Hicks, J. W. (2009). Atmospheric oxygen level affects growth trajectory, cardiopulmonary allometry and metabolic rate in the American alligator (*Alligator mississippiensis*). *J. Exp. Biol.* 212, 1237–1247. doi: 10.1242/jeb.023945

Pacifici, M., Visconti, P., Butchart, S. H. M., Watson, J. E. M., Cassola, F. M., and Rondinini, C. (2017). Species' traits influenced their response to recent climate change. *Nat. Clim. Chang.* 7, 205–208. doi: 10.1038/nclimate3223

Parmesan, C. (2006). Ecological and evolutionary responses to recent climate change. *Annu. Rev. Ecol. Evol. Syst.* 37, 637–669. doi: 10.1146/annurev.ecolsys.37.091305.110100

Parmesan, C., and Yohe, G. (2003). A globally coherent fingerprint of climate change impacts across natural systems. *Nature* 421, 37–42. doi: 10.1038/nature01286

Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D., and R Core Team (2021). *nlme: Linear and Nonlinear Mixed Effects Models*. Available online at: https://CRAN.R-project.org/package=nlme

Pintanel, P., Tejedo, M., Ron, S. R., Llorente, G. A., and Merino-Viteri, A. (2019). Elevational and microclimatic drivers of thermal tolerance in Andean Pristimantis frogs. *J. Biogeogr.* 46, 1664–1675. doi: 10.1111/jbi.13596

Pörtner, H. O., Peck, L. S., and Hirse, T. (2006). Hyperoxia alleviates thermal stress in the Antarctic bivalve, *Laternula elliptica*: evidence for oxygen limited thermal tolerance. *Polar Biol.* 29, 688–693. doi: 10.1007/s00300-005-0106-1

Pough, F. H., Magnusson, W. E., Ryan, M. J., Wells, K. D., and Taigen, T. L. (1992). "Behavioral energetics," in *Environmental Physiology of the Amphibians*, eds M. E. Feder and W. M. Burggren (Chicago, IL: The Univercity of Chicago Press), 395–436.

R Core Team (2020). *R: A Language and Environment for Statistical Computing*. Vienna: R Foundation for Statistical Computing.

Rall, B. C., Vucic-Pestic, O., Ehnes, R. B., Emmerson, M., and Brose, U. (2010). Temperature, predator-prey interaction strength and population stability. *Glob. Chang. Biol.* 16, 2145–2157. doi: 10.1111/j.1365-2486.2009.02124.x

Reidy, S. P., Nelson, J. A., Tang, Y. Y., and Kerr, S. R. (1995). Post-exercise metabolic rate in Atlantic cod and its dependence upon the method of exhaustion. *J. Fish Biol.* 47, 377–386. doi: 10.1111/j.1095-8649.1995.tb01907.x

Rezende, E. L., Tejedo, M., and Santos, M. (2011). Estimating the adaptive potential of critical thermal limits: methodological problems and evolutionary implications. *Funct. Ecol.* 25, 111–121. doi: 10.1111/j.1365-2435.2010.01778.x

Ribeiro, P. L., Camacho, A., and Navas, C. A. (2012). Considerations for assessing maximum critical temperatures in small ectothermic animals: Insights from leaf-cutting ants. *PLoS One* 7:e32083. doi: 10.1371/journal.pone.0032083

Rocha, P. L., and Branco, L. G. (1998). Seasonal changes in the cardiovascular, respiratory and metabolic responses to temperature and hypoxia in the bullfrog *Rana catesbeiana*. *J. Exp. Biol.* 201, 761–768. doi: 10.1242/jeb.201.5.761

Rohr, J. R., Civitello, D. J., Cohen, J. M., Roznik, E. A., Sinervo, B., and Dell, A. I. (2018). The complex drivers of thermal acclimation and breadth in ectotherms. *Ecol. Lett.* 21, 1425–1439. doi: 10.1111/ele.13107

Rollins-Smith, L. A. (2017). Amphibian immunity stress, disease, and climate change. *Dev. Comp. Immunol.* 66, 111–119. doi: 10.1016/j.dci.2016.07.002

Rome, L. C., Stevens, E. D., and John-Alder, H. B. (1992). "The influence of temperature and thermal acclimation on a physiological function," in *Environmental Physiology of the Amphibians*, eds M. E. Feder and W. W. Burggren (Chicago, IL: University of Chicago Press), 183–205.

Rosewarne, P. J., Wilson, J. M., and Svendsen, J. C. (2016). Measuring maximum and standard metabolic rates using intermittent-flow respirometry: a student laboratory investigation of aerobic metabolic scope and environmental hypoxia in aquatic breathers. *J.Fish Biol.* 88, 265–283. doi: 10.1111/jfb.12795

Ruthsatz, K., Dausmann, K. H., Peck, M. A., Drees, C., Sabatino, N. M., Becker, L. I., et al. (2018). Thyroid hormone levels and temperature during development alter thermal tolerance and energetics of *Xenopus laevis* larvae. *Conserv. Physiol.* 6, 1–15. doi: 10.1093/conphys/coy059

Sandblom, E., Clark, T. D., Gräns, A., Ekström, A., Brijs, J., Sundström, L. F., et al. (2016a). Physiological constraints to climate warming in fish follow principles of plastic floors and concrete ceilings. *Nat. Commun.* 7:11447. doi: 10.1038/ncomms11447

Sandblom, E., Ekström, A., Brijs, J., Sundström, L. F., Jutfelt, F., Clark, T. D., et al. (2016b). Cardiac reflexes in a warming world: thermal plasticity of barostatic control and autonomic tones in a temperate fish. *J. Exp. Biol.* 219, 2880–2887. doi: 10.1242/jeb.140319

Sandblom, E., Gräns, A., Axelsson, M., and Seth, H. (2014). Temperature acclimation rate of aerobic scope and feeding metabolism in fishes: implications in a thermally extreme future. *Proc. R. Soc. B* 281:20141490. doi: 10.1098/rspb.2014.1490

Sartori, M. R., Abe, A. S., Crossley, D. A., and Taylor, E. W. (2017). Rates of oxygen uptake increase independently of changes in heart rate in late stages of development and at hatching in the green iguana, *Iguana iguana*. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* 205, 28–34. doi: 10.1016/j.cbpa.2016.12.020

Schär, C., Vidale, P. L., Lüthi, D., Frei, C., Häberli, C., Liniger, M. A., et al. (2004). The role of increasing temperature variability in European summer heatwaves. *Nature* 427, 332–336. doi: 10.1038/nature02300

Schreiber, B., Monka, J., Drozd, B., Hundt, M., Weiss, M., Oswald, T., et al. (2017). Thermal requirements for growth, survival and aerobic performance of weatherfish larvae *Misgurnus fossilis*. *J. Fish Biol.* 90, 1597–1608. doi: 10.1111/jfb.13261

Schulte, P. M. (2015). The effects of temperature on aerobic metabolism: towards a mechanistic understanding of the responses of ectotherms to a changing environment. *J. Exp. Biol.* 218, 1856–1866. doi: 10.1242/jeb.118851

Seebacher, F., and Franklin, C. E. (2011). Physiology of invasion: cane toads are constrained by thermal effects on physiological mechanisms that support locomotor performance. *J. Exp. Biol.* 214, 1437–1444. doi: 10.1242/jeb.053124

Seebacher, F., and Grigaltchik, V. S. (2014). Embryonic developmental temperatures modulate thermal acclimation of performance curves in tadpoles of the frog *Limnodynastes peronei*. *PLoS One* 9:e106492. doi: 10.1371/journal.pone.0106492

Seebacher, F., White, C. R., and Franklin, C. E. (2015). Physiological plasticity increases resilience of ectothermic animals to climate change. *Nat. Clim. Chang.* 5, 61–66. doi: 10.1038/nclimate2457

Seibert, H. (1979). Thermal adaptation of heart rate and its parasympathetic control in the European eel *Anguilla anguilla* (L.). *Comp. Biochem. Physiol. Part C Comp. Pharmacol.* 64, 275–278. doi: 10.1016/0306-4492(79)90063-7

Settele, J., Scholes, R., Betts, S., Bunn, P., Leadley, D., Nepstad, J. T., et al. (2014). "Terrestrial and Inland Water Systems," in *Climate Change 2014: Impacts, Adaptation, and Vulnerability. Part A: Global and Sectoral Aspects. Contribution*

of Working Group II to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change, eds C. B. Field, V. R. Barros, D. J. Dokken, K. J. Mach, M. D. Mastrandrea, T. E. Bilir, et al. (Cambridge: Cambridge University Press), 271–359.

Simon, M. N., Ribeiro, P. L., and Navas, C. A. (2015). Upper thermal tolerance in tropical amphibian species from contrasting habitats: implications for warming impact prediction. *J. Exp. Biol.* 48, 36–44. doi: 10.1016/j.jtherbio.2014.12.008

Somero, G. N., and DeVries, A. L. (1967). Temperature tolerance of some Antarctic fishes. *Science* 156, 257–258. doi: 10.1126/science.156.3772.257

Steffensen, J. (1989). Some errors in respirometry of aquatic breathers: how to avoid and correct for them. *Fish Physiol. Biochem.* 6, 49–59. doi: 10.1007/BF02995809

Sureau, D., Lagardere, J. P., and Pennec, J. P. (1989). Heart rate and its cholinergic control in the sole (Solea vulgaris), acclimatized to different temperatures. *Comp. Biochem. Physiol. Part A* 92, 49–51. doi: 10.1016/0300-9629(89)90739-1

Svendsen, M. B. S., Bushnell, P. G., and Steffensen, J. F. (2016). Design and setup of intermittent-flow respirometry system for aquatic organisms. *J. Fish Biol.* 88, 26–50. doi: 10.1111/jfb.12797

Szdzuy, K., Fong, L. M., and Mortola, J. P. (2008). Oxygenation and establishment of thermogenesis in the avian embryo. *Life Sci.* 82, 50–58. doi: 10.1016/j.lfs.2007.10.007

Valdujo, P. H., Silvano, D. L., Colli, G., and Martins, M. (2012). Anuran species composition and distribution patterns in the Brazilian Cerrado, a Neotropical hotspot. *South Am. J. Herpetol.* 7, 63–78. doi: 10.2994/057.007.0209

Weerathunga, W. A. M. T., and Rajapaksa, G. (2020). The impact of elevated temperature and CO2 on growth, physiological and immune responses of Polypedates cruciger (common hourglass tree frog). *Front. Zool.* 17:3. doi: 10.1186/s12983-019-0348-3

Zena, L. A., da Silva, G. S. F., Gargaglioni, L. H., and Bícego, K. C. (2016). Baroreflex regulation affects ventilation in cururu toad Rhinella schneideri. *J. Exp. Biol.* 219, 3605–3615. doi: 10.1242/jeb.144774

Zena, L. A., Gargaglioni, L. H., and Bícego, K. C. (2015). Temperature effects on baroreflex control of heart rate in the toad, Rhinella schneideri. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* 179, 81–88. doi: 10.1016/j.cbpa.2014.09.027

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Two Locomotor Traits Show Different Patterns of Developmental Plasticity Between Closely Related Clonal and Sexual Fish

*Kate L. Laskowski[1,2], Frank Seebacher[3], Marie Habedank[1], Johannes Meka[1] and David Bierbach[1,4,5]**

[1] Department of Biology and Ecology of Fishes, Leibniz Institute of Freshwater Ecology and Inland Fisheries, Berlin, Germany, [2] Department of Evolution and Ecology, University of California, Davis, Davis, CA, United States, [3] School of Life and Environmental Sciences A08, The University of Sydney, Sydney, NSW, Australia, [4] Faculty of Life Sciences, Albrecht Daniel Thaer-Institute, Humboldt University of Berlin, Berlin, Germany, [5] Cluster of Excellence "Science of Intelligence," Technische Universität Berlin, Berlin, Germany

The capacity to compensate for environmental change determines population persistence and biogeography. In ectothermic organisms, performance at different temperatures can be strongly affected by temperatures experienced during early development. Such developmental plasticity is mediated through epigenetic mechanisms that induce phenotypic changes within the animal's lifetime. However, epigenetic modifiers themselves are encoded by DNA so that developmental plasticity could itself be contingent on genetic diversity. In this study, we test the hypothesis that the capacity for developmental plasticity depends on a species' among-individual genetic diversity. To test this, we exploited a unique species complex that contains both the clonal, genetically identical Amazon molly (*Poecilia formosa*), and the sexual, genetically diverse Atlantic molly (*Poecilia mexicana*). We predicted that the greater among-individual genetic diversity in the Atlantic molly may increase their capacity for developmental plasticity. We raised both clonal and sexual mollies at either warm (28°C) or cool (22°C) temperatures and then measured locomotor capacity (critical sustained swimming performance) and unforced movement in an open field across a temperature gradient that simulated environmental conditions often experienced by these species in the wild. In the clonal Amazon molly, differences in the developmental environment led to a shift in the thermal performance curve of unforced movement patterns, but much less so in maximal locomotor capacity. In contrast, the sexual Atlantic mollies exhibited the opposite pattern: developmental plasticity was present in maximal locomotor capacity, but not in unforced movement. Thus our data show that developmental plasticity in clones and their sexual, genetically more diverse sister species is trait dependent. This points toward mechanistic differences in how genetic diversity mediates plastic responses exhibited in different traits.

Keywords: developmental plasticity, swimming speed, thermal performance curve, *Poecilia formosa*, *Poecilia mexicana*, unisexual vertebrate

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***Correspondence:**
David Bierbach
bierbacd@hu-berlin.de

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INTRODUCTION

The early life environment can have pronounced and long-lasting effects on individual phenotypes (Atlasi and Stunnenberg, 2017; Hu and Barrett, 2017). Such developmental plasticity can allow an organism to better tailor their phenotypes for their future expected environments ["predictive adaptive hypothesis" (Bateson et al., 2014)] or better cope with rapid environmental perturbations later in life (Schulte et al., 2011). To understand the evolution of developmental plasticity, we need to understand when and how animals respond to early life environments and to what extent these responses allow animals to cope with later-in-life environmental conditions.

Organisms can respond to early life environments by adjusting their phenotype across numerous traits (Bozinovic et al., 2020). Reaction norms can be used to characterize the response of repeatedly expressed traits like behavioral or physiological traits across a range of environmental conditions (Sarkar and Fuller, 2003). The early developmental environment can lead to coordinated changes in whole suites of traits (Torres-Dowdall et al., 2012; Mateus et al., 2014); what is less clear is whether the plasticity underlying these traits is also matched. That is, will the reaction norm of one trait match the reaction norm of another in response to early environmental experiences? If some animals are more sensitive or better able to perceive environmental cues, then some authors have argued that plasticity should be consistent across different traits (Benus et al., 1987; Koolhaas et al., 1999; Whitman and Agrawal, 2009; Sih and Del Giudice, 2012; Forsman, 2015; Stamps and Biro, 2016). For example, in ectothermic animals, the thermal environment an animal experiences early in life can cause lifelong alterations to muscle contractile function resulting in coordinated effects on traits related to swimming capabilities (Hammill et al., 2004; Orczewska et al., 2010; Scott and Johnston, 2012; Le Roy et al., 2017). However, as different traits have different mechanistic bases it may instead be expected that there are differences in plasticity. Understanding whether and how patterns of plasticity are linked across different traits can therefore offer insight into the potential mechanistic underpinnings of these traits.

Epigenetic mechanisms are likely mediators of phenotypic changes such as developmental plasticity. For example, gene expression patterns can be altered by modifying access of transcriptional regulators to DNA (Whitfield et al., 2003; Aubin-Horth et al., 2005; Scott and Johnston, 2012; Ficz, 2015; Loughland et al., 2021), which can be mediated by changes in DNA-methylation patterns (Klose and Bird, 2006), histone binding (de Ruijter et al., 2003), or small RNA activity (Morris and Mattick, 2014). Even clonal, and hence genetically identical organisms often exhibit considerable phenotypic plasticity in response to variation in their environment (Doeringsfeld et al., 2004; Freund et al., 2013; Lynch and Kemp, 2014; Bierbach et al., 2017; Vogt, 2018). For example, the unisexual and genetically identical fish *Chrosomus eos-neogaeus* exhibited extensive variation in DNA methylation patterns across their genomes (Massicotte et al., 2011) that was correlated with environmental cues from their lake of origin (Massicotte and Angers, 2012). In the clonal Amazon molly (*Poecilia formosa*), several life history traits were strongly affected by salinity and temperature gradients experienced during developmental periods (Makowicz and Travis, 2020). Additionally, Amazon mollies raised in different social contexts developed different behavioral phenotypes (Bierbach et al., 2017). Developmental plasticity may therefore be especially relevant in such clonal organisms, as these animals do not have among-individual genetic variation to generate phenotypic variation.

However, even if phenotypic changes are mediated through epigenetic mechanisms, the shape of the reaction norm can also be altered in response to genotypic changes resulting from selection or genetic drift (Seebacher et al., 2012; Murren et al., 2014). Hence, variation in reaction norms can be mediated by variation in genetic and epigenetic mechanisms. For example, a single mutation determines whether *Manduca* caterpillars exhibit thermally sensitive pigmentation patterns (Suzuki and Nijhout, 2006) and if nematodes develop resource-sensitive variation in mouth morphologies (Bento et al., 2010). Additionally, the large number of proteins involved in the successful methylation (and demethylation) of DNA means that mutations at any number of nucleotides can alter the efficiency and/or specificity of this process (Klose and Bird, 2006; Campos et al., 2013). Natural selection or genetic drift may therefore influence the capacity for developmental plasticity in populations with greater among-individual genetic variation.

Here we test whether the capacity for developmental plasticity is linked across two phenotypic traits and whether plasticity depends on the presence of among-individual genetic variation. We raised closely related clonal and sexually reproducing fish species at two developmental temperatures to determine plasticity in thermal performance curves of swimming capacity and unforced movement. If functionally related traits are also mechanistically related, then we would predict that they would also show correlated patterns of developmental plasticity in response to early life environments. On the other hand, there is evidence for asymmetric thermal effects on thermal performance curves (Bozinovic et al., 2020), which instead predicts that patterns of plasticity are de-coupled. We investigated individual performance in two traits related to locomotion across a thermal gradient to compare the developmental plasticity: maximal swimming capacity measured as critical sustained swimming performance (U_{crit}) and unforced movement in an open field. Both traits are relevant ecologically for these fish; U_{crit} reflects maximal physiological swimming capacity that could limit more extended movement like dispersal (Svendsen et al., 2017) or escape capabilities (Irschick et al., 2008). However, animals rarely move at maximum speed so that it is also relevant to determine temperature effects on the movement speed actually selected by individuals (Wilson et al., 2015). Both traits rely on muscle-powered locomotion, but differ in that U_{crit} is determined by the physiological capacities of the cardiovascular system, mitochondria and muscle, and unforced movement also reflects behavioral decisions that are under cognitive control (Stewart et al., 2013).

To investigate whether and how among-individual genetic diversity may alter patterns of developmental plasticity, we took advantage of a unique species complex that contains both

clonal (genetically identical; the Amazon molly, *P. formosa*) and sexually reproducing (genetically diverse; Atlantic molly, *Poecilia mexicana*) fish (Laskowski et al., 2019). Amazon mollies are the first discovered clonal vertebrate (Hubbs and Hubbs, 1932; Schultz, 1973); they emerged from a single hybridization event between the Atlantic and sailfin molly (*Poecilia latipinna*) about 100,000 years ago and now reproduce gynogenetically (Lampert and Schartl, 2008; Stöck et al., 2010; Warren et al., 2018). The species requires sperm from one of their parental species (Atlantic or sailfin mollies) to stimulate embryonic development, but the paternal genetic material is not incorporated into the egg (but see Kallman, 1962; Rasch et al., 1965; Turner et al., 1980, for rare exceptions of male DNA fragment introgression). The offspring are therefore genetically identical to their mother and each other. The two species have essentially the same phylogenetic history, share half of their genome (Lampert and Schartl, 2008; Warren et al., 2018; Lu et al., 2021) and show a strong overlap in their ecological niche due to their sympatric occurrence as a result of the Amazon's dependence on either sailfin or Atlantic mollies' sperm for reproduction (Darnell and Abramoff, 1968; Schlupp et al., 2002; Scharnweber et al., 2011). This unique species complex thus allowed us to explore how the presence of among-individual genetic variation influences a species' capacity for developmental plasticity in species that are otherwise ecologically identical. On one hand, sexual species such as the Atlantic molly harbor significantly more among-individual genetic variation (Warren et al., 2018; Lu et al., 2021) allowing natural selection to be more effective in shaping the capacity for developmental plasticity and so we predicted that we may see larger shifts in their reaction norms in response to early life environments. On the other hand, the lack of among-individual genetic variation in Amazon mollies leaves epigenetically induced phenotypic plasticity as the major avenue to adjust phenotypes to changing environmental conditions, and so an alternative prediction is that this clonal species would exhibit greater sensitivity, and hence plasticity, to early life environments. We are well aware of the limitations of two-species comparisons (Garland and Adolph, 1994), and we treat this comparison as exploratory and do not intend to infer adaptation.

MATERIALS AND METHODS

Fish Breeding

We isolated several individual pregnant females of each species (housed at 25°C through their lifetime), and immediately after they gave birth, we split broods into groups of 4 sibs each; half of these groups were placed into a warm (28°C) treatment and half into a cool (22°C) treatment (**Figure 1**). Each group was maintained in a 38-liter aquarium with gravel and a plastic plant for shelter. These treatments were chosen as ecologically relevant temperatures the fish would experience seasonally in the wild (Schlupp et al., 2002; Costa and Schlupp, 2010). We generated a total of 16 groups of 4 sibs of the Amazon mollies (*P. formosa*, 8 groups per treatment) from 4 different broods (mothers that were sisters), and 18 groups (9 groups per treatment) of 4 sibs each for the Atlantic mollies (*P. mexicana*) from 5 different broods (mothers). Each treatment group was reared at their respective developmental thermal environment treatments until they were 25–27 weeks old. Fish were then acclimated to a common-garden, intermediate temperature (25°C) for 3 weeks. We conducted the common-garden acclimation treatment to reduce the effects of short-term reversible acclimation from that of the long-term effects of the developmental treatment that we were interested in here. Individuals were acclimated in individual clear plastic bottles (10 cm diameter) placed within a communal tank. The bottles had holes in their sides that were small enough to prevent fish from passing through while still allowing visual and chemical cues to pass among individuals. This set-up allowed us to follow individuals without the need to invasively mark them before phenotyping trials, and it limited handling stress because fish did not need to be netted for each trial (see below). After 3 weeks in acclimation, we measured unforced movement in an open field and locomotor capacity (see below) in each individual.

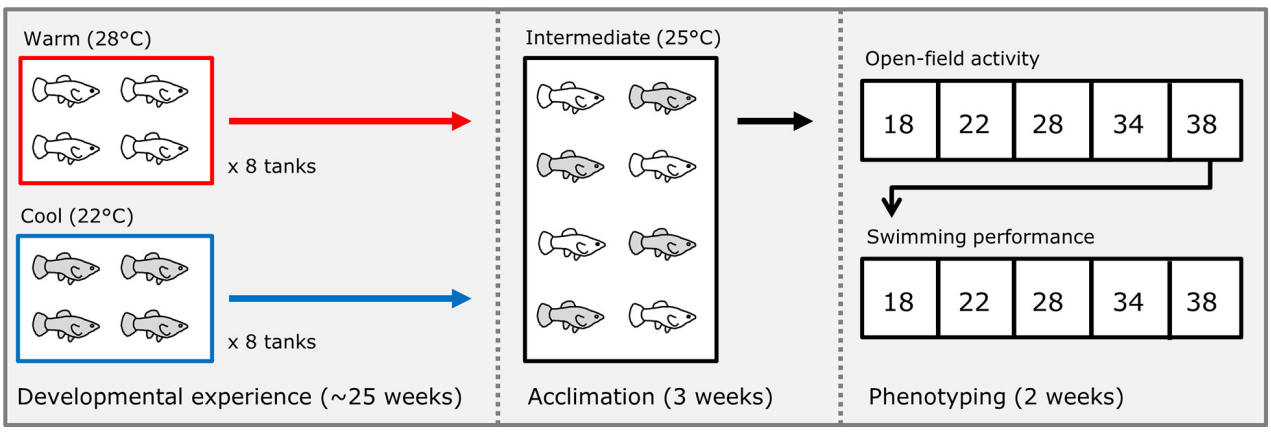

FIGURE 1 | Schematic of experimental design. Immediately after birth, broods of *Poecilia formosa* and *Poecilia mexicana* were split into either a "warm" or "cool" treatment where they developed for approximately 25 weeks. After this time, fish were then acclimated to an intermediate temperature for 3 weeks. After acclimation, each fish was tested for their open field movement and swimming performance at five different temperatures (in random order) over the course of 2 weeks.

Experimental fish of both species were lab-reared descendants of wild-caught fish bred in the laboratory for several generations. Founding individuals of both species were originally collected near the Mexican city of Tampico, where both species occur in sympatry. Regular molecular checks confirmed that all *P. formosa* individuals are clones (M. Schartl, personal communication), and the *P. mexicana* populations have been regularly supplied with new individuals from the wild to maintain levels of natural standing genetic variation; however, this procedure was stopped at least five generations ago to minimize uncontrollable cross-generational epigenetic effects (Kelley et al., 2021) brought in by differences in individual origin.

Throughout the experiment fish were fed twice daily on flake food (TetraMin, tropical fish flake food) and maintained on 12:12 L:D light cycle. On trial days, fish were not fed until after trials were completed. Measurements were staggered over the course of several weeks to ensure that individuals born at different times were of the same age at the time of measurement. We only included data from females of the Atlantic mollies (50 out of 69 experimental animals) as the Amazon molly is an all-female species. Additionally, males are generally considerably smaller and have a different body shape compared to females which could influence their swimming behaviors. In total we collected phenotypic data from 50 Atlantic mollies and 59 Amazon mollies. All behavioral protocols complied with German law and were approved by the Berlin Landesamt für Gesundheit und Soziales (GO 124/14).

Unforced Movement in an Open Field

Behavioral traits, such as unforced movement, are arguably some of the most plastic phenotypes an animal can exhibit and so might be especially sensitive to early environmental cues. Alternatively, because behavior is the result of many sensory, neural and cognitive inputs it may respond most strongly to the immediate environment and may not exhibit long-lasting shifts in response to early life environments. We measured unforced movement of each individual in an open field (white circular arena 48.5 cm diameter, water level 6 cm) (Bierbach et al., 2017) at 18, 22, 28, 34, and 38°C acute test temperatures. We chose these temperatures as they cover the range of temperatures that both species could encounter in the wild (Schlupp et al., 2002; Costa and Schlupp, 2010) and are well within the physiological tolerated range of each species (Bierbach et al., 2010). Fish were measured once per day for 5 days, each day at a different acute test temperature. The order of the temperatures was randomly assigned. In between trials, fish were returned to their individual bottles at the acclimation temperature. Before experiments, we familiarized each individual with the open field arena by conducting a single assay at 25°C to avoid confounding our measure of movement with an effect of novelty to the unfamiliar environment. We did not include data from these pre-trials in the analysis.

To perform an open field trial, an individual bottle containing a fish was removed from the acclimation tank and gently poured into a dark plastic cylinder at the center of the arena. The fish was allowed to rest for 1 min, after which time we gently lifted the cylinder and recorded the behavior of the fish for the next 5 min using a webcam (C920, Logitech, United States). After 5 min, we removed the fish and placed it back in its bottle in the acclimation tank. The water in the arena was replaced in between each trial to minimize chemical cues and to maintain the appropriate temperature by replacing water from a sump tank at the appropriate temperature for the day. Fish were tested in random order. The videos of the open field movement were analyzed using EthoVision 11TX software (Noldus Information Technologies, Inc., Netherlands) from which we extracted the mean velocity of the animal (in body lengths s^{-1}) over the 5-min trial as our measure of movement. Note that other measures such as total distance swam during the trial yielded essentially identical results; see **Supplementary Table 1**. After the trials, we took a digital photograph of each fish, from which we measured the standard length of each fish to the nearest mm.

Swimming Performance

Maximum locomotor capacity is a whole-animal performance trait that is determined to a large extent by muscle contractile function and there is evidence from multiple fish species (Hammill et al., 2004; Seebacher et al., 2012), including the closely related guppy, *P. reticulata* (Le Roy et al., 2017) that it responds plastically to early life environments. In the week following the open field tests, we measured maximal locomotor capacity as the critical sustained swimming speed (U_{crit}) (Kolok, 1999) of each individual at the same acute test temperatures (18, 22, 28, 34, and 38°C) in random order. We measured locomotor capacity because it integrates several underlying physiological systems, and it is closely related to fitness by increasing success in predator escape, prey capture, and increasing reproductive success (Irschick et al., 2008). U_{crit} was measured according to published protocols (Seebacher et al., 2015) in a Blazka-style swimming flume consisting of a cylindrical clear Perspex flume (150 mm length and 38 mm diameter). The flume was fitted tightly over the intake end of a submersible pump (12V DC, iL500, Rule, Hertfordshire, United Kingdom). A bundle of hollow straws at the inlet end of flume helped maintain laminar flow. The flume and pump were submerged in a plastic tank (38 cm by 62 cm) that contained water with the appropriate temperature for each trial. We controlled water flow speed by changing the voltage input into the pump with a variable DC power source (NP9615; Manson Engineering Industrial, Hong Kong, SAR China). The water flow in each flume was measured in real-time by a flow meter (DigiFlow 6710 M, Savant Electronics, Taichung, Taiwan) connected to the outlet of each pump. Fish swam at an initial flow rate of 0.06 m s^{-1} for 20 min followed by an increase in flow speed by 0.02 m s^{-1} every 5 min until the fish could no longer hold their position in the water column. When fish fell back onto the grid, the flow was stopped for 5–10 s before restarting and increasing the speed to the previous setting again. We terminated the trial when fish stopped swimming for the second time. Fish were rested for at least 24 h between swimming trials. We report U_{crit} as body length per second (BL s^{-1}).

Statistical Analysis

We tested for shifts in the reaction norms of locomotor capacity and movement due to the developmental thermal environment using linear mixed models. We first ran one model for each

trait (locomotor capacity and movement in an open field) to test for an overall three-way interaction between species (Atlantic or Amazon), developmental temperature (22 or 28°C) and acute test temperature (18, 22, 28, 34, and 38°C). To determine whether species differed, we tested for the three-way interaction ("species × developmental temperature × test temperature" and "species × developmental temperature × test temperature^2") of both linear and quadratic effects of test temperature; the quadratic effect captures the curvature in the performance curve of the traits, and the linear term indicates the slope of the reaction norm of the trait. We additionally included the fixed effects of observation order (day 1 – 5) and body length. Individual fish ID and Mother ID were included as random effects to account for the multiple observations per fish and brood.

After testing for the three-way interactions, we investigated differences in developmental plasticity within each species and each trait separately. In each model we included the effects of developmental temperature, linear and quadratic effects of test temperature, interactions between these and developmental temperature, and the effects of observation order and body length. Individual fish ID and Mother ID were included as random effects. In preliminary analyses we tested different random structures including random intercepts for each fish, tank (group of four siblings) and mother, random slopes and intercepts for each fish, and random curves, slopes and intercepts for each fish. However, in all cases the best random structure only contained random intercepts for each individual fish and mother, which we therefore used for all models (see **Supplementary Tables 2,3**). We additionally estimated both a marginal R^2 (proportion of total variance explained by the fixed effects) and conditional R^2 (proportion of total variance explained by the fixed and random effects) value for each model according to Nakagawa and Schielzeth (2017). We did not remove non-significant terms from our full models as we were interested *a priori* in all effects. We centered and scaled to unit variance the continuous variables (mean velocity, U_{crit}, and body length) before analysis to enable comparisons of effect estimates (Schielzeth, 2010). Acute test temperature was centered but not scaled to make the intercept more interpretable (Schielzeth, 2010). Inspection of the residuals confirmed that our models met the assumptions of a Gaussian error distribution with homogeneous variance. The significance of fixed and random effects was assessed using the log-likelihood ratio of a model that contained the effect of interest to a model that did not. Where interactions (e.g., between developmental temperature and test temperature) were significant, we did not test for the significance of the main effects (e.g., developmental temperature) as this would require removing the significant two-way interaction from the model. Models were run using the lme4 package in R (Bates et al., 2015).

To get an overall measure of each individual's swimming performance across all test temperatures, we analyzed thermal performance curves of U_{crit} by fitting quadratic equations to the data from each fish (Seebacher et al., 2015), and then setting the first differential to zero to obtain the mode of the curve (i.e., the temperature at which maximal U_{crit} occurred). We obtained the performance breadth (i.e., the temperature range over which U_{crit} was >80% of maximal) by reducing the maximum of the fitted curve for each fish to 80% and then calculating quadratic roots (Seebacher et al., 2015). We were unable to fit curves for movement in an open field as this behavior did not follow a typical quadratic curve shape (see section "Results").

Finally, to test whether and how the two traits were related to each other, we estimated among- and within-individual correlations between the two traits for each species separately. We used multivariate mixed models with U_{crit} and mean velocity as the response variables and individual included as a random effect. We attempted to include mother as an additional random effect, however, these models failed to converge likely due to the relatively small variance attributable to mother (see section "Results") and so was removed. Each trait was centered and scaled to unit variance prior to analysis so the resulting covariance estimates are equivalent to correlation coefficients (Dingemanse and Dochtermann, 2013). We used the MCMCglmm package in R (Hadfield, 2010) and ran chains of 400,000 iterations with a burn-in of 1,000 and thinning every 200 samples. We assumed Gaussian error distributions for each trait and used parameter-expanded priors and preliminary analyses indicated our results were not sensitive to prior specification. Inspection of the posterior plots of five independent chains indicated our models achieved good mixing. We interpreted a correlation coefficient as significantly different from zero if the resulting 95% credible interval did not overlap zero. Data and R code used to generate the results are provided as Supplementary material.

RESULTS

Maximal Locomotor Capacity

As predicted, there was an indication that the two species differed in their capacity for developmental plasticity in response to early life thermal environment (Dev.temp × Species × Test.temp^2: log-likelihood ratio (LLR) = 3.65, p = 0.055; see full results of the three-way interaction model in **Supplementary Table 4**). In general, the sexually reproducing Atlantic molly exhibited a greater capacity for developmental plasticity in U_{crit} compared to the clonal Amazon molly (**Figures 2A,C**).

When investigating U_{crit} within each species separately, we found that the curvature of the swimming thermal performance curve in sexual Atlantic mollies depended on the developmental temperature (Dev.temp × Test.temp^2 interaction, **Table 1**) indicating that early experience altered the thermal sensitivity of locomotor capacity later in life (**Figure 2A**). The combined fixed effects in our model explained nearly half of the total variation in U_{crit} (marginal R^2 = 0.43, **Table 1**), although there was still considerable variation among individuals and families (mothers) that explained an additional 31% of the total variance (conditional R^2 – marginal R^2, **Table 1**). As predicted, Atlantic mollies raised at the higher developmental temperature achieved peak performance at a higher temperature (mode, **Figure 3A**) and maintained performance across a broader range of temperatures (breadth, **Figure 3B**) compared to fish raised at the cooler developmental temperature (mode: t = 3.13, p = 0.003; breadth: t = 3.40, p = 0.001). However,

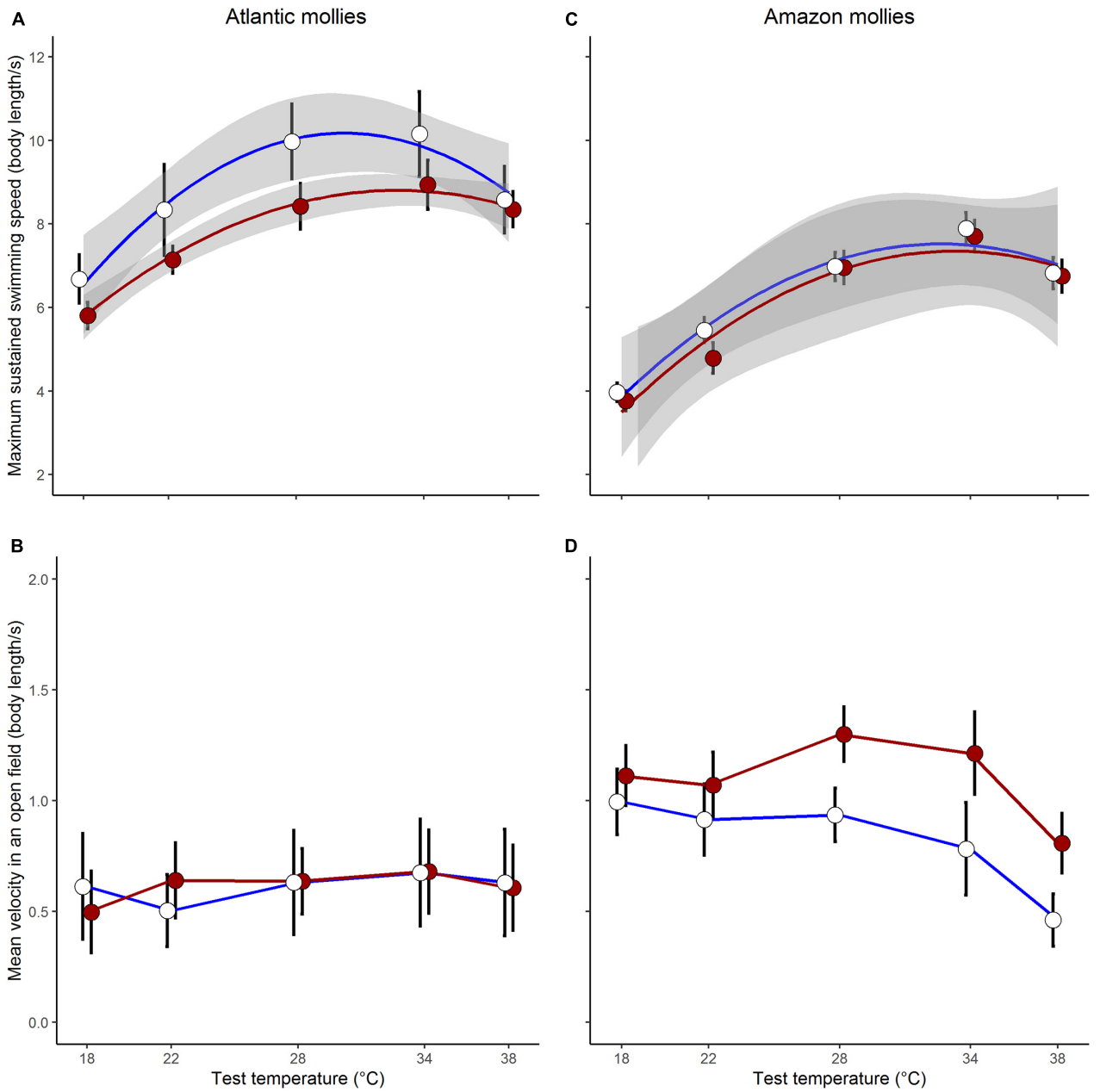

FIGURE 2 | Plasticity in locomotor traits. Sexually reproducing Atlantic mollies exhibited developmental plasticity in swimming performance **(A)** but not open field movement **(B)** in response to different developmental thermal environments. Clonal amazon mollies showed no developmental plasticity in swimming performance **(C)** but did in open field movement **(D)**. Shown are means ± SE; N = 26, 24 for cold, hot Atlantic mollies, respectively; and N = 28, 31 for cold, hot Amazon mollies, respectively. The gray bands in panel **(A,C)** indicate the 95% CI from the fitted quadratic curve; we were unable to fit similar curves to the movement data as it did not follow a typical quadratic form.

maximum performance was overall lower in fish from the high developmental temperatures compared to those from the low treatment (**Figure 2A** and **Table 1**).

In comparison, the U_{crit} of the clonal Amazon mollies was not significantly affected by their developmental thermal environment. Neither the linear nor the quadratic effects of test temperature interacted with developmental temperature (**Table 2** and **Figure 2C**). There was an overall effect of developmental temperature on U_{crit} (**Table 2**), but this effect was small biologically (**Figure 2C**). Also, there was no difference in U_{crit} mode (t = 0.65, p = 0.52) or breadth (t = 0.98, p = 0.33) between the different developmental temperatures (**Figure 3**). Our model explained a large portion of the total variance in swimming performance (marginal R^2 = 0.76), and the additional portion of variance explained by individual identity was low (4%; conditional R^2 – marginal R^2, **Table 2**).

TABLE 1 | Linear mixed effect model predicting mean velocity and critical sustained swimming speed (U_{crit}) in the **Atlantic mollies**.

Effect	Estimate (± s.e.)	*t*-value	LLR	*p*-value
Critical sustained swimming speed in a flume (marginal R^2 = 0.41; conditional R^2 = 0.72)[a]				
Intercept	1.26 (0.20)	6.27		
Length	**−0.17 (0.07)**	**−2.45**	**6.00**	**0.014**
Observation	−0.008 (0.03)	−0.31	0.15	0.70
Dev.temp (warm)	−0.67 (0.20)	−3.37		
Test.temp	**0.05 (0.007)**	**7.42**	**101.07**	**<0.001**
Test.temp^2	−0.01 (0.001)	−9.13		
Dev.temp × Test.temp	0.01 (0.009)	1.13	1.32	0.25
Dev.temp × Test.temp^2	**0.005 (0.002)**	**2.66**	**7.12**	**0.007**
Individual variance	0.212			
Mother variance	0.100			
Residual variance	0.293			
Adjusted repeatability[b]	0.35			
Mean velocity in an open field (marginal R^2 = 0.12, conditional R^2 = 0.55)[a]				
Intercept	−0.61 (0.20)	−2.99		
Length	**−0.28 (0.09)**	**−3.14**	**9.59**	**0.002**
Observation	−0.05 (0.03)	−1.71	3.17	0.07
Dev.temp (warm)	0.35 (0.25)	1.39	1.22	0.27
Test.temp	**0.01 (0.008)**	**1.35**	**3.76**	**0.05**
Test.temp^2	<0.001 (0.001)	0.11	0.66	0.42
Dev.temp × Test.temp	<0.001 (0.01)	0.07	0.004	0.94
Dev.temp × Test.temp^2	−0.002 (0.002)	−0.99	1.01	0.31
Individual intercepts variance	0.367			
Mother variance	0.053			
Residual	0.446			
Adjusted repeatability[b]	0.42			

Responses and length were centered and scaled to unit variance, and test temperature was centered prior to analysis. Significance of effects was estimated using a log-likelihood ratio test on nested models; in models where a two-way interaction was significant, we did not test the significance of an involved main effect (see section "Materials and Methods" for more details). Estimates significant at the $p < 0.05$ level are bolded.

[a]*Marginal R^2 describes the proportion of the total variance that is explained by the fixed effects in the model whereas conditional R^2 describes the proportion of total variance that is explained by the combined fixed and random effects in the model.*

[b]*Repeatability was estimated as the proportion of the remaining variance (not explained by the fixed effects) that was attributable to differences in individual intercepts.*

Movement

The effect of different developmental temperatures on unforced movement in an open field differed between the two species (Dev.temp × Species × Test.temp LLR = 4.24, p = 0.039, **Supplementary Table 5**). Interestingly, however, we found the opposite pattern to U_{crit} in the plasticity of movement in an open field: the clonal Amazon molly exhibited greater plasticity in response to the developmental environment compared to the sexual Atlantic molly. Movement of clonal Amazon mollies depended on the developmental temperature: fish raised in warmer environments exhibited greater movement at warmer temperatures compared to fish raised at cooler temperatures (Dev.temp × Test.temp interaction; **Table 2** and **Figure 2D**). The fixed effects in our model explained a lower proportion of the total variance (marginal R^2 = 0.30, **Table 2**) compared to that in the analysis of U_{crit}, although individual identity explained a much larger portion of variation in movement (26%, conditional R^2 – marginal R^2) compared to U_{crit}.

In contrast, the sexual Atlantic molly showed low levels of movement in an open field, and movement was only marginally affected by test temperature (**Figure 2B** and **Table 1**). There was no effect of developmental temperature. The fixed effects in our model explained only 12% of the total variation in movement (marginal R^2, **Table 1**), although there was considerable variation among individuals (43% of the total variation; conditional R^2 – marginal R^2, **Table 1**).

U_{crit} and movement were weakly correlated at the among-individual level showing that Atlantic mollies that had higher locomotor capacity on average also were more active in the open field (R = 0.25, 95% CI: [0.09, 0.44]). There was no relationship at the within-individual level (R = 0.02 [−0.06, 0.10]). In the Amazon mollies, there was no evidence that these two traits were correlated at either the among- (R = 0.03 [−0.01, 0.11]) or within-individual level (R = −0.03 [−0.12, 0.07]).

DISCUSSION

Here we show that the capacity for developmental plasticity differs between two related species and across two related traits within a species. We found clear evidence that the sexually reproducing Atlantic molly exhibited shifts in the reaction norm

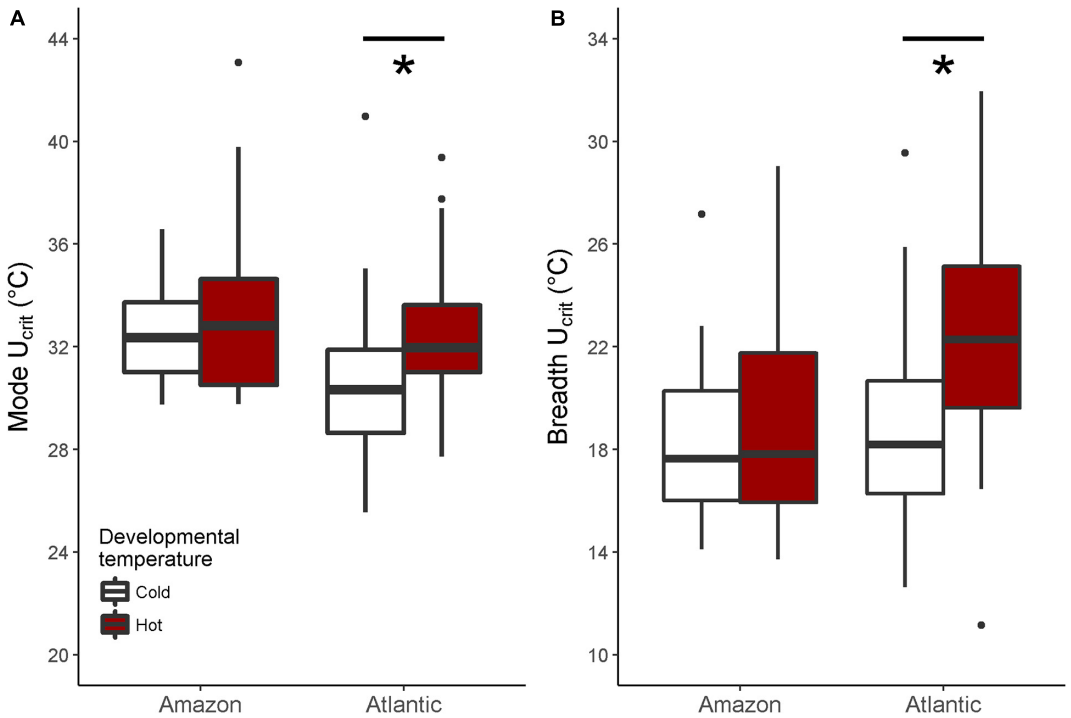

FIGURE 3 | Mode and breath of the thermal performance curves. In Atlantic mollies, different developmental temperatures shifted the mode (i.e., the temperature at which maximal swimming performance occurred) significantly **(A)** and increased the performance breadth (i.e., 80% maximal performance) **(B)**. Shown are boxplots where the box indicates the upper and lower quartiles, the dark middle line indicates the median, the whiskers indicate 1.5 times the inter-quartile range and asterixes denote significant differences. There were no significant effects of developmental temperature on performance curve mode or breadth in clonal Amazon mollies. Any values outside that range are shown as points. *N* = 26, 24 for cold, hot Atlantic mollies, respectively; and *N* = 28, 31 for cold, hot Amazon mollies respectively.

of their physiological swimming performance, but not of their movement levels. In contrast, the clonal Amazon molly exhibited the exact opposite pattern where their unforced movement was more plastic than their swimming performance. Therefore, we show that higher levels of among-individual genetic variation as seen in Atlantic mollies only leads to more pronounced developmental plasticity compared to the genetically identical Amazon mollies in a very trait specific manner even after fish had experienced long periods of very different thermal regimes (22 vs. 28°C for 25 weeks prior to testing). Furthermore, our data indicate that the mechanistic basis underlying these two traits might have different susceptibilities to epigenetic modifications and that there is possibly an interaction between genetic variation and epigenetic mechanisms, be that as a result of genetic diversity among individuals or genetic differences between the species.

Maximal locomotor capacity in the sexually reproducing Atlantic molly exhibited developmental plasticity, shifting location (mode) and shape (breadth) of their performance curves. This developmentally induced shift in swimming thermal performance curves meant that there was no difference in performance when developmental temperatures coincided with acute temperatures, that is, the 22°C developed fish measured at 22°C performed as well as 28°C developed fish measured at 28°C. Hence, developmental plasticity equalized performance so that it stayed constant at the anticipated environmental conditions later in life (Kawecki, 2000). Similar canalization occurred in guppies (*P. reticulata*), but only after two generations (Le Roy et al., 2017), and it may protect populations from environmental perturbations rather than matching phenotypes to prevalent environmental conditions as predicted by the "predictive adaptive hypothesis" (Bateson et al., 2014; Le Roy et al., 2017). Even though there were shifts in the performance curves in response to the early life thermal environment in the sexually reproducing fish, the maximum of each performance curve did not occur at the acute temperature that matched the developmental temperature as predicted by the "predictive adaptive hypothesis." Rather, both warm and cold reared fish achieved their greatest performance at temperatures warmer than their developmental temperatures.

In contrast, there was no shift in maximum locomotor performance in clonal Amazon mollies reared at different temperatures. We exposed fish to their respective developmental conditions over a relatively long period that would have included the developmental stages that are most sensitive to external temperature signals (Campos et al., 2012). Hence, lack of developmental plasticity is unlikely to be an artifact of the experimental treatment. The difference in the patterns of developmental plasticity may be due to genetic differences between the species even though the species share a large part

TABLE 2 | Linear mixed effect model predicting mean velocity and critical sustained swimming speed (U_{crit}) in the **Amazon mollies**.

Effect	Estimate (± s.e.)	*t*-value	LLR	*p*-value
Critical sustained swimming speed in a flume (marginal R^2 = 0.76, conditional R^2 = 0.80)[a]				
Intercept	0.07 (0.06)	1.10		
Length	**−0.34 (0.05)**	**−6.93**	**36.81**	**<0.001**
Observation	**0.06 (0.02)**	**3.45**	**11.79**	**<0.001**
Dev.temp (warm)	**−0.14 (0.08)**	**−1.79**	**4.35**	**0.04**
Test.temp	**0.07 (0.004)**	**17.73**	**345.92**	**<0.001**
Test.temp^2	**−0.007 (<0.001)**	**−8.47**	**96.94**	**<0.001**
Dev.temp × Test.temp	0.007 (0.006)	1.30	1.73	0.18
Dev.temp × Test.temp^2	<0.001 (0.001)	0.44	0.19	0.65
Individual variance	0.020			
Mother variance	0.001			
Residual variance	0.119			
Adjusted repeatability[b]	0.14			
Mean velocity in an open field (marginal R^2 = 0.30; conditional R^2 = 0.56)[a]				
Intercept	0.38 (0.13)	2.84		
Length	**−0.44 (0.13)**	**−3.47**	**11.58**	**<0.001**
Observation	−0.04 (0.03)	−1.42	2.07	0.35
Dev.temp (warm)	0.68 (0.03)	4.02		
Test.temp	−0.05 (0.007)	−6.71		
Test.temp^2	**−0.004 (0.001)**	**−3.12**	**32.39**	**<0.001**
Dev.temp × Test.temp	**0.03 (0.01)**	**3.08**	**9.45**	**0.002**
Dev.temp × Test.temp^2	−0.002 (0.002)	−1.31	1.76	0.18
Individual variance	0.207			
Mother variance	0.007			
Residual variance	0.358			
Adjusted repeatability[b]	0.36			

Responses and length were centered and scaled to unit variance, and test temperature was centered prior to analysis. Significance of effects was estimated using a log-likelihood ratio test on nested models; in models where a two-way interaction was significant, we did not test the significance of an involved main effect (see section "Materials and Methods" for more details). Estimates significant at the $p < 0.05$ level are bolded.

[a]*Marginal R^2 describes the proportion of the total variance that is explained by the fixed effects in the model whereas conditional R^2 describes the proportion of total variance that is explained by the combined fixed and random effects in the model.*

[b]*Repeatability was estimated as the proportion of the remaining variance (not explained by the fixed effects) that was attributable to differences in individual intercepts.*

of their genomes. Half of the Amazon molly's genome is from its Atlantic molly ancestor; the other half is from its sailfin molly ancestor (Lampert and Schartl, 2008; Stöck et al., 2010; Warren et al., 2018; Lu et al., 2021). Additionally, Amazon mollies require sperm from one of its parental species (Atlantic and sailfin molly), and Amazon and Atlantic mollies are sympatric for much of their ranges (Schlupp et al., 2002). The co-existence of clonal and sexually reproducing lineages is interesting, and it may be that partitioning of ecological niches facilitates this co-existence. However, there do not appear to be differences in their competitive abilities (da Barbiano et al., 2010, 2013; Scharnweber et al., 2011) or parasite loads (Tobler and Schlupp, 2005; Tobler et al., 2005). Hence, the major difference between these two species is that half of their genomes differ, and that they differ in among-individual genetic diversity. It is likely, therefore, that an interaction between genetic - at the (half)species and/or among-individual levels - and epigenetic mechanisms caused the differences in developmental plasticity in Amazon and Atlantic mollies.

Genetic and epigenetic mechanisms can interact at multiple levels (Ashe et al., 2021). Importantly, epigenetic mechanisms are themselves not independent from genetics, because epigenetic modifiers such as DNA methyltransferases and histone deacetylases are themselves encoded by DNA (Campos et al., 2012). Additionally, higher recombination rates are related to higher GC content (Stapley et al., 2017), which implies that a sexually reproducing species (with higher recombination), such as the Atlantic molly may have increased susceptibility to DNA methylation due to their increased GC content (Gelfman et al., 2013) compared to the clonal, non-recombining Amazon molly. Therefore, one possible explanation for the difference in the patterns of developmental plasticity in locomotor capacity in these two species is that developmental plasticity and canalization can be modulated by genetic diversity. The epigenome is now viewed as having at least as important an influence in shaping phenotypes as the DNA nucleotide sequence (Forsman, 2015; Ashe et al., 2021). Epigenetic modifications in response to different early life environments may not be independent from genetic diversity, and genetically mediated diversity in the molecular machinery that confers epigenetic changes (Taudt et al., 2016) could increase the efficacy of developmental plasticity at least for some traits. Epigenetic processes may also be linked to genetics and selection because epigenetic states can be heritable, and the resulting plasticity and

phenotypic variance can affect selection (Stajic and Jansen, 2021). Our data indicate that there are interactions between genetic and epigenetic mechanisms in determining developmental plasticity, but their exact manifestation and consequences must await further experimentation.

In addition to the difference in among-individual genetic diversity between Atlantic and Amazon mollies, there are other genetic factors that could play a role. In particular, while there was no genetic diversity among Amazon molly individuals within the same clonal lineage, there is high genetic diversity at the within-individual level (Lampert and Schartl, 2008; da Barbiano et al., 2013; Warren et al., 2018). The Amazon molly is a "frozen hybrid" that originated from a single hybridization event between a female Atlantic and a male sailfin molly and so exhibits extremely high heterozygosity, which is greater even than in either of its two parental species (Warren et al., 2018). This heterozygosity is one potential explanation for why clonal fish have persisted so long despite their inability to generate new genetic variation through sexual recombination. It is important to note, however, that the genome of the Amazon molly shows similar patterns of gene conversion, mutation accumulation and transposable element activity as genomes of both the Atlantic and sailfin molly (Warren et al., 2018). In addition to the accumulation of mutations, the rare introgression of paternal DNA can also generate diversity among clonal lineages (Schartl et al., 1995; Nanda et al., 2007; Warren et al., 2018). Genetic diversification between lineages generates clonal sorting, whereby the most fit clonal lineages are more likely to persist in a given environment (Vrijenhoek, 1979; Dawley and Bogart, 1989). These patterns of genetic diversification raise the possibility that differences in developmental plasticity between Atlantic and Amazon mollies are not just a by-product of differences in genetic diversity, but may have emerged as a result of selection or genetic drift (see Makowicz and Travis, 2020; Lu et al., 2021). To resolve these questions, it would be informative to also examine patterns of developmental plasticity in the second ancestral species, the sailfin molly (*Poecilia latipinna*), and in populations of the Amazon molly sampled across their geographic range.

We found no evidence for developmental plasticity in movement levels in the Atlantic molly demonstrating that the greater among-individual genetic diversity in this species is not the only contributor to developmental plasticity. As all fish will automatically swim when placed in running water, maximal locomotor capacity is principally constrained by intrinsic muscle function mediated by muscle fiber type expression and calcium cycling, for example (Josephson, 1993; Gordon et al., 2000; Seebacher and Walter, 2012), which are known to be modified epigenetically (McGee and Hargreaves, 2011; Campos et al., 2013; Simmonds and Seebacher, 2017). In contrast, behaviors such as unforced movement are likely modulated by a broader range of physiological systems including neuroendocrine and sensory inputs, nutritional state, metabolic rates, in addition to muscle function (Akre and Johnsen, 2014). Considering the different mechanisms underlying maximal locomotor capacity and movement in an open field, it is not surprising that these traits are only weakly correlated with one another at the among-individual level and not at all at the within-individual level. The two traits might respond differently to developmental inputs and other environmental cues might play a more important role in influencing this behavior in the Atlantic mollies (Forsman, 2015). For example, it is possible that the Atlantic mollies may have perceived the open field as riskier than the Amazon mollies did, and therefore maintained low movement levels regardless of temperature. We attempted to limit this possibility by giving the fish exposure to the arena the day before testing, and we would expect there to be evidence of some habituation effect as the fish became more familiar with the (initially) novel environment; however, there was no clear effect of repeated testing on movement levels. The complexity of behavioral traits is further underscored by the fact that individuals of both species consistently differed in their movement. This pattern of consistent behavioral differences was similar to previous findings showing differences in behavior among genetically identical Amazon molly individuals reared under identical environmental conditions (Bierbach et al., 2017).

In conclusion, we demonstrated that two seemingly related traits in two closely related species exhibit very different patterns of developmental plasticity. Our results suggest that greater (among-individual) genetic variation may enhance the capacity for developmental plasticity in a physiological trait but may not be necessary for plasticity in behavioral traits. These results have important implications on how animals respond to rapid environmental change, and how populations that face different environments may diverge genetically *via* genetic assimilation of epigenetically acquired characters.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

The animal study was reviewed and approved by the Berlin Landesamt für Gesundheit und Soziales (GO 124/14).

AUTHOR CONTRIBUTIONS

KL, FS, and DB developed the study and analyzed the data. DB, MH, and JM performed the experiments, KL, FS, and DB wrote the manuscript with input from all authors. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphys.2021.740604/full#supplementary-material

REFERENCES

Akre, K. L., and Johnsen, S. (2014). Psychophysics and the evolution of behavior. *Trends Ecol. Evol.* 29, 291–300. doi: 10.1016/j.tree.2014.03.007

Ashe, A., Colot, V., and Oldroyd, B. P. (2021). How does epigenetics influence the course of evolution? *Philos. Trans. R. Soc. B Biol. Sci.* 376:20200111. doi: 10.1098/rstb.2020.0111

Atlasi, Y., and Stunnenberg, H. G. (2017). The interplay of epigenetic marks during stem cell differentiation and development. *Nat. Rev. Genet.* 18, 643–658.

Aubin-Horth, N., Landry, C. R., Letcher, B. H., and Hofmann, H. A. (2005). Alternative life histories shape brain gene expression profiles in males of the same population. *Proc. Royal Soc. B Biol. Sci.* 272, 1655–1662.

Bates, D., Maechler, M., Bolker, B., and Walker, S. (2015). Fitting linear mixed-effects models using lme4. *J. Stat. Softw.* 67, 1–48.

Bateson, P., Gluckman, P., and Hanson, M. (2014). The biology of developmental plasticity and the predictive adaptive response hypothesis: developmental plasticity and the PAR response. *J. Physiol.* 592, 2357–2368. doi: 10.1113/jphysiol.2014.271460

Bento, G., Ogawa, A., and Sommer, R. J. (2010). Co-option of the hormone-signalling module dafachronic acid-DAF-12 in nematode evolution. *Nature* 466, 494–497.

Benus, R. F., Koolhaas, J. M., and Oortmerssen, G. A. v. (1987). Individual differences in behavioural reaction to a changing environment in mice and rats. *Behaviour* 100, 105–122.

Bierbach, D., Laskowski, K. L., and Wolf, M. (2017). Behavioural individuality in clonal fish arises despite near-identical rearing conditions. *Nat. Commun.* 8:15361.

Bierbach, D., Schleucher, E., Hildenbrand, P., Köhler, A., Arias-Rodriguez, L., Riesch, R., et al. (2010). Thermal tolerances in mollies (*Poecilia* spp.): reduced physiological flexibility in stable environments? *Bull. Fish Biol.* 12, 83–89.

Bozinovic, F., Cavieres, G., Martel, S. I., Alruiz, J. M., Molina, A. N., Roschzttardtz, H., et al. (2020). Thermal effects vary predictably across levels of organization: empirical results and theoretical basis. *Proc. R. Soc. B Biol. Sci.* 287:20202508. doi: 10.1098/rspb.2020.2508

Campos, C., Valente, L., Conceição, L., Engrola, S., and Fernandes, J. (2013). Temperature affects methylation of the myogenin putative promoter, its expression and muscle cellularity in Senegalese sole larvae. *Epigenetics* 8, 389–397. doi: 10.4161/epi.24178

Campos, C., Valente, L. M. P., and Fernandes, J. M. O. (2012). Molecular evolution of zebrafish dnmt3 genes and thermal plasticity of their expression during embryonic development. *Gene* 500, 93–100. doi: 10.1016/j.gene.2012.03.041

Costa, G. C., and Schlupp, I. (2010). Biogeography of the Amazon molly: ecological niche and range limits of an asexual hybrid species. *Glob. Ecol. Biogeogr.* 19, 442–451. doi: 10.1111/j.1466-8238.2010.00546.x

da Barbiano, L. A., Gompert, Z., Aspbury, A. S., Gabor, C. R., and Nice, C. C. (2013). Population genomics reveals a possible history of backcrossing and recombination in the gynogenetic fish *Poecilia formosa*. *PNAS* 110, 13797–13802. doi: 10.1073/pnas.1303730110

da Barbiano, L. A., Waller, J., and Gabor, C. R. (2010). Differences in competitive efficiency between a sexual parasite and its host provide insight into the maintenance of a sperm-dependent vertebrate species. *J. Freshw. Ecol.* 25, 523–530. doi: 10.1080/02705060.2010.9664401

Darnell, R. M., and Abramoff, P. (1968). Distribution of the gynogenetic fish, *Poecilia formosa*, with remarks on the evolution of the species. *Copeia* 1968, 354–361.

Dawley, R. M., and Bogart, J. P. (1989). *Evolution and Ecology of Unisexual Vertebrates*. New York, NY: New York State Museum Bulletin.

Dingemanse, N. J., and Dochtermann, N. A. (2013). Quantifying individual variation in behaviour: mixed-effect modelling approaches. *J. Anim. Ecol.* 82, 39–54. doi: 10.1111/1365-2656.12013

Doeringsfeld, M. R., Schlosser, I. J., Elder, J. F., and Evenson, D. P. (2004). Phenotypic consequences of genetic variation in a gynogenetic complex of Phoxinus eos-neogaeus clonal fish (pisces: Cyprinidae) inhabiting a heterogeneous environment. *Evolution* 58, 1261–1273. doi: 10.1111/j.0014-3820.2004.tb01705.x

Ficz, G. (2015). New insights into mechanisms that regulate DNA methylation patterning. *J. Exp. Biol.* 218, 14–20. doi: 10.1242/jeb.107961

Forsman, A. (2015). Rethinking phenotypic plasticity and its consequences for individuals, populations and species. *Heredity* 115, 276–284. doi: 10.1038/hdy.2014.92

Freund, J., Brandmaier, A. M., Lewejohann, L., Kirste, I., Kritzler, M., Krüger, A., et al. (2013). Emergence of individuality in genetically identical mice. *Science* 340, 756–759. doi: 10.1126/science.1235294

Garland, T., and Adolph, S. C. (1994). Why not to do two-species comparative studies: limitations on inferring adaptation. *Physiol. Zool.* 67, 797–828. doi: 10.1086/physzool.67.4.30163866

Gelfman, S., Cohen, N., Yearim, A., and Ast, G. (2013). DNA-methylation effect on cotranscriptional splicing is dependent on GC architecture of the exon–intron structure. *Genome Res.* 23, 789–799. doi: 10.1101/gr.143503.112

Gordon, A. M., Homsher, E., and Regnier, M. (2000). Regulation of contraction in striated muscle. *Physiol. Rev.* 80, 853–924. doi: 10.1152/physrev.2000.80.2.853

Hadfield, J. D. (2010). *MCMCglmm: Markov Chain Monte Carlo Methods for Generalised Linear Mixed Models. Tutorial for MCMCglmm package in R 125*.

Hammill, E., Wilson, R. S., and Johnston, I. A. (2004). Sustained swimming performance and muscle structure are altered by thermal acclimation in male mosquitofish. *J. Ther. Biol.* 29, 251–257. doi: 10.1016/j.jtherbio.2004.04.002

Hu, J., and Barrett, R. D. (2017). Epigenetics in natural animal populations. *J. Evol. Biolo.* 30, 1612–1632.

Hubbs, C. L., and Hubbs, L. C. (1932). APPARENT PARTHENOGENESIS IN NATURE, IN A FORM OF FISH OF HYBRID ORIGIN. *Science* 76, 628–630.

Irschick, D. J., Meyers, J. J., Husak, J. F., and Galliard, J.-F. L. (2008). How does selection operate on whole-organism functional performance capacities? A review and synthesis. *Evol. Ecol. Res.* 10, 177–196.

Josephson, R. K. (1993). Contraction dynamics and power output of skeletal muscle. *Annu. Rev. Physiol.* 55, 527–546. doi: 10.1146/annurev.ph.55.030193.002523

Kallman, K. D. (1962). Gynogenesis in the teleost,Mollienesia formosa (Girard), with a discussion of the detection of parthenogenesis in vertebrates by tissue transplantation. *J. Genet.* 58, 7–24.

Kawecki, T. J. (2000). The evolution of genetic canalization under fluctuating selection. *Evolution* 54, 1–12. doi: 10.1111/j.0014-3820.2000.tb00001.x

Kelley, J. L., Tobler, M., Beck, D., Sadler-Riggleman, I., Quackenbush, C. R., Arias-Rodriguez, L., et al. (2021). Epigenetic inheritance of DNA methylation changes in fish living in hydrogen sulfide–rich springs. *Proc. Natl. Acad. Sci. U.S.A.* 118:e2014929118. doi: 10.1073/pnas.2014929118

Klose, R. J., and Bird, A. P. (2006). Genomic DNA methylation: the mark and its mediators. *Trends Biochem. Sci.* 31, 89–97. doi: 10.1016/j.tibs.2005.12.008

Kolok, A. S. (1999). Interindividual variation in the prolonged locomotor performance of ectothermic vertebrates: a comparison of fish and herpetofaunal methodologies and a brief review of the recent fish literature. *Can. J. Fish. Aquat. Sci.* 56, 700–710. doi: 10.1139/f99-026

Koolhaas, J. M., Korte, S. M., De Boer, S. F., Van Der Vegt, B. J., Van Reenen, C. G., Hopster, H., et al. (1999). Coping styles in animals: current status in behavior and stress-physiology. *Neurosci. Biobehav. Rev.* 23, 925–935.

Lampert, K. P., and Schartl, M. (2008). The origin and evolution of a unisexual hybrid: *Poecilia formosa*. *Philos. Trans. R. Soc. B Biol. Sci.* 363, 2901–2909. doi: 10.1098/rstb.2008.0040

Laskowski, K. L., Doran, C., Bierbach, D., Krause, J., and Wolf, M. (2019). Naturally clonal vertebrates are an untapped resource in ecology and evolution research. *Nat. Ecol. Evol.* 3, 161–169. doi: 10.1038/s41559-018-0775-0

Le Roy, A., Loughland, I., and Seebacher, F. (2017). Differential effects of developmental thermal plasticity across three generations of guppies (*Poecilia reticulata*): canalization and anticipatory matching. *Sci. Rep.* 7:4313. doi: 10.1038/s41598-017-03300-z

Loughland, I., Little, A., and Seebacher, F. (2021). DNA methyltransferase 3a mediates developmental thermal plasticity. *BMC Biol.* 19:11. doi: 10.1186/s12915-020-00942-w

Lu, Y., Bierbach, D., Ormanns, J., Warren, W. C., Walter, R. B., and Schartl, M. (2021). Fixation of allelic gene expression landscapes and expression bias pattern shape the transcriptome of the clonal Amazon molly. *Gen. Res.* 31, 372–379. doi: 10.1101/gr.268870.120

Lynch, K. E., and Kemp, D. J. (2014). Nature-via-nurture and unravelling causality in evolutionary genetics. *Trends Ecol. Evol.* 29, 2–4. doi: 10.1016/j.tree.2013.10.001

Makowicz, A. M., and Travis, J. (2020). Are you more than the sum of your parents' genes? Phenotypic plasticity in a clonal vertebrate and F1 hybrids of its parental species. *Evolution* 74, 1124–1141.

Massicotte, R., and Angers, B. (2012). General-purpose penotype or how epigenetics extend the flexibility of a genotype. *Genet. Res. Int.* 2012, 1–7. doi: 10.1155/2012/317175

Massicotte, R., Whitelaw, E., and Angers, B. (2011). DNA methylation: a source of random variation in natural populations. *Epigenetics* 6, 421–427. doi: 10.4161/epi.6.4.14532

Mateus, A. R. A., Marques-Pita, M., Oostra, V., Lafuente, E., Brakefield, P. M., Zwaan, B. J., et al. (2014). Adaptive developmental plasticity: compartmentalized responses to environmental cues and to corresponding internal signals provide phenotypic flexibility. *BMC Biol.* 12:97. doi: 10.1186/s12915-014-0097-x

McGee, S. L., and Hargreaves, M. (2011). Histone modifications and exercise adaptations. *J.Appl. Physiol.* 110, 258–263. doi: 10.1152/japplphysiol.00979.2010

Morris, K. V., and Mattick, J. S. (2014). The rise of regulatory RNA. *Nat. Rev. Genet.* 15, 423–437. doi: 10.1038/nrg3722

Murren, C. J., Maclean, H. J., Diamond, S. E, Steiner, U. K., Heskel, M. A., Handelsman, C. A., et al. (2014). Evolutionary change in continuous reaction norms. *Am. Nat.* 183, 453–467. doi: 10.1086/675302

Nakagawa, S., and Schielzeth, H. (2017). A general and simple method for obtaining R2 from generalized linear mixed-effects models. *Methods Ecol. Evol.* 4, 133–142. doi: 10.1111/j.2041-210x.2012.00261.x

Nanda, I., Schlupp, I., Lamatsch, D. K., Lampert, K. P., Schmid, M., and Schartl, M. (2007). Stable inheritance of host species-derived microchromosomes in the gynogenetic fish *Poecilia formosa*. *Genetics* 177, 917–926. doi: 10.1534/genetics.107.076893

Orczewska, J. I., Hartleben, G., and O'Brien, K. M. (2010). The molecular basis of aerobic metabolic remodeling differs between oxidative muscle and liver of threespine sticklebacks in response to cold acclimation. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 299, R352–R364. doi: 10.1152/ajpregu.00189.2010

Rasch, E. M., Darnell, R. M., Kallman, K. D., and Abramoff, P. (1965). Cytophotometric evidence for triploidy in hybrids of the gynogenetic fish. *Poecilia formosa*. *J. Exp. Zool.* 160, 155–169.

de Ruijter, A. J., van Gennip, A. H., Caron, H. N., Kemp, S., and van Kuilenburg, A. B. (2003). Histone deacetylases (HDACs): characterization of the classical HDAC family. *Biochem. J.* 370, 737–749. doi: 10.1042/BJ20021321

Sarkar, S., and Fuller, T. (2003). Generalized norms of reaction for ecological developmental biology. *Evol. Dev.* 5, 106–115.

Scharnweber, K., Plath, M., Winemiller, K. O., and Tobler, M. (2011). Dietary niche overlap in sympatric asexual and sexual livebearing fishes *Poecilia* spp. *J. Fish Biol.* 79, 1760–1773. doi: 10.1111/j.1095-8649.2011.03114.x

Schartl, M., Wilde, B., Schlupp, I., and Parzefall, J. (1995). Evolutionary origin of a parthenoform, the Amazon molly. *Poecilia formosa*, on the basis of a molecular genealogy. *Evolution* 49, 827–835. doi: 10.1111/j.1558-5646.1995.tb02319.x

Schielzeth, H. (2010). Simple means to improve the interpretability of regression coefficients. *Methods Ecol. Evol.* 1, 103–113. doi: 10.1111/j.2041-210X.2010.00012.x

Schlupp, I., Parzefall, J., and Schartl, M. (2002). Biogeography of the Amazon molly. *Poecilia formosa*. *J. Biogeogr.* 29, 1–6. doi: 10.1046/j.1365-2699.2002.00651.x

Schulte, P. M., Healy, T. M., and Fangue, N. A. (2011). Thermal performance curves, phenotypic plasticity, and the time scales of temperature exposure. *Integr. Comp. Biol.* 51, 691–702. doi: 10.1093/icb/icr097

Schultz, R. J. (1973). *Origin and Synthesis of a Unisexual Fish. Pages 207-211*. Berlin: Springer Berlin Heidelberg.

Scott, G. R., and Johnston, I. A. (2012). Temperature during embryonic development has persistent effects on thermal acclimation capacity in zebrafish. *PNAS* 109, 14247–14252. doi: 10.1073/pnas.1205012109

Seebacher, F., Ducret, V., Little, A. G., and Adriaenssens, B. (2015). Generalist–specialist trade-off during thermal acclimation. *R. Soc. Open Sci.* 2:140251. doi: 10.1098/rsos.140251

Seebacher, F., Holmes, S., Roosen, N. J., Nouvian, M., Wilson, R. S., and Ward, A. J. W. (2012). Capacity for thermal acclimation differs between populations and phylogenetic lineages within a species. *Funct. Ecol.* 26, 1418–1428. doi: 10.1111/j.1365-2435.2012.02052.x

Seebacher, F., and Walter, I. (2012). Differences in locomotor performance between individuals: importance of parvalbumin, calcium handling and metabolism. *J. Exp. Biol.* 215, 663–670. doi: 10.1242/jeb.066712

Sih, A., and Del Giudice, M. (2012). Linking behavioural syndromes and cognition: a behavioural ecology perspective. Philosophical transactions of the Royal Society of London. *Ser. B Biol. Sci.* 367, 2762–2772.

Simmonds, A. I. M., and Seebacher, F. (2017). Histone deacetylase activity modulates exercise-induced skeletal muscle plasticity in zebrafish (*Danio rerio*). *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 313, R35–R43. doi: 10.1152/ajpregu.00378.2016

Stajic, D., and Jansen, L. E. T. (2021). Empirical evidence for epigenetic inheritance driving evolutionary adaptation. *Philos. Trans. R. Soc. B Biol. Sci.* 376:20200121. doi: 10.1098/rstb.2020.0121

Stamps, J. A., and Biro, P. A. (2016). Personality and individual differences in plasticity. *Curr. Opin. Behav. Sci.* 12, 18–23.

Stapley, J., Feulner, P. G. D., Johnston, S. E., Santure, A. W., and Smadja, C. M. (2017). Variation in recombination frequency and distribution across eukaryotes: patterns and processes. *Philos. Trans. R. Soc. B Biol. Sci.* 372:20160455. doi: 10.1098/rstb.2016.0455

Stewart, A. M., Cachat, J., Green, J., Gaikwad, S., Kyzar, E., Roth, A., et al. (2013). Constructing the habituome for phenotype-driven zebrafish research. *Behav. Brain Res.* 236, 110–117. doi: 10.1016/j.bbr.2012.08.026

Stöck, M., Lampert, K. P., Möller, D., Schlupp, I., and Schartl, M. (2010). Monophyletic origin of multiple clonal lineages in an asexual fish (*Poecilia formosa*). *Mol. Ecol.* 19, 5204–5215. doi: 10.1111/j.1365-294X.2010.04869.x

Suzuki, Y., and Nijhout, H. F. (2006). Evolution of a polyphenism by genetic accommodation. *Science* 311, 650–652.

Svendsen, J. C., Tirsgaard, B., Cordero, G. A., and Steffensen, J. (2017). Intraspecific variation in aerobic and anaerobic locomotion: gilthead sea bream (*Sparus aurata*) and Trinidadian guppy (*Poecilia reticulata*) do not exhibit a trade-off

between maximum sustained swimming speed and minimum cost of transport. *Front. Physiol.* 6:43. doi: 10.3389/fphys.2015.00043

Taudt, A., Colomé-Tatché, M., and Johannes, F. (2016). Genetic sources of population epigenomic variation. *Nat. Rev. Genet.* 17, 319–332. doi: 10.1038/nrg.2016.45

Tobler, M., and Schlupp, I. (2005). Parasites in sexual and asexual mollies (*Poecilia*, Poeciliidae, *Teleostei*): a case for the Red Queen? *Biol. Lett.* 1, 166–168. doi: 10.1098/rsbl.2005.0305

Tobler, M., Wahli, T., and Schlupp, I. (2005). Comparison of parasite communities in native and introduced populations of sexual and asexual mollies of the genus *Poecilia*. *J. Fish Biol.* 67, 1072–1082. doi: 10.1111/j.0022-1112.2005.00810.x

Torres-Dowdall, J., Handelsman, C. A., Reznick, D. N., and Ghalambor, C. K. (2012). Local adaptation and the evolution of phenotypic plasticity in trinidadian guppies (*Poecilia reticulata*). *Evolution* 66, 3432–3443. doi: 10.1111/j.1558-5646.2012.01694.x

Turner, B. J., Brett, B.-L. H., Rasch, E. M., and Balsano, J. S. (1980). Evolutionary genetics of a gynogenetic fish, *Poecilia formosa*, the amazon Molly. *Evolution* 34, 246–258.

Vogt, G. (2018). Investigating the genetic and epigenetic basis of big biological questions with the parthenogenetic marbled crayfish: a review and perspectives. *J. Biosci.* 43, 189–223. doi: 10.1007/s12038-018-9741-x

Vrijenhoek, R. C. (1979). Factors affecting clonal diversity and coexistence. *Am. Zool.* 19, 787–797. doi: 10.1093/icb/19.3.787

Warren, W. C., García-Pérez, R., Xu, S., Lampert, K. P., Chalopin, D., Stöck, M., et al. (2018). Clonal polymorphism and high heterozygosity in the celibate genome of the Amazon molly. *Nat. Ecol. Evol.* 2, 669–679. doi: 10.1038/s41559-018-0473-y

Whitfield, C. W., Cziko, A. M., and Robinson, G. E. (2003). Gene expression profiles in the brain predict behavior in individual honey bees. *Science* 302, 296–299.

