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Thermal conditions during embryogenesis influence metabolic rates of juvenile brown trout *Salmo trutta*

Richard D. Durtsche,[1,2,†] Bror Jonsson,[3] and Larry A. Greenberg[1]

[1]*River Ecology and Management Group, Department of Environmental and Life Sciences, Karlstad University, Karlstad S-651 88 Sweden*
[2]*Department of Biological Sciences, Northern Kentucky University, Highland Heights, Kentucky 41099 USA*
[3]*Norwegian Institute for Nature Research, Sognsveien 68, Oslo 0855 Norway*

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Abstract. The projected climate change and increase in thermal conditions in northern latitudes over the next 60 yr has the potential to alter the metabolic scope and potential fitness of aquatic ectotherms. Here, we experimentally tested if elevated egg incubation temperature affected metabolic scope in juvenile brown trout (*Salmo trutta*) as a phenotypically plastic response. Cohorts of brown trout from anadromous and resident crosses were raised through embryogenesis in either natural river temperatures (cold) or elevated (+3°C, warm) temperatures until they could feed exogenously. The standard metabolic rate (SMR), maximum metabolic rate (MMR), and aerobic scope (AS = MMR − SMR) of juveniles from four anadromous-resident crosses and from both incubation temperatures were tested at 13°C. We found that metabolic measures (SMR, MMR, AS) were lower in warm than cold-incubated fish. There was no difference in the metabolic rates of fish from different anadromous-resident crosses. The results of this experiment are consistent with the countergradient variation hypothesis (CGV) in which phenotypic variation, in this case variation in metabolic rates, is inversely related to thermal conditions, originally proposed in relation to altitudinal or latitudinal gradients. While previous studies have related CGV to genetic differences between populations, our study shows that thermal differences encountered at the embryonic stage can produce a phenotypic pattern consistent with CGV. It is difficult to predict the consequences of these metabolic changes in a future warmer climate, as lower metabolic rates indicate that brown trout will probably expend less energy, but a reduced aerobic scope may counteract this affect, limiting their ability as a top predator and in escaping predators. Our results suggest that there are mechanisms used to adjust to elevated water temperature that can be initiated during embryogenesis. Given that there were no differences among crosses, it is likely that temperature-induced differences are the result of plastic responses.

Key words: aerobic scope; brown trout; climate change; embryogenesis; incubation temperature; maximal metabolic rate; standard metabolic rate.

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† **E-mail:** durtsche@nku.edu

Introduction

Biological responses to temperature are the focus of much recent research, not the least because of ongoing, large-scale, human-influenced climate change (Norin and Metcalfe 2019). The climate is changing faster than ever before (IPCC 2013), and the ability of poikilothermic animals to cope with this depends, in part, on their phenotypic and genetic responses

to thermal changes. Temperature is a pervasive environmental factor that governs the biochemical and physiological reactions of poikilotherms, with subsequent effects on their behavior and ecology (Schulte 2015).

Metabolism is the sum of all reactions that yield energy, which organisms can use for different activities (Fry 1971). The magnitude of an organism's metabolic rate provides an overall description of its physiological status. There is a minimum metabolic rate needed to sustain resting, unfed individuals, typically denoted as standard metabolism or the standard metabolic rate (SMR). There is also a maximum sustainable rate of energetic expenditure or maximum metabolic rate (MMR) that cannot be exceeded without accumulating oxygen debt. For fish, MMR increases approximately proportionally to body mass (Brett and Groves 1979, Norin and Clark 2016). The difference between maximum and standard metabolism (MMR − SMR) is referred to as the aerobic scope (AS) for activity. These rates are important because they determine the well-being of organisms and their capacity to carry out their normal functions such as foraging and patrolling. As the rates are temperature-dependent and influence spatial distributions and change in phenology of populations, they are particularly important in climate change contexts (O'Connor et al. 2012).

