Within-Generation and Transgenerational Plasticity of a Temperate Salmonid in Response to Thermal Acclimation and Acute Temperature Stress

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ABSTRACT

The rise in temperature associated with climate change may threaten the persistence of stenothermal organisms with limited capacities for beneficial thermal acclimation. We investigated the capacity for within-generation and transgenerational thermal responses in brook trout (Salvelinus fontinalis), a cold-adapted salmonid. Adult fish were acclimated to temperatures within (10°C) and above (21°C) their thermal optimum for 6 mo before spawning, then mated in a full factorial breeding design to produce offspring from cold- and warm-acclimated parents and bi-directional crosses between parents from both temperature treatments. Offspring from families were subdivided and reared at two acclimation temperatures representing their current (15°C) and anticipated future (19°C) habitat temperatures. Offspring thermal physiology was measured as the rate of oxygen consumption (MO2) during an acute change in temperature (increase of 2°C h–1) to observe their MO2-temperature relationship. We recorded resting MO2, peak (highest achieved, thermally induced) MO2, and critical thermal maximum (CTM) as performance metrics. Although limited, within-generation plasticity was greater than transgenerational plasticity, with offspring warm acclimation elevating CTM by 0.5°C but slightly lowering peak thermally induced MO2. Transgenerational plasticity was evident as a slightly elevated resting MO2 and a shift of the MO2-temperature relationship to higher rates overall in offspring from warm-acclimated parents. Furthermore, offspring whose parents were warm acclimated were in worse condition than those whose parents were cold acclimated. Both parents contributed to offspring thermal responses; however, the paternal effect was stronger. Despite the existence of within-generation and transgenerational plasticity in brook trout, it is unlikely that these will be sufficient for coping with long-term changes to environmental temperatures.

Keywords: climate change, brook trout, metabolic rate, thermal tolerance, transgenerational acclimation, plasticity.

Introduction

Environmental warming as a result of climate change is adversely affecting the physiology and persistence of many species and populations globally (Moritz and Agudo 2013; Whitney et al. 2016). Species or populations that cannot migrate or are restricted to localized habitats are particularly vulnerable because they would be left to face temperatures higher than those they are physiologically capable of withstanding long-term (Somero 2010). Evolutionary change may provide the best option for long-term persistence for organisms; however, the accelerated rate of climate change is likely too rapid for most organisms to respond (Comte and Olden 2017). This is especially true for those with long generation times or limited standing genetic variation (Willi et al. 2006; Munday et al. 2013; Meier et al. 2014).

Thermal acclimation (phenotypic plasticity) may help to buffer temperature effects through physiological adjustments that can occur within a single generation or over multiple generations, potentially allowing some populations to compensate for short-term (within-generation) or long-term (transgenerational) environmental change (Jablonska et al. 1992; Somero 2010; Bonduriansky et al. 2012; Schulte 2015; Norouzitallab et al. 2019). Plasticity (within-generation and transgenerational) is thought to have evolved in populations that experience environmental variation over time (Leimar and McNamara 2015; Beaman et al. 2016), such as populations living in temperate regions. Here, we use the definition of transgenerational plasticity given in Bell and Hellmann (2019) and Bonduriansky (2021), which describe it as a form of plasticity where phenotypic changes occur over multiple
generations through nongenetic inheritance, which includes parental effects. Similar to within-generation plasticity, transgenerational plasticity may not always be beneficial, but if parents correctly anticipate their offspring’s environment, then they may precondition their offspring for future environmental conditions (Bonduriansky et al. 2012; Beaman et al. 2016; Norouzitallab et al. 2019). In this way, transgenerational plasticity could serve to buffer the impacts of environmental stressors and grant more time for the evolution of adaptive responses (Bernatchez 2016; Smith et al. 2016). To date, most studies of transgenerational plasticity of aquatic vertebrates have focused on temperate or tropical species (Donelson et al. 2012; Salinas and Munch 2012; Shama et al. 2014); however, cold-adapted stenothermal species are predicted to be most negatively impacted by climatic warming (Beitinger and Bennett 2000). For example, salmonid populations are currently predicted to be most negatively impacted by climatic warming (Beitinger and Bennett 2000). For example, salmonid populations are currently threatened by climate change, but their transgenerational responses to warming remain largely unexplored.

It is currently unclear how plasticity within and across generations operates or interacts in organisms that experience variable habitats or whether within-generation plasticity can override transgenerational plasticity (Shama et al. 2014; Leimar and Mcnamara 2015; Donelson et al. 2018). For example, lake trout (Salvelinus namaycush) inhabit a thermally stable environment (<10°C; Martin and Olver 1980; Wilson and Mandrak 2004) and have limited transgenerational thermal plasticity (Penney et al. 2021). In contrast to lake trout, brook trout (Salvelinus fontinalis) occupy different thermal habitats at different life stages (Biro et al. 2008; Smith and Ridgway 2019), experience relatively high levels of environmental variation (within-lake variation: 7.2°C–7.7°C; Smith et al. 2020), and exhibit within-generation thermal acclimation (McCormick et al. 1972; Stitt et al. 2014; Morrison et al. 2020). It is not yet known whether organisms, like brook trout, that display within-generation plasticity for thermal tolerance are more or less capable of transgenerational plasticity.

The brook trout is a cold-adapted salmonid native to eastern North America found in cold (10°C–16°C), well-oxygenated, freshwater habitats, such as streams and lakes (Power 1980; Smith and Ridgway 2019). Brook trout also have a poor tolerance for warm temperatures (Beitinger and Bennett 2000), making them highly vulnerable to climate change as temperatures become warmer and suitable habitat is lost (McKenna 2019). Thermal refugia in lakes are also being reduced as epilimnetic temperatures rise and the metalimnion shrinks (King et al. 1999); in some smaller lakes, brook trout populations already encounter temperatures that push them to their physiological limits (21°C–23°C; Smith et al. 2020) or prevent reproduction (>20°C; Warren et al. 2012).

In this study, we acclimated adult brook trout and their offspring to elevated temperatures to examine within-generation and transgenerational plasticity in offspring in response to a warming environment. We measured offspring thermal physiology as the rate of oxygen consumption (MO2) and critical thermal maximum (CTM) as performance metrics. We recorded resting MO2 at the offspring’s acclimation temperature (Tac, 15°C or 19°C), then increased temperature at a rate of 2°C h⁻¹ and recorded MO2 at every 1°C during this acute temperature increase to observe the MO2-temperature relationship. We also recorded the peak (highest achieved, thermally induced) MO2 observed as temperature increased, and we recorded the CTM as the temperature at which the fish lost equilibrium for each acclimation group (15°C or 19°C). We hypothesized that brook trout are capable of within-generation plasticity and transgenerational plasticity as responses to environmental temperatures and that transgenerational plasticity would enhance upper thermal tolerance (Stitt et al. 2014; Morrison et al. 2020; Penney et al. 2021).

