

Metabolic rates of embryos and alevin from a cold-adapted salmonid differ with temperature, population and family of origin: implications for coping with climate change

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Early developmental stages of cold-adapted ectotherms such as brook trout (*Salvelinus fontinalis*) are at higher risk of mortality with increasing water temperatures. To determine the amount of variation present in early life, which may allow for potential adaptation to increasing temperature, we examined the routine metabolic rates (RMR) of wild-origin brook trout embryos and alevins reared at normal (5°C) and elevated (9°C) temperatures. The experiment was structured to examine variation in RMR within and among several levels of biological organization (family, population and ancestral type (native vs. mixed ancestry)). As expected, family and temperature variables were most important for predicting RMR and body mass, although population-level differences also existed when family was excluded for more detailed analysis. Additionally, body mass strongly influenced RMR at all life stages except for eyed embryos. When family identity was removed from the analysis, population became the most significant variable. Variation in RMR and mass within and among populations may indicate existing adaptive potential within and among brook trout populations to respond to predicted warming under climate change scenarios.

Key words: Temperature, physiology, brook trout, *Salvelinus fontinalis*, thermal tolerance, acclimation, metabolism, climate change, adaptation, development

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Introduction

Increasing temperatures due to climate change are predicted to significantly stress aquatic systems globally. In freshwater fishes, habitat reduction and losses are expected to result in local and regional extirpations of cold-adapted species

(Jeppesen *et al.*, 2010; Deutsch *et al.*, 2015). Taxa that cannot shift their geographical range to better match their environment during periods of rapid climate change are at risk of extinction, especially if they are unable to adapt or physiologically compensate via acclimation (Calosi *et al.*, 2008; Gilman *et al.*, 2010). As dispersal opportunities for most

freshwater species are limited to suitable connected aquatic habitats, the persistence of many populations will likely be determined by their ability to keep pace with changing conditions by either or both mechanisms (Mantyka-Pringle *et al.*, 2014). Stenothermal coldwater species in the northern hemisphere such as charrs (*Salvelinus* sp.) are likely to be profoundly impacted by warming (Chu *et al.*, 2005), and are likely to experience geographical range contractions northward as more southern habitats become unsuitable (Jeppesen *et al.*, 2010).

Energetic costs for survival and growth are core parameters that influence life history, and vary within as well as among species (Clarke and Johnston, 1999; Burton *et al.*, 2011). Energy allocations to growth, reproduction and self-maintenance ultimately impact an individual's fitness, but allocation decisions may also be influenced by the organism's routine metabolic rate (RMR) (Arnott *et al.*, 2006; Burton *et al.*, 2011). The acclimation capacity of organisms can vary substantially among life stages, and can impact an individual's future phenotype as well as survival (Scott and Johnston, 2012; Mueller *et al.*, 2015). Given potential links among metabolic rate, life-history traits and temperature (Metcalf *et al.*, 1995; Burton *et al.*, 2011), variability in RMR at different environmental temperatures is likely to determine the local persistence of populations and species.

Brook trout (*Salvelinus fontinalis*) was one of the first North American freshwater fish species to be used to assess thermal stress and physiology (Huntsman, 1942, 1946; Fry *et al.*, 1946). Brook trout are native to cool, well-oxygenated streams and lakes in eastern North America, ranging from Georgia and New England to northern Ontario, Quebec and Labrador (Scott and Crossman, 1998). In mid- to late autumn, mature adults seek out nearshore spawning habitat with groundwater upwellings (Curry and Noakes, 1995), building redds by excavating gravel or rocky substrate over upwellings (Ridgway and Blanchfield, 1998). The groundwater maintains overwintering temperatures of 4–5°C for embryo and alevin development (Power, 1980; Meisner, 1990b; Curry *et al.*, 1995; Ridgway and Blanchfield, 1998; Franssen *et al.*, 2013). Following full yolk sac absorbance (end of the alevin life stage), emergent fry leave the redds (Snucins *et al.*, 1991) and spend several weeks foraging in warm (15–20°C) nearshore habitats (Biro, 1998). During the spring and summer months when temperatures exceed 20°C, brook trout tend to migrate to deeper waters or further upstream to cooler waters (Meisner, 1990b; Biro, 1998), with preferred summer temperatures ranging from 10 to 21°C (Graham, 1949; Baird *et al.*, 2006).

As with other species, early life stages of brook trout are the most vulnerable to changes in their environment (McCormick *et al.*, 1972; Power, 1980; Régnier *et al.*, 2010). Early developmental life stages of brook trout embryos to alevin are particularly sensitive to changes in temperature, as their window of thermal tolerance is much

smaller and lower than for adults (Fry *et al.*, 1946; Marten, 1992; Başçınar and Okumuş, 2002). Brook trout embryos and alevins can survive and develop normally within only a small range of temperatures (3–8°C) (Marten, 1992; Curry *et al.*, 1995; Başçınar and Okumuş, 2002) compared to yearling or adult life stages that can tolerate temperatures up to 25°C (Huntsman, 1942, 1946; Fry *et al.*, 1946; Stitt *et al.*, 2014). Evidence from long-term field data has shown that young of year (YOY) brook trout are sensitive to seasonal temperatures, and that YOY abundance is a key driver of population dynamics (Kanno *et al.*, 2016). Although early work showed little variation among populations for acute thermal stress (Sale, 1962), McDermid *et al.* (2012) showed both family-level variation and population differences for acute thermal stress in yearling brook trout. Other data on yearling brook trout from allopatric hatchery strains confirmed differences in standard metabolic rate among hatchery strains at 8°C, as well as substantial within-population variation and significant interaction between populations and acclimation temperature (Stitt *et al.*, 2014).

Looking forward, the distribution of brook trout across Canada is predicted to decrease 49% by 2050 (Chu *et al.*, 2005), in part due to increased environmental temperatures (Meisner, 1990a,b; Xu *et al.*, 2010). Global warming poses a significant threat to this cold-adapted species by both increasing the temperature and reducing the availability of groundwater, impacting essential habitat for early developmental life stages (Meisner, 1990b) and therefore recruitment (Kanno *et al.*, 2016). This sensitivity to warm waters is thought to have contributed to the ongoing northward retreat of the southern range limit of brook trout (Meisner *et al.*, 1987; Meisner, 1990a; DeWeber and Wagner, 2014). Whether there is capacity for early life stages to acclimate to warmer temperatures, and whether this capacity varies among populations at early life stages as it does for older life stages (Stitt *et al.*, 2014), is largely unexplored. Additionally, although hatchery strains originating from different wild populations have retained ancestral adaptations to thermal conditions in their original source habitats (Stitt *et al.*, 2014), it is not known whether these have resulted in differences in RMR between native and mixed-ancestry (hatchery introgressed) individuals in wild populations. If so, this could help inform management efforts to maintain wild populations through increasing their acclimation and/or adaptive capacity.

In this study, we examined variation in the RMR and body mass of brook trout at three different levels of biological organization (ancestry, population and family) to quantify their relative contributions to physiological variation at different life stages. To do this we used a nested design of early life stages (fertilized embryos and alevins through to yolk absorption) from multiple families within populations with differing ancestral categories (native and mixed-ancestry), rearing each family at the natural thermal optimum (5°C) (Power, 1980; Curry *et al.*, 1995; Ridgway

and Blanchfield, 1998) and near their upper thermal limit (9°C) (Marten, 1992). Our results provide insight into both the thermal plasticity and sensitivity of early life stages of brook trout, and the extent and structure of underlying variation in RMR within and among wild brook trout populations.

Methodology

This study was conducted in accordance of the Canadian Council of Animal Care and approved by the Institutional Animal Care Committee of Trent University (Protocol #13 033) and the Fish Animal Care Committee of the Ontario Ministry of Natural Resources and Forestry (Protocol #14–090).