Intraspecific variation in the relationship between AS for activity and temperature provides some evidence that genetic adaptation plays a role in metabolic capacities (Clarke 1993, Eliason et al. 2011). For instance, Eliason et al. (2011) reported that sockeye salmon *Oncorhynchus nerka* populations in the Fraser River that experienced more challenging migratory environments have greater AS than those with less arduous migrations and that the variation in AS is consistent with the historic river temperature ranges for each population. This suggests that physiological adaptation occurs at a very local scale, with population-specific thermal limits being set by physiological limitations in aerobic performance.

On the other hand, Elliott (1994), summarized studies of thermal adaptation in brown trout and found little if any controlled, experimental support for genetic variation in thermal performance of brown trout *Salmo trutta*. However, species variation need not be caused by changes in genetic structure, but instead, be a phenotypically plastic response, reducing variation among organisms growing up under different thermal conditions (Bossdorf et al. 2008). If so, this may be facilitated by adaptively programming of organisms in early life (Bateson et al. 2014). For instance, Cook et al. (2018) reported that temperatures experienced by brook trout *Salvelinus fontinalis* embryos affected body mass and routine metabolic rates of free-swimming fry. Furthermore, in the snapping turtle *Chelydra serpentine*, egg incubation temperature correlates negatively with later SMR (O'Steen and Janzen 1999, Steyermark and Spotila 2000). Turtles coming from eggs incubated at 21.5°C had a SMR that was 160% that of turtles from 30.5°C-incubated eggs. In this way, the environmental influence appeared to counteract the genetic influence. This finding accords with the countergradient variation hypothesis.

Countergradient variation (CGV) occurs when an environmental gradient counteracts the phenotypic variation influenced by the genetics of a population (Levins 1969, Conover and Schultz 1995). This can cause different populations of the same organism to display similar phenotypes regardless of their underlying genetics and differences in their environments. CGV can be useful in predicting responses of an organism to a change in climatic conditions (Conover and Schultz 1995). This is because the CGV hypothesis assumes that cold environments favor high metabolic rates to compensate for the negative effect of low temperatures, and/or that warm environments favor low metabolic rates, enabling organisms to conserve energy in an otherwise costly environment.

The CGV is not limited to geographical variation in environmental gradients, but may impact local populations that are influenced by changing conditions, such as changes in thermal conditions as a result of global climate change. These changes in populations could occur during the lifetime of the species, or even during a particular life stage as well as over relatively short evolutionary time periods (several generations; Bateson et al. 2014). For instance, Álvarez et al. (2006) reported a negative correlation between the temperature experienced during the first 2 months after yolk resorption and SMR for

brown trout from six streams. They suggested that this was due to selection caused by the different thermal regimes in the streams from which they originated. Similarly, Cook et al. (2018) reared embryos and alevins of brook trout *S. fontinalis* at either 5°C or 9°C and then acclimated the fry to either matched or mismatched temperature treatments once yolk sacs were resorbed. Variation in routine metabolism of the fish differed with acclimation temperature, which may indicate a phenotypic response in metabolic rate to temperatures experienced earlier in life. Furthermore, temperature during the embryo stage affects body growth of juveniles, as found for Atlantic salmon *Salmo salar* (Finstad and Jonsson 2012, Burgerhout et al. 2017) and Senegal sole *Solea senegalensis* (Carballo et al. 2018). Thus, there is reason to believe that temperature during early development affects metabolic rates and the scope for activity of fish later in life.

Brown trout is a partly migratory species (cf. Jonsson and Jonsson 1993) with populations exhibiting both freshwater resident phenotypes and anadromous phenotypes that migrate to the food-rich sea environment (Jonsson and Jonsson 2011). There is evidence to suggest that the decision to migrate has genetic but also phenotypically plastic components (Olsson et al. 2006, Lemopoulos et al. 2019). In addition, the trait is related to metabolic demands, where fast-growing fish constrained by resources in the natal habitat migrate sooner than slow-growing conspecifics (Forseth et al. 1999, Olsson et al. 2006, Wysujack et al. 2009).