Whitman, D., and Agrawal, A. (2009). "What is phenotypic plasticity and why is it omportant?," in *Phenotypic Plasticity of Insects: Mechanisms and Consequences*, eds D. Whitman and T. Ananthakrishnan (Hauppauge, NY: Science Publishers), doi: 10.1201/b10201-2

Wilson, R. S., Husak, J. F., Halsey, L. G., and Clemente, C. J. (2015). Predicting the movement speeds of animals in natural environments. *Integr. Comp. Biol.* 55, 1125–1141. doi: 10.1093/icb/icv106

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Body Temperature Frequency Distributions: A Tool for Assessing Thermal Performance in Endotherms?

D.L. Levesque[1], J. Nowack[2] and J.G. Boyles[3]*

[1]School of Biology and Ecology, University of Maine, Orono, ME, United States, [2]School of Biological and Environmental Sciences, Liverpool John Moores University, Liverpool, United Kingdom, [3]Cooperative Wildlife Research Laboratory, Center for Ecology, and School of Biological Sciences, Southern Illinois University, Carbondale, IL, United States

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Frank Seebacher,
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****Correspondence:***
D.L. Levesque
danielle.l.levesque@maine.edu

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There is increasing recognition that rather than being fully homeothermic, most endotherms display some degree of flexibility in body temperature. However, the degree to which this occurs varies widely from the relatively strict homeothermy in species, such as humans to the dramatic seasonal hibernation seen in Holarctic ground squirrels, to many points in between. To date, attempts to analyse this variability within the framework generated by the study of thermal performance curves have been lacking. We tested if frequency distribution histograms of continuous body temperature measurements could provide a useful analogue to a thermal performance curve in endotherms. We provide examples from mammals displaying a range of thermoregulatory phenotypes, break down continuous core body temperature traces into various components (active and rest phase modes, spreads and skew) and compare these components to hypothetical performance curves. We did not find analogous patterns to ectotherm thermal performance curves, in either full datasets or by breaking body temperature values into more biologically relevant components. Most species had either bimodal or right-skewed (or both) distributions for both active and rest phase body temperatures, indicating a greater capacity for mammals to tolerate body temperatures elevated above the optimal temperatures than commonly assumed. We suggest that while core body temperature distributions may prove useful in generating optimal body temperatures for thermal performance studies and in various ecological applications, they may not be a good means of assessing the shape and breath of thermal performance in endotherms. We also urge researchers to move beyond only using mean body temperatures and to embrace the full variability in both active and resting temperatures in endotherms.

Keywords: heterothermy, mammal, torpor, additive quantile regression, skew, acrophase, scotophase

INTRODUCTION

Thermoregulation and thermal sensitivity are vital to how an individual, population or species interacts with the environment. This has never been more true than under current shifts in environmental conditions associated with climate change (Huey et al., 2012). Research on thermoregulation and thermal sensitivity has been ongoing for decades, but a major turning point in our understanding of thermoregulation came with the conception of thermal performance curves (or thermal reaction norms) in the 1970s. Thermal performance curves relate some measure of performance to temperature (Huey and Slatkin, 1976; Angilletta, 2009; Huey and Stevenson, 2015) and allow for determination of the temperature at which the performance is maximised (T_{opt}) and estimation of the range of temperatures over which the species performs well (performance breath). It is a simple concept that has revolutionised the study of thermoregulation, especially in ectotherms.

The best uses of thermal performance curves are those that explicitly relate variation in function across body temperatures to some performance metric with direct fitness consequences (Angilletta et al., 2006; Schulte et al., 2011; Dowd et al., 2015) that might be virulence in bacteria (Ashrafi et al., 2018), growth and development in insects (Shi et al., 2015), or running performance in lizards (Hertz et al., 1983). At a biochemical and even tissue level, the same basic relationships between body temperature and function should hold for endotherms as well as they do for ectotherms (reviewed in Seebacher and Little, 2017). This realisation led to the proposal that thermal performance curves might also be useful in understanding the variation in body temperature among endotherms and consequences of that variation for coping with environmental conditions (Angilletta et al., 2010). Although the general idea should be transferable from ectotherms to endotherms, the specifics will necessarily be different because body temperature is highly modulated by endotherms through enhanced physiological thermoregulation. In practice, physiological thermoregulation has made measuring thermal performance curves in endotherms exceedingly difficult (Levesque and Marshall, 2021). A few researchers have managed to describe a thermal performance curve for a specific tissue group either *in vitro* (James et al., 2015; Seebacher and Little, 2017) or *in vivo* (Rummel et al., 2018, 2019). Even fewer have successfully measured whole animal performance across temperatures in endotherms (Seymour et al., 1998; Rojas et al., 2012; reviewed in Levesque and Marshall, 2021).

Because thermal performance is difficult to measure directly in endotherms, several authors (including us) have suggested that distributions of body temperature might serve as a proxy for thermal performance (Angilletta et al., 2010; Boyles and Warne, 2013; Levesque and Marshall, 2021). This suggestion is based on the idea that thermoregulation and thermal sensitivity have coadapted in endotherms, as appears to be the case in ectotherms. The coadaptation of thermoregulation and thermal sensitivity should lead to a generalist-specialist trade-off where individuals, populations and species fall along a continuum between strict thermoregulation with high peak performance at the chosen set point temperature and more flexible thermoregulation with moderate performance across a wide range of body temperatures (Angilletta et al., 2003; **Figure 1A**). Thus, the shape of a distribution of body temperature measurements could theoretically give some indication of the shape of a thermal performance curve (**Figure 1B**). The obvious benefit of this method of estimating endotherm thermal performance curves in this manner is the (relative) ease with which one can measure body temperature in free-ranging animals as well as the availability of existing datasets. As biologging technologies advance (Chmura et al., 2018; Hawkes et al., 2021), so does our collective ability to describe body temperature distributions. The use of body temperature distributions as a proxy for thermal performance curves could therefore facilitate large-scale examinations of thermal sensitivity across the mammalian and avian phylogenies, which would represent a huge advance in understanding how mammals and birds will respond to climate change.

It is telling that no such analyses exist. In fact, although researchers have found some utility in using thermal performance curves to address theoretical aspects of thermoregulation in endotherms (Boyles et al., 2011; Boyles and Warne, 2013; Levesque and Marshall, 2021), few researchers have used them in empirical research (but see Seebacher and Little, 2017). An inherent limitation of using body temperature distributions as a proxy for thermal performance is that the entire idea is based on an unverified and very difficult to test assumption proposed by Angilletta et al. (2010) that thermoregulation and thermal sensitivity are coadapted in endotherms. This is a significant hurdle, but there are some obvious pathways to advance this research. Physical or pharmacological manipulations of hypothalamic set point temperatures might offer an opportunity to manipulate body temperature and measure relevant functional traits (e.g. enzyme performance, digestive ability or muscle function) under a variety of body temperature conditions. Likewise, regional heterothermy throughout the body means that different tissue groups will be exposed to different thermal conditions and may therefore exhibit different levels of thermal sensitivity (James et al., 2015; Seebacher and Little, 2017). Finally, heterothermic species that have widely variable body temperatures over daily or annual cycles can serve as useful model taxa for studying thermal sensitivity at different body temperatures (Willis and Brigham, 2003; Rojas et al., 2012; Nowack et al., 2016b).

In this perspectives paper, we address the usefulness of body temperature distributions in assessing thermal performance or thermal sensitivity in endotherms. We first provide a brief overview of the primary drivers of variability in body temperature in endotherms and then attempt to find evidence of the potential utility of body temperature distributions to serve as a proxy for thermal performance curves. We do this by comparing the general shape of body temperature distributions from several small mammal species to see if they qualitatively conform to the classical shape of a thermal performance curve.

WHAT IS BODY TEMPERATURE?

Body temperature in endotherms is an emergent property of behavioural, morphological and physiological mechanisms that

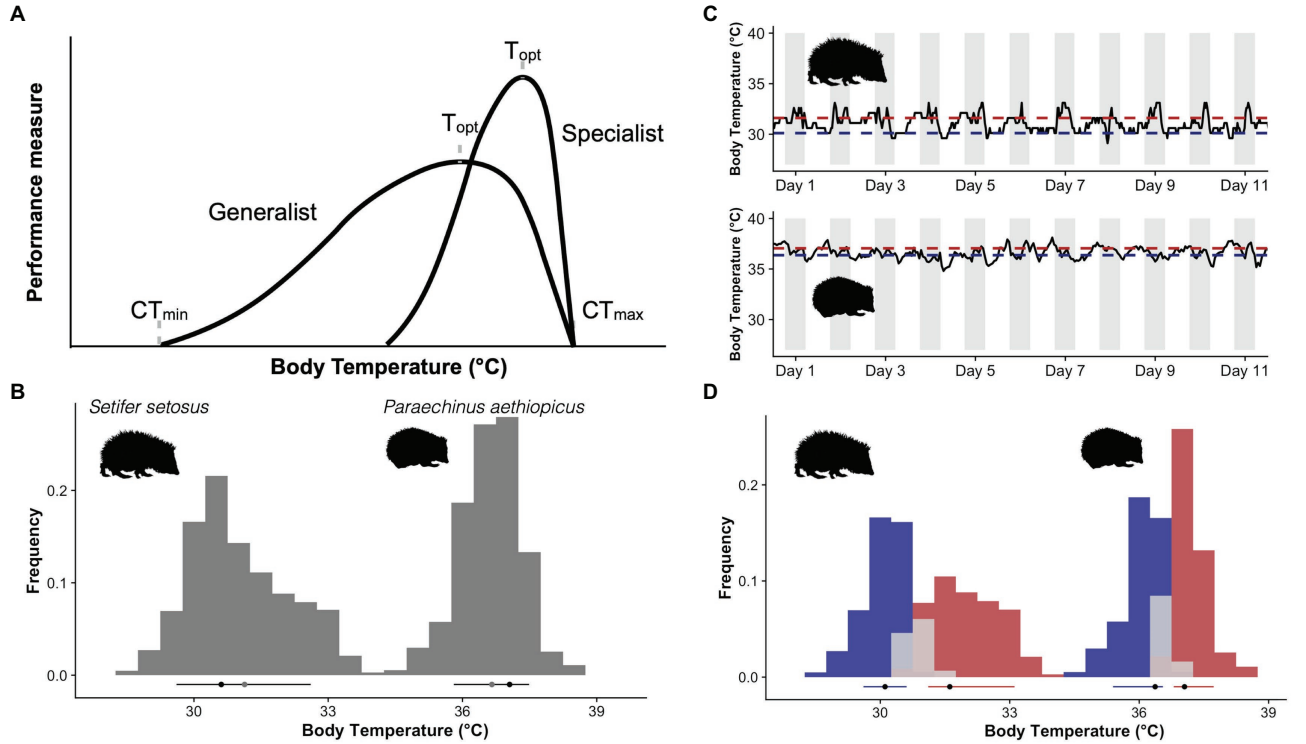

FIGURE 1 | (A) Hypothetical thermal performance curves for two ectotherms, a thermal generalist capable of performance over a wide range of temperatures and a specialist with higher performance but a narrower range. **(B)** Core body temperature frequency distribution curves from a greater hedgehog tenrec [*Setifer setosus* (Levesque et al., 2014)] and a desert hedgehog [*Paraechinus aethiopicus* (Boyles et al., 2017)]. Black dots indicate the mode, grey the mean and the lines the limits of the 10th and 90th quantiles of the body temperature distribution. **(C)** Free-ranging body temperature traces for the same two species, grey bars indicate the dark phase as both species are nocturnal, this also indicates the acrophase of their activity cycle. Red lines indicate the mode of the active phase and blue the resting. **(D)** Histograms generated by dividing the body temperature data into active (red) for data above the 55th quantile, resting (blue) for data below the 45th quantile or transitional (grey). Quantiles were generated using additive quantile regression smoothing (see text) to account for potential seasonal changes in the level of body temperature. Black dots indicate the mode, and the lines the 10th-90th quantiles of the distribution. Picture credits: Kerileigh Lobban (tenrec) and Claus Rebler (hedgehog, phylopic.org).

either generate heat or control its loss to the environment (Tattersall et al., 2012; Seebacher, 2020). Physiological heat generation occurs *via* metabolic activity at rest, muscular activity and shivering and non-shivering thermogenesis, among others (Humphries and Careau, 2011; Seebacher, 2018). The maintenance of body temperature within a narrow range of temperatures allows for sustained aerobic activity and independence from environmental conditions and is therefore believed to be the greatest benefit of the evolution of endothermy (Bennett, 1991; Farmer, 2000; Koteja, 2000; Clarke and Pörtner, 2010). Regulated in the hypothalamus, body temperature is defended at a set point by balancing heat production and heat retention with heat dissipation mechanisms (panting, changes in posture, etc.; Romanovsky, 2007; Zhao et al., 2017). Set points can be variable and difficult to assess outside of experiments directly manipulating hypothalamic temperatures (Heller et al., 1977). Furthermore, at any point in time, a particular value for body temperature could be the result of active control, passive cooling, a by-product of heat generated during activity or a transitional value between various states. Regional heterothermy adds an additional axis of variation in body temperature of endotherms and tissue temperature can vary depending on where the measurements are taken (Irving and Krog, 1955; Maloney et al., 2019). For the remainder of the paper, we will be referring predominantly to core body temperature as it is the most commonly measured form of internal body temperature, but we emphasise that core temperatures are not representative of all tissue temperatures.

Although we often speak about the 'near-constant' body temperature of endotherms, the range of temperatures shown by any one individual is often wider than realised and can be highly variable across both daily and annual cycles. Most endotherms show a pronounced circadian variation, with a higher body temperature during the active phase (acrophase) and a lower body temperature during the rest phase (scotophase) of their circadian cycle (Aschoff, 1983; Maloney et al., 2019; Refinetti, 2020). The amplitude of circadian rhythms is variable between species and also varies within species or individuals with water and food availability, ambient temperature and reproductive status (Poppitt et al., 1994; Scribner and Wynne-Edwards, 1994; Refinetti, 1999; Hetem et al., 2010; Levesque et al., 2014; Maloney et al., 2017). Differing pressures over evolutionary history have led to myriad thermoregulatory patterns

in endotherms of all sizes (Lovegrove, 2012). Thermoregulatory variation is most pronounced in species which reduce body temperature during energy-saving torpor (Grigg et al., 2004; Ruf and Geiser, 2015; Nowack et al., 2020), and the use of daily torpor during the rest phase combined with continued activity during the active phase can lead to pronounced daily amplitudes. Similarly, some endotherms inhabiting warm environments temporarily forgo water-costly cooling mechanisms and allow their body temperature to passively increase with ambient temperature during acute heat to save water (Degen, 2012; Gerson et al., 2019; Reher and Dausmann, 2021).

The vast majority of work on variation in body temperature in endotherms has been either in the context of describing heterothermic and homeothermic patterns or circadian and circannual rhythms. In both of those contexts, body temperature distributions contain useful information, but researchers have largely focused on measures of central tendencies (e.g. the mean of body temperature during normothermy or during the day). Due to the complexities of body temperature regulation in endotherms, assigning a single value (such as a mean) to a species can obscure important information about their underlying physiology. Understanding the distinction between the thermoregulatory physiology of an endotherm during the rest and active phases of their daily cycle (Aschoff, 1981; Wright et al., 2002) is essential to understanding the links between temperature and performance. Selection for high body temperatures during the activity phase (Koteja, 2000; Lovegrove and Mowoe, 2014; Stawski et al., 2017) would not preclude the additional adaptive benefits of energy savings during rest (from normothermic resting to nocturnal hypothermia through to deep hibernation). The distinction between these two very different physiological states (active and resting) as well as the differing determinants of variability in temperature during these states has been lost by focusing predominantly on mean body temperatures. For practical purposes, this thermolability means that mean body temperatures alone are unlikely to contain particularly useful information for understanding the evolution of thermal performance in endotherms. We therefore need methods that help us quantify more than just means.

DO BODY TEMPERATURE DISTRIBUTIONS CONTAIN USEFUL PERFORMANCE SIGNALS?

The very idea that body temperature distributions might tell us something about thermal sensitivity suggests the shape of the distributions is also important. Beginning with Angilletta et al. (2010), endothermic generalist and specialist thermoregulatory patterns have been depicted as left-skewed, unimodal distributions reminiscent of ectothermic thermal performance curves. This model served as a useful starting point in theoretical discussions of the coadaptation of thermoregulation and thermal sensitivity, but left-skewed, unimodal distributions of body temperatures are far from universal in endothermic species. Instead, bimodal distributions are common and right-skewed distributions do occur in some species (e.g. McKechnie et al., 2007; Levesque et al., 2018; **Figure 1B**). The disjunction between theoretical treatments of body temperature distributions and reality is obviously problematic. To address whether core body temperature distributions provide a useful analogue to thermal performance curves and to encourage a more biologically relevant means of describing endotherm body temperature distributions, we collated core (intraperitoneal) temperatures for single individuals of 13 different species of small eutherian and marsupial mammals (<300 g) and one monotreme (<4,000 g). This was not intended to be an exhaustive list but was chosen to represent species from different habitats and from across the mammalian phylogeny. We included only small mammals here to limit the influence of body mass, which should normally be taken into consideration, but is not fundamental to the point we hope to make. We also preferentially included data from active seasons (spring, summer or fall) to focus on times of year when increases in active body temperatures may be linked to increases in performance. We used non-stationary waveform analysis (Levesque et al., 2017) to split active and resting body temperatures in each species. This method has the advantage of being agnostic to both time and the level of body temperature and therefore can account for changes in the level and amplitude of body temperature cycles, such as those seen seasonally or during estrous cycles. We considered all measurements between the 45th and 55th quantiles estimated by the waveform analysis as transitory between active and resting. Measurements that fell above the 55th quantile were classified as active temperatures and those below the 45th quantile were classified as resting temperatures. This avoided problems of defining active periods based on external factors, such as day/night cycles (**Figure 1C**). For each phase, we then calculated modal temperature as a measure of central tendency, the 10th and 90th quantiles, and a measure of skewness (Bickel, 2002) of active and resting body temperature distributions for each species (**Figures 1D, 2**).

Despite the limited dataset, our analysis clearly reveals variation in the shape of body temperature distributions between mammalian species (**Figure 2**). The overall distributions for most (8/14) of the species were right-skewed, the opposite of the normal left skew thermal performance curves observed in ectotherms, tissue assays and protein function (Angilletta, 2009). A right-skewed performance curve indicates mammals have more leeway to allow body temperature to increase above set point than decrease below it. This fits with routine increases in body temperature that have been observed during locomotion in both small mammal (Bieber et al., 2017) and large mammals (Hetem et al., 2019). The shape of the body temperature distribution that combines both resting and active temperatures (e.g. **Figure 1B**) may, however, be irrelevant because as mentioned before, many mammals have distinctly bimodal body temperature distributions. We originally assumed that if we separate active from resting temperatures, a unimodal, left-skewed distribution may likely fit the active body temperatures of those species with a bimodal distribution. In our sample of small mammals, body temperature distributions display bimodality for most (8/14) of the species included (indicated by a Hartigan's dip test statistic <0.05). However, the resulting activity and resting distributions, while

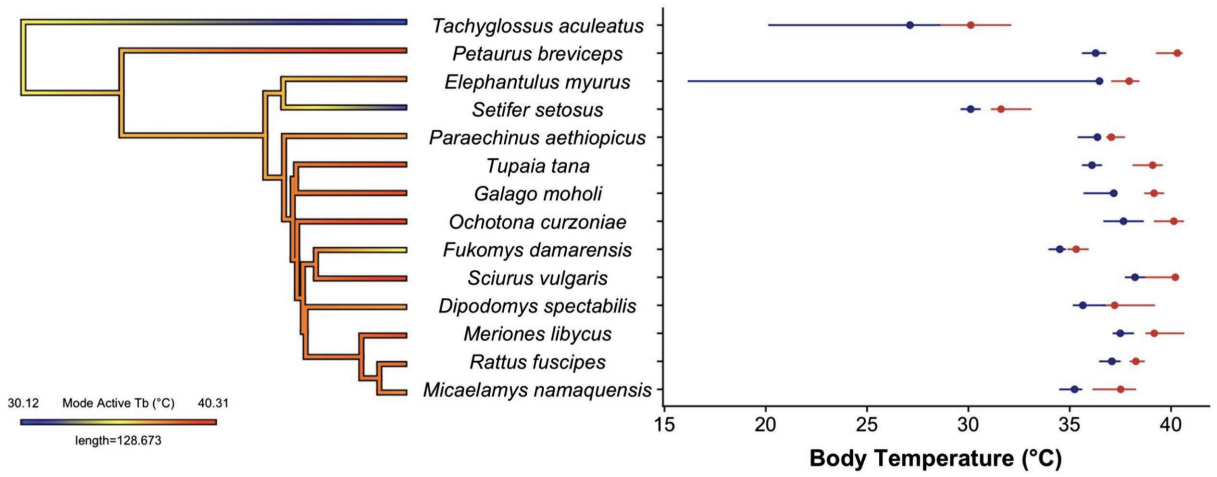

FIGURE 2 | Body temperature distributions for the 14 species of small mammals. The phylogeny shows estimated ancestral states for the mode of the active phase body temperatures. There was no relationship between body temperature and phylogenetic position in this sample. Phylogenetic tree was generated using the *contMap* function in the R package 'phytools' (Revell, 2012) using the mammal supertree from Upham et al. (2019). The spread of the active phase (red) and resting phase (blue) are shown for a single individual from each species. The dots indicate the modes of each phase and the daily cycle, and lines represent the 10th–90th quantiles of the distribution. The datasets for *Tachyglossus aculeatus* and *Elephantulus myurus* include torpor use, which leads to a clear left-skewed distribution. Body temperature traces were obtained from the following publications *Dipodomys* (Morales et al., in press), *Elephantulus* and *Micaelamys* (Boyles et al., 2012), *Fukomys* (Streicher et al., 2011), *Galago* (Nowack et al., 2013), *Meriones* (Alagaili et al., 2017), *Ochotona* (Speakman et al., 2021), *Paraechinus* (Boyles et al., 2017), *Petaurus* (Nowack and Geiser, 2016), *Rattus* (Nowack and Turbill unpublished data), *Sciurus* (Dausmann et al., 2013), *Setifer* (Levesque et al., 2014), *Tachyglossus* (Nowack et al., 2016a) and *Tupaia* (Levesque et al., 2018).

at least no longer bimodal, still did not follow the shape of a classic ectotherm thermal performance curve (**Figure 2**). Instead, most (6/8) of the species with a bimodal body temperature distribution also had a right-skewed body temperature distribution of active body temperatures. Clear left-skewed distributions were, however, observed for species with data spanning periods of torpor use (e.g. *Elephantulus, Galago and Tachyglossus*), which provides an interesting avenue for future research.

Our cursory inter-species comparison also revealed surprisingly comparable between-species variability in modal active and resting body temperatures (activity mode 37.2°C, mean 37.4°C, st. dev. 3.1°C, range 30.1–40.3°C; resting: mode 35.6°C, mean 35.4°C, st. dev. 3.1°C, range 27.1–38.2°C), although active temperatures were consistently higher than resting (**Figure 2**). This may, however, be influenced by our choice of species, and the time of year at which the recordings were taken, although our values do span the range of reported values for mammalian body temperatures (Clarke and Rothery, 2008). When given the option, we preferentially chose values from the active season to avoid additional complications of differences in torpor use, but in some species (*Elephantulus, Setifer and Tachyglossus*), torpor is unavoidable. Had we included data for the full annual cycle, we would expect to find additional differences in the level and potentially the shape of the distributions depending on factors, such as reproduction, time of year and environmental conditions. The intra-species difference for the daily amplitude between active and resting modes of our 14 species showed a wide variation (mode: 1.6°C, mean: 2.0°C, st. dev. 0.9°C, range 0.68–4.03°C), with the hedgehog (*Paraechinus aethiopicus*) showing the least amount of difference between active and resting and the sugar glider (*Petaurus breviceps*) displaying the largest daily amplitude, yet these might be expected to change had we focused on different times of year. These findings demonstrate the utility of body temperature distributions in generating points of comparison for eco-physiological or comparative studies.

CONCLUSION

Although our limited sample of species is small, mostly stems from small-bodied mammals and is biased towards our own study systems, we found no evidence to suggest that using distributions of core body temperatures is likely to be fruitful as proxies for ectotherm thermal performance curves. This does not mean that endotherms are free from the biophysical constraints that dictate temperature effects on performance, and therefore the classical shape of ectotherm thermal performance curves. Instead, it only suggests that core body temperature distributions are not a good proxy for the shape of thermal performance curves in endotherms. In fact, they might still be useful for estimating the single temperature at which most performances are maximised (the optimal temperature, possibly represented here by the mode of the active phase), just not for describing the thermal sensitivity of a species. We also still expect to find the left-skewed thermal performance curves, so common in ectotherms, in endotherms at cellular, tissue and whole organism levels (Seebacher and Little, 2017). We only conclude here that those performance curves will likely need to be measured directly at those levels instead of estimated indirectly *via* core body temperature distributions.

We also do not wish to discourage the description and use of body temperature distributions in the study of endotherm thermoregulation. Fully describing body temperature distributions will undoubtedly lead to advances that would not be possible by focusing only on measures of central tendencies. Body temperature distributions may be more valuable in addressing ecological questions about how a mammal or bird interacts with its environment than as direct analogues to ectotherm thermal performance curves. In fact, our cursory analysis hints that there is some benefit in distinguishing between the distinct active and resting phases of the daily body temperature cycle, at least at an interspecific comparative level, which should prove fruitful in evolutionary studies. There is also potential in the ability to analyse both the level and variability of active and resting temperatures separately which should prove beneficial in both within- and between-species studies. We strongly encourage the use of more detailed body temperature data so that we can move beyond the idea of endotherms as strict homeotherms and embrace the true heterothermic diversity of extant endotherms.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

DLL and JN conceived the idea and outlined the manuscript. DLL and JB designed the analysis and prepared the figures for the manuscript. All authors contributed to the article and approved the submitted version.

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REFERENCES

Alagaili, A. N., Bennett, N. C., Mohammed, O. B., Zalmout, I. S., and Boyles, J. G. (2017). Body temperature patterns of a small endotherm in an extreme desert environment. *J. Arid Environ.* 137, 16–20. doi: 10.1016/j.jaridenv.2016.10.010

Angilletta, M. J. Jr., Bennett, A. F., Guderley, H., Navas, C. A., Seebacher, F., and Wilson, R. S. (2006). Coadaptation: a unifying principle in evolutionary thermal biology. *Physiol. Biochem. Zool.* 79, 282–294. doi: 10.1086/499990

Angilletta, M. J. Jr. (2009). *Thermal Adaption: A Theoretical and Empricial Synthesis*. Oxford: Oxford University Press.

Angilletta, M. J. Jr., Cooper, B. S., Schuler, M. S., and Boyles, J. G. (2010). The evolution of thermal physiology in endotherms. *Front. Biosci.* 2, 861–881. doi: 10.2741/e148

Angilletta, M. J. Jr., Wilson, R. S., Navas, C. A., and James, R. S. (2003). Tradeoffs and the evolution of thermal reaction norms. *Trends Ecol. Evol.* 18, 234–240. doi: 10.1016/S0169-5347(03)00087-9

Aschoff, J. (1981). Thermal conductance in mammals and birds: its dependence on body size and crcadian phase. *Comp. Biochem. Physiol. A.* 69, 611–619. doi: 10.1016/0300-9629(81)90145-6

Aschoff, J. (1983). Circadian control of body temperature. *J. Therm. Biol.* 8, 143–147. doi: 10.1016/0306-4565(83)90094-3

Ashrafi, R., Bruneaux, M., Sundberg, L.-R., Pulkkinen, K., Valkonen, J., and Ketola, T. (2018). Broad thermal tolerance is negatively correlated with virulence in an opportunistic bacterial pathogen. *Evol. Appl.* 11, 1700–1714. doi: 10.1111/eva.12673

Bennett, A. F. (1991). The evolution of activity capacity. *J. Exp. Biol.* 160, 1–23. doi: 10.1242/jeb.160.1.1

Bickel, D. R. (2002). Robust estimators of the mode and skewness of continuous data. *Comput. Stat. Data Anal.* 39, 153–163. doi: 10.1016/S0167-9473(01)00057-3

Bieber, C., Cornils, J. S., Hoelzl, F., Giroud, S., and Ruf, T. (2017). The costs of locomotor activity? Maximum body temperatures and the use of torpor during the active season in edible dormice. *J. Comp. Physiol. B.* 187, 803–814. doi: 10.1007/s00360-017-1080-y

Boyles, J. G., Bennett, N. C., Mohammed, O. B., and Alagaili, A. N. (2017). Torpor patterns in desert hedgehogs (*Paraechinus aethiopicus*) represent another new point along a thermoregulatory continuum. *Physiol. Biochem. Zool.* 90, 445–452. doi: 10.1086/691542

Boyles, J. G., Seebacher, F., Smit, B., and Mckechnie, A. E. (2011). Adaptive thermoregulation in endotherms may alter responses to climate change. *Integr. Comp. Biol.* 51, 676–690. doi: 10.1093/icb/icr053

Boyles, J. G., Smit, B., Sole, C. L., and Mckechnie, A. E. (2012). Body temperature patterns in two syntopic elephant shrew species during winter. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* 161, 89–94. doi: 10.1016/j.cbpa.2011.09.007

Boyles, J. G., and Warne, R. W. (2013). A novel framework for predicting the use of facultative heterothermy by endotherms. *J. Theor. Biol.* 336, 242–245. doi: 10.1016/j.jtbi.2013.08.010

Chmura, H. E., Glass, T. W., and Williams, C. T. (2018). Biologging physiological and ecological responses to climatic variation: new tools for the climate change era. *Front. Ecol. Evol.* 6:92. doi: 10.3389/fevo.2018.00092

Clarke, A., and Pörtner, H.-O. (2010). Temperature, metabolic power and the evolution of endothermy. *Biol. Rev.* 85, 703–727. doi: 10.1111/j.1469-185X.2010.00122.x

Clarke, A., and Rothery, P. (2008). Scaling of body temperature in mammals and birds. *Funct. Ecol.* 22:10.1111/j.1365-2435.2007.01341.x, 58–67.

Dausmann, K. H., Wein, J., Turner, J. M., and Glos, J. (2013). Absence of heterothermy in the European red squirrel (*Sciurus vulgaris*). *Mamm. Biol.* 78, 332–335. doi: 10.1016/j.mambio.2013.01.004

Degen, A.A. (2012). *Ecophysiology of Small Mammals*. Berlin, Heidelberg: Springer Verlag.

Dowd, W. W., King, F. A., and Denny, M. W. (2015). Thermal variation, thermal extremes and the physiological performance of individuals. *J. Exp. Biol.* 218, 1956–1967. doi: 10.1242/jeb.114926

Farmer, C. G. (2000). Parental care: The key to understanding endothermy and other convergent features in birds and mammals. *Am. Soc. Nat* 155, 326–334. doi: 10.1086/303323

Gerson, A. R., Mckechnie, A. E., Smit, B., Whitfield, M. C., Smith, E. K., Talbot, W. A., et al. (2019). The functional significance of facultative hyperthermia varies with body size and phylogeny in birds. *Funct. Ecol.* 33, 597–607. doi: 10.1111/1365-2435.13274

Grigg, G., Beard, L., and Augee, M. (2004). The evolution of endothermy and its diversity in mammals and birds. *Physiol. Biochem. Zool.* 77, 982–997. doi: 10.1086/425188

Hawkes, L. A., Fahlman, A., and Sato, K. (2021). Introduction to the theme issue: measuring physiology in free-living animals. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 376:20200210. doi: 10.1098/rstb.2020.0210

Heller, H. C., Colliver, G. W., and Bread, J. (1977). Thermoregulation during entrance into hibernation. *Pflugers Arch.* 369, 55–59. doi: 10.1007/BF00580810

Hertz, P. E., Huey, R. B., and Nevo, E. (1983). Homage to Santa Anita: thermal sensitivity of sprint speed in agamid lizards. *Evolution* 37, 1075–1084. doi: 10.1111/j.1558-5646.1983.tb05634.x

Hetem, R. S., Mitchell, D., Ba, D. E. W., Fick, L. G., Maloney, S. K., Meyer, L. C. R., et al. (2019). Body temperature, activity patterns and hunting in free-living cheetah: biologging reveals new insights. *Integr. Zool.* 14, 30–47. doi: 10.1111/1749-4877.12341

Hetem, R. S., Strauss, W. M., Fick, L. G., Maloney, S. K., Meyer, L. C., Shobrak, M., et al. (2010). Variation in the daily rhythm of body temperature of free-living Arabian oryx (Oryx leucoryx): does water limitation drive heterothermy? *J. Comp. Physiol. B.* 180, 1111–1119. doi: 10.1007/s00360-010-0480-z

Huey, R. B., Kearney, M. R., Krockenberger, A., and Holtum, J.a.M., Jess, M., and Williams, S.E., (2012). Predicting organismal vulnerability to climate warming: roles of behaviour, physiology and adaptation. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 367, 1665–1679. doi: 10.1098/rstb.2012.0005

Huey, R., and Slatkin, M. (1976). Cost and benefits of lizard thermoregulation. *Q. Rev. Biol.* 51, 363–384. doi: 10.1086/409470

Huey, R. B., and Stevenson, R. D. (2015). Integrating thermal physiology and ecology of ectotherms: A discussion of approaches. *Am. Zool.* 19, 357–366. doi: 10.1093/icb/19.1.357

Humphries, M. M., and Careau, V. (2011). Heat for nothing or activity for free? Evidence and implications of activity-thermoregulatory heat substitution. *Integr. Comp. Biol.* 51, 419–431. doi: 10.1093/icb/icr059

Irving, L., and Krog, J. (1955). Temperature of skin in the arctic as a regulator of heat. *J. Appl. Physiol.* 7, 355–364. doi: 10.1152/jappl.1955.7.4.355

James, R. S., Tallis, J., and Angilletta, M. J. (2015). Regional thermal specialisation in a mammal: temperature affects power output of core muscle more than that of peripheral muscle in adult mice (*Mus musculus*). *J. Comp. Physiol. B.* 185, 135–142. doi: 10.1007/s00360-014-0872-6

Koteja, P. (2000). Energy assimilation, parental care and the evolution of endothermy. *Proc. R. Soc. Lond. Ser. Biol. Scie.* 267, 479–484. doi: 10.1098/rspb.2000.1025

Levesque, D. L., Lobban, K. D., and Lovegrove, B. G. (2014). Effects of reproductive status and high ambient temperatures on the body temperature of a free-ranging basoendotherm. *J. Comp. Physiol. B.* 184, 1041–1053. doi: 10.1007/s00360-014-0858-4

Levesque, D. L., and Marshall, K. E. (2021). Do endotherms have thermal performance curves? *J. Exp. Biol.* 224:jeb141309. doi: 10.1242/jeb.141309

Levesque, D. L., Menzies, A. K., Landry-Cuerrier, M., Larocque, G., and Humphries, M. M. (2017). Embracing heterothermic diversity: non-stationary waveform analysis of temperature variation in endotherms. *J. Comp. Physiol. B* 187, 749–757. doi: 10.1007/s00360-017-1074-9

Levesque, D. L., Tuen, A. A., and Lovegrove, B. G. (2018). Staying hot to fight the heat-high body temperatures accompany a diurnal endothermic lifestyle in the tropics. *J. Comp. Physiol. B* 188, 707–716. doi: 10.1007/s00360-018-1160-7

Lovegrove, B. G. (2012). The evolution of endothermy in Cenozoic mammals: a plesiomorphic-apomorphic continuum. *Biol. Rev.* 87, 128–162. doi: 10.1111/j.1469-185X.2011.00188.x

Lovegrove, B. G., and Mowoe, M. O. (2014). The evolution of micro-cursoriality in mammals. *J. Exp. Biol.* 217, 1316–1325. doi: 10.1242/jeb.095737

Maloney, S., Goh, G., Fuller, A., Vesterdorf, K., and Blache, D. (2019). Amplitude of the circadian rhythm of temperature in homeotherms. *CAB Rev.* 14, 1–30. doi: 10.1079/PAVSNNR201914019

Maloney, S. K., Marsh, M. K., Mcleod, S. R., and Fuller, A. (2017). Heterothermy is associated with reduced fitness in wild rabbits. *Biol. Lett.* 13:20170521. doi: 10.1098/rsbl.2017.0521

Mckechnie, A. E., Chetty, K., and Lovegrove, B. G. (2007). Phenotypic flexibility in the basal metabolic rate of laughing doves: responses to short-term thermal acclimation. *J. Exp. Biol.* 210, 97–106. doi: 10.1242/jeb.02615

Morales, J.O., Walker, N., Warne, R.W., and Boyles, J.G. (in press). *Heterothermy as a Mechanism to Offset Energetic Costs of Environmental and Homeostatic Perturbations*.

Nowack, J., Cooper, C. E., and Geiser, F. (2016a). Cool echidnas survive the fire. *Proc. R. Soc. Lond. B Biol. Sci.* 283:20160382. doi: 10.1098/rspb.2016.0382

Nowack, J., Delesalle, M., Stawski, C., and Geiser, F. (2016b). Can hibernators sense and evade fires? Olfactory acuity and locomotor performance during deep torpor. *Sci. Nat.* 103:73. doi: 10.1007/s00114-016-1396-6

Nowack, J., and Geiser, F. (2016). Friends with benefits: the role of huddling in mixed groups of torpid and normothermic animals. *J. Exp. Biol.* 219, 590–596. doi: 10.1242/jeb.128926

Nowack, J., Levesque, D. L., Reher, S., and Dausmann, K. H. (2020). Variable climates lead to varying phenotypes: "weird" mammalian torpor and lessons from non-holarctic species. *Front. Ecol. Evol.* 8:60. doi: 10.3389/fevo.2020.00060

Nowack, J., Mzilikazi, N., and Dausmann, K. H. (2013). Torpor as an emergency solution in *Galago moholi*: heterothermy is triggered by different constraints. *J. Comp. Physiol. B.* 183, 547–556. doi: 10.1007/s00360-012-0725-0

Poppitt, S. D., Speakman, J. R., and Racey, P. A. (1994). Energetics of reproduction in the lesser hedgehog tenrec, *Echinops telfairi* (Martin). *Physiol. Zool.* 67, 976–994. doi: 10.1086/physzool.67.4.30163874

Refinetti, R. (1999). Relationship between the daily rhythms of locomotor activity and body temperature in eight mammalian species. *Am. J. Phys. Regul. Integr. Comp. Phys.* 277, R1493–R1500. doi: 10.1152/ajpregu.1999.277.5.R1493

Refinetti, R. (2020). Circadian rhythmicity of body temperature and metabolism. *Temperature* 7, 321–362. doi: 10.1080/23328940.2020.1743605

Reher, S., and Dausmann, K. H. (2021). Tropical bats counter heat by combining torpor with adaptive hyperthermia. *Proc. R. Soc. B Biol. Sci.* 288:20202059. doi: 10.1098/rspb.2020.2059

Revell, L. J. (2012). Phytools: an R package for phylogenetic comparative biology (and other things). *Methods Ecol. Evol.* 3, 217–223. doi: 10.1111/j.2041-210X.2011.00169.x

Rojas, A. D., Körtner, G., and Geiser, F. (2012). Cool running: locomotor performance at low body temperature in mammals. *Biol. Lett.* 8, 868–870. doi: 10.1098/rsbl.2012.0269

Romanovsky, A. A. (2007). Thermoregulation: some concepts have changed. Functional architecture of the thermoregulatory system. *American journal of physiology-regulatory. Integr. Comp. Physiol.* 292, R37–R46. doi: 10.1152/ajpregu.00668.2006

Ruf, T., and Geiser, F. (2015). Daily torpor and hibernation in birds and mammals. *Biol. Rev.* 90, 891–926. doi: 10.1111/brv.12137

Rummel, A. D., Swartz, S. M., and Marsh, R. L. (2018). Low thermal dependence of the contractile properties of a wing muscle in the bat Carollia perspicillata. *J. Exp. Biol.* 221:jeb180166. doi: 10.1242/jeb.180166

Rummel, A. D., Swartz, S. M., and Marsh, R. L. (2019). Warm bodies, cool wings: regional heterothermy in flying bats. *Biol. Lett.* 15:20190530. doi: 10.1098/rsbl.2019.0530

Schulte, P. M., Healy, T. M., and Fangue, N. A. (2011). Thermal performance curves, phenotypic plasticity, and the time scales of temperature exposure. *Integr. Comp. Biol.* 51, 691–702. doi: 10.1093/icb/icr097

Scribner, S. J., and Wynne-Edwards, K. E. (1994). Disruption of body temperature and behavior rhythms during reproduction in dwarf hamsters (Phodopus). *Physiol. Behav.* 55, 361–369. doi: 10.1016/0031-9384(94)90147-3

Seebacher, F. (2018). The evolution of metabolic regulation in animals. *Comp. Biochem. Physiol. B: Biochem. Mol. Biol.* 224, 195–203. doi: 10.1016/j.cbpb.2017.11.002

Seebacher, F. (2020). Is Endothermy an evolutionary by-product? *Trends Ecol. Evol.* 35, 503–511. doi: 10.1016/j.tree.2020.02.006

Seebacher, F., and Little, A. G. (2017). Plasticity of performance curves can buffer reaction rates from body temperature variation in active endotherms. *Front. Physiol.* 8:575. doi: 10.3389/fphys.2017.00575

Seymour, R. S., Withers, P. C., and Weathers, W. W. (1998). Energetics of burrowing, running, and free-living in the Namib Desert golden mole (Eremitalpa namibensis). *J. Zool. (Lond.)* 244, 107–117. doi: 10.1111/j.1469-7998.1998.tb00012.x

Shi, P.-J., Reddy, G. V. P., Chen, L., and Ge, F. (2015). Comparison of thermal performance equations in describing temperature-dependent developmental rates of insects: (i) empirical models. *Ann. Entomol. Soc. Am.* 109, 211–215. doi: 10.1093/aesa/sav121

Speakman, J. R., Chi, Q., Ołdakowski, Ł., Fu, H., Fletcher, Q. E., Hambly, C., et al. (2021). Surviving winter on the Qinghai-Tibetan plateau: Pikas suppress energy demands and exploit yak feces to survive winter. *Proc. Natl. Acad. Sci. U. S. A.* 118:e2100707118. doi: 10.1073/pnas.2100707118

Stawski, C., Koteja, P., and Sadowska, E. T. (2017). A shift in the thermoregulatory curve as a result of selection for high activity-related aerobic metabolism. *Front. Physiol.* 8:1010. doi: 10.3389/fphys.2017.01070

Streicher, S., Boyles, J. G., Oosthuizen, M. K., and Bennett, N. C. (2011). Body temperature patterns and rhythmicity in free-ranging subterranean damaraland mole-rats, *Fukomys damarensis*. *PLoS One*. 6:e26346. doi: 10.1371/journal.pone.0026346

Tattersall, G. J., Sinclair, B. J., Withers, P. C., Fields, P. A., Seebacher, F., Cooper, C. E., et al. (2012). Coping with thermal challenges: Physiological adaptations to environmental temperatures. *Comprehensive Physiology 2,* 2151–2202. doi: 10.1002/cphy.c110055

Upham, N. S., Esselstyn, J. A., and Jetz, W. (2019). Inferring the mammal tree: species-level sets of phylogenies for questions in ecology, evolution, and conservation. *PLoS Biol*. 17:e3000494. doi: 10.1371/journal.pbio.3000494

Willis, C. K., and Brigham, R. M. (2003). Defining torpor in free-ranging bats: experimental evaluation of external temperature-sensitive radiotransmitters and the concept of active temperature. *J. Comp. Physiol. B.* 173, 379–389. doi: 10.1007/s00360-003-0343-y

Wright, K. P., Hull, J. T., and Czeisler, C. A. (2002). Relationship between alertness, performance, and body temperature in humans. *Am. J. Phys. Regul. Integr. Comp. Phys.* 283, R1370–R1377. doi: 10.1152/ajpregu.00205.2002

Zhao, Z. D., Yang, W. Z., Gao, C., Fu, X., Zhang, W., Zhou, Q., et al. (2017). A hypothalamic circuit that controls body temperature. *Proc. Natl. Acad. Sci. U. S. A.* 114, 2042–2047. doi: 10.1073/pnas.1616255114

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Thermal Performance Curves Are Shaped by Prior Thermal Environment in Early Life

Adriana P. Rebolledo, Carla M. Sgrò and Keyne Monro*

School of Biological Sciences, Monash University, Melbourne, VIC, Australia

Understanding links between thermal performance and environmental variation is necessary to predict organismal responses to climate change, and remains an ongoing challenge for ectotherms with complex life cycles. Distinct life stages can differ in thermal sensitivity, experience different environmental conditions as development unfolds, and, because stages are by nature interdependent, environmental effects can carry over from one stage to affect performance at others. Thermal performance may therefore respond to carryover effects of prior thermal environments, yet detailed insights into the nature, strength, and direction of those responses are still lacking. Here, in an aquatic ectotherm whose early planktonic stages (gametes, embryos, and larvae) govern adult abundances and dynamics, we explore the effects of prior thermal environments at fertilization and embryogenesis on thermal performance curves at the end of planktonic development. We factorially manipulate temperatures at fertilization and embryogenesis, then, for each combination of prior temperatures, measure thermal performance curves for survival of planktonic development (end of the larval stage) throughout the performance range. By combining generalized linear mixed modeling with parametric bootstrapping, we formally estimate and compare curve descriptors (thermal optima, limits, and breadth) among prior environments, and reveal carryover effects of temperature at embryogenesis, but not fertilization, on thermal optima at completion of development. Specifically, thermal optima shifted to track temperature during embryogenesis, while thermal limits and breadth remained unchanged. Our results argue that key aspects of thermal performance are shaped by prior thermal environment in early life, warranting further investigation of the possible mechanisms underpinning that response, and closer consideration of thermal carryover effects when predicting organismal responses to climate change.

Keywords: climate change, carryover effects, complex life cycles, developmental plasticity, fertilization, embryogenesis, larval development, thermal sensitivity

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University of California, San Diego, United States

****Correspondence:***
Adriana P. Rebolledo
adriana.rebolledonavarro@monash.edu

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INTRODUCTION

For ectotherms, accounting for the vast majority of animals, population resilience to climate change rests on the capacity to maintain critical physiological functions that buffer performance, and ultimately fitness (survival and reproduction), against variation in environmental temperature (Deutsch et al., 2008; Sinclair et al., 2016). Changes in temperature need not be detrimental

if they shift an organism's performance closer to its thermal optimum, or shift the optimum itself (Angilletta et al., 2010; Sørensen et al., 2018). Within generations, such shifts can emerge due to phenotypic plasticity, with evidence suggesting that ectotherms can often remodel their physiology to compensate for chronic or recurring changes in temperature (Seebacher et al., 2015; Sgrò et al., 2016), or to directional selection screening differences in survival or reproduction at different temperatures (Donelson et al., 2018). These mechanisms may often be inseparable (indeed, effects of selection may often be attributed to plasticity; Donelson et al., 2018) and both can result in environmental effects carrying over from one life stage to affect fitness outcomes at others (Donelson et al., 2018; Moore and Martin, 2019). Consequently, there is considerable interest in how such carryover effects might impact population resilience to climate change (Dupont et al., 2013; Seebacher et al., 2015; Campbell et al., 2020). Understanding their nature, direction, and strength, however, remains an ongoing challenge due to the complex life cycles of many ectotherms, and may benefit from new insights into thermal performance curves at understudied life stages that limit resilience (Sinclair et al., 2016; Kingsolver and Buckley, 2020; Rebolledo et al., 2020).

Temperature does not affect the same organism equally at all life stages (Angilletta, 2009). For ectotherms with complex life cycles, distinct developmental stages separated by days or even less can differ in thermal sensitivity due to multiple factors (e.g., evolved differences in thermal optima, along with rapid changes in complexity, size, or duration of exposure to thermal challenges), and thermal challenges can vary in intensity from one stage to the next (Kingsolver et al., 2011; Freda et al., 2017; Ezeakacha and Yee, 2019; Rebolledo et al., 2020). Nevertheless, most studies to date measure thermal performance and sensitivity at single life stages (Byrne et al., 2020) and predominantly in adults (Truebano et al., 2018; Pandori and Sorte, 2019). This is problematic in light of emerging evidence that reproductive stages and embryos tend to be more thermally sensitive and may better predict the vulnerability of ectotherms to climate warming (Dahlke et al., 2020; Rebolledo et al., 2020; Collin et al., 2021; Van Heerwaarden and Sgrò, 2021). Moreover, thermal performance at these critical stages is often incompletely characterized due to well-known challenges in gathering sufficient data, so that information about ontogenetic shifts in thermal limits and thermal optima, in particular, currently remains too limited to identify any general patterns (Kingsolver and Buckley, 2020).

Life stages are by nature interdependent, and there is growing evidence that prior thermal environments can have lasting effects on performance later in life (Arambourou et al., 2017; Ezeakacha and Yee, 2019; Carter and Sheldon, 2020). Evidence also suggests that these carryover effects can be more lasting and pervasive the earlier that they are induced in ontogeny, and especially when induced at embryogenesis (Watkins and Vraspir, 2006; Jonsson and Jonsson, 2014; Noble et al., 2018). This outcome possibly relates to the particular thermal sensitivity of embryos (Sanger et al., 2018; Rebolledo et al., 2020; Collin et al., 2021), and ample scope for thermal perturbation of cell division, differentiation, and regulatory pathways during this window of development to profoundly alter future form, function, and performance (Van Der Have, 2002; Hamdoun and Epel, 2007; Begasse et al., 2015). In general, however, the adaptive significance of carryover effects – at least those attributable to plasticity – remains contentious. Prior exposure to a given temperature is often assumed to optimize future performance at the same temperature (the so-called beneficial acclimation hypothesis), but this assumption has been subject to much debate (Huey et al., 1999; Loeschcke and Hoffmann, 2002; Wilson and Franklin, 2002; Deere and Chown, 2006), and evidence remains equivocal (e.g., Sgrò et al., 2016; Sørensen et al., 2016; Brahim et al., 2019; Van Heerwaarden and Kellermann, 2020). It might be that carryover effects are more nuanced and alter other aspects of thermal performance, but again, few studies have explored effects of early thermal environments on performance curves (but see Seebacher and Grigaltchik, 2014) and, to our knowledge, effects induced at fertilization – the key life stage linking one generation to the next – have received little attention in this context (Walsh et al., 2019; Chirgwin et al., 2021).

Thermal performance curves explicitly relate changes in temperature to performance, whether measured in terms of physiological rates, growth or development rates, or fitness components such as survival, fecundity, or fertility (Sinclair et al., 2016; Kingsolver and Buckley, 2020). Curve shape can vary with the measure considered, with curves for rates tending to be skewed and curves for survival tending toward symmetry (Van Der Have, 2002; Kingsolver et al., 2011). Regardless, thermodynamic effects on physiology see performance rise with increasing temperature from its lower thermal limit (CT_{min}) to a peak (P_{max}) at the thermal optimum (T_{opt}), before loss of metabolic efficiency or disruption of proteins and membranes at higher temperatures see it fall again to its upper thermal limit (CT_{max}). Thermal breadth (T_{br}, the range where performance is at least 50 or 80% of P_{max}) is then derived from these curve descriptors. Performance curves are key tools for assessing and predicting the responses of ectotherms to ongoing climate change, since the impacts of higher temperatures hinge on where, on the curve, conditions lie at present. Ectotherms may thrive, for example, if presently living below their thermal optima, or risk extinction if already living at or near their upper thermal limits (Seebacher et al., 2015; Sinclair et al., 2016; Pinsky et al., 2019).

Importantly, thermal performance curves are unlikely to be fixed for any performance measure, and determining how curves may themselves shift in response to environmental cues is also vital for understanding population responses to climate change (Angilletta, 2009; Sinclair et al., 2016). Multiple hypotheses have sought to explain coordinated shifts in curve shape and position along the temperature axis based on tension between thermodynamic constraints and mechanisms of thermal adaptation (Huey and Kingsolver, 1989; Huey et al., 1999; Izem and Kingsolver, 2005; Deere and Chown, 2006; Angilletta et al., 2010). Those hypotheses variously predict, for example, positive associations between peak performance and thermal optimum ("hotter-is-better" or "cooler-is-better") or between thermal

optimum and thermal limits ("hotter-colder"), and negative associations between peak performance and thermal breadth ("generalist-specialist"). Other hypotheses (including those centering on the benefits of acclimation or plasticity above) address the complex and diverse ways in which prior thermal experience may modify curve shape and position. To date, however, most evidence comes from plants, whereas responses for animals remain understudied (Angilletta, 2009; but see Deere and Chown, 2006; Seebacher and Grigaltchik, 2014) and so idiosyncratic as to evade prediction and synthesis (Sinclair et al., 2016). Hence, there is still a need to better understand how prior thermal experience affects thermal performance, particularly in early life for which knowledge is still scarce.

Here, we estimate and compare how thermal environments at fertilization and embryogenesis shape thermal performance curves at completion of planktonic development in an aquatic ectotherm – the externally-fertilizing tubeworm, *Galeolaria caespitosa*. Like most aquatic ectotherms, *Galeolaria* has planktonic gametes, embryos, and larvae that are dispersed passively by currents, undergo the key processes of fertilization and development in direct contact with the external environment, and are major bottlenecks for population resilience to climate change (Byrne, 2011; Pinsky et al., 2019; Walsh et al., 2019; Dahlke et al., 2020). These stages therefore present unique scope to assess how prior thermal experience alters performance at early life stages that govern adult abundances and dynamics. Using a split-cohort experimental design to standardize genetic backgrounds across stages, we factorially manipulate temperatures at fertilization (18 and 22°C) and embryogenesis (18, 20, and 22°C), then, for each combination of prior temperatures, measure thermal performance curves for survival of planktonic development (end of the larval stage). By combining generalized linear mixed modelling with parametric bootstrapping, we formally estimate and compare curve descriptors (thermal optima, limits, and breadth) among prior environments, and reveal new insights into the effects of those environments on thermal performance in early life.

MATERIALS AND METHODS

Study Species and Sampling

Galeolaria caespitosa (henceforth *Galeolaria*) is a calcareous tubeworm native to rocky shores of southeastern Australia, where it acts as an ecosystem engineer by forming dense colonies of tubes that provide habitat and reduce abiotic stress for associated communities (Wright and Gribben, 2017). Sessile adults breed year-round by releasing sperm and eggs into the sea for external fertilization (Chirgwin et al., 2020). Embryos develop into functionally-independent larvae ~24 h later, then larvae develop for another ~2–3 weeks until rapid changes in size, morphology, and behavior signal onset of metamorphosis (readiness to settle and recruit into sessile populations; Marsden and Anderson, 1981). These early life stages are bottlenecks for persistence under thermal stress (Byrne, 2011; Walsh et al., 2019), and exposure to stress at one stage can influence responses to the same level of stress later on (Chirgwin et al., 2021). However, the sensitivity of thermal performance curves to prior thermal environments in early life is unknown for organisms with complex life cycles like *Galeolaria*.

We sampled adult *Galeolaria* between March and July 2019 from a natural population at Brighton, Port Phillip Bay, Victoria, where water temperature ranges from 9°C in winter to 24°C in summer (Chirgwin et al., 2018). The region is a marine hotspot that has warmed at more than four times the global average rate in recent decades, and temperature is expected to increase by ~2–5°C by the century's end (Hobday and Lough, 2011; Hobday and Pecl, 2014). Adults were transferred in insulated aquaria to seawater tanks at Monash University, and acclimatized for 14 days at the mean annual temperature (17°C; Chirgwin et al., 2017) to reduce any effects of environmental differences among collection dates before obtaining gametes for experiments (gametogenesis is continuous and gametes can ripen in less than this time).

Experimental Overview

To explore how prior thermal environment alters thermal performance in early life, we factorially manipulated temperatures at fertilization (18 and 22°C) and embryogenesis (18, 20, and 22°C), then estimated thermal performance curves for survival of planktonic development (end of the larval stage). Survival to this point in the life cycle measures the proportion of initial offspring that ultimately become ready to settle and recruit to the adult population, and recruitment of new individuals is directly linked to population viability (Byrne, 2011). Temperatures at fertilization and embryogenesis were selected to bracket projected warming of 2–4°C by mid-to-late century (Hobday and Lough, 2011) and include the thermal optimum previously estimated for each stage (~21°C for fertilization and ~19°C for embryogenesis; Rebolledo et al., 2020). Thermal performance curves were based on 10 temperatures spanning the full performance range (10–28°C) and including the thermal optimum previously estimated for survival of larval development (~19°C; Rebolledo et al., 2020).

Thermal environment was manipulated, and performance assayed, in replicate vials of filtered, pasteurized seawater (loosely capped to allow oxygen flow) suspended upright in water baths. Baths were maintained at designated temperatures (±0.1 C) using controlled immersion heaters (Grant Optima TX150) for those ≥13°C and a refrigerated circulator (Julabo FP50) for 10°C. Four replicates were completed for each combination of temperatures with the exception of 27°C, for which two replicates were completed. Within each replicate, 30 individuals were evaluated for successful completion of development, giving an experiment-wide total of nearly 7,000 individuals. Replicates were generated in an incomplete block design with temperatures assigned haphazardly to blocks and unreplicated within them. Each block consisted of gametes, embryos, and larvae from the same cohort of parents used in one replicate per combination of temperatures at fertilization and embryogenesis, assayed at 2–5 temperatures at larval development (it was not logistically feasible to assay the full set of larval temperatures at once). Hence, all replicates per block were assayed concurrently using

different subsets of material from the same parents, under identical conditions aside from the manipulation of temperature (see details below). There were 10 blocks in total.

Gamete Collection and Manipulation of Temperature at Fertilization

Gametes were collected from 15 males and 15 females per block to minimize male-female compatibility effects at fertilization and development (Marshall and Evans, 2005; Chirgwin et al., 2017). To collect gametes, each mature adult was extracted from its tube and placed in a dish with ~1 ml of fresh filtered seawater at 17°C to spawn. Gametes were collected immediately after spawning, checked for quality based on appearance of eggs and motility of sperm, then pooled by sex and used within the hour before viability declines (Rebolledo et al., 2020). Pooled eggs were diluted to ~250 cells ml^{-1} before use. Pooled sperm were kept concentrated at ~10^7 cells ml^{-1} to minimize activity-related aging before use (Kupriyanova, 2006; Chirgwin et al., 2020). To initiate fertilizations, 45 ml of pooled eggs and 5 ml of pooled sperm were transferred separately to designated test temperatures (18 or 22°C), given 30 min to adjust, then combined at test temperatures. After 30 min of gamete contact (which maximizes fertilization success; Rebolledo et al., 2020), samples were rinsed through 0.25 µm mesh with seawater to remove excess sperm, then re-suspended in fresh seawater.

Manipulation of Temperature at Embryogenesis

About 1–2 h after fertilization (depending on temperature at fertilization), samples of two-cell embryos were transferred to designated test temperatures (18, 20, or 22°C) so that temperatures at this stage were fully crossed with temperatures at fertilization. We used two-cell embryos to ensure that all embryos were exposed to test temperatures at a similar point in development, and because this was the earliest point that they could be distinguished from unfertilized eggs under a stereomicroscope. Embryos were maintained in sufficient seawater to avoid oxygen-limitation (Chirgwin et al., 2018) until completing development into actively swimming, feeding larvae ~24 h later.

Assays of Thermal Performance at Completion of Planktonic Development

Thirty larvae were randomly allocated to each of 10–20 vials per designated test temperature (10, 13, 16, 18, 20, 22, 24, 26, 27, or 28°C, with fewer vials allocated to temperatures above 20°C), so that temperatures at this stage were fully crossed with temperatures at fertilization and embryogenesis. Larvae were maintained in sufficient seawater (10 ml) to avoid oxygen-limitation (Chirgwin et al., 2018) and fed a mix of microalgae *ad libitum* (~1×10^4 cells ml^{-1} every 2nd day). After the 1st week (larvae do not complete development in this time; Rebolledo et al., 2020), one vial was sampled destructively each day to monitor completion of development (normal onset of metamorphosis into the sessile form; Marsden and Anderson, 1981). Monitoring continued for up to 3 weeks depending on temperature, and ended when a final vial was observed in which all larvae had either died or successfully completed development. Each of the ~7,000 individuals in those final vials was then scored as 1 (denoting survival at completion of planktonic development) or 0 (denoting mortality beforehand), capturing the proportion of offspring ready to recruit to the adult population. No data came from individuals observed during monitoring, which was done simply to reliably identify the end of development, irrespective of development time.

Modeling Thermal Performance Curves

We fitted thermal performance curves to binary survival data (with 1 denoting survival or 0 denoting mortality) using a binomial mixed-effects regression model fitted with Laplace approximation in the *lme4* package (version 1.1-26; Bates et al., 2015) for *R* 4.0.5.[1] Based on the shape of unconstrained smoothers fitted to raw data (**Supplementary Figure S1**), survival was modeled as a cubic function of temperature using orthogonal polynomials. Prior temperatures at fertilization and embryogenesis, and all possible interactions with linear, quadratic, and cubic trends relating survival to temperature, were modeled as additional fixed effects. Block and final vial sampled within blocks were modeled as random effects. Model diagnostics were checked using the *DHARMa* package (version 0.4.1; Hartig, 2021) and showed no violations of assumptions. The significance of fixed effects was tested using Wald X^2 tests (Bolker et al., 2009) in the *car* package (version 3.0-10; Fox and Weisberg, 2019). For significant effects, estimates of linear, quadratic, and cubic trends in survival were extracted from the model, and contrasted between prior temperatures using Tukey-adjusted pairwise contrasts, in the *emmeans* package (version 1.6.0; Lenth et al., 2021).

Estimates and CIs of Curve Descriptors

We extracted standard descriptors of thermal performance curves for temperatures at embryogenesis from the binomial mixed-effects regression model (curves did not differ among temperatures at fertilization, so descriptors were not extracted at this level). Thermal optimum (T_{opt}) was calculated as the temperature of peak survival (P_{max}). Thermal breadth (T_{br}) was calculated as the temperature range at which survival was equal or above 50% of its peak (following Sinclair et al., 2016). Breadth is also commonly calculated at equal or above 80% of peak performance, but we chose 50% to capture more of the shapes of curves, and because results were qualitatively unchanged when 80% was used. Both calculations gave similar results to thermal tolerance (CT_{max}-CT_{min}), so only breadth is presented here. Critical thermal limits (CT_{min} and CT_{max}) were calculated as the lower and upper temperatures at which survival was 5% of its peak. This approach differs to classical measures based on acute limits, but was done because binary data may approach 0% *via* an asymptote and limit the biological meaning of CT_{min} and CT_{max} at complete mortality (Kellermann et al., 2019). Again, results were qualitatively unchanged when limits were calculated at complete mortality.

[1]https://www.r-project.org/

Last, to compare curve descriptors extracted from the regression model among prior temperatures at embryogenesis, we used parametric bootstrapping, implemented in the *boot* package (version 1.3-27; Canty and Ripley, 2021), to estimate the mean and 95% CI of each descriptor based on 1,000 bootstrap replicates of the regression model. We considered descriptors to differ significantly among prior temperatures if their 95% CIs did not overlap. Because this may be a conservative measure of differences between temperatures, we also calculated means and 95% CIs for pairwise comparisons of descriptors between temperatures (in this case, descriptors are significantly different if the 95% CI for their comparison excludes 0). Inferring significance this way gave similar results to inferring significance from overlapping intervals.

RESULTS

Modeling Thermal Performance Curves

The binomial mixed-effects regression model gave a good overall fit to the data, and detected a significant interaction between temperature at embryogenesis and thermal performance in terms of survival of planktonic development (**Figure 1**; **Table 1**). Linear, quadratic, and cubic trends in survival extracted from the model (**Figures 2A–C**), and compared between temperatures using Tukey-adjusted pairwise contrasts (**Figures 2D–F**), attributed this interaction to shifts in linear and cubic trends in survival. Linear trends (estimating the average slopes of curves in **Figure 1**) shifted from negative after embryogenesis at 18°C to positive after embryogenesis at 22°C (**Figure 2A**), and differed significantly between 18°C and both of the other temperatures (the contrast between 20 and 22°C was marginally non-significant at $p = 0.12$; **Figure 2D**). Cubic trends (estimating the degree to which slopes of curves in **Figure 1** are steeper or shallower initially) shifted from positive after embryogenesis at 18°C to negative after embryogenesis at 22°C (**Figure 2C**), capturing differences in curvature to the left of peaks in **Figure 1**. Again, trends in survival differed significantly between 18°C and both other temperatures (**Figure 2F**). Quadratic trends (estimating the concavity of curves in **Figure 1**) were consistently negative (**Figure 2B**) and did not differ between temperatures (**Figure 2E**). Temperature at embryogenesis did not affect curve height (peak survival), indicated by its non-significant main effect in **Table 1**.

Temperature at fertilization did not affect survival of planktonic development or thermal performance at this stage in any way (all effects involving it were non-significant; **Table 1**).

Estimates and CIs of Curve Descriptors

As suggested by linear and cubic trends relating survival to temperature above, estimates and CIs for curve descriptors (**Figure 3**) showed that thermal optima for survival of planktonic development shifted to track prior temperature at embryogenesis (**Figure 3B**). Specifically, the estimated thermal optimum after embryogenesis at 18°C increased by 1.4°C after embryogenesis at 20°C and by another 0.9°C after embryogenesis at 22°C, and CIs for estimates did not overlap between 18 and 22°C. Note that these results may be somewhat conservative, given our cubic model tended to underestimate the thermal optimum at 22°C (**Figure 1C**; **Supplementary Figure S1**). Peak performance, thermal breadth, and thermal limits were unaffected by temperature at embryogenesis (**Figures 3A,C–E**).

DISCUSSION

Linking thermal performance to prior thermal experience is necessary to better understand and predict organismal responses to climate change. For ectotherms with complex life cycles this

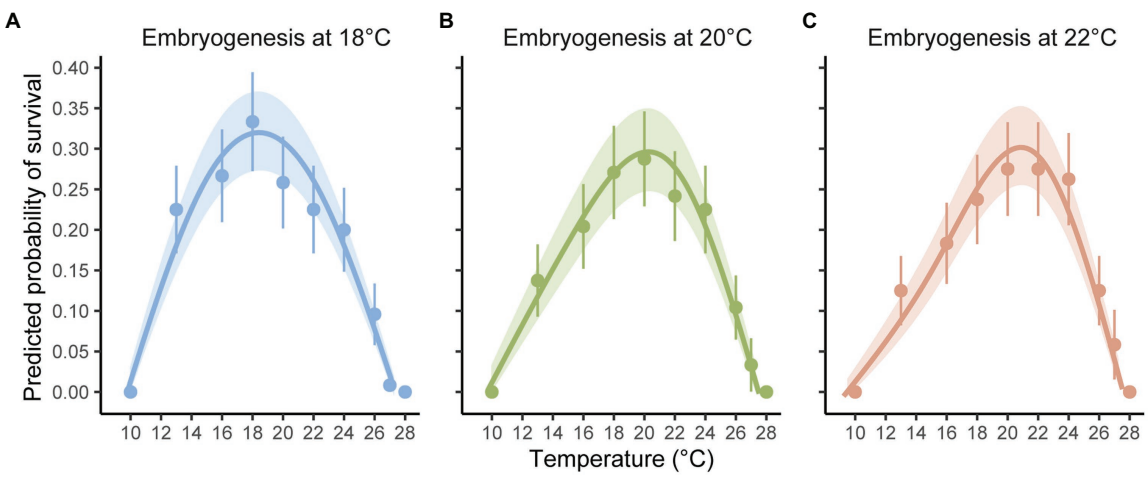

FIGURE 1 | Thermal performance curves showing the predicted probabilities of successfully surviving planktonic development after embryogenesis at **(A)** 18, **(B)** 20, and **(C)** 22°C. Points are observed success (mean and 95% CI) per temperature. Curves are predicted from a binomial mixed-effects regression of success on temperature, with shaded areas indicating 95% CIs of curve predictions. Temperature at embryogenesis was manipulated factorially with temperature at fertilization, but no effects of fertilization history on thermal performance were detected (see **Table 1**; **Supplementary Figure S1**).

TABLE 1 | Effects of prior temperatures at fertilization and embryogenesis on thermal performance in terms of survival at completion of planktonic development (modeled as a cubic function of temperature in a binomial mixed-effects regression model).

Fixed effects	X^2	d.f.	*p*
Temperature at fertilization	0.64	1	0.42
Temperature at embryogenesis	0.96	2	0.62
Temperature at fertilization × temperature at embryogenesis	1.30	2	0.53
Thermal performance at completion of planktonic development	460.35	3	**<0.001**
Temperature at fertilization × thermal performance	0.49	3	0.92
Temperature at embryogenesis × thermal performance	52.18	6	**<0.001**
Temperature at fertilization × temperature at embryogenesis × thermal performance	5.10	6	0.53

Significant effects are in bold. Linear, quadratic, and cubic trends in thermal performance are presented for prior temperatures at embryogenesis, and contrasted between temperatures, in ***Figure 2****.*

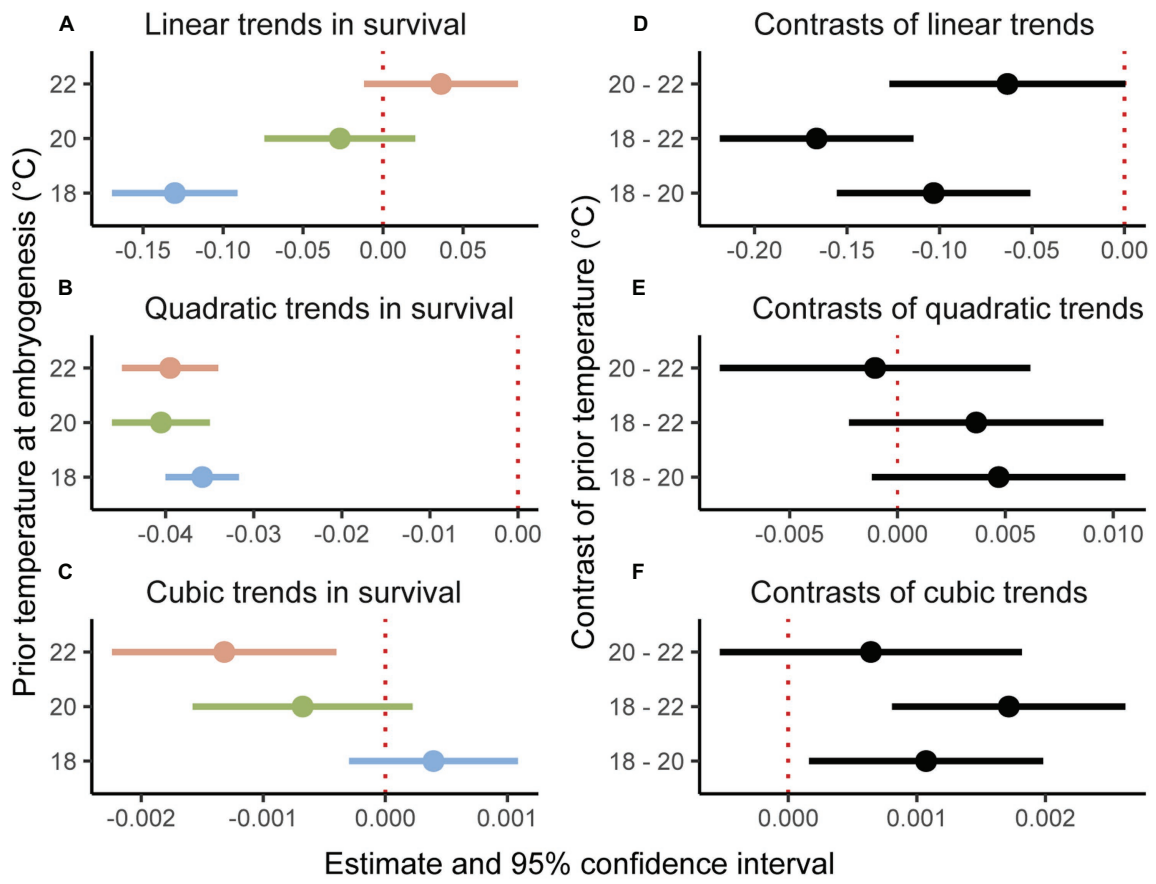

FIGURE 2 | Linear, quadratic, and cubic trends **(A–C)** of thermal performance curves in **Figure 1** contrasted **(D–F)** between prior temperatures at embryogenesis. Trends and contrasts differ significantly from 0 if their estimates have 95% credible intervals that exclude zero, marked by red dotted lines.

remains an ongoing challenge since life stages can differ in thermal sensitivity and experience different environmental conditions as development unfolds (Rebolledo et al., 2020). Seeing that life stages are by nature interconnected, environmental effects can carry over from one stage to affect performance at others (Arambourou et al., 2017; Lea et al., 2017; Ezeakacha and Yee, 2019). Thermal performance may therefore respond to carryover effects of prior thermal environments, yet detailed insights into the nature, strength, and direction of those responses are still lacking (Byrne et al., 2020). Here in *Galeolaria*, an aquatic ectotherm whose planktonic stages (gametes, embryos, and larvae) are considered most vulnerable to thermal stress (Pinsky et al., 2019; Walsh et al., 2019; Dahlke et al., 2020), we factorially manipulated temperatures at fertilization and embryogenesis, then, for each combination of prior temperatures, measured and compared thermal performance curves for survival at the end of planktonic development. Curves were unresponsive to temperature at fertilization, but temperature at embryogenesis caused shifts in

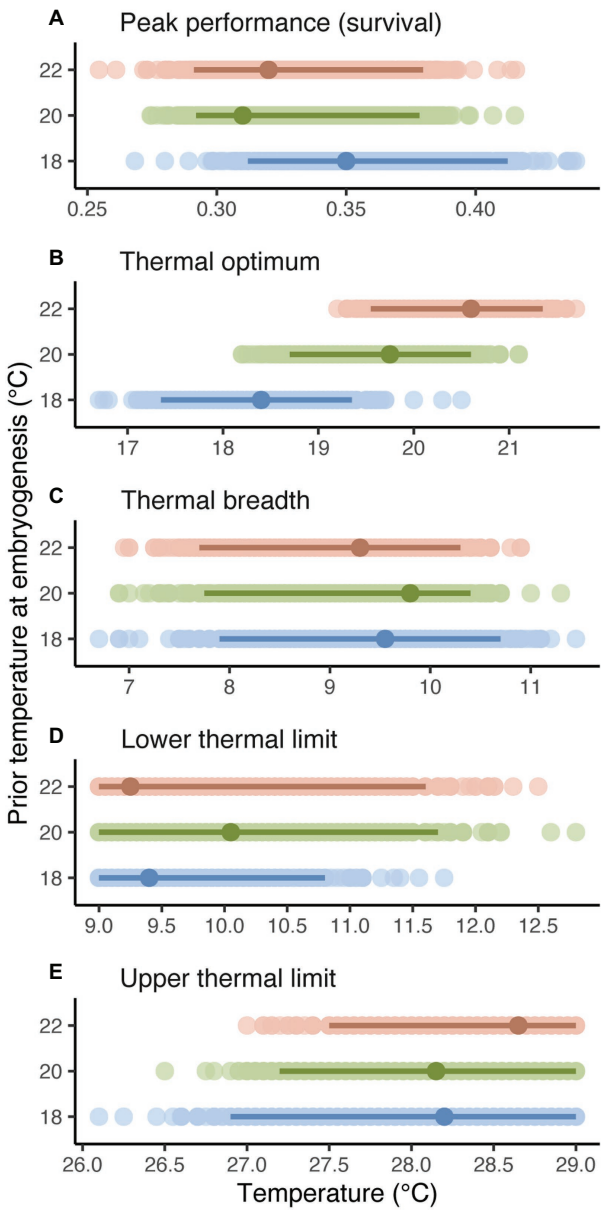

FIGURE 3 | Peak performance **(A)**, thermal optimum **(B)**, thermal breadth **(C)**, and thermal limits **(D,E)** for successful survival of planktonic development after embryogenesis at 18, 20, or 22°C (thermal performance was unaffected by fertilization at different temperatures before embryogenesis). Darker points and intervals are mean estimates and 95% CIs for curve descriptors, based on 1,000 bootstrap replicates (lighter points). See bootstrapping details in Materials and Methods.

larval thermal optima pointing to important carryover effects of thermal experience in this key window of development on survival.

Overall, the optimal temperature for completing 2–3 weeks of planktonic development tracked the temperature experienced in 24 h of embryogenesis beforehand, and did so without compromising peak performance (the proportion of larvae surviving development). To the extent that temperatures at embryogenesis and larval development match in nature, this carryover effect on thermal performance may increase individual fitness under modest levels of warming within the ~2–5°C range projected for the end the century (Hobday and Lough, 2011; Hobday and Pecl, 2014). Consequently, population viability may also be enhanced, since warming due to climate change is linked to changes in larval dispersal and recruitment that drive adult abundances and dynamics (Przeslawski et al., 2008). Enhanced population viability could further impact community structure, since *Galeolaria* is an ecosystem engineer that provides habitat for associated species (Wright and Gribben, 2017). Nevertheless, the extent to which prior thermal environment can buffer thermal performance in early life, and therefore have broader ecological impacts, seems to have its limitations, given that a 4°C increase in temperature at embryogenesis shifted the subsequent thermal optimum by only 2.2°C, and left thermal limits and breadth unchanged. Previous studies on terrestrial ectotherms have likewise reported limited scope for upper thermal limits of adults to increase in response to developmental temperature (Terblanche and Chown, 2006; Mitchell et al., 2011; Van Heerwaarden et al., 2016; Kellermann et al., 2017), although lower thermal limits tend to be more flexible (Araújo et al., 2013; Kingsolver and Buckley, 2020; Bennett et al., 2021). What exactly constrains thermal limits, and whether other descriptors of thermal performance are less constrained by comparison, remains actively debated (Schulte, 2015). Our results for *Galeolaria* show that the thermal optimum for planktonic development, at least, can respond to temperature at embryogenesis independently of other descriptors of thermal performance, and despite apparent constraints on upper limits.