Field procedures

In November 2013, gametes were collected from four wild brook trout populations in Algonquin Park, Ontario: two native populations (Charles Lake and Dickson Lake) and two populations with mixed ancestry resulting from introgression from historical stocking from provincial hatcheries (Scott Lake and Stringer Lake; geographical details of the lakes described in Table 1). Although Charles Lake has been stocked in the past with the Hills Lake hatchery strain (Ashford and Danzmann, 2001), brook trout in this lake maintained their native genetic ancestry and showed no evidence of introgression (Al-Shamliah, 2013).

Wild brook trout eggs were collected from each lake/population and fertilized in the field using the following procedures. Adult brook trout were non-lethally captured in 15.2 m × 2.4 m panels of 4.8 cm monofilament gill nets (Redden Custom Nets Ltd, Langley BC, Canada V3A 7C7). Nets were set close to spawning redds for 5–10 min and were monitored to avoid fish mortality. Adult fish were removed from the gill nets and placed in a floating corral. Once all fish were removed from the net they were sedated using TMSTM (tricaine methanesulfonate; 50 mg/L water; AquaLife Syndel Laboratories, Nanaimo BC, Canada V9S 4M9) and assessed for reproductive maturity (readiness to spawn). Fish that were ready to spawn were partially stripped of gametes (24–30 mL of eggs per female) and sampled for later genetic analysis by removing a small (0.25 cm²) tissue sample from the upper lobe of the caudal fin. Females were dry-spawned into separate 150 mL jars and fertilized with milt from single males, such that all matings were done as monogamous (single pair) crosses, with each reproductive adult used only once. Fertilization was initiated by adding 100 mL lake water to each jar and allowing the water to remain for one minute; fertilized eggs were then rinsed twice to remove excess milt and the jar was topped up with fresh oxygenated lake water. Eggs were stored overnight at 4°C before being transported to the Ontario Ministry of Natural Resources and Forestry (MNRF) Codrington Fisheries Research Facility (Codrington, Ontario; 44°08'49"N, 77°48'10"W). Upon arrival, eggs were externally

disinfected with 10% OvidineTM (Syndel Laboratories, Qualicum Beach BC, Canada V6K 1V5) solution for 10 min before being brought into the hatchery.

To record seasonal (overwintering) temperatures that wild embryos experience during development, HOBO Tidbit[®] temperature loggers (Onset Computer Corporation, Bourne, MA, USA) were placed in each lake on top of active brook trout spawning redds and ~2 m away from the redds to monitor the overwintering temperatures using a 90-min recording interval. Loggers were in place from mid-November 2013 until late May 2014; upon retrieval, temperature records were downloaded using HOBOWare Pro version 2.3.0 (Onset Computer Corporation, Bourne, MA, USA).

Experimental design

Within each of the four populations, six single-pair crosses (full-sibling families) of fertilized eggs were used for testing in this study, making a total of 24 families. For each family, the total egg volume (24–30 mL of eggs per female) was evenly divided into four separate steel-mesh egg boxes (9 cm × 9 cm × 7.5 cm) in four separate 200 L thermal acclimation tanks (Frigid Units Inc., Toledo, OH, USA). This was separately repeated for all families from each population, so that each of the tanks held identical replicates of all 24 families (6–8 mL family⁻¹ treatment⁻¹), with each family held separately within each tank.

The acclimation tanks were set up for rearing eggs and alevins at 5°C (cold) and 9°C (warm), with two tanks per temperature treatment. These temperatures were chosen to represent temperatures experienced by developing embryos in the wild (~5°C) and the upper thermal limit for brook trout embryos (9°C; Curry and Noakes, 1995). Tanks were initially held at ambient temperature (~6°C) and were gradually changed (~0.5°C every 12 h for 1–3 days) to reach the treatment temperatures. The tanks had a constant inflow of fresh water with temperatures regulated by electric submersible heaters (34 cm × 2.5 cm Finnex HC-810M cylindrical heater with digital temperature controller) or by first running the inflow water through chillers (33 cm × 30 cm × 68.5 cm Johnson Controls A419). Water was filtered and circulated throughout the tanks using 10 L Eheim filters (Type 2217) connected to spray bars placed at the bottom one end of the tank. Dissolved oxygen (DO) levels were maintained above 8 mg L⁻¹ using a 15 cm × 4 cm × 4 cm airstone and monitored using a YSI 55 DO meter (Fisher Scientific Ltd, Ottawa, Ontario, Canada). Tanks were exposed to natural photoperiod, but covered with opaque insulated lids for the duration of the experiment. Embryo and alevin survival was checked every second day, with all mortalities discarded.

Routine metabolic rate

RMR was defined as the lowest rate of metabolism needed to maintain basic body function measured at a specific temperature and accounting for sporadic, low levels of activity

Table 1: Details of the four source lakes in Algonquin Park, ON, Canada from which wild brook trout eggs were collected, showing the populations' genetic ancestries, locations, bathymetric characteristics and overwintering temperatures (mean \pm SE) from data loggers placed on brook trout redds and >2 m away from spawning areas in each source lake. Temperature loggers were in place from mid-November 2013 until late May 2014 and took readings every 90 min

Population	Ancestry	Historically stocked ^a	Lat. (°N)	Long. (°W)	Surface area (ha)	Max. depth (m)	Mean depth (m)	Overwinter temp (°C)
Charles Lake	Native	Yes ^b	45° 55'	78° 24'	12.3	8.2	3.4	n/a (loggers failed)
Dickson Lake	Native	No	45° 47'	78° 12'	974.7	16.8	5.5	3.02 \pm 4.35 (redd) 1.92 \pm 6.76 (off redd)
Scott Lake	Mixed	Yes	45° 29'	78° 43'	27.6	25	n/a	4.10 \pm 3.77 (redd) off red: logger failed
Stringer Lake	Mixed	Yes	45° 26'	78° 31'	33.5	21	6.5	4.13 \pm 4.04 (redd) 4.53 \pm 3.64 (off redd)

^aHistorically stocked (pre-1985) with the provincial Hills Lake hatchery strain, based on provincial stocking records (Ontario Ministry of Natural Resources and Forestry, unpublished data).

^bCharles Lake was stocked in the past, but brook trout currently in Charles Lake maintain their native genetic ancestry and show no evidence of introgression (Al-Shamli, 2013; Harbicht *et al.*, 2014).

(Burton *et al.*, 2011; Killen *et al.*, 2012). RMR was measured at four specific early life stages: eyed embryos, unhatched embryos at median (50%) hatch, alevins (newly hatched fish still feeding on yolk stores, generally immobile in wild redds) and at yolk absorption. To account for the effect of temperature on rates of growth, developmental stages were measured in degree days (Table 2). Degree days in this study were calculated as the sum of the average daily temperature above zero experienced each day throughout the life of the embryo/alevin (e.g. for embryo at 9°C: Day 1 average temperature = 9.3°C, Day 2 = 8.9°C, Day 3 = 9.1°C, therefore, the degree day of this embryo after three calendar days is 9.3 + 8.9 + 9.1 = 27.3 degree days). Temperatures were recorded in the rearing tanks every 90 min using HOBO TidbitT[®] data loggers.

To measure RMR, a closed respirometry setup with twelve identical custom-made glass metabolic chambers was used. Each chamber had an outer compartment (400 mL) where fresh water flowed around a separate inner compartment (20 mL), thereby maintaining a constant inner temperature during testing. The inner compartment contained the organism and was where measurements of oxygen consumption were performed. Submersible pumps (Marineland MaxiJet Submersible Utility Pump 400) were used to circulate the temperature regulated water from rearing tanks around the chambers during testing. Gas-proof tubing was used to connect the outer compartments of the chambers to each other and an acclimation tank to maintain a constant temperature. Chambers were placed on stir plates with micro-stir bars in the inner compartment to ensure water movement past the temperature-compensated DO probe (Vernier Technologies S120, Beaverton, OR, USA) during testing. A small piece of screen mesh was inserted into the inner compartment as a barrier to prevent the organisms contacting the stir bar. The 12 oxygen probes were connected to a laptop with LoggerPro 3.8.6.1 software (Vernier Technologies, Beaverton, OR, USA)

via LabPro Mini serial interfaces (six probes per interface; Vernier Technologies, Beaverton, OR, USA). Probes were calibrated daily using Vernier Technologies' recommended 2-point calibration of zero oxygen (sodium sulfite solution) and complete oxygen saturation based on water temperature and altitude of the facility.