Here, we experimentally test two hypotheses about the effects of egg incubation temperature and life-history pattern of the parent phenotype on metabolism of juvenile brown trout. First, we test the hypothesis that juveniles incubated as eggs in cold water will have higher SMR and MMR than conspecifics developed from eggs incubated in warm (+3°C) water. The relationship for AS is more difficult to predict, but we expect that warm-incubated fish will have aerobic scopes comparable to or narrower than cold-incubated fish. If our hypothesis regarding SMR and MMR is corroborated, this would suggest that countergradient variation in metabolic rates may be best explained as a phenotypic response, influenced by the thermal regime encountered prior to hatching, and not because of local adaptation through selection as originally hypothesized (Levins 1969, Conover and Schultz 1995). Second, we test the hypothesis that at warmer temperatures the offspring of freshwater resident trout will have a lower AS than offspring of anadromous trout, with intermediate values for anadromous × resident crosses. This hypothesis is based on the notion that the offspring of freshwater resident trout will have a lower AS to compensate for the limited resources of their freshwater environment, as selection should favor reduced metabolic rates in habitats with limited resources (Mueller and Diamond 2001). Alternatively, if the metabolic rates of the two life-history types are similar, this indicates that the life-history decision is chiefly environmentally determined (Ferguson et al. 2019). With the lack of trout-type effect on metabolic rate, migratory behavior may not be hard-wired genetically, but also environmentally induced as a knock-on effect of early environment. We test these two hypotheses by using a common garden experiment. Brown trout *S. trutta* offspring (parr) from all breeding cross combinations (male and female of anadromous and resident fish), held through embryogenesis at either ambient (cold) or +3°C (warm) temperatures, and then all maintained through a growth period at ambient river temperature, were evaluated for differences in their SMR, MMR and AS.

Materials and Methods

The experiment was conducted at the Ims Research Station, operated by the Norwegian Institute for Nature Research (NINA) in southwestern Norway (59° N, 6° E). The fish used in the experiment were the offspring from crosses of anadromous brown trout, collected in a fish trap 150 m upstream of the mouth of the River Imsa as they returned from the sea, and resident brown trout, collected by electrofishing in the Fossbekk Stream, a tributary to the River Imsa 1 km upstream from the sea. The anadromous sea trout were easily identified as they were all individually tagged when leaving the river as smolts (Jonsson and Jonsson 2009), and were also distinguished from freshwater resident fish by coloration, ectoparasites, and body form (cf. Jonsson 1985). All of the trout in the Fossbekk

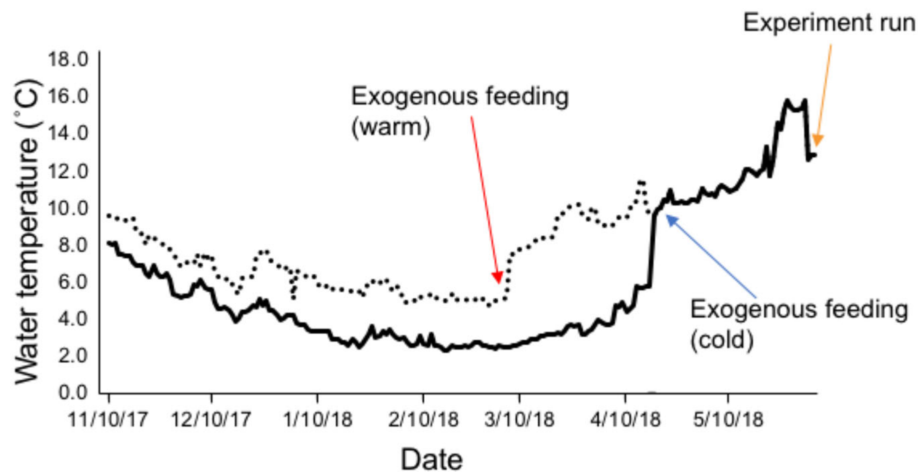

Fig. 1. Temperature (°C) during incubation and post-hatching of brown trout *Salmo trutta*, including the period in which respiration and ventilation rates were measured. Dashed line shows temperature for the warm water groups and the solid line for the cold-water groups.