Embryogenesis is the most formative life stage (Noble et al., 2018) and it is emerging as a critical threshold of thermal sensitivity in ectotherms whose embryos have no alternative but to develop in direct contact with the external environment (Van Der Have, 2002; Hamdoun and Epel, 2007; Begasse et al., 2015; Dahlke et al., 2020; Rebolledo et al., 2020). Consequently, carryover effects of temperature at this stage can be profound and persist across the life cycle (Watkins and Vraspir, 2006; Noble et al., 2018). The cellular mechanisms underlying such effects are poorly understood, but much attention has focused on inducible stress-response proteins that are differentially expressed in early life (Sørensen et al., 2003; Hammond and Hofmann, 2010; Burton and Metcalfe, 2014; Lockwood et al., 2017). Parents can load such proteins into waterborne gametes before release (Hamdoun and Epel, 2007; Hammond and Hofmann, 2010), potentially buffering gametes against direct thermal stress (Rebolledo et al., 2020) and explaining the lack of carryover effects of temperature at fertilization on thermal performance here. Embryos seem to downregulate these proteins when cell division is most active and overexpression is detrimental (Sørensen et al., 2003; Hamdoun and Epel, 2007), but shift to upregulation in response to thermal stress once cells start to differentiate and robust developmental pathways become vital (Leemans et al., 2000; Brown et al., 2004; Hamdoun and Epel, 2007; Hammond and Hofmann, 2010; Lockwood et al., 2017). Few studies, to our knowledge, have explicitly linked the induction of stress-response proteins at one life stage to carryover effects on thermal performance at others (Boon-Niermeyer et al., 1988; Hammond and Hofmann, 2010), but this is one mechanism by which prior exposure to stress may enhance performance under

future stress (Sørensen et al., 2003) and a plausible reason why higher temperatures at embryogenesis might prime larvae to have higher thermal optima here.

Whatever the underlying mechanism, carryover effects in early life are widely attributed to developmental plasticity – that is, changes in gene expression triggered by environmental cues at development and often interpreted as epigenetic in origin (Beldade et al., 2011; Beaman et al., 2016; Bonamour et al., 2019). Developmental plasticity can be adaptive if it enhances later fitness in the environment that triggered it, but can also be nonadaptive or maladaptive if, for example, cues are unpredictable, or organisms cannot sense and respond to cues fast enough for effective environmental matching (Beaman et al., 2016; Bonamour et al., 2019). Despite ongoing interest in thermal developmental plasticity as a means for ectotherms to cope with climate change (Sgrò et al., 2016; Donelson et al., 2018; Noble et al., 2018; Carter and Sheldon, 2020; Rodrigues and Beldade, 2020), evidence for adaptive plasticity in thermal performance triggered by temperature at embryogenesis rests primarily on physiological measures of performance (e.g., Scott and Johnston, 2012; Seebacher and Grigaltchik, 2014; Refsnider et al., 2019), while measures with closer links to fitness (survival and reproduction) are less studied. Here in *Galeolaria*, enhanced survival at temperatures experienced at embryogenesis appears to be broadly consistent with adaptive developmental plasticity, but also raises the prospect of viability selection as an alternative or added explanation.

In ectotherms with complex life cycles, episodes of selection in early life can potentially combine to shape genetic composition at later stages, allowing carryover effects to have fitness outcomes not purely driven by plasticity (Moore and Martin, 2019). This may be especially likely for external fertilizers like *Galeolaria*, which produce numerous propagules with high intrinsic mortality at successive planktonic stages, in addition to direct exposure to environmental stressors (Foo and Byrne, 2016; Chirgwin et al., 2020; Crean and Immler, 2021). It is therefore possible that our manipulations of temperature at fertilization and embryogenesis screened each stage by differential survival at different temperatures, and that subsequent increases in thermal optima for survival reflect shifts in allele frequencies, not just expression, driven by directional selection. *Galeolaria* may have limited scope to respond evolutionarily, however, based on recent evidence that genetic variation for survival to independence (capacity to swim and feed, overlapping our performance measure here) is negligible after fertilization and embryogenesis at 24°C (Chirgwin et al., 2021). Disentangling selection and developmental plasticity as candidate drivers of carryover effects is notoriously hard to do experimentally, and may ultimately require genomic approaches (Donelson et al., 2018; Fox et al., 2019). In the meantime, we cannot be certain whether plasticity or selection, or both drivers in combination, underpin the carryover effects on thermal optima detected here.

Overall, our work reveals carryover effects of temperature at embryogenesis (but not fertilization) on thermal performance in early life that may buffer vulnerable planktonic stages of aquatic ectotherms against climate change, and offers new insights into the responses of thermal performance curves to thermal history. In particular, curve descriptors did not respond to temperature in the coordinated manner predicted by hypotheses based on thermodynamic constraints – that is, higher thermal optimum did not coincide with higher peak performance (as suggested by the "hotter-is-better" hypothesis; Huey and Kingsolver, 1989; Angilletta et al., 2010; Sørensen et al., 2018), or with horizontal shifts in thermal limits (as suggested by the "hotter-colder" hypothesis; Izem and Kingsolver, 2005; Angilletta, 2009). Of similar "rules" (or variants on them) invoked to explain how curves respond to prior thermal experience (Huey et al., 1999; Deere and Chown, 2006), our results seem most consistent with an interpretation of the beneficial acclimation hypothesis that assumes no covariation between thermal optimum and peak performance. This is termed temperature compensation (partial or complete maintenance of physiological rates in the face of changing temperature) and may be the combined outcome of thermal adaptation and thermodynamic constraints (Clarke, 2003). Such an interpretation is of course speculative at this point. Our results do, however, add to mounting evidence pointing to embryogenesis as the most critical of early life stages in aquatic ectotherms, not only for the emergence of thermal sensitivity (Dahlke et al., 2020; Rebolledo et al., 2020; Collin et al., 2021), but also of thermal carryover effects. Although, our results suggest that fertilization matters less in this regard, the possibility remains that the environment at gametogenesis is more influential than the environment at fertilization, emphasising the need to better understand transgenerational effects on thermal performance. Further research is therefore needed to elucidate how parental and developmental environments interact to shape thermal performance in organisms with complex life cycles, and thereby gain a clearer picture of organismal responses and vulnerability to current and future climatic conditions.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

AR, CS, and KM conceived and designed the study. AR collected the data. AR and KM performed analyses and drafted the manuscript. KM created the graphics. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphys.2021.738338/full#supplementary-material

Supplementary Figure S1 | Proportions of individuals that successfully survived planktonic development after fertilization at 18 or 22°C and embryogenesis at 18, 20, or 22°C. Points are mean success (with bars indicating 95% CIs) and curves are locally-weighted smoothers (with shaded areas indicating 95% CIs) that assume no particular shape.

REFERENCES

Angilletta, M. J. (2009). *Thermal Adaptation: A Theoretical and Empirical Synthesis*. Oxford: Oxford University Press.

Angilletta, M. J., Huey, R. B., and Frazier, M. R. (2010). Thermodynamic effects on organismal performance: is hotter better? *Physiol. Biochem. Zool.* 83, 197–206. doi: 10.1086/648567

Arambourou, H., Sanmartín-Villar, I., and Stoks, R. (2017). Wing shape-mediated carry-over effects of a heat wave during the larval stage on post-metamorphic locomotor ability. *Oecologia* 184, 279–291. doi: 10.1007/s00442-017-3846-z

Araújo, M. B., Ferri-Yáñez, F., Bozinovic, F., Marquet, P. A., Valladares, F., and Chown, S. L. (2013). Heat freezes niche evolution. *Ecol. Lett.* 16, 1206–1219. doi: 10.1111/ele.12155

Bates, D., Maechler, M., Bolker, B., and Walker, S. (2015). Fitting linear mixed-effects models using lme4. *J. Stat. Softw.* 67, 1–48. doi: 10.18637/jss.v067.i01

Beaman, J. E., White, C. R., and Seebacher, F. (2016). Evolution of plasticity: mechanistic link between development and reversible acclimation. *Trends Ecol. Evol.* 31, 237–249. doi: 10.1016/j.tree.2016.01.004

Begasse, M. L., Leaver, M., Vazquez, F., Grill, S. W., and Hyman, A. A. (2015). Temperature dependence of cell division timing accounts for a shift in the thermal limits of *C. elegans* and *C. briggsae*. *Cell Rep.* 10, 647–653. doi: 10.1016/j.celrep.2015.01.006

Beldade, P., Mateus, A. R. A., and Keller, R. A. (2011). Evolution and molecular mechanisms of adaptive developmental plasticity. *Mol. Ecol.* 20, 1347–1363. doi: 10.1111/j.1365-294X.2011.05016.x

Bennett, J. M., Sunday, J., Calosi, P., Villalobos, F., Martínez, B., Molina-Venegas, R., et al. (2021). The evolution of critical thermal limits of life on earth. *Nat. Commun.* 12, 1–9. doi: 10.1038/s41467-021-21263-8

Bolker, B. M., Brooks, M. E., Clark, C. J., Geange, S. W., Poulsen, J. R., Stevens, M. H. H., et al. (2009). Generalized linear mixed models: a practical guide for ecology and evolution. *Trends Ecol. Evol.* 24, 127–135. doi: 10.1016/j.tree.2008.10.008

Bonamour, S., Chevin, L.-M., Charmantier, A., and Teplitsky, C. (2019). Phenotypic plasticity in response to climate change: the importance of cue variation. *Philos. Trans. R. Soc. B* 374:20180178. doi: 10.1098/rstb.2018.0178

Boon-Niermeyer, E. K., De Waal, A. M., Souren, J. E. M., and Van Wijk, R. (1988). Heat-induced changes in thermosensitivity and gene expression during development: (embryonic development/thermosensitivity/thermotolerance/heat shock response). *Develop. Growth Differ.* 30, 705–715.

Brahim, A., Mustapha, N., and Marshall, D. J. (2019). Non-reversible and reversible heat tolerance plasticity in tropical intertidal animals: responding to habitat temperature heterogeneity. *Front. Physiol.* 9:1909. doi: 10.3389/fphys.2018.01909

Brown, H. M., Briden, A., Stokell, T., Griffin, F. J., and Cherr, G. N. (2004). Thermotolerance and Hsp70 profiles in adult and embryonic California native oysters, Ostreola conchaphila (carpenter, 1857). *J. Shellfish Res.* 23, 135–142.

Burton, T., and Metcalfe, N. B. (2014). Can environmental conditions experienced in early life influence future generations? *Proc. R. Soc. B Biol. Sci.* 281:20140311. doi: 10.1098/rspb.2014.0311

Byrne, M. (2011). Impact of ocean warming and ocean acidification on marine invertebrate life history stages: vulnerabilities and potential for persistence in a changing ocean. *Oceanogr. Mar. Biol. Annu. Rev.* 20115434, 1–42. doi: 10.1201/b11009-2

Byrne, M., Foo, S. A., Ross, P. M., and Putnam, H. M. (2020). Limitations of cross-and multigenerational plasticity for marine invertebrates faced with global climate change. *Glob. Chang. Biol.* 26, 80–102. doi: 10.1111/gcb.14882

Campbell, H., Ledet, J., Poore, A. G., and Byrne, M. (2020). Thermal tolerance in the amphipod Sunamphitoe parmerong from a global warming hotspot, acclimatory carryover effects within generation. *Mar. Environ. Res.* 160:105048. doi: 10.1016/j.marenvres.2020.105048

Canty, A., and Ripley, B. (2021). Boot: Bootstrap R (S-Plus) Functions. R package version 1.3-28.

Carter, A. W., and Sheldon, K. S. (2020). Life stages differ in plasticity to temperature fluctuations and uniquely contribute to adult phenotype in *Onthophagus taurus* dung beetles. *J. Exp. Biol.* 223:jeb227884. doi: 10.1242/jeb.227884

Chirgwin, E., Connallon, T., and Monro, K. (2021). The thermal environment at fertilization mediates adaptive potential in the sea. *Evol. Lett.* 5, 154–163. doi: 10.1002/evl3.215

Chirgwin, E., Marshall, D. J., and Monro, K. (2020). Physical and physiological impacts of ocean warming alter phenotypic selection on sperm morphology. *Funct. Ecol.* 34, 646–657. doi: 10.1111/1365-2435.13483

Chirgwin, E., Marshall, D. J., Sgrò, C. M., and Monro, K. (2017). The other 96%: can neglected sources of fitness variation offer new insights into adaptation to global change? *Evol. Appl.* 10, 267–275. doi: 10.1111/eva.12447

Chirgwin, E., Marshall, D. J., Sgrò, C. M., and Monro, K. (2018). How does parental environment influence the potential for adaptation to global change? *Proc. R. Soc. B Biol. Sci.* 285:20181374. doi: 10.1098/rspb.2018.1374

Clarke, A. (2003). Costs and consequences of evolutionary temperature adaptation. *Trends Ecol. Evol.* 18, 573–581. doi: 10.1016/j.tree.2003.08.007

Collin, R., Rebolledo, A. P., Smith, E., and Chan, K. Y. K. (2021). Thermal tolerance of early development predicts the realized thermal niche in marine Ectotherms. *Funct. Ecol.* 35, 1679–1692. doi: 10.1111/1365-2435.13850

Crean, A. J., and Immler, S. (2021). Evolutionary consequences of environmental effects on gamete performance. *Philos. Trans. R. Soc. Lond. Ser. B Biol. Sci.* 376:20200122. doi: 10.1098/rstb.2020.0122

Dahlke, F. T., Wohlrab, S., Butzin, M., and Pörtner, H.-O. (2020). Thermal bottlenecks in the life cycle define climate vulnerability of fish. *Science* 369, 65–70. doi: 10.1126/science.aaz3658

Deere, J. A., and Chown, S. L. (2006). Testing the beneficial acclimation hypothesis and its alternatives for locomotor performance. *Am. Nat.* 168, 630–644. doi: 10.1086/508026

Deutsch, C. A., Tewksbury, J. J., Huey, R. B., Sheldon, K. S., Ghalambor, C. K., Haak, D. C., et al. (2008). Impacts of climate warming on terrestrial ectotherms across latitude. *Proc. Natl. Acad. Sci. U. S. A.* 105, 6668–6672. doi: 10.1073/pnas.0709472105

Donelson, J. M., Salinas, S., Munday, P. L., and Shama, L. N. (2018). Transgenerational plasticity and climate change experiments: where do we go from here? *Glob. Chang. Biol.* 24, 13–34. doi: 10.1111/gcb.13903

Dupont, S., Dorey, N., Stumpp, M., Melzner, F., and Thorndyke, M. (2013). Long-term and trans-life-cycle effects of exposure to ocean acidification in the green sea urchin *Strongylocentrotus droebachiensis*. *Mar. Biol.* 160, 1835–1843. doi: 10.1007/s00227-012-1921-x

Ezeakacha, N. F., and Yee, D. A. (2019). The role of temperature in affecting carry-over effects and larval competition in the globally invasive mosquito *Aedes albopictus*. *Parasit. Vectors* 12, 1–11. doi: 10.1186/s13071-019-3391-1

Foo, S. A., and Byrne, M. (2016). Acclimatization and adaptive capacity of marine species in a changing ocean. *Adv. Mar. Biol.* 74, 69–116. doi: 10.1016/bs.amb.2016.06.001

Fox, R. J., Donelson, J. M., Schunter, C., Ravasi, T., and Gaitán-Espitia, J. D. (2019). Beyond buying time: the role of plasticity in phenotypic adaptation to rapid environmental change. *Philos. Trans. R. Soc. B Biol. Sci.* 374:20180174. doi: 10.1098/rstb.2018.0174

Fox, J., and Weisberg, S. (2019). *An R Companion to Applied Regression*. *3rd Edn*. Thousand Oaks CA: Sage.

Freda, P. J., Alex, J. T., Morgan, T. J., and Ragland, G. J. (2017). Genetic decoupling of thermal hardiness across metamorphosis in *Drosophila melanogaster*. *Integr. Comp. Biol.* 57, 999–1009. doi: 10.1093/icb/icx102

Hamdoun, A., and Epel, D. (2007). Embryo stability and vulnerability in an always changing world. *Proc. Natl. Acad. Sci. U. S. A.* 104, 1745–1750. doi: 10.1073/pnas.0610108104

Hammond, L. M., and Hofmann, G. E. (2010). Thermal tolerance of *Strongylocentrotus purpuratus* early life history stages: mortality, stress-induced gene expression and biogeographic patterns. *Mar. Biol.* 157, 2677–2687. doi: 10.1007/s00227-010-1528-z

Hartig, F. (2021). DHARMa: residual diagnostics for hierarchical (Multi-Level/ Mixed) regression models. The Comprehensive R Archive Network (CRAN), R package version 0.4.1.

Hobday, A. J., and Lough, J. M. (2011). Projected climate change in Australian marine and freshwater environments. *Mar. Freshw. Res.* 62, 1000–1014. doi: 10.1071/MF10302

Hobday, A. J., and Pecl, G. T. (2014). Identification of global marine hotspots: sentinels for change and vanguards for adaptation action. *Rev. Fish Biol. Fish.* 24, 415–425. doi: 10.1007/s11160-013-9326-6

Huey, R. B., Berrigan, D., Gilchrist, G. W., and Herron, J. C. (1999). Testing the adaptive significance of acclimation: a strong inference approach. *Am. Zool.* 39, 323–336. doi: 10.1093/icb/39.2.323

Huey, R. B., and Kingsolver, J. G. (1989). Evolution of thermal sensitivity of ectotherm performance. *Trends Ecol. Evol.* 4, 131–135. doi: 10.1016/0169-5347(89)90211-5

Izem, R., and Kingsolver, J. G. (2005). Variation in continuous reaction norms: quantifying directions of biological interest. *Am. Nat.* 166, 277–289. doi: 10.1086/431314

Jonsson, B., and Jonsson, N. (2014). Early environment influences later performance in fishes. *J. Fish Biol.* 85, 151–188. doi: 10.1111/jfb.12432

Kellermann, V., Chown, S. L., Schou, M. F., Aitkenhead, I., Janion-Scheepers, C., Clemson, A., et al. (2019). Comparing thermal performance curves across traits: how consistent are they? *J. Exp. Biol.* 222:jeb193433. doi: 10.1242/jeb.193433

Kellermann, V., Van Heerwaarden, B., and Sgrò, C. M. (2017). How important is thermal history? Evidence for lasting effects of developmental temperature on upper thermal limits in *Drosophila melanogaster*. *Proc. R. Soc. B Biol. Sci. U. S. A.* 284:20170447. doi: 10.1098/rspb.2017.0447

Kingsolver, J. G., Arthur Woods, H., Buckley, L. B., Potter, K. A., Maclean, H. J., and Higgins, J. K. (2011). Complex life cycles and the responses of insects to climate change. *Integr. Comp. Biol.* 51, 719–732. doi: 10.1093/icb/icr015

Kingsolver, J. G., and Buckley, L. B. (2020). Ontogenetic variation in thermal sensitivity shapes insect ecological responses to climate change. *Curr. Opin. Insect Sci.* 41, 17–24. doi: 10.1016/j.cois.2020.05.005

Kupriyanova, E. K. (2006). Fertilization success in Galeolaria caespitosa (Polychaeta: Serpulidae): gamete characteristics, role of sperm dilution, gamete age, and contact time. *Sci. Mar.* 70, 309–317. doi: 10.3989/scimar.2006.70s3309

Lea, A. J., Tung, J., Archie, E. A., and Alberts, S. C. (2017). Developmental plasticity: bridging research in evolution and human health. *Evol. Med. Public Health* 2017, 162–175. doi: 10.1093/emph/eox019

Leemans, R., Egger, B., Loop, T., Kammermeier, L., He, H., Hartmann, B., et al. (2000). Quantitative transcript imaging in normal and heat-shocked Drosophila embryos by using high-density oligonucleotide arrays. *Proc. Natl. Acad. Sci. U. S. A.* 97, 12138–12143. doi: 10.1073/pnas.210066997

Lenth, R., Singmann, H., Love, J., Buerkner, P., and Herve, M. (2021). Emmeans: Estimated Marginal Means. R Package Version 1.6.0.

Lockwood, B. L., Julick, C. R., and Montooth, K. L. (2017). Maternal loading of a small heat shock protein increases embryo thermal tolerance in *Drosophila melanogaster*. *J. Exp. Biol.* 220, 4492–4501. doi: 10.1242/jeb.164848

Loeschcke, V., and Hoffmann, A. A. (2002). The detrimental acclimation hypothesis. *Trends Ecol. Evol.* 17, 407–408. doi: 10.1016/S0169-5347(02)02555-7

Marsden, J., and Anderson, D. (1981). Larval development and metamorphosis of the serpulid polychaete Galeolaria caespitosa Lamarck. *Mar. Freshw. Res.* 32, 667–680. doi: 10.1071/MF9810667

Marshall, D. J., and Evans, J. P. (2005). The benefits of polyandry in the free-spawning polychaete *Galeolaria caespitosa*. *J. Evol. Biol.* 18, 735–741. doi: 10.1111/j.1420-9101.2004.00873.x

Mitchell, K. A., Sgrò, C. M., and Hoffmann, A. A. (2011). Phenotypic plasticity in upper thermal limits is weakly related to *Drosophila* species distributions. *Funct. Ecol.* 25, 661–670. doi: 10.1111/j.1365-2435.2010.01821.x

Moore, M. P., and Martin, R. A. (2019). On the evolution of carry-over effects. *J. Anim. Ecol.* 88, 1832–1844. doi: 10.1111/1365-2656.13081

Noble, D. W., Stenhouse, V., and Schwanz, L. E. (2018). Developmental temperatures and phenotypic plasticity in reptiles: a systematic review and meta-analysis. *Biol. Rev.* 93, 72–97. doi: 10.1111/brv.12333

Pandori, L. L. M., and Sorte, C. J. B. (2019). The weakest link: sensitivity to climate extremes across life stages of marine invertebrates. *Oikos* 128, 621–629. doi: 10.1111/oik.05886

Pinsky, M. L., Eikeset, A. M., McCauley, D. J., Payne, J. L., and Sunday, J. M. (2019). Greater vulnerability to warming of marine versus terrestrial ectotherms. *Nature* 569, 108–111. doi: 10.1038/s41586-019-1132-4

Przeslawski, R., Ahyong, S., Byrne, M., Wörheide, G., and Hutchings, P. (2008). Beyond corals and fish: the effects of climate change on noncoral benthic invertebrates of tropical reefs. *Glob. Chang. Biol.* 14, 2773–2795. doi: 10.1111/j.1365-2486.2008.01693.x

Rebolledo, A. P., Sgrò, C. M., and Monro, K. (2020). Thermal performance curves reveal shifts in optima, limits and breadth in early life. *J. Exp. Biol.* 223:jeb233254. doi: 10.1242/jeb.233254

Refsnider, J. M., Clifton, I. T., and Vazquez, T. K. (2019). Developmental plasticity of thermal ecology traits in reptiles: trends, potential benefits, and research needs. *J. Therm. Biol.* 84, 74–82. doi: 10.1016/j.jtherbio.2019.06.005

Rodrigues, Y. K., and Beldade, P. (2020). Thermal plasticity in insects' response to climate change and to multifactorial environments. *Front. Ecol. Evol.* 8:271. doi: 10.3389/fevo.2020.00271

Sanger, T. J., Kyrkos, J., Lachance, D. J., Czesny, B., and Stroud, J. T. (2018). The effects of thermal stress on the early development of the lizard Anolis sagrei. *J. Exp. Zool. A Ecol. Integr. Physiol.* 329, 244–251. doi: 10.1002/jez.2185

Schulte, P. M. (2015). The effects of temperature on aerobic metabolism: towards a mechanistic understanding of the responses of ectotherms to a changing environment. *J. Exp. Biol.* 218, 1856–1866. doi: 10.1242/jeb.118851

Scott, G. R., and Johnston, I. A. (2012). Temperature during embryonic development has persistent effects on thermal acclimation capacity in zebrafish. *Proc. Natl. Acad. Sci. U. S. A.* 109, 14247–14252. doi: 10.1073/pnas.1205012109

Seebacher, F., and Grigaltchik, V. S. (2014). Embryonic developmental temperatures modulate thermal acclimation of performance curves in tadpoles of the frog *Limnodynastes peronii*. *PLoS One* 9:e106492. doi: 10.1371/journal.pone.0106492

Seebacher, F., White, C. R., and Franklin, C. E. (2015). Physiological plasticity increases resilience of ectothermic animals to climate change. *Nat. Clim. Chang.* 5, 61–66. doi: 10.1038/nclimate2457

Sgrò, C. M., Terblanche, J. S., and Hoffmann, A. A. (2016). What can plasticity contribute to insect responses to climate change? *Annu. Rev. Entomol.* 61, 433–451. doi: 10.1146/annurev-ento-010715-023859

Sinclair, B. J., Marshall, K. E., Sewell, M. A., Levesque, D. L., Willett, C. S., Slotsbo, S., et al. (2016). Can we predict ectotherm responses to climate change using thermal performance curves and body temperatures? *Ecol. Lett.* 19, 1372–1385. doi: 10.1111/ele.12686

Sørensen, J. G., Kristensen, T. N., and Loeschcke, V. (2003). The evolutionary and ecological role of heat shock proteins. *Ecol. Lett.* 6, 1025–1037. doi: 10.1046/j.1461-0248.2003.00528.x

Sørensen, J. G., Kristensen, T. N., and Overgaard, J. (2016). Evolutionary and ecological patterns of thermal acclimation capacity in *Drosophila*: is it important for keeping up with climate change? *Curr. Opin. Insect Sci.* 17, 98–104. doi: 10.1016/j.cois.2016.08.003

Sørensen, J. G., White, C. R., Duffy, G. A., and Chown, S. L. (2018). A widespread thermodynamic effect, but maintenance of biological rates through space across life's major domains. *Proc. Biol. Sci.* 285:20181775. doi: 10.1098/rspb.2018.1775

Terblanche, J. S., and Chown, S. L. (2006). The relative contributions of developmental plasticity and adult acclimation to physiological variation in the tsetse fly, *Glossina pallidipes* (Diptera, Glossinidae). *J. Exp. Biol.* 209, 1064–1073. doi: 10.1242/jeb.02129

Truebano, M., Fenner, P., Tills, O., Rundle, S. D., and Rezende, E. L. (2018). Thermal strategies vary with life history stage. *J. Exp. Biol.* 221:jeb171629. doi: 10.1242/jeb.171629

Van Der Have, T. (2002). A proximate model for thermal tolerance in ectotherms. *Oikos* 98, 141–155. doi: 10.1034/j.1600-0706.2002.980115.x

Van Heerwaarden, B., and Kellermann, V. (2020). Does plasticity trade off with basal heat tolerance? *Trends Ecol. Evol.* 35, 874–885. doi: 10.1016/j.tree.2020.05.006

Van Heerwaarden, B., Kellermann, V., and Sgrò, C. M. (2016). Limited scope for plasticity to increase upper thermal limits. *Funct. Ecol.* 30, 1947–1956. doi: 10.1111/1365-2435.12687

Van Heerwaarden, B., and Sgrò, C. M. (2021). Male fertility thermal limits predict vulnerability to climate warming. *Nat. Commun.* 12:2214. doi: 10.1038/s41467-021-22546-w

Walsh, B., Parratt, S., Hoffmann, A., Atkinson, D., Snook, R. R., Bretman, A., et al. (2019). The impact of climate change on fertility. *Trends Ecol. Evol.* 34, 249–259. doi: 10.1016/j.tree.2018.12.002

Watkins, T. B., and Vraspir, J. (2006). Both incubation temperature and posthatching temperature affect swimming performance and morphology of wood frog tadpoles (Rana sylvatica). *Physiol. Biochem. Zool.* 79, 140–149. doi: 10.1086/498182

Wilson, R. S., and Franklin, C. E. (2002). Testing the beneficial acclimation hypothesis. *Trends Ecol. Evol.* 17, 66–70. doi: 10.1016/S0169-5347(01)02384-9

Wright, J. T., and Gribben, P. E. (2017). Disturbance-mediated facilitation by an intertidal ecosystem engineer. *Ecology* 98, 2425–2436. doi: 10.1002/ecy.1932

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High-Elevation Populations of Montane Grasshoppers Exhibit Greater Developmental Plasticity in Response to Seasonal Cues

Julia M. Smith[1†], Rory S. Telemeco[1,2†], Bryan A. Briones Ortiz[1,3], César R. Nufio[4,5] and Lauren B. Buckley[1]*

[1] Department of Biology, University of Washington, Seattle, WA, United States, [2] Department of Biology, California State University, Fresno, Fresno, CA, United States, [3] School of Aquatic and Fishery Sciences, University of Washington, Seattle, WA, United States, [4] Howard Hughes Medical Institute, Chevy Chase, VA, United States, [5] University of Colorado Museum of Natural History, University of Colorado, Boulder, Boulder, CO, United States

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***Correspondence:**
Rory S. Telemeco
telemeco@csufresno.edu

† These authors have contributed equally to this work and share first authorship

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Populations of insects can differ in how sensitive their development, growth, and performance are to environmental conditions such as temperature and daylength. The environmental sensitivity of development can alter phenology (seasonal timing) and ecology. Warming accelerates development of most populations. However, high-elevation and season-limited populations can exhibit developmental plasticity to either advance or prolong development depending on conditions. We examine how diurnal temperature variation and daylength interact to shape growth, development, and performance of several populations of the montane grasshopper, *Melanoplus boulderensis,* along an elevation gradient. We then compare these experimental results to observed patterns of development in the field. Although populations exhibited similar thermal sensitivities of development under long-day conditions, development of high-elevation populations was more sensitive to temperature under short-day conditions. This developmental plasticity resulted in rapid development of high elevation populations in short-day conditions with high temperature variability, consistent with their observed capacity for rapid development in the field when conditions are permissive early in the season. Notably, accelerated development generally did not decrease body size or alter body shape. Developmental conditions did not strongly influence thermal tolerance but altered the temperature dependence of performance in difficult-to-predict ways. In sum, the high-elevation and season-limited populations exhibited developmental plasticity that enables advancing or prolonging development consistent with field phenology. Our results suggest these patterns are driven by the thermal sensitivity of development increasing when days are short early in the season compared to when days are long later in the season. Developmental plasticity will shape phenological responses to climate change with potential implications for community and ecosystem structure.

Keywords: climate change, development, physiology, temperature-size rule, thermal sensitivity

INTRODUCTION

Recent climate warming has advanced phenology including reproduction and adult emergence in a large (∼80%, Parmesan, 2006) proportion of species, but the fitness consequences of these phenological shifts are often unclear (Forrest and Miller-Rushing, 2010). Laboratory rearing experiments reveal developmental plasticity in growth and development rates in response to environmental cues (Atkinson, 1996; West-Eberhard, 2003). Developmental plasticity varies as a function of life-history strategy and the environment to which populations are adapted (Forrest, 2016). High temperatures generally reduce development time and decrease adult size in insects and other ectotherms due to development being more thermally sensitive than growth (Bale et al., 2002; Sgro et al., 2016). However, short growing seasons at high latitudes or high elevations can drive a reversal of this temperature-size rule, such that higher rearing temperatures produce larger adult size (Dingle et al., 1990; Hodkinson, 2005; Berner and Blanckenhorn, 2006). What are the implications of these growth and developmental tradeoffs for phenological shifts observed in the field? The combination of greater warming and greater thermal sensitivity of development for high elevation populations may flatten phenological gradients occurring along elevational clines (Chmura et al., 2018; Vitasse et al., 2018; Nufio and Buckley, 2019).

We examine how growth and development rates of montane grasshopper populations respond to seasonal cues- diurnal temperature variation and daylength- as well as how these changes affect adult performance. We then interpret our experimental, laboratory observations in light of historical, and recent phenology observed for the grasshopper populations in the field. We focus on grasshopper populations spanning a 1,500 m elevation gradient along the 40th N parallel in Boulder County, CO, United States. Weekly survey data from the Gordon Alexander Project reveal that the first appearance of adults has generally advanced between initial surveys in 1959–1960 and resurveys conducted since 2006 (Nufio et al., 2010). Phenological advancements and phenological variation across elevation were most pronounced for early-season species, such as our focal species (Nufio and Buckley, 2019). Progression through juvenile developmental stages has also generally advanced, but development proceeds more slowly and developmental stages persist longer in warm conditions for the high-elevation, season-limited populations of early-season species (Buckley et al., 2015; Nufio and Buckley, 2019). Broader phenology can increase overlap among species with potential implications for interactions in communities (Buckley et al., 2021).

Under controlled laboratory conditions, we further test the hypothesis that higher elevation populations exhibit greater developmental plasticity due to occupying more variable, season-limited environments. Daylength often cues developmental rate when conditions are permissive in time constrained environments (Bradshaw and Holzapfel, 2007). We build on a previous study for a generalist, late-season grasshopper species, *M. sanguinipes* (Buckley et al., 2015). Higher-elevation *M. sanguinipes* populations displayed greater phenotypic plasticity in development rate in response to developmental temperature than lower-elevation populations (Buckley et al., 2015). This resulted in higher-elevation populations advancing their phenology more than lower-elevation populations in warm conditions (Buckley et al., 2015). Studies on univoltine, high-latitude damselflies found that short daylengths consistent with seasonal time limitations accelerated development (Sniegula et al., 2016; Norling, 2018). However, they found less, rather than our hypothesized more, developmental plasticity among high-latitude populations (Sniegula et al., 2016).

Here we examine how the development plasticity of an early-season species, *Melanoplus boulderensis*, responds to naturalistic variation in both temperature and daylength. We intended these factors as naturalistic seasonal cues but note that greater temperature variability also exposes grasshoppers to higher temperatures and fixed daylengths throughout development would not be experienced in nature. *M. boulderensis* has a restricted, montane distribution (Otte, 2012) and is cool adapted (Buckley et al., 2013b; Buckley and Nufio, 2014). Dispersal ability is limited by short wings and the species exhibits genetic differentiation along the elevation gradient (Slatyer et al., 2020). The species is univoltine, which excludes phenological advancements from increasing fitness by adding an additional seasonal generation.

We assess growth and development responses of three elevationally distinct populations to temperature variability and daylength. In addition to the hypothesis of greater developmental plasticity for high-elevation populations, we hypothesized that long-day conditions experienced at later developmental stages, indicative of a release from seasonal time constraints, would slow development. To further assess the fitness consequences of growth and development, we assess plasticity in thermal sensitivity and the temperature dependence of performance resulting from rearing conditions. These data allow us to test for potential trade-offs between accelerated development and performance, as well as for potential matching of performance to developmental conditions. For example, we might expect grasshoppers that rapidly develop to incur tradeoffs that reduce their peak performance capacity as adults, or for grasshoppers that develop under more variable temperatures to display more generalist thermal performance. Finally, we review field phenology across the elevation gradient in light of our findings on how temperature and daylength influence development rates.

MATERIALS AND METHODS

Rearing Experiments

We examined development rates among *M. boulderensis* populations inhabiting three montane or subalpine sites along the 40th N parallel in Boulder County, CO: A1 (2,195 m, 40.01N, 105.37W), B1 (2,591 m, 40.02N, 105.43W), C1 (3,048 m, 40.03N, 105.55W) (descriptions[1]). The sites are all grassy meadows, with somewhat denser vegetation at the lower-elevation sites. *M. boulderensis* overwinters in an egg diapause with eggs deposited in pods just below the soil surface. The species is

[1]https://nwt.lternet.edu/explore-the-ridge

a generalist consumer of forbs so vegetation phenology is not expected to substantially constrain its phenology.

Eggs were collected by allowing individual, labeled females, collected from the three field sites in mid-summer, to oviposit in damp sand, and then sieving the sand. Eggs were stored in damp vermiculite within 2oz polyurethane containers. The surface was periodically coated with 0.25% methyl-p-hydroxy benzoate to inhibit fungal or microbial growth. The eggs first developed for 3 weeks in incubators at near ambient conditions (25–30°C), which is required to enable an obligate diapause (Dingle et al., 1990), and were then stored in diapause conditions at 2°C for ∼110 days. Following diapause, the eggs were moved to our experimental treatments within incubators and individuals were maintained in these treatments throughout hatching and development to adulthood (details below). Upon hatching, the egg containers were enclosed within rectangular 2.25L polyurethane containers and lettuce and wheat bran were provided. The grasshoppers were reared together until they reached 3rd instar. Subsequently, grasshoppers were reared individually in 0.47L polyurethane containers, which were changed every other day and supplied with romaine lettuce and wheat bran. We checked for eclosion when containers were changed. For newly eclosed grasshoppers, we noted the date and stage, and measured mass (g, Mettler Toledo AL104 balance). For adults, we additionally measured pronotum and femur length (mm) using digital calipers.

Melanoplus boulderensis post-diapause eggs and juvenile instars from each of the three populations were reared in factorial combinations of high (24 $\pm$ 4°C, HV treatment) or low (24 $\pm$ 2°C, LV treatment) temperature variability and long (14 h:10 h light:dark cycle) or short (12 h:12 h light:dark cycle) daylength. The daylengths were chosen to correspond to mid-summer versus early (March) or late (September) conditions. Temperature varied diurnally as a step function aligned with the 12 h:12 h photoperiod. Temperatures were chosen based on previous fixed temperature experiments, to allow for rapid development while maintaining high survival. A side-effect of our thermal treatment design was that faster development occurred at higher temperatures in the high-variance thermal treatment than the low-variance treatment even though their mean temperatures were the same. This difference is most easily interpreted by calculating the constant temperature equivalent (CTE) which is the median developmental temperature after accounting for development speed increasing with temperature (for CTE equations and additional details see Georges, 1989; Georges et al., 2004; Telemeco et al., 2013). The CTE for our treatments differed by 1°C, with the HV treatment having a CTE of 26°C and the LV treatment having a CTE of 25°C. Grasshoppers were reared in Percival I-36VL incubators with 32W fluorescent bulbs (Phillips F32T8/TL741). There was no indication that the grasshoppers were able to use the lights to thermoregulate. The final analysis included grasshoppers that survived to maturity from populations at 2,195 m (n = 104), 2,591 m (n = 63), and 3,048 m (n = 69). We assessed adult age and mass for an average of 10 individuals for each treatment, population, and sex combination (median = 9, range 3–19).

Thermal Sensitivity of Reared Grasshoppers

Most individuals were measured for all thermal traits with measurements occurring in the following order: hopping performance at four temperatures, feeding performance at three temperatures, preferred body temperatures (PBT), critical thermal minimum (CT_{min}), and critical thermal maximum (CT_{max}). We selected this order to minimize the potential for earlier measurements to bias later measurements, although this potential cannot be completely removed.

Hopping Performance

To assess the temperature dependence of hopping performance, we acclimated grasshoppers for 1 h at one of four temperatures (10, 17, 25, or 35°C) in incubators (same type as for rearing treatments) after which we immediately measured the distance of five jumps. To control for potential exposure order effects, we measured each grasshopper at each temperature in one of four orders (10-35-25-17; 25-17-10-35; 17-10-35-25; or 10-17-35-25). All measurements occurred across 2 days. After acclimation, grasshoppers were removed individually from the incubators and immediately placed in the center of the experimental arena at room temperature. The arena consisted of a 1.8 m × 1.8 m sheet of fabric with a checkered pattern at an interval of 2.5 cm (methods follow Harrison et al., 1991). Hopping was induced by manual prodding if necessary. We marked the position of the grasshopper after each of five jumps and subsequently recorded the x and y locations to an x and y resolution of 2.5 cm. We assessed hopping performance for an average of 7 individuals for each treatment, population, and sex combination (median = 7, range 3–18).

Feeding Performance

Grasshoppers were fasted prior to each feeding trial for 12 h, a sufficient period to complete digestion and absorption (Harrison and Fewell, 1995), and provided with a damp paper towel for humidity during trials. Feeding trials were conducted at three temperatures (10, 20, and 40°C). We assessed feeding performance for an average of 8 individuals for each treatment, population, and sex combination (median = 7.5, range 3–17).

The order of temperature trials was randomized. Grasshoppers were acclimated to the test temperature for 1 h prior to being provided with organic, baby romaine leaves at the start of two consecutive feeding periods each day. The first feeding period lasted for 2 h (reflecting rates of ingestion and of crop and mid-gut filling) and the later feeding period lasted an additional 6 h [reflecting rates of ingestion, crop filling and gut throughput; (Harrison and Fewell, 1995)]. The initial feeding trials commenced between 07:00 and 09:00 h. We used a flatbed scanner (Canon LiDE 100) to photograph the leaves before and after each of the feeding trials. We estimated leaf areas using ImageJ software[2].

[2]http://rsbweb.nih.gov/ij/

Preferred Body Temperatures and Critical Thermal Limits

We first measured PBT using a thermal gradient constructed on an aluminum sheet ($0.125'' \times 24'' \times 48''$). We placed one end in an ice bath and the other on a hotplate (Springate and Thomas, 2005), which created a temperature gradient spanning 5–50°C. Grasshoppers were placed within 5-cm-wide lanes created by corrugated plastic dividers running longitudinally across the thermal gradient. A clear acrylic lid was then placed above the gradient with holes for circulation and thermocouple measurements, and the grasshoppers were allowed to acclimate for 30 min. We then used an Extech type K thermocouple to monitor the thermal gradient and record the temperatures associated with the position of grasshoppers every 10 min over a 50-min period (Forsman, 2000; Springate and Thomas, 2005). During the acclimation period, grasshoppers moved freely throughout their lane on the thermal gradient before reducing activity. Most grasshoppers spent the duration of the observation period resting in one position. We assessed PBT for an average of 7 individuals for each treatment, population, and sex combination (median = 7, range 2–15).

We measured critical thermal limits, CT_{min} and CT_{max}, which were defined as the lower and upper temperatures at which the grasshoppers were no longer able to right themselves. Grasshoppers were placed individually into 50ml centrifuge tubes, which were slowly ($\sim$0.2°C min^{-1}) cooled or heated in a water bath. Given that warming rates may influence estimates of critical thermal limits, we chose an intermediate rate of warming (Chown et al., 2009). To minimize stress, we first cooled body temperatures for CT_{min} estimates and then began heating body temperatures for CT_{max} estimates at least an hour after the conclusion of the CT_{min} assays. We assessed CT_{min} for an average of 5 individuals for each treatment, population, and sex combination (median = 3.5, range 0–11). For CT_{max} the average was 4 (median = 3.5, range 0–10).

Field Phenology

For comparison to our experimental results, we analyzed historic (1958–1960) and recent (2006–2016) phenology data for the same *M. boulderensis* populations that sourced individuals for our rearing experiment as part of the Gordon Alexander Project. Surveys consisted of 1 person-hour of sweep netting and 0.5 person-hours of searching for adults and juveniles that may have been missed by sweep netting (Nufio et al., 2010; Nufio and Buckley, 2019). Data and analyses are as in Nufio and Buckley (2019).

We calculated degree days as the accumulated product of time and temperature above the lower developmental temperature (LDT). The calculation employed a single-sine approximation (Allen, 1976) based on daily minimum and maximum temperatures and a fixed spacing of 12 h between temperature minima and maxima. We used daily maximum and minimum temperature data from weather stations at our study sites (McGuire et al., 2012; Nufio and Buckley, 2019). For the field analysis, we calculated degree days based on air temperature to avoid assumptions regarding thermoregulatory behavior, radiation, windspeed, and soil temperatures. We use an LDT of 0°C, which corresponds to an estimate based on rearing *M. boulderensis* in constant temperatures and regressing development time against temperature (Trudgill, 1995). This differs from previous analyses (Nufio et al., 2010; Nufio and Buckley, 2019) that used an LDT of 12.0°C based on a fit to field phenology data pooled across multiple species. Our estimation of degree days for field populations are intended as an approximate translation of environmental temperature into physiological time.

We used a development index (DI), which represents the average development stage of the population and ranges from 1 (all first instars) to 6 (all adults), to describe the developmental stage of communities sampled through field surveys. We also used the DI to estimate the timing of adulthood, because DI generally exhibits a smooth increase through the season whereas counts of individual development stages can be variable. DI also allows interpolating between the weekly survey intervals. We quantified phenology both in terms of day of year (doy) and growing degree days (GDDs). We fit a spline (R function smooth.spline) to DI data for each combination of species, site, and year. We used the splines to estimate the timing of adulthood as the doy or GDDs when DI = 5.5 (R predict function). We selected DI = 5.5 as it tended to approximately correspond to the inflection point before the DI curve reached the asymptote at DI = 6.

Analyses

Our analyses primarily used linear mixed-effects (LME) models and ANOVAs in R (lmer function from Lme4 library; Bates et al., 2007). We used an LME model to evaluate the (categorical) effects of temperature variance, photoperiod, site, sex, and their interactions up to the fourth order. We checked for normality of the response variables and subsequently assumed a normal distribution. We used Akaike information criterion corrected for small sample size (AICc) to compare the full model to models restricted to a subset of terms and interactions. We used model selection whereby models were preferred if $\Delta AICc > 2$. However, as described below, for some dependent variables we selected slightly less-preferred models for ease of comparison to other dependent variables. Models included the identity of the grasshopper's mother as a random effect except where indicated.

The full model was preferred for development time (days from removal from diapause conditions to adulthood). Several simpler models were preferred to the full model for adult mass, but we used the full model to facilitate comparison to the model for development time. We repeated the analyses for development time and mass, additionally including developmental stage (instar) as a numeric predictor (3rd to 6th = adult) to assess whether effects on age and mass shifted over development in a repeated-measures ANOVA. We assessed correlations between femur length and $mass^{1/3}$ and between pronotum length and $mass^{1/3}$ to determine whether rearing conditions altered body shape.

We used the model for PBT receiving the most support, which included only the main effects. The model we selected for critical thermal minimum and maximum included temperature

variation, photoperiod, and the interaction between the two. No single model received support for CT_{min} or CT_{max}, so we chose the most complex model with ΔAICc < 2. We also omitted the random effect since it led to slightly higher AICc values and singularity issues.

To examine rearing effects on hopping and feeding performance, the full model was preferred based on AICc scores. For hopping performance, we included a random effect to account for the grasshopper measured (nested within the identity of the mother) since each individual performed five replicate hops. We accounted for test temperature as a second-order polynomial for hopping, due to the unimodal shape and improved AICc scores, and as a linear term for feeding given that we had only three test temperatures and no obvious non-linearity of response. Our dependent variable was consumed leaf area scaled to the mass of the grasshopper over the full 8 h test period (visual inspection showed similar trends existed at 2 h).

We quantified the temperature dependence of growth and development rates using Q10 values, which describe the shift in rates with a 10-degree temperature increase as $Q10 = (R_2/R_1)^{[10/(T_2-T_1)]}$, where, R_1 and R_2 are rates at temperatures T_1 and T_2 (Seebacher et al., 2015). $Q10$ values represent the effects of temperature on physiological rates that are normalized for comparison, and are commonly used to compare the thermal sensitivity of processes such as growth and development. We normalized $R_1 = 1$ at $T_1 = 24°C$, the mean rearing temperature. We estimated the number of development days, d, to reach adulthood as $d = c/[Q10^{(T_{var}/10)}+Q10^{(-T_{var}/10)}]$, where, T_{var} is the temperature variance from $T_1 = 20°C$ and c is a constant that accounts for 12 h at each temperature and assumes that a fixed number of units of development are required to reach adulthood. Similarly, we estimate the Q10 for growth using the following expression for adult mass, M (g) as $M = c\ d\ [Q10^{(T_{var}/10)}+Q10^{(-T_{var}/10)}]$, where, d is the number of days of development. We estimate development and growth Q10s for each site, daylength, and sex using the generalized least squares model fitting function gnls() from the nlme package for R (Pinheiro et al., 2020) and plot the estimates as relative Q10s by normalizing the highest Q10 estimate to 1. We then used these Q10 values to graphically assess the effects of elevation-of-origin and daylength on the thermal sensitivity of development rate.

For the field phenology data, we used linear mixed-effects (LME) models and ANOVAs in R to examine how the development index responds to the 2nd-degree polynomial of day of year or cumulative degree days, season warmth, site, and their interaction. We controlled for survey year as a random variable.

RESULTS

Growth and Development

Temperature variation and photoperiod interacted with site and sex to determine time to adulthood (**Figure 1**, top row). The high-temperature variation treatment (HV) which had a CTE 1°C higher than the low-temperature variation treatment (LV) substantially accelerated development (**Table 1**, top row, coefficients: **Supplementary Table 1**, model plot: **Supplementary Figure 1**). Long-days accelerated development in HV conditions but decelerated development in LV conditions, particularly at low elevations and for males (model plot: **Supplementary Figure 1**). Although higher elevation populations had reduced development times for most treatments, this effect of elevation-of-origin disappeared when animals were reared under HV, long-day conditions (**Figure 1**). Significant lesser-order interactions are largely consistent with the four-way interaction (**Table 1**; coefficients: **Supplementary Table 1**). Analyzing time to each instar indicates that the effects of photoperiod and temperature variability on development become most apparent late in development (**Supplementary Figure 2**). The four-way interaction described above also significantly interacts with instar (**Supplementary Tables 2, 3**).

Estimating the thermal sensitivity of development using Q10 values suggests similar thermal sensitivity across elevation for long-day conditions (**Figure 2A**). However, Q10 values, and thus physiological responsiveness to temperature increases, are estimated to increase with elevation under short-day conditions, which suggest the capacity for rapid growth when conditions are permissive. Consistent with the Q10 estimates, high-elevation populations in short-day conditions exhibited the fastest development (**Figure 1**). By contrast, Q10s estimated for growth were relatively flat across elevation regardless of daylength, although females had higher Q10s than males (**Figure 2B**).

Shifts in developmental rates and times influenced adult mass, but in a manner that diverges from the simple expectation that prolonged development increases mass. Interestingly, there is not a significant linear relationship between development time and adult mass across all individuals from all treatments (coefficient = 0.0014 $\pm$ 0.0011 SE, $t_{[1,234]} = 1.3$, $p = 0.2$, $r^2 = 0.003$). The LV thermal treatment, which prolonged development (**Figure 1**, top row), did not increase mass, and, in fact, substantially decreased mass in females from higher-elevation sites (**Figure 1**, bottom row, $\chi^2_2 = 6.0$, $p < 0.05$, **Table 1** and **Supplementary Figure 3**). Comparing just the lowest and highest elevation sites suggests that lower masses at higher elevations induced by the LV thermal treatment were extenuated by long-day conditions ($t = 2.4$, $p < 0.05$, **Supplementary Table 1** and **Supplementary Figure 4**). Body shape was not influenced by temperature variability or daylength, as adult femur length and pronotum length each have a strong linear relationship with $mass^{1/3}$ (femur coefficient = 11.7 $\pm$ 0.8 SE, $t = 15.0$, $p < 0.001$, $r^2 = 0.49$; pronotum coefficient = 7.0 $\pm$ 0.4 SE, $t = 18.4$, $p < 0.001$, $r^2 = 0.59$). Analyzing the mass of each instar does not show significant interactions between instar and these variables, except that the mass differential between males and females emerges and grows as development proceeds (see **Supplementary Figure 5** and **Supplementary Tables 2, 3**).

Thermal Sensitivity

Preferred body temperature was not significantly affected by our rearing treatments, but there were non-significant trends for both the LV ($\chi^2_1 = 3.0$, $p = 0.08$) and long-day treatments ($\chi^2_1 = 3.1$, $p = 0.08$) reducing preferred temperature by up to

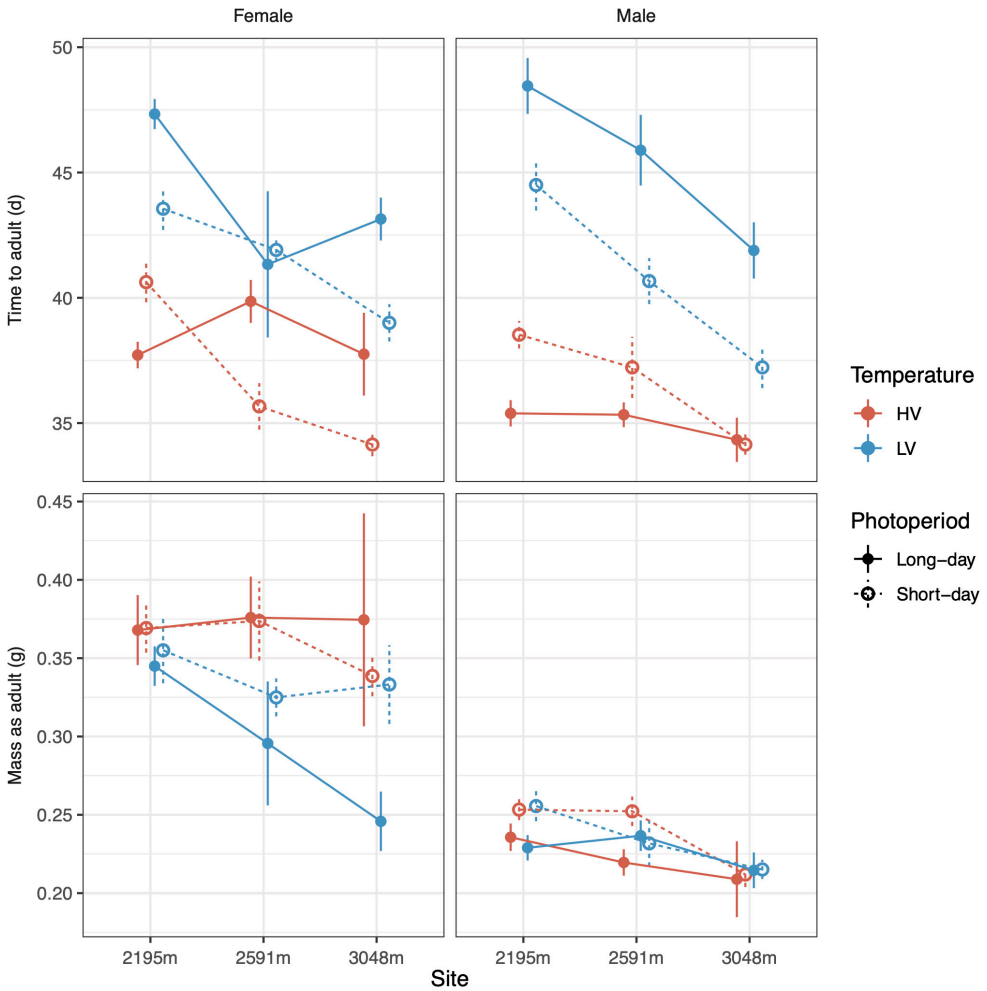

FIGURE 1 | Time to adulthood **(top row)** and adult mass **(bottom row)** vary as a function of photoperiod, temperature variance, site, and sex. High temperature variance (red color) leads to faster development, and males develop faster than females (column) across sites (*X*-axis). Photoperiod (line type) interacts with temperature to determine development rates. Low temperature variance at higher elevation sites leads to lower mass, especially in females. Error bars represent standard error.

∼3°C (**Figure 3** and **Table 2**; coefficients: and **Supplementary Table 4**). LV, long-day rearing decreased grasshopper CT_{min} by up to ∼4°C (mean = 4.46°C, F = 4.1, $p < 0.05$), while we found no effects of rearing treatments on the grasshoppers' CT_{max} (mean = 49.2°C, **Figure 3** and **Table 2**; coefficients: **Supplementary Table 5**).

The temperature sensitivity of hopping performance responded to both temperature variability and photoperiod during rearing in a manner that depended on sex and site: the female thermal performance curve was narrow (indicated by $TestTemp^2$ in **Supplementary Table 6**) when reared under HV, long-day conditions, and was especially narrow for the highest elevation site (five-way interaction: $\chi^2_4 = 13.8$, $p < 0.05$, **Figure 4A** and **Table 3**). Hopping performance was typically highest when reared under short days ($\chi^2_1 = 11.7$, $p < 0.001$), especially for grasshoppers reared in the HV thermal treatment ($\chi^2_1 = 14.9$, $p < 0.001$, **Table 3**). Low-temperature hopping performance declined when grasshoppers were reared in both HV, long-day conditions and LV, short-day conditions (test temperature x temperature variance x photoperiod: $\chi^2_2 = 22.1$, $p < 0.001$, **Figure 4A** and **Table 3**; coefficients: **Supplementary Table 6**).

Grasshopper feeding rate increased up to our highest test temperature (40°C) for all rearing conditions and populations (**Figure 4B**). This positive effect of temperature on feeding rate was strongest for grasshoppers raised in HV conditions ($\chi^2_1 = 5.0$, $p < 0.05$, **Figure 4B** and **Table 3**). Additionally, as elevation increased, the temperature dependence of males' feeding increased while that of females decreased, leading to a performance differential at high test temperatures ($\chi^2_2 = 6.4$, $p < 0.05$, **Table 3**). With increasing elevation, LV grasshoppers' feeding performance at high test temperatures transitioned from worse than HV grasshoppers to better ($\chi^2_2 = 8.5$, $p < 0.05$, **Table 3**; coefficients: **Supplementary Table 6**).

TABLE 1 | Wald 3 ANOVA for linear mixed effects models of grasshopper time to adulthood (days) and mass at adulthood (g).

	Time to adult			Mass at adult		
	χ^2	*df*	*p*	χ^2	*df*	*p*
(Intercept)	2348.7	1	<0.001***	768.6	1	<0.001***
Sex	6.4	1	0.011*	56.3	1	<0.001***
Site	1.6	2	0.460	0.1	2	0.933
Temperature	66.8	1	<0.001***	1.2	1	0.273
Photoperiod	6.4	1	0.011*	0.0	1	0.943
Sex:Site	1.4	2	0.505	1.0	2	0.619
Sex:Temperature	4.7	1	0.031*	0.3	1	0.568
Site:Temperature	17.8	2	<0.001***	8.4	2	0.015*
Sex:Photoperiod	0.0	1	0.850	0.4	1	0.506
Site:Temperature	16.3	2	<0.001***	1.3	2	0.533
Temperature:Photoperiod	15.1	1	<0.001***	0.1	1	0.774
Sex:Site:Temperature	8.8	2	0.012*	6.0	2	0.049*
Sex:Site:Photoperiod	6.0	2	0.0497*	0.3	2	0.857
Sex:Temperature:Photoperiod	0.0	1	0.928	0.0	1	0.991
Site:Temperature:Photoperiod	17.0	2	<0.001***	5.9	2	0.053
Sex:Site:Temperature:Photoperiod	12.4	2	0.002**	3.6	2	0.164

"Temperature" refers to temperature variance and "Site" indicates three source locations at different elevations. Stars indicate significant effects (: $p < 0.05$, **: $p < 0.01$, and ***: $p < 0.001$).*

Field Phenology

At each elevation, grasshoppers developed and matured into adults faster during warmer seasons, although development through late instars appeared to slow for some warm seasons (**Figure 5A**). Season warmth interacted with day of year to determine the developmental progression ($\chi^2_2 = 22.3, p < 0.001$), but the response to season warmth did not vary significantly across sites. Several warm seasons exhibited slow development and several cool seasons exhibited rapid development in response to the accumulation of growing degree days at the 3,048 m site (**Figure 5B**). Phenological differences with season warmth were less apparent at the lower elevation sites. The accumulation of degree days, season warmth, and site significantly interacted in determining the progression of development ($\chi^2_4 = 18.0$, $p < 0.001$).

The day of year of adulthood accelerated in warmer seasons ($F_{[2,23]} = 6.0$, $p < 0.001$) and varied across sites ($F_{[2,23]} = 5.0$, $P < 0.05$), but the response to season warmth was similar across sites ($F_{[2,23]} = 0.1$, $P = 0.87$, **Figure 5C**). The cumulative degree days at adulthood did not shift significantly in warmer seasons ($F_{[2,23]} = 2.7$, $P = 0.11$, **Figure 5D**), with the relationship being particularly flat for the 3,048 m site. The cumulative degree days at adulthood varied across sites ($F_{[2,23]} = 28.1$, $p < 0.001$) but did not significantly interact with season warmth ($F_{[2,23]} = 1.7$, $P = 0.21$, **Figure 5D**). See Nufio and Buckley (2019) for further analysis and comparison with other sites and species.

DISCUSSION

Our rearing experiment builds on past inferences, based on field phenology, of greater developmental plasticity in response to temperature for season-limited high-elevation populations of *M. boulderensis* grasshoppers compared to low-elevation populations. Rearing temperature and daylength interacted to cue plastic variation in developmental rate consistent with phenological patterns observed in the field. Somewhat surprisingly, we did not detect evidence for trade-offs between accelerated development and other fitness-relevant traits such as body size, thermal tolerance, and performance, although thermal sensitivities for performance were affected by rearing treatment in complex ways.

Grasshoppers from high-elevation populations generally developed into adults more rapidly than those from low-elevation populations, but the size of this effect depended strongly on rearing treatment. For example, the treatment that most closely modeled mid-summer, high-elevation conditions (long-day, HV) was least affected by population of origin, with all populations developing quickly. By contrast, we observed the greatest effect of elevation-of-origin for the treatment most closely modeling springtime, low-elevation conditions (short-day, LV), with high-elevation animals developing much more rapidly than low-elevation animals. These differences appear to be driven by short daylengths, typical of spring, increasing the thermal sensitivity of development (i.e., Q10) for the highest-elevation population but not the lower-elevation populations. This plasticity suggests that high-elevation populations have the capacity to facultatively increase their rate of development when conditions are permissive early in the season.

Additionally, grasshoppers reared in the HV temperature treatment developed more rapidly than those reared in the LV treatment. This effect can be partially explained by HV animals developing at warmer temperatures with a constant temperature equivalent (CTE) 1°C higher than animals reared in the LV treatment. However, a 1°C average difference in developmental temperature appears insufficient to fully explain the ∼10-day difference in development time between HV and LV treatments. For example, in *M. sanguinipes*, another grasshopper species from this community, a 6°C difference in constant rearing temperature (24°C vs. 30°C) is needed to induce a similar effect on development time (Buckley et al., 2015). These results suggest that differences in temperature variation between the HV and LV treatments affected development rate independent of the direct effect of temperature on development, although additional data are needed to confirm this conclusion. Observations of low *M. boulderensis* survival in high, constant temperatures in preliminary rearing experiments led to our examination of fluctuating temperatures. We intended thermal variance as an indicator of seasonality but selected a constant mean temperature for tractability. Shifting both the mean and variance of treatments so that the CTE is controlled across treatments could help disentangle these effects. Further, in most studies of developmental plasticity including ours there is a need to consider more realistic environmental variation both to capture the variation during a single day and to represent environmental shifts during the course of development (Sniegula et al., 2016; Burggren, 2018).

Examining daylength in this study allowed us to refine previous conclusions that developmental plasticity at high elevation extended development when environmental conditions

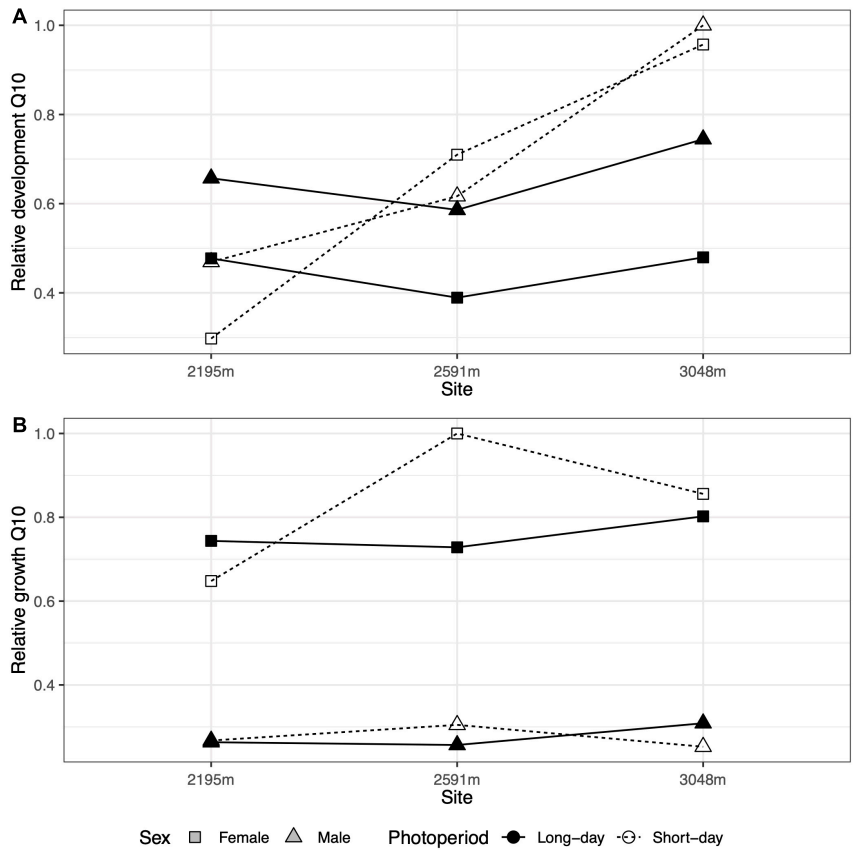

FIGURE 2 | (A) The relative thermal sensitivity (Q10) of development (normalized from 0 to 1) is relatively flat across site elevations for long-day conditions (filled symbols and solid lines) but increases with elevation for short-day conditions (open symbols and dashed lines) for both sexes (symbols). **(B)** The relative thermal sensitivity (Q10) of growth does not vary consistently with photoperiod.

allowed (Buckley et al., 2015; Nufio and Buckley, 2019). The thermal sensitivity of development (Q10s) suggests a role of photoperiod in accelerating development when days are short, indicative of early- or late- season conditions. While the consequences are similar, this suggests selection to complete development in season-limited environments rather than selection to extend development when conditions allow and days are long. Similar roles of short photoperiod in accelerating development have been observed for other grasshopper (Dingle et al., 1990) and insect populations (Lopatina et al., 2011; Lindestad et al., 2019). However, a study including low-elevation populations of the late-season *M. sanguinipes* implicated seasonal constraints but detected daylength-x-temperature interactions in sea-level but not high-altitude populations (Dingle et al., 1990). Similarly, short days indicative of seasonal time constraints accelerated damselfly development but there was little variation in development time among high-latitude, time-constrained populations (Sniegula et al., 2016).

We did not detect a cost of accelerating development in short-day conditions such as reduced size. LV led to both delayed development and either unchanged or decreased mass, depending upon other variables, and adult mass and development time were not significantly correlated. Other studies finding similar mass invariance (Sniegula et al., 2016; Norling, 2018) suggest the need to refine understanding of tradeoffs between growth and development.

Selection for elevated thermal sensitivity in short-day conditions is often associated with ensuring the completion of a generation in the late summer in time constrained, high-elevation, or latitude environments (Dingle et al., 1990; Abrams et al., 1996; Johansson et al., 2021). However, *M. boulderensis* generally reaches adulthood in the early season before daylength declines. The latest that all members of *M. boulderensis* populations reached adulthood at any elevation across resurveys approximated the summer solstice when daylength is longest (day of year 171–173). Although cool, early-season conditions may limit development acceleration, grasshoppers can effectively use solar radiation to elevate their body temperatures once they reach sufficient size (Buckley et al., 2013a). Thus, high-elevation populations should generally develop in conditions analogous to our HV, short-day treatment which maximized development rate. *M. boulderensis* grasshoppers can lay egg pods every few days and individuals can persist as adults for at least a month at 3,048 m site (Nufio unpublished data), so faster development may allow production of more egg pods. Higher-elevation populations of *M. boulderensis* exhibit smaller eggs and

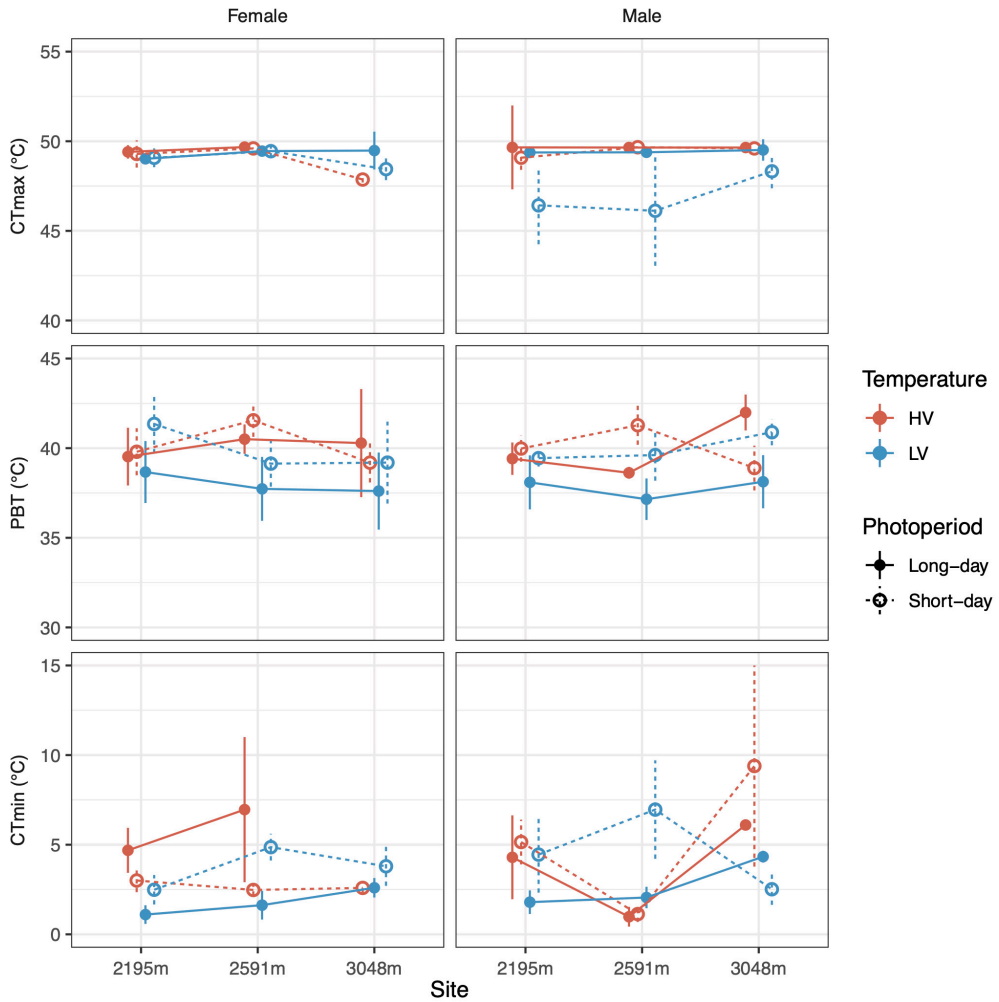

FIGURE 3 | Photoperiod (line type) and temperature variance (colors) alter CT_{min} **(bottom row)** but not CT_{max} **(top row)** of male and female (columns) grasshoppers from different populations (*X*-axis). Mean preferred body temperatures **(middle row)** of lab-reared grasshoppers do not differ significantly based on sex, photoperiod, temperature variance, or population. Error bars represent standard error.

TABLE 2 | Wald 3 ANOVA of our linear mixed effects model of (A) preferred body temperature, and (B) CTmin and CTmax (°C) as a function of temperature variance, photoperiod, sex, site, and interactions.

(A)

	χ^2	*df*	*p*
(Intercept)	4143.1	1	<0.001***
Sex	0.0	1	0.854
Site	0.1	2	0.966
Temperature	3.0	1	0.081
Photoperiod	3.1	1	0.077

(B)

	CT_{min}				CT_{max}			
	Sum Sq	***df***	***F value***	***p***	***Sum Sq***	***df***	***F value***	***p***
(Intercept)	468.9	1	44.9	<0.001***	46629.0	1	12215.9	<0.001***
Temperature	89.1	1	8.5	0.004**	0.4	1	0.1	0.737
Photoperiod	4.2	1	0.4	0.529	1.4	1	0.4	0.544
Temperature:Photoperiod	43.0	1	4.1	0.045*	4.2	1	1.1	0.295
Residuals	1107.2	106			385.5	101		

Stars indicate significant effects (: $p < 0.05$, **: $p < 0.01$, and ***: $p < 0.001$).*

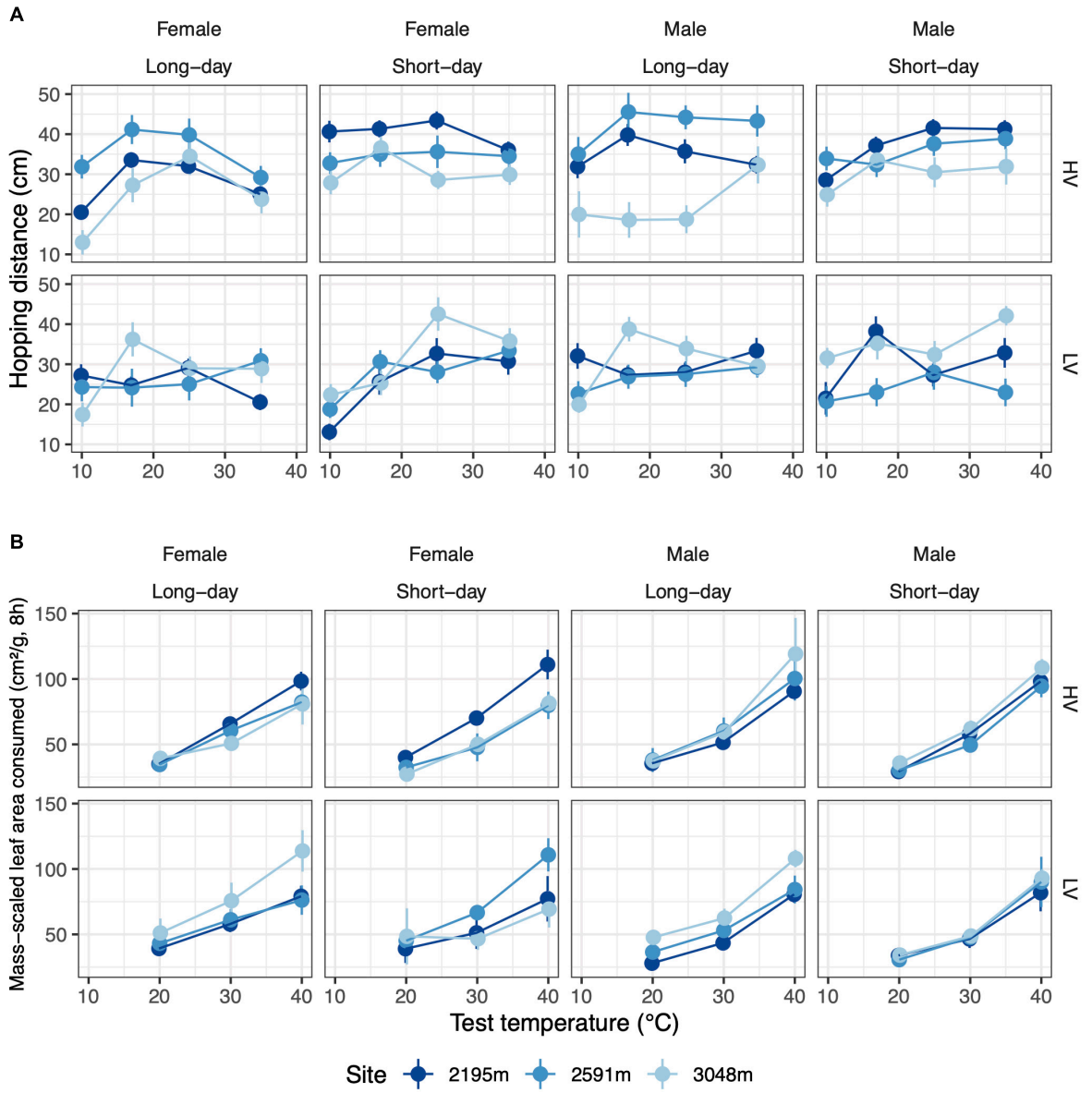

FIGURE 4 | (A) The temperature dependence of hopping performance varies with developmental temperature variation (rows) and photoperiod (columns). **(B)** The temperature dependence of feeding performance varies with temperature variability (rows) but not photoperiod (columns). Error bars represent standard error.

clutches (Levy and Nufio, 2014; Slatyer et al., 2020), consistent with laying more clutches. Alternatively, observed developmental plasticity may enable rapid early-season development to avoid resource competition with other species when conditions are permissive. Selection for the developmental plasticity could also precede the species and its seasonal timing.

Our developmental analyses are broadly consistent with field observations of phenology (Nufio et al., 2010; Nufio and Buckley, 2019; Buckley et al., 2021). Phenological advancements of *M. boulderensis* are less apparent when considering physiological time (degree days) than calendar dates (**Figure 5**). This reflects the temperature dependence of development and indicates that phenology indeed responds to environmental conditions. However, comparing phenology between seasons that differ in warmth suggests greater developmental plasticity at the high-elevation site: some warm years yield slow developmental progression and some cool years yield rapid developmental progression. Daylength altering the thermal sensitivity of development can explain divergences in the relationship between growing degree days and development rate, especially at high elevation. When suitable temperatures occur early in the season when days are short, development progresses rapidly (i.e., steep slopes in **Figure 5B**), but when suitable temperatures only occur later in the season development progresses more slowly (i.e., shallow slopes in **Figure 5B**). This developmental pattern will occur regardless of the total number of growing degrees days

TABLE 3 | Wald 3 ANOVA of our linear mixed effects model of hopping distance (cm) and feeding performance (cm^2/g).

	Hopping distance			Leaf area consumed		
	χ^2	*df*	*p*	χ^2	*df*	*p*
(Intercept)	0.3	1	0.603	8.6	1	0.003**
f(TestTemp)	26.4	2	<0.001***	114.5	1	<0.001***
Sex	6.1	1	0.013*	0.1	1	0.704
Site	4.2	2	0.120	1.4	2	0.478
Temperature	4.0	1	0.046*	2.5	1	0.113
Photoperiod	11.7	1	<0.001***	0.1	1	0.778
f(TestTemp):Sex	3.8	2	0.148	1.0	1	0.328
f(TestTemp):Site	5.9	4	0.205	3.6	2	0.162
Sex:Site	3.8	2	0.146	2.4	2	0.304
f(TestTemp):Temperature	6.6	2	0.037*	5.0	1	0.026*
Sex:Temperature	0.0	1	0.927	1.9	1	0.166
Site:Temperature	0.0	2	0.979	1.2	2	0.537
f(TestTemp):Photoperiod	6.5	2	0.039*	0.7	1	0.395
Sex:Photoperiod	9.6	1	0.002*(*)	0.5	1	0.495
Site:Photoperiod	2.0	2	0.373	0.4	2	0.801
Temperature:Photoperiod	14.9	1	<0.001***	0.0	1	0.884
f(TestTemp):Sex:Site	10.6	4	0.0314*	6.4	2	0.042
f(TestTemp):Sex:Temperature	2.3	2	0.317	2.2	1	0.137
f(TestTemp):Site:Temperature	7.3	4	0.122	5.1	2	0.077
Sex:Site:Temperature	4.3	2	0.115	2.8	2	0.253
f(TestTemp):Sex:Photoperiod	16.2	2	<0.001***	0.2	1	0.621
f(TestTemp):Site:Photoperiod	6.1	4	0.194	0.5	2	0.785
Sex:Site:Photoperiod	4.2	2	0.123	1.3	2	0.511
f(TestTemp):Temperature: Photoperiod	22.1	2	<0.001***	0.3	1	0.558
Sex:Temperature:Photoperiod	2.8	1	0.092	0.5	1	0.493
Site:Temperature:Photoperiod	1.4	2	0.494	3.4	2	0.181
f(TestTemp):Sex:Site:Temperature	13.8	4	0.008**	5.6	2	0.060
f(TestTemp):Sex:Site:Photoperiod	13.7	4	0.008**	1.3	2	0.527
f(TestTemp):Sex:Temperature: Photoperiod	13.8	2	0.001**	0.2	1	0.679
f(TestTemp):Site:Temperature: Photoperiod	8.1	4	0.088	8.5	2	0.015*
Sex:Site:Temperature:Photoperiod	4.1	2	0.132	3.4	2	0.183
f(TestTemp):Sex:Site:Temperature: Photoperiod	13.8	4	0.008**	4.5	2	0.105

f(TestTemp) = TestTemp + $TestTemp^2$ in the case of hopping distance and f(TestTemp) = TestTemp in the case of feeding performance. Stars indicate significant effects (: $p < 0.05$, **: $p < 0.01$, and ***: $p < 0.001$).*

that occur throughout the season, particularly when suitable temperatures progress late into the summer or fall. The effect of daylength on the thermal sensitivity of development can also explain why field development frequently progresses more rapidly early in the season and then slows as the population approaches all adults (i.e., asymptotic curves in **Figure 5B**). During the early stages of development, days are short and induce fast development, whereas days grow longer late in development thereby slowing development. Such developmental dynamics are consistent with observations of broader phenologies in warm years (Buckley et al., 2021). It is plausible that such variable developmental rates cued by daylength are adaptive, facilitating optimal development and high relative fitness, but additional experiments varying daylength across development are needed to test this hypothesis. Including more realistic seasonal photoperiod shifts is likewise needed (Sniegula et al., 2016; Norling, 2018). These studies suggest that absolute photoperiod is only part of the picture and whether the days are lengthening or shortening over time is another important cue for phenology which can help distinguish early season from late season.