We predicted that CTM and peak MO2 would increase with offspring warm acclimation, demonstrating within-generation plasticity (Mackey et al. 2020; Morrison et al. 2020). We also predicted that, through transgenerational plasticity, offspring with warm-acclimated parents would have a higher CTM and a higher peak MO2 compared with offspring with cold-acclimated parents because transgenerational warm acclimation would improve thermal tolerance at elevated temperatures (Donelson et al. 2012, 2018; Shama et al. 2014). The effect of transgenerational plasticity on offspring resting MO2 and whether the effect would be beneficial or detrimental was more difficult to predict: transgenerational warm acclimation could result in a lower resting MO2 in offspring from warm-acclimated parents, as seen in some other fish species (Donelson et al. 2012, 2018; Shama et al. 2014). Alternatively, resting MO2 could be higher, as seen with transgenerational warming in lake trout (Penney et al. 2021). To date, parental contributions to transgenerational plasticity have largely focused on the maternal environment (Shama et al. 2014; Best et al. 2018); however, paternal contributions are increasingly being reported across taxa (Hellmann et al. 2020; Rutkowska et al. 2020). Our experimental design provided us with the opportunity to assess both maternal and paternal contributions to offspring thermal responses. We predicted that offspring and parental warm acclimation would interact (i.e., parents anticipate their offspring’s environment) to strengthen the effect of transgenerational plasticity on CTM, resting and peak MO2, and the MO2-temperature relationship.

**Methods**

All experiments were approved by the Trent University Animal Care Committee (protocol 24794) and the Ontario Ministry of Northern Development, Mines, Natural Resources and Forestry (OMNDRNRF) Aquatic Animal Care Committee (protocol FACC 136). Experiments were conducted according to the guidelines outlined by the Canadian Council on Animal Care.

The brook trout used for this study originated from wild spawn collections from a native population in Dickson Lake, Algonquin Provincial Park in south central Ontario (45°47’N, 78°12’W). Dickson Lake has a maximum depth of 18.5 m and stratifies in the summer, with temperatures ranging between approximately 8°C and 21°C and the uppermost 10 m, where brook trout venture to feed, reaching 20°C–21°C (Smith 2017; D. A. Smith 2022, personal communication). Overwintering (November to May) temperatures range between 1.9°C and 3.0°C (Cook et al. 2018b).

The captive brook trout population has been kept in the OMNDRNRF hatchery system since 2002 under conditions...
to minimize hatchery selection, including equalizing family sizes as well as rotational line crossing (Kincaid 1977) to maintain original genetic variation and minimize inbreeding (OMNR 2005; OMNDMNRF, unpublished data). The holding facility circulated water through the tanks using a flow-through system with water from a nearby lake, and the fish experienced ambient water temperatures, which were monitored daily, with water temperature generally increasing from approximately 3.0°C to 12°C from midwinter to early spring.

Experimental Design: Adult Trout Acclimation and Breeding

Adult brook trout (age 5; 0.3–0.9 kg) from the Dickson Lake hatchery brood stock were transported to the OMNDMNRF White Lake Fish Culture Station (Sharbot Lake, Ontario) in the spring of 2015 and implanted with passive integrated transponder tags. A small caudal fin clip (<0.25 cm²) was taken from each individual and separately stored in 95% ethanol to enable subsequent genetic parentage analysis of offspring families (described in the appendix). In May 2015, adults were divided into two groups (n = 8 and 9; four or five per sex), acclimated to one of two temperatures (10°C ± 0.5°C and 21°C ± 0.5°C, respectively), and held until fall reproduction. The lower temperature was based on the temperature requirements for brook trout spawning, while the warmer temperature was selected to induce thermal stress without exceeding their physiological limits or compromising reproductive success (Hokanson et al. 1973; Blanchfield and Ridgway 1997). Each group was kept in a 6,000-L flow-through tank covered with opaque acrylic lids to reduce stress to the fish, with light allowed in at the inflow and outflow to provide natural photoperiod cues. The tanks received water from White Lake (44°46’N, 78°45’W), and the target temperatures (10°C ± 0.5°C and 21°C ± 0.5°C) were achieved by mixing inflows from above and below the lake’s thermocline. Fish were acclimated to these temperatures from mid-July to September, after which the temperature of each tank followed the lake’s seasonal cooling beginning from September and reaching 5.2°C by mid-December for both treatment tanks. Tank water temperature and oxygen levels were checked daily, with temperature also logged every hour using two HOBO TidbiT loggers (Onset, Bourne, MA) per tank for the duration of the adult acclimation period. The temperature data were collected from the loggers with a HOBO USB optic reader and HOBOware Pro (ver. 2.3.0; Onset) after spawning to track acclimation temperatures throughout the duration of the experiment.

Beginning in early October, the reproductive status of the trout was checked weekly by visual inspection following mild anesthesia (0.1 g L⁻¹ MS-222; Aqualife, Syndel Laboratories, Nanaimo, British Columbia), and all adults were reproductive by mid-December. As males and females came into reproductive condition, fish were dry spawned by collecting gametes from anesthetized fish, subdividing eggs from individual ripe females into two glass jars, and fertilizing them with milt from separate males. In total, we used two males and two females from each of the two temperature treatments (eight adult fish in total) in two 2 × 2 factorial crosses (fig. 1), where the offspring were from parents of matched or mismatched thermal histories (C indicates cold, and W indicates warm): C♀ × C♂, C♀ × W♂, W♀ × C♂, and W♀ × W♂. Egg numbers for all families were equalized so that 140 mL of eggs from each female were sired by each of the four males, resulting in 16 families. Fertilized egg families were transported in insulated jars packed inside a cooler with ice packs to the OMNDMNRF Codrington Fisheries Research Facility (Codrington, Ontario), where they were transferred to Heath trays receiving freshwater at ambient temperature (5°C–6°C) under constant dim light for development.