Stream are resident as an artificial waterfall, constructed in 1993, prevents anadromous trout from reaching this stream (Jonsson and Jonsson 2017). Four different crosses were created: anadromous male × anadromous female, anadro mous male × resident female, resident male × resident female, and resident male × anadromous female. Eggs were collected from four anadromous (mean ± 1 standard deviation [SD], 754 ± 642 g; range 257–1443 g) and four resident females (mean ± 1 SD, 170 ± 18 g; range 133–171 g) and fertilized with milt collected from four anadromous (mean ± 1 SD, 423 ± 253 g; range 167–659 g) and four resident males (mean ± 1 SD, 156 ± 70 g, range 64–233 g) on 10 November 2017. Each adult was used in two different crosses, one by partnering it with a resident trout and the other with an anadromous trout. In total, 16 different family groups were created, representing four replicates of each of the four crossings. Two replicates of each of the four crossings were incubated using water piped in from the River Imsa to the NINA station at either ambient (cold) temperature conditions or at temperatures elevated approximately +3°C above ambient (warm) conditions (mean ± 1 SD, 3.1 ± 1.5°C) to simulate the potential climate impact on embryogenesis and lifetime condition of the fish (Fig. 1). Water temperature in the Imsa is recorded daily all year round, and annual mean minimum and maximum temperatures are 2°C in winter and 18°C in summer (cf. Fig. 1 in Jonsson and Jonsson 2017). There are no water temperature records for the surface-fed Fossbekk tributary, but based on the 20–25 m elevational difference between Fossbekk Stream and the River Imsa, differences in water temperatures should be marginal.

A 2 × 4 factorial design (two incubation temperatures and four crossings) was used, resulting in eight treatments, with each treatment replicated twice. Fish raised at ambient conditions are hereafter referred to as cold fish and fish raised in heated water as warm fish. Approximately 100 eggs were acquired from each cross, and cross cohort was raised separately in incubation trays with constant water flow until the start of exogenous feeding. On 6 March and 17 April 2018, the warm and cold trout were moved into 60-L tanks, respectively, to coincide with their ability feed on external food. The following day, exogenous feeding was initiated, using commercial trout pellets (Ewos, Cargill, Norway). From 18 April 2018 onwards, all trout were maintained at ambient water temperature conditions from the River Imsa. River temperatures increased with the progression of spring. While fish growth may be influenced by incubation temperature, it is unclear if growth of these trout is influenced by post-hatching differences in temperature. Finstad and Jonsson (2012) tested if temperature after hatching influenced growth of juvenile Atlantic

salmon, but no effect was found. The only effect on growth was temperature prior to hatching.