Six replicate measurements of each of the 24 families were performed. This resulted in 36 replicates at the population level (six replicates \times six families per population) and 72 replicates for each ancestry category (six replicates \times six families \times two populations per ancestral category). Because of the small size of the embryos and resolution limits of the equipment, five embryos (or two alevins) were measured simultaneously in the same chamber to ensure detectable levels of oxygen consumption during the trials (Miller *et al.*, 2008). Preliminary trials with 1, 2, 3 and 5 embryos or alevins ensured that observed oxygen decline was due to the brook trout and not solely microbes that could have been present (data not shown). Embryos and alevin were transferred from rearing tanks to the testing chambers in water using a turkey baster. Once in the chamber, organisms were acclimated for 60 min in the inner compartment. Oxygen consumption was not monitored during the acclimation period. At the end of the acclimation period the water was refreshed, the probe was inserted, ensuring no air bubbles were present, and testing began. Measurement of oxygen consumption rate continued for 90 min or until the DO reached the critical limit of 3.5 mg L⁻¹ (Graham, 1949), well above the lethal limit of 1.25 mg L⁻¹ for brook trout (Graham, 1949; Shepard, 1955), at which point the test was stopped, embryos/alevins were removed, chambers were refilled with fresh water and sealed once again for 60 min to measure microbial oxygen consumption. Slopes for oxygen consumption were taken as oxygen depletion in mg L⁻¹ by time in minutes. The microbial oxygen consumption rate was then subtracted from the

Table 2: Life stages and ages (in degree days and calendar days) of brook trout at which routine metabolic rate measurements were performed at either 5 or 9°C. The mean spawning date of adults used to make these families was 02 November 2013

Developmental life stage	Age at 5°C in degree days (Mean ± SE, ^a n)	Age at 9°C in degree days (Mean ± SE, n)	Mean calendar day (5°C, 9°C)
Eyed embryo	477 ± 4.58, 22	452 ± 4.54, 22	29 January 2014, Day 89; 24 December 2013, Day 53
Embryo at median hatch	497 ± 1.35, 132	497 ± 1.26, 120	02 February 2014, Day 93; 29 December 2013, Day 58
Alevin	758 ± 1.77, 120	680 ± 1.48, 96	06 March 2014, Day 125; 17 January 2014, Day 77
Yolk absorption	928 ± 2.38, 132	873 ± 1.94, 132	10 April 2014; Day 160; 10 February 2014, Day 101

^an (i.e. sample size) refers to the number of trials. The number of trials was lower for the eyed embryo life stage because we did not test for effects of family of origin.

original consumption of the embryos/fish. Upon removal from the chamber, individuals were euthanized in an overdose of MS-222 (Sigma-Aldrich, St. Louis, MO, USA), patted dry with paper towel for 30 s to ensure all excess water was removed, and then weighed (± 0.1 mg). Total mass and oxygen consumption of the pooled embryos or alevins in a chamber were treated as a single replicate measurement, averaged to avoid pseudoreplication, and expressed as micrograms of oxygen consumed per hour per individual embryo or individual alevin.

Statistical analyses

All statistical analyses were conducted in JMP 11 (SAS Institute Inc., Cary, NC) using an information theoretic approach (Akaike's Information Criterion corrected value for small sample sizes, AICc) to select the strongest yet simplest model representing the data. Parameters included in AICc models were ancestry (native vs. mixed), population (Charles, Dickson, Scott and Stringer Lakes), family identity (one of six families per population, except for Charles Lake which only had four families due to early mortality in all temperature treatments), temperature and mass (as a covariate in RMR analysis only), including two-way interactions. Population and family levels were treated as random effects and nested within the larger level(s) of ancestry, and ancestry/population, respectively, when those larger levels occurred in the model. It should be noted that due to limited equipment availability early in the experiment (only three respirometry chambers were initially available, prior to another nine being manufactured), family-level variation in RMR and mass was not assessed at the eyed embryo life stage. Instead, each family was tested only once, and families were considered as replicates within populations, with each family representing one replicate (six replicates per population). Approximately 20 candidate models were used for AICc model selection for mass and RMR based on *a priori* knowledge of the working system, and included all single parameter models. The best model(s) for each life stage were selected based on their simplicity using their AICc value (lower is better), Δ AICc, Akaike weight and evidence ratio (ER) (Burnham and Anderson, 2002). The Δ AICc is the difference in AICc scores between the model of interest and the top model. Generally, models with a Δ AICc of < 2 are all considered to

have strong support accounting for the variation found within the dataset; those with Δ AICc's of 2–6 cannot be disregarded as they have moderate support, but models with Δ AICc values > 6 are generally discarded (Burnham and Anderson, 2002; Burnham *et al.*, 2011). The Akaike weight (W_i) describes the probability that out of the candidate models, the focal model was the best approximating model and the ER associated with the focal model identified how many times less likely that model is to be the best approximating model over the top model. In this way, AIC was used to assess which parameters (ancestry, population, family, temperature and mass) had the greatest impact on RMR at different life stages of young brook trout. It should be noted that the AIC strategy for data analysis could only be used to compare models from the same dataset or life stage, and not between them. In and of themselves, AICc values for a single model are uninformative until used in combination with others in the dataset.

Following our initial model selection of the factors best describing mass and RMR, AICc analysis was used again but excluding family as a variable. We excluded the family of origin as it was expected to show considerable variation due to maternal effects, which may have masked other patterns concerning ancestry and population of origin. Traditional statistical analysis [e.g. general linear models (GLM)] were subsequently run on the top models for each life stage, using post-hoc Tukey HSD tests to compare among treatments when statistically significant differences were detected. Where mass was detected in the top model for RMR, the metabolic rates are said to be 'mass-adjusted,' different from 'mass-specific' in that mass differences related to RMR were accounted for statistically and not by simply dividing the metabolic rates by each individual's mass.

A two-way ANOVA and post-hoc Tukey HSD test were used to test for potential population and temperature effects on percent mortality, as well as potential interactions between population and temperature.

Results

Lake and experimental temperatures

No temperature data were obtained from Charles Lake, as the data loggers deployed in the lake failed and no other

temperature data were available. In the remaining three lakes, overwinter temperatures on active wild redds from the wild source populations ranged from 3.0 to 4.1°C (Table 1), slightly below the ‘cold’ study temperature of 5°C. Mean water temperatures off the redds differed significantly among the three study lakes with temperature data ($F_{2,8453} = 2040.04$, $P = 0.0001$), with Dickson Lake having a significantly lower mean overwintering temperature than Scott and Stringer Lakes (Tukey HSD test; $Q = 2.34$, $P < 0.05$). Temperatures in the rearing tanks at the hatchery were successfully maintained at $5.0 \pm 0.84^\circ\text{C}$ (mean temperature \pm SD) for the cold treatment and $9.4 \pm 0.12^\circ\text{C}$ for the warm treatment from the beginning of November to the middle of May.

Mass and RMR of eyed embryos

Due to equipment constraints early on, family-level variation was not studied at the eyed embryo life stage. Variation in mass of eyed embryo brook trout was best accounted for by ancestry differences, and to a lesser extent temperature, indicated by two strongly supportive models ($\Delta\text{AICc} < 2$) and two of three moderately supportive models (ΔAICc between 2 and 6; Table 3). The top model of ancestry with population as a nested random effect (Model i) was almost two times more likely the best model than Model ii (ER = 1.92), and was 44% the most likely top model out of the candidate models ($W_i = 0.44$). Despite Model i as the most strongly supportive model, there was no statistically significant evidence of ancestral differences impacting the mass of brook trout at this life stage ($F_{1,1.91} = 0.26$, $P = 0.66$) as all populations across both temperature treatments were statistically similar (Fig. 1a).