One caveat of transgenerational studies is that parental effects cannot be accurately assessed unless a full factorial breeding design is used (Uller et al. 2013). We used a full factorial design, but we recognize that we used only four males and four females in the crosses (technically, 16 breeding pairs; four families in each of the C♀ × C♂, C♀ × W♂, W♀ × C♂, and W♀ × W♂ groups). One of the challenges of working with larger, nonmodel organisms is providing adequate space. We opted to use fewer adults and test more offspring per family to ensure that we had enough replicates from each family to test individually. A limited number of breeding adults could mean that any effects seen in offspring Mo2 may not be entirely due to parental acclimation temperature (transgenerational plasticity) but potentially due to differences in parental or family fitness. For brook trout, however, this seems somewhat unlikely based on the limited variation for standard metabolic rate observed within and among brook trout populations (Stitt et al. 2014) and the consistency of brook trout aerobic scope across independent studies that controlled for thermal acclimation (Smith and Ridgway 2019).

Experimental Design: Offspring Temperature Acclimation

When fry reached the exogenous feeding stage, we randomly chose 20 offspring from each of the 16 families and divided them into two groups for acclimation to two different temperatures (15°C and 19°C). We chose the cooler acclimation temperature according to the optimal growth temperature reported for brook trout (McCormick et al. 1972), whereas the warm temperature simulated the potential warming due to climate change in the Great Lakes region by the end of the century (Hayhoe et al. 2010). The Tω’s, while different from their parents’ acclimation temperatures, were chosen because they are ecologically relevant for the adults and juveniles (Smith et al. 2020). Furthermore, the temperatures between the generations need not be identical for tests of transgenerational plasticity (Uller et al. 2013). Each group of 10 was moved into one of four larger (200-L) tanks: two tanks were designated for 15°C and the other two for 19°C so that each family had 10 representatives acclimated to each temperature. Each tank was divided into four sections to keep the families separate, but because of space constraints, two families were kept in each tank section where the families sharing a section had a father in common. Individuals were identified to family after measurement trials by microsatellite genotyping (described in the appendix).

Temperature acclimation began after the offspring were transferred to the larger tanks. We increased the water temperature at
a rate of 1°C d⁻¹ using titanium heaters (500 W, model TH-0500, Finnex, Countryside, IL) with digital temperature controllers (model 192 HC 810M, Finnex) until the water in each tank reached its designated temperature (15°C ± 0.6°C or 19°C ± 0.6°C). The temperatures were checked and recorded twice daily. During this time the fish were fed five or six times a day at 2%–3% their body weight. The experiments began after the fish had been acclimated for at least 3–4 wk.
**Respirometry Setup**

We explored the influence of parental thermal history on the MO2-temperature relationship, resting metabolic rate, peak (highest achieved, thermally induced) metabolic rate, and upper thermal tolerance of the offspring. The metabolic rate of the offspring was measured as MO2 using closed respirometry. We began the respirometry trial by measuring resting MO2 at the T0 (15°C or 19°C). The temperature was increased 2°C h⁻¹, and MO2 was measured at every 1°C increase until the fish lost equilibrium, which was recorded as the CTM. From this data set (MO2 as temperature increased), we recorded the highest MO2 achieved by each fish during the acute temperature increase. This peak (highest achieved, thermally induced) MO2 may not necessarily occur at or immediately before loss of equilibrium (CTM), as MO2 could potentially plateau at temperatures below the CTM. Peak MO2 also differs from maximum metabolic rate (MMR) in that peak MO2 is the highest MO2 observed with an acute temperature increase (2°C h⁻¹), whereas MMR is usually tested with exhaustive exercise. Thus, peak MO2 may not be the absolute maximum rate each offspring was actually capable of.

We used the same respirometry setup and general protocol reported previously for lake trout (Penney et al. 2021). Each experimental trial used eight custom-built respirometers. Respirometers were made from an 8-cm-diameter glass tube that was cut at a length of 4.5 cm and sealed at one end (i.e., the floor of the chamber) for a total volume of 226 cm³. The respirometer lids were made of acrylic. Each lid contained a fitting in the center for an O2 probe and valves on opposite sides of the probe fitting to allow water to circulate through the respirometer chamber. Two respirometers were placed in each of four transparent plastic tubs, and the tubs were seated on top of two side-by-side stir plates (one plate per respirometer). The plates were used to spin a magnetic stir bar in each respirometer at approximately 60 rpm to prevent the establishment of O2 gradients in the chambers and to keep water moving past an O2 probe (Clark-type polarographic electrode, model DO-BTA, Vernier Software and Technology, Beaverton, OR) that was inserted into the lid of each respirometer. The O2 probes were connected to a LabPro interface (Vernier Software and Technology), and O2 concentration within the respirometers was recorded every second using Logger Pro software (ver. 3.8.6; Vernier Software and Technology). Each respirometer also contained a perforated steel grid to separate the fish from the stir bar. Water from the tub was circulated through the respirometer at 4.5 L min⁻¹ using a submersible pump (universal 1005, EHEIM, Deizisau, Germany), and the water in each tub was also circulated with aerated, temperature-controlled freshwater from a source tank.

**Respirometry and Critical Thermal Maximum Protocol**

Respirometry trials were conducted from August 9 to September 15, 2016. The night before a trial, eight fish (mass: range, 1.1–55 g median, 30 g) were individually transferred to clean respirometers where they received a continuous flow of freshwater maintained at their acclimation temperature and delivered via vinyl tubing. They were left to adjust to the experimental apparatus overnight, and a thin sheet of black plastic covered each tub to minimize visual disturbance to the fish during the adjustment period and experimental trial. Fish were fasted for at least 12 h before each trial to eliminate the physiological effects of digestion on the experimental results (Millidine et al. 2009).

We began measuring MO2 in each individual fish the next morning at 7:00 a.m. To measure MO2, the respirometer chambers were sealed by manually closing the respirometer valves and switching off the pumps that circulated water through the chambers. After a 30-s wait period, the reduction in chamber O2 concentration was recorded for 10 min. Afterward, the flow valves were reopened to restore water circulation. Water temperature was then increased at a rate of 2°C h⁻¹, and the MO2 of each fish was measured at each 1°C increase with 30 min between the repeated MO2 measurements. MO2 was calculated using the formula

\[
MO2 = \frac{\text{(rate of decline in } [O2] \text{]}(V_R - V_F) \times 60}{h},
\]

where “rate of decline in [O2]” is the decline in water oxygen concentration (mg O₂ L⁻¹ min⁻¹) during the 10-min measurement period, \(V_R\) is the volume (L) of the respirometers, \(V_F\) is the volume of the fish (L), and \(h\) is the time in hours. The background microbial respiration, measured at the end of the respirometry trials, was nil. The rate of decline was determined with Logger Pro, and we measured the linear fit of the drop in respirometer O₂ concentration over time. If the linear correlation coefficient (r) was below 0.8, the data point was excluded from the analysis. This resulted in the exclusion of 225 of 2,845 total data points collected. Some of these excluded values were measures of resting and peak MO2 (of 230 individuals, 43 resting MO2 and 5 peak MO2 were not included in the analysis).