As with many grasshopper species (Verberk et al., 2021), *M. boulderensis* reverses the temperature-size rule. One explanation for the reversal in grasshoppers is that warm adaptation of many physiological processes related to feeding leads to greater increases in growth than development at warm temperatures (Miller et al., 2009). This is consistent with our observations that feeding rates increase up to high temperatures. However, our Q10 analysis suggests high thermal sensitivity of development and less thermal sensitivity of growth, at least for the high elevation population. Higher developmental Q10s but roughly constant growth Q10s with increasing elevation and short-day conditions may contribute to the temperature-size rule reversal observed. Further examination of the relative slopes and intercepts of the thermal dependence of development and growth is needed to assess the mechanisms underlying the temperature-size rule for *M. bouldernesis* (Walters and Hassall, 2006).

Despite differences in the thermal sensitivity of development, we found no evidence that preferred body temperatures or critical thermal limits varied among populations. Somewhat surprisingly, CT_{min} was lower for grasshoppers reared in LV and long-day conditions which should generally model animals developing at low elevation during mid-summer when the risk of exposure to critically low temperatures is reduced. However, delayed development in these conditions may allow for broader thermal tolerance which could be useful for producing egg clutches late into fall. The greater plasticity of CT_{min} especially for the highest elevation populations is unsurprising since there is more variability in low temperatures with elevation, while hot spikes will occur regardless of elevation.

In addition to influencing development rate, temperature variance and daylength during development influenced the temperature dependence of adult hopping and feeding performance in a manner that varied among sites in difficult-to-predict ways. Some combinations of temperature variance and daylength narrowed the thermal breadth of hopping performance, but the narrowing is not readily interpretable in terms of environmental exposure. That said, development under HV, short-day conditions, which result in the fastest development across populations, also resulted in increased peak hopping performances, suggesting rearing conditions beneficial for development rate are also beneficial for performance. Increases in feeding rates with temperature up to 40°C are also consistent with faster development in HV conditions. Increased feeding performance at high temperatures in grasshoppers reared in HV conditions may be one driver of the observed developmental plasticity in development rate and hopping performance.

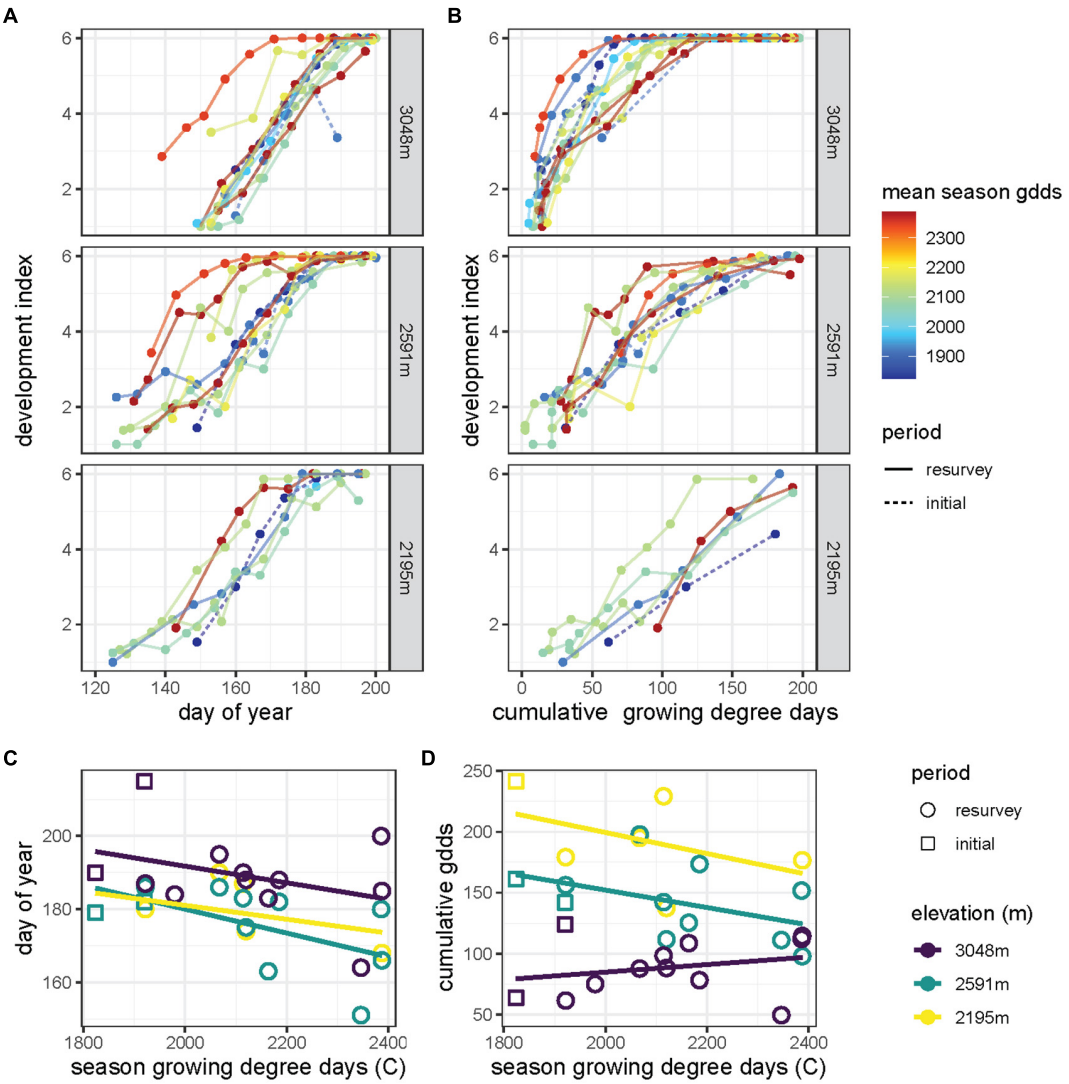

FIGURE 5 | The development index, which represents the average developmental stage of the population and ranges from 1 (all first instars) to 6 (all adults), increases **(A)** with day of year and **(B)** as growing degree-days accumulate through the season for sites across elevation (rows). Lines correspond to years of the initial survey (dashed) and resurvey (solid) with colors indicating the seasonal growing degree-days averaged along the elevational gradient (red, warm years to blue, cool years). Adult phenology of *Melanoplus boulderensis* (estimated using the development index) quantified as **(C)** day of year and **(D)** cumulative growing degree days (gdds) varies as a function of seasonal cumulative growing degree days. We distinguish years during the initial survey (squares) and resurvey (circles) for each site elevation (color).

The evolution of rapid development at smaller size in season-limited environments is supported by fitness optimization models (Abrams et al., 1996). A study of damselflies revealed pronounced plasticity in response to photoperiod (which was changed weekly to best mimic shifting environmental conditions during growth and development), with a northern photoperiod indicative of time constraints resulting in faster development and smaller body size (Johansson et al., 2021). As with our study, the photoperiod response was more apparent for development than body mass. An analysis of genetic covariance suggested that the alignment of photoperiod with strong seasonal time constraints can promote the evolution of developmental plasticity (Johansson et al., 2021).

Our experimental results reveal complex interactions between rearing temperature, daylength, and population of origin that refute simple interpretation such as "warmer is better" or "longer is better." Given the uncoupling of adult mass and development time, further work is needed to understand the phenotypic and fitness consequences of developmental plasticity associated with seasonal time constraints. A further complication is that thermal performance curves for hopping and feeding were affected by multiple developmental treatments and their interactions in difficult to predict ways. These observations highlight the challenges in identifying the physiological mechanisms underlying variable responses to climate change among populations and species (Buckley et al., 2018). Considering

how developmental plasticity in response to seasonal cues can alter environmental responses may be central to predicting phenological and other biological implications of climate change (Forrest, 2016; Chmura et al., 2018).

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: https://github.com/HuckleyLab/GrasshopperDev.

AUTHOR CONTRIBUTIONS

RT and LB designed the experiment. RT led laboratory data collection. BB conducted feeding experiments. CN led field data collection. JS led analysis. JS, RT, and LB contributed to analyses and wrote the first draft of the manuscript. All authors edited the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphys.2021.738992/full#supplementary-material

REFERENCES

Abrams, P. A., Leimar, O., Nylin, S., and Wiklund, C. (1996). The effect of flexible growth rates on optimal sizes and development times in a seasonal environment. *Am. Nat.* 147, 381–395. doi: 10.1086/285857

Allen, J. C. (1976). A modified sine wave method for calculating degree days. *Environ. Entomol.* 5, 388–396. doi: 10.1093/ee/5.3.388

Atkinson, D. (1996). Ectotherm life-history responses to developmental temperature. *Anim. Temp.* 1996, 183–204. doi: 10.1017/CBO9780511721854.009

Bale, J. S., Masters, G. J., Hodkinson, I. D., Awmack, C., Bezemer, T. M., Brown, V. K., et al. (2002). Herbivory in global climate change research: direct effects of rising temperature on insect herbivores. *Glob. Chang. Biol.* 8, 1–16. doi: 10.1046/j.1365-2486.2002.00451.x

Bates, D., Sarkar, D., Bates, M. D., and Matrix, L. (2007). The lme4 package. *R Package Version* 2:74.

Berner, D., and Blanckenhorn, W. U. (2006). Grasshopper ontogeny in relation to time constraints: adaptive divergence and stasis. *J. Anim. Ecol.* 75, 130–139. doi: 10.1111/j.1365-2656.2005.01028.x

Bradshaw, W. E., and Holzapfel, C. M. (2007). Evolution of Animal Photoperiodism. *Annu. Rev. Ecol. Evol. Syst.* 38, 1–25. doi: 10.1146/annurev.ecolsys.37.091305.110115

Buckley, L. B., Cannistra, A. F., and John, A. (2018). Leveraging organismal biology to forecast the effects of climate change. *Integr. Comp. Biol.* 58, 38–51. doi: 10.1093/icb/icy018

Buckley, L. B., Graham, S. I., and Nufio, C. R. (2021). Grasshopper species' seasonal timing underlies shifts in phenological overlap in response to climate gradients, variability and change. *J. Anim. Ecol.* 90, 1252–1263. doi: 10.1111/1365-2656.13451

Buckley, L. B., Nufio, C. R., and Kingsolver, J. G. (2013b). Phenotypic clines, energy balances, and ecological responses to climate change. *J. Anim. Ecol.* 83, 41–50.

Buckley, L. B., Miller, E. F., and Kingsolver, J. G. (2013a). Ectotherm thermal stress and specialization across altitude and latitude. *Integr. Comp. Biol.* 53, 571–581.

Buckley, L. B., and Nufio, C. R. (2014). Elevational clines in the temperature dependence of insect performance and implications for ecological responses to climate change. *Conserv. Physiol.* 2:cou035. doi: 10.1093/conphys/cou035

Buckley, L. B., Nufio, C. R., Kirk, E. M., and Kingsolver, J. G. (2015). Elevational differences in developmental plasticity determine phenological responses of grasshoppers to recent climate warming. *Proc. R. Soc. Lond. Ser. Biol. Sci.* 282:20150441. doi: 10.1098/rspb.2015.0441

Burggren, W. (2018). Developmental phenotypic plasticity helps bridge stochastic weather events associated with climate change. *J. Exp. Biol.* 221:jeb161984. doi: 10.1242/jeb.161984

Chmura, H. E., Kharouba, H. M., Ashander, J., Ehlman, S. M., Rivest, E. B., and Yang, L. H. (2018). The mechanisms of phenology: the patterns and processes of phenological shifts. *Ecol. Monogr.* 89:e01337.

Chown, S. L., Jumbam, K. R., Sørensen, J. G., and Terblanche, J. S. (2009). Phenotypic variance, plasticity and heritability estimates of critical thermal limits depend on methodological context. *Funct. Ecol.* 23, 133–140. doi: 10.1111/j.1365-2435.2008.01481.x

Dingle, H., Mousseau, T. A., and Scott, S. M. (1990). Altitudinal variation in life cycle syndromes of California populations of the grasshopper, *Melanoplus sanguinipes* (F.). *Oecologia* 84, 199–206. doi: 10.1007/BF00318272

Forrest, J., and Miller-Rushing, A. J. (2010). Toward a synthetic understanding of the role of phenology in ecology and evolution. *Philos. Trans. R. Soc. B Biol. Sci.* 365, 3101–3112. doi: 10.1098/rstb.2010.0145

Forrest, J. R. (2016). Complex responses of insect phenology to climate change. *Curr. Opin. Insect Sci.* 17, 49–54. doi: 10.1016/j.cois.2016.07.002

Forsman, A. (2000). Some like it hot: intra-population variation in behavioral thermoregulation in color-polymorphic pygmy grasshoppers. *Evol. Ecol.* 14, 25–38. doi: 10.1023/A:1011024320725

Georges, A. (1989). Female turtles from hot nests: is it duration of incubation or proportion of development at high temperatures that matters? *Oecologia* 81, 323–328.

Georges, A., Doody, S., Beggs, K., and Young, J. (2004). "Thermal models of TSD under laboratory and field conditions," in *Temperature-Dependent Sex Determination in Vertebrates*, eds N. Valenzuela and V. Lance (Washington, DC: Smithsonian Institute), 79–89.

Harrison, J. F., and Fewell, J. H. (1995). Thermal effects on feeding behavior and net energy intake in a grasshopper experiencing large diurnal fluctuations in body temperature. *Physiol. Zool.* 68, 453–473. doi: 10.1086/physzool.68.3.30163779

Harrison, J. F., Phillips, J. E., and Gleeson, T. T. (1991). Activity physiology of the two-striped grasshopper, *Melanoplus bivittatus*: gas exchange, hemolymph acid-base status, lactate production, and the effect of temperature. *Physiol. Zool.* 64, 451–472. doi: 10.1086/physzool.64.2.30158185

Hodkinson, I. D. (2005). Terrestrial insects along elevation gradients: species and community responses to altitude. *Biol. Rev.* 80, 489–513. doi: 10.1017/S1464793105006767

Johansson, F., Watts, P. C., Sniegula, S., and Berger, D. (2021). Natural selection mediated by seasonal time constraints increases the alignment between

evolvability and developmental plasticity. *Evolution* 75, 464–475. doi: 10.1111/evo.14147

Levy, R. A., and Nufio, C. N. (2014). Dispersal potential impacts size clines of grasshoppers across an elevation gradient. *Oikos* 124, 610–619. doi: 10.1111/oik.01615

Lindestad, O., Wheat, C. W., Nylin, S., and Gotthard, K. (2019). Local adaptation of photoperiodic plasticity maintains life cycle variation within latitudes in a butterfly. *Ecology* 100:e02550. doi: 10.1002/ecy.2550

Lopatina, E. B., Kipyatkov, V. E., Balashov, S. V., and Kutcherov, D. A. (2011). Photoperiod-temperature interaction-a new form of seasonal control of growth and development in insects and in particular a Carabid Beetle, *Amara communis* (Coleoptera: Carabidae). *J. Evol. Biochem. Phys.* 47, 578–592. doi: 10.1134/S002209301106010X

McGuire, C. R., Nufio, C. R., Bowers, M. D., and Guralnick, R. P. (2012). Elevation-dependent temperature trends in the rocky mountain front range: changes over a 56-and 20-year record. *PLoS One* 7:e44370. doi: 10.1371/journal.pone.0044370

Miller, G. A., Clissold, F. J., Mayntz, D., and Simpson, S. J. (2009). Speed over efficiency: locusts select body temperatures that favour growth rate over efficient nutrient utilization. *Proc. R. Soc. Lond. B Biol. Sci.* 276, 3581–3589. doi: 10.1098/rspb.2009.1030

Norling, U. (2018). Constant and shifting photoperiods as seasonal cues during larval development of the univoltine damselfly *Lestes sponsa* (Odonata: Lestidae). *Int. J. Odonatol.* 21, 129–150. doi: 10.1080/13887890.2018.1462263

Nufio, C. R., and Buckley, L. B. (2019). Grasshopper phenological responses to climate gradients, variability, and change. *Ecosphere* 10:e02866. doi: 10.1002/ecs2.2866

Nufio, C. R., McGuire, C. R., Bowers, M. D., and Guralnick, R. P. (2010). Grasshopper community response to climatic change: variation along an elevational gradient. *PLoS One* 5:e12977. doi: 10.1371/journal.pone.0012977

Otte, D. (2012). Eighty new *Melanoplus* species from the United States (Acrididae: Melanoplinae). *Trans. Am. Entomol. Soc.* 138, 73–167.

Parmesan, C. (2006). Ecological and evolutionary responses to recent climate change. *Annu. Rev. Ecol. Evol. Syst.* 37, 637–669. doi: 10.1146/annurev.ecolsys.37.091305.110100

Pinheiro, J., Bates, D., DebRoy, S., and Sarkar, D., and R Core Team. (2020). *nlme: Linear and Nonlinear Mixed Effects Models*. R package version 3. 1–149. Available online at: https://CRAN.R-project.org/package=nlme

Seebacher, F., White, C. R., and Franklin, C. E. (2015). Physiological plasticity increases resilience of ectothermic animals to climate change. *Nat. Clim. Chang.* 5, 61–66. doi: 10.1038/nclimate2457

Sgro, C. M., Terblanche, J. S., and Hoffmann, A. A. (2016). What can plasticity contribute to insect responses to climate change? *Annu. Rev. Entomol.* 61, 433–451. doi: 10.1146/annurev-ento-010715-023859

Slatyer, R. A., Schoville, S. D., Nufio, C. R., and Buckley, L. B. (2020). Do different rates of gene flow underlie variation in phenotypic and phenological clines in a montane grasshopper community? *Ecol. Evol.* 10, 980–997.

Sniegula, S., Golab, M. J., Drobniak, S. M., and Johansson, F. (2016). Seasonal time constraints reduce genetic variation in life-history traits along a latitudinal gradient. *J. Anim. Ecol.* 85, 187–198. doi: 10.1111/1365-2656.12442

Springate, S., and Thomas, M. B. (2005). Thermal biology of the meadow grasshopper, *Chorthippus parallelus*, and the implications for resistance to disease. *Ecol. Entomol.* 30, 724–732. doi: 10.1111/j.0307-6946.2005.00743.x

Telemeco, R. S., Abbott, K. C., and Janzen, F. J. (2013). Modeling the effects of climate change–induced shifts in reproductive phenology on temperature-dependent traits. *Am. Nat.* 181, 637–648. doi: 10.1086/670051

Trudgill, D. L. (1995). Why do tropical poikilothermic organisms tend to have higher threshold temperatures for development than temperate ones? *Funct. Ecol.* 9, 136–137.

Verberk, W. C. E. P., Atkinson, D., Hoefnagel, K. N., Hirst, A. G., Horne, C. R., and Siepel, H. (2021). Shrinking body sizes in response to warming: explanations for the temperature–size rule with special emphasis on the role of oxygen. *Biol. Rev.* 96, 247–268. doi: 10.1111/brv.12653

Vitasse, Y., Signarbieux, C., and Fu, Y. H. (2018). Global warming leads to more uniform spring phenology across elevations. *Proc. Natl. Acad. Sci. U.S.A.* 115, 1004–1008. doi: 10.1073/pnas.1717342115

Walters, R. J., and Hassall, M. (2006). The temperature-size rule in ectotherms: may a general explanation exist after All? *Am. Nat.* 167, 510–523. doi: 10.1086/501029

West-Eberhard, M. J. (2003). *Developmental Plasticity and Evolution*. Oxford: Oxford University Press. doi: 10.1093/oso/9780195122343.001.0001

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The Potential for Physiological Performance Curves to Shape Environmental Effects on Social Behavior

Shaun S. Killen[1], Daphne Cortese[1], Lucy Cotgrove[1], Jolle W. Jolles[2], Amelia Munson[1] and Christos C. Ioannou[3]*

[1]*Institute of Biodiversity, Animal Health and Comparative Medicine, University of Glasgow, Glasgow, United Kingdom,*
[2]*Center for Ecological Research and Forestry Applications (CREAF), Campus de Bellaterra (UAB), Barcelona, Spain,*
[3]*School of Biological Sciences, University of Bristol, Bristol, United Kingdom*

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***Correspondence:**
Shaun S. Killen
shaun.killen@glasgow.ac.uk

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As individual animals are exposed to varying environmental conditions, phenotypic plasticity will occur in a vast array of physiological traits. For example, shifts in factors such as temperature and oxygen availability can affect the energy demand, cardiovascular system, and neuromuscular function of animals that in turn impact individual behavior. Here, we argue that nonlinear changes in the physiological traits and performance of animals across environmental gradients—known as physiological performance curves—may have wide-ranging effects on the behavior of individual social group members and the functioning of animal social groups as a whole. Previous work has demonstrated how variation between individuals can have profound implications for socially living animals, as well as how environmental conditions affect social behavior. However, the importance of variation between individuals in how they respond to changing environmental conditions has so far been largely overlooked in the context of animal social behavior. First, we consider the broad effects that individual variation in performance curves may have on the behavior of socially living animals, including: (1) changes in the rank order of performance capacity among group mates across environments; (2) environment-dependent changes in the amount of among- and within-individual variation, and (3) differences among group members in terms of the environmental optima, the critical environmental limits, and the peak capacity and breadth of performance. We then consider the ecological implications of these effects for a range of socially mediated phenomena, including within-group conflict, within- and among group assortment, collective movement, social foraging, predator-prey interactions and disease and parasite transfer. We end by outlining the type of empirical work required to test the implications for physiological performance curves in social behavior.

Keywords: physiology, environmental change, individual heterogeniety, individual differences, phenotypic plasticity, social grouping, individual variation

INTRODUCTION

Within species there exists considerable among-individual variation in numerous physiological traits associated with energy demand (Burton et al., 2011; Metcalfe et al., 2016), cardiorespiratory systems (Walsberg et al., 1986; Brijs et al., 2019), and neuromuscular function and movement (Wilson et al., 2004; Marras et al., 2010). A major aim in the field of ecophysiology is to understand how these traits are linked with organismal performance and behavior in an ecological context, including the ability to escape predators and obtain resources (Jablonszky et al., 2017; Mathot et al., 2017; Killen et al., 2017a). More recently, there has been growing interest in how among-individual heterogeneity in physiological traits can modulate animal social behavior, including social hierarchies (Kochhann, 2017), social networks (Moyers et al., 2018), and emergent collective behavior and group functioning (Jolles et al., 2017, 2020a). While there is a growing appreciation for the physiological underpinnings of social behavior (Seebacher and Krause, 2017), a more nuanced understanding of how environmental factors influence differences between individuals across a gradient is needed to accurately predict how social groups will respond to changing environments.

Social grouping ranges from pairs of animals to large scale communities and enormous aggregations consisting of millions of individuals. Variation in this tendency to group, both at the individual and species level, can be explained by the balance between the benefits of reducing predation risk, improving foraging and saving energy during locomotion, vs. the costs of competition within groups over food and the opportunity to breed, and a greater exposure to socially-transmitted diseases. These benefits and costs can be shifted, however, by individuals' behavior within groups, with effects on social interactions and group functioning (Jolles et al., 2017; del Mar Delgado et al., 2018). However, increasing evidence suggests that social behavior is also related to physiological traits associated with individual's metabolic phenotype (Killen et al., 2017b; Cooper et al., 2018), stress responsiveness (Spencer, 2017), cognition (Wascher et al., 2018), locomotor performance and movement speed (Jolles et al., 2017; Hansen et al., 2020), and immune function (Raulo et al., 2018). Physiological traits associated with bioenergetics and locomotion may be especially important in this regard because they are sensitive to environmental factors and can also influence performance in a social context, affecting both the capacity and motivation to express various behaviors. Metabolic rate, for example, has been linked with dominance and risk-prone behaviors (Mathot et al., 2019), which in turn have links with individual sociability (Jolles et al., 2017). There is also evidence of direct links between metabolic demand and sociability, with individuals with a higher metabolic rate being perhaps less social and therefore less likely to associate with conspecifics (Killen et al., 2016b; Cooper et al., 2018; but see Killen et al., 2021).

Social interactions can be influenced by environmental factors such as food abundance and potential predation risk (Beauchamp, 2004; Schaerf et al., 2017), but also by many aspects of the abiotic environment, including light levels (Ginnaw et al., 2020), temperature (Bartolini et al., 2015), hypoxia (Domenici et al., 2017), turbidity (Chamberlain and Ioannou, 2019), and habitat structure (Takada and Minami, 2021), as well as by anthropogenic changes such as acoustic noise (Currie et al., 2020), and pollutants (Armstrong et al., 2011). While environmental factors can impact behavior through the masking of cues and signals (McNett et al., 2010) and shifting attention to other tasks (Chan et al., 2010), environmental conditions can also affect behavior *via* physiological changes. The effects of environmental variables on social behavior *via* physiological changes can be indirect by inducing stress *via* stress hormones, or can directly affect the physiological traits associated with locomotor performance and movement speed, such as muscular function and aerobic and anaerobic capacity (Ord and Stamps, 2017). Because movement speed plays a fundamental role in leadership, cohesion, and alignment (Pettit et al., 2015; Jolles et al., 2020b), these aspects of social behavior may be sensitive to environmental perturbations. Over various timescales, changing environmental conditions will influence physiological trait expression, which will in turn affect social behavior and the degree of among-individual trait variation and trait repeatability (Killen et al., 2016a; Huang et al., 2020). These effects of environmental conditions on social behavior are becoming increasingly important to understand due to human-induced rapid environmental change (Sih, 2013; Barrett et al., 2019; Fisher et al., 2021).

Breakthroughs in our understanding of the mechanistic underpinnings of sociality could be facilitated by studying the effects of individual performance curves on social dynamics. Functionally, performance curves are a type of reaction norm, but specifically depict shifts in fitness or a putative fitness proxy—often a physiological variable—across a continuous gradient of an environmental variable (as opposed to discrete measurements at specific environmental conditions), and generally exclude developmental effects on phenotypes to instead focus on relatively recent changes in environmental conditions (Kingsolver et al., 2014). Performance curves are therefore usually nonlinear—though they may appear linear within narrow environmental ranges—with their exact shape depending on the trait and environmental variable being considered (Kingsolver et al., 2014; **Figure 1A**). Such curves are generally determined for specific physiological traits or performance indices, expressed as biological rates, such as maximum locomotor speed or aerobic capacity, with performance defined as the capacity to express a given trait across a range of environmental conditions. As an example, in ectotherms a typical performance curve for maximum locomotor speed would be a gradual increase with temperature, a peak level of performance at an optimal temperature, followed by a decline in performance capacity with further warming (yellow line in **Figure 1A**). Performance curves often depict the change of a physiological trait in response to the environment and can therefore reflect environmental sensitivity (Kingsolver and Gomulkiewicz, 2003; Lefevre, 2016; Jutfelt et al., 2018). This sensitivity may, in turn, affect the capacity or motivation to perform specific behaviors, but these links are often uncertain and the focus of study to provide insight into intra- and

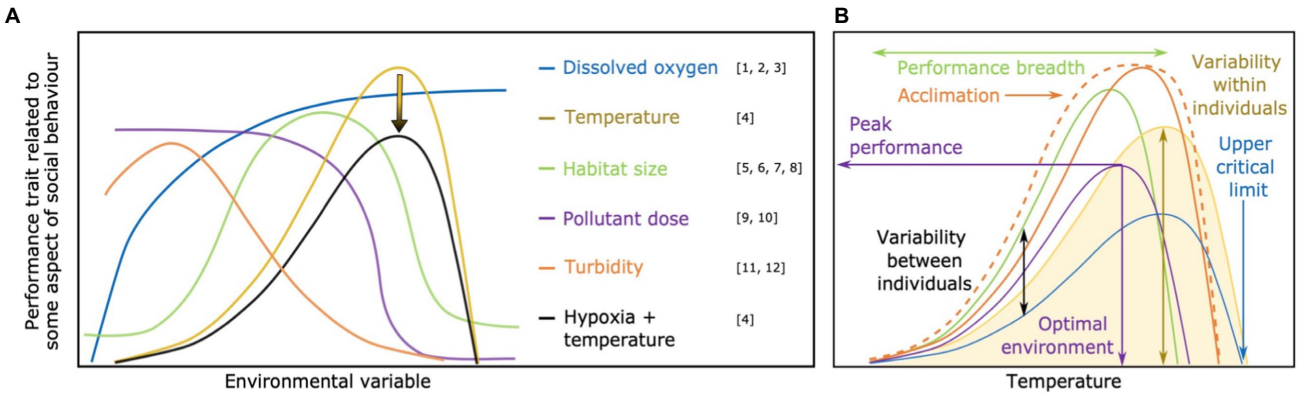

FIGURE 1 | (A) Performance curve shape is heavily dependent on the environmental factor being examined. In this panel, different types of environmental factors are represented by different colors. The arrow represents an overall depression of trait expression when potential effects of hypoxia are combined with the effects of temperature. Note, when habitat size increases, greater protection/space to hide from predators and/or increase food availability may enhance performance, thus reducing endocrine stress level (Breves and Specker, 2005; Bauer et al., 2013). However, when territory is very large the performance traits may be reduced again in territorial animals (e.g., anemonefish; Ross, 1978) due to increased stress and/or energy investment to protect a larger area from competitors or predators. **(B)** Potential effects of among-individual variation in performance curves for a trait related to the expression of social behavior (e.g., aerobic capacity, cognitive ability, locomotor capacity, muscular function) in response to temperature (environmental variable). In this panel, the performance curve of different individuals within a social group are represented in different colors. The dashed orange line shows variation in the performance curve (solid orange line) caused by acclimation to the environmental variable (temperature in this example). Acclimation generally results in an overall "flattening" of the performance curve, but may also cause an increase in the peak performance. Arrows illustrate the different points of individual variation in performance curve that have implications for animal social behavior, especially in ectotherms. Each point and its consequence on social behavior is highlighted in **Figures 2**–**7**. References: [1] Barrionuevo and Burggren (1999); [2] Fry (1971); [3] Pörtner (2010); [4] Pörtner and Farrell (2008); [5] Maierdiyali et al. (2020); [6] Bauer et al. (2013); [7] Breves and Specker (2005); [8] Ross (1978); [9] Gomez Isaza et al. (2020); [10] McKenzie et al. (2007); [11] Meager et al. (2006); [12] Chamberlain and Ioannou (2019).

intergenerational responses to environmental stressors (Metcalfe et al., 2016; Norin and Metcalfe, 2019).

Here, we argue that performance curves, and especially individual variation in performance curves within groups (**Figure 1B**), may be key in understanding how social behaviors are affected by shifting environmental conditions. In their natural environment, socially grouping animals can experience environmental changes at a scale of minutes, days, or months, but will also experience environmental changes over more protracted timeframes in response to broadscale phenomena such as climate change. For example, many animal species accommodate seasonal changes in temperature that are consistent across years, but due to human-induced climate change, such changes are becoming more extreme (IPCC, 2019). A more mechanistic, physiologically-based approach to the study of social behavior will be key for understanding how routine environmental shifts affect social behaviors as well as predict how social behavior may change or evolve in response to anthropogenic disturbances.

The study of animal social systems and particularly the study of collective behavior has transitioned from a focus on uncovering universal mechanisms underpinning emergent behavior and self-organization (Couzin et al., 2002), to an increasing recognition that among-individual heterogeneity plays a critical role in these processes (del Mar Delgado et al., 2018; Jolles et al., 2020a). We suggest that a promising next step in this line of research will be to examine how the degree of heterogeneity *itself* can change depending on the environment—as is dictated by individual performance curves—and how this will influence various dimensions of animal social behavior. We first discuss the broad effects that individual variation in performance curves within social groups may have on the relative physiological capacity and behavioral motivation of individuals within social groups. Next, we discuss the specific consequences of these effects for an array of ecological phenomena related to social behavior including within-group conflict, leader-follower dynamics, predator avoidance, and social foraging. Our aim is to highlight the enormous potential for performance curves to alter social behavior at the individual, group, and community level and outline priority areas for future research.

INDIVIDUAL VARIATION IN PERFORMANCE CURVES

A key factor to consider when assessing the impact of performance curves on social behavior is among-individual variation in how animals physiologically respond to changes in their environment (Bulté and Blouin-Demers, 2006). For example, different individuals can show different physiological sensitivities to factors such as temperature (Navas et al., 1999), or requirements in terms of oxygen (Killen et al., 2012b; Pang et al., 2015) or nutrition (Killen et al., 2011), with direct effects on among-individual variation in bioenergetics and capacity for locomotor performance. Such variation has traditionally been examined in the context of reaction norms whereby individuals are repeatedly measured for traits at around 2 or 3 environmental levels and modelled using mixed-models with random slopes (Dingemanse et al., 2010). However, assumptions of linearity may not be appropriate for all traits and particularly

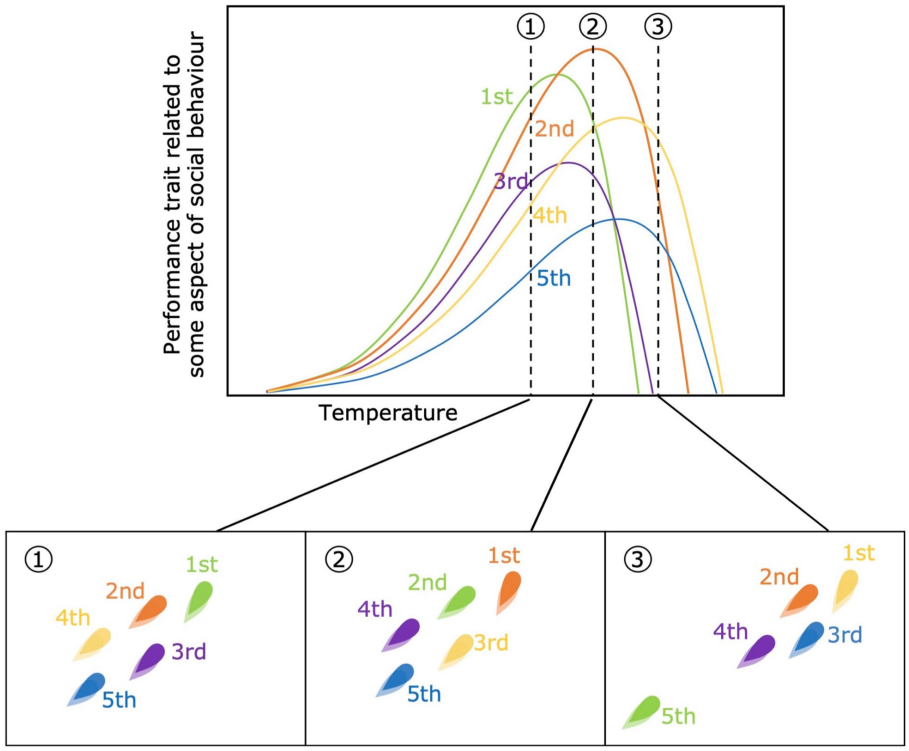

FIGURE 2 | Changes in the rank order of performance capacity across three different temperatures (top panel). Each color refers to an individual within the same social group. In the bottom panels the rank-assortment within the group is shown for each temperature (1, 2 and 3), assuming that higher-ranked individuals are positioned on the front of the group. For example, the green individual is the highest rank-individual (leader) at temperature 1, but a follower with 2nd rank position at temperature 2, and is no longer part of the group at temperature 3, given that the individual's performance capacity decreases to 0 before temperature 3, while the rest of the groups has not.

over broader environmental ranges. Therefore, to properly assess intra-individual variation in environmental sensitivity, the assessment of individual performance curves may be required (Gilbert and Miles, 2017). This work is still in its infancy, but investigations to date indicate that, similar to the case with linear reaction norms (van de Pol, 2012; Roche et al., 2016), individuals within species show variation in performance curves (Careau et al., 2014; Bartheld et al., 2017; Childress and Letcher, 2017; Nowakowski et al., 2020). There is also evidence that there may be within-individual variation in performance curves, in response to factors such as recent feeding history (Gilbert and Miles, 2016), which adds an extra layer of complexity.

If individual animals show variable degrees of behavioral and physiological plasticity in response to environmental variables, this has a wide range of potential consequences for social behavior. To illustrate this, consider among-individual variability in performance curves for a physiological trait (e.g., aerobic capacity or optimum movement speed) relevant to social behavior, in relation to some environmental variable (e.g., temperature; **Figure 1B**; van Berkum, 1988). There are numerous effects that emerge from individual variation in environmental sensitivity that could have important consequences for how individuals interact with each other within social groups, which we discuss in detail below. Important to consider for any of these effects is the influence of acclimation to environmental conditions. During acute environmental changes, such as in temperature or oxygenation, individual animals tend to show much stronger changes in the expression of their physiology or behavior (Guderley, 1990). These responses generally dampen with physiological acclimation to the new conditions (i.e., specific points along a performance curve), resulting in an overall "flattening" of the performance curve. Depending on the acclimation response of each individual groupmate and on the timescale of exposure to a given environment, the relative importance of each of the following considerations may change in prominence.

Changes in the Rank Order of Performance Capacity

Differences in sensitivity to the environmental variable in question may generate differences in the rank order of performance capacity among individuals within a social group that directly depends on where along the environmental gradient performance is being measured (**Figure 2**). All else being equal, differences in this rank order could mean that, for example, the individual most likely to be dominant or a leader at one temperature may be subordinate or a follower at another temperature. Aside from having a direct effect on the social behaviors displayed by individuals, changes in the rank order

of traits will also decrease their repeatability and, potentially, the ability of that trait to be a target for selection in a social context. Another key consideration is that, if relative differences in energy demand (related to food-acquisition) or locomotor ability (related to predator avoidance) change among individuals, then the fundamental costs and benefits of sociality and group membership could change differently for individual group members depending on the current environmental conditions (Cooper et al., 2018). As an example, at higher temperatures all individuals may be expected to become less social due to an increased escape ability, *via* increased muscle contractile ability and nervous stimulation (Johnson and Bennett, 1995), and decreased motivation to share or compete for discovered resources. Thereby differences in the steepness of individual performance curves could influence when specific individuals no longer benefit from staying in a group.

Change in Among-Individual Variation

As individual performance curves diverge or converge along the environmental gradient, the amount of phenotypic variation among individuals will correspondingly change. At a low temperature, for example, there may be a modest degree of among-individual variation in movement speed while at a higher temperature there may be wider variation (Killen et al., 2013; **Figure 3**). This change in the degree of variation among-individuals within a social group could have consequences for group coordination, cohesion, or intra-group conflict (Jolles et al., 2020a). Changing environmental conditions and among-individual variation may therefore cause groups to split or merge, which in turn may impact the degree of phenotypic differences among groups. In particular, at environmental extremes, suitable habitats may become scarce, causing groups to merge and potentially homogenize. Importantly, changes in the amount of among-individual variation are fundamental in exposing traits to selective pressures in the social context (Farine et al., 2015).

Change in Within-Individual Variation

The effects of environmental conditions on variation among individuals may extend to physiological and behavioral flexibility within individuals. Depending on the environment, individuals may become more or less flexible in their behavioral expression. Physiological constraints at very low or high temperatures, for example, may limit the behavioral options available to individuals. At temperatures around their individual optimum, however, individuals should be less constrained and more able to express behavior based on moment-to-moment changes in their motivation (Jolles et al., 2020a; **Figure 4**). Changes in

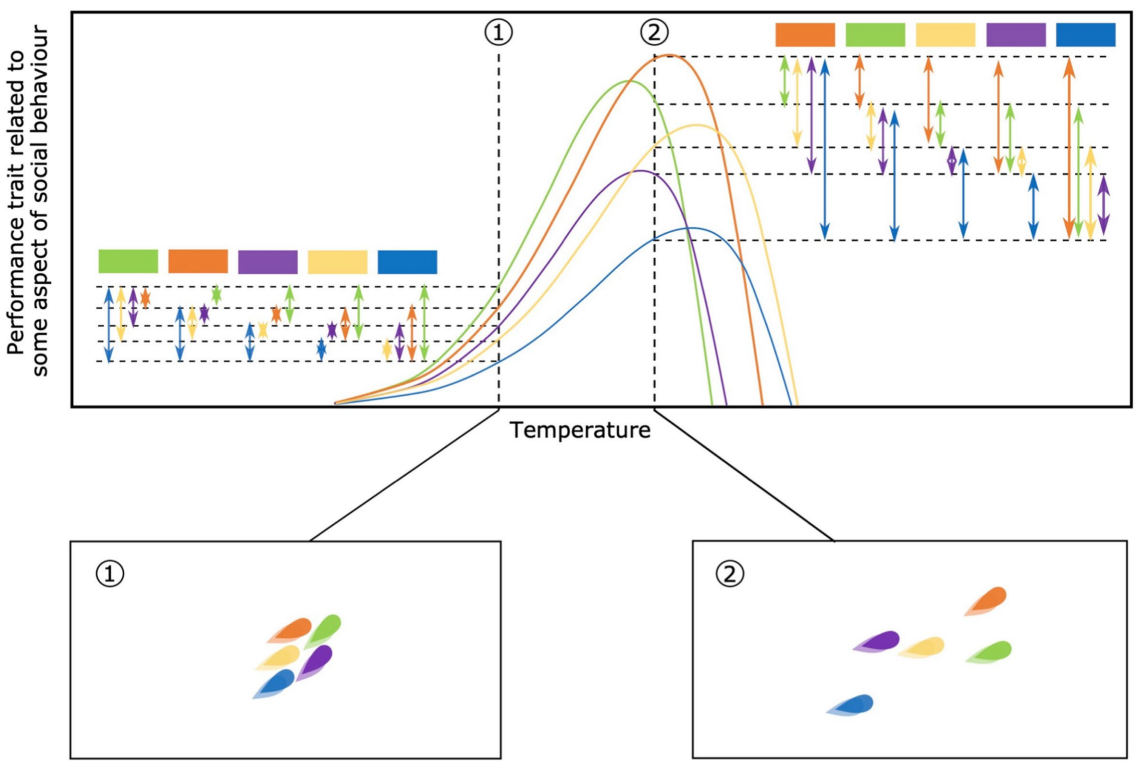

FIGURE 3 | Change in among-individual variation along the environmental gradient. On the top panel the among-individual variation is highlighted at 2 different temperatures, when individual performance curves converge (temperature 1) or diverge (temperature 2). Each color refers to an individual within the same social group. Square boxes represent individuals used as a reference to show the amount of variation. Arrows show the amount of variation between individuals. In the bottom panels is shown an example of the consequences of among-individual variation in performance curves on social groups. Wider variation could lead to less cohesion, i.e., higher distances among individuals within the same group, here shown at temperature 2 compared to temperature 1.

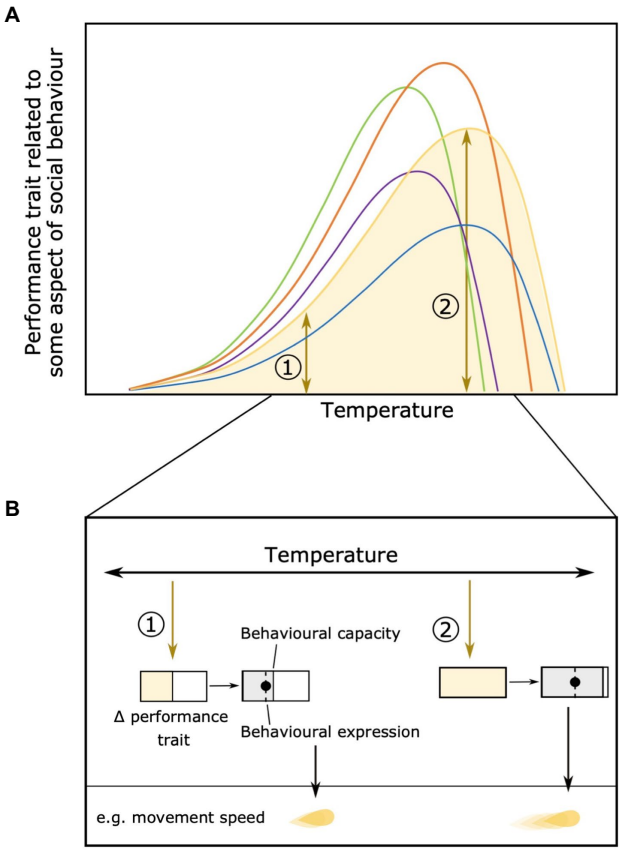

FIGURE 4 | Change in within-individual variation across an environmental gradient (e.g., temperature). The area below the performance curve indicates the variation in individual performance [**(A)**, individual in yellow used as an example]. Differences in individual variation in performance trait at two different temperatures [1 and 2 **(B)**] can result in different behavioral capacity and expression. For example, at temperature 1 the yellow individual has only little variation in performance and its behavior is only expressed as low movement speed, while at temperature 2 (close to its optimum) the same individual has a higher variation of movement and can move up to very high speeds. Panel **(B)** reproduced from Jolles et al. (2020a).

within-individual variation along performance curves could also have consequences for the ability of natural selection to act on that trait if there are changes in across- or within-context trait repeatability (Killen et al., 2016a).

Among-Individual Differences in Optimal Environments

Different individuals within a social group are likely to have different environmental conditions at which their individual performance is optimized (green and blue lines in **Figure 5A**). It is also possible that the environmental conditions selected by the individual (or the group as a whole) may have nothing to do with optimizing their performance within a social group. For example, a group may choose to occupy a given location based solely on the availability of food or some other resource. In that case, the environmental conditions present at that point in space and time will determine how close each individual is operating relative to their individually optimal conditions and maximum capacity (**Figures 5A,B**). While individuals may be able to acclimate to environmental conditions, differences in rate of acclimation could still lead to very different trait values among individuals within the same environment. One possible consequence is that individuals may fit into vastly different social niches depending on the physiological constraints they end up facing within the (local) environment of the social group.

Among-Individual Differences in Peak Performance Regardless of Environmental Optima

Even if measured at their optimum environmental conditions, individual group members will show different absolute peak levels of performance (orange and purple lines in **Figure 6**). Individuals are likely to try and take advantage of an increased performance potential and consequently influence their behavior and decision making within the context of the group. For example, an individual may choose to occupy a microhabitat within their group that brings that individual closer to its own peak performance capacity, or direct group movements to areas where that individual will derive an advantage due the local environmental conditions. For example, an individual that is relatively robust to variation in environmental oxygen availability (i.e., hypoxia; Killen et al., 2012b) could conceivably thrive socially in a moderately hypoxic environment if the competitive ability of its group-mates are reduced (although, the overall benefits of grouping for predator avoidance may decrease if overall group cohesion is impaired).

Among-Individual Differences in Performance Breadth and Critical Limits

Differences in performance curve shape may generate differences in the breadth over which individuals can function above particular thresholds of performance. For example, some individuals may be specialists (green individual in **Figure 7A**) and able to perform at a high level but only within a narrow environmental range, while others may be generalists (blue individual in **Figure 7A**) and able to perform over a wider range of environments but at a reduced absolute peak level of performance. The evidence for this trade-off between performance breadth and peak performance is however limited (Nati et al., 2016). There may also be among-individual differences in environmental tolerances of animals within a social group. Some individuals may simply be incapable of occupying the same environments as their conspecifics and even before this extreme point, have a sharper decline in performance (green and purple individual in **Figure 7B**). This variation in the breadth of environmental tolerance and critical thresholds for performance or survival should limit the habitats or environmental "options" available to groups with wide individual variation in such thresholds and may promote among-group assortment.

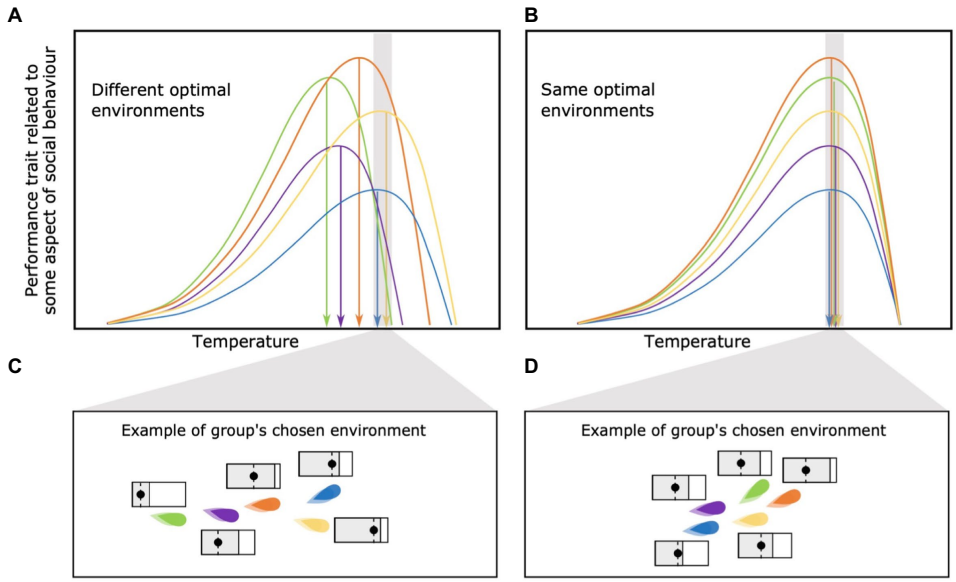

FIGURE 5 | (A) Among-individual differences in optimal environments vs. **(B)** equal optimal environment among individuals belonging the same social group. One of the consequences of among-individual differences in optimal environments is that individuals may fit into different social "niches," each with a different behavioral capacity and expression, depending on the physiological constraints they end up facing within the group's chosen environment **(C)**. On the other hand, an similar optimal environments may lead to behavioral conformity among individuals **(D)**.

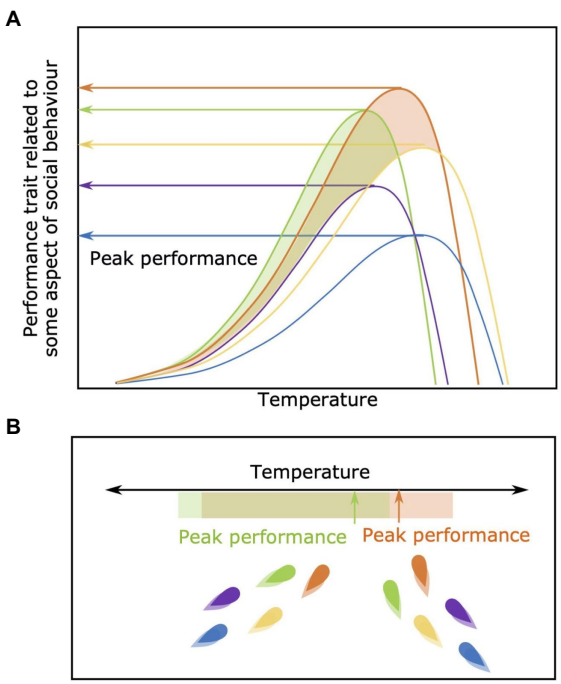

FIGURE 6 | Among-individual differences in peak performance regardless of optima. In panel **(A)** individuals show variation in their absolute performance capacity across temperatures. In panel **(B)** individuals green and orange have a higher peak performance compared to the other individuals within the group, regardless of the optimal temperature for each. This elevated peak allows these two individuals to have a higher relative capacity for performance even if they are deviating from their own specific optimal temperature.

EFFECTS ON SOCIAL INTERACTIONS

Within-Group Competition and Conflict

Many social systems include dominance hierarchies, whereby individuals with greater resource holding potential have improved access to food, mates and/or other resources, and can often be found in locations within the group that reduce their risk of predation (Ward and Webster, 2016). Physiological traits are known to be important in the contests that establish dominance, as they correlate with competitive ability and also constrain the frequency, duration and intensity of contests, due to the build-up of lactic acid, for example, which limits anaerobic capacity (Briffa and Sneddon, 2007). A higher dominance status in contests has been shown to be associated with higher heart rate (Turbill et al., 2013), metabolic rate (Mccarthy, 2001), and aerobic scope (Killen et al., 2014). In turn, environmental variables can affect aggressive interactions *via* effects on physiology. For example, in cooler water, the cichlid fish *Cichlasoma paranaense* reduces aggressive interactions (Brandão et al., 2018), and the duration of fights between shore crabs (*Carcinus maenas*) is reduced in hypoxic conditions, associated with a greater accumulation of lactic acid during fights in hypoxia (Sneddon et al., 1998). Individuals experiencing cooler temperatures can compensate for reduced locomotor performance, however, through elevated aggression and be just as likely to win contests, as demonstrated in velvet geckos (*Oedura lesueurii*; Kondo and Downes, 2007).

Although these previous studies have shown that environmental variables can affect average levels of antagonistic

interactions, variation in performance curves suggests that differences between individuals in resource holding potential and other forms of competitive ability (e.g., the ability to detect food sooner than others) is plastic, being dependent on the prevailing environmental conditions. This may mean, for example, that under some environmental conditions, individuals are more closely matched in fighting ability, which tends to result in more frequent, longer, and more intense contests (Schmitz and Baldassarre, 1992; Hack et al., 1997). Under other environmental conditions, differences in competitive ability between individuals may be magnified, resulting in clear winners, where contests are infrequent and easily won before they escalate. In cases where environmental changes over time are large enough to alter the rank order of physiological performance that determines dominance status, aggression may be more frequent and the dominance hierarchy less stable, which may explain changes in hierarchy stability with temperature (Kochhann et al., 2015). Changes in dominance may also be delayed or may not occur at all if there are carryover effects whereby a dominant individual is more likely to stay dominant (Hock and Huber, 2009), even if the environmental conditions become less favorable for its own phenotype. For example, established social rank can weaken the importance of physiological performance for competitive interactions in some species (Miln et al., 2021).

Even without dominance hierarchies and aggression, individuals within groups also consistently differ in their ability to exploit resources, such as food, during scramble competition (David et al., 2011). These individual differences can be explained by physiological processes that affect motivation for the resource, sensory perception of the resource and locomotory performance to acquire the resource before competitors (Miln et al., 2021). Differences among individuals in physiological performance curves will affect how resources are distributed within groups depending on the current environmental conditions. In environments where the variability in physiological performance of individuals is reduced, the distribution of resources to individuals will be more even, and the opposite effect is expected when environmental conditions magnify inter-individual variation within groups in physiological performance. If environmental conditions are varying at an appropriate scale over time, changes in the physiological performance rank of individuals may mean that average levels of resource use over time are more similar within the group than at any single time point. It is worth noting that under natural conditions, the availability of some resources is also dependent on environmental conditions. For example, scramble competition increases in colobus monkeys (*Colobus vellerosus*) within groups during the wet season when preferred foods are scarce (Teichroeb and Sicotte, 2018). Thus, the abundance of a resource over an environmental gradient must be considered alongside the physiological performances over that gradient. If, for example, the variability between individuals in performance during scramble competition is greatest when resources are highly abundant and not limiting, we may not expect to see a relationship between food intake and physiological performance, which may instead be seen when variability between individuals is reduced, but resources are highly limited.

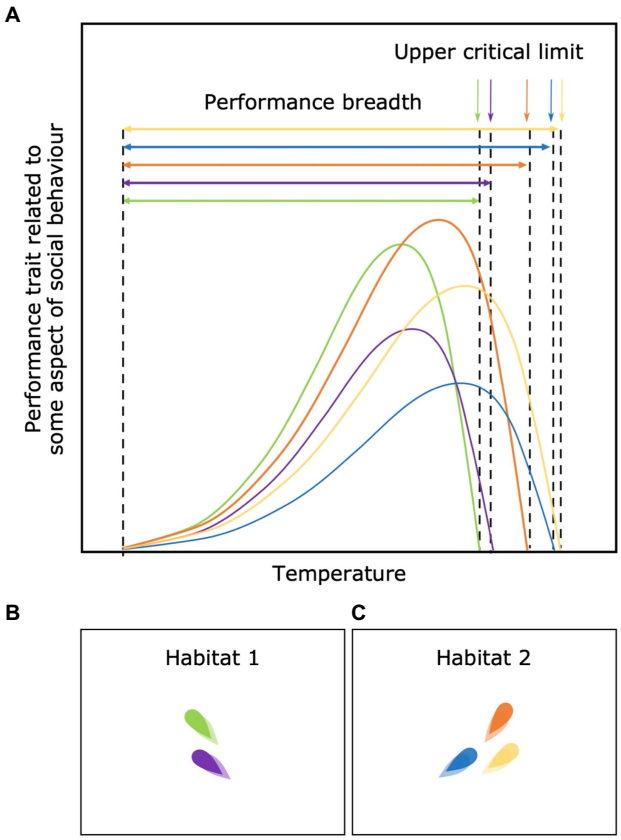

FIGURE 7 | Among-individual differences in performance breadth and critical limits **(A)** and its consequences **(B,C)**. Variation in the breadth and critical thresholds limit the options of habitats available for each individual and promote among-group assortment [e.g. **(B,C)**].

In groups without clear dominance hierarchies, more subtle forms of conflict can occur without obvious aggression or scramble competition. Groups often make decisions regarding when, where and how to move, which requires coordination to maintain cohesion of the group. Multiple sources of variation between individuals within groups, whether short-term and transient (Kerth et al., 2006) or long-term and consistent (Bevan et al., 2018), have the potential to result in conflict over these collective decisions that require consensus (Conradt, 2012). In contexts such as when behaviors should be performed, compromise can be reached; in others where behavioral decisions are mutually exclusive, such as where to travel to, compromise is not possible (Wade et al., 2020). In this latter case, the 'consensus costs' paid by individuals who do not get their preferred outcome should, on average, be higher than when compromise is possible (Conradt and Roper, 2009). If such consensus costs are too high relative to the benefit of remaining with the group, groups can split (Ioannou et al., 2015). As the extent of variation between individuals often determines the extent of conflicting preferences within groups, variation in physiological performance curves would mean that the degree of conflicting preferences will be sensitive to environmental

conditions. When environmental conditions result in reduced variation between individuals in physiological performance, preferences should be similar and this reduced within-group conflict should result in fast decisions and more cohesive, coordinated groups. In contrast, if greater physiological differences result in conflicting preferences, decisions are predicted to be slower, and the group may change their decision more frequently or even split. For example, the speed of travel of a group can be determined by the physiological performance of the group members, and a consensus decision on that speed will be easier when preferred speeds, based on physiological performance capacities, are similar (Sankey et al., 2019). A potential outcome is that groups may be quicker to make consensus decisions in relatively harsh or extreme environments when performance capacity is limited or among-individual variation is constrained.

Social Niches and Social Conformity

While performance curves typically represent the maximum capacity that an individual has for a given physiological performance metric, individuals do not always opt to perform at their maximum capacity. This is partly because individuals within groups may need to coordinate behavior by either conforming to the group average or matching the behavior of a particularly influential individual (Brown and Irving, 2014; McCune et al., 2018). Alternatively, competition within groups can cause initial individual heterogeneity among group members to become amplified over time due to character displacement (the "social niche hypothesis"; Bergmüller and Taborsky, 2010; Montiglio et al., 2013, Jolles et al., 2020a). Previous research has attempted to determine whether conformity or the social niche hypothesis is a larger driver of behavior within social groups (Munson et al., 2021), however, changes in the environmental context can either constrain or expose phenotypic variation such that behavioral conformity or differentiation within a group is more or less possible in different environments. For example, behaviors may appear to conform if interindividual variation in performance curves is low and there are limited differences in potential performance. Alternatively, social niche formation should be optimized in environments where the differences in performance curves are the highest because there are the greatest initial differences in individual capacity for behavior.

Social dynamics may influence behavior to such an extent that individuals do not perform at their optimum across environmental contexts *via* behavioral conformity and the formation of social niches. This could have important feedbacks on differences in responses to changing environments despite individual performance curves. Even as the environment changes, individuals may be constrained by social dynamics to behaving similarly (or dissimilarly) from other group members, the predicted changes in performance based on individual performance curves may not be evident. For example, if fish conform to slower individuals in a group that also do not change as rapidly in their swim speed in response to changes in the environment, then the whole group will be limited in how much they respond to changes in the environment. Similarly, behavioral conformity and social niche formation should limit acclimation to environmental change within an individual. Even if an individual's potential performance in one environmental context changes over time, they may not change their behavior if they are constrained to behaving similarly (or dissimilarly) from group members. Experiments that test performance curves for individuals alone and in groups would help to elucidate the influence of the group on individual performance across changing environments.

Among and Within-Group Assortment

Animal groups are generally not randomly composed in nature, with individuals tending to assort according to various characteristics including body size, sex, age, or morphology (Krause et al., 2000; Jolles et al., 2020a). Animals both assort at the among-group level, with different phenotypes occurring in different groups, and the within-group level, with individuals occupying different spatial locations according to their phenotype and/or non-randomly interacting with similar individuals within the group. Furthermore, animals assort both actively, with individuals selecting which individuals they associate with, or passively, with individuals exhibiting spatiotemporal overlap due to shared habitat selection or attraction to a resource (Killen et al., 2017b). The potential influence of individual metabolic traits and locomotor capacity on among- and within-group assortment have been discussed in depth elsewhere (Killen et al., 2017b), sometimes in relation to sex-based differences in physiology and associated locomotor capacity and habitat preferences (Conradt and Roper, 2000; Ruckstuhl and Neuhaus, 2006), but there are a range of circumstances where the performance curves in particular could play an important role in these processes.

As environmental conditions change, differences in individual performance curves could lead to an increase or decrease in within-group variation in performance capacity. For example, environmental conditions may increase group movement speed and thereby lead to more within-group spatial assortment, such as slower individuals occupying posterior positions within the group. This has been observed in fish schools, in which the flow of water increasingly leads to individuals with lower aerobic scope to occupy positions in the back of the group (Killen et al., 2012a). Such effects could be further amplified or reduced depending on interactions among multiple environmental factors, such as that faster flowing water may carry more oxygen, which may thereby partly reduce assortment effects caused by the increased water flow rate. In contrast, an increase in water temperature may generate increased variation in locomotor capacity among group members and thereby enhance such assortment effects. In environments that produce greater amounts of variation among individuals within groups, groups may even split according to performance capacity, essentially leading to among-group assortment based on individual sensitivities to a particular environmental variable.

Among individual differences in environmental optima, tolerance breadths, or habitat preferences may also cause

among-group assortment. For example, individual sensitivity to hypoxia stemming from performance curves may dictate which individuals occupy specific habitats or depths in aquatic environments (Joyce et al., 2016), and thus which conspecifics are available for them to interact with socially. Differences in energy requirements due to performance curves may also cause individuals to select different habitats and therefore spatially segregate (Michelangeli et al., 2018). Among-individual variation in changes in maintenance or active metabolism at different temperatures could cause individuals with a lower energy demand to select safer habitats, even if it means less access to food. Individuals with steeper increase in energy demand in response to temperature, however, may choose riskier habitats if it grants them increased access to food, and thereby group with individuals with a similar physiological and behavioral phenotype.

Leader/Follower Dynamics

Choices in social group behavior (e.g., movement or a feeding event) can be reached by egalitarianism where all individuals reach consensus, or can be initiated by one or few individuals (i.e., leaders; Conradt and Roper, 2009). Leaders are only successful if followed by other group members, instigated voluntarily or as a result of hierarchical influence or dominance. Leaders in these groups often have better access to resources and make decisions for the group which may be at cost to others (King et al., 2008; although see McComb et al., 2001). In self-organized moving groups, leadership has been shown to propagate from the front of the group (Bumann and Krause, 1993; Nagy et al., 2010). Front positions are thought to be occupied by individuals who have more information about the surrounding environment or a greater need for resources and motivation to locate preferable environments (Ioannou et al., 2015), therefore leadership can depend on resource requirements linked to body size and sex (Fischhoff et al., 2007; Bierbach et al., 2020). The group members that successfully lead others and achieve their preferred outcome may be those with the highest physiological performance, for example those with the greatest aerobic capacity (Killen et al., 2012a) who can sustain more energetically-demanding positions or be better able to escape from attacks by predators, both costs of leadership associated with being at the front of moving groups (Ioannou et al., 2019). The ability to lead through spatial position or behavioral signaling could thus be constrained by physiological capacity, governed by an individual's performance curve. However, the optimal leader may differ across environmental conditions. For example, it has been proposed that under benign conditions, individuals with the lowest metabolic rate and aerobic scope may become leaders as a way for the group to maintain high levels of cohesion, whereas under environmental stress individuals with a higher performance capacity take on leadership roles (Ward et al., 2018).

What is particularly interesting when considering group movement and physiological performance curves is that group movement may result in substantial changes to the environment that individuals experience. Those with greater influence on group movement may lead the group to locations with environmental conditions that improves (either absolutely or relatively to others in the group) their physiological performance, which may reinforce their position as leader. On the other hand, leaders' preferred locations may be driven by factors other than their physiological performance, and due to inter-individual variation in physiological performance curves, a changed environment may shift which individual is most physiologically capable to lead subsequent group decisions. If groups are moving between locations which vary considerably in environmental parameters, individuals with narrower environmental tolerances may have the greatest motivation to lead, as they are likely to experience greater consensus costs if collective decisions take the group into locations of unpreferred environmental conditions. Additionally, other group members with wider tolerances may be less affected by environmental conditions, and may have less motivation to lead the group, despite potentially having a higher peak performance in changing environments. As the group encounters a less optimal environmental gradient then a leader's capacity to lead may decrease due to variation in environmental tolerance. Moreover, if individual capacity to lead changes with performance curves, individuals may be more influential in different environments and could cause a switch in leadership from one individual to another. Alternatively, multiple individuals with similar performance curves could have the capacity to lead when experiencing a change in environment, causing a disruption to hierarchy and may lead to group splitting if the cost to staying with a group is too large (Ioannou et al., 2015).

Collective Dynamics

Collective patterns, including the speed, alignment, synchronization, and movement tendency of animal groups, emerge *via* self-organizing mechanisms from the behavior and interactions of the individual group members (Couzin et al., 2002; Couzin and Krause, 2003, 2002). Hence, the phenotypic composition of groups, including the average behavior of and heterogeneity among group members, and its change over time, may strongly impact on collective dynamics (del Mar Delgado et al., 2018; Jolles et al., 2020a). Furthermore, changes in individual behavior and the interactions among grouping individuals in response to their environment coincides with changes in group-level patterns (Schaerf et al., 2017). Both the movement speed and social responsiveness of individuals are strongly linked to a range of physiological characteristics that may change depending on the environment, and thereby impact collective dynamics. For example, at higher temperatures, ectothermic animals may have less aerobic scope available, reducing their optimal and preferred movement speed and in turn result in slower, but potentially more cohesive groups. Alternatively, temperatures colder than optimal may also increase cohesion if overall activity is reduced *via* effects on individual performance curves (Bartolini et al., 2015). Similarly, changes in oxygen availability may differently impact the muscular functioning of individuals and, by changes in movement speed, impact collective dynamics.

Importantly, if individuals are far from their performance optimum, this could negatively impact their social responsiveness as they may be less able to and/or motivated to cognitively focus on their group mates. If environmental conditions push groups further from their physiological optima, this could then result in less synchronized groups and potentially cause groups to break apart. In a similar way, differences in metabolic requirements may, across changing resource availability in the environment, cause relative changes in individuals' focus on goal-oriented vs. socially-oriented movements (i.e., motivation to stay together) and thereby impact the cohesion, speed, and alignment of groups. In many cases, social responsiveness is affected by sensory input, such as the extent to which individuals can see each other, and conditions such as increased water turbidity or habitat complexity will require individuals to slow down and be more socially responsive to not break social contact. This in turn may actually provide more scope for individuals with different physiological optima or different breadths of performance curves to stay together. Finally, the limits of group members' physiological performance curves (or environmental tolerances) will determine how well they will be able to stay together and move across increasingly extreme conditions, as individuals may simply differ in the upper limits they can survive, such as in refuge pools of streams during extreme droughts.

EFFECTS ON THE COSTS AND BENEFITS OF GROUPING

Social Foraging

Individuals in groups can benefit by increased access to food sources and the potential to exploit food resources discovered by others, but grouping can also result in competition (Ranta et al., 1993). As discussed earlier when considering within-group conflict, differences in physiological performance can allow some individuals to have disproportionately greater access to food. When physiological performance curves differ between individuals, the variability in how food is distributed between individuals should be driven by variation in physiological performance under the current environmental conditions. This could favor less competitively able individuals to actively leave groups, and the reduction in group size to potentially impact foraging efficiency and anti-predator benefits experienced by those group members that remain (Krause and Ruxton, 2002).

Predicting the role of physiological performance curves on social foraging may be dependent on the feedback between individuals' physiological performance and changes in physiological state that occur during foraging. If the intake of food and time to satiation differs between individuals (Gifford et al., 2014; MacGregor et al., 2021), which could be determined by differences in physiological performance in the current environment, there may be conflict in the optimal time to stop foraging at that patch. If those with higher physiological performance have both faster food intake and greater influence over group decisions, then other individuals in the group will be less likely to forage for an adequate duration. This may act as a positive feedback which magnifies differences in physiological performance between individuals over the longer term. Because of variation in physiological performance curves, such a feedback would however be suppressed if foraging occurs under variable environmental conditions, favoring food intake of different individuals at different times.

The metabolic cost of digestion (Norin and Clark, 2017), which can impact physiological traits such as locomotion (Dupont-Prinet et al., 2009), may alter the spatial distribution of individuals within groups and their behavior during social foraging. For example, in common minnows (*Phoxinus phoxinus*) there are consistent among-individual differences in the time spent at the front of a shoal, with some fish spending more time in front than others and individuals in front tending to ingest most food items (McLean et al., 2018). After feeding however, individuals at the front move toward the back of the shoal, as a result of the reduction in aerobic metabolic scope available due to digestion (McLean et al., 2018). Satiated individuals may also reduce foraging and increase anti-predator vigilance to the benefit of others in the group (Arbon et al., 2020), dampening differences between individuals in food intake. Thus, both changing environmental conditions and inter-individual variation in physiological performance curves have potential to disrupt positive and negative feedback and thereby result in either a reduction or strengthening of inter-individual variation in food intake.

Feedbacks among physiological performance, environmental conditions and social behavior can be informed by recent research exploring how individual differences based on state can drive behavior, and how behavior can in turn drive differences in state (i.e., state-behavior feedbacks; Sih et al., 2015). Experimental tests with sticklebacks (*Gasterosteus aculeatus*) support the existence of feedbacks between risk-taking behavior and satiation, but even in this relatively simple case, these studies show that these feedbacks are unpredictable, without strong evidence in favor of negative or positive feedbacks (MacGregor et al., 2021). This suggests that integrating feedbacks into the interaction between physiological performance curves and social foraging will be challenging. Simulation modelling based on assumptions and parameters that are empirically determined may thus be an essential tool in this endeavor.

While there is strong evidence that group living improves rates of finding and exploiting food sources (Cvikel et al., 2015; Ioannou, 2017), if an individual's success during collective foraging is related to their physiological performance, then performance these curves are likely to impact group-level performance when groups foraging in different environments or microhabitats. If groups are reliant on a small proportion of individuals to lead, for example those with information regarding the presence and location of food (Ioannou et al., 2015), and the ability of these individuals to lead is positively associated with their physiological (e.g., locomotory) performance, group foraging success will be greatest when environmental conditions are optimal for leading individuals. In contrast, if foraging is dependent on pooling information from many individuals in the group, such as in many eusocial insect colonies (Detrain and Deneubourg, 2009), then

environmental conditions which favor the greatest average physiological performance may maximize foraging success. The environmental conditions that optimize group performance in foraging may thus be dependent on whether influence on foraging performance is distributed between many individuals or a few.

Predator Avoidance

Reduced predation risk has been proposed as one of the main drivers for why most animals live in social groups (Krause and Ruxton, 2002). Importantly, the environmental context may alter predation risk for grouping animals, both by affecting predator behavior (Grigaltchik et al., 2012) as well as effects on group behavior. For example, if in a particular environment, phenotypic variance is high due to among-individual variation in performance curves, this may result in less cohesive groups, potentially reducing the anti-predator benefits for those individuals (Sogard and Olla, 1997). Groups that are more cohesive with less phenotypic variance benefit from the confusion effect whereby visual predators have reduced targeting accuracy when prey are phenotypically homogenous (Jeschke and Tollrian, 2007). Because of this, phenotypically different individuals can experience increased risk of predation relative to their group mates (the oddity effect; Theodorakis, 1989). As individual behavior and group composition are important aspects of predator avoidance (Farine et al., 2015; Blake et al., 2018), this suggests that not only should groups differ in their anti-predator success across environments as performance curves converge and diverge, but that individuals may prefer different groups as environments change. Different individuals are affected by the oddity effect to different extents (Rodgers et al., 2015). For example, an individual with particularly high-performance capacity in a given environment may be less susceptible to predation than an individual who has a low performance capacity relative to its groupmates, especially if these differences in physiological capacity manifest in behavioral differences (e.g., activity level) that make them more of less obvious to predators. Thus, as environments change, there may be differences in group membership, as individuals opt to forego or receive the full anti-predator benefits of being in a group. Additionally, there may be important ramifications on group level success if group predator avoidance is influenced by a leader, and if the identity or influence of a leader changes across an environmental gradient due to variation in performance curves.

Social Learning and the Spread of Information

Many animals rely on social learning as a shortcut for behaviors linked to predation avoidance, migration, foraging, and reproduction (Brown and Laland, 2003; Mueller et al., 2013). The efficiency and benefits of social learning may change across an environmental gradient because of changes in the transmission of information from demonstrators, and perception and processing of information from learners. Information is mainly transmitted *via* sensory signals (cues), perceived, and transduced *via* sensory organs and processed *via* neurological pathways. Variation in the transmission, perception and processing of information may arise from alteration of the sensory signals themselves, which may be disrupted directly by changes in the environment, such as acoustic cues masked by human noise pollution (Radford et al., 2014), or visual cues reduced by increased water turbidity (Nieman and Gray, 2019). Physiological changes across environments can also impact the perception and processing of cues, as well as indirectly by changes in group cohesion and coordination, which will influence how well information will spread within groups (MacGregor et al., 2020).

Although in extreme environments sensory organs may even be directly damaged, less dramatic changes may occur in response to environmental changes that lead to physiological effects and impact individual signaling and perception. An example is hormonal disruptions such as modification of melatonin rhythms in birds with variation in night lighting (Dominoni et al., 2020). Neural transmission, brain functioning, and cognition may also vary across an environmental gradient with impacts on social learning capacities. A well-known example is honey bees exposed to pesticides, which have reduced brain functioning (Klein et al., 2017) that may translate into a weaker ability to learn how to localize food from waggle dances (von Frisch, 1967). As with the development of social niches and leader and follower behaviors, greater within-group variation in individuals' physiological performance should favor more distinct demonstrator and learner roles, which can result in conflict over preferred group dynamics (MacGregor et al., 2020). Furthermore, variation in rank order across environments, such as a change in rank order of performance capacity at higher temperature (**Figure 2**), may result in a change in which individuals are demonstrators and which are learners. If relative changes in physiological performance and preferences promote a less stable group composition, reduced familiarity with the demonstrator and other individuals belonging to the group may affect the social transmission of information (Hasenjager and Dugatkin, 2017; Barrett et al., 2019).

Group-level behaviors and dynamics are likely to vary across environments (e.g., increased water temperature and hypoxia may decrease group cohesion in aquatic ectotherms), which can strongly affect how social information is transmitted (e.g., visual information, MacGregor et al., 2020). Any changes in group cohesion could in turn alter the potential for information transfer among groupmates due to changes in spatial distances among individuals and their ability to give and receive social cues (Pineda et al., 2020). In addition, the extent that individuals use social learning can be dependent on group behavioral composition. For example, using network-based diffusion analysis it has been found that, in guppies, social learning rate is higher in both bold and risk averse individuals when they are part of groups dominated by risk-averse individuals or mixed groups and there is a bold demonstrator (Hasenjager et al., 2020). Across gradients of environmental variation, among- and within-individual differences in behavioral expression in relation to performance curves may therefore lead to variation in social learning. If, across such gradients, the risks and benefits associated with social learning change (e.g., different reliability and efficiency

of the transmission and perception of information within groups), non-optimal environments may lead to changes in social learning (e.g., l'Anson Price et al., 2019).

Disease and Parasite Transfer

Disease transfer and parasite load can both be affected by the environmental context (Aeby and Santavy, 2006) and social behavior of animals (Hawley et al., 2011). Social behavior can increase risk of disease and parasite transfer between individuals (Ezenwa, 2004), especially when groups are more cohesive because of the closer proximity between individuals (Bull et al., 2012). This is detrimental because parasites can have both direct costs to infected individuals and indirect costs to group members (Granroth-Wilding et al., 2015). Indeed, avoidance of disease and parasite transfer has been proposed as one of the key factors keeping group sizes small in some species (Alexander, 1974; Huffman and Chapman, 2009; Patterson and Ruckstuhl, 2013), although "socially transferred" infection resistance can in certain cases improve immune abilities in group dwelling organisms (Traniello et al., 2002; Ugelvig and Cremer, 2007).

However, the relationship between social behavior and disease transfer may be influenced by individual differences in performance curves. For example, if group cohesion is altered due to changes in phenotypic variance in performance curves, rates of disease and parasite transfer could also change. Furthermore, if shifting environmental conditions affect optimal group membership due to altered physiological performance and individuals move between groups, this could increase disease transfer between groups. Previous work suggests that increased space use relates to parasite load (Boyer et al., 2010) and that this can be influenced by the environmental context (Spiegel et al., 2015). If environmental conditions change rapidly, this could cause decreased group stability and more rapid transfer of individuals (and their diseases) between groups as individuals spread out.

Additionally, infection may alter an individuals' behavior such that it becomes less social. This could be for a variety of reasons including active avoidance by healthy individuals (Poirotte et al., 2017), a response to reduce disease transmission and increase inclusive fitness (Heinze and Walter, 2010), or manipulation by the infecting agent (Hughes et al., 2004). However, an apparent decrease in sociability could also occur because the physiological costs of disease make maintaining group membership challenging. Importantly, susceptibility to disease can change across environmental gradients, both because of differences in parasite performance curves (Sheets et al., 2021) and because of changes in the potential host's immune function (Adamo and Lovett, 2011; Makrinos and Bowden, 2016). Further, differences in individual performance curves may mean that individuals are differentially susceptible to disease or parasite infection (Kurtz et al., 2000), which could relate to position within a social hierarchy or leader-follower dynamics (Larcombe et al., 2013; Snyder-Mackler et al., 2016). Different individuals may thus be more susceptible to disease or parasite transmission across an environmental gradient which could influence their social behavior and potentially group dynamics, particularly if susceptible individuals are leaders. Finally, impairments caused by diseases or parasites can reduce individual speed and mobility, which in turn may influence their ability and motivation to be social (Jolles et al., 2020c). There may therefore be synergistic effects on social behavior between disease or parasitic infection and other factors that affect locomotor performance, such as temperature or hypoxia.