The RMR of eyed embryos was dependent on the population from which they originated from, and the temperature they were reared in (Table 3). Two strong models ($\Delta\text{AICc} < 2$) contained population, and the top model also contained temperature (Table 3). The top model (Model a; population and temperature) was 2.5 times more likely the best model than Model b (ER = 2.51). If mass were included in the model as it was in Model c (Table 3) mass was not a significant predictor of RMR ($F_{1,38} = 0.02$, $P = 0.90$) and did not alter the model outcomes. Both population ($F_{3,39} = 6.17$, $P = 0.002$) and temperature ($F_{1,39} = 4.23$, $P = 0.047$) significantly impacted the variability of RMR. Their interaction, however, was not present until the seventh model with little support for the data ($W_i = 0.01$). Charles and Dickson populations had higher RMRs than Scott and Stringer lakes, however, Dickson Lake did not differ significantly from Stringer Lake nor from Scott Lake which may be why ancestry did not appear in the top model. As expected, average RMRs were higher in eyed embryos reared at 9°C than at 5°C (LSM \pm SEM; $2.24 \pm 0.16 \mu\text{g O}_2/\text{h}$ and $1.78 \pm 0.16 \mu\text{g O}_2/\text{h}$, respectively, Fig. 1b). Moderately strong models with a ΔAICc between 2 and 6 also contained mass as a predictive variable (Table 3). Figure 1b shows the mean RMR for

individuals from each population and temperature treatment, although there was no significant relationship between the mass of eyed embryos and RMR based on their regression ($F_{1,42} = 0.0045$, $P = 0.95$).

Mass and RMR of embryos at median hatch

Variation in mass of embryos at median hatch was best explained by family, temperature, and a family \times temperature interaction, as these terms appeared in the only strongly supportive model (Model 1; Table 4). Once family was excluded from the analysis, population took its place in the top model, which now contained population, temperature and their interaction (Model i; Table 4). This was the best supporting model as it had a 99.6% likelihood of being the top model (W_i ; Table 4) and was more than 520 times more likely (ER) to explain the variability in mass than the next best model, which contained only population (not shown in Table 4). When statistically controlling for temperature, the populations showed significant differences in their mass with Charles Lake individuals being smaller than the other three populations which were all similar in mass ($F_{3,221} = 9.26$, $P < 0.0001$; Tukey HSD $P < 0.05$). Temperature alone did not significantly contribute to the variability in mass ($F_{1,221} = 3.19$, $P = 0.076$), however, in addition to population, the population \times temperature interaction did ($F_{3,221} = 7.02$, $P = 0.0002$, respectively), as can be seen in Fig. 2a. When the analysis was split by temperature, mass of embryos reared at 5°C did not differ significantly among populations (Fig. 2a). In contrast, embryos reared at 9°C showed significant differences among populations, with embryos from Charles Lake weighing less than all other populations (Tukey’s HSD, $P < 0.05$; Fig. 2a).

Differences in RMR of embryos at median hatch was also best explained by a single strongly supportive model containing family, temperature, and a family \times temperature interaction, which was nearly 1000 times more likely to be the best model, than the next ‘best’ one according to AICc (ER for Model B = 9897.13, not shown in Table 4). When family was excluded and the analysis was re-run, there was a single strongly supportive model (Model a, $\Delta\text{AICc} < 2$), and one moderately supportive model (Model b, ΔAICc between 2 and 6; Table 4). Both models contained temperature, mass and their interaction, but model a also included population (Table 4). The ANCOVA conducted on Model a showed significant differences in mass-adjusted RMR among populations ($F_{3,222} = 3.63$, $P = 0.014$) with Charles Lake having the highest mass-adjusted RMR and Dickson Lake the lowest (Tukey HSD, $P < 0.05$; Fig. 2b). Temperature and mass as individual variables were not significant in the model ($F_{1,222} = 0.65$, $P = 0.42$ and $F_{1,222} = 1.24$, $P = 0.27$, respectively), but their interaction was ($F_{1,222} = 18.16$, $P < 0.0001$). The significant interaction between mass and temperature showed that small embryos at median hatch reared at 9°C had higher mass-adjusted RMRs than small embryos reared at 5°C whereas the opposite was true for large embryos.

Table 3: Summary of Akaike’s Information Criteria (AIC) models for predicting the mass and routine metabolic rate (RMR) of brook trout eyed embryos

Eyed embryo life stage	Models	<i>K</i>	AICc	ΔAICc	ER	<i>W_i</i>
Mass	i. An + Pop[An]&Random	4	315.38	0.00	1.00	0.44
	ii. An + Pop[An]&Random + Temp	5	316.68	1.30	1.92	0.23
	iii. Temp	3	317.54	2.16	2.95	0.15
	iv. An + Pop[An]&Random + Temp + An × Temp	6	317.73	2.35	3.24	0.14
	v. Pop	3	320.87	5.49	15.56	0.03
RMR	a. Pop + Temp	4	106.7	0.00	1.00	0.52
	b. Pop	3	108.54	1.84	2.51	0.21
	c. Pop + Mass + Temp	5	109.53	2.83	4.12	0.13
	d. Pop + Mass	4	111.11	4.41	9.07	0.06
	e. Pop + Mass + Temp + Mass × Temp	6	112.01	5.31	14.22	0.04

Note: Candidate models are ordered by ascending AICc value. The roman numeral or letter identifying each model corresponds with that model’s location within the set of 6 and 17 candidate models for mass and RMR. Bolded models have strong support for predicting variability as they had ΔAICc scores of 0–2 and models in regular font have moderate support (ΔAICc between 2 and 6). RMR = routine metabolic rate; *K* = number of parameters in the model plus two (for the intercept and variance); AICc = Akaike’s Information Criteria corrected for small sample sizes; ΔAICc = difference in AICc score between focal model and best model; ER (evidence ratio) = measure of how much more likely the best model is than the focal model; *W_i* (Akaike weight) = probability that focal model is the best approximating model; An = ancestry; Pop = population; Pop[An] = population nested within ancestry and is a random effect; Fam = family nested within population and ancestry; mass = mass of single organism; Temp = temperature at which brook trout were reared and tested.

Mass and RMR of alevins

Variation in mass of alevins was best explained by family, temperature and their interaction, with this being the only strongly supportive model (Table 5, Model 1; *W_i* = 1.00): the next best model was 3 × 10⁶ times less likely to be the best supporting model. Again, when family was removed from the analysis, population took its place in the top model (Model i, Table 5) with a 99% probability of being the best model out of the 7 candidate models considered (*W_i* = 0.99). All three parameters were significant in accounting for mass differences in alevins (population: $F_{3,208} = 28.75$, $P < 0.0001$, temperature: $F_{1,208} = 68.47$, $P < 0.0001$, population × temperature: $F_{3,208} = 5.72$, $P = 0.0009$), with largest individuals being those from Charles Lake reared at 5°C, and the smallest being from Dickson Lake at 9°C (Fig. 3a). Differences in alevin mass at the two rearing temperatures were apparent for all populations as were population-level differences (Fig. 3a).

Alevin RMR also varied with family, temperature and their interaction, and was once again the only supportive model (Model A: ΔAICc < 6, ER = 1, *W_i* = 1.00, Table 5). When family was removed from the model and the analysis was re-run, population, mass and the population × mass interaction was the sole supportive model with a 95% likelihood of being the best supporting model (*W_i* = 0.95, Model a; Table 5). Mass-adjusted RMR significantly varied with mass ($F_{1,208} = 10.28$, $P = 0.002$), but the degree to which mass-adjusted RMR varied with mass was dependent on source population (population × mass: $F_{3,208} = 7.33$, $P = 0.0001$). For example, while there was no significant relationship between mass-adjusted RMR and body mass for Scott Lake, the relationship was positive for the other

three populations. The mass-adjusted RMR of alevin reared at different temperatures showed generally similar metabolic rates across all populations, with the exception of Charles Lake fish reared in 5°C which had much higher mass-adjusted RMRs than all other populations and treatments (Fig. 3b). Because the interaction between population and temperature was not in the top AICc model, however, this relationship was not statistically tested.