The CTM for each fish was recorded as the temperature when it lost its righting response (i.e., equilibrium), and this was recognized as the point at which the fish could no longer maintain an upright position within the respirometer. All fish were closely monitored as temperature increased, and when a fish lost equilibrium, it was quickly removed from the respirometer and euthanized with 0.3 g L⁻¹ of tricaine methanesulphonate (MS-222; Aqualife, Syndel Laboratories). Euthanized fish were immediately blotted dry on paper towels, and mass (nearest 0.1 g) and fork length (mm) were measured using a digital balance and calipers, respectively. Measurements of mass and length were used to calculate the condition factor using the following formula:

\[
\text{condition factor} = \frac{\text{mass}}{\text{fork length}^3} \times 100.
\]

A tissue sample (caudal fin clip) was taken from the euthanized fish and individually stored in 95% ethanol for microsatellite genotyping to identify each offspring to their respective family (described in the appendix). Twelve of the 230 fish used for this experiment died at or just before collecting CTM, so they were not included in the analysis of CTM.

To ensure that O2 would not become limiting at warmer temperatures, we monitored O2 saturation of the water throughout
Calculations and Statistical Analysis

The $\text{MO}_2$ measured at the fish’s acclimation temperature before the temperature began to rise with the acute temperature challenge was considered the fish’s resting $\text{MO}_2$. We report peak $\text{MO}_2$ as the highest $\text{MO}_2$ achieved during the respirometry trial. We do not report aerobic scope here because our measurement of peak $\text{MO}_2$ may not necessarily represent the absolute maximum $\text{MO}_2$ achievable by the offspring; maximum $\text{MO}_2$ is typically obtained using exhaustive exercise protocols, which we did not use in this study. We analyze whole-animal rates of $\text{O}_2$ consumption with mass as a covariate rather than perform the analysis on mass-specific values because the former is statistically more appropriate (Hayes and Shonkwiler 1996). The mean values reported from these models are referred to as mass-adjusted $\text{MO}_2$; how- ever, we also provide the data plotted as mass-specific $\text{MO}_2$ in the appendix (fig. S1).

The effect of $T_{o}$ and parental acclimation temperature on mass and condition factor was assessed using a general linear mixed effects model (GLMM). The models for mass and condition factor included $T_{o}$ (cold or warm) and parental acclimation temperature (both parents combined into a single parental group: $C_{i} \times C_{j}$, $C_{i} \times W_{i}$, $W_{i} \times C_{j}$, or $W_{i} \times W_{j}$) as fixed effect predictors. An interaction term between $T_{o}$ and parental acclimation temperature was also included as a fixed effect predictor to determine whether parental acclimation temperature had differential effects on offspring mass and condition depending on whether the offspring were acclimated to a cold or warm temperature. Degree days were included as a random intercept to account for the potential effects of age on mass and condition factor. Degree days were calculated for each fish as the cumulative temperature experienced above $0°C$ (Chezik et al. 2014; Cook et al. 2018a) until the beginning of the experimental trial. A Tukey’s honestly significant difference post hoc analysis was performed if the test determined a significant effect of fixed predictors on mass or condition to uncover where differences occurred among pairwise comparisons.

To identify factors contributing to variation in resting $\text{MO}_2$, peak $\text{MO}_2$, and CTM, we evaluated competing statistical models using an Akaike information criterion corrected for small sample size ($\text{AICc}$). The possible model terms included $T_{o}$, maternal acclimation temperature ($T_{a}$), and paternal acclimation temperature ($T_{p}$) as fixed effect predictors, with interactions between all factors. Including maternal and paternal effects as separate terms (instead of as single parental groups: $C_{i} \times C_{j}, C_{i} \times W_{i}, W_{i} \times C_{j},$ or $W_{i} \times W_{j}$) allows us to investigate the relative parental contribution to offspring resting $\text{MO}_2$, peak $\text{MO}_2$, and CTM. An additive effect of parental acclimation would be detected as both $T_{a}$ and $T_{p}$ appearing in the models ($T_{a} + T_{p}$). Models also included offspring mass as a covariate because the warm-acclimated offspring grew heavier than cold-acclimated ones and because metabolic rate scales with mass. The effects of maternal ID ($\text{ID}_{m}$) and paternal ID ($\text{ID}_{p}$) were included as random intercepts to control for statistical nonindependence of offspring relatedness, as some were full siblings or half-siblings based on the 2 × 2 factorial mating design. From the model $\text{AICc}$ values, we calculated the $\Delta \text{AIC}$, evidence ratio (ER), and Akaike weight ($\text{W}$) for each model and considered the best models as those with a $\Delta \text{AIC} \leq 2$ (Burnham and Anderson 2002). All models with a $\Delta \text{AIC} \leq 2$ were therefore included in the results. We used the calculated $\text{AIC}$ metrics to compare the models and identify common parameters among the models that explained variation in resting $\text{MO}_2$, peak $\text{MO}_2$, and CTM. We generated figures using the residuals from a model containing the natural log of mass (fixed effect) and $\text{ID}_{m}$ and $\text{ID}_{p}$ (random effects) to compare the direction of the effects of $T_{o}$, $T_{a}$, and $T_{p}$ on CTM and resting and peak $\text{MO}_2$ from a presumed population mean of 0 ($y = 0$).

To detect within-generation plasticity and transgenerational plasticity in the metabolic response of the offspring to an acute temperature challenge, we tested whether $T_{a}$, $T_{o}$, and $T_{p}$ influenced the effect of acute temperature exposure on an offspring’s $\text{MO}_2$. If the relationship between acute temperature exposure and an offspring’s $\text{MO}_2$ were linear (or conformed with a lower-order polynomial function), we would achieve this end by using a GLMM with offspring $\text{MO}_2$ as a dependent variable and temperature exposure, parental acclimation treatment, $T_{o}$, and interactions between each parameter as independent variables. Across all offspring, however, the relationship between temperature exposure and $\text{MO}_2$ was not linear, and it could not be explained by a simple polynomial function. To account for this nonlinearity, we first modeled the effect of acute temperature exposure alone on offspring $\text{MO}_2$ using a generalized additive model (GAM) with $\text{MO}_2$ as a dependent variable and temperature exposure, parental acclimation treatment, $T_{o}$, and interactions between each parameter as independent variables. We then tested whether parental or offspring acclimation treatments (or any combination of each) could explain the remaining variation between an offspring’s true $\text{MO}_2$ at a given temperature exposure and that explained by temperature exposure alone ($\beta_{t}$, predicted by our GAM) using a GLMM (similar to Penney et al. 2021). This approach is similar to using residual $\text{MO}_2$ as a dependent variable and allowed us to test for broad differences in the nonlinear effect of acute temperature exposure on $\text{MO}_2$ among acclimation treatments. Unlike using residual $\text{MO}_2$ as a dependent variable, however, our approach allowed us to test for the influence of $T_{o}$ and parental acclimation temperature on the slope of the nonlinear $\text{MO}_2$-temperature relationship and not just its vertical position.