Migrations and Range Expansions

Group movement occurs at different spatial and temporal scales. At small scales, within a population's distribution, group movement is generally driven by organisms' motivation and necessity to find resources or shelter. Such movements, from one resource patch to another or from one tree to the other for cover, often relies on social interactions where the presence of more experienced individuals or with knowledge for specific information such as the location of food resources can guide naïve individuals or transmit the information to the other group members (Mueller et al., 2013; Berdahl et al., 2018). At a larger scale, movements are associated with migration or range expansion (Cote et al., 2017) and social interactions still have a central role. Indeed social interactions can improve the accuracy of group navigation (Simons, 2004; Berdahl et al., 2018) and reduces energy expenditures (Herskin and Steffensen, 1998; Marras et al., 2015). However, despite numerous advantages there are also potential costs to individuals associated with group movement, including coordination (Nagy et al., 2010) and consensus costs (Conradt and Roper, 2009) such as adjustment of individual performance to match the group performance and individual differences in lower or upper limits of physiological performance across environmental gradients (**Figures 1, 7**). Therefore, as groups move across various spatial scales and environments, environmental effects on performance curves will continuously modulate group functioning and performance of individuals within the group.

One response of organisms to unsuitable environmental conditions is to relocate into more favorable habitats. However, relocation is strictly linked to movement behavior including group movement and to the ability to settle. If individual variation in performance curves affects group movement then reduced relocation opportunities may be expected under certain environments. For example, during drought, especially in mediterranean climates, parts of rivers dry up completely, requiring individuals from fish populations in those rivers to move to deeper safe refuges that do not dry up. In those conditions individual physiological and behavioral traits may be essential for group movement (see Box 1 for more details). However, not all individuals perform equally well in new environments and even if large scale movements occur, they may come at the cost of group re-arrangement.

EXPERIMENTAL APPROACHES

While gaining a better understanding of the relationships between performance curves and social behavior is critically

important in a changing world, these are not easy relationships to decipher. Ideally, we need performance data for individuals tested repeatedly across an environmental gradient and then in groups across the same range. Acquiring detailed data to be able to construct individual performance curves requires many repeated measures of the same individuals across a range of conditions of the same environmental variable. Accurate and precise estimates of individual variation in a reaction norm require relatively large sample sizes and each individual tested multiple times (Martin et al., 2011; van de Pol, 2012; Allegue et al., 2017). Estimating performance curves can be even more sample intensive, particularly because the important variation is typically greater in estimating higher order parameters associated with curve shape than for those associated with offset or slope (Murren et al., 2014). To then consider the social axis as we discuss here, the number of individuals required for a study will be even larger.

Still, these studies are possible, particularly with the advent of automated techniques and low cost open source electronics (Jolles, 2021). Experiments with social groups that directly examine the influence of food availability and predator presence across environmental gradients may help address these issues, as will validation of detailed patterns seen in lab studies with less granular studies done in wild populations. Emerging technologies that allow high-resolution tracking of animals in the wild (Guzzo et al., 2018), combined with transmitters or loggers that acquire physiological data (e.g., heart rate; Williams et al., 2021) across habitats and environments will be particularly useful for allowing researchers to examine these questions. The general approach begins with measuring the same individuals repeatedly for a physiological trait and their behavior (e.g., locomotor capacity, temperature preference, spatial position) across a range of conditions (e.g., temperature, oxygen availability, turbidity) to construct individual performance curves. It is important to consider that, due to the large number of measurements required, not all traits can be easily investigated, especially those that are relatively invasive such as those relating to tissues or organ level physiological performance. Notedly, because lab studies often test animals when they are otherwise at relatively benign conditions, there have been recent calls to improve ecological relevance by confirming laboratory studies of performance curves with field data (Childress and Letcher, 2017). This may be particularly important when seeking to understand group behavior, the patterns of which are often the result of trade-offs between individual foraging needs and the benefits of groups for predator protection.

After repeatedly measuring individual performance curves in isolation, animals should be assigned to groups. The method for group assignment should be considered carefully depending on the exact question being asked. For example, if researchers are interested in how performance in a given environmental context affects group assortment, animals should be allowed to assort themselves. However, if the question relates more to how groups manage performance of different individuals as conditions change, group assignment can be done by the experimenter. This also requires careful consideration such as whether to optimize the performance of all individuals, the performance of the group as a whole or the differences between individuals.

Additionally, experimenters will need to decide whether they are going to measure the performance of a few focal individuals or all individuals in the social groupings. Due to the time and work involved in collecting performance curves on each additional animal, this is a serious consideration. While measuring every individual in a group provides more information, it can functionally limit the number of groups that can reasonably be measured. Whether fewer individuals per group can be measured depends on the exact question being asked. Importantly, even if the ultimate question relates to individual performance, it may be important to construct performance curves for all individuals in a group if the question focuses on how the individual relates to group performance and whether the important metric is average group performance or individual rank. While this type of experiment can be time intensive, without a better understanding of how individual performance curves influence social behavior traits and group performance, we will be unable to adequately predict how animal groups respond to changing environmental conditions.

BOX 1 | Methodological Case Study: Using performance curves and social dynamics to understand how fish deal with droughts

Many freshwater ecosystems are characterized by natural seasonal fluctuations of their water cycle, including droughts and floods (Lennox et al., 2019). Despite being an integral part of the ecosystem, droughts have strong impacts on fish and other aquatic biota by increases in water temperature, deoxygenation, and reducing habitat availability and connectivity by reductions in water flow (Magoulick and Kobza, 2003; Mas-Martí et al., 2010). In fluvial systems in particular, severe droughts can result in complete sections of rivers to dry up, confining fish to few refugia with very extreme abiotic conditions, intense competition, and high predation risk (Magoulick and Kobza, 2003). Physiological performance curves are likely to directly affect how individual fish cope with these strong environmental changes, but also indirectly through various social effects, whereby the responses and capabilities of individual animals to drought may be compromised or enhanced, influenced by the phenotypic composition of groups (see main text; Killen et al., 2017b; Jolles et al., 2020a). For example, fish more sensitive to temperature increases may be the first to leave areas that may dry up later and thereby could act as leaders that "rescue" individuals with broader performance curves and correspondingly wider thermal tolerances. It is also possible that, in pools with low oxygen availability and warm water, competitive interactions change considerably relative to non-drought conditions, putting individuals with narrower performance curves (e.g., in terms of aerobic scope) at risk.

To better understand the above types of scenarios in terms of how fish may deal with the severe effects of droughts, we first need to understand how individual fish cope with changes in their environment related to drought at both the behavioral and physiologic levels. To start, one could decide to focus on hypoxia linked to drought and determine the physiological performance curves in terms of metabolic capacity and activity change across decreasing levels of dissolved oxygen the water. To do this, a replicated setup of 16 respirometry chambers could be used to measure the standard metabolic rate (SMR) and aerobic scope (AS) of fish during acute exposure to various levels of oxygen availability observed in the wild, e.g., 100, 75, 50, and 25% air saturation. Fish would be tested in a random order in

terms of oxygen treatment to avoid temporal effects, and could be tested on alternative days such that two batches could be run on following days. In that way it would be possible to test 32 fish on all four treatment levels in approximately 8 days.

Physiological experiments could be complemented with automated behavioral experiments to determine how fish behaviorally respond to different levels of oxygenation, particularly spontaneous activity, air-breathing, and potential escape (longer directed movement) behavior. For this, fish could be tested individually in arenas, filled with water at a specific oxygen level and containing rocks and partitions to provide structure. A system of replicated setups could be used with automated recording (e.g., pirecorder) and tracking of the fishes' movements, such that all 32 fish could be tested on one treatment level per day (randomized).

After acquiring the individual measures, fish could be tested for social behavior in larger arenas in small groups of different compositions in terms of their physiological performance. A range of different questions could be investigated, each requiring a different type of homo- and heterogenization. To start, one could focus on understanding the effects of individuals' breadth of performance curve in terms of metabolic phenotype on competitiveness in a social foraging scenario. Thereby groups, such as with a group size of 6 fish, could be composed of individuals with small and large performance breadths and exposed to an open arena with hidden foraging patches and repeatedly tested across the four oxygen treatment levels. Manual video observations will help determine the cumulative food intake of the individual fish with automated tracking linking this to changes in the individual movement and social interaction rules (see, e.g., Jolles et al., 2017; MacGregor et al., 2020). Additional experiments could be performed in which social trials are run at differing levels of hypoxia such that among-individual variation in performance capacity and behavior could be manipulated according to each individual's performance curves, and the resulting effects on social behavior observed.

With careful planning of the physiological and behavioral measurements, while properly accounting for acclimatization and randomizing for order and treatment effects, it should be feasible, following the above, to get a sample size of 96 fish tested within 8 weeks. In the foraging experiment described above, the dataset would have 384 unique individual scores in terms of SMR, AS, individual activity, and social activity to determine individual physiological performance curves and heterogeneity therein as well as the effects of this heterogeneity on group functioning in terms of social foraging (at the baseline foraging condition, presumably at normoxia). Note that this experimental design only considers acute exposures to the various levels of oxygen availability. A study could also start with fish acclimated (for at least 2 weeks) to the various hypoxia treatments, but this would obviously increase the amount of time needed for the project if individual performance curves are to be constructed after acclimation and subsequent testing at each condition.

CONCLUDING REMARKS

It is becoming increasingly clear that: (1) animal social behavior is linked with the physiological performance capacity of individuals; and (2) physiological performance is strongly influenced by environmental factors. Accordingly, it is apparent that a research approach that involves estimation of performance curves is required to fully understand how environmental factors influence social behavior. Conversely, the measurement of performance curves has been a central feature of the study of comparative physiology and ecophysiology during the last several decades, but in virtually all cases has only been applied to individual animals and devoid of any social context. As individual heterogeneity within groups is a known driver of leadership, conflict, cohesion and coordination, environmental effects on phenotypic variation should ultimately influence behaviors at the group level. As wild animals are being exposed to increasing environmental changes, an integration of physiological performance curves with the measurement of social behavior will be key for understanding how such changes affect group living and associated ecological phenomena. We therefore encourage increased collaboration among ecophysiologists and researchers that investigate animal social behavior to achieve a more complete understanding of how species will respond to environmental change.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

SK and CI contributed to conception and design of the manuscript. DC, LC, JJ, and AM contributed to further idea development and refinement. SK coordinated manuscript writing and compiled manuscript drafts. All authors drafted specific sections of the manuscript. DC, SK, JJ, and CI designed and produced figures with additional input from LC and AM. All authors contributed to manuscript revision, read, and approved the submitted version.

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REFERENCES

Adamo, S. A., and Lovett, M. M. (2011). Some like it hot: the effects of climate change on reproduction, immune function and disease resistance in the cricket *Gryllus texensis*. *J. Exp. Biol.* 214, 1997–2004. doi: 10.1242/jeb.056531

Aeby, G. S., and Santavy, D. L. (2006). Factors affecting susceptibility of the coral Montastraea faveolata to black-band disease. *Mar. Ecol. Prog. Ser.* 318, 103–110. doi: 10.3354/meps318103

Alexander, R. D. (1974). The evolution of social behavior. *Annu. Rev. Ecol. Syst.* 5, 325–383. doi: 10.1146/annurev.es.05.110174.001545

Allegue, H., Araya-Ajoy, Y. G., Dingemanse, N. J., Dochtermann, N. A., Garamszegi, L. Z., Nakagawa, S., et al. (2017). Statistical quantification of individual differences (SQuID): an educational and statistical tool for understanding multilevel phenotypic data in linear mixed models. *Methods Ecol. Evol.* 8, 257–267. doi: 10.1111/2041-210X.12659

Arbon, J. J., Kern, J. M., Morris-Drake, A., and Radford, A. N. (2020). Context-dependent contributions to sentinel behaviour: audience, satiation and danger effects. *Anim. Behav.* 165, 143–152. doi: 10.1016/j.anbehav.2020.04.021

Armstrong, J. D., Millidine, K. J., and Metcalfe, N. B. (2011). Ecological consequences of variation in standard metabolism and dominance among salmon parr. *Ecol. Freshw. Fish* 20, 371–376. doi: 10.1111/j.1600-0633.2011.00486.x

Barrett, B., Zepeda, E., Pollack, L., Munson, A., and Sih, A. (2019). Counter-culture: does social learning help or hinder adaptive response to human-induced rapid environmental change? *Front. Ecol. Evol.* 7:183. doi: 10.3389/fevo.2019.00183

Barrionuevo, W. R., and Burggren, W. W. (1999). O2 consumption and heart rate in developing zebrafish (Danio rerio): influence of temperature and ambient O2. *Am. J. Phys. Regul. Integr. Comp. Phys.* 276, R505–R513. doi: 10.1152/ajpregu.1999.276.2.R505

Bartheld, J. L., Artacho, P., and Bacigalupe, L. (2017). Thermal performance curves under daily thermal fluctuation: a study in helmeted water toad tadpoles. *J. Therm. Biol.* 70, 80–85. doi: 10.1016/j.jtherbio.2017.09.008

Bartolini, T., Butail, S., and Porfiri, M. (2015). Temperature influences sociality and activity of freshwater fish. *Environ. Biol. Fish* 98, 825–832. doi: 10.1007/s10641-014-0318-8

Bauer, C. M., Skaff, N. K., Bernard, A. B., Trevino, J. M., Ho, J. M., Romero, L. M., et al. (2013). Habitat type influences endocrine stress response in the degu (*Octodon degus*). *Gen. Comp. Endocrinol.* 186, 136–144. doi: 10.1016/j.ygcen.2013.02.036

Beauchamp, G. (2004). Reduced flocking by birds on islands with relaxed predation. *Proceedings of the Royal Society of London. Series B Biologic. Sci.* 271, 1039–1042. doi: 10.1098/rspb.2004.2703

Berdahl, A. M., Kao, A. B., Flack, A., Westley, P. A. H., Codling, E. A., Couzin, I. D., et al. (2018). Collective animal navigation and migratory culture: from theoretical models to empirical evidence. *Philosophic. Trans. Royal Society B Biologic. Sci.* 373:20170009. doi: 10.1098/rstb.2017.0009

Bergmüller, R., and Taborsky, M. (2010). Animal personality due to social niche specialisation. *Trends Ecol. Evol.* 25, 504–511.

Bevan, P. A., Gosetto, I., Jenkins, E. R., Barnes, I., and Ioannou, C. C. (2018). Regulation between personality traits: individual social tendencies modulate whether boldness and leadership are correlated. *Proc. R. Soc. B Biol. Sci.* 285:20180829. doi: 10.1098/rspb.2018.0829

Bierbach, D., Mönck, H. J., Lukas, J., Habedank, M., Romanczuk, P., Landgraf, T., et al. (2020). Guppies prefer to follow large (robot) leaders irrespective of own size. *Front. Bioeng. Biotechnol.* 8:441. doi: 10.3389/fbioe.2020.00441

Blake, C. A., Andersson, M. L., Hulthén, K., Nilsson, P. A., and Brönmark, C. (2018). Conspecific boldness and predator species determine predation-risk consequences of prey personality. *Behav. Ecol. Sociobiol.* 72:133. doi: 10.1007/s00265-018-2544-0

Boyer, N., Réale, D., Marmet, J., Pisanu, B., and Chapuis, J.-L. (2010). Personality, space use and tick load in an introduced population of Siberian chipmunks Tamias sibiricus. *J. Anim. Ecol.* 79, 538–547. doi: 10.1111/j.1365-2656.2010.01659.x

Brandão, M. L., Colognesi, G., Bolognesi, M. C., Costa-Ferreira, R. S., Carvalho, T. B., and Gonçalves-de-Freitas, E. (2018). Water temperature affects aggressive interactions in a Neotropical cichlid fish. *Neotropic. Ichthyol.* 16:e170081. doi: 10.1590/1982-0224-20170081

Breves, J. P., and Specker, J. L. (2005). Cortisol stress response of juvenile winter flounder (*Pseudopleuronectes americanus*, Walbaum) to predators. *J. Exp. Mar. Biol. Ecol.* 325, 1–7. doi: 10.1016/j.jembe.2005.04.019

Briffa, M., and Sneddon, L. U. (2007). Physiological constraints on contest behaviour. *Funct. Ecol.* 21, 627–637. doi: 10.1111/j.1365-2435.2006.01188.x

Brijs, J., Sandblom, E., Rosengren, M., Sundell, K., Berg, C., Axelsson, M., et al. (2019). Prospects and pitfalls of using heart rate bio-loggers to assess the welfare of rainbow trout (*Oncorhynchus mykiss*) in aquaculture. *Aquaculture* 509, 188–197. doi: 10.1016/j.aquaculture.2019.05.007

Brown, C., and Irving, E. (2014). Individual personality traits influence group exploration in a feral guppy population. *Behav. Ecol.* 25, 95–101.

Brown, C., and Laland, K. N. (2003). Social learning in fishes: a review. *Fish Fish.* 4, 280–288. doi: 10.1046/j.1467-2979.2003.00122.x

Bull, C. M., Godfrey, S. S., and Gordon, D. M. (2012). Social networks and the spread of salmonella in a sleepy lizard population. *Mol. Ecol.* 21, 4386–4392. doi: 10.1111/j.1365-294X.2012.05653.x

Bulté, G., and Blouin-Demers, G. (2006). Cautionary notes on the descriptive analysis of performance curves in reptiles. *J. Therm. Biol.* 31, 287–291. doi: 10.1016/j.jtherbio.2005.11.030

Bumann, D., and Krause, J. (1993). Front individuals lead in shoals of three-spined sticklebacks (*Gasterosteus aculeatus*) and juvenile roach (*Rutilus rutilus*). *Behaviour* 125, 189–198. doi: 10.1163/156853993X00236

Burton, T., Killen, S. S., Armstrong, J. D., and Metcalfe, N. B. (2011). What causes intraspecific variation in resting metabolic rate and what are its ecological consequences? *Proc. R. Soc. B Biol. Sci.* 278, 3465–3473. doi: 10.1098/rspb.2011.1778

Careau, V., Biro, P. A., Bonneaud, C., Fokam, E. B., and Herrel, A. (2014). Individual variation in thermal performance curves: swimming burst speed and jumping endurance in wild-caught tropical clawed frogs. *Oecologia* 175, 471–480. doi: 10.1007/s00442-014-2925-7

Chamberlain, A. C., and Ioannou, C. C. (2019). Turbidity increases risk perception but constrains collective behaviour during foraging by fish shoals. *Anim. Behav.* 156, 129–138. doi: 10.1016/j.anbehav.2019.08.012

Chan, A. A. Y.-H., Giraldo-Perez, P., Smith, S., and Blumstein, D. T. (2010). Anthropogenic noise affects risk assessment and attention: the distracted prey hypothesis. *Biol. Lett.* 6, 458–461. doi: 10.1098/rsbl.2009.1081

Childress, E. S., and Letcher, B. H. (2017). Estimating thermal performance curves from repeated field observations. *Ecology* 98, 1377–1387. doi: 10.1002/ecy.1801

Conradt, L. (2012). Models in animal collective decision-making: information uncertainty and conflicting preferences. *Interface Focus* 2, 226–240. doi: 10.1098/rsfs.2011.0090

Conradt, L., and Roper, T. J. (2000). Activity synchrony and social cohesion: a fission-fusion model. *Proc. Royal Soc. London Series B Biologic. Sci.* 267, 2213–2218. doi: 10.1098/rspb.2000.1271

Conradt, L., and Roper, T. J. (2009). Conflicts of interest and the evolution of decision sharing. *Philosophic. Trans. Royal Soc. B Biologic. Sci.* 364, 807–819. doi: 10.1098/rstb.2008.0257

Cooper, B., Adriaenssens, B., and Killen, S. S. (2018). Individual variation in the compromise between social group membership and exposure to preferred temperatures. *Proc. R. Soc. B Biol. Sci.* 285:20180884. doi: 10.1098/rspb.2018.0884

Cote, J., Bocedi, G., Debeffe, L., Chudzińska, M. E., Weigang, H. C., Dytham, C., et al. (2017). Behavioural synchronization of large-scale animal movements – disperse alone, but migrate together? *Biol. Rev.* 92, 1275–1296. doi: 10.1111/brv.12279

Couzin, I. D., Krause, J., James, R., Ruxton, G. D., and Franks, N. R. (2002). Collective memory and spatial sorting in animal groups. *J. Theor. Biol.* 218, 1–11. doi: 10.1006/jtbi.2002.3065

Couzin, I. D., and Krause, J. (2003). Self-organization and collective behavior in vertebrates. *Adv. Study Behav.* 32, 10–1016.

Currie, H. A. L., White, P. R., Leighton, T. G., and Kemp, P. S. (2020). Group behavior and tolerance of Eurasian minnow (Phoxinus phoxinus) in response to tones of differing pulse repetition rate. *J. Acoustical Soc. America* 147, 1709–1718. doi: 10.1121/10.0000910

Cvikel, N., Egert Berg, K., Levin, E., Hurme, E., Borissov, I., Boonman, A., et al. (2015). Bats aggregate to improve prey search but might be impaired when their density becomes too high. *Curr. Biol.* 25, 206–211. doi: 10.1016/j.cub.2014.11.010

David, M., Cézilly, F., and Giraldeau, L.-A. (2011). Personality affects zebra finch feeding success in a producer–scrounger game. *Anim. Behav.* 82, 61–67. doi: 10.1016/j.anbehav.2011.03.025

del Mar Delgado, M., Miranda, M., Alvarez, S. J., Gurarie, E., Fagan, W. F., Penteriani, V., et al. (2018). The importance of individual variation in the dynamics of animal collective movements. *Philosophic. Trans. Royal Soc. B Biologic. Sci.* 373:20170008. doi: 10.1098/rstb.2017.0008

Detrain, C., and Deneubourg, J. L. (2009). "Social cues and adaptive foraging strategies in ants," in *Food exploitation by social insects*. eds. S. Jarau and M. Hrncir (Boca Raton, FL: CRC Press), 29–54.

Dingemanse, N. J., Kazem, A. J., Réale, D., and Wright, J. (2010). Behavioural reaction norms: animal personality meets individual plasticity. *Trends Ecol. Evol.* 25, 81–89. doi: 10.1016/j.tree.2009.07.013

Domenici, P., Steffensen, J. F., and Marras, S. (2017). The effect of hypoxia on fish schooling. *Philosophic. Trans. Royal Soc. B Biologic. Sci.* 372:20160236. doi: 10.1098/rstb.2016.0236

Dominoni, D. M., Halfwerk, W., Baird, E., Buxton, R. T., Fernández-Juricic, E., Fristrup, K. M., et al. (2020). Why conservation biology can benefit from sensory ecology. *Nat. Ecol. Evol.* 4, 502–511. doi: 10.1038/s41559-020-1135-4

Dupont-Prinet, A., Claireaux, G., and McKenzie, D. J. (2009). Effects of feeding and hypoxia on cardiac performance and gastrointestinal blood flow during critical speed swimming in the sea bass Dicentrarchus labrax. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* 154, 233–240. doi: 10.1016/j.cbpa.2009.06.015

Ezenwa, V. O. (2004). Host social behavior and parasitic infection: a multifactorial approach. *Behav. Ecol.* 15, 446–454. doi: 10.1093/beheco/arh028

Farine, D. R., Montiglio, P.-O., and Spiegel, O. (2015). From individuals to groups and Back: The evolutionary implications of group phenotypic composition. *Trends Ecol. Evol.* 30, 609–621. doi: 10.1016/j.tree.2015.07.005

Fischhoff, I. R., Sundaresan, S. R., Cordingley, J., Larkin, H. M., Sellier, M.-J. J., and Rubenstein, D. I. (2007). Social relationships and reproductive state influence leadership roles in movements of plains zebra, Equus burchellii. *Anim. Behav.* 73, 825–831. doi: 10.1016/j.anbehav.2006.10.012

Fisher, D. N., Kilgour, R. J., Siracusa, E. R., Foote, J. R., Hobson, E. A., Montiglio, P.-O., et al. (2021). Anticipated effects of abiotic environmental change on intraspecific social interactions. *Biol. Rev.* doi: 10.1111/brv.12772 [Ahead of preprint]

Fry, F. E. J. (1971). "The effect of environmental factors on the physiology of fish," in *Fish physiology, Environmental relations and behavior. Vol. 6.* eds. W. S. Hoar and D. J. Randall (New York: Academic Press), 1–98.

Gifford, M. E., Clay, T. A., and Careau, V. (2014). Individual (co)variation in standard metabolic rate, feeding rate, and exploratory behavior in wild-caught semiaquatic salamanders. *Physiol. Biochem. Zool.* 87, 384–396. doi: 10.1086/675974

Gilbert, A. L., and Miles, D. B. (2016). Food, temperature and endurance: effects of food deprivation on the thermal sensitivity of physiological performance. *Funct. Ecol.* 30, 1790–1799. doi: 10.1111/1365-2435.12658

Gilbert, A. L., and Miles, D. B. (2017). Natural selection on thermal preference, critical thermal maxima and locomotor performance. *Proc. R. Soc. B Biol. Sci.* 284:20170536. doi: 10.1098/rspb.2017.0536

Ginnaw, G. M., Davidson, I. K., Harding, H. R., Simpson, S. D., Roberts, N. W., Radford, A. N., et al. (2020). Effects of multiple stressors on fish shoal collective motion are independent and vary with shoaling metric. *Anim. Behav.* 168, 7–17. doi: 10.1016/j.anbehav.2020.07.024

Gomez Isaza, D. F., Cramp, R. L., and Franklin, C. E. (2020). Simultaneous exposure to nitrate and low pH reduces the blood oxygen-carrying capacity and functional performance of a freshwater fish. *Conservation physiol.* 8:coz092. doi: 10.1093/conphys/coz092

Granroth-Wilding, H. M., Burthe, S. J., Lewis, S., Herborn, K. A., Takahashi, E. A., Daunt, F., et al. (2015). Indirect effects of parasitism: costs of infection to other individuals can be greater than direct costs borne by the host. *Proc. R. Soc. B Biol. Sci.* 282:20150602. doi: 10.1098/rspb.2015.0602

Grigaltchik, V. S., Ward, A. J. W., and Seebacher, F. (2012). Thermal acclimation of interactions: differential responses to temperature change alter predator–prey relationship. *Proc. R. Soc. B Biol. Sci.* 279, 4058–4064. doi: 10.1098/rspb.2012.1277

Guderley, H. (1990). Functional significance of metabolic responses to thermal acclimation in fish muscle. *Am. J. Phys. Regul. Integr. Comp. Phys.* 259, R245–R252. doi: 10.1152/ajpregu.1990.259.2.R245

Guzzo, M. M., Van Leeuwen, T. E., Hollins, J., Koeck, B., Newton, M., Webber, D. M., et al. (2018). Field testing a novel high residence positioning system for monitoring the fine-scale movements of aquatic organisms. *Methods Ecol. Evol.* 9, 1478–1488.

Hack, M. A., Thompson, D. J., and Fernandes, D. M. (1997). Fighting in males of the autumn spider, Metellina segmentata: effects of relative body size, prior residency and female value on contest outcome and duration. *Ethology* 103, 488–498. doi: 10.1111/j.1439-0310.1997.tb00162.x

Hansen, M. J., Ligocki, I. Y., Zillig, K. E., Steel, A. E., Todgham, A. E., and Fangue, N. A. (2020). Risk-taking and locomotion in foraging threespine sticklebacks (*Gasterosteus aculeatus*): the effect of nutritional stress is dependent on social context. *Behav. Ecol. Sociobiol.* 74:12. doi: 10.1007/s00265-019-2795-4

Hasenjager, M. J., and Dugatkin, L. A. (2017). Familiarity affects network structure and information flow in guppy (*Poecilia reticulata*) shoals. *Behav. Ecol.* 28, 233–242. doi: 10.1093/beheco/arw152

Hasenjager, M. J., Hoppitt, W., and Dugatkin, L. A. (2020). Personality composition determines social learning pathways within shoaling fish. *Proc. R. Soc. B Biol. Sci.* 287:20201871. doi: 10.1098/rspb.2020.1871

Hawley, D. M., Etienne, R. S., Ezenwa, V. O., and Jolles, A. E. (2011). Does animal behavior underlie covariation between hosts' exposure to infectious agents and susceptibility to infection? Implications for disease dynamics. *Integr. Comp. Biol.* 51, 528–539. doi: 10.1093/icb/icr062

Heinze, J., and Walter, B. (2010). Moribund ants leave their nests to die in social isolation. *Curr. Biol.* 20, 249–252. doi: 10.1016/j.cub.2009.12.031

Herskin, J., and Steffensen, J. F. (1998). Energy savings in sea bass swimming in a school: measurements of tail beat frequency and oxygen consumption at different swimming speeds. *J. Fish Biol.* 53, 366–376. doi: 10.1111/j.1095-8649.1998.tb00986.x

Hock, K., and Huber, R. (2009). Models of winner and loser effects: a cost-benefit analysis. *Behaviour* 146, 69–87. doi: 10.1163/156853908X390931

Huang, Y., Fu, S., Cooke, S. J., and Xia, J. (2020). Is repeatability of metabolic rate influenced by social separation? A test with a teleost fish. *Biol. Lett.* 16:20190825. doi: 10.1098/rsbl.2019.0825

Huffman, M. A., and Chapman, C. A. (2009). Primate parasite ecology: the dynamics and study of host-parasite relationships. Cambridge, UK: Cambridge University Press.

Hughes, D. P., Kathirithamby, J., Turillazzi, S., and Beani, L. (2004). Social wasps desert the colony and aggregate outside if parasitized: parasite manipulation? *Behav. Ecol.* 15, 1037–1043. doi: 10.1093/beheco/arh111

l'Anson Price, R., Dulex, N., Vial, N., Vincent, C., and Grüter, C. (2019). Honeybees forage more successfully without the "dance language" in challenging environments. *Scientific Advan.* 5:eaat0450. doi: 10.1126/sciadv.aat0450

Ioannou, C. C. (2017). Swarm intelligence in fish? The difficulty in demonstrating distributed and self-organised collective intelligence in (some) animal groups. *Behav. Process.* 141, 141–151. doi: 10.1016/j.beproc.2016.10.005

Ioannou, C. C., Rocque, F., Herbert-Read, J. E., Duffield, C., and Firth, J. A. (2019). Predators attacking virtual prey reveal the costs and benefits of leadership. *Proc. Natl. Acad. Sci.* 116, 8925–8930. doi: 10.1073/pnas.1816323116

Ioannou, C. C., Singh, M., and Couzin, I. D. (2015). Potential leaders trade off goal-oriented and socially oriented behavior in mobile animal groups. *Am. Nat.* 186, 284–293. doi: 10.1086/681988

Jablonszky, M., Szász, E., Markó, G., Török, J., Herczeg, G., and Garamszegi, L. Z. (2017). Escape ability and risk-taking behaviour in a Hungarian population of the collared flycatcher (Ficedula albicollis). *Behav. Ecol. Sociobiol.* 71, 1–12. doi: 10.1007/s00265-017-2276-6

Jeschke, J. M., and Tollrian, R. (2007). Prey swarming: which predators become confused and why? *Anim. Behav.* 74, 387–393. doi: 10.1016/j.anbehav.2006.08.020

Johnson, T., and Bennett, A. (1995). The thermal acclimation of burst escape performance in fish: an integrated study of molecular and cellular physiology and organismal performance. *J. Exp. Biol.* 198, 2165–2175. doi: 10.1242/jeb.198.10.2165

Jolles, J. W. (2021). Broad-scale applications of the raspberry pi: A review and guide for biologists. *Methods Ecol. Evol.* 12, 1562–1579. doi: 10.1111/2041-210X.13652

Jolles, J. W., Boogert, N. J., Sridhar, V. H., Couzin, I. D., and Manica, A. (2017). Consistent individual differences drive collective behavior and group functioning of schooling fish. *Curr. Biol.* 27, 2862–2868.e7. doi: 10.1016/j.cub.2017.08.004

Jolles, J. W., King, A. J., and Killen, S. S. (2020a). The role of individual heterogeneity in collective animal behaviour. *Trends Ecol. Evol.* 35, 278–291. doi: 10.1016/j.tree.2019.11.001

Jolles, J. W., Mazué, G. P., Davidson, J., Behrmann-Godel, J., and Couzin, I. D. (2020c). Schistocephalus parasite infection alters sticklebacks' movement ability and thereby shapes social interactions. *Sci. Rep.* 10, 1–11. doi: 10.1038/s41598-020-69057-0

Jolles, J. W., Weimar, N., Landgraf, T., Romanczuk, P., Krause, J., and Bierbach, D. (2020b). Group-level patterns emerge from individual speed as revealed by an extremely social robotic fish. *Biol. Lett.* 16:20200436. doi: 10.1098/rsbl.2020.0436

Joyce, W., Ozolina, K., Mauduit, F., Ollivier, H., Claireaux, G., and Shiels, H. A. (2016). Individual variation in whole-animal hypoxia tolerance is associated with cardiac hypoxia tolerance in a marine teleost. *Biol. Lett.* 12:20150708. doi: 10.1098/rsbl.2015.0708

Jutfelt, F., Norin, T., Ern, R., Overgaard, J., Wang, T., McKenzie, D. J., et al. (2018). Oxygen- and capacity-limited thermal tolerance: blurring ecology and physiology. *J. Exp. Biol.* 221:jeb169615. doi: 10.1242/jeb.169615

Kerth, G., Ebert, C., and Schmidtke, C. (2006). Group decision making in fission–fusion societies: evidence from two-field experiments in Bechstein's bats. *Proc. R. Soc. B Biol. Sci.* 273, 2785–2790. doi: 10.1098/rspb.2006.3647

Killen, S. S., Adriaenssens, B., Marras, S., Claireaux, G., and Cooke, S. J. (2016a). Context dependency of trait repeatability and its relevance for management and conservation of fish populations. *Conservation Physiol.* 4:cow007. doi: 10.1093/conphys/cow007

Killen, S. S., Calsbeek, R., and Williams, T. D. (2017a). The ecology of exercise: mechanisms underlying individual variation in behavior, activity, and performance: an introduction to symposium. *Integr. Comp. Biol.* 57, 185–194. doi: 10.1093/icb/icx083

Killen, S. S., Fu, C., Wu, Q., Wang, Y.-X., and Fu, S.-J. (2016b). The relationship between metabolic rate and sociability is altered by food deprivation. *Funct. Ecol.* 30, 1358–1365. doi: 10.1111/1365-2435.12634

Killen, S. S., Marras, S., and McKenzie, D. J. (2011). Fuel, fasting, fear: routine metabolic rate and food deprivation exert synergistic effects on risk-taking in individual juvenile European sea bass. *J. Anim. Ecol.* 80, 1024–1033. doi: 10.1111/j.1365-2656.2011.01844.x

Killen, S. S., Marras, S., Metcalfe, N. B., McKenzie, D. J., and Domenici, P. (2013). Environmental stressors alter relationships between physiology and behaviour. *Trends Ecol. Evol.* 28, 651–658. doi: 10.1016/j.tree.2013.05.005

Killen, S. S., Marras, S., Nadler, L., and Domenici, P. (2017b). The role of physiological traits in assortment among and within fish shoals. *Philosophic. Trans. Royal Soc. B Biologic. Sci.* 372:20160233. doi: 10.1098/rstb.2016.0233

Killen, S. S., Marras, S., Ryan, M. R., Domenici, P., and McKenzie, D. J. (2012b). A relationship between metabolic rate and risk-taking behaviour is revealed during hypoxia in juvenile European sea bass. *Funct. Ecol.* 26, 134–143. doi: 10.1111/j.1365-2435.2011.01920.x

Killen, S. S., Marras, S., Steffensen, J. F., and McKenzie, D. J. (2012a). Aerobic capacity influences the spatial position of individuals within fish schools. *Proc. R. Soc. B Biol. Sci.* 279, 357–364. doi: 10.1098/rspb.2011.1006

Killen, S. S., Mitchell, M. D., Rummer, J. L., Chivers, D. P., Ferrari, M. C. O., Meekan, M. G., et al. (2014). Aerobic scope predicts dominance during early life in a tropical damselfish. *Funct. Ecol.* 28, 1367–1376. doi: 10.1111/1365-2435.12296

Killen, S. S., Nadler, L. E., Grazioso, K., Cox, A., and McCormick, M. I. (2021). The effect of metabolic phenotype on sociability and social group size preference in a coral reef fish. *Ecol. Evol.* 11, 8585–8594. doi: 10.1002/ece3.7672

King, A. J., Douglas, C. M. S., Huchard, E., Isaac, N. J. B., and Cowlishaw, G. (2008). Dominance and affiliation mediate despotism in a social primate. *Curr. Biol.* 18, 1833–1838. doi: 10.1016/j.cub.2008.10.048

Kingsolver, J., Diamond, S., and Gomulkiewicz, R. (2014). "Curve-thinking: understanding reaction norms and developmental trajectories as traits," in *Integrative Organismal Biology*. eds. L. B. Martin, C. K. Ghalambor and H. A. Woods (New Jersey: John Wiley & Sons Ltd.), 39–53.

Kingsolver, J. G., and Gomulkiewicz, R. (2003). Environmental variation and selection on performance curves. *Integr. Comp. Biol.* 43, 470–477. doi: 10.1093/icb/43.3.470

Klein, S., Cabirol, A., Devaud, J.-M., Barron, A. B., and Lihoreau, M. (2017). Why bees are so vulnerable to environmental stressors. *Trends Ecol. Evol.* 32, 268–278. doi: 10.1016/j.tree.2016.12.009

Kochhann, D. (2017). Social hierarchy and resting metabolic rate in the dwarf cichlid Apistogramma agassizii: the role of habitat enrichment. *Hydrobiologia* 789, 123–131. doi: 10.1007/s10750-016-2806-7

Kochhann, D., Campos, D. F., and Val, A. L. (2015). Experimentally increased temperature and hypoxia affect stability of social hierarchy and metabolism of the Amazonian cichlid Apistogramma agassizii. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* 190, 54–60. doi: 10.1016/j.cbpa.2015.09.006

Kondo, J., and Downes, S. J. (2007). Does social behaviour reliably reflect temperature-dependent physiological capacity in geckos? *Anim. Behav.* 74, 873–880. doi: 10.1016/j.anbehav.2006.10.030

Krause, J., Hoare, D. J., Croft, D., Lawrence, J., Ward, A., Ruxton, G. D., et al. (2000). Fish shoal composition: mechanisms and constraints. *Proceedings of the Royal Society of London. Series B Biologic. Sci.* 267, 2011–2017. doi: 10.1098/rspb.2000.1243

Krause, J., and Ruxton, G. D. (2002). *Living in Groups*. New York: Oxford University Press.

Kurtz, J., Wiesner, A., Götz, P., and Sauer, K. P. (2000). Gender differences and individual variation in the immune system of the scorpionfly *Panorpa vulgaris* (Insecta: Mecoptera). *Dev. Comp. Immunol.* 24, 1–12. doi: 10.1016/S0145-305X(99)00057-9

Larcombe, S. D., Bedhomme, S., Garnier, S., Cellier-Holzem, E., Faivre, B., and Sorci, G. (2013). Social interactions modulate the virulence of avian malaria infection. *Int. J. Parasitol.* 43, 861–867. doi: 10.1016/j.ijpara.2013.05.008

Lefevre, S. (2016). Are global warming and ocean acidification conspiring against marine ectotherms? A meta-analysis of the respiratory effects of elevated temperature, high CO2 and their interaction. *Conservation Physiol.* 4:cow009. doi: 10.1093/conphys/cow009

Lennox, R. J., Crook, D. A., Moyle, P. B., Struthers, D. P., and Cooke, S. J. (2019). Toward a better understanding of freshwater fish responses to an increasingly drought-stricken world. *Rev. Fish Biol. Fish.* 29, 71–92. doi: 10.1007/s11160-018-09545-9

MacGregor, H. E. A., Cottage, A., and Ioannou, C. C. (2021). Suppression of personality variation in boldness during foraging in three-spined sticklebacks. *Behav. Ecol. Sociobiol.* 75:71. doi: 10.1007/s00265-021-03007-2

MacGregor, H. E. A., Herbert-Read, J. E., and Ioannou, C. C. (2020). Information can explain the dynamics of group order in animal collective behaviour. *Nat. Commun.* 11:2737. doi: 10.1038/s41467-020-16578-x

Magoulick, D. D., and Kobza, R. M. (2003). The role of refugia for fishes during drought: a review and synthesis. *Freshw. Biol.* 48, 1186–1198. doi: 10.1046/j.1365-2427.2003.01089.x

Maierdiyali, A., Wang, L., Luo, Y., and Li, Z. (2020). Effect of tank size on zebrafish behavior and physiology. *Animals* 10:2353. doi: 10.3390/ani10122353

Makrinos, D. L., and Bowden, T. J. (2016). Natural environmental impacts on teleost immune function. *Fish Shellfish Immunol.* 53, 50–57. doi: 10.1016/j.fsi.2016.03.008

Marras, S., Claireaux, G., McKenzie, D. J., and Nelson, J. A. (2010). Individual variation and repeatability in aerobic and anaerobic swimming performance of European sea bass, *Dicentrarchus labrax*. *J. Exp. Biol.* 213, 26–32. doi: 10.1242/jeb.032136

Marras, S., Killen, S. S., Lindström, J., McKenzie, D. J., Steffensen, J. F., and Domenici, P. (2015). Fish swimming in schools save energy regardless of their spatial position. *Behav. Ecol. Sociobiol.* 69, 219–226. doi: 10.1007/s00265-014-1834-4

Martin, J. G. A., Nussey, D. H., Wilson, A. J., and Réale, D. (2011). Measuring individual differences in reaction norms in field and experimental studies: a power analysis of random regression models. *Methods Ecol. Evol.* 2, 362–374. doi: 10.1111/j.2041-210X.2010.00084.x

Mas-Martí, E., García-Berthou, E., Sabater, S., Tomanova, S., and Muñoz, I. (2010). "Comparing fish assemblages and trophic ecology of permanent and intermittent reaches in a mediterranean stream," in *Global Change and River Ecosystems—Implications for Structure, Function and Ecosystem Services*. eds. R. J. Stevenson and S. Sabater (Dordrecht: Springer Netherlands), 167–180.

Mathot, K. J., Dekinga, A., and Piersma, T. (2017). An experimental test of state–behaviour feedbacks: gizzard mass and foraging behaviour in red knots. *Funct. Ecol.* 31, 1111–1121. doi: 10.1111/1365-2435.12827

Mathot, K. J., Dingemanse, N. J., and Nakagawa, S. (2019). The covariance between metabolic rate and behaviour varies across behaviours and thermal types: meta-analytic insights. *Biol. Rev.* 94, 1056–1074. doi: 10.1111/brv.12491

Mccarthy, I. D. (2001). Competitive ability is related to metabolic asymmetry in juvenile rainbow trout. *J. Fish Biol.* 59, 1002–1014. doi: 10.1111/j.1095-8649.2001.tb00167.x

McComb, K., Moss, C., Durant, S. M., Baker, L., and Sayialel, S. (2001). Matriarchs as repositories of social knowledge in african elephants. *Science* 292, 491–494. doi: 10.1126/science.1057895

McCune, K., Jablonski, P., Lee, S., and Ha, R. (2018). Evidence for personality conformity, not social niche specialization in social jays. *Behav. Ecol.* 29, 910–917. doi: 10.1093/beheco/ary055

McKenzie, D. J., Garofalo, E., Winter, M. J., Ceradini, S., Verweij, F., Day, N., et al. (2007). Complex physiological traits as biomarkers of the sub-lethal toxicological effects of pollutant exposure in fishes. *Philosophic. Trans. Royal Soc. B Biologic. Sci.* 362, 2043–2059. doi: 10.1098/rstb.2007.2100

McLean, S., Persson, A., Norin, T., and Killen, S. S. (2018). Metabolic costs of feeding predictively alter the spatial distribution of individuals in fish schools. *Curr. Biol.* 28, 1144–1149.e4. doi: 10.1016/j.cub.2018.02.043

McNett, G. D., Luan, L. H., and Cocroft, R. B. (2010). Wind-induced noise alters signaler and receiver behavior in vibrational communication. *Behav. Ecol. Sociobiol.* 64, 2043–2051. doi: 10.1007/s00265-010-1018-9

Meager, J. J., Domenici, P., Shingles, A., and Utne-Palm, A. C. (2006). Escape responses in juvenile Atlantic cod *Gadus morhua L.*: the effects of turbidity and predator speed. *J. Exp. Biol.* 209, 4174–4184. doi: 10.1242/jeb.02489

Metcalfe, N. B., Van Leeuwen, T. E., and Killen, S. S. (2016). Does individual variation in metabolic phenotype predict fish behaviour and performance? *J. Fish Biol.* 88, 298–321. doi: 10.1111/jfb.12699

Michelangeli, M., Goulet, C. T., Kang, H. S., Wong, B. B. M., and Chapple, D. G. (2018). Integrating thermal physiology within a syndrome: locomotion, personality and habitat selection in an ectotherm. *Funct. Ecol.* 32, 970–981. doi: 10.1111/1365-2435.13034

Miln, C., Ward, A. J., and Seebacher, F. (2021). Social rank and not physiological capacity determines competitive success in zebrafish (Danio rerio). *R. Soc. Open Sci.* 8:210146. doi: 10.1098/rsos.210146

Montiglio, P. O., Ferrari, C., and Reale, D. (2013). Social niche specialization under constraints: personality, social interactions and environmental heterogeneity. *Philos. Trans. R. Soc. B, Biol. Sci.* 368:20120343.

Moyers, S. C., Adelman, J. S., Farine, D. R., Moore, I. T., and Hawley, D. M. (2018). Exploratory behavior is linked to stress physiology and social network centrality in free-living house finches (*Haemorhous mexicanus*). *Horm. Behav.* 102, 105–113. doi: 10.1016/j.yhbeh.2018.05.005

Mueller, T., O'Hara, R. B., Converse, S. J., Urbanek, R. P., and Fagan, W. F. (2013). Social Learning of migratory performance. *Science* 341, 999–1002. doi:10.1126/science.1237139

Munson, A., Michelangeli, M., and Sih, A. (2021). Stable social groups foster conformity and among-group differences. *Anim. Behav.* 174, 197–206. doi: 10.1016/j.anbehav.2021.02.011

Murren, C. J., Maclean, H. J., Diamond, S. E., Steiner, U. K., Heskel, M. A., Handelsman, C. A., et al. (2014). Evolutionary change in continuous reaction norms. *Am. Nat.* 183, 453–467. doi: 10.1086/675302

Nagy, M., Ákos, Z., Biro, D., and Vicsek, T. (2010). Hierarchical group dynamics in pigeon flocks. *Nature* 464, 890–893. doi: 10.1038/nature08891

Nati, J. J. H., Lindström, J., Halsey, L. G., and Killen, S. S. (2016). Is there a trade-off between peak performance and performance breadth across temperatures for aerobic scope in teleost fishes? *Biol. Lett.* 12:20160191. doi: 10.1098/rsbl.2016.0191

Navas, C., James, R., Wakeling, J., Kemp, K., and Johnston, I. (1999). An integrative study of the temperature dependence of whole animal and muscle performance during jumping and swimming in the frog *Rana temporaria*. *J. Comp. Physiol. B* 169, 588–596. doi: 10.1007/s003600050259

Nieman, C. L., and Gray, S. M. (2019). Visual performance impaired by elevated sedimentary and algal turbidity in walleye Sander vitreus and emerald shiner *Notropis atherinoides*. *J. Fish Biol.* 95, 186–199. doi: 10.1111/jfb.13878

Norin, T., and Clark, T. D. (2017). Fish face a trade-off between 'eating big' for growth efficiency and 'eating small' to retain aerobic capacity. *Biol. Lett.* 13:20170298. doi: 10.1098/rsbl.2017.0298

Norin, T., and Metcalfe, N. B. (2019). Ecological and evolutionary consequences of metabolic rate plasticity in response to environmental change. *Philosophic. Trans. Royal Society B Biologic. Sci.* 374:20180180. doi: 10.1098/rstb.2018.0180

Nowakowski, A. J., Peaden, J. M., Tuberville, T. D., Buhlmann, K. A., and Todd, B. D. (2020). Thermal performance curves based on field movements reveal context-dependence of thermal traits in a desert ectotherm. *Landsc. Ecol.* 35, 893–906. doi: 10.1007/s10980-020-00986-x

Ord, T. J., and Stamps, J. A. (2017). Why does the rate of signal production in ectotherms vary with temperature? *Behav. Ecol.* 28, 1272–1282. doi: 10.1093/beheco/arx089

Pang, X., Fu, S.-J., and Zhang, Y.-G. (2015). Individual variation in metabolism and swimming performance in juvenile black carp (*Mylopharyngodon piceus*) and the effects of hypoxia. *Mar. Freshw. Behav. Physiol.* 48, 431–443. doi: 10.1080/10236244.2015.1090205

Patterson, J. E., and Ruckstuhl, K. E. (2013). Parasite infection and host group size: a meta-analytical review. *Parasitology* 140, 803–813. doi: 10.1017/S0031182012002259

Pettit, B., Akos, Z., Vicsek, T., and Biro, D. (2015). Speed determines leadership and leadership determines learning during pigeon flocking. *Curr. Biol.* 25, 3132–3137. doi: 10.1016/j.cub.2015.10.044

Pineda, M., Aragao, I., McKenzie, D. J., and Killen, S. S. (2020). Social dynamics obscure the effect of temperature on air breathing in Corydoras catfish. *J. Exp. Biol.* 223:jeb222133. doi: 10.1242/jeb.222133

Poirotte, C., Massol, F., Herbert, A., Willaume, E., Bomo, P. M., Kappeler, P. M., et al. (2017). Mandrills use olfaction to socially avoid parasitized conspecifics. *Sci. Adv.* 3:e1601721. doi: 10.1126/sciadv.1601721

Pörtner, H. O. (2010). Oxygen-and capacity-limitation of thermal tolerance: a matrix for integrating climate-related stressor effects in marine ecosystems. *J. Exp. Biol.* 213, 881–893. doi: 10.1242/jeb.037523

Pörtner, H. O., and Farrell, A. P. (2008). Physiology and climate change. *Science* 322, 690–692. doi: 10.1126/science.1163156

Radford, A. N., Kerridge, E., and Simpson, S. D. (2014). Acoustic communication in a noisy world: can fish compete with anthropogenic noise? *Behav. Ecol.* 25, 1022–1030. doi: 10.1093/beheco/aru029

Ranta, E., Rita, H., and Lindstrom, K. (1993). Competition vs. cooperation: success of individuals foraging alone and in groups. *Am. Nat.* 142, 42–58. doi: 10.1086/285528

Raulo, A., Ruokolainen, L., Lane, A., Amato, K., Knight, R., Leigh, S., et al. (2018). Social behaviour and gut microbiota in red-bellied lemurs (*Eulemur rubriventer*): In search of the role of immunity in the evolution of sociality. *J. Anim. Ecol.* 87, 388–399. doi: 10.1111/1365-2656.12781

Roche, D. G., Careau, V., and Binning, S. A. (2016). Demystifying animal 'personality' (or not): why individual variation matters to experimental biologists. *J. Exp. Biol.* 219, 3832–3843. doi: 10.1242/jeb.146712

Rodgers, G. M., Downing, B., and Morrell, L. J. (2015). Prey body size mediates the predation risk associated with being "odd". *Behav. Ecol.* 26, 242–246. doi: 10.1093/beheco/aru185

Ross, R. M. (1978). Territorial behavior and ecology of the anemonefish *Amphiprion melanopus* on Guam 1. *Z. Tierpsychol.* 46, 71–83. doi: 10.1111/j.1439-0310.1978.tb01439.x

Ruckstuhl, K., and Neuhaus, P. (2006). *Sexual Segregation in Vertebrates*. New York: Cambridge University Press.

Sankey, D. W. E., Shepard, E. L. C., Biro, D., and Portugal, S. J. (2019). Speed consensus and the 'goldilocks principle' in flocking birds (Columba livia). *Anim. Behav.* 157, 105–119. doi: 10.1016/j.anbehav.2019.09.001

Schaerf, T. M., Dillingham, P. W., and Ward, A. J. W. (2017). The effects of external cues on individual and collective behavior of shoaling fish. *Sci. Adv.* 3:e1603201. doi: 10.1126/sciadv.1603201

Schmitz, R. A., and Baldassarre, G. A. (1992). Contest asymmetry and multiple bird conflicts during foraging among nonbreeding American flamingos in Yucatan, Mexico. *Condor* 94, 254–259. doi: 10.2307/1368814

Seebacher, F., and Krause, J. (2017). Physiological mechanisms underlying animal social behaviour. *Philosophic. Trans. Royal Society B Biologic. Sci.* 372:20160231. doi: 10.1098/rstb.2016.0231

Sheets, C. N., Schmidt, D. R., Hurtado, P. J., Byrne, A. Q., Rosenblum, E. B., Richards-Zawacki, C. L., et al. (2021). Thermal performance curves of multiple isolates of batrachochytrium dendrobatidis, a lethal pathogen of amphibians. *Front. Vet. Sci.* 8:648. doi: 10.3389/fvets.2021.687084

Sih, A. (2013). Understanding variation in behavioural responses to human-induced rapid environmental change: a conceptual overview. *Anim. Behav.* 85, 1077–1088. doi: 10.1016/j.anbehav.2013.02.017

Sih, A., Mathot, K. J., Moirón, M., Montiglio, P.-O., Wolf, M., and Dingemanse, N. J. (2015). Animal personality and state–behaviour feedbacks: a review and guide for empiricists. *Trends Ecol. Evol.* 30, 50–60. doi: 10.1016/j.tree.2014.11.004

Simons, A. M. (2004). Many wrongs: the advantage of group navigation. *Trends Ecol. Evol.* 19, 453–455. doi: 10.1016/j.tree.2004.07.001

Sneddon, L. U., Huntingford, F. A., and Taylor, A. C. (1998). Impact of an ecological factor on the costs of resource acquisition: fighting and metabolic physiology of crabs. *Funct. Ecol.* 12, 808–815. doi: 10.1046/j.1365-2435.1998.00249.x

Snyder-Mackler, N., Sanz, J., Kohn, J. N., Brinkworth, J. F., Morrow, S., Shaver, A. O., et al. (2016). Social status alters immune regulation and response to infection in macaques. *Science* 354, 1041–1045. doi: 10.1126/science.aah3580

Sogard, S. M., and Olla, B. L. (1997). The influence of hunger and predation risk on group cohesion in a pelagic fish, walleye Pollock *Theragra chalcogramma*. *Environ. Biol. Fish* 50, 405–413. doi: 10.1023/A:1007393307007

Spencer, K. A. (2017). Developmental stress and social phenotypes: integrating neuroendocrine, behavioural and evolutionary perspectives. *Philosophic. Trans. Royal Society B Biologic. Sci.* 372:20160242. doi: 10.1098/rstb.2016.0242

Spiegel, O., Leu, S. T., Sih, A., Godfrey, S. S., and Bull, C. M. (2015). When the going gets tough: behavioural type-dependent space use in the sleepy lizard changes as the season dries. *Proc. R. Soc. B Biol. Sci.* 282:20151768. doi: 10.1098/rspb.2015.1768

Takada, H., and Minami, M. (2021). Open habitats promote female group formation in a solitary ungulate: the Japanese serow. *Behav. Ecol. Sociobiol.* 75:60. doi: 10.1007/s00265-021-02999-1

Teichroeb, J. A., and Sicotte, P. (2018). Cascading competition: the seasonal strength of scramble influences between-group contest in a folivorous primate. *Behav. Ecol. Sociobiol.* 72:6. doi: 10.1007/s00265-017-2418-x

Theodorakis, C. W. (1989). Size segregation and the effects of oddity on predation risk in minnow schools. *Anim. Behav.* 38, 496–502. doi: 10.1016/S0003-3472(89)80042-9

Traniello, J. F., Rosengaus, R. B., and Savoie, K. (2002). The development of immunity in a social insect: evidence for the group facilitation of disease resistance. *Proc. Natl. Acad. Sci.* 99, 6838–6842. doi: 10.1073/pnas.102176599

Turbill, C., Ruf, T., Rothmann, A., and Arnold, W. (2013). Social dominance is associated with individual differences in heart rate and energetic response to food restriction in female Red Deer. *Physiol. Biochem. Zool.* 86, 528–537. doi: 10.1086/672372

Ugelvig, L. V., and Cremer, S. (2007). Social prophylaxis: group interaction promotes collective immunity in ant colonies. *Curr. Biol.* 17, 1967–1971. doi: 10.1016/j.cub.2007.10.029

van Berkum, F. H. (1988). Latitudinal patterns of the thermal sensitivity of sprint speed in lizards. *Am. Nat.* 132, 327–343. doi: 10.1086/284856

van de Pol, M. (2012). Quantifying individual variation in reaction norms: how study design affects the accuracy, precision and power of random regression models. *Methods Ecol. Evol.* 3, 268–280. doi: 10.1111/j.2041-210X.2011.00160.x

von Frisch, K. (1967). *The Dance Language and Orientation of Bees*. Harvard, USA: Harvard University Press.

Wade, A. S. I., Ramnarine, I. W., and Ioannou, C. C. (2020). The effect of group size on the speed of decision making depends on compromise and predation risk across populations in the guppy *Poecilia reticulata*. *Behaviour* 157, 1173–1192. doi: 10.1163/1568539X-bja10044

Walsberg, G. E., Lea, M. S., and Hillman, S. S. (1986). Individual variation in maximum aerobic capacity: cardiovascular and enzymatic correlates in *Rana catesbeiana*. *J. Exp. Zool.* 239, 1–5. doi: 10.1002/jez.1402390102

Ward, A. J. W., Herbert-Read, J. E., Schaerf, T. M., and Seebacher, F. (2018). The physiology of leadership in fish shoals: leaders have lower maximal metabolic rates and lower aerobic scope. *J. Zool.* 305, 73–81. doi: 10.1111/jzo.12534

Ward, A., and Webster, M. (2016). "Sociality" in *Sociality: The Behaviour of Group-Living Animals*. eds. A. Ward and M. Webster (Cham: Springer International Publishing), 1–8.

Wascher, C. A. F., Kulahci, I. G., Langley, E. J. G., and Shaw, R. C. (2018). How does cognition shape social relationships? *Philosophic. Trans. Royal Society B Biologic. Sci.* 373:20170293. doi: 10.1098/rstb.2017.0293

Williams, H. J., Shipley, J. R., Rutz, C., Wikelski, M., Wilkes, M., and Hawkes, L. A. (2021). Future trends in measuring physiology in free-living animals. *Philos. Trans. R. Soc. B* 376:20200230. doi: 10.1098/rstb.2020.0230

Wilson, R. S., James, R. S., Kohlsdorf, T., and Cox, V. M. (2004). Interindividual variation of isolated muscle performance and fibre-type composition in the toad *Bufo viridus*. *J. Comp. Physiol. B* 174, 453–459. doi: 10.1007/s00360-004-0431-7

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Physiological Performance Curves: When Are They Useful?

Alexander G. Little[1]** *and Frank Seebacher*[2]

[1]*Department of Biology, Biosciences Complex, Queen's University, Kingston, ON, Canada,* [2]*School of Life and Environmental Sciences, University of Sydney, Darlington, NSW, Australia*

This review serves as an introduction to a special issue of Frontiers in Physiology, focused on the importance of physiological performance curves across phylogenetic and functional boundaries. Biologists have used performance curves to describe the effects of changing environmental conditions on animal physiology since the late 1800s (at least). Animal physiologists have studied performance curves extensively over the past decades, and there is a good foundation to understanding how the environment affects physiological functions of individuals. Our goal here was to build upon this research and address outstanding questions regarding the mutability and applicability of performance curves across taxonomic groups and levels of biological organization. Performance curves are not fixed at a taxonomic, population, or individual level – rather they are dynamic and can shift in response to evolutionary pressures (e.g., selection) and epigenetic programming (e.g., plasticity). The mechanisms underlying these shifts are being increasingly used to predict the efficacy with which plasticity and heritability of performance curves can render individuals and populations less vulnerable to climate change. Individual differences in physiological performance curves (and plasticity of performance curves) can also have cascading effects at higher levels of biological organization. For instance, individual physiology likely influences group behaviors in non-additive ways. There is a need therefore to extend the concept of performance curves to social interactions and sociality. Collectively, this special issue emphasizes the power of how within- and between-individual shifts in performance curves might scale up to the population-, species-, and community-level dynamics that inform conservation management strategies.

Keywords: reaction norm, adaptation, developmental plasticity, reversible acclimation, climate change, environment

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Dieter Blottner,
Charité University Medicine Berlin, Germany

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Gary Sieck,
Mayo Clinic, United States

****Correspondence:***
Alexander G. Little
alexander.little@queensu.ca

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INTRODUCTION

Physiological performance curves describe changes in physiological rates with respect to an environmental gradient. The y-axis can represent any level of physiological performance, from single-molecule (e.g., enzyme kinetics) to whole-animal measures (e.g., locomotion, metabolic rate, and reproductive output). Similarly, the x-axis can describe a wide range of environmental gradients, including those associated with abiotic (e.g., temperature, salinity, and oxygen-concentration) and biotic factors (e.g., infection, competition, and predation). The shapes of physiological performance curves thereby describe physiological rates as a

function of the acute environment. The precise shapes can vary within and between taxonomic and functional boundaries. Determining the mechanisms that drive these changes is important for understanding the diverse strategies and limitations that shape animal responses to their environments.

A comparative approach has been particularly powerful to help trace the cascading effects of environmental change across levels of biological organization. Reductionist approaches focusing on single-enzyme thermal performance curves, for instance, indicate that breakdowns in animal performance at high temperatures cannot simply be ascribed to protein denaturation, as was once thought. Rather, the shapes of these single-enzyme curves suggest that decreases in whole-animal performance likely reflect more nuanced shifts in enzyme microstates and activation energies (see Schulte, 2015). Thus, understanding how lower levels of performance change across environmental gradients can signal important consequences for emergent traits at higher levels of organization. Thermal sensitivity of heart rate scope, for instance, can indicate whole-animal and population-level performance, such as migration success in Pacific salmon (Eliason et al., 2011).

In an even broader sense, a comparative approach has also helped identify the adaptive and plastic (i.e., developmental plasticity and reversible acclimation) responses that animals use to overcome environmental variability. By comparing performance curves *between* populations and species, for instance, differences in shape can point to adaptations that promote fitness in local environments. Comparing how performance curves shift as a function of the environment *within* individuals and populations, on the other hand, can indicate whether plasticity allows animals to defend performance against environmental change. These comparative approaches can also help to assess tradeoffs associated with specific physiological strategies.

In this Special Issue, we focus on molecular and physiological mechanisms underlying performance curves and how performance curves may shift in response to an environmental change, by both genetic and epigenetic mechanisms. Responses to the environment are likely to differ between phylogenetic groups, and we are particularly interested in differences between ectotherms and endotherms. Along these lines, we also aim to extend the concept of performance curves from individuals to groups of animals to ask how the physiology of performance curves of individuals can impact emergent properties, like social behavior.

History of Physiological Performance Curves

In the simplest terms, physiological performance curves effectively represent dose-response relationships – an increasing "dose" of some environmental factor alters the response of a physiological system. Many ancient societies clearly recognized the phenomenon of dose-dependence. Ancient Greeks, for instance, recognized that wine in excess would dull the senses (e.g., Homer's Odyssey; Papakonstantinou, 2009), and herbal remedies in Ancient China were recommended to be doubled daily, then increased 10-fold if a cure was not yet achieved (Waddell, 2010). It was Paraclesus (c. 16th century), however, who synthesized our modern appreciation for dose-response relationships by stating that "all things are poison, and nothing is without poison; the dosage alone makes it so a thing is not a poison" (see Waddell, 2010 for review). Paraclesus was originally generous in his definition of a poison, even including compounds like oxygen and water. It is therefore little reach to extrapolate his model to include grades of other environmental factors, including temperature, UV-radiation, or even social stress.

Some of the first ecologically focused physiological performance curves came in the form of thermal performance curves in the late 19th century (**Figure 1**). Vernon (1894), for instance, plotted the thermal sensitivity of metabolism (as CO_2-production) in a frog warmed from 2 to 32°C, then cooled back down to 2°C again the following day (**Figure 1A**). Martin (1903) plotted body temperatures of various animals as a function of ambient temperature to separate animals that thermoconformed (ectotherms) from those that thermoregulated (endotherms; **Figure 1B**). He subsequently plotted thermal performance curves for metabolic rate (CO_2-production) between these various taxonomic groups to investigate the metabolic properties that correspond to these distinct evolutionary strategies (**Figures 1C**,**D**). Later, Snyder (1908) plotted terrapin and cat heart rates over a thermal gradient to investigate the basis of dynamic changes in their thermal sensitivities. This work was particularly novel in that Snyder compared the shapes of these curves with solubility curves for various physiological salts (e.g., Na_2CO_3; **Figure 1E**) in an attempt to identify the mechanisms that limit performance at high temperatures. While his hypothesis on "ion-proteid" interference was subsequently deemed incorrect, his approach may have been the first to compare such curves down levels of organization in search of a mechanistic explanation.

Since these early experiments on thermal sensitivity, physiological performance curves have been used to describe animal responses to a wide array of environmental factors. These types of curves have been useful not only in understanding animal responses to environmental stress (i.e., conformers vs. regulators), but also understanding the physiological strategies and limitations that bound these responses. The critical oxygen tension (P_{crit}), for instance, represents the lowest PO_2 at which an oxyregulator can regulate some rate of metabolic oxygen consumption (MO_2), below which it oxyconforms (Tang, 1933). Comparing inflection points in performance at lower (e.g., hemoglobin-binding) and higher (e.g., locomotion) levels of organization has garnered great insights for mechanisms contributing to oxyregulation (e.g., McBryan et al., 2013).

Performance: A Caveat

The concept of performance is to some degree subjective and does not necessarily correlate well with measures of fitness. During development, for instance, elevated resting metabolic rates (RMR) may promote higher fitness by implying increased rates of growth and development (Burton et al., 2011). In other cases, however, increased RMRs may reflect lower fitness

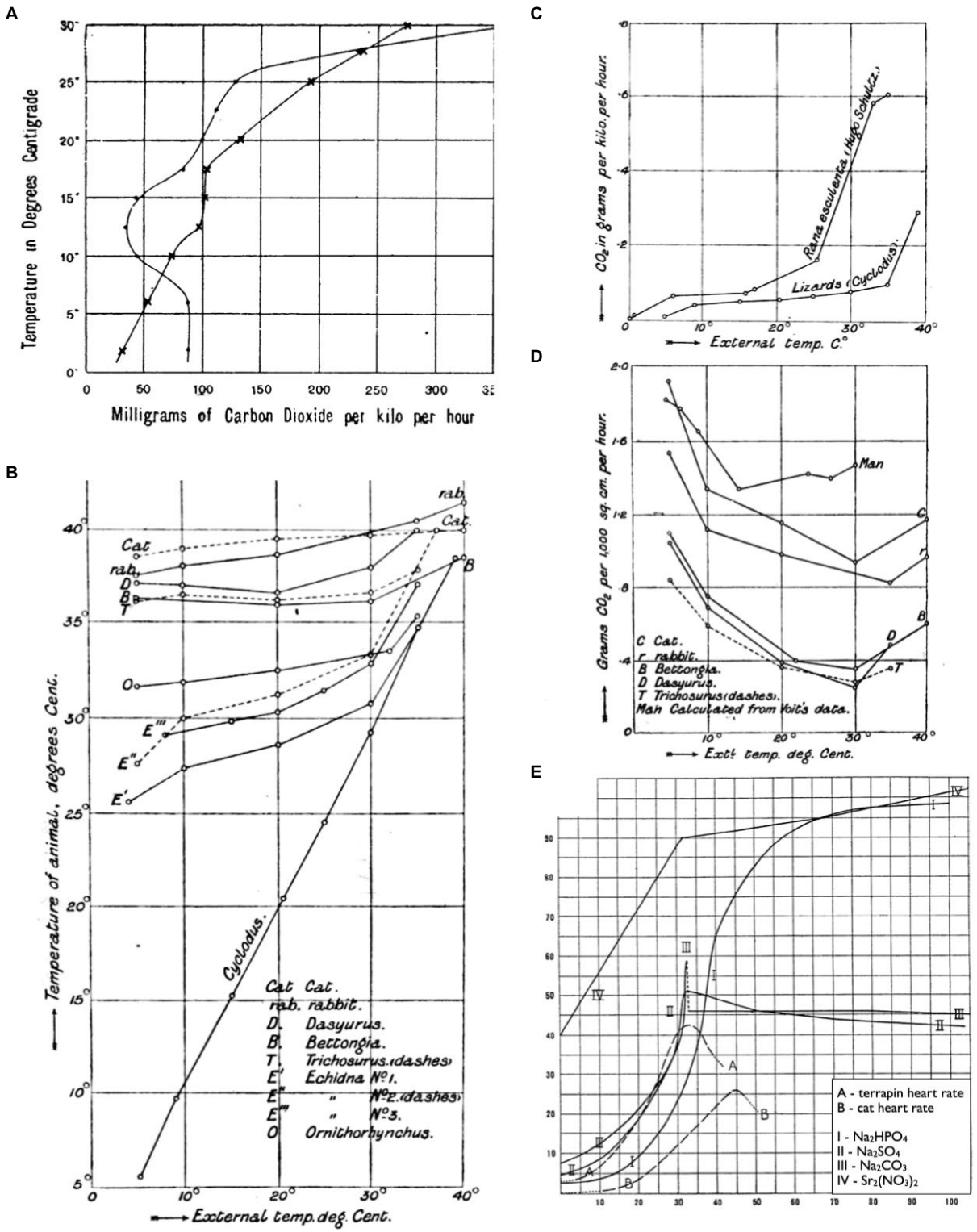

FIGURE 1 | Thermal reaction norms as early examples of performance curves. CO_2-production of a frog warmed from 2 to 32°C, then cooled back down to 2°C again the following day (**A**; reprinted from Vernon, 1894). Body temperatures of thermoregulators and thermoconformers respond differently to changes in ambient temperature (**B**; reprinted from Martin, 1903). CO_2-production of thermoconformers **(C)** and thermoregulators **(D)** reveal that different metabolic properties underlie each evolutionary strategy (reprinted from Martin, 1903). Thermal sensitivities of heart rates for terrapin ventricle and a cat heart alongside thermal solubility curves for Na_2HPO_4, Na_2SO_4, Na_2CO_3, and $Sr_2(NO_3)_2$ (**E**; adapted from Snyder, 1908).

as a consequence of reductions in aerobic scope (the oxygen use available for fitness-related activities; see McKenzie, 2011 for review). It is therefore important to consider specific contexts when assigning meaning to measures of performance. The disconnect between performance and fitness can be especially apparent when performance is measured at lower levels of biological organization, which are further removed from the emergent traits on which selection acts. An increase in the maximum activity of a single enzyme may mean very little unless it represents an important regulatory step in its broader biochemical pathway. Even seemingly direct links between lower-level processes and animal fitness can be obscured by tradeoffs within and between levels of organization. Such tradeoffs may mean that performance fails to track with fitness in intuitive ways. Thus, an important caveat is that high levels of performance at one level of organization do not necessarily translate to high levels of performance at another. It is also important to recognize that without empirical support, which is quite rare, it is very difficult to link measures of performance, even at the whole-animal level, with fitness outcomes in animal systems.

THE CURVES

Curve Shapes

Performance curves take a number of shapes that can vary greatly across environmental and physiological contexts (**Figure 2**). The *inverted U-shape* arguably represents the most common shape for a physiological performance curve, where performance is maximized at some intermediate environmental state and decreases as the environment changes in either direction. Thermodynamic effects on enzymatic rate processes, for instance, mean that catalytic activity is often optimized at some temperature that may be reflective of local environmental conditions. Cooler temperatures reduce performance by limiting the free energy available for activation, whereas warm temperatures ultimately limit performance by shifting the distribution of active enzyme microstates and potentially raising activation energies (see Schulte, 2015). The shapes of these thermal performance curves also tend to be mirrored at higher levels of biological organization, like muscle function (e.g., Rall and Woledge, 1990), aerobic scope (see McKenzie, 2011 for review), and locomotor performance (e.g., Brett, 1967). Some curves, on the other hand, may appear to be more *U-shaped*. However, these curves tend to describe rates that are inversely proportional to performance. RMRs in marine osmoregulators, for instance, tend to be lowest in isosmotic conditions and increase as salinity changes in either direction. While the subsequent curve is invariably U-shaped, rising RMRs reflect metabolic costs of osmoregulation, which ultimately act as loading factors on aerobic scope (e.g., Behrens et al., 2017). Increasing rates of reactive oxygen species (ROS) production at thermal extremes represent another example where increased rates are likely to correspond with decreased performance.

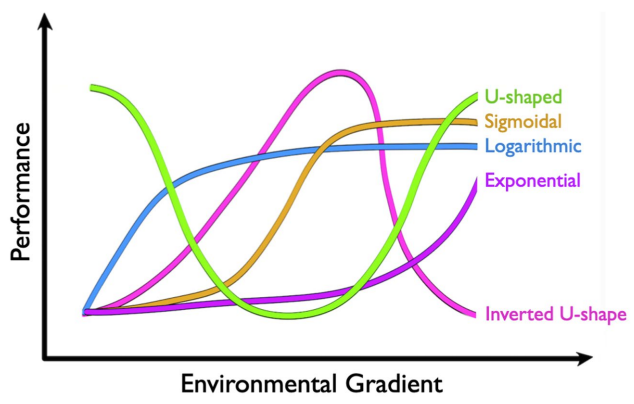

FIGURE 2 | Common shapes of physiological performance curves include U-shape, sigmoidal, logarithmic, exponential, and inverted U-shape.

Physiological performance curves can also be exponential, logarithmic, or sigmoidal in shape, although it may be important to restrict our discussion to ecologically relevant conditions. "All things are poison," as Paraclesus stated, and performance will ultimately suffer when any variable exists in excess. Some physiological rates increase *exponentially* over environmental gradients. The thermal sensitivity of MR in ectotherms, for instance, often follows an exponential curve (see McKenzie, 2011 for review). Increases in free energy drive metabolic reactions forward with temperature coefficients (Q_{10}) typically between two and three for biological systems. In other words, physiological reaction rates double to triple for every 10°C increase, and are halved or third for every 10°C decrease (Rogers, 1911). While RMRs ultimately tend to fail with extreme heat, ectotherms typically succumb to thermal stress first. Pcrit curves, on the other hand, tend to be *logarithmic* (e.g., Richards, 2011; Stoffels, 2015), where oxyregulators defend RMR over an impressive range of PO_2 but ultimately conform to oxygen limitations at some critical PO_2 threshold (Pcrit). Another common type of performance curve in physiology is dose-response curves that are classically *sigmoidal* in shape (Meddings et al., 1989; but see Calabrese and Baldwin, 1999). Here, increasing doses (i.e., treatment concentration or time) of a drug, toxin, or pollutant may alter performance exponentially across a threshold range, beyond which increases in dose have ever-decreasing effects on performance. Rats treated with methamphetamine, for instance, increase heart rate by approximately 60 beats per minute (bpm) as doses rise between 0.1 and 1 mg/kg. Outside this range, however, increases or decreases in dose have diminishing effects on heart rate (Hassan et al., 2016).

Curve Shifts

Adaption

Performance curves that differ between populations (intraspecific) and species (interspecific) can reflect adaptive differences in environmental tolerance (e.g., **Figures 3A–C**). This is particularly true when populations are genetically distinct (i.e., minimal geneflow). Many field-based studies show compelling evidence for local adaption. However, without controlled common-garden experiments, local adaption is often difficult to disentangle

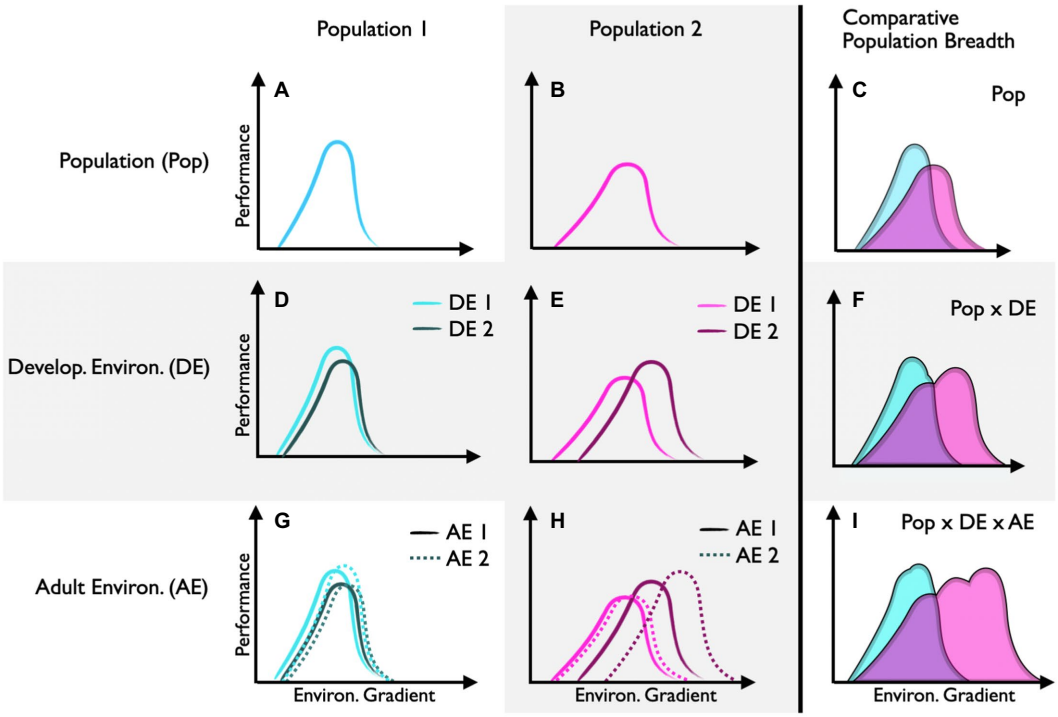

FIGURE 3 | Performance curve shifts between and within populations. Performance curves for population 1 **(A)** and population 2 **(B)** are shifted **(C)**, suggesting that each population is locally adapted (assuming common-garden conditions). Population 1 has low capacity for developmental plasticity **(D)**, whereas population 2 has high capacity for developmental plasticity **(E)**, which increases its potential performance breadth **(F)**. Population 1 has low capacity for reversible acclimation regardless of the developmental environment **(G)**. Population 2 has high capacity for reversible acclimation, but only when individuals develop in environment 2 **(H)**. This relatively high capacity for reversible acclimation further increases the potential performance breadth of population 1 **(I)**.

from the potential effects of developmental plasticity. Likewise, it is often difficult to demonstrate that the trait in question is truly adaptive in animal systems (but see lizard running paper) or that the trait was actively selected for (rather than the consequence of bottleneck events or drift). Despite these distinct challenges, recent work across eight populations of marine snail (*Urosalpinx cinerea*) suggested that shifts in thermal performance curves for growth represented local adaptation to seasonal growth periods (Villeneuve et al., 2021). Specifically, high-latitude populations of snails had higher thermal optima, presumably to achieve large body sizes over a shorter seasonal growth window. Here, common-garden experiments suggest these patterns reflect an adaptive trait (as opposed to developmental plasticity) and insights into the underlying genetic diversity of similar gastropod populations indicate selection (as opposed to a founder event; Villeneuve et al., 2021).