Mass and RMR of alevins at yolk absorption

As was the case with the previous two life stages, mass of alevins at the yolk absorption life stage was solely dependent on family-level variation and temperature, along with their interaction (Model 1, Table 6). However, unlike the previous two life stages when family was excluded, only population and temperature (and not their interaction) were contained in the best supportive model (Model i: ΔAICc < 2) followed by two moderately supportive models which additionally included ancestry (Model ii: ΔAICc between 2 and 6) and the population × temperature interaction (Model iii: ΔAICc between 2 and 6; Table 6). The probability that Model i was in fact the best approximating model (*W_i*) was 86% compared with 7 and 4% for Models ii and iii, respectively. Using Model i for further analysis revealed that mass varied with populations ($F_{3,259} = 25.09$, $P < 0.0001$), with Dickson being lighter than all others (Fig. 4a). When separated by temperature only, alevin at 5°C were significantly heavier than those from 9°C (LSM ± SEM; 97.20 ± 0.96 mg and 92.49 ± 0.99 mg, respectively; $F_{1,259} = 12.27$, $P = 0.0005$).

RMR of alevins at yolk absorption varied with family and temperature, as well as their interaction (Model A,

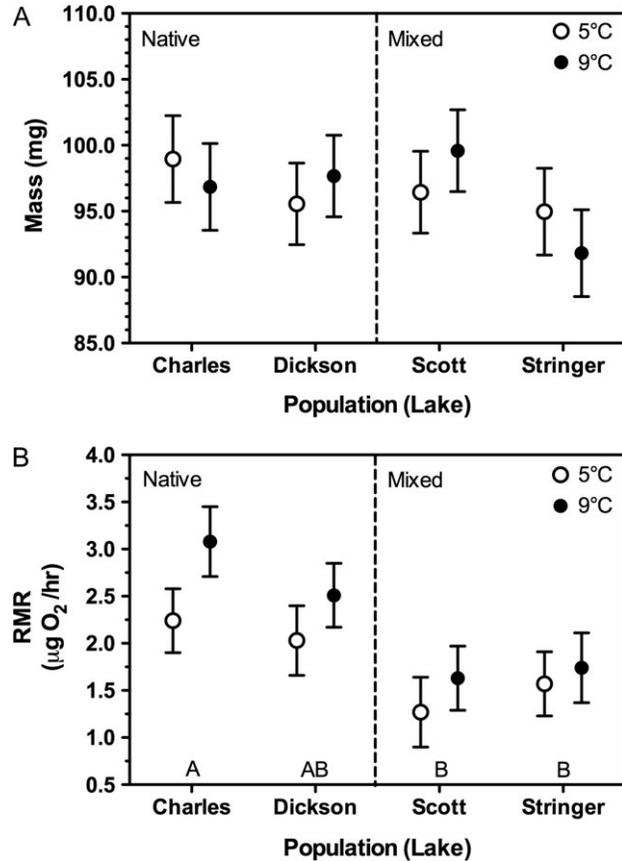


Figure 1: Eyed embryos. Differences in the (A) mass and (B) routine metabolic rate (LSM ± SEM) of single eyed embryos among four populations of brook trout, reared at either 5 or 9°C. Populations originated from either native or hatchery-introgressed mixed ancestry. In (A, mass), no significant differences were found between ancestries nor populations based on the top AICc model (in which temperature was not a variable). In (B, routine metabolic rate), only population and temperature appeared in the top AICc model. Routine metabolic rate was consistently higher at 9°C than at 5°C ($P < 0.05$). Populations separated by different letters are significantly different (Tukey's HSD, $P < 0.05$). Routine metabolic rate is not expressed in mass-adjusted terms because mass did not occur in the top model.

Table 6). When family was excluded, population, mass and temperature all accounted for variation in RMR in Model a. In a second strongly supportive model, Model b also contained the interaction between mass and temperature (Table 6). Two models with moderate support existed as well ($\Delta AICc$ between 2 and 6) with the probability of each actually being the best model ranging from 14 to 10% (models c and d; W_i in Table 6) compared with 44 and 32% for models a and b, respectively. Using Model a for statistical analysis (as the interaction in Model b was not significant: $F_{1,257} = 1.48, P = 0.22$) all three parameters were significant (population: $F_{3,258} = 2.86, P = 0.038$; mass: $F_{1,258} = 24.70, P < 0.0001$; temperature: $F_{1,258} = 44.13, P < 0.0001$). Alevin from Charles and Scott lakes had similar mass-adjusted RMRs at yolk absorption, as did Dickson and Stringer lakes, with those from Charles and Scott lakes having lower rates than those from Dickson and Stringer lakes (Fig. 4b). Controlling for families and populations, individuals reared at 9°C (LSM ± SEM; $20.53 \pm 0.41 \mu\text{g O}_2/\text{h}$) had significantly higher mass-adjusted RMR than those reared at 5°C (LSM ± SEM; $16.64 \pm 0.41 \mu\text{g O}_2/\text{h}$; $F_{1,258} = 44.13, P < 0.0001$, Fig. 4b), and did not differ among populations.

Mortality

Early on in the study many embryos were lost, apparently due to a combination of gamete quality and increased incubation temperature. Two of the six Charles Lake families died out prior to testing (Supplementary Table S1); across populations, some families experienced higher mortality than others but enough individuals survived for continuation of the study. The two-way ANOVA showed significant effects of both population and temperature on mortality (population: $F_{3,88} = 11.408, P < 0.001$; temperature: $F_{1,88} = 6.183, P = 0.015$). Among the four populations, percent mortality was highest for Charles Lake ($0.616^A \pm 0.065$), followed in descending order by Stringer Lake ($0.351^C \pm 0.065$), Dickson Lake ($0.279^B \pm 0.065$), and Scott Lake ($0.086^{BC} \pm 0.065$). Tukey's HSD (shown in superscripts) indicated that mortality was significantly higher in Charles Lake families; despite Scott Lake families experiencing the lowest mortality, no categorical difference in mortality was observed between the

Table 4: Summary of Akaike's Information Criteria (AIC) models for predicting the mass and routine metabolic rate (RMR) of brook trout embryos at median hatch

Embryos at median hatch:	Models	K	AICc	$\Delta AICc$	ER	W_i
Mass	1. Fam + Temp + Fam × Temp	5	1385.06	0.00	1.00	1.00
	i. Pop + Temp + Pop × Temp	5	1720.26	0.00	1.00	0.99
RMR	A. Fam + Temp + Fam × Temp	5	552.41	0.00	1.00	0.99
	a. Pop + Mass + Temp + Mass × Temp	6	566.22	0.00	1.00	0.90
	b. Temp + Mass + Temp × Mass	5	570.81	4.59	9.92	0.09

Note: Candidate models are ordered by ascending AICc value. The number or letter identifying each model corresponds with that model's location within the set of 10, 7, 23 and 17 candidate models for mass and RMR both with and without family, respectively.