Here, our GLMM included the true $\text{MO}_2$ of offspring as the dependant variable, with the offspring’s expected $\text{MO}_2$ at a
given temperature ($\beta_{\tau}$), $T_0$ (cold and warm), $T_\beta$ (cold and warm), $T_o$ (cold and warm), mass, and all interactions between $\beta_{\tau}$, $T_0$, $T_\beta$, and $T_o$ as independent variables. As with our previously described models, our GLMM also included random intercepts for $ID_{\beta}$, $ID_o$, and offspring ID. Finally, we included a type I autoregressive correlation structure ($\rho = 0.221$) in our model to correct for autocorrelation between MO$_2$ measurements, as they occur at adjacent points (temperatures) during the acute temperature challenge (according to Penney et al. 2021).

In our GLMM, a significant effect of $\beta_{\tau}$ would indicate that changes in MO$_2$ across the acute temperature challenge could be reliably explained by the nonlinear relationship modeled in our GAM (i.e., the expected values of offspring MO$_2$ correlate with their true values at each temperature). Significance of other independent factors would suggest that they shift the nonlinear MO$_2$-temperature relationship up or down, while significant interactions between $\beta_{\tau}$ and the other factors mean that they tilt the MO$_2$-temperature relationship.

All statistical analyses were conducted in JMP 13 (ver. 18.1) or R (ver. 3.5.2), with the level of significance set to 0.05. Linearity, homogeneity of variance, sample independence, and residual normality were confirmed visually and with the Shapiro-Wilk $W$, Levene’s, and Brown-Forsythe tests. The factors that contributed to variation in body mass and condition factor were investigated using JMP 13. Statistical analyses of the resting and peak MO$_2$, CTM, and MO$_2$ during the temperature challenge were conducted using R with the MuMIn (ver. 1.43.15; Barton 2019), lme4 (Bates et al. 2015), nlme (ver. 3.1-143; Pinheiro et al. 2019), and mgcv (Wood 2011) packages. We discovered that one of the peak MO$_2$ data points was 5 SDs below the mean; therefore, this data point was not included in the final analysis.

## Results

### Mass and Condition Factor

Warm-acclimated offspring were heavier overall compared with cold-acclimated offspring (19°C offspring: 3.31 ± 0.08 g; 15°C offspring: 2.73 ± 0.08 g; GLMM: $F_{1,24.60} = 25.22, P < 0.01$). Parental acclimation temperature had a significant effect on offspring mass (GLMM: $F_{3,52.71} = 13.78, P < 0.01$) where offspring from parental groups $C_r \times C_r$ and $W_r \times W_r$ (3.50 ± 0.11 vs. 3.22 ± 0.11 g) were significantly heavier ($P < 0.05$) than those from the $C_r \times W_r$ and $W_r \times C_r$ parental groups (2.66 ± 0.12 vs. 2.69 ± 0.11 g), indicating that offspring with cold-acclimated fathers ($C_r \times C_r$ and $W_r \times W_r$) were heavier than those with warm-acclimated fathers. No other parental group comparisons were significantly different. There was no interaction between offspring acclimation and parental acclimation group (GLMM: $F_{3,52.71} = 0.34, P = 0.80$).

Warm-acclimated offspring had a higher condition factor than cold-acclimated offspring (1.0 ± 0.01 vs. 0.96 ± 0.01; GLMM: $F_{1,23.25} = 27.16, P < 0.01$), and the condition factor of offspring was significantly affected by parental acclimation temperature (GLMM: $F_{1,53.89} = 6.10, P < 0.01$). There was a transgenerational effect of parental temperature acclimation on offspring condition factor: offspring from parents that were both cold acclimated ($C_r \times C_r$) were in significantly better condition than offspring from parents that were both warm acclimated ($W_r \times W_r; 1.0 \pm 0.01$ vs. $0.95 \pm 0.01$, respectively; $P < 0.05$). No other parent groups differed significantly from each other. There was no significant interaction between $T_0$ and parental acclimation group (GLMM: $F_{3,53.89} = 0.13, P = 0.94$).

### Critical Thermal Maximum

CTM was influenced by $T_0$ (within-generation plasticity) but not transgenerational (i.e., parental) acclimation. $T_0$ along with $ID_{\beta}$ and $ID_o$ (random effects) best explained the variation in offspring CTM ($\Delta$AIC ≤ 2; table 1); no other model was within a $\Delta$AIC ≤ 2. The effect of $T_0$, resulted in an approximately 0.5°C higher average CTM in warm-acclimated offspring versus cold-acclimated offspring (28.6 ± 0.03 vs. 29.1 ± 0.02; fig. 2A, 2B).

### Resting and Peak Metabolic Rate

The brook trout MO$_2$ values were within the expected range reported for similar-sized trout (Myrick 2003). Resting MO$_2$ was affected by $T_0$ and parental acclimation temperature. The AICc revealed four models that best explained variation in resting MO$_2$, and each included mass and $T_0$, with $ID_{\beta}$ and $ID_o$ as random effects (table 1). The first model contained only these factors, while $T_0$ appeared in models 2 and 3, with an interaction occurring between $T_0$ and $T_{\beta}$ in model 3, suggesting that resting MO$_2$ depends on whether offspring and maternal environment are consistent with each other (table 1). $T_0$ appeared only once in the top four models and occurred in model 4, which was 2.11 (ER) less likely to best explain variation in the data compared with model 1 (table 1). We plotted the resting MO$_2$ residuals to observe the direction of the effects and saw that warm-acclimated offspring tended to have residual resting MO$_2$ values slightly below the population mean (fig. 3A, 3B). With regard to parental acclimation temperature, the residual resting MO$_2$ of cold-acclimated offspring from warm-acclimated mothers and fathers (fig. 3A, 3B) was higher than the population mean. In contrast, resting MO$_2$ was lower than the population mean for warm-acclimated offspring from warm-acclimated mothers (fig. 3A) and warm-acclimated fathers (fig. 3B). Together, this suggests a transgenerational effect of lowering resting MO$_2$ when parents and offspring each experience warming. The interaction between $T_{\beta}$ and $T_0$ ($T_0 \times T_{\beta}$) was evident in the residual plot (fig. 3A): when mothers were cold acclimated, the residual resting MO$_2$ of their cold-acclimated offspring was slightly lower than that of their warm-acclimated offspring, and this trend reversed when the mothers were warm acclimated. Although figure 3 shows the direction of the effects detected by the AICc, it is important to note that in each case the confidence intervals overlap zero, suggesting that the effect size is small.