Developmental Plasticity

Performance curves that differ as a function of the developmental environment can reflect developmental plasticity (e.g., **Figures 3D–F**). Here, epigenetic modifiers regulate traits that are generally thought to be irreversible (but see Burggren, 2020) in response to early environmental cues. In this special issue, Rebolledo et al. (2021) show that planktonic survival of developing Australian tubeworms (*Galeolaria caespitosa*) is optimized to the temperatures experienced during embryogenesis. Specifically, there was a warmward skew in thermal survival curves with increasing embryonic temperature. Together, these findings demonstrate that specific aspects of the thermal performance curve (in this case thermal optima, but not thermal limits or thermal breadths) are shaped differently by developmental conditions.

Clonal organisms represent a particularly powerful tool for studies in developmental plasticity because genetic backgrounds are easily controlled. In this special issue, Laskowski et al. (2021) show that the clonal Amazon molly (*Poecilia formosa*) adjusts the thermal sensitivity of open-field swimming behavior, but not maximal swimming performance, as a function of developmental temperature. Interestingly, the authors find the opposite pattern in the closely related but sexually reproducing Atlantic molly (*Poecilia mexicana*), where performance curves for maximal swimming performance, but not open-field swimming behavior, shift as a factor of developmental temperature. This divergence between species suggests that genetic mechanisms are important in mediating the strength of epigenetic responses.

Developmental plasticity can itself represent an adaptive trait. That is, the capacity for developmental plasticity can vary between individuals and populations (e.g., **Figures 3D**,**E**). In theory, developmental plasticity is thought to be favored in variable environments where early developmental conditions can project future environments. In this special issue, Smith

et al. (2021) found that populations of the grasshopper (*Melanoplus boulderensis*) from higher elevations have greater capacity for developmental plasticity in response to seasonal changes (thermal variability and photoperiod) in the environment. Here, shifts in thermal performance curves for hopping distance and feeding rates revealed that different populations (and sexes) responded to developmental changes in day-length and thermal variability in complex ways. Together this work suggests that the interaction between genotype (populations) and epigenetics (responses to the developmental environment) shape ecologically important responses.

Reversible Acclimation

Performance curves that shift temporally within individuals as a function of the environment can reflect acclimation (e.g., **Figures 3G–I**). While acclimation is a physiological remodeling that is reversible over time, most studies use population-level sampling (as opposed to an individual-level approach) to quantify mean acclimation responses. In other words, studies often acclimate different subsamples of individuals from the same population to different environmental conditions to compare means, as opposed to reversibly acclimating the same individuals (but see x). Often, logistics, life-histories, and the delopmental stages of a focal animal can make experimental designs that acclimate the same individual to multiple environmental conditions challenging if not impossible. In this special issue, for instance, Longhini et al. (2021) analyzed thermal performance curves for aerobic scope in tadpoles of the stream-breeding savanna tree frog (*Bokermannohyla ibitiguara*) to determine their capacity for reversible acclimation. Here, a within-individual approach would likely not be possible because temporal responses to the changing thermal environment would be confounded with developmental stage. While the authors found that thermal performance curves for aerobic scope shifted with acclimation temperature, the effect is likely to reflect a pathology of high acclimation temperature rather than a regulated compensatory response. That is, aerobic scope collapsed in tadpoles acclimated to the upper boundary of temperatures naturally experienced in their microhabitat (i.e., 25°C). Thus, not all curve shifts within and between individuals represent adaptive plastic responses – a comprehensive understanding of the performance parameter in question, the life history of the focal species, and the acute effects of the environmental input(s) used represent important points of interpretation.

An increasing body of work has also revealed that there can be substantial inter-individual variation in the capacity for acclimation; that is, some individuals have phenotypes that are particularly plastic, and others have phenotypes that are more fixed (e.g., x). While some of this variation is likely to be genetically determined, the capacity for reversible acclimation itself also appears to be a plastic trait that can be programmed in response to the developmental environment. An implication of individual variation in the capacity for acclimation is that mean acclimation responses cannot predict population responses to environmental change in meaningful ways. With a bet-hedging strategy, for instance, only a subsection of the population may persist under conditions that favor plastic over fixed phenotypes. In this special issue, Seebacher and Little (2021) collate published data from 608 mosquitofish (*Gambusia holbrooki*) that were each reversibly acclimated to both a cool and warm temperature to show that focusing on mean values can mask underlying variation and obscure bet-hedging dynamics – particularly when populations are undersampled. Further, by focusing on plasticity at an individual level, Seebacher and Little (2021) also investigate tradeoffs (and potential costs) associated with plasticity, including a tradeoff between plasticity and maximal performance.

Curves Beyond Boundaries

Reductionist Mechanisms

Shapes of performance curves across functional boundaries and levels of biological organization can be particularly useful to determine potential mechanisms underlying higher-level traits. In this special issue, Rollwitz and Jastroch (2021) used plate-based respirometry to investigate the mechanisms compromising oxygen consumption rates of zebrafish (*Danio rerio*) embryos at thermal extremes. Specifically, the authors used a targeted pharmacological approach to generate thermal reaction norms for mitochondrial (e.g., basal mitochondrial respiration, ATP-linked respiration, and coupling efficiency) and cardiac (heart rate) performance in embryos pre-exposed to a range of five temperatures. Collectively, their work demonstrates that comparing performance curves down levels of biological organization can help elucidate mechanisms driving whole-animal responses. A note of particular interest is that the mechanisms that modulate mitochondrial performance with changing temperatures are non-linear – mitochondrial oxygen consumption is constrained by reduced rates of ATP production at low temperatures and amplified by increasing rates of proton leak at high temperatures.

Emergent Properties

In the same way performance curves can help uncover mechanisms underlying physiological responses to the environment, they can also be used to make predictions about emergent properties of a system at higher levels of organization. In this special issue, Killen et al. (2021) explore how within- and between-individual variation in physiological performance curves can shape social behavior, and higher order interactions like collective movement, disease and parasite transfer, and predator-prey relationships. Specifically, Killen et al. (2021) use thermal performance curves to model how differences in individual responses to temperature (e.g., the rank order of performance capacity as temperatures change) can impact group dynamics. Their work is comprehensive in the sense that they consider how the different parameters that comprise the thermal performance curve (i.e., peak performance, optimal performance, performance breadth, and critical limits) may shift between individuals as environments change. This type of approach is particularly important to link changes in abiotic stressors, which typically act at the molecular-, cell-, or tissue-level, with their environmental, economic, and cultural impacts, which are

typically assessed at population-, community-, and ecosystem-levels. While their work here is primarily theoretical, the authors make specific recommendations for empirical studies testing the effects of individual variation in physiological performance curves on social dynamics.

CONCLUSION

Performance curves represent a powerful tool to understand the implications of dynamic traits in changing environments. While the approach itself has been around for more than a century, this special issue showcases underused or emerging ways in which performance curves can be applied in comparative fields. We partitioned curve shapes, curve shifts, and functional boundaries into distinct subsections for simplicity, but we acknowledge that biological systems are rarely so defined. The complexity of natural systems is reflected by the studies included in this special issue, where comparisons within and between individuals, populations, endotherms and ectotherms (Levesque et al., 2021), and phylogenetic differences between species highlight the complex interactions that exist between evolutionary forces and epigenetic mechanisms underlying plasticity. Focusing on multiple traits across functional boundaries can also help elucidate mechanisms that underlie animal responses to environmental change and make predictions about the impacts of lower-level responses on higher levels of biological organization. Combined, these approaches are particularly powerful to identify how individual variation in environmental responses might scale up to the population-, species-, and community-level dynamics that inform conservation management strategies.

AUTHOR CONTRIBUTIONS

AL and FS conceived the ideas. AL wrote the manuscript. FS edited the manuscript. All authors contributed to the article and approved the submitted version.

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REFERENCES

Behrens, J. W., van Deurs, M., and Christensen, E. A. (2017). Evaluating dispersal potential of an invasive fish by the use of aerobic scope and osmoregulation capacity. *PLoS One* 12:e0176038. doi: 10.1371/journal.pone.0176038

Brett, J. R. (1967). Swimming performance of sockeye salmon (Oncorhynchus nerka) in relation to fatigue time and temperature. *J. Fish. Board Canada* 24, 1731–1741. doi: 10.1139/f67-142

Burggren, W. W. (2020). Phenotypic switching resulting from developmental plasticity: fixed or reversible? *Front. Physiol.* 10:1634. doi: 10.3389/fphys.2019.01634

Burton, T., Killen, S. S., Armstrong, J. D., and Metcalfe, N. B. (2011). What causes intraspecific variation in resting metabolic rate and what are its ecological consequences? *Proc. Biol. Sci.* 278, 3465–3473. doi: 10.1098/rspb.2011.1778

Calabrese, E. J., and Baldwin, L. A. (1999). Reevaluation of the fundamental dose–response relationship: a new database suggests that the U-shaped, rather than the sigmoidal, curve predominates. *Bioscience* 49, 725–732. doi: 10.2307/1313596

Eliason, E. J., Clark, T. D., Hague, M. J., Hanson, L. M., Gallagher, Z. S., Jeffries, K. M., et al. (2011). Differences in thermal tolerance among sockeye salmon populations. *Science* 332, 109–112. doi: 10.1126/science.1199158

Hassan, S. F., Wearne, T. A., Cornish, J. L., and Goodchild, A. K. (2016). Effects of acute and chronic systemic methamphetamine on respiratory, cardiovascular and metabolic function, and cardiorespiratory reflexes. *J. Physiol.* 594, 763–780. doi: 10.1113/JP271257

Killen, S., Cortese, D., Cotgrove, L., Jolles, J., Munson, A., and Ioannou, C. (2021). The potential for physiological performance curves to shape environmental effects on social behaviour. *Front. Physiol.* 12:719. doi: 10.3389/fphys.2021.754719

Laskowski, K. L., Seebacher, F., Habedank, M., Meka, J., and Bierbach, D. (2021). Two locomotor traits show different patterns of developmental plasticity between closely related clonal and sexual fish. *Front. Physiol.* 12:740604. doi: 10.3389/fphys.2021.740604

Levesque, D. L., Nowack, J., and Boyles, J. G. (2021). Body temperature frequency distributions: a tool for assessing thermal performance in endotherms? *Front. Physiol.* 12:760797. doi: 10.3389/fphys.2021.760797

Longhini, L. S., Zena, L. A., Polymeropoulos, E. T., Rocha, A. C., da Silva Leandro, G., Prado, C., et al. (2021). Thermal acclimation to the highest natural ambient temperature compromises physiological performance in tadpoles of a stream-breeding savanna tree frog. *Front. Physiol.* 12:726440. doi: 10.3389/fphys.2021.726440

Martin, C. J. (1903). I. Thermal adjustment and respiratory exchange in monotremes and marsupials—a study in the development of homæothermism. *Philos. Trans. R. Soc. Lond. B* 195, 1–37. doi: 10.1098/rstb.1903.0001

McBryan, T. L., Anttila, K., Healy, T. M., and Schulte, P. M. (2013). Responses to temperature and hypoxia as interacting stressors in fish: implications for adaptation to environmental change. *Integr. Comp. Biol.* 53, 648–659. doi: 10.1093/icb/ict066

McKenzie, D. J. (2011). Swimming and other activities. *Encycl. Fish. Physiol.* 3, 1636–1644.

Meddings, J. B., Scott, R. B., and Fick, G. H. (1989). Analysis and comparison of sigmoidal curves: application to dose-response data. *Am. J. Physiol.* 257, G982–G989. doi: 10.1152/ajpgi.1989.257.6.G982

Papakonstantinou, Z. (2009). Wine and wine drinking in the homeric world. *L'Antiquité Class.* 78, 1–24. doi: 10.3406/antiq.2009.3735

Rall, J. A., and Woledge, R. C. (1990). Influence of temperature on mechanics and energetics of muscle contraction. *Am. J. Physiol.* 259, R197–R203. doi: 10.1152/ajpregu.1990.259.2.R197

Rebolledo, A., Monro, K., and Sgrò, C. (2021). Thermal performance curves are shaped by prior thermal environment in early life. *Front. Physiol.* 12:738338. doi: 10.3389/fphys.2021.738338

Richards, J. G. (2011). Physiological, behavioral and biochemical adaptations of intertidal fishes to hypoxia. *J. Exp. Biol.* 214, 191–199. doi: 10.1242/jeb.047951

Rogers, C. G. (1911). "Studies upon the temperature coefficient of the rate of heart beat in certain living animals," in *Am. J. Phys. Legacy Content*. 28, 81–93.

Rollwitz, E., and Jastroch, M. (2021). Plate-based respirometry to assess thermal sensitivity of zebrafish embryo bioenergetics in situ. *Front. Physiol.* 12:746367. doi: 10.3389/fphys.2021.746367

Schulte, P. M. (2015). The effects of temperature on aerobic metabolism: towards a mechanistic understanding of the responses of ectotherms to a changing environment. *J. Exp. Biol.* 218, 1856–1866. doi: 10.1242/jeb.118851

Seebacher, F., and Little, A. G. (2021). Plasticity of performance curves in ectotherms: individual variation modulates population responses to environmental change. *Front. Physiol.* 12:733305. doi: 10.3389/fphys.2021.733305

Smith, J., Telemeco, R., Ortiz, B. B., Nufio, C. R., and Buckley, L. (2021). Greater seasonal developmental plasticity in higher-elevation grasshopper populations. *Front. Physiol.* 12:738992. doi: 10.3389/fphys.2021.738992

Snyder, C. D. (1908). A comparative study of the temperature coefficients of the velocities of various physiological actions. *Am. J. Physiol.* 22, 309–334. doi: 10.1152/ajplegacy.1908.22.3.309

Stoffels, R. J. (2015). Physiological trade-offs along a fast-slow lifestyle continuum in fishes: what do they tell us about resistance and resilience to hypoxia? *PLoS One* 10:e0130303. doi: 10.1371/journal.pone.0130303

Tang, P. S. (1933). On the rate of oxygen consumption by tissues and lower organisms as a function of oxygen tension. *Q. Rev. Biol.* 8, 260–274. doi: 10.1086/394439

Vernon, H. M. (1894). The relation of the respiratory exchange of cold-blooded animals to temperature. *J. Physiol.* 17, 277–292. doi: 10.1113/jphysiol.1894.sp000531

Villeneuve, A. R., Komoroske, L. M., and Cheng, B. S. (2021). Environment and phenology shape local adaptation in thermal performance. *Proc. Biol. Sci.* 288:20210741. doi: 10.1098/rspb.2021.0741

Waddell, W. J. (2010). History of dose response. *J. Toxicol. Sci.* 35, 1–8. doi: 10.2131/jts.35.1

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Interacting Effects of Cell Size and Temperature on Gene Expression, Growth, Development and Swimming Performance in Larval Zebrafish

Iris Louise Eleonora van de Pol[1], Adam Hermaniuk[2] and Wilhelmus Cornelis Egbertus Petrus Verberk[1]*

[1] Department of Animal Ecology and Physiology, Radboud Institute for Biological and Environmental Sciences, Radboud University, Nijmegen, Netherlands, [2] Department of Evolutionary and Physiological Ecology, Faculty of Biology, University of Białystok, Białystok, Poland

Cell size may be important in understanding the thermal biology of ectotherms, as the regulation and consequences of cell size appear to be temperature dependent. Using a recently developed model system of triploid zebrafish (which have around 1.5-fold larger cells than their diploid counterparts) we examine the effects of cell size on gene expression, growth, development and swimming performance in zebrafish larvae at different temperatures. Both temperature and ploidy affected the expression of genes related to metabolic processes (*citrate synthase* and *lactate dehydrogenase*), growth and swimming performance. Temperature also increased development rate, but there was no effect of ploidy level. We did find interactive effects between ploidy and temperature for gene expression, body size and swimming performance, confirming that the consequences of cell size are temperature dependent. Triploids with larger cells performed best at cool conditions, while diploids performed better at warmer conditions. These results suggest different selection pressures on ectotherms and their cell size in cold and warm habitats.

Keywords: triploidy, genome size, flow cytometry, thermal biology, ectotherm

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****Correspondence:***
Iris Louise Eleonora van de Pol
i.vandepol@science.ru.nl

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INTRODUCTION

Cell size is emerging as an important trait in understanding the thermal biology of ectothermic animals, as the regulation and consequences of cell size appear to be dependent on temperature (Dufresne and Jeffery, 2011; Hessen et al., 2013; Alfsnes et al., 2017). The predominant factor governing cell size is genome size as the cytoplasmic to nuclear ratio appears to be relatively constant (Gregory, 2001a). Among different animal groups, a large variation in genome sizes is found (Horner and Macgregor, 1983; Gregory, 2001b; Jalal et al., 2015) and in extant fish, the variation in genome size spans the entire breadth of vertebrate genome sizes (Hardie and Hebert, 2004). This large variation in genome size can be partially attributed to whole-genome duplication (WGD) events in the teleost lineage after the divergence of tetrapods (Amores et al., 1998) and polyploidization events in chondrichtheans (cartilaginous fishes) (Stingo and Rocco, 2001), chondrosteans (sturgeons and bichirs) (Blacklidge and Bidwell, 1993) and in certain actinopterygians (ray-finned fishes) (Uyeno and Smith, 1972). However, genome size reductions

have also taken place following WGDs as genome size did not exponentially increase DNA content after consecutive WGDs, indicating that genome size and hence cell size is under selection.

Evidence from field studies comparing animals (and plants) with a larger genome and especially polyploids suggests that organisms with a larger genome perform better in colder environments (Bennett, 1976; Gregory and Hebert, 1999): Polyploid amphibians are more abundant at high altitudes or latitudes (Otto et al., 2007), and polyploid *Daphnia pulex* mature faster than their diploid conspecifics, but only at low temperatures (Dufresne and Hebert, 2016). Fish species living in deep, cold water also possess larger cells and genomes than those living in warmer waters (Hardie and Hebert, 2003; Ebeling et al., 2015). Laboratory studies comparing performance of fish with different ploidy levels and cell sizes also indicate that fish with larger cells tend to favor lower temperatures (Atkins and Benfey, 2008; Sambraus et al., 2017). In addition, ectotherms often grow to a larger size in cold environments and this pattern may be especially prominent for aquatic animals with a larger cell size (Verberk et al., 2021). As cell size may be an important determinant for body size, advancing our understanding of cell size adaptations will contribute to understanding the thermal biology of ectotherms.

A role for oxygen in shaping the thermal response in growth is likely (Forster et al., 2012; Horne et al., 2015; Hoefnagel and Verberk, 2015). Oxygen is more likely to become limiting in water than in air, because of the lower diffusion rates in water and its higher viscosity (Verberk et al., 2011). At high temperatures, the metabolic rate of ectotherms increases, which can eventually cause the oxygen demand to exceed supply, which may impair performance (i.e., reduced growth, heat tolerance, fecundity, feeding) (Pörtner, 2010; Verberk et al., 2011). To a certain extent, ectotherms such as fish are able to remodel their physiology to increase oxygen supply, for example by enlarging their gill surface or by increasing hematocrit or ventilating rates (Nilsson, 2007).

Cell size may also affect oxygen supply, as oxygen diffuses more easily across lipid membranes than through aqueous cytosol (Subczynski et al., 1989). Therefore, animals composed of smaller cells may be less susceptible to oxygen limitation, as their membrane surface area is greater than in similarly sized animals composed of large cells. The capacity of individual cells to take up oxygen may be especially relevant in fish larvae that do not have fully developed gills yet and rely heavily on cutaneous oxygen uptake (Rombough, 2002). On the other hand, the larger membrane-to-surface volume ratio in smaller cells makes them more energetically costly to maintain, due to higher costs for lipid turnover and the maintenance of electrochemical gradients across the cell membranes (Szarski, 1983; Hulbert and Else, 2000). Indeed, smaller cells exhibit higher resting metabolic rates on a per mass basis than larger cells (Kozłowski et al., 2003). A previous study on zebrafish larvae with different cell sizes and exposed to different rearing and testing temperatures in a full factorial design found that being composed of larger cells could provide metabolic advantages in the cold, whereas smaller cells are metabolically more beneficial in warm water (Hermaniuk et al., 2021).

This study examined the effects of cell size on gene expression, growth, development and swimming performance in zebrafish larvae reared at different temperatures. We used a triploid zebrafish model that we developed in a previous study (van de Pol et al., 2020). We demonstrated a 1.64 (+0.18 SD) fold increase in erythrocyte cell volume and a 1.72 (+0.70 SD) fold decrease in total nuclei number when comparing triploid larvae to similar sized diploid larvae. Thus, these differences between diploids and triploids closely agree with a 1.5-fold increase in cell size and a 1.5-fold reduction in cell number, that would be expected under a constant nuclear-cytoplasmic ratio. This general pattern of triploid zebrafish larvae having larger but fewer cells need not necessarily apply to all cell types, as we only measured erythrocyte volume directly, but increases in cell volume in polyploid fish have been shown in a range of tissues, including muscle (Suresh and Sheehan, 1998; Vargas et al., 2015), brain and liver (reviewed by Benfey, 1999). Furthermore, triploid zebrafish larvae have been found to be very similar to their diploid counterparts under non-demanding conditions (van de Pol et al., 2020; Small et al., 2021), which makes triploid zebrafish a good model to study the effects of cell size. For this study, we tested whether the consequences of cell size are temperature dependent by rearing and testing the zebrafish at different temperatures. We hypothesize that cell size affects oxygen uptake and transport capacity and therefore triploids with larger cells have a lower energy budget than diploids with smaller cells, especially under conditions where oxygen demand is high (i.e., high temperatures), but not when oxygen demand is lower (i.e., low temperatures), where their greater efficiency can allow them to outperform diploids. Although cold water holds more oxygen, it is also more viscous and oxygen diffusion rates are slower, resulting in a lower bioavailability of oxygen. In addition, and more importantly, the metabolic rate of larvae is reduced in the cold, which means they require less oxygen. This effect of temperature on oxygen demand outweighs the effect on oxygen supply (Verberk et al., 2011). Thus, in the cold the requirement for small cells with high capacity for oxygen uptake is absent, and the larger cells are more beneficial in the cold, as they are more energy efficient. We measured gene expression of metabolic genes, which we considered useful proxies to assess differences in energy budget. We also tested our predictions by comparing diploids and triploids in terms of growth, development, and swimming performance at different temperatures.

MATERIALS AND METHODS

Zebrafish Husbandry, Egg Collection and Triploidy Induction

Maintenance of adult zebrafish and triploidy induction have been described in detail in van de Pol et al. (2020). In brief, zebrafish from the AB strain (supplied by ZIRC, ZFIN ID: ZDB-GENO-960809-7) were kept in 4-L tanks at a density of approximately 30 fish per tank, provided with recirculating tap water (temperature 27°C, pH 7.5–8) under a 14 h:10 h light:dark photoperiod.

Eggs were collected by both natural spawning in mass breeding tanks and *in vitro* fertilization. Triploidy was induced by using a cold shock treatment; 3 min after fertilization the collected eggs were immersed in 4°C E2 medium (5 mmol l^{-1} NaCl, 0.17 mol l^{-1} KCl, and 0.33 mmol l^{-1} $MgSO_4$) for

20 min. Eggs were fertilized with a pooled sperm solution of 8–12 males to ensure a heterogeneous offspring. In addition, eggs of different females were randomly distributed over the experimental treatments. Different batches of eggs were harvested from multiple rounds of fertilization, alternating between different parental tanks with similar genetic background. We did not divide the egg batches in half to produce diploids and triploids, due to the vulnerability of the eggs right after fertilization. However, we do not expect family effects as we obtained diploids and triploids from similar parental tanks, although not in the same round of fertilization. Multiple batches of eggs were reared for each experiment: seven for genome size and DNA condensation, two for gene expression, four for development rates, and for growth and swimming performance six batches were reared.

All experiments were carried out in accordance with the Dutch Animals Act[1], the European guidelines for animal experiments (Directive 2010/63/EU)[2] and institutional regulations.

Larval Rearing

Within 2 h after fertilization, diploid and cold shocked embryos were divided over three temperature treatments: 23.5°C, 26.5° (control temperature), and 29.5°C. The embryos were kept in 48-wells plates (Greiner Bio-One, Kremsmünster, Austria) with a mesh bottom and placed in a rearing tank containing E3 medium (E2 medium with addition of 10^{-5}% methylene blue). During development, these tanks were constantly aerated and maintained in water baths connected to a circulating heating/cooling system (Grant LT ecocool 150) at the aforementioned temperatures ±0.5°C. Dead embryos or larvae were removed from the setup daily. Survival was affected by ploidy level, but not rearing temperature [see also Hermaniuk et al. (2021) who used the same setup]. We measured length for all larvae used in the swimming trials, enabling us to include length as a co-variate in our statistical models on swimming velocity.

To ensure that larvae were in the same developmental stage in physiological time when performing our experiments, larvae at 23.5°C were reared until 6 days post-fertilization (dpf) and larvae at 29.5°C were reared until 4 dpf, at which point they reached the same developmental stage as 26.5°C larvae reared until 5 dpf. At this time, larvae have almost fully resorbed their yolk sack and are able to produce a proper escape response. Throughout the manuscript, when we indicate 5 dpf, we refer to the developmental stage of 5 dpf for larvae reared at the three different temperatures.

Genome Size Verification

Triploidy induction was verified in cold shocked larvae at 5 dpf using a propidium iodide (PI) staining to quantify the amount of nuclear DNA. This method has been described in detail in van de Pol et al. (2020). Briefly, a cold shocked and a diploid larva were pooled in one sample, where the diploid serves as an internal control. After homogenization in lysis buffer, the suspension was filtered using a 70 μm cell strainer (pluriSelect Life Science, Leipzig, Germany) to obtain single cell nuclei. These nuclei were stained using PI staining buffer and samples were analyzed with a FC500 5-color Flow Cytometer (Beckman Coulter Life Science, Indianapolis, IN, United States). To verify induction of triploidy, ploidy level was measured on the individual larvae used for the swimming performance trials and growth measurements. Larvae that were cold shocked but turned out to be diploids were excluded from our analyses. The larvae that we used for gene expression and development rate measurements were not verified individually. Instead, triploidy induction efficiency was based on the measurements of 20–30 individual larvae from the same batch. These batch efficiencies ranged from 95.8 to 100%.

In addition to triploidy verification, we also compared the PI staining within diploid and triploid larvae reared at different temperatures. Therefore, we also measured samples containing two diploid larvae for each temperature treatment. Lastly, we calculated the G2/G1 ratio of the cells for each condition, which is a measure for the dividing potential of the cells. The R package flowPloidy (Smith et al., 2018) was used to analyze genome size.

Gene Expression

Diploid and cold shocked larvae of all three temperature treatments were collected for qPCR analysis at 5 dpf. Two or three larvae were pooled in 2 mL Eppendorf tubes and immediately frozen in liquid nitrogen, with at least five replicate samples per treatment. Details about the qPCR preparation can be found in van de Pol et al. (2020). In short, total RNA isolation with TRIzol (Thermo Fisher Scientific, Waltham, MA, United States) was performed according to the manufacturer's instruction with some minor changes. RNA concentrations were measured by NanoDrop spectrophotometry (ND-1000; Isogen Life Science B.V., De Meern, Netherlands) to obtain equal amounts of RNA. For cDNA synthesis 500 ng total RNA was used, which was diluted 10x in DEPC H_2O for qPCR.

The expression levels of six housekeeping genes and four genes related to metabolic processes and temperature responses were analyzed: ribosomal protein S11 (*rps11*, previously known as *40S*); actin, beta 1 (*actb1*); eukaryotic translation elongation factor 1 alpha 1, like 1 (*eef1a1| 1*); polymerase (RNA) II (DNA directed) polypeptide D (*polr2d*); ribosomal protein L13a (*rpl13a*) and TATA box binding protein (*tbp)*. These housekeeping genes are frequently used to normalize gene expression of genes of interest, as they are involved in a range of basic cellular processes. The genes of interest are: citrate synthase, mitochondrial (*cs*); L-lactate dehydrogenase A chain (*ldha*); L-lactate dehydrogenase B-A chain (*ldhba*) and heat shock cognate 70-kd protein, tandem duplicate 1 (*hsp70.1*). Primer sequences and the involvement of these genes in cellular and physiological processes can be found in **Table 1**.

To normalize the expression values of the housekeeping genes, the relative quantity of the five other housekeeping genes was used as a combined index. For the genes of interest, relative quantities of all six housekeeping genes were used to normalize expression values, to average out possible variation in housekeeping gene expression caused by the temperature treatments.

[1]https://wetten.overheid.nl/BWBR0003081/2019-01-01

[2]https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:32010L0063

TABLE 1 | Primer sequences for qPCR.

Gene	Source	Process	Fw primer sequence (5′-3′)	Rv primer sequence (5′-3′)
rps11	NM_213377.1	Translation	GCTTCAAAACCCCCAGAGAA	TCAGGACGTTGAACCTCACA
actb1	NM_131031.1	Cytoskeleton integrity	CTTGCTCCTTCCACCATGAA	CTGCTTGCTGATCCACATCT
eef1a1l1	NM_131263.1	Cell cycle	CTGGAGGCCAGCTCAAACAT	TCAAGAAGAGTAGTACCGCTAGCATTAC
polr2d	NM_001002317.2	Transcription	CCAGATTCAGCCGCTTCAAG	CAAACTGGGAATGAGGGCTT
rpl13a	NM_212784.1	Translation	TCTGGAGGACTGTAAGAGGTATGC	AGACGCACAATCTTGAGAGCAG
tbp	NM_200096.1	Transcription	CTTACCCACCAGCAGTTTAGCAG	CCTTGGCACCTGTGAGTACGACTTTG
cs	NM_199598.1	Aerobic metabolism	ACTCCAACCTGGACTGGTCA	ACGTTTCCACCCTCATGGTC
ldha	NM_131246.1	Anaerobic metabolism	GAGACATTCCAGCCCATCCT	ACACCAACCACTGTCACCTT
ldhba	NM_131247.1	Anaerobic metabolism	AATCGGGATCATGGCCTCAG	ATCTGCAAGTTCCCGAAGCA
hsp70.1	NM_001362359.1	Cellular stress response	GACATCGACGCCAACGGG	GCAGAAATCTTCTCTCTCTGC

Growth and Development

Development of the embryos was followed up until 72 hours post-fertilization (hpf), by determining their developmental stage according to Kimmel et al. (1995) at fixed time points using a Leica MZ FLIII stereomicroscope (Leica Microsystems, Wetzlar, Germany). For all temperature treatments, staging was performed at 6, 24, 30, 48, and 72 hpf in real-time, to be able to observe temperature effects on development. These time points were chosen because of the clearly distinguishable developmental features, namely: embryonic shield, heart beat and early pigmentation, weak circulation, tapering yolk extension and protruding mouth.

At each time point, five diploid and five cold shocked embryos were staged of all temperature treatments. In our final analysis, we only included development of embryos that morphologically appeared to have developed normally at 5 dpf (e.g., straight body axis, no pericardial edema and normally sized head and eyes). Length was measured at the developmental stage of 5 dpf (protruding mouth stage), using pictures of larvae taken with a Leica MZ FLIII stereomicroscope equipped with a Leica DFC450 C camera and the segmented line tool of the ImageJ program[3].

Swimming Performance

Maximum swimming velocity of diploid and cold shocked larvae of the three rearing temperatures was assessed at 5 dpf using a DanioVision system (Noldus Information Technology B.V., Wageningen, Netherlands). Details of the procedure can be found in van de Pol et al. (2020). Briefly, larvae were placed individually in a well of a 24-wells plate (Greiner Bio-One, Kremsmünster, Austria), containing 1 mL of E3 medium. They were presented with 10 tap stimuli with an interval of 20 s [a startle protocol, described in van den Bos et al. (2017)]. The measurement started 10 min after transferring the larvae to the setup. Then they were tracked for 10 min in which no startles were presented, followed by the 10 tap stimuli. In total, this measurement takes 23 min. Their maximum velocity during the startle response was used as a readout, as well as the proportion of responders (velocity above 15 mm/s) and non-responders (velocity below 15 mm/s). Larvae that never showed a response to the tap stimulus were excluded from further analyses. The larvae were measured at the same temperature they were reared at (23.5°C, 26.5°C, and 29.5°C). In a previous study we found the most pronounced differences in metabolic rate between diploids and triploids in larvae reared at 29.5°C and tested at 23.5°C (Hermaniuk et al., 2021). Therefore, we also included this fourth temperature combination in our measurements of swimming performance.

Statistical Analyses

The collected data were analyzed using RStudio version 1.1.383, respecting a significance threshold of $\alpha = 0.05$. To compare genome sizes and to test for the effect of rearing temperature on DNA condensation for diploid and triploid larvae, we used a general linear model and subsequent ANOVA. Effects of ploidy and rearing temperature and the interaction between these factors on the ratio of cells in the G2 and G1 phase were also tested with ANOVA. Tukey's *post hoc* test was performed to compare between the different ploidy and temperature groups.

Gene expression data were analyzed for each gene separately, using a general linear model of which the frequency distribution of the residuals was visually checked for normality. We used a subsequent ANOVA to test for the effects of ploidy level and rearing temperature and the interaction thereof. Tukey's *post hoc* test was used to annotated differences between ploidy levels and temperature groups.

Development rates of diploid and triploid larvae reared at different temperatures were compared using a general linear model, after visually checking the frequency distribution of the residuals as being normally distributed. We tested for the effects of ploidy level, rearing temperature, development time and the interaction of these factors. Within temperature treatments we tested for the effect of ploidy level, again using general linear models and subsequent ANOVA. Length measurements were analyzed using a general linear model to test for the effects of ploidy level, rearing temperature and the interaction of these factors, as the residuals were normally distributed. We used ANOVA and a subsequent Tukey's *post hoc* test to compare between the different ploidy and temperature groups.

For the swimming performance data, we first calculated the proportion of diploid and triploid responders vs. non-responders for each temperature treatment and stimulus number, as we observed a bimodality in startle responses

[3]https://imagej.nih.gov/ij/

(**Supplementary Figures 1–3**). A linear mixed effects model with a negative binomial structure was used to test for the effects of ploidy level, stimulus number and temperature treatment, including trial as a random factor (a trial was a measurement of a 24-wells plate with diploid or triploid larvae reared at a given temperature). We then analyzed maximum swimming velocity, only for those larvae that showed a startle response at a given stimulus, again using a linear mixed effects model in which we also included length as a factor. For both the proportion of responders and the swimming velocity, we found interactions with temperature treatment. We therefore also analyzed the effect of ploidy level for each temperature treatment by creating subsets of the data. For each subset, we then fitted two models, one with and one without ploidy and used likelihood ratio tests to assess whether the model with ploidy was a significant improvement over the model without ploidy. ANOVA tables for all analyses can be found in **Supplementary Tables 1–10**.

RESULTS

DNA Content and Dividing Potential

Flow cytometry clearly distinguished between cells in the G1 phase and cells in the G2 phase (**Supplementary Figure 4**), with fluorescence intensity mirroring the 1.5 difference in DNA content between diploids and triploids ($F_{1,59}$ = 650.18, $p < 0.001$, **Figure 1**). The fluorescence intensity not only reflects genome size, but also the degree of DNA condensation: less propodium iodide can bind to condensed DNA. Focusing on the G1 phase, we found that fluorescence intensity increased with rearing temperature in both diploids and triploids (rearing temperature: $F_{1,59}$ = 9.57, p = 0.003, **Figure 1**). We did not find an interaction between ploidy and rearing temperature on fluorescence intensity.

The ratio between G2 and G1 phase cells reflects how many cells are in the process of dividing. At the lowest rearing temperature, we found a higher G2/G1 ratio in diploids compared to triploids, but at the highest temperature this pattern was reversed, reflecting a significant interaction between ploidy and rearing temperature ($F_{1,58}$ = 46.32, $p < 0.001$, **Figure 2**).

Expression Levels of Housekeeping Genes and Metabolism Related Genes

Expression levels of the housekeeping genes was used to normalize gene expression of genes of interest (**Supplementary Figure 5**). Since for some housekeeping genes we also found differences in their expression with rearing temperature and ploidy, we used two normalizations, one based on all housekeeping genes (i.e., *rps11*, *actb1*, *eef1a1l1*, *polr2d*, *rpl13a*, and *tbp*; results shown below) and one based on only those genes whose expression did not vary with ploidy and temperature (i.e., *rps11* and *actb1;* results shown in **Supplementary Figure 6**).

Expression levels of *cs* were significantly affected by both rearing temperature and ploidy level ($F_{2,41}$ = 6.11, p = 0.004 and $F_{1,41}$ = 4.19, p = 0.047, respectively, **Figure 3A**), such that the expression of *cs* increased at low temperature in both diploid and triploid larvae. At all temperatures, *cs* expression was lower in triploids. The interaction between temperature and ploidy was not significant. A significant interaction between rearing temperature and ploidy was shown for *ldha* ($F_{2,41}$ = 9.76,

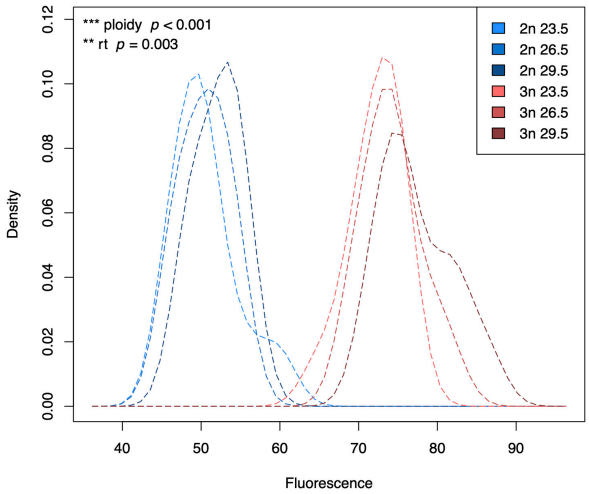

FIGURE 1 | Kernel density plot of the fluorescence of G1 phase cells of diploid and triploid larvae reared at different temperatures. Light, medium, and dark blue lines represent densities for diploid larvae reared at 23.5°C, 26.5°C, and 29.5°C, respectively. Light, medium, and dark red lines represent densities for triploid larvae reared at 23.5°C, 26.5°C, and 29.5°C, respectively. The density is scaled to 1 (100%), which does not have a unit. Fluorescence also does not have a unit, which is why we always add a standard when measuring fluorescence of our samples. Effects of rearing temperature and ploidy were significant (ANOVA, p = 0.003 and ANOVA, $p < 0.001$, respectively, n = 63).

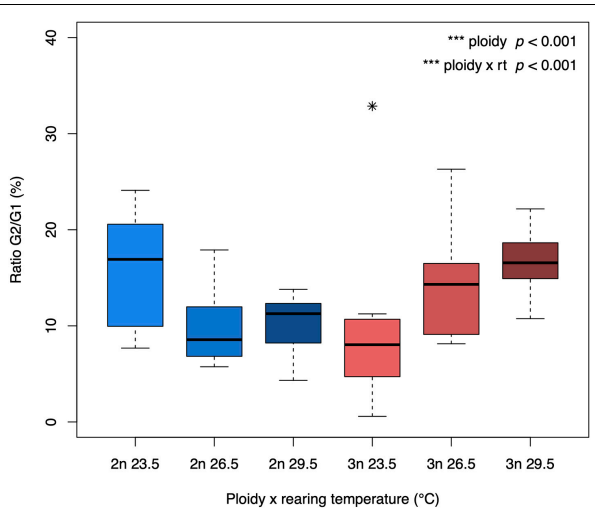

FIGURE 2 | G2/G1 ratio of diploid and triploid larvae reared at different temperatures. The ratio of cells in the G1 and G2 phase was calculated as G2/G1*100 for each larva. The box extends from the lower quartile to the upper quartile of the data, spanning the inter-quartile range (IQR). The thick line within the box represents the median. Whiskers extend to minima and maxima, but are limited to data points 1.5 times outside the IQR. The asterisk represents an outlier. The effect of ploidy and the interaction between ploidy level and rearing temperature were significant (ANOVA, $p < 0.001$, n = 64).

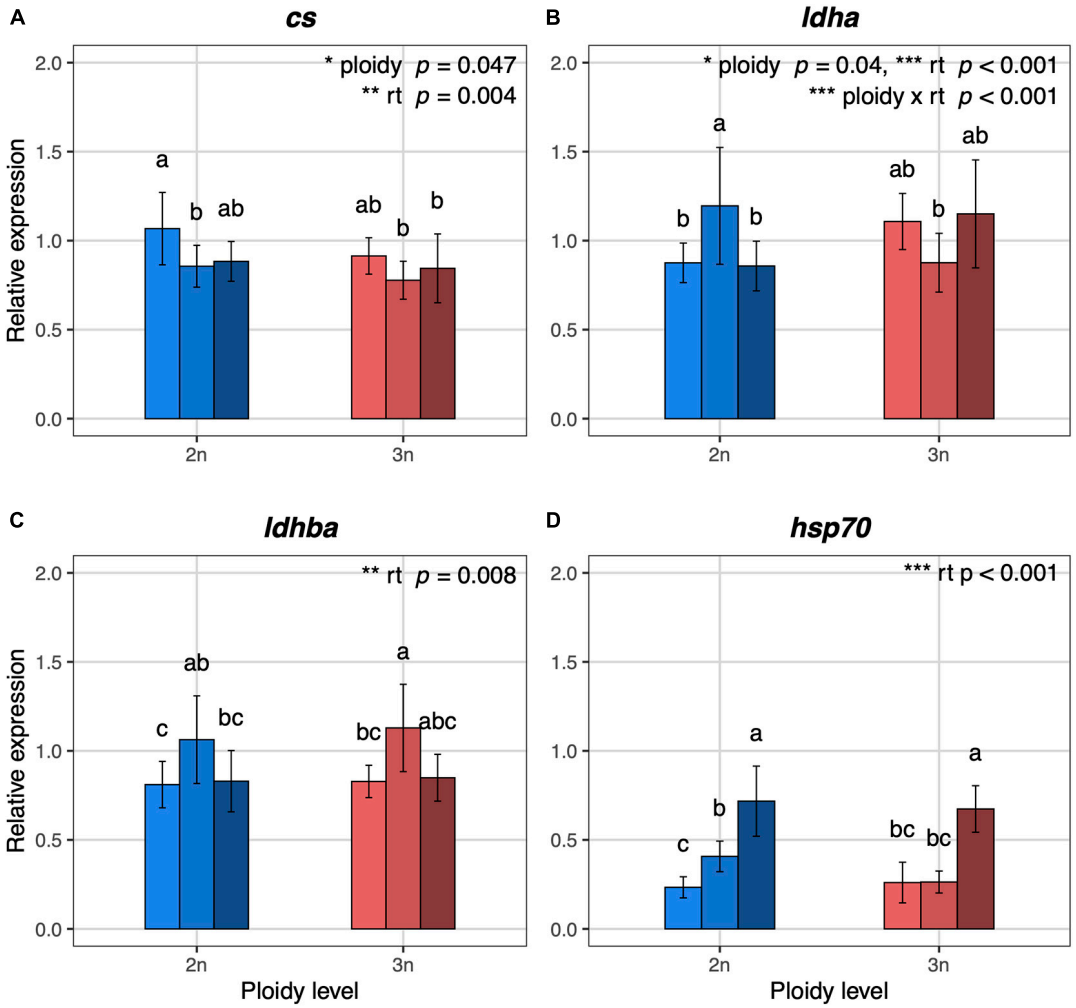

FIGURE 3 | Relative expression values of metabolism and temperature related genes in 5 dpf diploid and triploid larvae reared at different temperatures. **(A)** *cs*, citrate synthase, mitochondrial. **(B)** *ldha*, L-lactate dehydrogenase A chain. **(C)** *ldhba*, L-lactate dehydrogenase B-A chain. **(D)** *hsp70.1*, heat shock cognate 70-kd protein, tandem duplicate 1. Light, medium, and dark blue bars represent expression values for diploid larvae reared at 23.5°C, 26.5°C, and 29.5°C, respectively. Light, medium, and dark red bars represent expression values for triploid larvae reared at 23.5°C, 26.5°C, and 29.5°C, respectively. For each gene, the expression values are normalized using a combined index of the relative quantity of the six housekeeping genes shown in **Supplementary Figure 5**. Values are represented as means with standard deviations. Rearing temperature and ploidy were significant for *cs* (ANOVA, $p = 0.004$ and $p = 0.047$, respectively, $n = 48$). The interaction between rearing temperature and ploidy, rearing temperature and ploidy were significant for *ldha* (ANOVA, $p < 0.001$, $p = 0.001$, and $p = 0.04$, respectively, $n = 48$). Rearing temperature was significant for *ldhba* and *hsp70.1* (ANOVA, $p = 0.008$ and $p < 0.001$, respectively, $n = 48$; $n = 47$ for hsp70.1). Different letters indicate significant differences between groups (Tukey's *post hoc* test, $p < 0.05$, $n = 48$).

$p < 0.001$, **Figure 3B**), one of the genes coding for a subunit of the lactate dehygrogenase enzyme, with a reverse pattern for diploids and triploids at the different rearing temperatures. For the expression of *ldhba*, only the effect of rearing temperature was significant ($F_{2,41} = 5.48$, $p = 0.008$, **Figure 3C**), where the lowest expression was found at the higher and lower temperatures. A significant effect of rearing temperature was also found for the expression levels of *hsp70.1* ($F_{2,40} = 41.20$, $p < 0.001$, **Figure 3D**), which increased with temperature.

Development and Body Length

Both diploid and triploid larvae progressed faster through the different developmental stages at higher temperatures ($F_{1,386} = 55.98$, $p < 0.001$, **Figure 4**). There was no significant effect of ploidy ($F_{1,386} = 0.02$, $p = 0.88$, **Figure 4**), and the interaction between rearing temperatures and ploidy level was also not significant ($F_{1,386} = 0.03$, $p = 0.86$), indicating that the stimulating effect of warm conditions on development was similar for diploids and triploids.

The body length reached at the developmental stage of 5 dpf differed between diploids and triploids, depending on rearing temperature (temperature × ploidy: $F_{1,559} = 23.90$, $p < 0.001$, **Figure 5**). Body length decreased with increasing temperature only in triploids. As a result, triploids tended to reach a larger size in the coldest rearing temperature, but a smaller size in the warmest rearing temperature.

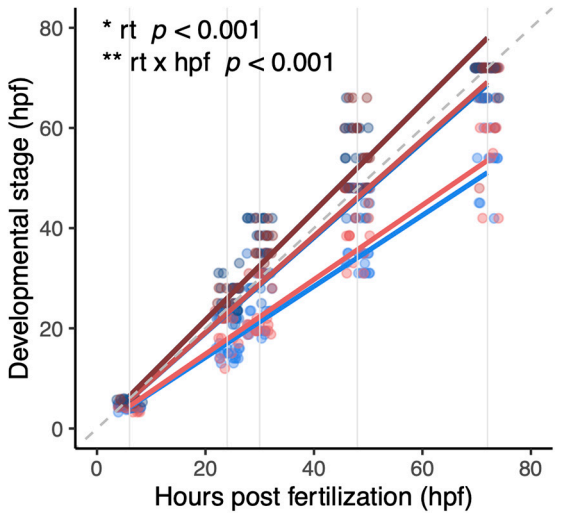

FIGURE 4 | Development of diploid and triploid larvae up to 72 hpf reared at different temperatures. Light, medium, and dark blue lines represent development of diploid larvae reared at 23.5°C, 26.5°C, and 29.5°C, respectively. Light, medium, and dark red lines represent development of triploid larvae reared at 23.5°C, 26.5°C, and 29.5°C, respectively. Note that at 26.5°C and 29.5°C the red and blue lines almost entirely overlap. In this graph, the dashed gray line is the reference x = y. Solid gray lines are the hours post-fertilization at which the embryos and larvae were staged, namely: 6, 24, 30, 48, and 72 h. Effects of rearing temperature and the interaction between hpf and rearing temperature were significant (ANOVA, $p < 0.001$, $n = 393$).

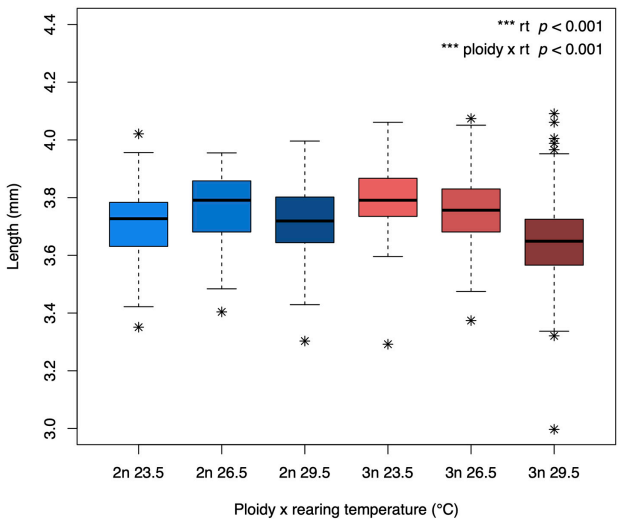

FIGURE 5 | Body length of diploid and triploid larvae reared at different temperatures. Body length was determined at the developmental stage of 5 dpf. The box extends from the lower quartile to the upper quartile of the data, spanning the inter-quartile range (IQR). The thick line within the box represents the median. Whiskers extend to minima and maxima, but are limited to data points 1.5 times outside the IQR. The asterisks represent outliers. The effect of rearing temperature and the interaction between rearing temperature and ploidy level were significant (ANOVA, $p < 0.001$, $n = 565$).

Swimming Performance

Upon being startled, most individuals responded by exhibiting an escape response. The proportion of larvae that responded was highest upon the first stimulus and decreased with subsequent stimuli for both ploidy levels and in all temperature treatments ($z = -13.10$, $p < 0.001$, **Figure 6**). The drop in the proportion of responders was stronger in triploids for the two coolest rearing temperatures, such that diploids were more responsive than triploids ($p = 0.04$ and $p = 0.01$, respectively). This pattern seemed to be reversed at an acute low temperature, although the effect of ploidy was not significant ($p = 0.06$, **Figure 6D**). All larvae were confirmed to be alive after these trials, as we observed them to swim when transferring them to Eppendorf tubes.

When larvae responded to a stimulus and exhibited an escape response, they could generate an escape velocity of about 63 mm/s (note that the y-axis on **Figure 7** are log transformed). Swimming velocity of larvae also decreased with increasing stimuli and this effect was small albeit significant ($F_{1,3547} = 155.96$, $p < 0.001$, **Figure 7**). While the model accounted for potential size differences between triploids and diploids, triploid larvae swam faster when tested at an acute low temperature than diploid larvae ($p < 0.001$, **Figure 7D**).

DISCUSSION

Genome size matters for the biology of species. Comparative studies document associations between a species' genome size and its performance, including a faster development in copepods (Wyngaard et al., 2005), a smaller egg size in fish (Hardie and Hebert, 2003), a higher metabolic rate in amphibians (Gregory, 2003) and a reduced cold tolerance in ectotherms (Leiva et al., 2019). The effects of genome size likely act *via* its correlate cell size, as cell size has consequences for the cellular energy budget: the greater surface area to volume ratio of small cells allows for better cellular uptake of oxygen to fuel aerobic metabolism, but at the same time more energy needs to be expended on maintaining electrochemical gradients. In this study, we tested if the consequences of cell size were dependent on temperature, using triploid zebrafish larvae as a model for zebrafish with larger cells (van de Pol et al., 2020). Our general hypothesis is that having larger cells is advantageous in the cold when energy demand is low, but not in warm conditions that increase energy and hence oxygen demand. Across different levels of biological organization, we indeed found evidence of interactions between rearing temperature and ploidy, confirming the growing idea in the literature that consequences of cell size are temperature dependent.

At the organismal level, we found differences in swimming performance between diploids and triploids. For the first stimulus, almost all larvae exhibited an escape response, but on subsequent stimuli, the proportion of larvae that responded declined, especially in triploids under control conditions at 26.5°C. This suggests that triploid larvae run out of energy sooner. The smaller surface area to volume ratio of their larger cells could limit oxygen transport by erythrocytes and oxygen transport across cell membranes, causing a shortage of oxygen

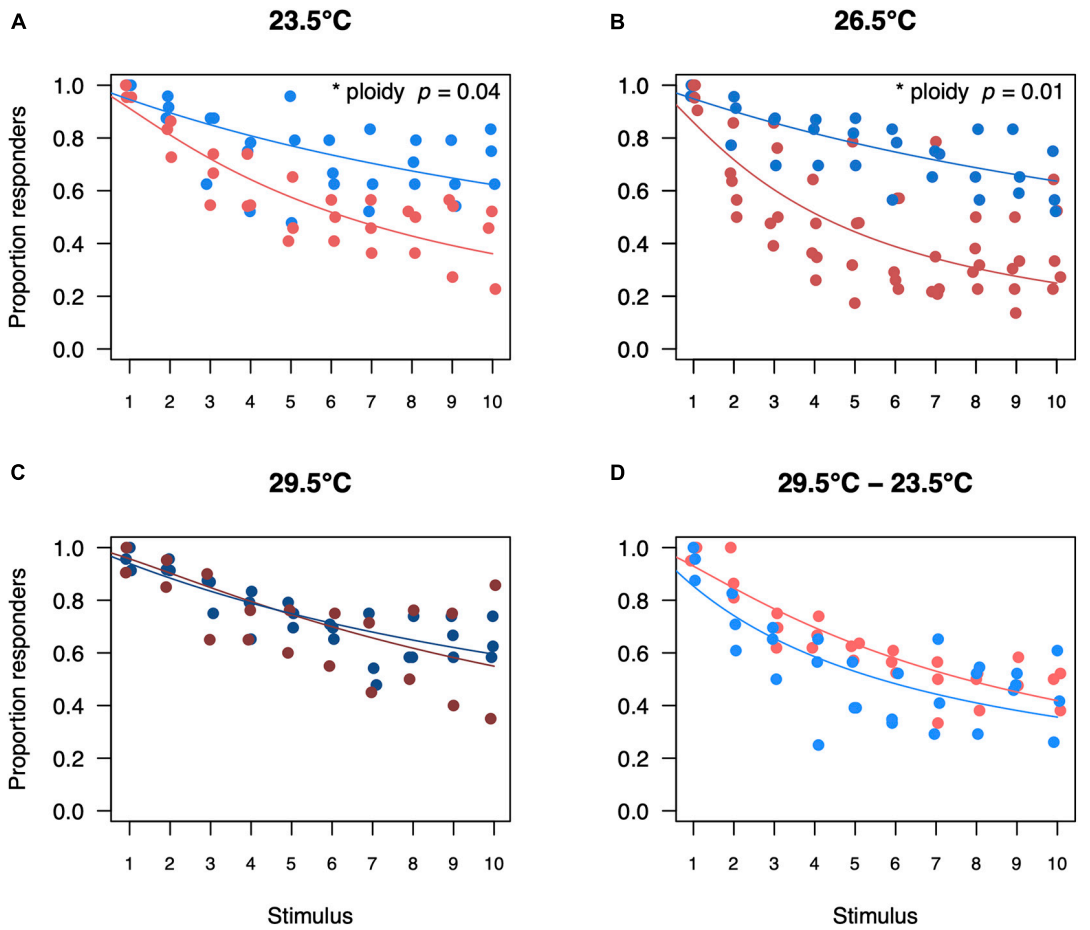

FIGURE 6 | Proportion of diploid and triploid responders for each startle stimulus per temperature treatment. Proportion of responders for diploid and triploid larvae reared and measured at 23.5°C **(A)**, 26.5°C **(B)**, 29.5°C **(C)** or reared at 29.5°C and measured at 23.5°C **(D)**. Depicted values are the proportion of responders per trial, where blue points represent diploid larvae and red points represent triploid larvae. Over all the effect of stimulus number was significant (ANOVA, $p < 0.001$, $n = 250$) as was the interaction between ploidy level and stimulus number (ANOVA, $p < 0.05$, $n = 250$). The effect of ploidy was also analyzed for a subset of each temperature treatment, comparing a model with and without ploidy level included. Ploidy was significant for larvae reared and measured at 23.5°C (ANOVA, $p = 0.04$, $n = 60$) and for larvae reared and measured at 26.5°C (ANOVA, $p = 0.01$, $n = 80$). Ploidy was not significant for larvae reared and measured at 29.5°C (ANOVA, $p = 0.87$, $n = 50$) and for larvae reared at 29.5°C and measured at 23.5°C (ANOVA, $p = 0.06$, $n = 60$).

supply to the mitochondria. Although the startle response is a form of burst activity, it is likely fueled predominantly by aerobic metabolism in larval zebrafish. In contrast to adult fish, larval fish have only one layer of red muscle fibers, which show strong cytochrome oxidase activity, instead of a 2-gear muscle system with white and red fibers (El-Fiky et al., 1987; Hunt von Herbing, 2002). In addition, enzyme activity related to glycolysis, indicative of anaerobic metabolism, develops later in ontogeny (El-Fiky et al., 1987; Hinterleitner et al., 1987). The responsiveness after multiple stimuli differed across temperature treatments. In the trials where the larvae were exposed to an acute lower temperature (i.e., reared at 29.5°C, tested at 23.5°C) the triploids were more responsive. Interestingly, at this same temperature combination we previously reported that triploid zebrafish larvae were able to maintain higher metabolic rates than diploids (Hermaniuk et al., 2021). In salmonids (*Salmo salar* and *Salvelinus fontinalis*) triploids are also reported to maintain higher routine metabolic rate at low temperatures compared with diploids (Atkins and Benfey, 2008). Because of their larger cells, triploids should have lower energetic costs for maintaining ionic gradients across cell membranes. The fact that they nevertheless exhibit higher metabolic rates at low temperatures suggests that they have higher energy budgets. Our results seem to confirm this line of thought, as the higher responsiveness of triploids at the acute low measurement temperature indicates that they had more energy available to exhibit a startle response. Moreover, when they exhibited a startle response, the triploids appeared to be slightly faster at the colder test temperatures.

Growth and size attained is another important performance metric (Lefevre et al., 2021; Verberk et al., 2021). Warmer temperatures are known to speed up growth and development such that ectotherms grow faster but reach a smaller size when comparing them at the same stage (Verberk et al., 2021). The effect of temperature on development was taken into

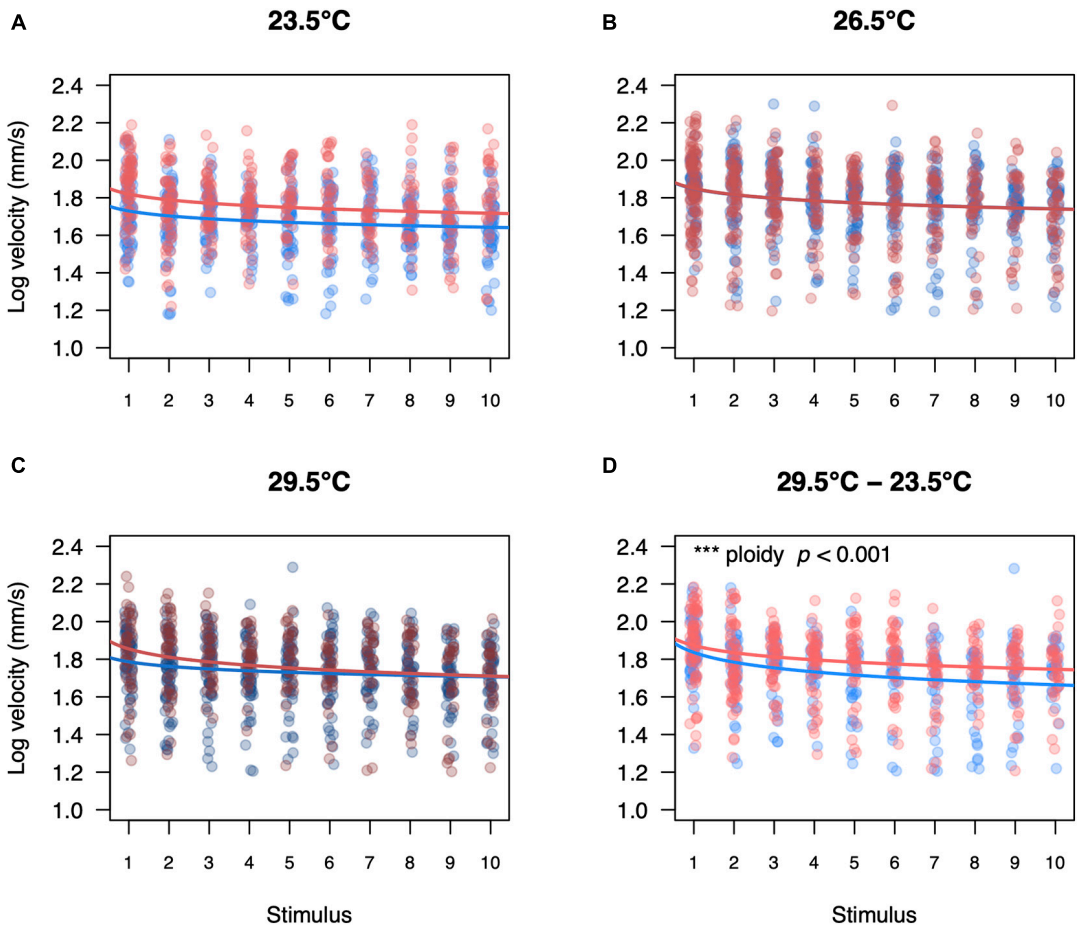

FIGURE 7 | Swimming velocity of diploid and triploid responders for each startle stimulus per temperature treatment. Swimming velocity of diploid and triploid larvae reared and measured at 23.5°C **(A)**, 26.5°C **(B)**, 29.5°C **(C)** or reared at 29.5°C and measured at 23.5°C **(D)**. Each value represents an individual larva that responded to the startle stimulus, where blue points represent diploid larvae and red points represent triploid larvae. Over all the effects of ploidy level (ANOVA, $p < 0.05$), temperature treatment (ANOVA, $p < 0.05$), stimulus number (ANOVA, $p < 0.001$), and length (ANOVA, $p < 0.001$) were significant. The effect of ploidy was also analyzed for a subset of each temperature treatment, comparing a model with and without ploidy level included. Ploidy was significant for larvae reared at 29.5°C and measured at 23.5°C ($p < 0.001$, $n = 60$).

account in our experimental design so we could compare larvae reared at different temperatures at the same developmental stage (i.e., 5 dpf at 26.5°C). For a given temperature, there were no clear differences in development rate with ploidy. Similarly, no differences in development rate were reported between developing diploid and triploid tadpoles (*Pelophylax esculentus*) (Hermaniuk et al., 2016). Indeed, developmental processes appear to be remarkably robust at the organismal level when cell sizes are altered (Fankhauser, 1945; Henery et al., 1992; Neufeld et al., 1998). In contrast to development, the effects of temperature on growth and the resultant size did differ with ploidy. Especially triploids attained a larger size at the coldest temperature and vice versa, consistent with a higher energy budget of triploids in the cold (Hermaniuk et al., 2021). A stronger temperature-size response has been previously reported under hypoxia, i.e., conditions were oxygen is more likely to be limiting (Frazier et al., 2001; Hoefnagel and Verberk, 2015). Similarly, comparisons between diploids and triploids have found triploids to be more susceptible to hypoxia (Sambraus et al., 2017) and exhibit stronger temperature-size responses in Hermaniuk et al. (2016). Thus, the stronger temperature-size response in larger celled, aquatic organisms may result from an increased susceptibility to oxygen limitation (Verberk et al., 2021).

Growth and development are ultimately determined by the rate of cell proliferation and cellular differentiation. Given that the timing of development was largely unaffected by ploidy, it follows that size differences likely reflect rates of cell division. In a previous study we found more cells being in the G2 phase in triploids, giving rise to a higher ratio of G2/G1 phases than in diploids (van de Pol et al., 2020). Here we show that this ratio is temperature dependent, and that the relationship with temperature differs between diploids and triploids. In triploids, the G2/G1 ratio was lowest in the cold, while in diploids, the lowest ratios were observed in the two warmest temperatures. We suggest that lower G2/G1 ratios indicate faster growth with

less cells being "stuck" in the G2 phase. This would be consistent with the results on size obtained, and suggests that growth was more optimal in cold conditions for triploids and vice versa. Also, for gene expression, we found increased expression levels of the genes *eef1a1| 1* and *rpl13* with higher rearing temperature, both of which are involved in protein synthesis during translation. Although expression levels of genes cannot be directly interpreted as different amounts of protein product from these genes, due to differences in translation and post-translational modifications, the higher expression levels would suggest increased protein synthesis to fuel the faster growth and development at higher temperatures. Interestingly, gene expression of *polr2d*, coding for RNA polymerase II, and *tbp*, coding for TATA box binding protein, were higher at lower temperatures. Possibly, transcription, rather than translation was a rate limiting step in colder temperatures and the higher gene expression helps to maintain transcription and thus enhance cell growth even at lower temperatures (Hessen et al., 2013). There was a tendency for both these genes to be expressed at higher levels in diploids (significant for *tbp*), perhaps compensating for their lower DNA content. Moreover, fluorescence intensity of stained DNA decreased with temperature, suggesting that DNA might be more condensed at lower temperatures. It is possible that the DNA configuration of the larvae at 29.5°C is easier accessible for PI, which is a small molecule, due to more flexible bended DNA (Driessen et al., 2014). Jalal et al. (2015) also reported more condensed DNA in fruit flies reared under low temperature than those raised at high temperature.

Both ploidy and rearing temperature had significant effects on the expression levels of citrate synthase, which is the pacemaker enzyme in the TCA cycle and thus a marker for the aerobic metabolism (Ciccarone et al., 2018). The expression of *cs* increased at low temperature in both diploid and triploid larvae, but this could reflect either a higher activity of the enzyme or constitute a compensatory mechanism to maintain high metabolic rates in cold conditions. For example, McClelland et al. (2006) found that cold-acclimation in adult zebrafish for 4 weeks increased the enzymatic activity of citrate synthase in muscle, but they did not find a significant increase in *cs* expression levels. At all rearing temperatures, *cs* was slightly lower expressed in triploids, which is in line with the lower mass-specific metabolic rate of larger cells (Goniakowska, 1970; Monnickendam and Balls, 1973).

Lactate dehydrogenase is generally used as a marker for anaerobic metabolism, as it is a key enzyme in maintaining cellular homeostasis when oxygen is short in supply by converting pyruvate to lactate. The interaction between rearing temperature and ploidy was significant for *ldha*, the gene coding for the subunit which is predominantly present in muscle and liver tissue (Markert et al., 1975). Taken together, our results could indicate a difference in cellular metabolism and energy fluxes in diploid compared to triploid zebrafish larvae, but the exact mechanisms require further investigation. Finally, expression of *hsp70.1* was significantly elevated at the highest rearing temperature. Although, this increase was similar in triploids and diploids, this suggests that zebrafish already experience thermal stress at 29.5°C, which is quite a lot lower than the critical thermal maximum of 41°C reported by Morgan et al. (2018), but note that these trials were rapid (approx. 45 min) and the upper temperature that an animal can tolerate will be lower at longer timescales (Rezende et al., 2014; Semsar-Kazerouni and Verberk, 2018).

In summary, our study demonstrates that zebrafish larvae with larger cells respond differently to different rearing temperatures than diploids, in terms of their gene expression, growth, development and swimming performance. Likely, this is caused by a different energy budget of their larger cells. A role for oxygen is plausible, as we measured differential expression of citrate synthase, and a previous study demonstrated that oxygen consumption was affected by cell size and temperature in this model system (Hermaniuk et al., 2021). As we studied larvae up until 5 dpf and used relatively short trials to measure swimming performance, some of the subtle differences found in this study may have a larger cumulative effect later in ontogeny. Across all metrics, a general picture emerged, with large celled triploids performing better at low temperatures than small celled diploids. This is consistent with the idea that being composed of larger cells is energetically more efficient at low temperatures. This suggests that the cellular trade-off between high capacity for performance and efficiency is dependent on temperature, and suggest different selection pressures operate on ectotherms and their cell size in cold and warm habitats.

DATA AVAILABILITY STATEMENT

All data files will be made available in the DANS EASY archive (https://easy.dans.knaw.nl/ui/home, doi: 10.17026/dans-z6g-75da).

ETHICS STATEMENT

Ethical review and approval was not required for the animal study because the experiments were performed with larvae up to a developmental stage of 5 days post-fertilization, a stage where larvae are not yet dependent on external feeding.

AUTHOR CONTRIBUTIONS

IP and WV conceived the experimental design and analyzed the data. IP performed the experiments and wrote the first draft of the manuscript. AH participated in collecting data on development rates. WV and AH edited the manuscript. All authors contributed to the article and approved the final version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphys.2021.738804/full#supplementary-material

REFERENCES

Alfsnes, K., Leinaas, H. P., and Hessen, D. O. (2017). Genome size in arthropods; different roles of phylogeny, habitat and life history in insects and crustaceans. *Ecol. Evol.* 7, 5939–5947. doi: 10.1002/ece3.3163

Amores, A., Force, A., Yan, Y. L., Joly, L., Amemiya, C., Fritz, A., et al. (1998). Zebrafish hox clusters and vertebrate genome evolution. *Science* 282, 1711–1714. doi: 10.1126/science.282.5394.1711

Atkins, M. E., and Benfey, T. J. (2008). Effect of acclimation temperature on routine metabolic rate in triploid salmonids. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* 149, 157–161. doi: 10.1016/j.cbpa.2007.11.004

Benfey, T. J. (1999). The physiology and behavior of triploid fishes. *Rev. Fish Sci.* 7, 39–67.

Bennett, M. D. (1976). DNA amount, latitude, and crop plant distribution. *Environ. Exp. Bot.* 16, 93–108. doi: 10.1016/0098-8472(76)90001-0

Blacklidge, K. H., and Bidwell, C. A. (1993). Three ploidy levels indicated by genome quantification in acipenseriformes of North America. *J. Hered.* 84, 427–430. doi: 10.1093/oxfordjournals.jhered.a111367

Ciccarone, F., Di Leo, L., and Ciriolo, M. R. (2018). "TCA cycle aberrations and cancer," in *Encyclopedia of Cancer*, eds P. Boffetta and P. Hainaut (Cambridge, MA: Academic Press), 429–436. doi: 10.1016/B978-0-12-801238-3.65066-3

Driessen, R. P. C., Sitters, G., Laurens, N., Moolenaar, G. F., Wuite, G. J. L., Goosen, N., et al. (2014). Effect of temperature on the intrinsic flexibility of DNA and its interaction with architectural proteins. *Biochemistry* 53, 6430–6438. doi: 10.1021/bi500344j

Dufresne, F., and Hebert, P. D. N. (2016). Temperature-related differences in life-history characteristics between diploid and polyploid clones of the *Daphnia pulex* complex. *Écoscience* 5, 433–437. doi: 10.1080/11956860.1998.11682481

Dufresne, F., and Jeffery, N. (2011). A guided tour of large genome size in animals: what we know and where we are heading. *Chromosome Res.* 19, 925–938. doi: 10.1007/s10577-011-9248-x

Ebeling, A. W., Atkin, N. B., and Setzer, P. Y. (2015). Genome sizes of teleostean fishes: increases in some deep-sea species. *Am. Nat.* 105, 549–561. doi: 10.1086/282744

El-Fiky, N., Hinterleitner, S., and Wieser, W. (1987). Differentiation of swimming muscles and gills, and development of anaerobic power in the larvae of cyprinid fish (Pisces, Teleostei). *Zoomorphology* 107, 126–132. doi: 10.1007/BF00312122

Fankhauser, G. (1945). Maintenance of normal structure in heteroploid salamander larvae, through compensation of changes in cell size by adjustment of cell number and cell shape. *J. Exp. Zool.* 100, 445–455. doi: 10.1002/jez.1401000310

Forster, J., Hirst, A. G., and Atkinson, D. (2012). Warming-induced reductions in body size are greater in aquatic than terrestrial species. *Proc. Natl. Acad. Sci. U.S.A.* 109, 19310–19314. doi: 10.1073/pnas.1210460109

Frazier, M. R., Woods, H. A., and Harrison, J. F. (2001). Interactive effects of rearing temperature and oxygen on the development of *Drosophila melanogaster*. *Physiol. Biochem. Zool.* 74, 641–650.

Goniakowska, L. (1970). The respiration of erythrocytes of some amphibians in vitro. *Bull. Acad. Pol. Sci. Biol.* 18, 793–797.

Gregory, R. T. (2003). Variation across amphibian species in the size of the nuclear genome supports a pluralistic, hierarchical approach to the C-value enigma. *Biol. J. Linn. Soc.* 79, 329–339. doi: 10.1046/j.1095-8312.2003.00191.x

Gregory, T. R. (2001a). Coincidence, coevolution, or causation? DNA content, cell size, and the C-value enigma. *Biol. Rev.* 76, 65–101. doi: 10.1017/S1464793100005595

Gregory, T. R. (2001b). The bigger the C-value, the larger the cell: genome size and red blood cell size in vertebrates. *Blood Cells Mol. Dis.* 27, 830–843. doi: 10.1006/bcmd.2001.0457

Gregory, T. R., and Hebert, P. D. (1999). The modulation of DNA content: proximate causes and ultimate consequences. *Genome Res.* 9, 317–324. doi: 10.1101/gr.9.4.317

Hardie, D. C., and Hebert, P. D. (2003). The nucleotypic effects of cellular DNA content in cartilaginous and ray-finned fishes. *Genome* 46, 683–706. doi: 10.1139/g03-040

Hardie, D. C., and Hebert, P. D. (2004). Genome-size evolution in fishes. *Can. J. Fish. Aquat. Sci.* 61, 1636–1646. doi: 10.1139/f04-106

Henery, C. C., Bard, J. B., and Kaufman, M. H. (1992). Tetraploidy in mice, embryonic cell number, and the grain of the developmental map. *Dev. Biol.* 152, 233–241. doi: 10.1016/0012-1606(92)90131-y

Hermaniuk, A., Rybacki, M., and Taylor, J. R. E. (2016). Low temperature and polyploidy result in larger cell and body size in an ectothermic vertebrate. *Physiol. Biochem. Zool.* 89, 118–129. doi: 10.1086/684974

Hermaniuk, A., van de Pol, I. L. E., and Verberk, W. C. E. P. (2021). Are acute and acclimated thermal effects on metabolic rate modulated by cell size? A comparison between diploid and triploid zebrafish larvae. *J. Exp. Biol.* 224:jeb227124.

Hessen, D. O., Daufresne, M., and Leinaas, H. P. (2013). Temperature-size relations from the cellular-genomic perspective. *Biol. Rev.* 88, 476–489. doi: 10.1111/brv.12006

Hinterleitner, S., Platzer, U., and Wieser, W. (1987). Development of the activities of oxidative, glycolytic and muscle enzymes during early larval life in three families of freshwater fish. *J. Fish Biol.* 30, 315–326.

Hoefnagel, K. N., and Verberk, W. C. E. P. (2015). Is the temperature-size rule mediated by oxygen in aquatic ectotherms? *J. Therm. Biol.* 54, 56–65. doi: 10.1016/j.jtherbio.2014.12.003

Horne, C. R., Hirst, A. G., and Atkinson, D. (2015). Temperature-size responses match latitudinal-size clines in arthropods, revealing critical differences between aquatic and terrestrial species. *Ecol. Lett.* 18, 327–335. doi: 10.1111/ele.12413

Horner, H. A., and Macgregor, H. C. (1983). C value and cell volume: their significance in the evolution and development of amphibians. *J. Cell Sci.* 63, 135–146.

Hulbert, A. J., and Else, P. L. (2000). Mechanisms underlying the cost of living in animals. *Annu. Rev. Physiol.* 62, 207–235. doi: 10.1146/annurev.physiol.62.1.207

Hunt von Herbing, I. (2002). Effects of temperature on larval fish swimming performance: the importance of physics to physiology. *J. Fish Biol.* 61, 865–876.

Jalal, M., Andersen, T., and Hessen, D. O. (2015). Temperature and developmental responses of body and cell size in *Drosophila*; effects of polyploidy and genome configuration. *J. Therm. Biol.* 51, 1–14. doi: 10.1016/j.jtherbio.2015.02.011

Kimmel, C. B., Ballard, W. W., Kimmel, S. R., Ullmann, B., and Schilling, T. F. (1995). Stages of embryonic development of the zebrafish. *Dev. Dyn.* 203, 253–310. doi: 10.1002/aja.1002030302

Kozłowski, J., Konarzewski, M., and Gawelczyk, A. T. (2003). Cell size as a link between noncoding DNA and metabolic rate scaling. *Proc. Natl. Acad. Sci. U.S.A.* 100, 14080–14085. doi: 10.1073/pnas.2334605100

Lefevre, S., Wang, T., and McKenzie, D. J. (2021). The role of mechanistic physiology in investigating impacts of global warming on fishes. *J. Exp. Biol.* 224:jeb238840. doi: 10.1242/jeb.238840

Leiva, F. P., Calosi, P., and Verberk, W. C. E. P. (2019). Scaling of thermal tolerance with body mass and genome size in ectotherms: a comparison between water- and air-breathers. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 374:20190035. doi: 10.1098/rstb.2019.0035

Markert, C. L., Shaklee, J. B., and Whitt, G. S. (1975). Evolution of a gene. Multiple genes for LDH isozymes provide a model of the evolution of gene structure, function and regulation. *Science* 189, 102–114. doi: 10.1126/science.1138367

McClelland, G. B., Craig, P. M., Dhekney, K., and Dipardo, S. (2006). Temperature- and exercise-induced gene expression and metabolic enzyme changes in skeletal muscle of adult zebrafish (*Danio rerio*). *J. Physiol. (Lond.)* 577, 739–751. doi: 10.1113/jphysiol.2006.119032

Monnickendam, M. A., and Balls, M. (1973). Amphibian organ culture. *Experientia* 29, 1–17. doi: 10.1007/BF01913222

Morgan, R., Finnøen, M. H., and Jutfelt, F. (2018). CT max is repeatable and doesn't reduce growth in zebrafish. *Sci. Rep.* 8:7099. doi: 10.1038/s41598-018-25593-4

Neufeld, T. P., de la Cruz, A. F., Johnston, L. A., and Edgar, B. A. (1998). Coordination of growth and cell division in the *Drosophila* wing. *Cell* 93, 1183–1193. doi: 10.1016/s0092-8674(00)81462-2

Nilsson, G. E. (2007). Gill remodeling in fish–a new fashion or an ancient secret? *J. Exp. Biol.* 210, 2403–2409. doi: 10.1242/jeb.000281

Otto, C. R. V., Snodgrass, J. W., Forester, D. C., Mitchell, J. C., and Miller, R. W. (2007). Climatic variation and the distribution of an amphibian polyploid complex. *J. Anim. Ecol.* 76, 1053–1061. doi: 10.1111/j.1365-2656.2007.01300.x

Pörtner, H. O. (2010). Oxygen- and capacity-limitation of thermal tolerance: a matrix for integrating climate-related stressor effects in marine ecosystems. *J. Exp. Biol.* 213, 881–893. doi: 10.1242/jeb.037523

Rezende, E. L., Castañeda, L. E., and Santos, M. (2014). Tolerance landscapes in thermal ecology. *Funct. Ecol.* 28, 799–809. doi: 10.1111/1365-2435.12268

Rombough, P. (2002). Gills are needed for ionoregulation before they are needed for O_2 uptake in developing zebrafish, *Danio rerio*. *J. Exp. Biol.* 205, 1787–1794. doi: 10.1242/jeb.205.12.1787

Sambraus, F., Olsen, R. E., Remen, M., Hansen, T. J., Torgersen, T., and Fjelldal, P. G. (2017). Water temperature and oxygen: the effect of triploidy on performance and metabolism in farmed Atlantic salmon (*Salmo salar* L.) post-smolts. *Aquaculture* 473, 1–12.