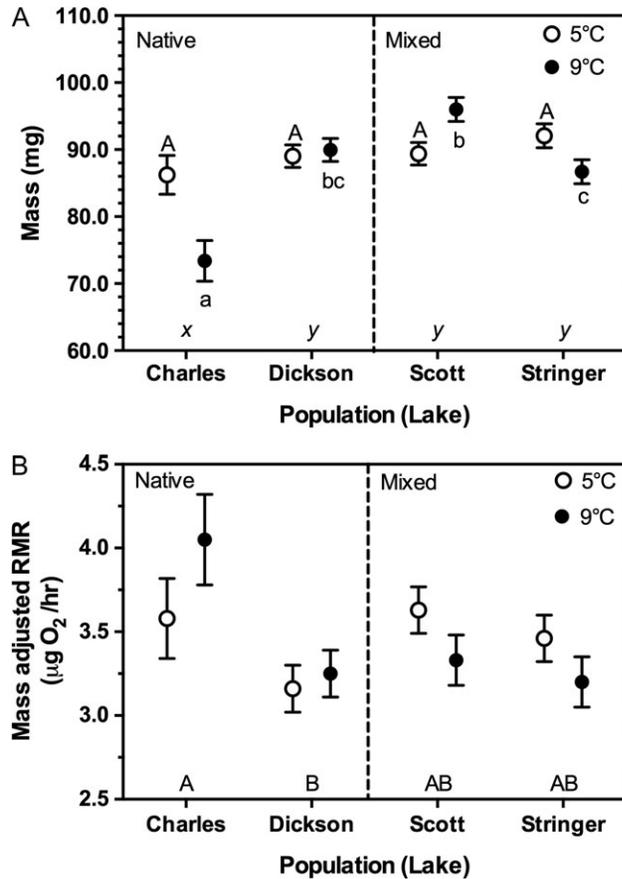


Figure 2: Embryos at median hatch. Differences in the (A) mass (LSM ± SEM) and (B) mass-adjusted routine metabolic rate (RMR, LSM ± SEM) of single brook trout embryos at median hatch reared at 5 or 9°C. Populations originated from either native or hatchery-introgressed mixed ancestry. In (A, Mass), Charles Lake (indicated by an asterisks above its name) was significantly smaller than the other populations, which did not differ (Tukey's HSD, $P < 0.05$). Uppercase letters above error bars show significant differences (Tukey's HSD, $P < 0.05$) among populations at 5°C and lowercase letters below the error bars show differences at 9°C (as a significant interaction between population and temperature occurred in the top AICc model). In (B, RMR), mass was included as a covariate in the analysis. Letters above population names indicate significant differences (Tukey's HSD, $P < 0.05$) between populations. Although temperature appeared in the top model for RMR, it was not statistically significant. Ancestry was not in the top model for either mass or RMR.

native and mixed-ancestry population pairs. Mortality was higher in the 9°C treatment (0.447 ± 0.032) than at 5°C (0.333 ± 0.032) across all four populations. No significant interaction between population and temperature was detected ($F_{3,88} = 0.510$, $P = 0.676$).

Discussion

The experiment demonstrated that brook trout mass and RMR vary within and among each of the levels of biological

organization examined [family, population, and ancestral type (native vs. mixed ancestry)], and that mass and RMR are highly influenced by family- and population-level differences as well as environmental thermal regimes. Variation in mass was accounted for by family (or population when excluding family) and temperature at all life stages studied, except for the earliest eyed embryo life stage, where ancestry and population (nested within ancestry) were alone in the strongest predictive model according to AICc. Variation in RMR was observed at the family level at all life stages for which family could be tested (e.g. embryos at median hatch, alevins and alevins at yolk absorption), suggesting the presence of parental effects. When family was excluded from the RMR analyses, population and temperature occurred in the top AIC for each life stage. As predicted, embryos and alevins reared at 5°C generally showed higher mass and lower metabolic rates than those reared at 9°C, suggesting that the experimental temperatures used in this study had biologically meaningful effects on growth and metabolic rates of early brook trout life stages.

Increasing model complexity with organism complexity

As organismal complexity increased throughout the studied life stages, the AICc models became more complex as well for both mass and RMR. The increased model complexity for the eyed embryo life stage was minimal, including only temperature and ancestry (including population); however, the models best explaining mass and RMR variability in alevins at yolk absorption were much more complex including temperature, family/population and mass (for RMR) as well as interactions of these parameters. This trend makes sense because developing embryos have less architectural complexity and a limited number of active developmental and physiological pathways compared with more advanced life stages. For example, in the anemonefish (*Amphiprion melanopus*), paternal effects did not impact egg size, but upon hatching, maternity, paternity and temperature all influenced the size and growth rates of larvae (Green and McCormick, 2005). The increased biological complexity after hatching, when individual metabolic demands increase, includes effects caused by the incorporation of male genetic contributions (Green and McCormick, 2005). As alevins progress to free-swimming, actively foraging fry, they are not only exposed to a more complex environment, but are also able to seek out thermally optimum environments (Biro, 1998). This active interaction between individuals and environmental temperatures and conditions maximizes foraging opportunities and lowers energy use in brook trout (Biro, 1998).

Family effects

Although not tested in our study, it is highly probable that maternal effects contributed to the observed variation in mass and RMR. Maternal investment is well known to influence offspring size and quality in fish and amphibians

Table 5: Summary of Akaike's Information Criteria (AIC) models for predicting the mass and routine metabolic rate (RMR) of brook trout alevin

Alevin life stage	Models	K	AICc	Δ AICc	ER	W_i
Mass	1. Fam + Temp + Fam \times Temp	5	1194.24	0.00	1.00	1.00
	i. Pop + Temp + Pop \times Temp	5	1495.84	0.00	1.00	0.99
RMR	A. Fam + Temp + Fam \times Temp	5	1057.43	0.00	1.00	1.00
	a. Pop + Mass + Pop \times Mass	5	1106.04	0.00	1.00	0.95

Note: Candidate models are ordered by ascending AICc value. The number or letter identifying each model corresponds with that model's location within the set of 10, 7, 23 and 17 candidate models for mass and RMR both with and without family, respectively.

(Einum and Fleming, 1999; Burton *et al.*, 2013; Moore *et al.*, 2015), as well as RMR (Régnier *et al.*, 2010; Moore *et al.*, 2015). In many fish species, egg size is an important determinant of embryo and larval quality (Atlantic salmon, Kazakov, 1981; rainbow trout, *Oncorhynchus mykiss*, Springate and Bromage, 1985; multiple other species of Pacific salmon, Murray and McPhail, 1988; Braun *et al.*, 2013), and is correlated with temperature (Braun *et al.*, 2013, Jonsson and Jonsson, 2016) and survival during early exogenous feeding (Braun *et al.*, 2013). Other studies that have looked at survival and metabolic rates of early developmental stages of coldwater fish species at different temperatures have shown that both survival and metabolic rate of alevins differed among families at a single rearing temperature (Huuskonen *et al.*, 2003, Pakkasmaa *et al.*, 2006, Régnier *et al.*, 2010).

Although equipment constraints prevented the assessment of family-level variation in RMR at the eyed embryo stage, variation among families contributed greatly to the differences in RMR observed in embryos at median hatch. As parentage has been shown to have a strong influence on temperature effects on early life history across multiple fish species (Burt *et al.*, 2011), it seems likely that family would have also appeared in the top model at the eyed embryo life stage instead of (or in addition to) ancestry. Pakkasmaa *et al.* (2006) found strong family differences in RMR of Arctic charr at the eyed embryo stage, suggesting that genetic and/or maternal effects influence embryonic metabolism. Similar to our study, mass was not correlated with RMR at the eyed embryo life stage (Pakkasmaa *et al.*, 2006). Although egg size and mass are well known to influence RMR in many species (Clarke and Johnston, 1999; Clarke *et al.*, 2010; Rogers *et al.*, 2016), it is highly probable that additional forms of maternal investment such as lipid and/or protein provisioning contribute to RMR variability in eyed embryos and earlier developmental stages (Pakkasmaa *et al.* 2006). However, the poor relationship between RMR and mass in eyed embryos in our study may also reflect the sensitivity of the oxygen probes used and the small size of the embryos, as other studies have utilized more sensitive DO probes for fish embryos (Eme *et al.* 2015; Mueller *et al.*, 2015). Using micro-respirometry, Régnier *et al.* (2010) found differences in maternal investment by female brown trout to their offspring, leading to variation in embryo and larval RMR. For

the embryos at median hatch in our study, the mass-related increase in RMR of individual embryos was consistent with other studies that reported a positive relationship between mass and metabolic rate in brown trout (*Salmo trutta*) embryos and alevin (Régnier *et al.*, 2012) and free-swimming Atlantic salmon fry (Van Leeuwen *et al.*, 2016).