Peak MO$_2$ was also affected by $T_0$ and parental acclimation temperature. Three models best explained variation in peak MO$_2$ ($\Delta$AIC ≤ 2), each with the random effects of $ID_{\beta}$ and $ID_o$ (table 1). Mass and $T_0$ were the best predictors of peak MO$_2$, as
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Table 1: Summary of the top models determined with Akaike information criterion corrected for small sample size (AICc) to explain variation in brook trout offspring resting rate of oxygen consumption (MO2), peak MO2, and critical thermal maximum (CTM) with transgenerational acclimation

| Measure, model no. | ΔAIC | ER | \( R^2 \) | Model |
|-------------------|------|----|---------|-------|
| CTM:              |      |    |         |       |
| 1                 | 0    | 1  | .51     | \( T_O + ID_M + ID_P \) |
| Resting MO2:      |      |    |         |       |
| 1                 | 0    | 1  | .39     | Mass + \( T_M + ID_M + ID_P \) |
| 2                 | 1.10 | 1.73 | .22     | Mass + \( T_O + T_M + ID_M + ID_P \) |
| 3                 | 1.26 | 1.88 | .21     | Mass + \( T_O + T_M + (T_O \times T_M) + ID_M + ID_P \) |
| 4                 | 1.49 | 2.11 | .18     | Mass + \( T_M + T_P + ID_M + ID_P \) |
| Peak MO2:         |      |    |         |       |
| 1                 | 0    | 1  | .48     | Mass + \( T_O + ID_M + ID_P \) |
| 2                 | 1.02 | 1.67 | .29     | Mass + \( T_O + T_P + ID_M + ID_P \) |
| 3                 | 1.53 | 2.15 | .23     | Mass + \( T_O + T_M + ID_M + ID_P \) |

Note. Offspring (age: 5 mo) were from parents acclimated to either a cold or warm temperature and were similarly acclimated to cold or warm temperature. \( T_P, T_M, \) and \( T_O \) are the paternal, maternal, and offspring acclimation temperatures, respectively, and ID_M and ID_P are the maternal and paternal individual identification (random effects). All models with \( \Delta AIC \leq 2 \) were included. ER = evidence ratio; \( W_i = \) Akaike weight.

Metabolic Response of Offspring to an Acute Temperature Challenge

Offspring MO2 increased with challenge temperature, and the correlation between acute challenge temperature and MO2 (GAM) was supported (\( \beta_T: P < 0.001 \); table 2; fig. 4). Offspring mass had a significant effect on oxygen consumption, as rates were higher in heavier fish (mass: \( P < 0.001 \); table 2), but the MO2-temperature relationship was similar between offspring....

Figure 2. Effect of maternal (A) and paternal (B) acclimation temperature on the critical thermal maximum (CTM) of brook trout offspring (age: 5 mo) acclimated to a cold (all 15°C-acclimated offspring, \( n = 100 \)) or warm (all 19°C-acclimated offspring, \( n = 116 \)) temperature. On average, CTM was approximately 0.5°C higher in warm-acclimated offspring versus cold-acclimated offspring (28.6°C ± 0.03°C vs. 29.1°C ± 0.02°C). Values are the residuals (± confidence intervals) from a model containing the natural log of mass (fixed effect) and maternal and paternal identity (random effects).
of cold and warm acclimation temperatures \((T_O: P = 0.880; \text{table } 2)\).

There was a transgenerational effect of parental acclimation temperature on offspring \(M_O\) responses to an acute temperature challenge. The acclimation temperature of the mothers and fathers (fig. 4A, 4B, respectively) each significantly affected the offspring’s metabolic response to the challenge temperature. That is, the \(M_O\) of the offspring from warm-acclimated parents was elevated compared with offspring from cold-acclimated parents (fig. 4A, 4B). While the effect was significant for both parents, a stronger effect occurred on the paternal side \((T_{M}: P = 0.042; T_{P}: P = 0.010; \text{table } 2)\).

There was a significant statistical interaction between \(\beta_{Ta}\) and \(T_P\) \((\beta_{Ta} \times T_P: P = 0.007; \text{table } 2)\), indicating a tilt in the offspring’s \(M_O\)-temperature curve depending on the acclimation temperature of the father. Offspring mass-adjusted \(M_O\) was lower in offspring from cold-acclimated fathers compared with those from warm-acclimated fathers when challenge temperatures

Figure 3. Effect of maternal (A, C) and paternal (B, D) acclimation temperature on the resting rate of oxygen consumption (\(M_O\)) and peak \(M_O\) of brook trout offspring (age: 5 mo) acclimated to a cold (all 15°C-acclimated offspring) or warm (all 19°C-acclimated offspring) temperature \((n = 85–122)\). Values are the residuals (± confidence intervals) from a model containing the natural log of mass (fixed effect) and maternal and paternal identity (random effects).
Within-Generation Plasticity

Within-generation plasticity was observed in the CTM, in that it increased with $T_o$. CTM was in the expected range for brook trout acclimated to our temperatures (Wehrly et al. 2007; O’Donnell et al. 2020); however, it increased by only ~0.5°C in warm-acclimated offspring despite the 4°C difference in temperature between the two groups of offspring. We cannot be certain whether this modest increase in CTM was genuine within-generation plasticity or an artifact of experimental starting temperature. Although the rate of heating used in our experiment (2°C h⁻¹) has been deemed an appropriate rate for measuring CTM in brook trout (Galbreath et al. 2004), it is unclear whether significant differences in CTM would have occurred with a slower rate of heating. For example, Morrison et al. (2020) found that the CTM of 20°C-acclimated brook trout was significantly higher than that of 15°C-acclimated trout (approximately 31.7°C vs. 30.5°C) using a heating rate of 0.3°C min⁻¹ (i.e., 18°C h⁻¹).