Semsar-Kazerouni, M., and Verberk, W. C. E. P. (2018). It's about time: linkages between heat tolerance, thermal acclimation and metabolic rate at different temporal scales in the freshwater amphipod *Gammarus fossarum* Koch, 1836. *J. Therm. Biol.* 75, 31–37. doi: 10.1016/j.jtherbio.2018.04.016

Small, C. D., Davis, J. P., Crawford, B. D., and Benfey, T. J. (2021). Early, nonlethal ploidy and genome size quantification using confocal microscopy in zebrafish embryos. *J. Exp. Zool. B Mol. Dev. Evol.* 336, 496–510. doi: 10.1002/jez.b.23069

Smith, T. W., Kron, P., and Martin, S. L. (2018). flowPloidy: an R package for genome size and ploidy assessment of flow cytometry data. *Appl. Plant Sci.* 6:e01164. doi: 10.1002/aps3.1164

Stingo, V., and Rocco, L. (2001). Selachian cytogenetics: a review. *Genetica* 111, 329–347. doi: 10.1023/a:1013747215866

Subczynski, W. K., Hyde, J. S., and Kusumi, A. (1989). Oxygen permeability of phosphatidylcholine-cholesterol membranes. *Proc. Natl. Acad. Sci. U.S.A.* 86, 4474–4478. doi: 10.1073/pnas.86.12.4474

Suresh, A. V., and Sheehan, R. J. (1998). Muscle fibre growth dynamics in diploid and triploid rainbow trout. *J. Fish Biol.* 52, 570–587.

Szarski, H. (1983). Cell size and the concept of wasteful and frugal evolutionary strategies. *J. Theor. Biol.* 105, 201–209.

Uyeno, T., and Smith, G. R. (1972). Tetraploid origin of the karyotype of catostomid fishes. *Science* 175, 644–646. doi: 10.1126/science.175.4022.644

van de Pol, I. L. E., Flik, G., and Verberk, W. C. E. P. (2020). Triploidy in zebrafish larvae: effects on gene expression, cell size and cell number, growth, development and swimming performance. *PLoS One* 15:e0229468. doi: 10.1371/journal.pone.0229468

van den Bos, R., Mes, W., Galligani, P., Heil, A., Zethof, J., Flik, G., et al. (2017). Further characterisation of differences between TL and AB zebrafish (*Danio rerio*): gene expression, physiology and behaviour at day 5 of the larval stage. *PLoS One* 12:e0175420. doi: 10.1371/journal.pone.0175420

Vargas, C. C., Peruzzi, S., and Hagen, Ø (2015). Growth and muscle cellularity of diploid and triploid Atlantic cod (*Gadus morhua* Linnaeus, 1758) larvae. *J. Appl. Ichthyol.* 31, 687–694.

Verberk, W. C. E. P., Atkinson, D., Hoefnagel, K. N., Hirst, A. G., Horne, C. R., and Siepel, H. (2021). Shrinking body sizes in response to warming: explanations for the temperature-size rule with special emphasis on the role of oxygen. *Biol. Rev.* 96, 247–268. doi: 10.1111/brv.12653

Verberk, W. C. E. P., Bilton, D. T., Calosi, P., and Spicer, J. I. (2011). Oxygen supply in aquatic ectotherms: partial pressure and solubility together explain biodiversity and size patterns. *Ecology* 92, 1565–1572. doi: 10.1890/10-2369.1

Wyngaard, G. A., Rasch, E. M., Manning, N. M., Gasser, K., and Domangue, R. (2005). The relationship between genome size, development rate, and body size in copepods. *Hydrobiologia* 532, 123–137. doi: 10.1007/s10750-004-9521-5

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Rapid Adjustments in Thermal Tolerance and the Metabolome to Daily Environmental Changes – A Field Study on the Arctic Seed Bug *Nysius groenlandicus*

Natasja Krog Noer[1], Mathias Hamann Sørensen[1], Hervé Colinet[2], David Renault[2,3], Simon Bahrndorff[1†] and Torsten Nygaard Kristensen[1†]*

[1] Department of Chemistry and Bioscience, Aalborg University, Aalborg, Denmark, [2] UMR 6553, CNRS, Ecosystèmes, Biodiversité, Évolution, University of Rennes 1, Rennes, France, [3] Institut Universitaire de France, Paris, France

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***Correspondence:**
Natasja Krog Noer
nkn@bio.aau.dk

† These authors share last authorship

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Laboratory investigations on terrestrial model-species, typically of temperate origin, have demonstrated that terrestrial ectotherms can cope with daily temperature variations through rapid hardening responses. However, few studies have investigated this ability and its physiological basis in the field. Especially in polar regions, where the temporal and spatial temperature variations can be extreme, are hardening responses expected to be important. Here, we examined diurnal adjustments in heat and cold tolerance in the Greenlandic seed bug *Nysius groenlandicus* by collecting individuals for thermal assessment at different time points within and across days. We found a significant correlation between observed heat or cold tolerance and the ambient microhabitat temperatures at the time of capture, indicating that *N. groenlandicus* continuously and within short time-windows respond physiologically to thermal changes and/or other environmental variables in their microhabitats. Secondly, we assessed underlying metabolomic fingerprints using GC-MS metabolomics in a subset of individuals collected during days with either low or high temperature variation. Concentrations of metabolites, including sugars, polyols, and free amino acids varied significantly with time of collection. For instance, we detected elevated sugar levels in animals caught at the lowest daily field temperatures. Polyol concentrations were lower in individuals collected in the morning and evening and higher at midday and afternoon, possibly reflecting changes in temperature. Additionally, changes in concentrations of metabolites associated with energetic metabolism were observed across collection times. Our findings suggest that in these extreme polar environments hardening responses are marked and likely play a crucial role for coping with microhabitat temperature variation on a daily scale, and that metabolite levels are actively altered on a daily basis.

Keywords: arctic, climate change, diurnal environmental variation, GC-MS metabolomics, insects, phenotypic plasticity, temperature variation, thermal tolerance

INTRODUCTION

Terrestrial ectotherms are subject to large spatial and temporal variability in their thermal environment (Kearney and Porter, 2009). In terrestrial ecosystems, daily changes in temperature can be substantial, and vary greatly with microhabitat characteristics such as topography and orientation, vegetation cover, shading and more (Sears et al., 2019; Kearney et al., 2020; Lembrechts and Lenoir, 2020). Some of the most extreme environments are found in the polar regions where the winters are long and cold, and the summers short and periodically hot (Bahrndorff et al., 2021b). During the arctic summer, daily temperatures can vary by $>30°C$ and reach subzero temperatures at night (Convey et al., 2018; Davey et al., 2021). Organisms, including insects, living in these environments must therefore be able to survive and reproduce over a wide range of temperatures (Deere et al., 2006; Bahrndorff et al., 2021a). This can be achieved by evolutionary adaptation to the local thermal conditions across generations, or by fast adjustments of the physiology within the lifetime of an organism *via* phenotypic plasticity (Scheiner, 1993; Fusco and Minelli, 2010; Kristensen et al., 2020). Evolutionary adaptation to changing and periodically stressful temperatures can be slow, and are sometimes constrained by genetic trade-offs or lack of adaptive genetic variation (Araújo et al., 2013; Hoffmann et al., 2013). Conversely, rapid plastic adaptive changes can rescue individuals exposed to biotic and abiotic challenges at a shorter timescale, including daily environmental fluctuations (Colinet and Hoffmann, 2012; Noer et al., 2022). Plastic changes might therefore be particularly relevant for arctic species exposed to unpredictable and rapid changes in the environment.

Organisms can respond plastically to short-term exposure to sub-optimal temperatures through hardening or by acclimation at longer term exposures (Colinet and Hoffmann, 2012; Schou et al., 2017). Hardening responses to extreme or acute temperatures are thought to counter rapid thermal stress, such as daily temperature extremes and stochastic events (Koveos, 2001; Hoffmann et al., 2003; Kelty, 2007; Overgaard and Sørensen, 2008; Jensen et al., 2019). The other form of more gradual acclimation includes seasonal acquisition of cold or heat tolerance that is induced by changes in temperature and photoperiod interacting with other abiotic factors (reviewed by Chown and Terblanche, 2006; Teets and Denlinger, 2013). There are several published examples of cold acclimation and rapid cold hardening in arctic arthropods (e.g., Bahrndorff et al., 2007; Everatt et al., 2013), but very few studies have investigated physiological acclimation of polar terrestrial arthropods to high temperatures (Sørensen et al., 2019; Bahrndorff et al., 2021b). Traditionally, thermal plasticity of insects has been investigated using model-organisms kept and hardened/acclimated to constant controlled temperatures in the laboratory (Angilletta, 2009; Colinet et al., 2015; Javal et al., 2016; Ketola and Kristensen, 2017). However, recent work on the impacts of temperature variability on thermal tolerance have emphasized that thermal performance based on constant temperatures do not always accurately predict performance under variable conditions in the laboratory (reviewed by Colinet et al., 2015; Vázquez et al., 2017), nor in the field (see e.g., Kingsolver and Nagle, 2007; Loeschcke and Hoffmann, 2007; Kristensen et al., 2008; Ketola and Kristensen, 2017). This potential mismatch in the conclusions arising from investigations based on constant *versus* fluctuating temperatures partly results from the non-linear impact of temperatures on thermal performance (Jensen's inequality) (Ruel and Ayres, 1999; Colinet et al., 2015), time-by-temperature interactions (Foray et al., 2013; Kingsolver et al., 2015), and methodology (Chown et al., 2009; Mitchell and Hoffmann, 2010; Bahrndorff et al., 2016). Based on such results, the potential for transferring the knowledge obtained from the laboratory to field conditions, and thus forecast reliable predictions of the effects of climate change on the responses and geographic distribution of insects, is being increasingly questioned (Fischer et al., 2011; Kingsolver et al., 2015; Kinzner et al., 2019; Taylor et al., 2021).

The physiological and molecular mechanisms enabling arthropods to tolerate temperature stress has previously focused on controlled laboratory studies (but see Tomanek and Somero, 1999, 2002; Buckley et al., 2001; Gracey et al., 2008; Kristensen et al., 2012; Vasquez et al., 2019). Studies on temperate and polar species suggest (causation is typically lacking in such studies) that metabolites such as sugars, free amino acids and polyols can be associated with changes in cold tolerance measures (Zachariassen, 1985; Fields et al., 1998; Sømme, 1999; Holmstrup et al., 2002; Michaud and Denlinger, 2007, 2010; Overgaard et al., 2007, 2014). Changes in polyols on the other hand have been associated with changes in heat tolerance (Hendrix and Salvucci, 1998; Wolfe et al., 1998; Salvucci et al., 2000; Benoit et al., 2009). However, few have attempted to describe how these metabolites are affected by dynamic and fluctuating temperatures as encountered in nature (but see Kristensen et al., 2012; Noer et al., 2020; Sheldon et al., 2020).

In this study, we examined the effects of daily variation in the microhabitat temperatures on plastic adjustments of heat and cold tolerance of the Greenlandic seed bug *Nysius groenlandicus* (Zetterstedt) during summer in Southern Greenland. *Nysius groenlandicus* is a univoltine species, and widespread and abundant in Arctic and sub-Arctic regions. Previous work on the species have revealed that it can rapidly increase heat tolerance when exposed to high and stressful temperatures under laboratory conditions (Sørensen et al., 2019), and thus *N. groenlandicus* represents a valuable polar insect model for field-based description of daily changes in individuals' thermal tolerance. Further, we examined the metabolic fingerprints within days with high and low temperature variation using a quantitative targeted gas chromatography-mass spectrometry (GC-MS) approach. We hypothesized that the ability to tolerate low and high temperatures is constantly fine-tuned to respond to temporally fluctuating temperatures in field-collected individuals of *N. groenlandicus* as an adaptation to the highly variable environmental conditions within and across days. Thus, we expected specimens collected in early morning and late evening to have the highest cold tolerance, while those collected at midday exhibiting the highest heat tolerance. Finally, we expected to see daily changes in metabolites known to improve cold tolerance (sugars and amino acids) in individuals collected in early morning and late evening, while metabolites enhancing

heat tolerance (polyols) would show higher concentrations in individuals sampled during the warm periods of the day.

MATERIALS AND METHODS

Field Collection and Experimental Design

The field work was conducted in July–August 2018 in Narsarsuaq (Southern Greenland, 61.160°N, 45.424°W). This region is characterized by cool temperatures, long winters and short and thermally variable summers (Böcher and Nachman, 2001; Bahrndorff et al., 2021b). The study site was a heath-like, grass-covered area, where adult *N. groenlandicus* were collected from the grasses using a sweep net (**Figure 1**). All individuals used in the experiment were caught in a 50 m × 50 m area less than 100 m from the laboratory facilities.

Adult individuals of *N. groenlandicus* were collected at four time points (08:00 am, 12:00 pm, 04:00 pm, and 08:00 pm) during each of 5 days (depending on weather conditions and the abundance of *N. groenlandicus*; see **Supplementary Table 1** for exact collection times and dates), thereafter referred to as day 1 to day 5. At the time of field-collection, each individual was placed in a 4 mL screw-cap glass vial (45 mm × 14.7 mm) and placed in the shade on the ground to prevent abrupt changes in the thermal conditions. The sex of individuals was then assessed by eye, and the vials transferred to the laboratory within 30–45 min of collection. Immediately after returning, the heat and cold tolerances were scored using 20 females and 20 males for each assay (see next section).

Additional individuals were collected for subsequent metabolomics analysis at the same four sampling times at two dates (22/08/18 and 27/08/18, representing day 4 and 5, respectively, see **Supplementary Table 1**), representing days with either high or low observed temperature variation. At each collection time, eight samples of five females (only females were used for the metabolomics studies) were collected, transferred directly into ice cold RNAlater, and stored at −20°C for approximately 1 week. Then the samples were transferred to our laboratory in Denmark where they were stored at −80°C and later used for metabolomic fingerprinting. Collection of

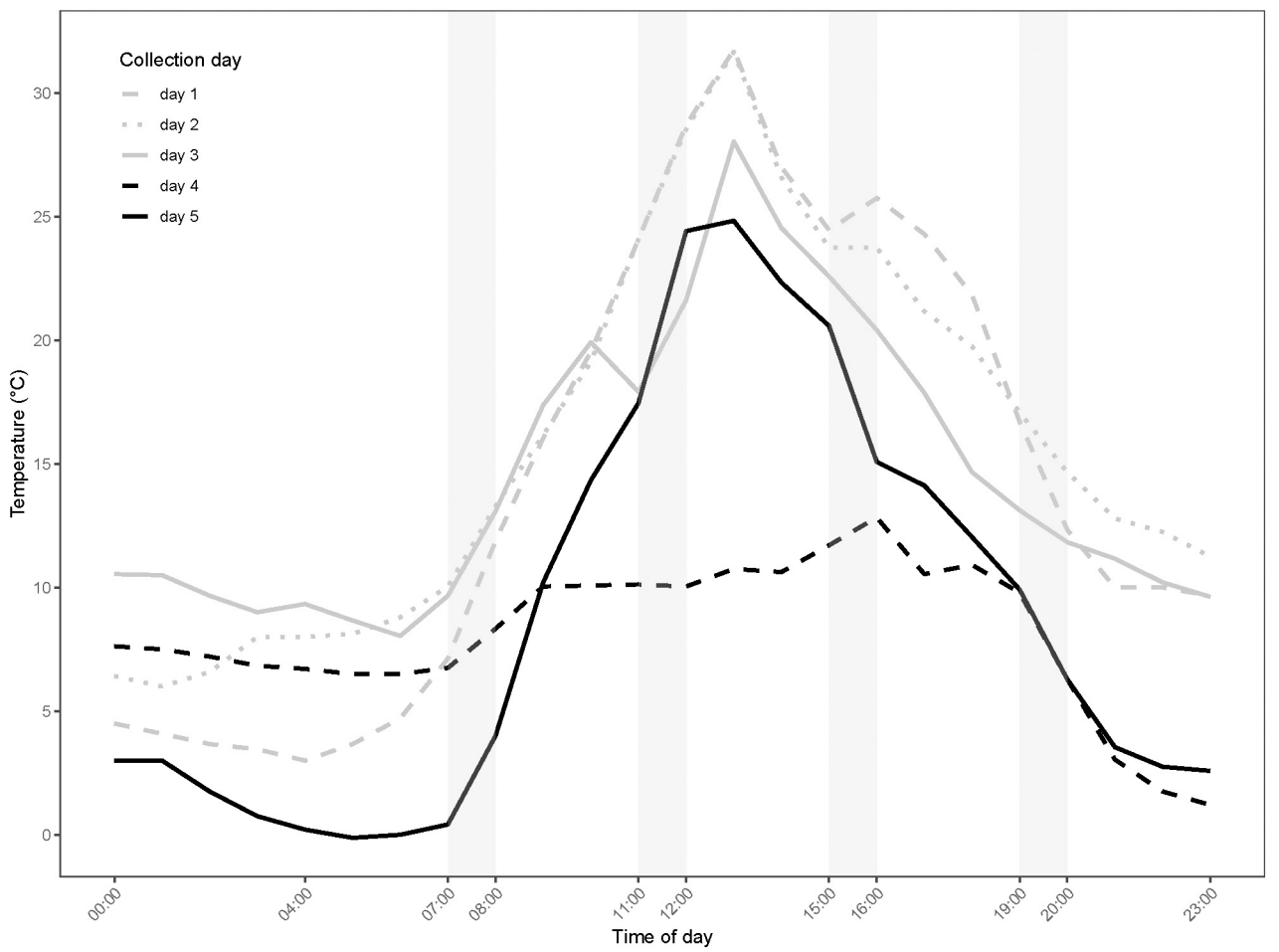

FIGURE 1 | Microhabitat temperature (°C) averaged in 1-h bins across the five experimental days. The temperatures were measured at 15 cm above the soil surface in the shade every 5 min. Samples for GC-MS analysis were collected on day 4–5 (black lines). Shaded columns indicate the timespans where specimen are collected.

samples in RNAlater is amenable for downstream metabolomics analysis if stored at subzero temperatures (van Eijsden et al., 2013; Harris, 2018).

The air temperature at the collection site was continuously recorded with 5-min intervals in the shade using Easylog USB data loggers (LASCAR Electronics, EL-USB-2^+). The temperature was measured in the shade to avoid warm temperature-spikes in the measurements caused by direct solar radiation (Maclean et al., 2021) and the loggers were placed 15 cm above the soil surface to reflect the thermal environment at the top of the grasses where *N. groenlandicus* was most abundant, and was caught with the sweeping net. Based on these recordings, the mean temperature was calculated for the 1-h timespan prior to testing thermal tolerances. The mean temperature immediately prior to thermal assessment has been shown to be highly correlated with the heat tolerance in a range of insect species collected in the field (Noer et al., 2022).

Heat and Cold Tolerance Assays

Heat Knockdown Time

Heat tolerance was measured as heat knockdown time (HKDT), i.e., a measure of the time before individuals go into a heat-induced coma/die at a high stressful temperature (Terblanche et al., 2007; Bak et al., 2020). For *N. groenlandicus* HKDT constitutes a direct measure of heat tolerance as we register the time individuals can withstand before they die. The vials containing field-collected individuals were mounted to a rack and submerged into a temperature-controlled water bath (PolyScience MX Immersion Circulator: MX-CA12E) maintained at 48°C. The individuals were then observed and stimulated with flashes of light and gentle taps on the vial caps with a metal rod. The time until movement ceased was noted for each individual. The chosen HKDT temperature was based on experiences from previous work on the species (Sørensen et al., 2019), and on unpublished preliminary results showing that *N. groenlandicus* individuals went into coma within 20–40 min at 48°C. Earlier results suggest a fast heat hardening response for this species and since we wanted a measure of their acute heat tolerance, and aimed for reducing hardening responses induced while testing, this HKDT was found relevant.

Chill Coma Recovery

Cold tolerance was measured as the temperature at which the bugs regained the ability to move after being knocked down by exposure to a low temperature ($T_{recovery}$) following a modified procedure of the method described in Overgaard et al. (2011). Thus, we used a proxy rather than direct measures of cold tolerance. The glass vials containing the individuals were mounted to a rack and submerged into a glycol-water solution that was kept at −3°C using a thermostat (LAUDA Proline Edition X RP 1845-C, LAUDA DR. R. WOBSER GMBH & CO., KG, Germany). This temperature was based on the lower critical temperature (CTmin) which induces a cold coma for the species (Bahrndorff et al., 2021b). Immediately after submersion, the temperature of the solution was increased at a rate of 0.2°C/min. Pilot studies showed that this temperature (−3°C) was sufficient to induce chill coma within a few minutes with full survival upon returning to room temperature (data not presented). Following the HKDT procedure, the individuals were observed and provoked using light flashes and gentle taps, and the temperature at which individuals first moved any body part was noted as their chill coma recovery temperature ($T_{recovery}$). A low $T_{recovery}$ is interpreted as high cold tolerance.

Metabolomic Fingerprinting

We adapted the methods detailed in Thiébaut et al. (2020) for detecting and quantifying the metabolite content from whole-body extracts of female *N. groenlandicus*. For each field sampling time, eight replicates were used, each consisting of five pooled females to obtain sufficient biomass (∼4 mg dry mass per sample). Each sample was vacuum dried (Speed Vac Concentrator, miVac; Genevac Ltd., Ipswich, England) and weighed (Mettler Toledo UMX2, accurate to 0.001 mg) before extractions so that metabolite concentrations could be reported according to dry mass. The samples were first homogenized for 90 s with two tungsten beads in 450 μL of a solution of ice cold methanol-chloroform (ratio 2:1, v:v) using a bead beater (Qiagen MM301; Retsch GmbH, Haan, Germany) set at 25 Hz. To separate the homogenate in two distinct phases (lipid-rich containing phase, and aqueous phase containing metabolites), 300 μL of cold ultrapure water was added to each tube before centrifugation (Sigma 2-16K, Sigma GmbH, Harz, Germany) for 10 min at 4000 *g* at 4°C. The supernatants containing metabolites were transferred to new tubes and stored at −80°C until analysis by GC-MS. Prior to the analysis, 120 μL of the metabolite extract was transferred to glass vials and vacuum dried.

The derivatization of the samples (dry residues) was automatized with a CTC CombiPAL autosampler (CTC Analytics AG, Zwingen, Switzerland). Dried samples were re-suspended in 30 μL of 25 mg mL^{-1} methoxyamine-hydrochloride (CAS Number: 593-56-6, SIGMA-ALDRICH, St. Louis, MO, United States) in pyridine prior to incubation under orbital shaking at 40°C for 60 min. Following incubation, 30 μL of N-methyl-bis(trifluoroacetamine) (BSTFA, CAS Number: 685-27-8) was added, and derivatization was conducted at 40°C for 60 min under agitation. An Agilent 7890B gas chromatograph coupled to a 5977B mass spectrometer was used for the separation and detection of the metabolites. For each sample, 1 μL was injected (Injector temperature: 250°C; split mode with a split ratio of 25:1); the temperature of the oven increased from 70 to 170°C at a rate of 5°C/min, from 170 to 280°C at 7°C/min, and from 280 to 320°C at 15°C/min, and then remained at 320°C for 4 min. We used a 30-m fused silica column (HP5 MS 30 m, I.D. 0.25 mm, thickness 0.25 μm, 5% Diphenyl/95% Dimethylpolysiloxane, Agilent Technologies), and the gas carrier (Helium) had a flow of 1 mL per min. The temperatures of the transfer line and ion source were 280 and 230°C, respectively. Metabolite fragmentation and ionization were carried out by electronic impact (electron energy: 70 eV) and detected with the full scan mode. The detected peaks were identified and annotated with MassHunter (Agilent). Most detected metabolites were identified, and calibration curves were used with pure compounds for calculating the concentration of each metabolite.

Statistical Analysis

Differences in thermal tolerance with sampling time were examined using two-way ANOVAs for male and female *N. groenlandicus* separately with "time to knockdown" or "chill coma recovery temperature" as dependent variables, and "time of day" and "day" as independent variables. The tests were run on individuals. To ensure normal distribution, the data were transformed using rank inverse transformation and the models were run on both transformed and non-transformed data for validation. In addition, we used Pearson's correlations to examine if microhabitat temperature affected thermal tolerances rather than sampling time alone. The Pearson's correlations were calculated using the mean field temperatures observed in the 1 h preceding each collection round and the mean HKDT and $T_{recovery}$ for each assay and sex ($n = 20$).

We used one-way ANOVAs to examine if individual metabolite concentrations within each day differed according to collection time. The concentrations of all quantified metabolites were then scaled and mean centered. For each sampling day, between-class PCA (R-package "ade4"), were run to explore the daily temporal structure of the metabolomic profiles. Monte Carlo tests (1000 permutations) were used to examine the significance of differences in metabolite profiles among classes of individuals from the four sampling times. Further, the metabolites that contributed the most to separation of groups were identified and ranked by their correlation to the principal components that described most of the inertia in the data. All analyses were carried out using the software "R" (R Core Team, 2020). Raw temperature and GC-MS files used for the analyses are available in the **Supplementary Material**.

RESULTS

Microhabitat Temperatures

Observed microhabitat temperatures of the different sampling periods are shown in **Figure 1**. The largest daily amplitude recorded was 28.8°C on day 1, while the lowest of 11.6°C was on day 4. The warmest average temperature in the 1-h timespan prior to thermal tolerance tests was 26.3°C (see **Supplementary Table 1** for exact temperatures) and was recorded at midday on day 2; the coldest average temperature prior to tests was 1.0°C and was recorded in the morning of day 5.

The microhabitat temperatures varied markedly between the two experimental days where samples were collected for metabolomic profiling (day 4 and 5; **Figure 1**). Within day 4, the temperatures were relatively constant with only 4.9°C difference in the average temperatures at the four daily collection times. Day 4 was also characterized by the lowest recorded daily amplitude. Day 5 was characterized by a high temperature variation with an amplitude of 25°C; the average temperatures at the four collection times differed by 21°C (**Figure 1** and **Supplementary Table 1**).

Thermal Tolerance

Thermal tolerances varied significantly within and between days for both male and female individuals (**Supplementary Figure 2** and ANOVA **Supplementary Table 2**). The average HKDT at each collection time and day was correlated with the average field temperature observed 1 h prior to heat tolerance assessment. The correlation was significant for females but not for males (**Figure 2A**). The regression slope, representing the change in HKDT per °C change in field temperature, was 0.36 min/°C for females and 0.18 min/°C for males. In addition, HKDT was overall higher for females than for males at any given field temperature with a difference of 5 min at the intercept. The maximal difference in mean HKDT measured across all temperatures was 23 min for females and 12 min for males. The relation between mean $T_{recovery}$ and the average microhabitat temperature preceding cold assays revealed a significant positive relationship for both females and males (**Figure 2B**). The increase in recovery temperature per °C change in field temperature were 0.09 and 0.07°C for females and males, respectively. The largest difference in $T_{recovery}$ found across all days was 3.3°C for females and 3.4°C for males.

For the days where females were collected for metabolomic fingerprinting, the HKDT and $T_{recovery}$ varied markedly across the thermally variable day (day 5), and less so on the thermally stable day (day 4) (**Supplementary Figure 2**). Thus, the correlation between the field temperature and heat tolerance of the females collected only at the time points used for metabolomic profiling was strong and highly significant ($R = 0.94$, $p = <0.001$). The relationship between field temperature and $T_{recovery}$ of females collected on day 4 and 5 was also positive and directional, however, not significant ($R = 0.52$, $p = 0.19$).

Metabolomic Profiling

Description of the Quantified Metabolites in the Samples

The full scan monitoring allowed us to identify and quantify 33 metabolites with the quadrupole GC-MS platform (**Supplementary Table 3**; and see raw metabolite concentrations in **Supplementary Table 4**). We quantified 13 free amino acids, five sugars, six polyols, six metabolic intermediates, and three other metabolites. Xylitol and ethanolamine concentrations were below the quantification limit (i.e., below the lowest concentration of the calibration curve) in many samples and were therefore excluded from the quantitative profiling. The most abundant metabolites across all sampling times and days were phosphoric acid, proline, glutamate, and tyrosine.

Effects of Collection Time on Metabolic Fingerprints

Individual metabolite concentrations for each sampling time are displayed in **Figure 3**. We observed that a large number of metabolites varied in concentrations across collection times on day 5. There was a significant effect of "time of day" on the levels of eight metabolites (**Supplementary Table 5**). Conversely, the metabolite concentrations varied less on collection day 4, though there was a significant effect of "time of day" on concentration for seven of these (**Supplementary Table 5**). To examine whether the concentration levels varied similarly across collection times for the two collection days, we ran 2-way ANOVAs for each metabolite. Six metabolites varied distinctively across the four collection times for the 2 days, and these are depicted as

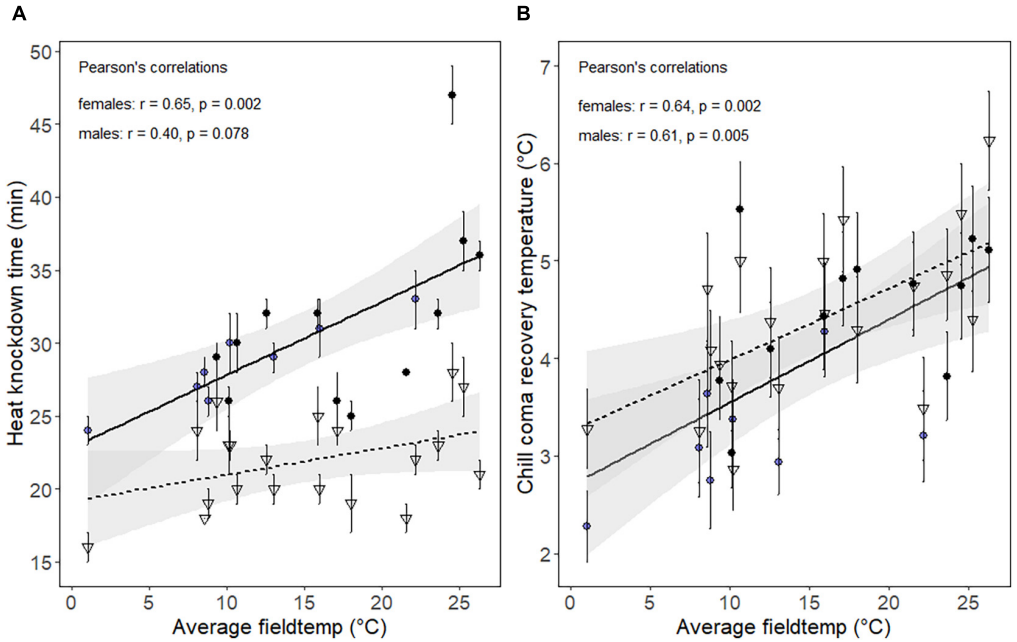

FIGURE 2 | Scatter plot showing the mean **(A)** heat knockdown time in minutes, and **(B)** chill coma recovery temperature ($T_{recovery}$) of females (circles) and males (triangles), as a function of the average microhabitat temperature 1 h prior to assay start. At eight sampling times, females were collected for metabolomic fingerprints simultaneously with individuals used for the thermal assays (blue circles). Black points are data points with no metabolomic fingerprints associated. Solid (female) and dotted (male) lines represent regressions between field temperature and thermal tolerances. Bars are standard errors of the mean.

the interaction between collection day and time on **Figure 3**. Finally, the sugars glucose, fructose, and galactose and the polyol glycerol occurred in larger concentrations in individuals collected on day 5.

Between-class PCAs were run separately on the metabolite concentrations from individuals sampled on day 4 (**Figure 4A**) and day 5 (**Figure 4B**). PC1 and PC2 cumulated 80 and 83% inertia on day 4 and 5, respectively; thus, on both days, the first two PCs explained most of the between-class variation. All classes showed a clear-cut separation meaning that metabolomic fingerprints differed among the different sampling times. The separation appeared stronger on day 5 (**Figure 4B**) than on day 4 (**Figure 4A**). Monte-Carlo randomization tests confirmed differences in metabolomic profiles among classes on day 5 ($p < 0.001$) and day 4 ($p = 0.026$) and the significance levels underpins that metabolomic profiles differed more markedly between sampling times on the thermally variable day 5 compared to on the less thermal variable day 4, as evidenced by much lower ellipses overlap.

Effects of Low Daily Thermal Fluctuations on Metabolic Fingerprints

On day 4, which was characterized by low temperature variation, the metabolomic profiles of individuals collected in the morning and evening differed from the profiles from midday and afternoon along PC1 (**Figure 4A**). Further, the metabolic fingerprints of individuals collected during midday and afternoon separated along PC2.

The metabolites most positively correlated to PC1 were the sugars galactose and glucose (**Figure 4A**). These two sugars were more abundant in individuals sampled in the morning and evening compared to those sampled at midday and afternoon. Lactic acid and citrulline were the metabolites that were most negatively correlated to PC1, and they were more abundant in individuals sampled at midday and afternoon. The polyol glycerol-3-phosphate and succinic acid had the highest positive associations with PC2 (**Supplementary Figure 3**) and were characterized by higher concentrations in individuals sampled in the morning and afternoon. Only one metabolite, glucose-6-phosphate, were negatively associated with PC2 and thus more abundant in the morning.

Effects of High Daily Thermal Fluctuations on Metabolic Fingerprints

On day 5, metabolomic profiles of individuals collected in the morning separated strongly from individuals sampled in the afternoon (**Figure 4B**). These two collection times were also the two thermal extremes of the day (i.e., the lowest and highest temperatures of the day, see **Figure 1** and **Supplementary Table 1**); this observation suggests that PC1 explained metabolic changes correlated to high diel thermal variation. Further, the metabolic fingerprints of the individuals collected at these time points separated from the fingerprints of individuals collected at midday and in the evening along PC2.

The metabolites that were positively associated with PC1 on day 5 were phenylalanine, glutamic acid, citric acid, and to a lesser extent other amino acids, including valine, leucine, serine, and alanine (**Figure 4B**). These were more abundant in individuals sampled in the morning compared to individuals from the other collection times. The metabolites that were most negatively

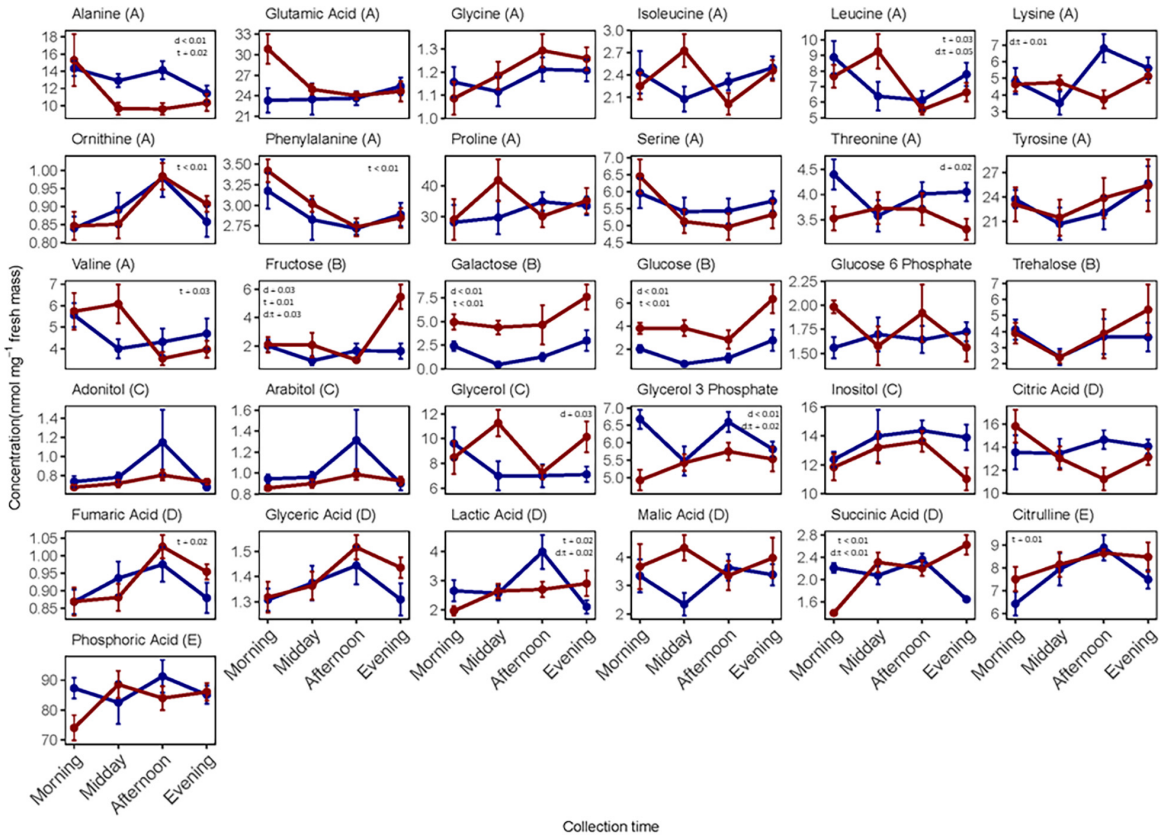

FIGURE 3 | Individual metabolite concentrations (nmol mg^{-1} dry mass) measured in whole-body extracts of female *N. groenlandicus* collected from the field at four consecutive time points (morning, midday, afternoon, and evening) during day 4 (blue) and day 5 (red). Each point represent mean concentration (n = 8) and bars are standard errors of the mean. Differences between concentrations (log-transformed) and collection days (d), collection times (t), and the interaction between day and time (d:t) was investigated using 2-way ANOVAs and significance p-values are shown on each plot if significant. The metabolites are classified from A to E in the header according to functional group; A, amino acids; B, sugars; C, polyols; D, metabolic intermediates; and E, other metabolites.

correlated to PC1 were succinic acid, fumaric acid, glyceric acid and ornithine. Further, negative correlations included several polyols (adonitol, glycerol-3-phosphate, arabitol) whose concentrations were higher in individuals collected in the afternoon than in the morning. The sugars fructose, glucose and galactose, and the amino acids isoleucine and lysine, as well as succinic acid and glycerol were positively associated with PC2 (**Supplementary Figure 3**), and were thus more abundant in individuals sampled in the evening. Negative associations were few, but the most negatively associated metabolite was the sugar glucose-6-phosphate which was more abundant in the morning and afternoon.

DISCUSSION

Diel Variations in the Thermal Tolerance of Field-Sampled Insects

In our study, we showed a linear relationship between ambient microhabitat temperature and measures of both cold and heat tolerance of field-collected specimens of *N. groenlandicus*. Given the temperatures observed in the field during summer in Narsarsuaq includes subzero night temperatures and peak day temperatures above 40°C (Sørensen et al., 2019; Bahrndorff et al., 2021b) we advocate that the ability to withstand and remain active at high temperatures and recover fast from low temperature coma is ecologically important, especially for a univoltine species such as *N. groenlandicus*. This allows the species to, e.g., forage and mate in a transient environment and short summer season. Thus, our results point to plasticity in thermal tolerances being of strong importance for survival of insects in this region. Recently, the heat hardening capacity of *N. groenlandicus* has been examined in the laboratory, showing that heat tolerance can be increased within 45 min when the insects are exposed to high temperatures (Sørensen et al., 2019). It was also found that the heat hardening effect was reverted within 2 h when the insects were returned to cooler temperatures. Our results are consistent with these former observations, and the rapid adjustments that we observed in both heat and cold tolerance from field-sampled *N. groenlandicus* additionally support the assumption that hardening responses are important for coping with rapid changes in ambient temperature in the field. This is in contrast to the slower hardening responses observed in some temperate

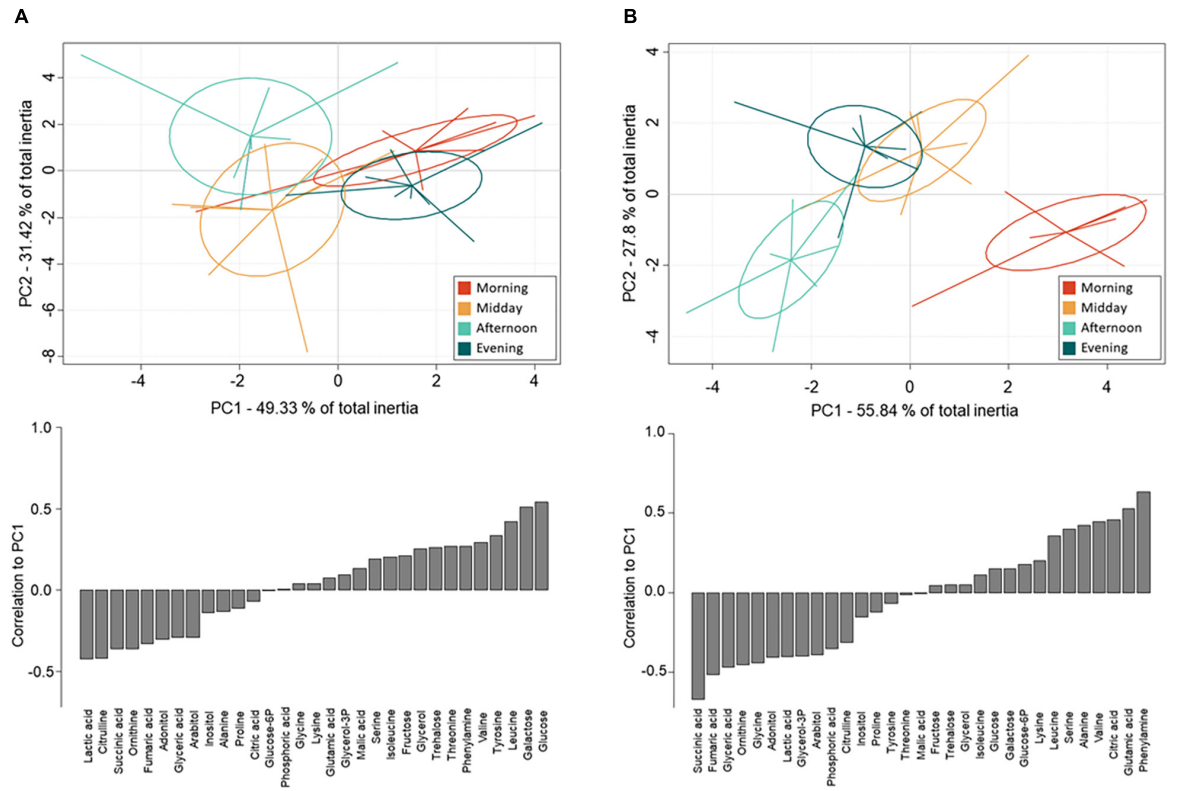

FIGURE 4 | Between-class Principal Component Analyses (top) and metabolite correlations to PC1 (bottom) based on GC-MS quantification of metabolites from whole-body extracts of female *N. groenlandicus* sampled in the field at four consecutive sampling time points (8:00 am, 12:00 pm, 4:00 pm, 8:00 pm) on **(A)** the thermally stable sampling day (day 4) and **(B)** the thermally variable sampling day (day 5). Lines represents individual sample positions respective to centroids (n = 8).

insect species and indicates that high thermal variability of the environment can be a selective agent for rapid plastic responses (Dahlgaard et al., 1998; Bahrndorff et al., 2009; Alemu et al., 2017).

Interestingly, the adjustments occurred not only at extreme temperatures, but also at temperatures that are not considered as stressful or sub-lethal to the species. The body temperatures experienced by *N. groenlandicus,* like other insect species, might differ from the temperatures measured in the shade due to behavioral thermoregulation (e.g., Stevenson, 1985; Kearney et al., 2009), such as seeking microhabitat temperatures that deviate from air temperatures (Stevenson, 1985; Böcher and Nachman, 2001; Danks, 2004; Kearney et al., 2009). However, as sampled individuals are collected in the same microhabitat as where air temperatures are measured, we do argue that the difference between air and body temperatures is likely to be minor. The adult life-stage of *N. groenlandicus* seemingly has a high preferred body temperature of approximately 30°C (Böcher and Nachman, 2001) and an extreme thermal tolerance breadth spanning from critical lower limits (CTmin) of −3.4°C to critical upper thermal limits (CTmax) of 48.5–52°C (Böcher and Nachman, 2001; Sørensen et al., 2019; Bahrndorff et al., 2021b). Thus, only the lowest temperatures recorded in the field approximated sub-lethal conditions for the species. Typically, hardening responses are described as being induced by stressful conditions (Angilletta, 2009). For example it has been described from laboratory and field studies performed on *Drosophila* spp. that hardening temperatures ca. 10–15°C above optimal rearing temperatures are needed to induce adaptive increases in heat tolerance, and cause upregulation of heat shock proteins (Sørensen et al., 2003; King and MacRae, 2015). Here, despite exposure to temperatures well within their thermal comfort zone, we show that heat and cold tolerance changed daily in *N. groenlandicus*. This finding might represent an evolutionary adaptation to the extreme climatic variations of arctic environments, but may also suggest that temperature variation act in concert with changes in air humidity and/or other climate variables to affect thermal tolerances as found for several other polar and sub-polar species (Block et al., 1994; Hodkinson et al., 1996; Benoit et al., 2009; Everatt et al., 2015).

Another important discovery was that the patterns of plastic changes in cold tolerance were similar for males and females, while distinct patterns were seen for heat tolerance for the two sexes (**Figure 2**). Females had a higher HKDT (+5 min HKDT) compared to males and tended to exhibit a stronger plasticity for that trait when field temperatures varied. Often, studies on thermal plasticity in insects find that the variation in upper thermal limits is constrained, and less plastic, compared to lower thermal limits (reviewed by Hoffmann et al., 2013; see also Chown, 2001; Overgaard et al., 2011; Alford et al., 2012). This

is likely resulting from the difficulty of insects to seek shelter from cold temperatures, thus resulting in a stronger selection pressure for plasticity of cold tolerance (Hoffmann et al., 2013). Our findings suggest that the selection pressure for cold tolerance may have been similar in male and female *N. groenlandicus* because no differences were observed in $T_{recovery}$. Conversely, heat stress is often countered by behavioral thermoregulation in ectotherms, for instance by seeking shadow or migrating below-ground (Huey and Tewksbury, 2009; Kearney et al., 2009). The higher heat tolerance and plasticity for this trait in females could be explained by the univoltine life history and the short arctic summers requiring females to seek out warm temperatures to rapidly complete their life cycle (Bahrndorff et al., 2021a). Further, our results indicate a trade-off between heat and cold tolerance. Thus, individuals sampled at middays and afternoons are overall more heat tolerant and less cold tolerant compared to individuals sampled during mornings and evenings. Similar results have been found for thermal tolerance of the fruit fly *Drosophila melanogaster* kept under natural and semi-natural conditions (Overgaard and Sørensen, 2008; Schou et al., 2015). A consequence might be maladaptive plastic responses to environmental cues, as climatic conditions are prospected to become more unpredictably variable in the future (Kingsolver and Huey, 1998; Huey et al., 1999; Manenti et al., 2014).

Daily Thermal Variations and Metabolic Fingerprints

The separation of the metabolic fingerprints from field-sampled *N. groenlandicus* was much stronger when individuals were collected during the thermally variable day (day 5), as compared with the less temperature variable day (day 4). This finding supports our hypothesis that microhabitat environmental conditions have a strong impact on diurnal changes of the physiology of adult *N. groenlandicus*. It also suggests that the observed diurnal variation in metabolic fingerprints cannot be explained by circadian clock regulations alone because similar patterns would be expected on the two collection days if that was the case.

On the less variable day, the average temperature prior to testing the insects was rather similar for the four collection periods (maximal temperature difference of 4.9°C; **Figure 1**). The measured changes in metabolite concentrations on this day were thus mostly independent of temperature, and rather reflected adjustments in energetic metabolism over the day or circadian regulated responses independent of temperature. This assumption is supported by the grouping pattern of the metabolomic profiles of individuals collected at the four different time points of day 4. Metabolomic profiles in the morning and evening were more similar and separated from those of individuals collected on the midday and afternoon. This pattern may reflect that the activity of the individuals was higher during midday and afternoon and in turn increased the energetic needs and metabolism in general. Consistently, sugars (glucose, fructose, galactose), some metabolic intermediates (citric and fumaric acid), and a range of free amino acids (phenylalanine, valine, serine, glutamine, and tyrosine), all being important substrates for glycolysis and Krebs cycle, varied in rhythmic patterns on both day 4 and 5 (**Figure 3**). These patterns might constitute circadian clock mechanisms that are regulated independently of temperature, humidity and other variable abiotic factors as seen, e.g., in *D. melanogaster* (Rhoades et al., 2018).

On the temperature variable day, the pattern of separation was markedly different than the one reported for day 4. The groups separating the strongest and explaining most of the variation in the data belonged to the individuals collected in the morning and afternoon, representing the time points with the lowest and the highest temperatures of day 5. Throughout this day, the sugars fructose, glucose and galactose, occurred in higher quantities compared to day 4 and especially fructose and glucose accumulated 2–3 fold in the evening in the individuals sampled on day 5, despite the temperature not being different from the temperature on day 4 at this time point (**Figures 1**, **3**). Typically, sugar accumulation is associated with cold shock responses (Jagdale et al., 2005; Lalouette et al., 2007; Michaud and Denlinger, 2007; Overgaard et al., 2007; Holmstrup et al., 2010; Teets et al., 2011) and seasonal preparation for diapause (Koštál et al., 2001; Watanabe, 2002; Vasquez et al., 2019). Sugars have osmoprotective properties that may play important protective roles in cold tolerance possibly through stabilization of cell membranes and macromolecular structures even at low concentration (Gekko and Timasheff, 1981; Yancey, 2005; Koštál et al., 2016) or by maintaining haemolymph osmolality despite low [Na+] and [K+] due to cold exposure (MacMillan et al., 2015). Thus, sugars might be accountable for the higher cold tolerance observed in individuals on days characterized by large temperature amplitude.

Polyols accumulated during the warmest periods of the days and especially inositol, fluctuated on day 5. Accumulation of the polyols mannitol and sorbitol in whiteflies and aphids with daily warm peaks has been associated with increased heat tolerance under natural and semi-natural conditions (Hendrix and Salvucci, 1998; Wolfe et al., 1998; Salvucci et al., 2000). This could indicate that polyols contribute to regulation of heat tolerance in *N. groenlandicus*.

It is possible that oscillations of sugars and polyols were affected by temperature-dependent activity patterns such as feeding, mating and general metabolism, and this might confound the effects of temperature and humidity alone on thermal tolerance. Foraging or feeding rates are partly governed by upper and lower activity thresholds of organisms (Everatt et al., 2013). Our own unpublished data on the activity of *N. groenlandicus* show that the species is virtually inactive at temperatures below 15°C and activity peaks at 35–40°C. This might suggest that feeding is constricted to the warmest periods of the day (typically between 20 and 30°C at the given study site). *Nysius* species feed on phloem sap and plant seeds (Böcher, 1972; Broadle et al., 1986; Böcher et al., 2015; Tiwari and Wratten, 2019; Maharjan et al., 2020), and therefore ingest large quantities of sucrose, which is produced by photosynthesis in plants. In other hemipteran phloem-feeders, sucrose is hydrolyzed to glucose and fructose when ingested and rapidly converted to trehalose or polyols, which are less toxic compounds to store

in the hemolymph at high concentrations (Becker et al., 1996; Hendrix and Salvucci, 1998). Thus, there might be a direct link between feeding behavior and sugar and polyol levels. For instance, trehalose concentrations increased more in the afternoon/evening on the warm day when feeding rates are expected to be higher (**Figure 3**).

Additionally, selective feeding on protein- and lipid rich diets impact on thermal tolerance in several insect species. For instance, the dung beetle *Thorectes lusitanicus* has been found to selectively supplement its diet with acorn, and experiments showed that beetles that were fed on acorn had a hemolymph supercooling point that was 5°C lower than individuals fed on cow-dung (Verdú et al., 2010). The shift was associated with alterations in hemolymph cryoprotectant content. Likewise, Rho and Lee (2017) showed that the beetle *Tenebrio molitor* selectively chose a carbohydrate rich diet at cold and warm temperatures, opposed to a more balanced protein-carbohydrate diet at intermediate temperatures. Switches in feeding preference with cold stress have also been found for *Drosophila* species (Brankatschk et al., 2018; Strassburger and Teleman, 2018). These aspects should be examined further in future studies.

Whether the oscillations of sugars and polyols constitute protective responses rather than consequences of altered energetic metabolism or temperature-dependent feeding is not evident from our results. However, the contrasting patterns of sugar and polyol oscillation may indicate that polyols are converted to sugars during the coldest times of the thermally variable day, maybe as a protective response. It might also explain the negative trade-off observed between heat and cold tolerance in this study. However, this is speculative and should be examined further in future studies along with common garden experiments revealing the importance of circadian rhythm regulation of *N. groenlandicus* behavior and physiology.

CONCLUSION

Here, we showed that thermal tolerance was correlated with ambient microhabitat temperature in the Greenlandic seed bug, *N. groenlandicus*. Thus, we show that hardening responses observed under constant laboratory conditions in a previous study (Sørensen et al., 2019) occur also in field settings for this species. Interestingly, we found that the plastic adjustments of thermal tolerance occurred at relative benign temperatures contrasting experimental evidence from laboratory studies on primarily model species, suggesting that these responses occur at sub- or supra lethal temperatures. Thus, plasticity of thermal tolerance is likely affected by multiple factors under natural conditions and constitute an example of evolutionary adaption to the extreme and variable Arctic and sub-Arctic habitats of *N. groenlandicus*. Further, we showed that field heat hardening causes increased heat tolerance and reduced cold tolerance and *vice versa* with cold acclimation, suggesting a trade-off. GC-MS investigation of field collected individuals revealed candidate metabolites that are regulated according to thermal and other abiotic and biotic conditions that vary on a diurnal basis. Distinct metabolic fingerprints associated with temperature on the thermally variable day (day 5) were not observed at the thermal stable day (day 4). This suggests that our results cannot be explained by circadian clock regulated mechanisms alone, but that these metabolites are partly regulated by temperature variability (or variation in correlated environmental or physiological variables) and constitute important physiological mechanisms controlling diurnal variability in thermal tolerances in the species. The strong plastic responses observed in heat and cold tolerance and in the metabolomic profiles in our study suggests ongoing strong selection for plasticity in this highly fluctuating polar environment. The genetic architecture of these traits are mainly investigated in model organisms where studies on *D. melanogaster* suggest significant heritable variation for plasticity of thermal stress tolerance traits and metabolite profiles (Gerken et al., 2015; Hangartner and Hoffmann, 2015; Ørsted et al., 2017; Rohde et al., 2021).

DATA AVAILABILITY STATEMENT

All data, including raw field temperature files and GC-MS data, are presented in this article/**Supplementary Material**.

AUTHOR CONTRIBUTIONS

SB, TNK, and NKN conceived the ideas and designed the methodology. MHS, SB, TNK, and NKN collected the data. DR, HC, and NKN processed samples for metabolomics analysis and analyzed the metabolomics data. TNK and NKN led the writing of the manuscript. All authors contributed to the drafts and gave final approval for publication.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphys.2022.818485/full#supplementary-material

REFERENCES

Alemu, T., Alemneh, T., Pertoldi, C., Ambelu, A., and Bahrndorff, S. (2017). Costs and benefits of heat and cold hardening in a soil arthropod. *Biol. J. Linn. Soc.* 122, 765–773. doi: 10.1093/biolinnean/blx092

Alford, L., Blackburn, T. M., and Bale, J. S. (2012). Effect of latitude and acclimation on the lethal temperatures of the peach-potato aphid *Myzus persicae*. *Agric. For. Entomol.* 14, 69–79. doi: 10.1111/j.1461-9563.2011.00553.x

Angilletta, M. J. (2009). "Thermal acclimation," in *Thermal Adaptation: A Theoretical and Empirical Synthesis*, ed. M. J. Angilletta (New York, NY: Oxford University Press), 126–156.

Araújo, M. B., Ferri-Yáñez, F., Bozinovic, F., Marquet, P. A., Valladares, F., and Chown, S. L. (2013). Heat freezes niche evolution. *Ecol. Lett.* 16, 1206–1219. doi: 10.1111/ele.12155

Bahrndorff, S., Gertsen, S., Pertoldi, C., and Kristensen, T. N. (2016). Investigating thermal acclimation effects before and after a cold shock in *Drosophila melanogaster* using behavioural assays. *Biol. J. Linn. Soc.* 117, 241–251. doi: 10.1111/bij.12659

Bahrndorff, S., Lauritzen, J. M. S., Sørensen, M. H., Noer, N. K., and Kristensen, T. N. (2021b). Responses of terrestrial polar arthropods to high and increasing temperatures. *J. Exp. Biol.* 224:jeb230797. doi: 10.1242/jeb.230797

Bahrndorff, S., Alemu, T., Kristensen, T. N., Sørensen, M. H., Høye, T., and Holmstrup, M. (2021a). Thermal adaptations of adults and eggs in the arctic insect, *Nysius groenlandicus*. *Polar Biol.* 44, 491–498. doi: 10.1007/s00300-021-02807-6

Bahrndorff, S., Mariën, J., Loeschcke, V., and Ellers, J. (2009). Dynamics of heat-induced thermal stress resistance and hsp70 expression in the springtail, *Orchesella cincta*. *Funct. Ecol.* 23, 233–239. doi: 10.1111/j.1365-2435.2009.01541.x

Bahrndorff, S., Petersen, S. O., Loeschcke, V., Overgaard, J., and Holmstrup, M. (2007). Differences in cold and drought tolerance of high arctic and sub-arctic populations of *Megaphorura arctica* Tullberg 1876 (Onychiuridae: Collembola). *Cryobiology* 55, 315–323. doi: 10.1016/j.cryobiol.2007.09.001

Bak, C. W., Bahrndorff, S., Noer, N. K., Jørgensen, L. B., Overgaard, J., and Kristensen, T. N. (2020). Comparison of static and dynamic assays when quantifying thermal plasticity of drosophilids. *Insects* 11:537. doi: 10.3390/insects11080537

Becker, A., Schlöder, P., Steele, J. E., and Wegener, G. (1996). The regulation of trehalose metabolism in insects. *Experientia* 52, 433–439.

Benoit, J. B., Lopez-martinez, G., Elnitsky, M. A., Lee, R. E., and Denlinger, D. L. (2009). Dehydration-induced cross tolerance of *Belgica antarctica* larvae to cold and heat is facilitated by trehalose accumulation. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* 152, 518–523. doi: 10.1016/j.cbpa.2008.12.009

Block, W., Webb, N. R., Coulson, S., Hodkinson, I. D., and Worland, M. R. (1994). Thermal adaptation in the Arctic collembolan *Onychiurus arcticus* (Tullberg). *J. Insect Physiol.* 40, 715–722. doi: 10.1016/0022-1910(94)90099-X

Böcher, J. (1972). Feeding biology of *Nysius groenlandicus* (Heteroptera: Miridae) in Greenland. With a note on oviposition in relation to food-source and dispersal of the species. *Medd. Grønland* 191, 1–41.

Böcher, J., Kristensen, N. P., Pape, T., and Vilhelmsen, L. (2015). *The Greenland Entomofauna: An Identification Manual of Insects, Spiders and Their Allies (Fauna Entomologica Scandinavica)*. Leiden: Brill. doi: 10.1163/ej.9789004157705.i-265.29

Böcher, J., and Nachman, G. (2001). Temperature and humidity responses of the arctic-alpine seed bug *Nysius groenlandicus*. *Entomol. Exp. Appl.* 99, 319–330. doi: 10.1046/j.1570-7458.2001.00831.x

Brankatschk, M., Gutmann, T., Knittelfelder, O., Palladini, A., Prince, E., Grzybek, M., et al. (2018). A temperature-dependent switch in feeding preference improves *Drosophila* development and survival in the cold. *Dev. Cell* 46, 781–793.e4. doi: 10.1016/J.DEVCEL.2018.05.028

Broadle, R., Simpson, B., and Beavis, C. (1986). Damage by "*Nysius*" spp. (Hemiptera: Lygaeidae) in non-stressed sunflower ('*Helianthus annus*' L.) crops. *Gen. Appl. Entomol.* 18, 17–24.

Buckley, B. A., Owen, M. E., and Hofmann, G. E. (2001). Adjusting the thermostat: the threshold induction temperature for the heat-shock response in intertidal mussels (genus *Mytilus*) changes as a function of thermal history. *J. Exp. Biol.* 204, 3571–3579.

Chown, S. L. (2001). Physiological variation in insects: hierarchical levels and implications. *J. Insect Physiol.* 47, 649–660. doi: 10.1016/S0022-1910(00)00163-3

Chown, S. L., Jumbam, K. R., Sørensen, J. G., and Terblanche, J. S. (2009). Phenotypic variance, plasticity and heritability estimates of critical thermal limits depend on methodological context. *Funct. Ecol.* 23, 133–140. doi: 10.1111/J.1365-2435.2008.01481.X

Chown, S. L., and Terblanche, J. S. (2006). Physiological diversity in insects: ecological and evolutionary contexts. *Adv. Insect Phys.* 33, 50–152. doi: 10.1016/S0065-2806(06)33002-0

Colinet, H., and Hoffmann, A. A. (2012). Comparing phenotypic effects and molecular correlates of developmental, gradual and rapid cold acclimation responses in *Drosophila melanogaster*. *Funct. Ecol.* 26, 84–93. doi: 10.1111/j.1365-2435.2011.01898.x

Colinet, H., Sinclair, B. J., Vernon, P., and Renault, D. (2015). Insects in fluctuating thermal environments. *Ann. Rev. Entomol.* 60, 123–140. doi: 10.1146/annurev-ento-010814-021017

Convey, P., Coulson, S. J., Worland, M. R., and Sjöblom, A. (2018). The importance of understanding annual and shorter-term temperature patterns and variation in the surface levels of polar soils for terrestrial biota. *Polar Biol.* 41, 1587–1605.

Dahlgaard, J., Loeschcke, V., Michalak, P., and Justesen, J. (1998). Induced thermotolerance and associated expression of the heat-shock protein Hsp70 in adult *Drosophila melanogaster*. *Funct. Ecol.* 12, 786–793. doi: 10.1046/j.1365-2435.1998.00246.x

Danks, H. V. (2004). Seasonal adaptations in Arctic insects. *Integr. Comp. Biol.* 44, 85–94. doi: 10.1093/ICB/44.2.85

Davey, M. C., Pickup, J., and Block, W. (2021). Temperature variation and its biological significance in fellfield habitats on a maritime Antarctic island. *Antarct. Sci.* 4, 383–388. doi: 10.1017/S0954102092000567

Deere, J. A., Sinclair, B. J., Marshall, D. J., and Chown, S. L. (2006). Phenotypic plasticity of thermal tolerances in five oribatid mite species from sub-Antarctic Marion Island. *J. Insect Physiol.* 52, 693–700. doi: 10.1016/j.jinsphys.2006.03.009

Everatt, M. J., Bale, J. S., Convey, P., Worland, M. R., and Hayward, S. A. L. (2013). The effect of acclimation temperature on thermal activity thresholds in polar terrestrial invertebrates. *J. Insect Physiol.* 59, 1057–1064. doi: 10.1016/j.jinsphys.2013.08.003

Everatt, M. J., Convey, P., Bale, J. S., Worland, M. R., and Hayward, S. A. (2015). Responses of invertebrates to temperature and water stress: a polar perspective. *J. Therm. Biol.* 54, 118–132. doi: 10.1016/j.jtherbio.2014.05.004

Fields, P. G., Fleurat-Lessard, F., Lavenseau, L., Febvay, G., Peypelut, L., and Bonnot, G. (1998). The effect of cold acclimation and deacclimation on cold tolerance, trehalose and free amino acid levels in *Sitophilus granarius* and *Cryptolestes ferrugineus* (Coleoptera). *J. Insect Physiol.* 44, 955–965. doi: 10.1016/S0022-1910(98)00055-9

Fischer, K., Kölzow, N., Höltje, H., and Karl, I. (2011). Assay conditions in laboratory experiments: is the use of constant rather than fluctuating temperatures justified when investigating temperature-induced plasticity? *Oecologia* 166, 23–33. doi: 10.1007/s00442-011-1917-0

Foray, V., Desouhant, E., Voituron, Y., Larvor, V., Renault, D., Colinet, H., et al. (2013). Does cold tolerance plasticity correlate with the thermal environment and metabolic profiles of a parasitoid wasp? *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* 164, 77–83. doi: 10.1016/j.cbpa.2012.10.018

Fusco, G., and Minelli, A. (2010). Phenotypic plasticity in development and evolution: facts and concepts. *Philos. Trans. R. Soc. B* 365, 547–556. doi: 10.1098/rstb.2009.0267

Gekko, K., and Timasheff, S. N. (1981). Mechanism of protein stabilization by glycerol: preferential hydration in glycerol-water mixtures. *Biochemistry* 20, 4667–4676. doi: 10.1021/bi00519a023

Gerken, A. R., Eller, O. C., Hahn, D. A., and Morgan, T. J. (2015). Constraints, independence, and evolution of thermal plasticity: probing genetic architecture of long-and short-term thermal acclimation. *Proc. Natl. Acad. Sci. U.S.A.* 112, 4399–4404.

Gracey, A. Y., Chaney, M. L., Boomhower, J. P., Tyburczy, W. R., Connor, K., and Somero, G. N. (2008). Rhythms of gene expression in a fluctuating intertidal environment. *Curr. Biol.* 14, 1501–1507. doi: 10.1016/j.cub.2008.08.049

Hangartner, S., and Hoffmann, A. A. (2015). Evolutionary potential of multiple measures of upper thermal tolerance in *Drosophila melanogaster*. *Funct. Ecol.* 30, 442–452. doi: 10.1111/1365-2435.12499

Harris, D. T. (2018). Biobanking and omics. *Front. Biol.* 13:287–292. doi: 10.1007/s11515-018-1505-3

Hendrix, D. L., and Salvucci, M. E. (1998). Polyol metabolism in homopterans at high temperatures: accumulation of mannitol in aphids (Aphididae:

Homoptera) and sorbitol in whiteflies (Aleyrodidae: Homoptera). *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* 120, 487–494. doi: 10.1016/S1095-6433(98)10058-2

Hodkinson, I. D., Coulson, S. J., Webb, N. R., and Block, W. (1996). Can high Arctic soil microarthropods survive eleveated summer temperatures? *Funct. Ecol.* 10, 314–321. doi: 10.2307/2390278

Hoffmann, A. A., Chown, S. L., and Clusella-Trullas, S. (2013). Upper thermal limits in terrestrial ectotherms: how constrained are they? *Funct. Ecol.* 27, 934–949. doi: 10.1111/j.1365-2435.2012.02036.x

Hoffmann, A. A., Sørensen, J. G., and Loeschcke, V. (2003). Adaptation of *Drosophila* to temperature extremes: bringing together quantitative and molecular approaches. *J. Therm. Biol.* 28, 175–216. doi: 10.1016/S0306-4565(02)00057-8

Holmstrup, M., Bayley, M., Pedersen, S. A., and Zachariassen, K. E. (2010). "Interactions between cold, desiccation and environmental toxin," in *Low Temperature Biology of Insects*, eds D. L. Denlinger and R. E. Lee (Cambridge: Cambridge University Press), 166–190.

Holmstrup, M., Hedlund, K., and Boriss, H. (2002). Drought acclimation and lipid composition in *Folsomia candida*: implications for cold shock, heat shock and acute desiccation stress. *J. Insect Physiol.* 48, 961–970. doi: 10.1016/S0022-1910(02)00175-0

Huey, R. B., Berrigan, D., Gilchrist, G. W., and Herron, J. C. (1999). Testing the adaptive significance of acclimation: a strong inference approach. *Am. Zool.* 39, 323–336. doi: 10.1093/icb/39.2.323

Huey, R. B., and Tewksbury, J. J. (2009). Can behavior douse the fire of climate warming? *Proc. Natl. Acad. Sci. U.S.A.* 106, 3647–3648. doi: 10.1073/pnas.0900934106

Jagdale, G. B., Grewal, P. S., and Salminen, S. O. (2005). Both heat-shock and cold-shock influence trehalose metabolism in an entomopathogenic nematode. *J. Parasitol.* 91, 988–994. doi: 10.1645/GE-504R.1

Javal, M., Renault, D., and Colinet, H. (2016). Impact of fluctuating thermal regimes on *Drosophila melanogaster* survival to cold stress. *Anim. Biol.* 66, 427–444. doi: 10.1163/15707563-00002510

Jensen, A., Alemu, T., Alemneh, T., Pertoldi, C., and Bahrndorff, S. (2019). Thermal acclimation and adaptation across populations in a broadly distributed soil arthropod. *Funct. Ecol.* 33, 833–845. doi: 10.1111/1365-2435.13291

Kearney, M., Gillingham, P. K., Bramer, I., Duffy, J. P., and MacLean, I. M. D. (2020). A method for computing hourly, historical, terrain-corrected microclimate anywhere on earth. *Methods Ecol. Evol.* 11, 38–43. doi: 10.1111/2041-210X.13330

Kearney, M., and Porter, W. (2009). Mechanistic niche modelling: combining physiological and spatial data to predict species' ranges. *Ecol. Lett.* 12, 334–350. doi: 10.1111/j.1461-0248.2008.01277.x

Kearney, M., Shine, R., and Porter, W. P. (2009). The potential for behavioral thermoregulation to buffer "cold-blooded" animals against climate warming. *Proc. Natl. Acad. Sci. U.S.A.* 106, 3835–3840. doi: 10.1073/pnas.0808913106

Kelty, J. (2007). Rapid cold-hardening of *Drosophila melanogaster* in a field setting. *Physiol. Entomol.* 32, 343–350. doi: 10.1111/j.1365-3032.2007.00584.x

Ketola, T., and Kristensen, T. N. (2017). Experimental approaches for testing if tolerance curves are useful for predicting fitness in fluctuating environments. *Front. Ecol. Evol.* 5:129. doi: 10.3389/fevo.2017.00129

King, A. M., and MacRae, T. H. (2015). Insect heat shock proteins during stress and diapause. *Ann. Rev. Entomol.* 60, 59–75. doi: 10.1146/annurev-ento-011613-162107

Kingsolver, J. G., Higgins, J. K., and Augustine, K. E. (2015). Fluctuating temperatures and ectotherm growth: distinguishing non-linear and time-dependent effects. *J. Exp. Biol.* 218, 2218–2225. doi: 10.1242/jeb.120733

Kingsolver, J. G., and Huey, R. B. (1998). Evolutionary analyses of morphological and physiological plasticity in thermally variable environments. *Am. Zool.* 38, 545–560. doi: 10.1093/icb/38.3.545

Kingsolver, J. G., and Nagle, A. (2007). Evolutionary divergence in thermal sensitivity and diapause of field and laboratory populations of *Manduca sexta*. *Physiol. Biochem. Zool.* 80, 473–479. doi: 10.1086/519962

Kinzner, M. T., Kinzner, M. C., Kaufmann, R., Hoffmann, A. A., Arthofer, W., Schlick-Steiner, B. C., et al. (2019). Is temperature preference in the laboratory ecologically relevant for the field? The case of *Drosophila nigrosparsa*. *Glob. Ecol. Conserv.* 18:e00638. doi: 10.1016/j.gecco.2019.e00638

Koštál, V., Korbelová, J., Poupardin, R., Moos, M., and Šimek, P. (2016). Arginine and proline applied as food additives stimulate high freeze tolerance in larvae of *Drosophila melanogaster*. *J. Exp. Biol.* 219, 2358–2367. doi: 10.1242/jeb.142158

Koštál, V., Šlachta, M., and Šimek, P. (2001). Cryoprotective role of polyols independent of the increase in supercooling capacity in diapausing adults of *Pyrrhocoris apterus* (Heteroptera: Insecta). *Comp. Biochem. Physiol. B Biochem. Mol. Biol.* 130, 365–374. doi: 10.1016/S1096-4959(01)00441-9

Koveos, D. S. (2001). Rapid cold hardening in the olive fruit fly *Bactrocera oleae* under laboratory and field conditions. *Entomol. Exp. Appl.* 101, 257–263. doi: 10.1046/j.1570-7458.2001.00910.x

Kristensen, T. N., Hoffmann, A., Overgaard, J., Sørensen, J. G., Hallas, R., and Loeschcke, V. (2008). Costs and benefits of cold acclimation in field-released *Drosophila*. *Proc. Natl. Acad. Sci. U.S.A.* 105, 216–221. doi: 10.1073/pnas.0708074105

Kristensen, T. N., Ketola, T., and Kronholm, I. (2020). Adaptation to environmental stress at different timescales. *Ann. N. Y. Acad. Sci.* 1476, 5–12. doi: 10.1111/nyas.13974

Kristensen, T. N., Overgaard, J., Hoffmann, A. A., Nielsen, N. C., and Malmendal, A. (2012). Inconsistent effects of developmental temperature acclimation on low-temperature performance and metabolism in *Drosophila melanogaster*. *Evol. Ecol. Res.* 14, 821–837.

Lalouette, L., Kostal, V., Colinet, H., Gagneul, D., and Renault, D. (2007). Cold exposure and associated metabolic changes in adult tropical beetles exposed to fluctuating thermal regimes. *FEBS J.* 274, 1759–1767. doi: 10.1111/j.1742-4658.2007.05723.x

Lembrechts, J. J., and Lenoir, J. (2020). Microclimatic conditions anywhere at any time! *Glob. Change Biol.* 26, 337–339. doi: 10.1111/gcb.14942

Loeschcke, V., and Hoffmann, A. A. (2007). Consequences of heat hardening on a field fitness component in *Drosophila* depend on environmental temperature. *Am. Nat.* 169, 175–183. doi: 10.1086/510632

Maclean, I. M. D., Duffy, J. P., Haesen, S., Govaert, S., De Frenne, P., Vanneste, T., et al. (2021). On the measurement of microclimate. *Methods Ecol. Evol.* 12, 1397–1410. doi: 10.1111/2041-210x.13627

MacMillan, H. A., Ferguson, L. V., Nicolai, A., Donini, A., Staples, J. F., and Sinclair, B. J. (2015). Parallel ionoregulatory adjustments underlie phenotypic plasticity and evolution of *Drosophila* cold tolerance. *J. Exp. Biol.* 218, 423–432. doi: 10.1242/jeb.115790

Maharjan, R., Yoon, Y., Jang, Y., Jeong, M., Jung, T. W., Ha, T. J., et al. (2020). Oviposition and development response of perilla seed bugs (*Nysius* sp.) (Heteroptera: Lygaeidae) to five crop seeds. *J. Appl. Entomol.* 144, 806–816. doi: 10.1111/JEN.12814

Manenti, T., Sørensen, J. G., Moghadam, N. N., and Loeschcke, V. (2014). Predictability rather than amplitude of temperature fluctuations determines stress resistance in a natural population of *Drosophila simulans*. *J. Evol. Biol.* 27, 2113–2122. doi: 10.1111/jeb.12463

Michaud, M. R., and Denlinger, D. L. (2007). Shifts in the carbohydrate, polyol, and amino acid pools during rapid cold-hardening and diapause-associated cold-hardening in flesh flies (*Sarcophaga crassipalpis*): a metabolomic comparison. *J. Comp. Physiol. B Biochem. Syst. Environ. Physiol.* 177, 753–763. doi: 10.1007/s00360-007-0172-5

Michaud, R., and Denlinger, D. L. (2010). "Genomics, proteomics and metabolomics: finding the other players in insect cold-tolerance," in *Low Temperature Biology of Insects*, eds D. L. Denlinger and R. E. Lee (New York, NY: Cambridge University Press), 91–115.

Mitchell, K. A., and Hoffmann, A. A. (2010). Thermal ramping rate influences evolutionary potential and species differences for upper thermal limits in *Drosophila*. *Funct. Ecol.* 24, 694–700. doi: 10.1111/j.1365-2435.2009.01666.x

Noer, N. K., Ørsted, M., Schiffer, M., Hoffmann, A. A., Bahrndorff, S., and Kristensen, T. N. (2022). Into the wild – a field study on the evolutionary and ecological importance of thermal plasticity in ectotherms across temperate and tropical regions. *Philos. Trans. R. Soc. B.* 376:20210004. doi: 10.1098/rstb.2021.0004

Noer, N. K., Pagter, M., Bahrndorff, S., Malmendal, A., and Kristensen, T. N. (2020). Impacts of thermal fluctuations on heat tolerance and its metabolomic basis in *Arabidopsis thaliana*, *Drosophila melanogaster*, and *Orchesella cincta*. *PLoS One* 15:e0237201. doi: 10.1371/journal.pone.0237201

Ørsted, M., Rohde, P. D., Hoffmann, A. A., Sørensen, P., and Kristensen, T. N. (2017). Environmental variation partitioned into separate heritable components. *Evolution* 72, 136–152. doi: 10.1111/evo.13391

Overgaard, J., Kristensen, T. N., Mitchell, K. A., and Hoffmann, A. A. (2011). Thermal tolerance in widespread and tropical *Drosophila* species: does phenotypic plasticity increase with latitude? *Am. Nat.* 178, S80–S96. doi: 10.1086/661780

Overgaard, J., Malmendal, A., Sørensen, J. G., Bundy, J. G., Loeschcke, V., Nielsen, N. C., et al. (2007). Metabolomic profiling of rapid cold hardening and cold shock in *Drosophila melanogaster*. *J. Insect Physiol.* 53, 1218–1232. doi: 10.1016/j.jinsphys.2007.06.012

Overgaard, J., and Sørensen, J. G. (2008). Rapid thermal adaptation during field temperature variations in *Drosophila melanogaster*. *Cryobiology* 56, 159–162. doi: 10.1016/j.cryobiol.2008.01.001

Overgaard, J., Sørensen, J. G., Com, E., and Colinet, H. (2014). The rapid cold hardening response of *Drosophila melanogaster*: complex regulation across different levels of biological organization. *J. Insect Physiol.* 62, 46–53. doi: 10.1016/j.jinsphys.2014.01.009

R Core Team (2020). *R: A Language and Environment for Statistical Computing*. Vienna: R Foundation for Statistical Computing.