Part of our interest in looking for family-level variation in mass or RMR was to seek possible evidence for heritability, which could be used to infer potential for evolutionary change in response to climatic warming. Evidence of heritability of RMR has been shown in other fish taxa (e.g. coral reef damselfish, *Acanthochromis polyacanthus*, Munday *et al.*, 2017), but the extent to which RMR is heritable in salmonid fishes is largely unexplored. Although the design of our experiment precluded estimating heritability of the observed variation, future studies using diallel or factorial mating designs to assess heritable variation in RMR and other physiological traits would be useful to quantify trait heritability and additive and non-additive genetic variance components.

Population and ancestral effects on brook trout RMR

We detected population-level differences in RMR, consistent with previous reports of local adaptation across small geographic scales (Eliason *et al.*, 2011, 2017; Chen *et al.*, 2013). Although data for population-level differences in RMR are limited for early life stages in fish (but see Seppänen *et al.*, 2009), studies of older individuals are abundant (Billerbeck *et al.*, 2000, Álvarez and Nieceza, 2005; Arnott *et al.*, 2006; Kelly *et al.*, 2014). For example, metabolic rates vary among populations of Atlantic silverside (*Menidia menidia*), when reared in similar environments (Billerbeck *et al.*, 2000), suggesting a heritable component. In contrast, lake trout show little variability in RMR within and among allopatric populations (Kelly *et al.*, 2014).

Several mechanisms likely contribute to variation among populations in RMR (Burton *et al.*, 2011, and references within). For example, populations of cold-adapted fish species have been shown to differ in their cardiorespiratory physiology and aerobic scope (sockeye salmon, Eliason *et al.*, 2011, 2017; lake trout, Kelly *et al.*, 2014; Atlantic

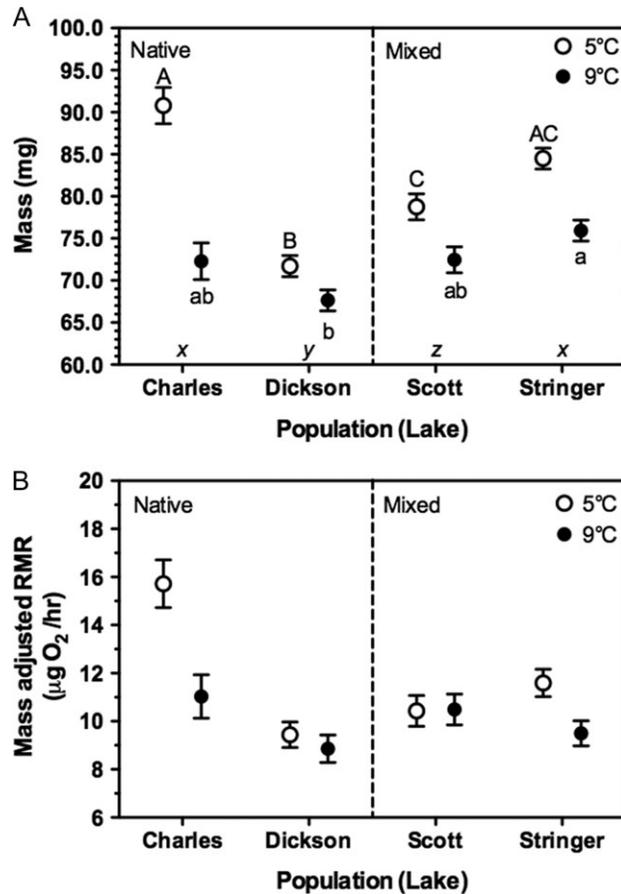


Figure 3: Alevin. Differences in (A) mass and (B) mass-adjusted routine metabolic rate (RMR, LSM \pm SEM) of single brook trout alevin from four populations, with either native or mixed ancestry, and reared at either 5 or 9°C. Ancestry (native/mixed) did not appear in the top models but is shown here for clarity purposes. In (A, mass), populations separated by italicized letters above their name on the x-axis are significantly different from one another (Tukey's HSD, $P < 0.05$). Uppercase letters above error bars show significant differences (Tukey's HSD, $P < 0.05$) among populations at 5°C and lowercase letters below the error bars show differences at 9°C (as a significant interaction between population and temperature occurred in the top AICc model; see text for statistics). Alevin from all populations were larger at 5°C than 9°C ($P < 0.05$). In (B, RMR) mass was included as a covariate in the analysis (see text for statistics). Temperature was not found in the top AICc model, and were therefore not tested. Although population occurred in the top model for mass-adjusted RMR, populations were not significantly different.

salmon, Gradil *et al.*, 2016) as well as feeding efficiency and growth rates (rainbow trout, Hartman and Porto, 2014). As these studies looked at $>1+$ year old fish, however, the mechanisms contributing to variability in RMR at early life stages remain less clear.

Contrary to expectations, ancestry (native vs. hatchery-introgressed) had minimal effects in our study, influencing only the mass of eyed embryos and not affecting the

observed variation in RMR at any life stage in this study. Stitt *et al.* (2014) similarly found no significant differences in standard metabolic rates (SMR) among yearling (age 1+) brook trout from hatchery strains with allopatric origins.

Temperature effects on brook trout RMR

Rearing temperature appeared in every model as an important variable in predicting variability in RMR. As expected, rearing temperature had an effect on embryo and alevin growth and mortality. Rearing temperature is known to greatly impact growth and metabolic rates as well as survival of fish embryos (Beacham and Murray, 1990; Baxter and McPhail, 1999; Simčič *et al.*, 2015). Brook trout embryos grew much faster in the warm water treatment than their siblings in the coldwater treatment, consistent with previous studies of other species showing individuals in warmer environments had earlier hatch dates (Whitney *et al.*, 2014; Hu *et al.*, 2017). Also consistent with previous studies on thermal requirements of brook trout embryos (Zeigler *et al.*, 2013; Réalis-Doyelle *et al.*, 2016), families held at 9°C temperature exhibited higher mortality than in the 5°C (optimal) treatment, reinforcing the evidence that brook trout embryos have a small thermal tolerance window.

The effect of rearing temperature on alevin mass and RMR, as well as survival, changed markedly between life stages. For all test populations, alevins reared at 9°C were smaller and had similar or lower RMRs compared with their siblings reared at 5°C. These patterns shifted noticeably for alevin at full yolk absorption, however, with reduced differences in mass between the two temperature treatments and higher RMR at 9°C across all populations. Although this latter pattern is expected based on a Q_{10} effect, the marked changes between the two alevin stages is noteworthy. The observed trend of reduced mass in sub-optimal environments was consistent with previous studies, which showed that salmonid alevins reared at high temperatures hatch and emerge earlier, are smaller in size due to a decrease in the yolk absorption period, have an increase in the metabolic costs of self-maintenance, and have decreased survival (Chinook salmon, *Oncorhynchus tshawytscha*, Heming, 1982; Atlantic salmon, *Salmo salar*, Ojanguren *et al.*, 1999). These results emphasize the importance of appropriate thermal environments in ensuring the survival of young fish. Food to energy conversion efficiency has been found to be much higher in coldwater fry feeding at test temperatures emulating natural conditions rather than at higher temperatures (Sadler *et al.*, 1986). Similarly, brook trout alevins had a higher feeding efficiency at temperatures near those that they experience under natural conditions (McCaughey and Casselman, 1981; Amin *et al.*, 2014).