We detected an effect of thermal acclimation on offspring resting $M_O_2$; however, the effect was small (table 1). Resting metabolic rate typically increases with acclimation temperatures until the individual reaches its pejus temperature (approximately 20°C for brook trout; Hartman and Cox 2008). Thus, we anticipated that resting $M_O_2$ would be higher in warm-acclimated offspring compared with cold-acclimated offspring, largely because they were being measured at a warmer temperature, but we did not see this trend. Further analysis revealed that resting $M_O_2$ also did not differ at common temperatures (19°C-acclimated offspring at 19°C vs. 15°C-acclimated offspring at 19°C). A previous study on variation in upper thermal tolerance and metabolic rate of brook trout found that individuals originating from Dickson Lake (the same lake from which our brook trout originated) had a higher standard metabolic rate following acclimation to 16°C compared with 20°C, though the authors did not suggest that the lower standard metabolic rate at 20°C was due to the fish reaching their pejus temperature (Stitt et al. 2014). Stitt et al.’s (2014) experimental temperatures were comparable to ours (15°C–16°C and 19°C–20°C); however, the fish tested were of different life stages (yearling vs. adult).
Figure 4. Influence of maternal (A) and paternal (B) acclimation temperature on the change in the rate of oxygen consumption (MO2) of cold-acclimated (15°C, n = 105) and warm-acclimated (19°C, n = 125) brook trout offspring (age: 5 mo) given an acute temperature challenge of 2°C h⁻¹. Plots show means and 95% confidence intervals for cold- and warm-acclimated parents as estimated from the general linear mixed effects model where challenge temperature corresponds to a spline. MO2 was statistically adjusted for effects of body mass (see text).

T₀ had a modest effect on peak MO2, demonstrating some within-generation plasticity in this parameter. Some fish species are capable of extending the upper limit of MO2 (i.e., peak MO2) when acclimated to warmer temperatures (reviewed by Schulte 2015). For example, exercise-induced MMR increased by 20%–30% in lake trout acclimated from 8°C to 15°C (Kelly et al. 2014). It is important to note that peak MO2 and MMR differ in that peak MO2 is the highest MO2 observed with an acute temperature increase (2°C h⁻¹), whereas MMR is usually tested with an exhaustive exercise. Although related to MMR, peak MO2 may not be the absolute maximum rate each offspring was actually capable of achieving. The small effect of T₀ could suggest that brook trout peak MO2 is not capable of further increases. This generally agrees with the idea that metabolic ceilings, like peak MO2 or CTM, are relatively thermally (acclimation) insensitive (Sandblom et al. 2016; Norin and Metcalfe 2019; Morrison et al. 2020).

T₀ did not influence the offspring’s MO2 response to an acute temperature challenge (2°C h⁻¹). Interestingly, offspring MO2 did not begin to rise until the challenge temperature exceeded 23°C. Although this is unusual, we are confident that this result was not an experimental artifact. We used the same respirometry setup and experimental protocol to address parallel questions in lake trout, which displayed an increase in MO2 with increasing temperature, as would be predicted (Penney et al. 2021). For this same reason, we do not suspect an effect of thermal inertia in brook trout in our study. The sudden increase in MO2 at ~23°C in brook trout could be due to physiological stress responses being initiated at this temperature, especially considering that 23°C is near the upper incipient lethal temperature recorded for these fish (24°C; Fry et al. 1946; Wehrly et al. 2007). Such a rapid increase could also occur with hypoxia stress. Although we monitored O2 concentration throughout each respirometry trial to ensure that levels did not reach the limit that would induce a hypoxia stress response (3.5 mg O2 L⁻¹; Graham 1949; Doudoroff and Shumway 1970), we acknowledge that hypoxemia could have occurred. Identifying the physiological processes that result in the increased MO2 at ~23°C in brook trout would require further study. Chadwick et al. (2015) saw that levels of HSP70 and glucose increased in juvenile brook trout when challenge temperatures reached approximately 21°C. It is possible that stress responses, such as induction of molecular chaperones or mobilization of energetic resources, were initiated at 23°C in the juvenile brook trout in our study, thus increasing metabolic rate at this temperature. It is also unclear whether metabolic compensation may have been occurring in our brook trout to keep O2 consumption at a steady rate up to the point of 23°C; however, further experimentation would be required to confirm this.

Transgenerational Plasticity

Offspring condition factor was reduced overall with transgenerational warm acclimation. We cannot confirm with certainty that this is a condition transfer effect (Bonduriansky and Crean 2018). However, a reduced condition factor could potentially have negative downstream effects on fecundity, though it is unclear whether a low condition factor would persist into adulthood.

Both Tₛ and T₀ affected the offspring’s MO2-temperature relationship, with an overall upward shift for offspring from warm-acclimated parents. This was also reflected in offspring resting MO2. A higher resting metabolic rate could indicate faster growth (especially if food is plentiful), meaning that fish may mature faster. Similar to our findings for lake trout (Penney et al. 2021), however, parental warm acclimation did not contribute to faster growth in brook trout when fed in amounts of 2%–3%
their body weight; offspring from warm-acclimated parents were not larger than those from cold-acclimated parents. A higher \( \text{MO}_2 \) could also mean that physiological systems are upregulated to respond to stressors, keeping the fish alive until the stressors subside (Norin and Metcalfe 2019; Rosenfeld et al. 2020). While this may benefit short-term survival, prolonged elevated resting \( \text{MO}_2 \) due to environmental stressors could reduce the energetic resources necessary for growth and, later in life, reproduction (Somero 2010; Rosenfeld et al. 2020). Our results suggest that brook trout offspring will incur a higher cost of living (Norin and Metcalfe 2019; Rosenfeld et al. 2020). Our results suggest that brook trout offspring will incur a higher cost of living (Norin and Metcalfe 2019) when their parents experience warmer summers.

It is thought that transgenerational plasticity is adaptive when the environment varies across generations and parents can correctly anticipate their offspring’s environment (Metcalfe 2019; Metcalfe et al. 2019). On the basis of this idea, transgenerational plasticity would be predicted to be weak in stenothermal organisms that have adapted to habitats that are thermally stable across generations. The limited available evidence supports this: in lake trout (Salvelinus namaycush), a cold-adapted stenothermal congener of brook trout, transgenerational plasticity was limited and most evident as elevated \( \text{MO}_2 \) in warm-acclimated offspring from warm-acclimated parents (Penney et al. 2021). In contrast, in eurythermal or warm-adapted fish species, metabolic rates are reduced in warm-acclimated offspring from warm-acclimated parents compared with cold-acclimated parents (Donelson et al. 2012, 2018; Shama et al. 2014). How changes in \( \text{MO}_2 \) through transgenerational plasticity influence fitness in future generations is not immediately clear. An increase in \( \text{MO}_2 \) with warming could indicate an increase in the use of energy for certain physiological processes like protein synthesis for growth or repair. This may be sustainable if food is plentiful and there is sufficient metabolic scope remaining for reproduction (Schulte 2015; White and Wahl 2020). Conversely, energy reallocation is also possible where more energetic resources are diverted to thermal responses (i.e., survival), potentially reducing growth or reproduction. In this case, a change in \( \text{MO}_2 \) may not be observed, but its effects on body size and fecundity could be apparent later in life.