Rho, M. S., and Lee, K. P. (2017). Temperature-driven plasticity in nutrient use and preference in an ectotherm. *Oecologia* 185, 401–413. doi: 10.1007/S00442-017-3959-4/FIGURES/4

Rhoades, S. D., Nayak, K., Zhang, S. L., Sehgal, A., and Weljie, A. M. (2018). Circadian- and light-driven metabolic rhythms in *Drosophila melanogaster*. *J. Biol. Rhythms* 33, 126–136. doi: 10.1177/0748730417753003

Rohde, P. D., Kristensen, T. N., Sarup, P., Muñoz, J., and Malmendal, A. (2021). Prediction of complex phenotypes using the *Drosophila melanogaster* metabolome. *Heredity* 126, 717–732. doi: 10.1038/s41437-021-00404-1

Ruel, J. J., and Ayres, M. P. (1999). Jensen's inequality predicts effects of environmental variation. *Trends Ecol. Evol.* 14, 361–366. doi: 10.1016/S0169-5347(99)01664-X

Salvucci, M. E., Stecher, D. S., and Henneberry, T. J. (2000). Heat shock proteins in whiteflies, an insect that accumulates sorbitol in response to heat stress. *J. Therm. Biol.* 25, 363–371. doi: 10.1016/S0306-4565(99)00108-4

Scheiner, S. M. (1993). Genetics and evolution of phenotypic plasticity. *Ann. Rev. Ecol. Evol. Syst.* 24, 35–68. doi: 10.1146/annurev.es.24.110193.000343

Schou, M. F., Kristensen, T. N., Pedersen, A., Karlsson, G., Loeschcke, V., and Malmendal, A. (2017). Metabolic and functional characterization of effects of developmental temperature in *Drosophila melanogaster*. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 312, R211–R222. doi: 10.1152/ajpregu.00268.2016

Schou, M. F., Loeschcke, V., and Kristensen, T. N. (2015). Strong costs and benefits of winter acclimatization in *Drosophila melanogaster*. *PLoS One* 10:e0130307. doi: 10.1371/journal.pone.0130307

Sears, M. W., Riddell, E. A., Rusch, T. W., and Angilletta, M. J. (2019). The world still is not flat: lessons learned from organismal interactions with environmental heterogeneity in terrestrial environments. *Integr. Comp. Biol.* 59, 1049–1058. doi: 10.1093/icb/icz130

Sheldon, K. S., Padash, M., Carter, A. W., and Marshall, K. E. (2020). Different amplitudes of temperature fluctuation induce distinct transcriptomic and metabolomic responses in the dung beetle *Phanaeus vindex*. *J. Exp. Biol.* 223(Pt 23):jeb233239. doi: 10.1242/jeb.233239

Sømme, L. (1999). The physiology of cold hardiness in terrestrial arthropods. *Eur. J. Entomol.* 96, 1–10.

Sørensen, J. G., Kristensen, T. N., and Loeschcke, V. (2003). The evolutionary and ecological role of heat shock proteins. *Ecol. Lett.* 6, 1025–1037. doi: 10.1046/j.1461-0248.2003.00528.x

Sørensen, M. H., Kristensen, T. N., Lauritzen, J. M. S., Noer, N. K., Høye, T. T., and Bahrndorff, S. (2019). Rapid induction of the heat hardening response in an Arctic insect. *Biol. Lett.* 15:20190613. doi: 10.1098/rsbl.2019.0613

Stevenson, R. D. (1985). The relative importance of behavioral and physiological adjustments controlling body temperature in terrestrial ectotherms. *Am. Nat.* 126, 362–386. doi: 10.1086/284423

Strassburger, K., and Teleman, A. A. (2018). Flies eat their veggies to survive the cold. *Dev. Cell* 46, 671–672. doi: 10.1016/j.devcel.2018.05.030

Taylor, E. N., Diele-Viegas, L. M., Gangloff, E. J., Hall, J. M., Halpern, B., Massey, M. D., et al. (2021). The thermal ecology and physiology of reptiles and amphibians: a user's guide. *J. Exp. Zool. A Ecol. Integr. Physiol.* 335, 13–44. doi: 10.1002/jez.2396

Teets, N. M., and Denlinger, D. L. (2013). Physiological mechanisms of seasonal and rapid cold-hardening in insects. *Physiol. Entomol.* 38, 105–116. doi: 10.1111/phen.12019

Teets, N. M., Kawarasaki, Y., Lee, R. E., and Denlinger, D. L. (2011). Survival and energetic costs of repeated cold exposure in the Antarctic midge, *Belgica antarctica*: a comparison between frozen and supercooled larvae. *J. Exp. Biol.* 214, 806–814. doi: 10.1242/jeb.051912

Terblanche, J. S., Deere, J. A., Clusella-Trullas, S., Janion, C., and Chown, S. L. (2007). Critical thermal limits depend on methodological context. *Proc. R. Soc. B Biol. Sci.* 274, 2935–2942. doi: 10.1098/rspb.2007.0985

Thiébaut, G., Tarayre, M., Jambon, O., Le Bris, N., Colinet, H., and Renault, D. (2020). Variation of thermal plasticity for functional traits between populations of an invasive aquatic plant from two climatic regions. *Hydrobiologia* 848, 2077–2091. doi: 10.1007/s10750-020-04452-2

Tiwari, S., and Wratten, S. D. (2019). Profile biology and management of the New Zealand endemic wheat bug, *Nysius huttoni* (Hemiptera: Lygaeidae). *J. Integr. Pest Manag.* 10, 34–35. doi: 10.1093/jipm/pmz032

Tomanek, L., and Somero, G. N. (1999). Evolutionary and acclimation-induced variation in the heat-shock responses of congeneric marine snails (genus *Tegula*) from different thermal habitats: implications for limits of thermotolerance and biogeography. *J. Exp. Biol.* 202, 2925–2936.

Tomanek, L., and Somero, G. N. (2002). Interspecific- and acclimation-induced variation in levels of heat-shock proteins 70 (hsp70) and 90 (hsp90) and heat-shock transcription factor-1 (HSF1) in congeneric marine snails (genus *Tegula*): implications for regulation of hsp gene expression. *J. Exp. Biol.* 205, 677–685. doi: 10.1242/jeb.205.5.677

van Eijsden, R. G. E., Stassen, C., Daenen, L., Van Mulders, S. E., Bapat, P. M., Siewers, V., et al. (2013). A universal fixation method based on quaternary ammonium salts (RNAlater) for omics-technologies: *Saccharomyces cerevisiae* as a case study. *Biotechnol. Lett.* 35, 891–900. doi: 10.1007/s10529-013-1163-0

Vasquez, M. C., Lippert, M. R., White, C., Walter, R. K., and Tomanek, L. (2019). Proteomic changes across a natural temperature gradient in a marine gastropod. *Mar. Environ. Res.* 149, 137–147. doi: 10.1016/j.marenvres.2019.06.002

Vázquez, D. P., Gianoli, E., Morris, W. F., and Bozinovic, F. (2017). Ecological and evolutionary impacts of changing climatic variability. *Biol. Rev.* 92, 22–42. doi: 10.1111/brv.12216

Verdú, J. R., Casas, J. L., Lobo, J. M., and Numa, C. (2010). Dung beetles eat acorns to increase their ovarian development and thermal tolerance. *PLoS One* 5:e10114. doi: 10.1371/journal.pone.0010114

Watanabe, M. (2002). Cold tolerance and myo-inositol accumulation in overwintering adults of a lady beetle, *Harmonia axyridis* (Coleoptera: Coccinellidae). *Eur. J. Entomol.* 99, 5–9. doi: 10.14411/eje.2002.002

Wolfe, G. R., Hendrix, D. L., and Salvucci, M. E. (1998). A thermoprotective role for sorbitol in the silverleaf whitefly, *Bemisia argentifolii*. *J. Insect Physiol.* 44, 597–603. doi: 10.1016/s0022-1910(98)00035-3

Yancey, P. H. (2005). Organic osmolytes as compatible, metabolic and counteracting cytoprotectants in high osmolarity and other stresses. *J. Exp. Biol.* 208, 2819–2830. doi: 10.1242/jeb.01730

Zachariassen, K. E. (1985). Physiology of cold tolerance in insects. *Physiol. Rev.* 65, 799–832. doi: 10.1152/physrev.1985.65.4.799

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frontiers in Physiology

REVIEW
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The Physiological and Evolutionary Ecology of Sperm Thermal Performance

Wayne Wen-Yeu Wang and Alex R. Gunderson*

Department of Ecology and Evolutionary Biology, Tulane University, New Orleans, LA, United States

Ongoing anthropogenic climate change has increased attention on the ecological and evolutionary consequences of thermal variation. Most research in this field has focused on the physiology and behavior of diploid whole organisms. The thermal performance of haploid gamete stages directly tied to reproductive success has received comparatively little attention, especially in the context of the evolutionary ecology of wild (i.e., not domesticated) organisms. Here, we review evidence for the effects of temperature on sperm phenotypes, emphasizing data from wild organisms whenever possible. We find that temperature effects on sperm are pervasive, and that above normal temperatures in particular are detrimental. That said, there is evidence that sperm traits can evolve adaptively in response to temperature change, and that adaptive phenotypic plasticity in sperm traits is also possible. We place results in the context of thermal performance curves, and encourage this framework to be used as a guide for experimental design to maximize ecological relevance as well as the comparability of results across studies. We also highlight gaps in our understanding of sperm thermal performance that require attention to more fully understand thermal adaptation and the consequences of global change.

Keywords: climate change, fertility, heat stress, postcopulatory selection, spermatogenesis, spermatozoa, thermal adaptation, thermal plasticity

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University of East Anglia,
United Kingdom
Matthew Gifford,
University of Central Arkansas,
United States
Abigail Kimmitt,
University of Michigan, United States

****Correspondence:***
Wayne Wen-Yeu Wang
wwang16@tulane.edu

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INTRODUCTION

Global climate change is expected to have broad effects on the biodiversity of Earth, and our knowledge about how organisms will respond to ongoing environmental disturbance still needs considerable improvement (IPCC, 2014). Many studies have measured the thermal performance and lethal heat thresholds of species to examine the ecological and evolutionary impact of rising global temperature, with the ultimate goal of making inferences about how population processes will be affected (summarized in Seebacher et al., 2014; Gunderson and Stillman, 2015; Sinclair et al., 2016; Pinsky et al., 2019; Buckley and Kingsolver, 2021). However, these studies tend to focus on diploid adult or sub-adult individuals. Far fewer studies have focused on how sublethal temperatures impact reproductive cells, even though they have direct influence on fertility and fitness. For example, a recent study on 43 *Drosophila* species showed that male sterility temperatures better predict species global distributions than the upper lethal temperatures of adults (Parratt et al., 2021). This result emphasizes the significant role male gametes can play in shaping

ecological pattern and process, and their likely importance in dictating how biodiversity will react to global warming.

Sperm are one of the only cell types that are released into a foreign environment after maturing, traveling through and interacting with the external physical environment and/or the female reproductive tract (Pitnick et al., 2009, 2020). During the life cycle of sperm, from development in the testis to egg fertilization, sperm may experience a wide range of environmental conditions including variation in temperature, pH, ionic state, and viscosity (Reinhardt et al., 2015). Here we focus on temperature, one of the most significant environmental factors relevant to environmental adaptation and climate change that could impact sperm traits and their ability to fertilize. In general, studies of temperature-dependent sperm performance are concentrated on model organisms used for medical or domestic breeding purposes, and therefore lack ecological and evolutionary context (Reinhardt et al., 2015; Lüpold and Pitnick, 2018; Walsh et al., 2019). From these studies, we know the impact of thermal stress on sperm traits can include morphological and behavioral changes, decreased sperm count and longevity, and increased DNA damage (Cameron and Blackshaw, 1980; Foldesy and Bedford, 1982; Flowers, 1997, 2015; Blanckenhorn and Hellriegel, 2002; Reinhardt et al., 2015; Parrish et al., 2017; Peña et al., 2021). Therefore, as thermal niches of populations diverge, we expect the temperature-dependence of sperm performance to undergo adaptive evolutionary change in concert with other aspects of organismal performance. However, considerably less is known about ecologically relevant sperm thermal biology in wild populations. This is slowly changing, however, and exciting results are beginning to emerge.

Here, we review empirical evidence for how temperature, and especially high temperature, affect sperm traits. We focus on data from wild animal populations whenever possible, but include data from plants and domesticated organisms when appropriate. We also attempt to place data within the "thermal performance curve" framework (Huey and Kingsolver, 1989). Thermal performance curves describe how rates of biological processes change with temperature, and are applicable to everything from enzymatic catalysis to population growth rates (Angilletta, 2009; Somero et al., 2017). The crux of thermal performance curves is that they are unimodal and asymmetrical: performance peaks at a temperature closer to the heat tolerance limit than to the cold tolerance limit (**Figure 1A**). This means that organisms tend to live at body temperatures close to their heat thresholds, making them vulnerable to warming (Deutsch et al., 2008; Huey et al., 2009). Data on sperm thermal traits are rarely discussed within the performance curve context (**Figure 1B**), but we believe doing so is beneficial for understanding the evolutionary ecology of sperm traits. Throughout, we attempt to synthesize emerging patterns from the available data, and also highlight gaps in our understanding that require greater attention.

Our review includes morphological and performance aspects of sperm phenotypes. We apply the term "performance" broadly, including any aspect of sperm-related phenotypes (number, percent motility, velocity, and fertilization success) that is not explicitly morphological.

HOW TEMPERATURE AFFECTS SPERM TRAITS

Temperature can affect sperm traits in many ways, both pre- and post-ejaculation (**Figure 2**). Below, we step through examples of how temperature can affect sperm traits at different stages of the sperm life cycle.

Pre-ejaculate Thermal Effects

Male developmental temperature plays an important role in testis development (Blanckenhorn and Henseler, 2005; Sales et al., 2021), which could subsequently affect sperm traits (Blanckenhorn and Hellriegel, 2002; Vasudeva et al., 2014; Sales et al., 2021; but see Iglesias-Carrasco et al., 2020). In yellow dung flies (*Scathophaga stercoraria*), individuals produced the longest sperm after undergoing development at either intermediate or high temperature (Blanckenhorn and Hellriegel, 2002). In the pseudoscorpion (*Cordylochernes scorpiodes*), fewer sperm were produced and fertility declined when reared at higher temperature (Zeh et al., 2012). The effects of heat stress can also vary across development stage, and male fertility may be more sensitive to heat during testicular development relative to earlier developmental stages. For example, red flour beetles (*Tribolium castaneum*) exposed to heat shock during the pupa and immature adult stage when testis are developing had to decreased testis volume, sperm number, sperm viability, and fertility; conversely, heat shock during an earlier larval stage had no effect on reproductive traits (Saxena et al., 1992; Sales et al., 2021).

Studies capable of isolating the effect of temperature on sperm through effects on testis development are rare (Sales et al., 2021). This is because many studies maintain males at their developmental temperature through sexual maturity, at which point sperm analysis occurs. As a result, it is not possible to disentangle effects that emerge due to testis development from direct effects on spermatogenesis or stored sperm. Regardless of the specific mechanism, studies often find that pre-ejaculate temperatures affect sperm traits. For instance, fewer and shorter sperm were produced by bruchid beetles (*Callosobruchus maculatus*) reared until sperm analysis at the highest and lowest experimental temperatures (Vasudeva et al., 2014), while field crickets (*Gryllus bimaculatus*) produced the most sperm when they developed at high temperature (Gasparini et al., 2018). In Trinidadian guppies (*Poecilia reticulata*), sperm length and swimming velocity were lowest for males reared until sperm analysis in the warmest treatment groups (Breckels and Neff, 2013, 2014).

Once males have reached sexual maturity, the temperature they experience can greatly affect sperm. For example, adult boar (*Sus scrofa domestica*) exposed to high temperature produce sperm with higher levels of DNA damage (e.g., decreased DNA integrity), more morphological abnormalities, and lower motility than sperm from boar housed under normal or cooler thermal conditions (Cameron and Blackshaw, 1980; Flowers, 1997, 2015; Parrish et al., 2017; Peña et al., 2021). Similar effects occur in bull (*Bos taurus*; Cheng et al., 2016; Morrell, 2020), ram

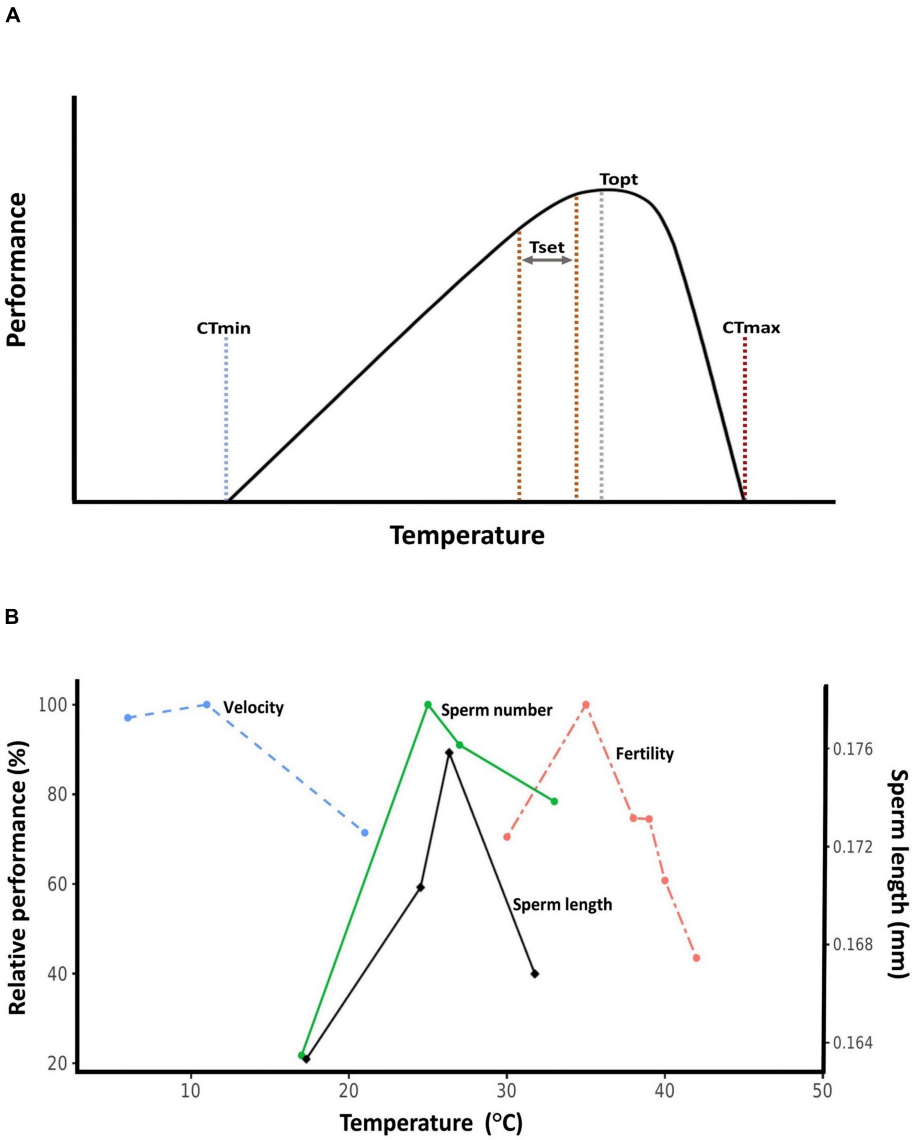

FIGURE 1 | Thermal performance curves. **(A)** Hypothetical thermal performance curve. CTmin: critical thermal minimum, the cold tolerance limit. CTmax: critical thermal maximum, the heat tolerance limit. Set point range (Tset): also known as the preferred temperature range, this is the target range of body temperatures ectothermic organisms seek out in a thermally variable environment. Topt: thermal optimum, the temperature at which performance is maximized. **(B)** Relative thermal performance (maximum value set to 100%) of ejaculated sperm velocity (Atlantic cod, *Gadus morhua*, Purchase et al., 2010), developmental sperm length (bruchid beetle, *Callosobruchus maculatus*, Vasudeva et al., 2014), sperm number (bruchid beetle, *C. maculatus*, Vasudeva et al., 2014), and male fertility (red flour beetle, *Tribolium castaneum*, Sales et al., 2018).

(*Ovis aries*; Rathore, 1970; Küçük and Aksoy, 2020), zebra finch (*Taeniopygia guttata*; Hurley et al., 2018), and chicken (*Gallus gallus domesticus*; Karaca et al., 2002). Such studies in wild endotherms are rare. To the best of our knowledge, the only applicable data come from the African lion (*Panthera leo*). Male lions with darker and thicker manes have higher body and testicular temperature and produce more morphologically abnormal sperm than lions with blonde manes (West and Packer, 2002).

Studies that increase testicular temperature specifically have also found large impacts on ejaculated sperm traits. For example, raising the testis temperature of laboratory rats to core body temperature by sealing the testis into the abdomen leads to a significant decrease in sperm motility and male fertility (Foldesy and Bedford, 1982). Negative effects are also observed in experiments in which the scrotum is insulated to increase temperature. For example, scrotal insulation studies in livestock and humans have found decreased sperm concentration, motility, and viability, as well as increased morphological abnormalities and DNA damage (Vogler et al., 1993; Abdelhamid et al., 2019; Garcia-Oliveros et al., 2020; Shahat et al., 2020).

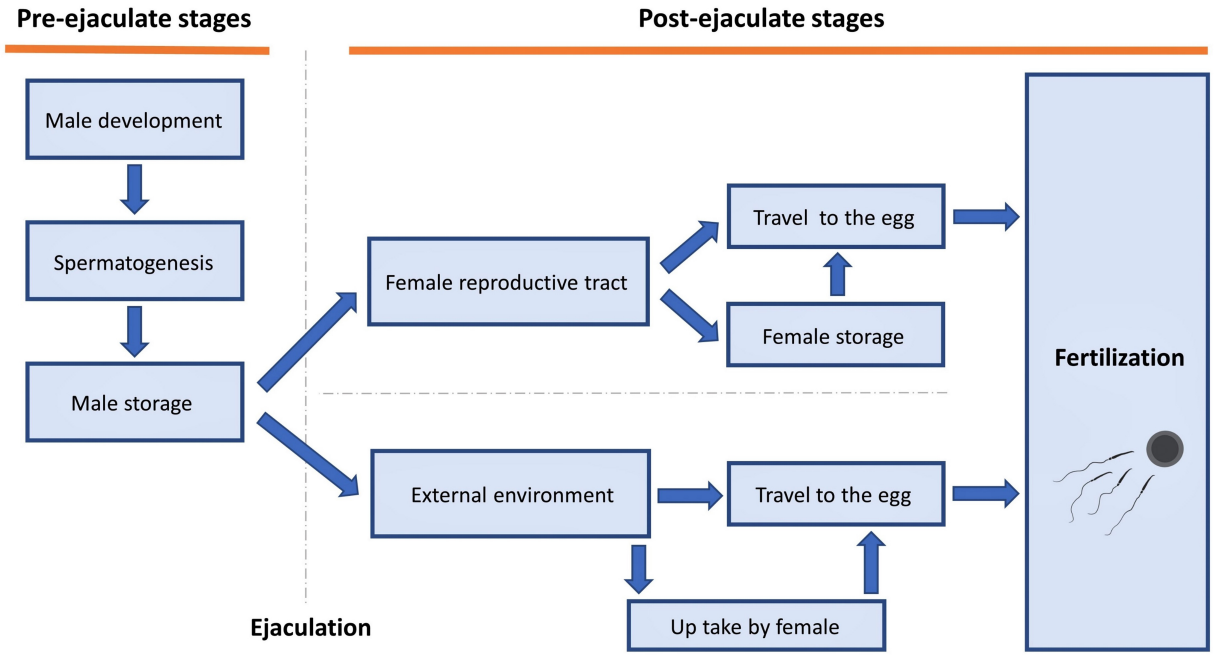

FIGURE 2 | Stages at which temperature could impact sperm traits.

The above examples focus on endotherms, but heat exposure after maturity also has a dramatic effect on ectotherm sperm traits. For instance, male red flour beetles (*T. castaneum*) produced 75% less sperm and had decreased sperm viability after exposure to a simulated heatwave (Sales et al., 2018, 2021; Vasudeva et al., 2021). Similar effects on sperm number and motility occur in field crickets (*G. bimaculatus*; Gasparini et al., 2018). In Brown trout (*Salmo trutta*), males exposed to warmer temperature showed no change in the proportion of motile cells, but produced significantly slower sperm (Fenkes et al., 2017). In some organisms, temperatures do not need to be much higher than "normal" for sperm development to be affected. For example, in the coral (*Acropora digitifera*), adults produced significantly fewer sperm during spawning after experiencing only a 2°C elevation of water temperature above ambient for 1 month (Paxton et al., 2015).

These effects can have subsequent repercussions for reproduction. For example, in the flesh fly (*Sarcophaga crassipalpis*), males became completely infertile after heat shock, and very few sperm were found in females mated to heat shocked males (Rinehart et al., 2000). Similar findings have been shown in red flour beetles (*T. castaneum*; Sales et al., 2018, 2021) and *C. elegans* (Aprison and Ruvinsky, 2014; Poullet et al., 2015). Trans-generational effects of temperature exposure have also been found in field crickets (*G. bimaculatus*), where adult males that experienced a warm treatment produced lower quality sperm and offspring with lower survival rate compared with cool treated males (Gasparini et al., 2018).

Though lacking information on specific sperm traits, several additional studies have found that male fertility is affected by temperatures they experience after maturity. For example, in many fruits flies (*Drosophila*), heat stressed males produce significantly fewer offspring, and the effect increases with the duration of heat exposure (David et al., 2005). Although these males were usually able to regain fertility through time, many were permanently sterile after exposure to prolonged or more extreme heat stress (Jørgensen et al., 2006; Sutter et al., 2019; van Heerwaarden and Sgrò, 2021). Similar effects of heat on male fertility have also been shown in Diamondback Moths (*Plutella xylostella*; Zhang et al., 2013). Male sterility after heat exposure was likely caused by a change in sperm traits, since normal mating behavior was observed in these studies (Jørgensen et al., 2006; Zhang et al., 2013; Sutter et al., 2019). In response to heat-induced male sterility, females may engage in mating with more males to maintain their own fecundity. By accumulating stored sperm through multiple mating, females can potentially restore their fecundity to normal levels (*Drosophila pseudoobscura*; Sutter et al., 2019; red flour beetles, *T. castaneum*; Vasudeva et al., 2021).

It is worth noting that male reproductive traits seem to be more sensitive to heat than female reproductive traits, based on studies in which the effects of temperature can be inferred for both sexes. In field crickets and flour beetles, heat treatments that affected males sperm traits did not affect female egg number (Paxton et al., 2015) or reproductive output (Sales et al., 2018, 2021). Furthermore, in the nematodes *C. elegans* and *C. briggsae*, infertility after heat stress is mainly due to defective sperm function (Prasad et al., 2011; Aprison and Ruvinsky, 2014; Poullet et al., 2015; but see Janowitz and Fischer, 2011). More work is required to determine the generality of these findings.

Overall, the evidence indicates that high temperatures can have major consequences for sperm quality and performance. Indeed, this is highlighted by the many thermoregulatory

mechanisms that organisms have evolved to protect sperm from heat. For example, most mammals maintain testis temperatures 1–6°C below core body temperature, facilitated by morphological and physiological specialization in the testicles (Cowles, 1958; Rommel et al., 1992; Pabst et al., 1995; Thundathil et al., 2012).

In ectotherms, thermoregulation is primarily behavioral. Taxa have a target range of body temperatures (the preferred temperature range, Tpref) that they seek out within their habitats. Tpref is expected to evolve in concert with other physiological adaptations to temperature such that animals prefer body temperatures that confer high physiological performance (Huey et al., 1999; Angilletta et al., 2006). Classic early papers in reptiles contended that Tpref evolution is tied to the thermal dependence of testis health in males (reviewed in Dawson, 1975). For example, exposing mature males to temperatures 1–2°C above Tpref led to testicular tissue damage in the lizards *Urosaurus ornatus*, *Sceloporus virgatus*, and *S. graciosus* (Licht, 1965). Similar results were found using cultured testicular tissue from the lizards *Anolis carolinensis* and *Uma scoparia* (Licht and Basu, 1967). These observations highlight that thermoregulation plays a central role in the ecology and evolution of temperature-dependent sperm performance and warrants further attention within that context.

Post-ejaculate Thermal Effect: Direct Effect on Mature Sperm Cells

After ejaculation, the temperature of the environment has a direct effect on sperm performance (reviewed in Alavi and Cosson, 2005; Revathy and Benno Pereira, 2016). For example, the sperm of external fertilizers may experience a wide range of ambient temperatures before reaching the eggs (Chirgwin et al., 2019; Walsh et al., 2019). Several studies have shown increased sperm velocity and/or flagellar beat frequency at higher water temperatures (Kupriyanova and Havenhand, 2005; Lahnsteiner and Mansour, 2012; Fenkes et al., 2017; Iglesias-Carrasco et al., 2020). That said, studies that include a wide range of test temperatures usually find that velocity is maximized at intermediate temperatures, consistent with a thermal performance curve (Alavi and Cosson, 2005; Purchase et al., 2010; Lahnsteiner and Mansour, 2012).

In contrast, high temperature tends to decrease sperm longevity, likely due to increased metabolic rates that rapidly deplete finite energy reserves (Schlenk and Kahmann, 1938; Alavi and Cosson, 2005; Bombardelli et al., 2013). For example, southern hake (*Merluccius australis*) sperm longevity was greatest when incubated at 4°C and decreased progressively with incubation at 10 and 15°C (Effer et al., 2013; but see Kekäläinen et al., 2018; Lymbery et al., 2020). The consequences of variation in environmental temperature for sperm performance can translate to fertilization success. For example, fertilization capacity decreases significantly if ejaculated sperm are exposed to heat in multiple sea urchin species (Rahman et al., 2009).

Exposing ejaculated sperm to heat may also have trans-generational effects. In European whitefish (*Coregonus lavaretus*), offspring sired by heat exposed sperm were smaller and had poorer swimming ability (Kekäläinen et al., 2018). Conversely, positive effects have been found in mussels (*Mytilus galloprovincialis*), where offspring survival rate was higher when sired by heat exposed sperm (Lymbery et al., 2021). These trans-generational effects may be caused by epigenetic changes or selection on sperm haplotypes (Lymbery et al., 2020, 2021). However, the underlying mechanisms are far from clear and need further testing.

Fertilization success changes with water temperature in external fertilizers, although it is difficult to determine the relative contribution of sperm and egg to this pattern. For instance, in tube worms (*Galeolaria caespitosa*), the thermal performance curve for fertilization rate shows maximum fertilization success at 21°C, which is the typical water temperature in their native habitat during summer (Kupriyanova and Havenhand, 2005). In a different study, fertilization rates of three-spined sticklebacks (*Gasterosteus aculeatus*) were lowest when gametes were exposed to the higher of two temperatures (Mehlis and Bakker, 2014).

Temperature also affects sperm performance in internal fertilizers, and high temperature in particular tends to result in negative effects (Monterroso et al., 1995; Chandolia et al., 1999). For example, the motility of boar (*S. scrofa domestica*) sperm decreased by 32% when incubated at 40 versus 38.5°C, the latter temperature closer to body temperature (Calle-Guisado et al., 2017; Gong et al., 2017). In New Zealand white rabbits (*Oryctolagus cuniculus*), sperm motility and metabolic activity decreased significantly after incubation above body temperature for 3 h (Sabés-Alsina et al., 2016). Available evidence is broadly consistent in ectotherms (Sales et al., 2018). For example, decreased motility and velocity of ejaculated sperm at high temperature has been observed in lizards (Rossi et al., 2021) and snakes (Tourmente et al., 2011).

An important consideration when analyzing the performance of ejaculated sperm in internal fertilizers is the influence of the female reproductive tract. The female reproductive system interacts with sperm and influences sperm performance and fertilization success by dictating the physical and chemical environment that sperm experience (Pitnick et al., 2009; Rosengrave et al., 2009; Sasanami et al., 2013; Lüpold and Pitnick, 2018; Rossi et al., 2021). Though rarely studied, available evidence suggests this interaction is important for sperm thermal performance. For example, oviductal fluid extracted from females reduced the negative effect of high incubation temperature on sperm in spiny lava lizards (*Tropidurus spinulosus*). Specifically, sperm incubated at high temperature with oviductal fluid had significantly higher motility and velocity compared to those without oviductal fluid (Rossi et al., 2021).

That said, protection provided by the female likely only goes so far. Ejaculated sperm stored within the female reproductive system can still be effected by temperature. In the spiny lizard example, the performance of sperm incubated with oviductal fluid still decreased at high temperature (Rossi et al., 2021). Indirect evidence for limits on female capacity to buffer sperm from high temperature damage is found in a study of the red flour beetle (*T. castaneum*). They found that heat exposure dramatically reduced female fertility; however, that was only true if females were mated beforehand. Females mated after heat exposure did not experience reduced fertility. Though not conclusive, this pattern is consistent with

high temperature negatively affecting sperm after insemination (Sales et al., 2018; Vasudeva et al., 2021). If sperm are influenced by female body temperature after insemination, female thermoregulation will also play a key role in the ecology and evolution of sperm performance, especially in species with long-term female storage.

IS PHENOTYPIC PLASTICITY IN SPERM TRAITS ADAPTIVE OR NOT?

Phenotypic plasticity refers to the ability of a genotype to express different phenotypes based on environmental conditions (Pigliucci, 2001). If plasticity occurs in a manner that improves performance, it is considered adaptive (Huey et al., 1999; Wilson and Franklin, 2002). Adaptive phenotypic plasticity is thought to be an important mechanism that can help organisms cope with temperature fluctuations (Somero, 2010; Seebacher et al., 2014; Gunderson and Stillman, 2015).

Explicit tests of adaptive phenotypic plasticity in sperm traits are rare, but there is compelling evidence that it can occur. Perhaps the best evidence comes from a study of red flour beetles (*T. castaneum*). Male beetles were acclimated to either standard or warm conditions, and were then mated to standard-reared females under the male acclimation temperature. After mating, females were move into either standard or warm conditions to reproduce. The results showed that females were able to produce significantly more offspring when their post-mating temperature matched male acclimation temperature. In other words, sperm performance was greatest under temperatures to which males were acclimated (Vasudeva et al., 2019). However, adaptive sperm thermal plasticity has not been supported in some other studies. In brown trout (*S. trutta*) and European whitefish (*C. lavaretus*), adult males acclimated to warm temperatures did not produce sperm with improved thermal tolerance or relative motility under warm temperatures (Fenkes et al., 2017; Kekäläinen et al., 2018). Similarly, no adaptive plasticity has been found in response to either rearing or adult acclimation temperature in mosquitofish (*G. holbrooki*; Adriaenssens et al., 2012; Iglesias-Carrasco et al., 2020).

Another approach to inferring adaptive plasticity is to compare the direction of plastic phenotypic change to the direction of evolutionary phenotypic change under the same conditions (Ghalambor et al., 2015). Interestingly, two studies that have compared evolutionary and plastic responses of sperm traits to temperature within the same taxa found that they occur in opposite directions: decreased sperm length was the plastic response to heat, while increased sperm length was the evolutionary response to heat (Breckels and Neff, 2013; Vasudeva et al., 2019). This suggests that some plasticity in temperature-dependent sperm traits could be maladaptive, and evolution acts to overcome that maladaptive response (Ghalambor et al., 2015).

Phenotypic plasticity is typically underlain by changes in gene regulation, and temperature-dependent transcript plasticity has been shown when individuals or their ejaculated sperm experience heat stress. For example, male domesticated bulls (*B. taurus*) produce sperm with an increased abundance of transcripts for heat shock protein genes *hsp60* and *hsp70* after exposure to heat (Cheng et al., 2016). In contrast, lower levels of *hsp90* but not *hsp70* mRNA were found in ejaculated sperm of mussels (*M. galloprovincialis*) after ejaculated sperm were exposed heat, though no phenotypic changes in sperm motility were observed (Lymbery et al., 2020). Whether differences in transcript abundance were due to gene regulation, degradation of specific RNAs, and/or changes in translation remain unclear (Lymbery et al., 2020). Heat-induced changes in transcript profiles, particularly for *hsp* genes, could imply an adaptive response since these genes code for molecular chaperones that maintain cellular function under thermal stress and are important in sperm development and function (Huang et al., 2000; Dun et al., 2012; Meccariello et al., 2014). However, further studies will need to be done to establish any adaptive significance.

EVIDENCE OF EVOLUTIONARY DIVERSIFICATION IN SPERM THERMAL PERFORMANCE

From an evolutionary perspective, sperm traits are expected to be selected to maximize fertilization success under prevailing environmental conditions (Pitnick et al., 2009; Reinhardt et al., 2015). This view has been supported by studies on sperm morphology. For example, in many species, sperm length co-evolves with different aspects of female reproductive tract (Miller and Pitnick, 2002; Pitnick et al., 2009; Breckels and Neff, 2013). However, we know relatively little about adaptive divergence in sperm physiological traits with respect to abiotic environmental factors such as temperature (Reinhardt et al., 2015). From our review of the literature, there seems to be a correlation between adult thermal tolerance and the heat tolerance of sperm production and/or post-ejaculate performance. For example, several studies have compared sperm thermal performance between two or three species with divergent thermal niches and have found that performance at high temperature is greater in the warmer niche species (Rahman et al., 2009; Tourmente et al., 2011; Lahnsteiner and Mansour, 2012; Poullet et al., 2015; Revathy and Benno Pereira, 2016).

Of course, evolutionary adaptation cannot be inferred from two-species studies (Garland and Adolph, 1994). More compelling evidence comes from studies of divergence in male thermal sterility thresholds. For example, male whole-organism heat tolerance, a proxy for thermal niche, highly corelates with male sterility temperature across many Drosophilinae fly species (**Figure 3**; David et al., 2005; Parratt et al., 2021; van Heerwaarden and Sgrò, 2021). In addition, male fertility limits were found to correlate strongly with geographic distribution (and thus thermal environment) across many *Drosophila* species, providing further evidence for adaptive divergence in temperature-dependent male reproductive traits (Parratt et al., 2021). Interestingly, in almost every species, male fertility thermal (heat) limits (FTL) are lower than the adult heat limits (**Figure 4**), indicating that male fertility is more sensitive to heat than the whole organism (David et al., 2005; Jørgensen et al., 2006; Parratt et al., 2021; van Heerwaarden and Sgrò, 2021).

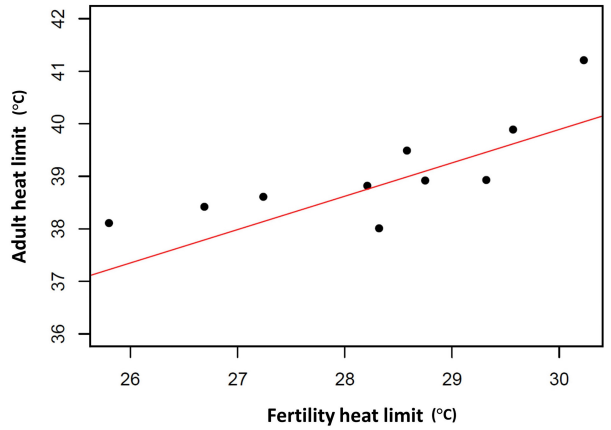

FIGURE 3 | Association between adult critical thermal maximum (CTmax) and male fertility thermal limits (FTL50) among 10 *Drosophila* species. Red line represents the fitted phylogenetic generalized least squares (PGLS) model fitted using the R package nlme (R^2 = 0.59, t = 5.59, $d.f.$ = 10, p < 0.001). CTmax and FTL50 data from van Heerwaarden and Sgrò (2021). Phylogenetic information from Finet et al. (2021).

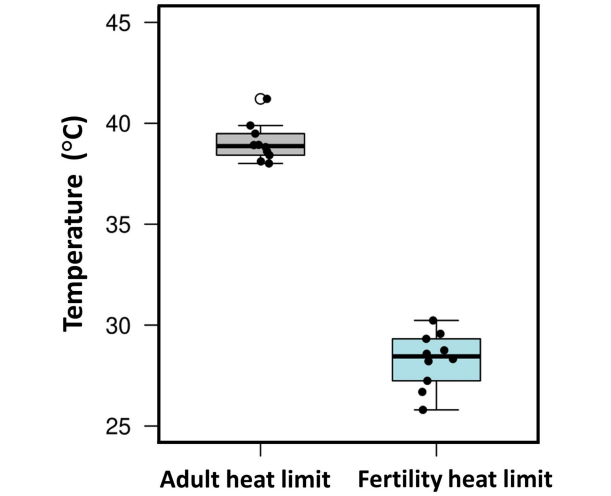

FIGURE 4 | Male fertility is more sensitive to heat than adult thermal tolerance. Box plot showing male fertility thermal limits (FTLs) and critical thermal maximum (CTmax) from 10 *Drosophila* species (data from van Heerwaarden and Sgrò, 2021). Boxes include the interquartile range, the whiskers span the range of the data, and the thick bars indicate the median values. Each point refers to data for a single species.

At the population level, divergence in sperm morphological traits is commonly reported (Kuramoto, 1996; Pitnick et al., 2003; Hettyey and Roberts, 2006; Minoretti and Baur, 2006; Kustra et al., 2019). Data on population-level divergence in environment-dependent performance is much more limited. For example, Green et al. (2019) found evidence of adaptive sperm evolution in response to local salinity conditions in two invasive populations of round goby (*Neogobius melanostomus*). Sperm from the high salinity population had greater sperm motility under high salinity conditions than those from the low salinity population (Green et al., 2019). Few studies have tested for intraspecies divergence in sperm thermal performance, but examples do exist. Sperm from a low latitude (i.e., warmer climate) population of *Drosophila subobscura* were less affected by heat than those from a high latitude population (Porcelli et al., 2017). Similarly, intraspecific variation in the heat sensitivity of fertility is largely consistent with habitat temperature in *D. melanogaster* (Rohmer et al., 2004) and the nematodes *C. briggsae* and *C. tropicalis* (Prasad et al., 2011; Poullet et al., 2015).

For sperm traits to evolve under different thermal regimes, there must be genetic variation in the traits among individuals. Individual-level variation in sperm thermal performance has been reported in Atlantic cod (*Gadus morhua*), where a genotype by temperature interaction was shown for sperm swimming speed (Purchase et al., 2010). Similarly, individual variation in sperm trait thermal plasticity has been reported in domesticated animals that experience scrotal insulation (Vogler et al., 1991; Barth and Bowman, 1994). Individual variation in sperm performance opens the door for temperature-dependent sperm competition. Furthermore, if adult thermal traits are genetically linked to sperm thermal traits, selection *via* sperm competition could increase rates of thermal adaptation when habitat temperatures change. Although rarely tested in animals, temperature selection on the gamete level has been applied in plants. For example, pollen selection has been shown to increase chilling tolerance in chickpea (*Cicer arietinum*; Clarke et al., 2004) and heat tolerance in maize (*Zea mays* L.; Mohapatra et al., 2020).

Laboratory experimental evolution experiments have shown that sperm traits can rapidly evolve under divergent thermal regimes, though these experiments are rare. In the two studies we are aware of, evolution under warm temperature led to a decrease in sperm length over time in guppies (8 generations, Breckels and Neff, 2013) and in red flour beetles (over 50 generations, Vasudeva et al., 2019). Whether the evolutionary response of sperm length is adaptive, or is simply linked with other adaptive traits, remains unclear. In contrast, laboratory selection experiments in *Drosophila* found no evidence for evolution in male fertility thermal limits, and heat-induced infertility often leads to population extinction (van Heerwaarden and Sgrò, 2021).

IMPLICATION UNDER GLOBAL CLIMATE CHANGE

The evidence reviewed above has obvious implications for the ability of species to tolerate anthropogenic global change. High temperatures can have strong negative effects on sperm performance. Importantly, these effects can develop at temperatures not far above normal body temperatures, and far below the lethal body temperatures of mature adults that are often used to model susceptibility to warming (**Figure 4**). The ability of sperm traits to rapidly evolve under high temperatures does provide some hope for adaptation to rapid climate change. So too does the potential for adaptive thermal plasticity in

sperm traits. However, the relative dearth of studies that have investigated questions of thermal evolvability and adaptive plasticity in sperm traits leaves us with much to learn before we can draw any solid conclusions.

One approach that could greatly increase our understanding of how climate change and sperm thermal performance interact is greater integration of ecologically meaningful temperature data into experimental design. The conditions that organisms and sperm are experimentally exposed to should reflect what they experience in the wild, as well as what they may experience in the future (Helmuth et al., 2010; Sunday et al., 2014; Buckley et al., 2018). In many of the papers described above, habitat-specific environmental or, more importantly, body temperature data were not presented nor discussed with respect to experimental treatments. As a result, it is difficult to interpret the results in an ecological context. Studies that use unrealistically high treatment temperatures could be biased toward finding a negative effect of warming, pushing organisms above the thermal optimum of their thermal performance curve. Similarly, studies that use unrealistically cool temperatures could incorrectly predict that warming will increase sperm performance if they span temperatures below the thermal optimum of the thermal performance curve. Ideally, experiments will occur across a wide range of temperature such that a thermal performance curves can be estimated and ecologically relevant temperatures can be mapped onto that curve. Then, one can predict the effects of warming within populations and estimate relative vulnerability to warming among taxa (Huey et al., 2009).

Furthermore, treatments should subject experimental units (individuals or sperm) to ecologically relevant thermal fluctuations, not just constant temperatures. Constant-temperature treatments are common in physiological experiments (Gunderson et al., 2016), but exposure to more ecologically relevant fluctuating temperatures often elicit different physiological responses (Colinet et al., 2015; Marshall and Sinclair, 2015; Marshall et al., 2021). Only when ecologically relevant thermal data are more consistently incorporated into studies of sperm thermal physiology will we be able to determine how close wild organisms are to experiencing sperm performance detriments, how great performance detriments may be when temperatures change, and how the effects of warming may differ geographically and taxonomically.

CONCLUSION

The weight of evidence makes it clear that temperature has a profound effect on sperm traits. These effects can manifest at many points in the life cycle of sperm and the tissues that produce and support them. Given the thermal sensitivity of sperm combined with the fact that they are so directly tied to reproductive fitness, we expect sperm traits to be under intense selection as species adapt to new thermal environments. Studies on the evolution of temperature-dependent sperm traits are relatively rare, but the data available suggest that divergence is common and may often be adaptive. Furthermore, experimental evolution experiments demonstrate that sperm traits can evolve rapidly when thermal conditions change.

Most studies of temperature-dependent sperm performance measured traits at only two or three temperatures, making inferences about thermal performance curve difficult or impossible. However, studies that applied a wide range of temperature treatments often found the classic thermal performance curve response for traits related to both morphology and performance (**Figure 1B**). Moving forward, we recommend that the thermal performance curve framework be used as a guide to design studies of thermal effects on sperm traits. Doing so will allow us to better understand the full spectrum of thermal effects on sperm and elucidate the ecological and evolutionary consequences of sperm phenotypes, as is the case for whole-organism thermal performance (Sinclair et al., 2016). In addition, application of the thermal performance curve perspective may help resolve inconsistencies observed among studies. For example, whether a study finds that warming increases, decreases, or has no effect on a trait of interest will depend on whether the treatment temperatures chosen fall on the rising (left), falling (right), or plateau section of the traits thermal performance curve. Ultimately, greater integration of sperm thermal responses to a wide range of ecologically relevant temperatures will be crucial to predict the effects of global warming on sperm and the subsequent consequences for reproduction and population processes.

AUTHOR CONTRIBUTIONS

WW and AG conceived the project and wrote the manuscript. Both authors contributed to the article and approved the submitted version.

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REFERENCES

Abdelhamid, M., Esquerre-Lamare, C., Walschaerts, M., Ahmad, G., Mieusset, R., Hamdi, S., et al. (2019). Experimental mild increase in testicular temperature has drastic, but reversible, effect on sperm aneuploidy in men: a pilot study. *Reprod. Biol.* 19, 189–194. doi: 10.1016/j.repbio.2019.06.001

Adriaenssens, B., van Damme, R., Seebacher, F., and Wilson, R. S. (2012). Sex cells in changing environments: can organisms adjust the physiological function of gametes to different temperatures? *Glob. Chang. Biol.* 18, 1797–1803. doi: 10.1111/j.1365-2486.2012.02672.x

Alavi, S. M., and Cosson, J. (2005). Sperm motility in fishes. I. Effects of temperature and pH: a review. *Cell Biol. Internat.* 29, 101–110. doi: 10.1016/j.cellbi.2004.11.021

Angilletta, M. J. Jr., Bennett, A. F., Guderley, H., Navas, C. A., Seebacher, F., and Wilson, R. S. (2006). Coadaptation: a unifying principle in evolutionary thermal biology. *Physiolog. Biochem. Zool.* 79, 282–294. doi: 10.1086/499990

Angilletta, M. J. (2009). *Thermal Adaptation: A Theoretical and Empirical Synthesis*. Oxford: University Press.

Aprison, E. Z., and Ruvinsky, I. (2014). Balanced trade-offs between alternative strategies shape the response of *C. elegans* reproduction to chronic heat stress. *PLoS One* 9:e105513–e105513. doi: 10.1371/journal.pone.0105513

Barth, A. D., and Bowman, P. A. (1994). The sequential appearance of sperm abnormalities after scrotal insulation or dexamethasone treatment in bulls. *Can. Vet. J.* 35, 93–102.

Blanckenhorn, W. U., and Hellriegel, B. (2002). Against Bergmann's rule: fly sperm size increases with temperature. *Ecol. Lett.* 5, 7–10. doi: 10.1046/j.1461-0248.2002.00298.x

Blanckenhorn, W. U., and Henseler, C. (2005). Temperature-dependent ovariole and testis maturation in the yellow dung fly. *Entomol. Exp. Appl.* 116, 159–165. doi: 10.1111/j.1570-7458.2005.00316.x

Bombardelli, R. A., Sanches, E. A., Baggio, D. M., Sykora, R. M., Souza, B. E., de Tessaro, L., et al. (2013). Effects of the spermatozoa: oocyte ratio, water volume and water temperature on artificial fertilization and sperm activation of cascudo-preto. *Revista Brasileira de Zootecnia* 42, 1–6. doi: 10.1590/S1516-35982013000100001

Breckels, R. D., and Neff, B. D. (2013). The effects of elevated temperature on the sexual traits, immunology and survivorship of a tropical ectotherm. *J. Exp. Biol.* 216(Pt 14), 2658–2664. doi: 10.1242/jeb.084962

Breckels, R. D., and Neff, B. D. (2014). Rapid evolution of sperm length in response to increased temperature in an ectothermic fish. *Evol. Ecol.* 28, 521–533. doi: 10.1007/s10682-014-9692-0

Buckley, L. B., Cannistra, A. F., and John, A. (2018). Leveraging organismal biology to forecast the effects of climate change. *Integr. Comp. Biol.* 58, 38–51. doi: 10.1093/icb/icy018

Buckley, L. B., and Kingsolver, J. G. (2021). Evolution of thermal sensitivity in changing and variable climates. *Annu. Rev. Ecol. Evol. Syst.* 52, 563–586. doi: 10.1146/annurev-ecolsys-011521-102856

Calle-Guisado, V., Bragado, M. J., García-Marín, L. J., and González-Fernández, L. (2017). HSP90 maintains boar spermatozoa motility and mitochondrial membrane potential during heat stress. *Anim. Reprod. Sci.* 187, 13–19. doi: 10.1016/j.anireprosci.2017.09.009

Cameron, R. D. A., and Blackshaw, A. W. (1980). The effect of elevated ambient temperature on spermatogenesis in the boar. *J. Reprod. Fert.* 59, 173–179. doi: 10.1530/jrf.0.0590173

Chandolia, R. K., Reinertsen, E. M., and Hansen, P. J. (1999). Lack of breed differences in responses of bovine spermatozoa to heat shock. *J. Dairy Sci.* 82, 2617–2619. doi: 10.3168/jds.S0022-0302(99)75517-7

Cheng, Y., Liu, S., Zhang, Y., Su, D., Wang, G., Lv, C., et al. (2016). The effect of heat stress on bull sperm quality and related HSPs expression. *Anim. Biol.* 66, 321–333. doi: 10.1163/15707563-00002507

Chirgwin, E., Marshall, D. J., and Monro, K. (2019). Physical and physiological impacts of ocean warming alter phenotypic selection on sperm morphology. *Funct. Ecol.* 34, 646–657. doi: 10.1111/1365-2435.13483

Clarke, H. J., Khan, T. N., and Siddique, K. H. M. (2004). Pollen selection for chilling tolerance at hybridisation leads to improved chickpea cultivars. *Euphytica* 139, 65–74. doi: 10.1007/s10681-004-2466-y

Colinet, H., Sinclair, B. J., Vernon, P., and Renault, D. (2015). Insects in fluctuating thermal environments. *Annu. Rev. Entomol.* 60, 123–140. doi: 10.1146/annurev-ento-010814-021017

Cowles, R. B. (1958). The evolutionary significance of the scrotum. *Evolution* 12, 417–418. doi: 10.1111/j.1558-5646.1958.tb02970.x

David, J. R., Araripe, L. O., Chakir, M., Legout, H., Lemos, B., Pétavy, G., et al. (2005). Male sterility at extreme temperatures: a significant but neglected phenomenon for understanding *Drosophila* climatic adaptations. *J. Evol. Biol.* 18, 838–846. doi: 10.1111/j.1420-9101.2005.00914.x

Dawson, W. R. (1975). On the Physiological significance of the preferred body temperatures of reptiles. *Perspect. Biophys. Ecol.* 12, 443–473. doi: 10.1007/978-3-642-87810-7_25

Deutsch, C. A., Tewksbury, J. J., Huey, R. B., Sheldon, K. S., Ghalambor, C. K., Haak, D. C., et al. (2008). Impacts of climate warming on terrestrial ectotherms across latitude. *Proc. Natl. Acad. Sci.* 105, 6668–6672. doi: 10.1073/pnas.0709472105

Dun, M. D., Aitken, R. J., and Nixon, B. (2012). The role of molecular chaperones in spermatogenesis and the post-testicular maturation of mammalian spermatozoa. *Hum. Reprod. Update* 18, 420–435. doi: 10.1093/humupd/dms009

Effer, B., Figueroa, E., Augsburger, A., and Valdebenito, I. (2013). Sperm biology of *Merluccius australis*: Sperm structure, semen characteristics and effects of pH, temperature and osmolality on sperm motility. *Aquaculture* 40, 147–151. doi: 10.1016/j.aquaculture.2013.05.040

Fenkes, M., Fitzpatrick, J. L., Ozolina, K., Shiels, H. A., and Nudds, R. L. (2017). Sperm in hot water: direct and indirect thermal challenges interact to impact on brown trout sperm quality. *J. Exp. Biol.* 220, 2513–2520. doi: 10.1242/jeb.156018

Finet, C., Kassner, V. A., Carvalho, A. B., Chung, H., Day, J. P., Day, S., et al. (2021). DrosoPhyla: resources for Drosophilid Phylogeny and Systematics. *Genome Biol. Evol.* 13:8. doi: 10.1093/gbe/evab179

Flowers, W. L. (1997). Management of boars for efficient semen production. *J. Reprod. Fert.* 52, 67–78.

Flowers, W. L. (2015). Factors affecting the efficient production of boar sperm. *Reprod. Dom. Anim.* 50, 25–30. doi: 10.1111/rda.12529

Foldesy, R. G., and Bedford, J. M. (1982). Temperature and androgen as determinants of the sperm storage capacity of the rat cauda epididymidis. *Biol. Reprod.* 26, 673–682. doi: 10.1095/biolreprod26.4.673

Garcia-Oliveros, L. N., de Arruda, R. P., Batissaco, L., Gonzaga, V. H. G., Nogueira, V. J. M., Florez-Rodriguez, S. A., et al. (2020). Heat stress effects on bovine sperm cells: a chronological approach to early findings. *Internat. J. Biometeorol.* 64, 1367–1378. doi: 10.1007/s00484-020-01917-w

Garland, T. Jr., and Adolph, S. C. (1994). Why not to do two-species comparative studies: limitations on inferring adaptation. *Physiol. Zool.* 67, 797–828. doi: 10.1086/physzool.67.4.30163866

Gasparini, C., Lu, C., Dingemanse, N. J., Tuni, C., and Sinclair, B. (2018). Paternal-effects in a terrestrial ectotherm are temperature dependent but no evidence for adaptive effects. *Funct. Ecol.* 32, 1011–1021. doi: 10.1111/1365-2435.13022

Ghalambor, C. K., Hoke, K. L., Ruell, E. W., Fischer, E. K., Reznick, D. N., and Hughes, K. A. (2015). Non-adaptive plasticity potentiates rapid adaptive evolution of gene expression in nature. *Nature* 525, 372–375. doi: 10.1038/nature15256

Gong, Y., Guo, H., Zhang, Z., Zhou, H., Zhao, R., and He, B. (2017). Heat stress reduces sperm motility via activation of glycogen synthase kinase-3α and inhibition of mitochondrial protein import. *Front. Phys.* 8, 718–718. doi: 10.3389/fphys.2017.00718

Green, L., Havenhand, J. N., and Kvarnemo, C. (2019). Evidence of rapid adaptive trait change to local salinity in the sperm of an invasive fish. *Evol. Appl.* 13, 533–544. doi: 10.1111/eva.12859

Gunderson, A. R., Armstrong, E. J., and Stillman, J. H. (2016). Multiple stressors in a changing world: the need for an improved perspective on physiological responses to the dynamic marine environment. *Annu. Rev. Mar. Sci.* 8, 357–378. doi: 10.1146/annurev-marine-122414-033953

Gunderson, A. R., and Stillman, J. H. (2015). Plasticity in thermal tolerance has limited potential to buffer ectotherms from global warming. *Proc. R. Soc. B* 282, 20150401–20150401. doi: 10.1098/rspb.2015.0401

Helmuth, B., Broitman, B. R., Yamane, L., Gilman, S. E., Mach, K., Mislan, K. A. S., et al. (2010). Organismal climatology: analyzing environmental variability at scales relevant to physiological stress. *J. Exp. Biol.* 213, 995–1003. doi: 10.1242/jeb.038463

Hettyey, A., and Roberts, J. D. (2006). Sperm traits of the quacking frog, *Crinia georgiana*: intra- and interpopulation variation in a species with a high risk of sperm competition. *Behav. Ecol. Sociobiol.* 59, 389–396. doi: 10.1007/s00265-005-0062-3

Huang, S. Y., Kuo, Y. H., Lee, Y. P., Tsou, H. L., Lin, E. C., Ju, C. C., et al. (2000). Association of heat shock protein 70 with semen quality in boars. *Anim. Reproduct. Sci.* 63, 231–240. doi: 10.1016/S0378-4320(00)00175-5

Huey, R. B., Berrigan, D., Gilchrist, G. W., and Herron, J. C. (1999). Testing the adaptive significance of acclimation: a strong inference approach. *Integr. Compar. Biol.* 39, 323–336. doi: 10.1093/icb/39.2.323

Huey, R. B., Deutsch, C., Tewksbury, J., Vitt, L., Hertz, P., Pérez, H., et al. (2009). Why tropical forest lizards are vulnerable to climate warming. *Proc. R. Soc. B Biol. Sci.* 276, 1939–1948. doi: 10.1098/rspb.2008.1957

Huey, R. B., and Kingsolver, J. G. (1989). Evolution of thermal sensitivity of ectotherm performance. *Trends Ecol. Evol.* 4, 131–135. doi: 10.1016/0169-5347(89)90211-5

Hurley, L. L., McDiarmid, C. S., Friesen, C. R., Griffith, S. C., and Rowe, M. (2018). Experimental heatwaves negatively impact sperm quality in the zebra finch. *Proc. R. Soc. B Biol. Sci.* 285:20172547. doi: 10.1098/rspb.2017.2547

Iglesias-Carrasco, M., Harrison, L., Jennions, M. D., and Head, M. L. (2020). Combined effects of rearing and testing temperatures on sperm traits. *J. Evol. Biol.* 33, 13710. doi: 10.1111/jeb.13710

IPCC (2014). "Climate Change 2014: Synthesis report," in *contribution of working groups I, II and III to the fifth assessment report of the intergovernmental panel on climate change*, eds R. K. Pachauri and L. A. Meyer (Geneva: IPCC), 151.

Janowitz, S. A., and Fischer, K. (2011). Opposing effects of heat stress on male versus female reproductive success in *Bicyclus anynana* butterflies. *J. Therm. Biol.* 36, 283–287. doi: 10.1016/j.jtherbio.2011.04.001

Jørgensen, K. T., Sørensen, J. G., and Bundgaard, J. (2006). Heat tolerance and the effect of mild heat stress on reproductive characters in *Drosophila buzzatii* males. *J. Ther. Biol.* 31, 280–286. doi: 10.1016/j.jtherbio.2005.11.026

Karaca, A. G., Parker, H. M., Yeatman, J. B., and McDaniel, C. D. (2002). The effects of heat stress and sperm quality classification on broiler breeder male fertility and semen ion concentrations. *Br. Poult. Sci.* 43, 621–628. doi: 10.1080/0007166022000004552

Kekäläinen, J., Oskoei, P., Janhunen, M., Koskinen, H., Kortet, R., and Huuskonen, H. (2018). Sperm pre-fertilization thermal environment shapes offspring phenotype and performance. *J. Exp. Biol.* 221:181412. doi: 10.1242/jeb.181412

Küçük, N., and Aksoy, M. (2020). Effect of environmental heat stress on Kıvırcık ram sperm parameters. *J. Hell. Vet. Med. Soc.* 71, 2073–2080. doi: 10.12681/jhvms.22968

Kupriyanova, E. K., and Havenhand, J. N. (2005). Effects of temperature on sperm swimming behaviour, respiration and fertilization success in the serpulid polychaete, *Galeolaria caespitosa* (Annelida: Serpulidae). *Inverteb. Reprod. Dev.* 48, 7–17. doi: 10.1080/07924259.2005.9652166

Kuramoto, M. (1996). Generic differentiation of sperm morphology in treefrogs from Japan and Taiwan. *J. Herpetol.* 30, 437–443. doi: 10.2307/1565190

Kustra, M. C., Kahrl, A. F., Reedy, A. M., Warner, D. A., and Cox, R. M. (2019). Sperm morphology and count vary with fine-scale changes in local density in a wild lizard population. *Oecologia* 191, 555–564. doi: 10.1007/s00442-019-04511-z

Lahnsteiner, F., and Mansour, N. (2012). The effect of temperature on sperm motility and enzymatic activity in brown trout Salmo trutta, burbot *Lota lota* and grayling *Thymallus thymallus*. *J. Fish Biol.* 81, 197–209. doi: 10.1111/j.1095-8649.2012.03323.x

Licht, P. (1965). The relation between preferred body temperatures and testicular heat sensitivity in lizards. *Copeia* 1965, 428–436. doi: 10.2307/1440991

Licht, P., and Basu, S. L. (1967). Influence of temperature on lizard testes. *Nature* 213, 672–674. doi: 10.1038/213672a0

Lüpold, S., and Pitnick, S. (2018). Sperm form and function: what do we know about the role of sexual selection? *Reproduction* 155, R229–R243. doi: 10.1530/REP-17-0536

Lymbery, R. A., Evans, J. P., and Kennington, W. J. (2020). Post-ejaculation thermal stress causes changes to the RNA profile of sperm in an external fertilizer. *Proc. R. Soc. B* 287:20202147. doi: 10.1098/rspb.2020.2147

Lymbery, R. A., Kennington, W. J., and Evans, J. P. (2021). The thermal environment of sperm affects offspring success: a test of the anticipatory paternal effects hypothesis in the blue mussel. *Biol. Lett.* 17:20210213. doi: 10.1098/rsbl.2021.0213

Marshall, K. E., Anderson, K. M., Brown, N. E. M., Dytnerski, J. K., Flynn, K. L., Bernhardt, J. R., et al. (2021). Whole-organism responses to constant temperatures do not predict responses to variable temperatures in the ecosystem engineer *Mytilus trossulus*. *Proc. R. Soc. . B* 288, 20202968–20202968. doi: 10.1098/rspb.2020.2968

Marshall, K. E., and Sinclair, B. J., (2015). The relative importance of number, duration and intensity of cold stress events in determining survival and energetics of an overwintering insect. *Funct. Ecol.* 29, 357–366. doi: 10.1111/1365-2435.12328

Meccariello, R., Chianese, R., Ciaramella, V., Fasano, S., and Pierantoni, R. (2014). Molecular chaperones, cochaperones, and ubiquitination/deubiquitination system: Involvement in the production of high quality spermatozoa. *BioMed Res. Internat.* 2014, 561426–561410. doi: 10.1155/2014/561426

Mehlis, M., and Bakker, T. C. M. (2014). The influence of ambient water temperature on sperm performance and fertilization success in three-spined sticklebacks (*Gasterosteus aculeatus*). *Evol. Ecol.* 28, 655–667. doi: 10.1007/s10682-014-9707-x

Miller, G. T., and Pitnick, S. (2002). Sperm-female coevolution in *Drosophila*. *Science* 298, 1230–1233. doi: 10.1126/science.1076968

Minoretti, N., and Baur, B. (2006). Among- and within-population variation in sperm quality in the simultaneously hermaphroditic land snail *Arianta arbustorum*. *Behav. Ecol. Sociob.* 60, 270–280. doi: 10.1007/s00265-006-0165-5

Mohapatra, U., Singh, A., and Ravikumar, R. L. (2020). Effect of gamete selection in improving of heat tolerance as demonstrated by shift in allele frequency in maize (*Zea mays* L.). *Euphytica* 216:5. doi: 10.1007/s10681-020-02603-z

Monterroso, V. H., Drury, K. C., Ealy, A. D., Edwards, J. L., and Hansen, P. J. (1995). Effect of heat shock on function of frozen/thawed bull spermatozoa. *Theriogenology* 44, 947–961. doi: 10.1016/0093-691X(95)00282-D

Morrell, J. M. (2020). Heat stress and bull fertility. *Theriogenology* 153, 62–67. doi: 10.1016/j.theriogenology.2020.05.014

Pabst, D. A., Rommel, S. A., McLellan, W. A., Williams, T. M., and Rowles, T. K. (1995). Thermoregulation of the intra-abdominal testes of the bottlenose dolphin (*Tursiops truncatus*) during exercise. *J. Exp. Biol.* 198(Pt 1), 221–226. doi: 10.1242/jeb.198.1.221

Parratt, S. R., Walsh, B. S., Metelmann, S., White, N., Manser, A., Bretman, A. J., et al. (2021). Temperatures that sterilize males better match global species distributions than lethal temperatures. *Nat. Clim. Chang.* 11, 481–484. doi: 10.1038/s41558-021-01047-0

Parrish, J. J., Willenburg, K. L., Gibbs, K. M., Yagoda, K. B., Krautkramer, M. M., Loether, T. M., et al. (2017). Scrotal insulation and sperm production in the boar. *Mole. Reprod. Dev.* 84, 969–978. doi: 10.1002/mrd.22841

Paxton, C. W., Baria, M. V. B., Weis, V. M., and Harii, S. (2015). Effect of elevated temperature on fecundity and reproductive timing in the coral *Acropora digitifera*. *Zygote* 24, 511–516. doi: 10.1017/S0967199415000477

Peña, F. J., Ortiz-Rodríguez, J. M., Gaitskell-Phillips, G. L., Gil, M. C., Ortega-Ferrusola, C., and Martín-Cano, F. E. (2021). An integrated overview on the regulation of sperm metabolism (glycolysis-Krebs cycle-oxidative phosphorylation). *Anim. Reprod. Sci.* 2021, 106805. doi: 10.1016/j.anireprosci.2021.106805

Pigliucci, M. (2001). *Phenotypic Plasticity: Beyond Nature and Nurture*. London: The Johns Hopkins University Press.

Pinsky, M. L., Eikeset, A. M., McCauley, D. J., Payne, J. L., and Sunday, J. M. (2019). Greater vulnerability to warming of marine versus terrestrial ectotherms. *Nature* 569, 108–111. doi: 10.1038/s41586-019-1132-4

Pitnick, S., Hosken, D. J., and Birkhead, T. R. (2009). *Sperm Biology: An Evolutionary Perspective*. San Diego: Academic Press.

Pitnick, S., Miller, G. T., Schneider, K., and Markow, T. A. (2003). Ejaculate-female coevolution in *Drosophila mojavensis*. *Proc. R. Soc. B* 270, 1507–1512. doi: 10.1098/rspb.2003.2382

Pitnick, S., Wolfner, M. F., and Dorus, S. (2020). Post-ejaculatory modifications to sperm (PEMS). *Biolog. Rev. Camb. Philosoph. Soc.* 95, 365–392. doi: 10.1111/brv.12569

Porcelli, D., Gaston, K. J., Butlin, R. K., and Snook, R. R. (2017). Local adaptation of reproductive performance during thermal stress. *J. Evol. Biol.* 30, 422–429. doi: 10.1111/jeb.13018

Poullet, N., Vielle, A., Gimond, C., Ferrari, C., and Braendle, C. (2015). Evolutionarily divergent thermal sensitivity of germline development and fertility in hermaphroditic *Caenorhabditis nematodes*. *Evol. Dev.* 17, 380–397. doi: 10.1111/ede.12170

Prasad, A., Croydon-Sugarman, M. J. F., Murray, R. L., and Cutter, A. D. (2011). Temperature-dependent fecundity associate with latitude in *Caenorhabditis briggsae*. *Evolution* 65, 52–63. doi: 10.1111/j.1558-5646.2010.01110.x

Purchase, C. F., Butts, I. A. E., Alonso-Fernandez, A., and Trippel, E. A. (2010). Thermal reaction norms in sperm performance of Atlantic cod (*Gadus morhua*). *Can. J. Fish. Aquat. Sci.* 67, 498–510. doi: 10.1139/F10-001

Rahman, M. S., Tsuchiya, M., and Uehara, T. (2009). Effects of temperature on gamete longevity and fertilization success in two sea urchin species, *Echinometra mathaei* and *Tripneustes gratilla*. *Zoolog. Sci.* 26, 1–8. doi: 10.2108/zsj.26.1

Rathore, A. K. (1970). Aosomal abnormality in ram spermatozoa due to heat stress. *Br. Vet. J.* 126, 440–443. doi: 10.1016/S0007-1935(17)48252-2

Reinhardt, K., Dobler, R., and Abbott, J. (2015). An ecology of sperm: sperm diversification by natural selection. *Annu. Rev. Ecol. Evol. Syst.* 46, 435–459. doi: 10.1146/annurev-ecolsys-120213-091611

Revathy, S., and Benno Pereira, F. G. (2016). effect of temperature on sperm motility in fishes. *Internat. J. Sci. Res. Methodol.* 5, 81–89.

Rinehart, J. P., Yocum, G. D., and Denlinger, D. L. (2000). Thermotolerance and rapid cold hardening ameliorate the negative effects of brief exposures to high or low temperatures on fecundity in the flesh fly, *Sarcophaga crassipalpis*: Thermotolerance in *Sarcophaga crassipalpis*. *Physiol. Entomol.* 25, 330–336. doi: 10.1111/j.1365-3032.2000.00201.x

Rohmer, C., David, J. R., Moreteau, B., and Joly, D. (2004). Heat induced male sterility in Drosophila melanogaster: adaptive genetic variations among geographic populations and role of the Y chromosome. *J. Exp. Biol.* 207, 2735–2743. doi: 10.1242/jeb.01087

Rommel, S. A., Pabst, D. A., McLellan, W. A., Mead, J. G., and Potter, C. W. (1992). Anatomical evidence for a countercurrent heat exchanger associated with dolphin testes. *Anatom. Rec.* 232, 150–156. doi: 10.1002/ar.1092320117

Rosengrave, P., Taylor, H., Montgomerie, R., Metcalf, V., McBride, K., and Gemmell, N. J. (2009). Chemical composition of seminal and ovarian fluids of chinook salmon (*Oncorhynchus tshawytscha*) and their effects on sperm motility traits. *Compar. Biochem. Physiol.* 152, 123–129. doi: 10.1016/j.cbpa.2008.09.009

Rossi, N., Lopez Juri, G., Chiaraviglio, M., and Cardozo, G. (2021). Oviductal fluid counterbalances the negative effect of high temperature on sperm in an ectotherm model. *Biol. Open* 10:058593. doi: 10.1242/bio.058593

Sabés-Alsina, M., Tallo-Parra, O., Mogas, M. T., Morrell, J. M., and Lopez-Bejar, M. (2016). Heat stress has an effect on motility and metabolic activity of rabbit spermatozoa. *Anim. Reprod. Sci.* 173, 18–23. doi: 10.1016/j.anireprosci.2016.08.004

Sales, K., Vasudeva, R., Dickinson, M. E., Godwin, J. L., Lumley, A. J., Michalczyk, L., et al. (2018). Experimental heatwaves compromise sperm function and cause transgenerational damage in a model insect. *Nat. Comm.* 9, 4771–4771. doi: 10.1038/s41467-018-07273-z

Sales, K., Vasudeva, R., and Gage, M. J. G. (2021). Fertility and mortality impacts of thermal stress from experimental heatwaves on different life stages and their recovery in a model insect. *R. Soc. Open Sci.* 8, 201717–201717. doi: 10.1098/rsos.201717

Sasanami, T., Matsuzaki, M., Mizushima, S., and Hiyama, G. (2013). Sperm storage in the female reproductive tract in birds. *J. Reprod. Dev.* 59, 334–338. doi: 10.1262/jrd.2013-038

Saxena, B. P., Sharma, P. R., Thappa, R., and Tikku, K. (1992). Temperature induced sterilization for control of three stored grain beetles. *J. Stored Prod. Res.* 28, 67–70. doi: 10.1016/0022-474x(92)90031-k

Schlenk, W., and Kahmann, H. (1938). The chemical composition of seminal fluids and their physiological importance study with trout sperm. *Biochem. Zool.* 295, 283–301.

Seebacher, F., White, C. R., and Franklin, C. E. (2014). Physiological plasticity increases resilience of ectothermic animals to climate change. *Nat. Clim. Chang.* 5, 61–66. doi: 10.1038/nclimate2457

Shahat, A. M., Rizzoto, G., and Kastelic, J. P. (2020). Amelioration of heat stress-induced damage to testes and sperm quality. *Theriogenology* 158, 84–96. doi: 10.1016/j.theriogenology.2020.08.034

Sinclair, B. J., Marshall, K. E., Sewell, M. A., Levesque, D. L., Willett, C. S., Slotsbo, S., et al. (2016). Can we predict ectotherm responses to climate change using thermal performance curves and body temperatures? *Ecol. Lett.* 19, 1372–1385. doi: 10.1111/ele.12686

Somero, G. N. (2010). The physiology of climate change: how potentials for acclimatization and genetic adaptation will determine "winners" and "losers.". *J. Exp. Biol.* 213, 912–920. doi: 10.1242/jeb.037473

Somero, G. N., Lockwood, B. L., and Tomanek, L. (2017). *Biochemical Adaptation: Response to Environmental Challenges from Life's Origins to the Anthropocene*. Sunderland, MA: Sinauer Associates, Inc.

Sunday, J. M., Bates, A. E., Kearney, M. R., Colwell, R. K., Dulvy, N. K., Longino, J. T., et al. (2014). Thermal-safety margins and the necessity of thermoregulatory behavior across latitude and elevation. *Proc. Natl. Acad. Sci. PNAS* 111, 5610–5615. doi: 10.1073/pnas.1316145111

Sutter, A., Travers, L. M., Oku, K., Delaney, L., Store, S., Price, T. A. R., et al. (2019). Flexible polyandry in female flies is an adaptive response to infertile males. *Behav. Ecol.* 30, 1715–1724. doi: 10.1093/beheco/arz140

Thundathil, J. C., Rajamanickam, G. D., Kastelic, J. P., and Newton, L. D. (2012). The effects of increased testicular temperature on testis-specific isoform of Na+/K+-atpase in sperm and its role in spermatogenesis and sperm function. *Reproduct. Domest. Anim.* 47, 170–177. doi: 10.1111/j.1439-0531.2012.02072.x

Tourmente, M., Giojalas, L. C., and Chiaraviglio, M. (2011). Sperm parameters associated with reproductive ecology in two snake species. *Herpetologica* 67, 58–70. doi: 10.1655/HERPETOLOGICA-D-10-00052.1

van Heerwaarden, B., and Sgrò, C. M. (2021). Male fertility thermal limits predict vulnerability to climate warming. *Nat. Comm.* 12, 2214–2211. doi: 10.1038/s41467-021-22546-w

Vasudeva, R., Deeming, D. C., and Eady, P. E. (2014). Developmental temperature affects the expression of ejaculatory traits and the outcome of sperm competition in *Callosobruchus maculatus*. *J. Evol. Biol.* 27, 1811–1818. doi: 10.1111/jeb.12431

Vasudeva, R., Dickinson, M., Sutter, A., Powell, S., Sales, K., and Gage, M. J. G. (2021). Facultative polyandry protects females from compromised male fertility caused by heatwave conditions. *Anim. Behav.* 178, 37–48. doi: 10.1016/j.anbehav.2021.05.016

Vasudeva, R., Sutter, A., Sales, K., Dickinson, M. E., Lumley, A. J., and Gage, M. J. (2019). Adaptive thermal plasticity enhances sperm and egg performance in a model insect. *eLife* 8:49452. doi: 10.7554/eLife.49452

Vogler, C. J., Bame, J. H., DeJarnette, J. M., McGilliard, M. L., and Saacke, R. G. (1993). Effects of elevated testicular temperature on morphology characteristics of ejaculated spermatozoa in the bovine. *Theriogenology* 40, 1207–1219. doi: 10.1016/0093-691X(93)90291-C

Vogler, C. J., Saacke, R. G., Bame, J. H., Dejarnette, J. M., and McGilliard, M. L. (1991). Effects of scrotal insulation on viability characteristics of cryopreserved bovine semen. *J. Dairy Sci.* 74, 3827–3835. doi: 10.3168/jds.S0022-0302(91)78575-5

Walsh, B. S., Parratt, S. R., Hoffmann, A. A., Atkinson, D., Snook, R. R., Bretman, A., et al. (2019). The impact of climate change on fertility. *Trends Ecol. Evol.* 34, 249–259. doi: 10.1016/j.tree.2018.12.002

West, P. M., and Packer, C. (2002). Sexual selection, temperature, and the lion's mane. *Science* 297, 1339–1343. doi: 10.1126/science.1073257

Wilson, R. S., and Franklin, C. E. (2002). Testing the beneficial acclimation hypothesis. *Trends Ecol. Evol.* 17, 66–70. doi: 10.1016/S0169-5347(01)02384-9

Zeh, J. A., Bonilla, M. M., Su, E. J., Padua, M. V., Anderson, R. V., Kaur, D., et al. (2012). Degrees of disruption: projected temperature increase has catastrophic consequences for reproduction in a tropical ectotherm. *Glob. Chang. Biol.* 18, 1833–1842. doi: 10.1111/j.1365-2486.2012.02640.x

Zhang, W., Zhao, F., Hoffmann, A. A., and Ma, C.-S. (2013). A single hot event that does not affect survival but decreases reproduction in the diamondback moth, *Plutella xylostella*. *PLoS One* 8:e75923–e75923. doi: 10.1371/journal.pone.0075923

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