Early developmental life stages (i.e. embryos and alevin) are thought to be the most sensitive to environmental temperatures, as individuals are limited in their capacity to move to more suitable habitats (Meisner, 1990a; Régner *et al.*, 2010; Mueller *et al.*, 2015). As acclimation capacity varies

Table 6: Summary of Akaike's Information Criteria (AIC) models for predicting the mass and routine metabolic rate (RMR) of brook trout alevin at yolk absorption

Alevin life stage	Models	K	AICc	Δ AICc	ER	W_i
Mass	1. Fam + Temp + Fam \times Temp	5	1691.11	0.00	1.00	0.99
	i. Pop + Temp	4	2019.05	0.00	1.00	0.86
	ii. An + Pop[An]&Random + Temp	5	2024.00	4.95	11.88	0.07
	iii. Pop + Temp + Pop \times Temp	5	2025.00	5.95	19.59	0.04
RMR	A. Fam + Temp + Fam \times Temp	5	1534.94	0.00	1.00	1.00
	a. Pop + Mass + Temp	5	1568.32	0.00	1.00	0.44
	b. Pop + Mass + Temp + Mass \times Temp	6	1568.93	0.61	1.36	0.32
	c. Temp + Mass	4	1570.66	2.34	3.22	0.14
	d. Temp + Mass + Temp \times Mass	5	1571.20	2.88	4.22	0.10

Note: Candidate models are ordered by ascending AICc value. The number or letter identifying each model corresponds with that model's location within the set of 10, 7, 23 and 17 candidate models for mass and RMR both with and without family, respectively.

among life stages, carry-on effects from sensitive life stages can affect individuals' future phenotype and survival (Scott and Johnston, 2012; Mueller *et al.*, 2015). Lake whitefish (*Coregonus clupeaformis*) exposed to different temperatures at critical windows during early development generally showed reduced costs of development at low temperatures as eggs (from fertilization to organogenesis), but higher costs later in egg development just prior to hatching (Mueller *et al.*, 2015). This implies that these cold-adapted fish are more energy efficient overall at cooler temperatures, and that changes in water temperature during development may impact the individual later in life (Mueller *et al.*, 2015). Similar findings were observed in the warm-water zebrafish (*Danio rerio*) where individuals performed best when tested at the temperature to which they had previously been acclimated during a previous life stage (Scott and Johnston, 2012).

In our study, some life stages showed a family by temperature interaction, indicating that some families responded to changes in temperature differently than others. The expressed family-level differences in Q_{10} at early life stages may therefore enable adaptive responses within local populations in response to changing environmental conditions. Perhaps having the ability to increase or decrease RMR throughout the alevin life stage with warming or cooling waters is beneficial to preserving yolk stores for timing complete absorption and emergence from the redd when food is most abundant. However, those with higher RMRs are also more likely to die when experiencing food reductions, as they use their reserved resources more quickly than their counterparts with low RMRs (Santos *et al.*, 2010; Burton *et al.*, 2011). Auer *et al.* (2015) found metabolic rate to adjust with food availability in brown trout young-of-year: fish that were able to increase their metabolic rates when food was abundant were able to grow faster, as did those that could depress their metabolism in times of low food

availability (Auer *et al.*, 2015). This inter-individual flexibility in metabolic rates may greatly benefit young fish when faced with variable food levels, and provide context-dependent fitness outcomes in wild populations (Santos *et al.*, 2010; Burton *et al.*, 2011; Moore *et al.*, 2015).

Conservation and management implications

As a cold-adapted species, brook trout are highly vulnerable to extreme temperature fluctuations (Jentsch *et al.*, 2007; Gunn and Snucins, 2010; Mantua *et al.*, 2010), and impacts on early life stages can negatively affect population productivity (Kanno *et al.* 2016). Our study identified differences in RMR of brook trout within and among populations, suggesting potential adaptive resources for responding to changing environmental conditions. However, the heritability and potential genetic architecture underlying the observed variation is yet to be determined. The lack of significant differences in RMR between native and mixed-ancestry wild populations contrasted with results from subadult life stages (McDermid *et al.*, 2012; Stitt *et al.*, 2014), reinforcing the vulnerability and reduced plasticity of early life history stages in response to habitat changes. Nonetheless, whereas other coldwater species have shown little ability to cope with increasing water temperatures (lake trout, McDonald *et al.*, 1996; Kelly *et al.*, 2014; Arctic charr, Gerdeaux, 2011), observed levels of variation within brook trout families and populations at multiple life stages suggests that they have at least some ability to respond to changing environmental conditions (this study; McDermid *et al.*, 2012; Stitt *et al.*, 2014), providing that suitable habitat persists. For now, protecting groundwater recharge zones to provide stable overwintering habitat for embryo development and thermal refugia (Meisner, 1990a,b) may be the most prudent option for helping to ensure population persistence.

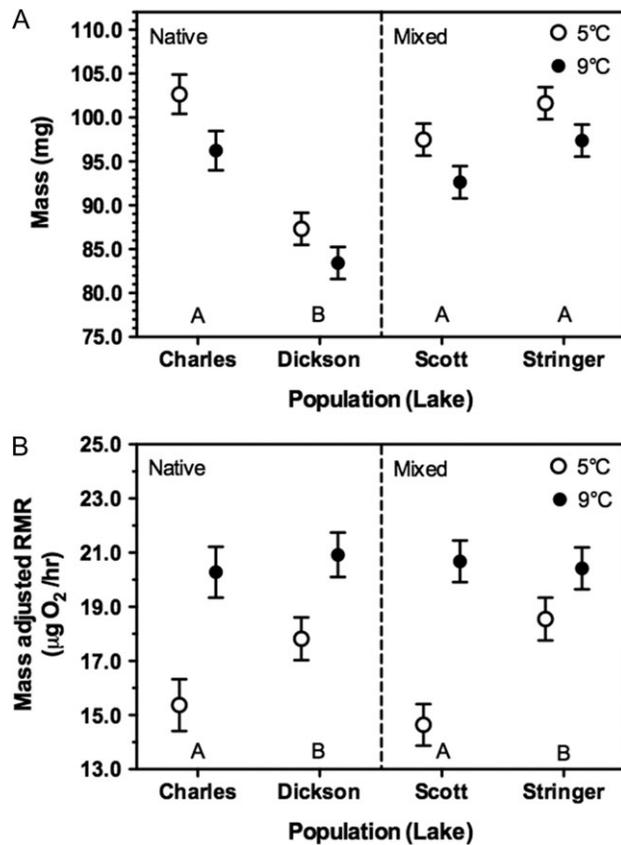


Figure 4: Alevin at yolk absorption. Differences in (A) mass and (B) mass-adjusted routine metabolic rate (RMR, LSM \pm SEM) of single brook trout alevin at yolk absorption from four populations, with either native or mixed ancestry, and reared at either 5 or 9°C. In (A, mass) letters above population names indicate significant differences (Tukey's HSD, $P < 0.05$) between populations. Individuals were larger when reared at 5°C compared to those at 9°C ($P < 0.05$). In (B, RMR), mass was included as a covariate in the analysis (see text for statistics). Letters above population names indicate significant differences in mass-adjusted RMR (Tukey's HSD, $P < 0.05$) between populations. Individuals reared at 9°C had consistently higher mass-adjusted RMRs than those reared at 5°C ($P < 0.05$). In (A) and (B), neither ancestry (native/mixed), nor the interaction between population and temperature were in the top models.

Assisted migration and 'evolutionary rescue' have been proposed as potential options to increase adaptive capacity of wild populations by facilitating gene flow from better-adapted populations (Aitken and Whitlock, 2013; Carlson *et al.*, 2014). With the inability to migrate beyond local watersheds, brook trout may benefit from assisted migration by potentially increasing their adaptive gene pool and resilience, accelerating adaptation to emerging selective pressures (Willi *et al.*, 2006; Aitken and ,2013; Carlson *et al.* 2014). Regardless of the potential efficacy of infusions of new genetic material for coping with other emerging stressors, however, the similar RMR responses to increased rearing temperature across populations regardless of ancestry

suggests that metabolic processes in early brook trout life stages are canalized and unlikely to benefit from assisted gene flow. It would therefore be advisable to experimentally test the efficacy and potential benefits of evolutionary rescue for thermal performance and fitness before implementing this approach as a potential mitigation tool for climate change effects.

Supplementary material

Supplementary material is available at *Conservation Physiology* online.

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Author contributions

C.C.W. and G.B. designed the experiment; C.J.C. collected and analyzed the data; C.J.C., C.C.W. and G.B. wrote the article.

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