In our study, peak \( \text{MO}_2 \) varied only slightly with \( T_w \) or \( T_p \), and no transgenerational effect on offspring CTM was detected. Our results agree with the limited number of studies on the transgenerational effects in temperate fish (Sandblom et al. 2016; White and Wahl 2020) and cold-adapted fish (Penney et al. 2021). Together, these studies suggest transgenerational plasticity is unlikely to significantly alter CTM or peak \( \text{MO}_2 \) in response to increased environmental temperatures over relatively short multigeneration time spans, reinforcing evidence that these metabolic ceilings are likely to be exceeded in ecological time frames (Sandblom et al. 2016; Norin and Metcalfe 2019; Morrison et al. 2020).

Relative Parental Contributions

Although both maternal and paternal thermal history (temperature acclimation) each contributed to offspring thermal physiology by elevating the \( \text{MO}_2 \) of their warm-acclimated offspring, we did not find strong evidence that transgenerational effects were additive (i.e., stronger when the offspring had a warm mother and a warm father; \( T_M + T_F \)). \( T_M \) and \( T_F \) appeared in the same model only once for resting \( \text{MO}_2 \) (model 4; table 1) but not for peak \( \text{MO}_2 \) or CTM. Similarly, each parent contributed to their offspring’s \( \text{MO}_2 \) response to an acute temperature increase.

Paternal effects have received less attention relative to maternal effects (Rutkowska et al. 2020), and the size of the epigenetic paternal contribution to such changes relative to the maternal contribution is still debated (reviewed by Best et al. 2018). In the few studies that have tested relative parental contributions to transgenerational plasticity in metabolic traits in fish, the paternal contribution is either less than (Shama et al. 2014) or comparable to the maternal contribution (Penney et al. 2021). In this study, fathers surprisingly appeared to have greater contributions to transgenerational plasticity than did mothers. Paternal effects are complex, can depend on the sex of the offspring, and can vary depending on the environment experienced by paternal grandparents (Crane and Bonduriansky 2014; Hellmann et al. 2020). Environmentally mediated epigenetic changes do occur in sperm (Immler 2018; Ord et al. 2020), and these along with cytoplasmic components can influence offspring phenotypes (summarized by Donkin and Barrès 2018). Parents can also have opposing effects on gene expression in their offspring despite both parents having received the same treatment, in that a gene may be maternally downregulated but paternally upregulated in the offspring (Bautista et al. 2020). While epigenetic regulation of gene expression may be an underlying factor in the paternal contribution we observed in our study, we are not aware of another study showing such a large paternally mediated transgenerational plasticity contribution to thermal responses relative to the maternal contribution.

Summary and Perspectives

While within-generation plasticity was evident in peak \( \text{MO}_2 \) and CTM, it was through transgenerational plasticity that warm-acclimated parents elevated resting \( \text{MO}_2 \) and affected the \( \text{MO}_2 \)-temperature relationship in offspring. The importance of transgenerational plasticity relative to within-generation plasticity may depend on life stage and variation in the habitat experienced at each life stage. It is possible that transgenerational effects are strongest in early-juvenile life stages (Yin et al. 2019) but only when the environment is stable. In contrast, within-generation plasticity may be favored when temperatures are more variable (Leimar and McNamara 2015). In fact, in situations where environmental temperature variation exists, transgenerational plasticity effects may be overridden by within-generation plasticity, as found in stickleback (Shama 2017). Our study examined juvenile brook trout 5–6 mo after hatching, at a time when they would be feeding in shallow depths near shore and near the surface in warmer water (Biro et al. 2008). Experiments examining within-generation and transgenerational plasticity at multiple life stages could be very informative, though we are not aware of any such studies to date.
Brook trout exhibited less of a response to within-generation and transgenerational acclimation than expected. It is thought that plasticity occurs in populations experiencing predictable environmental variation over time (Bonduriansky et al. 2012; Beaman et al. 2016; Norouzitallab et al. 2019). For example, compared with brook trout, lake trout live in a more thermally stable habitat and have little variation in within-generation thermal plasticity (Kelly et al. 2014) and limited transgenerational plasticity (Penney et al. 2021). As such, we had expected to detect greater plasticity in brook trout given the greater degree of thermal variation experienced by brook trout over their lifetime (McCormick et al. 1972; Stitt et al. 2014; Morrison et al. 2020). Although our results represent the response of brook trout to anticipated warming as a result of climate change (4°C; Hayhoe et al. 2010), it is possible that a within-generation plastic response may have been stronger with acclimation temperatures that differ by more than 4°C.

Transgenerational effects on offspring phenotypes depend on genotype or ecotype (Verhoeven and van Gurp 2012; Vayda et al. 2018), and transgenerational plasticity is predicted to arise in populations that experience variation in temperature over multiple generations (Beaman et al. 2016; Yin et al. 2019). Given that different populations of brook trout display variation in thermal tolerance and capacity for acclimation across populations (McDermid et al. 2012; Stitt et al. 2014), it would be informative to assess whether transgenerational responses to warming vary among stream and lake populations of brook trout and across the species’ range. One might predict, for example, that daily as well as seasonal thermal variation in stream environments (Chadwick and McCormick 2017) would select for increased transgenerational plasticity compared with lake habitats. Family can also be an important contributor to variation in the metabolic response to temperature (Cook et al. 2018a). We simply accounted for this variation by including family as a random effect in our analyses, but we acknowledge that this existing variation among families could serve as potential substrate for selection.

Transgenerational plasticity may be adaptive for some species of tropical or eurythermal fish (Donelson et al. 2012, 2018; Shama et al. 2014), buffering the impact of environmental stressors associated with climate change (Bonduriansky et al. 2012; Bernatchez et al. 2016; Smith et al. 2021), but this may not be true for some temperate or stenothermal fish species (S. namaycush [Penney et al. 2021]; brook trout [this study]). Our results underscore the importance of conservation programs and environmental monitoring to protect species that are threatened by climate change and have no opportunity for migration, have long generation times or limited standing genetic variation, and have limited plasticity (within and across generations).

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