From 30 May to 4 June 2018, respiration (oxygen consumption) and ventilation rates were measured for trout from the eight treatments. For measures of respiration, two static flow respirometry systems with four fiber-optic dissolved oxygen sensors per system were used (two 4-channel FireStingO_2 Optical Oxygen Meters, PyroScience, Aachen, Germany). Each system consisted of four 50 mL respirometry chambers. Each system had its own external digital thermocouple (Pt100-TSUB21 Temperature Probe, PyroScience) positioned at the center of each set of chambers during the trials to enable automatic temperature compensation of the oxygen sensor signals by the FireStingO_2 software. These two systems were submerged in a single 500-L fiberglass tank (water depth 30 cm), continuously supplied with fresh river water via a flow-through system. Chambers were placed on submerged gray cinder blocks within the tank, as preliminary observations showed that the fish moved less when these were used, presumably because of reduced stress. With two systems of four chambers each, we could measure oxygen consumption for one trout from each of the eight treatments simultaneously. The two systems were programmed so that oxygen concentrations were measured every 15 s for 15 min under static flow (i.e., no flow), followed by a 15 min long water recharge period (Clark et al. 2013, Rosewarne et al. 2016, Svendsen et al. 2016). It should be noted that these chambers, when closed, were too small (50 mL) to use water mixing devices, though opercular breathing movements and occasional fish movements in the chamber contributed to mixing during the respiration measurement periods. Respiration and recharge periods (15 min each) were determined by the limitations posed by the 24-h timer used to turn on and off the flushing pump. The respirometry system was typically started 5–15 min before fish were added to the chambers and stopped after completion of the SMR and MMR measurement recordings, ~5–7 h later. After a trout was placed in a 50 mL chamber, 9–10 oxygen consumption measurements were recorded for SMR measurements. If oxygen levels remained <80% after three measurement periods these fish were omitted (n = 8), otherwise fish trials were retained for analysis. All fish were only tested once. At the end of the SMR respiration trial, each fish was removed from its chamber, placed in a circular chase tank (~500 mL), and kept swimming at maximum speed to allow burst-glide swimming. This was done by lightly touching the fish's caudal fin with the handle end of a small dipnet, keeping the fish swimming at maximum speed for 2 min. This method of maintaining maximum swim speed has been previously used as a measure of MMR (Reidy et al. 1995, Norin and Malte 2011, Clark et al. 2013, Norin and Clark 2016). After 2 min, when the fish was exhausted and did not respond to touch, the fish was immediately (<10 s) returned to its respiration chamber, and a final measure of oxygen consumption was made, representing its maximum respiration, MMR. Post-chase oxygen consumption was measured for 10–15 min, ensuring that MMR was attained, and this typically occurred within the initial 5 min of measurements. A total of 11 replicate trials for SMR and MMR were conducted for each breeding cross × incubation temperature treatment. Even though water from the Imsa River was constantly flowing through the 500-L tank, ambient water temperature was consistent across respiratory chambers and trials (mean ± 1 SD, 13.44 ± 0.02°C; range 12.9–13.8°C; Table 1). At the completion of a trial, the trout were removed from the chambers and total length was measured in a measurement tray, where the fish could be kept moist and under low stress (Table 1). Ideally, the mass of each fish should be measured after the trial, but because sample sizes for some treatments were low and the fish were needed for subsequent experiments, only lengths were measured (such small fish would need to be sacrificed to obtain accurate measures of mass). To convert the total lengths (mm) to mass (g), the total length and mass of 772 fish, representing fish from all treatments, were measured, producing a regression of mass on total length ($F_{1,\,770}$ = 149,298.9, P < 0.001, R^2 = 0.995; $\log_{10}(\text{Mass}) = -2.229 + 3.28 \times \log_{10}(\text{Length})$).
There was no relationship between oxygen consumption rates (mg O_2/min) and body mass (g; $F_{1,78}$ = 2.304, P = 0.133, R^2 = 0.029), suggesting that there are no allometric effects of body mass on metabolic rate for the fish used in this study. For each replicate, the mass-specific metabolic rate was calculated using the following formula:

Table 1. Measurements of the size range and sample size of brown trout used in each sample run.

Trial	Chamber temperature (°C)	n	Total length (mm)			Calculated mass (g)		
			Mean ± 1 SD	Min	Max	Mean ± 1 SD	Min	Max
1	13.18 ± 0.19	8	42.6 ± 6.61	35	54	0.74 ± 0.40	0.36	1.48
2	13.38 ± 0.05	8	41.9 ± 6.35	33	48	0.68 ± 0.27	0.29	1.01
3	13.39 ± 0.04	8	42.6 ± 4.55	34	52	0.73 ± 0.35	0.33	1.31
4	13.59 ± 0.12	8	41.1 ± 1.61	37	50	0.63 ± 0.25	0.43	1.16
5	13.48 ± 0.10	8	45.4 ± 5.66	36	54	0.88 ± 0.35	0.39	1.49
6	13.43 ± 0.09	8	43.1 ± 3.31	38	47	0.72 ± 0.18	0.47	0.94
7	13.31 ± 0.08	8	44.1 ± 3.44	39	49	0.78 ± 0.20	0.51	1.08
8	13.66 ± 0.09	8	42.6 ± 5.10	36	50	0.72 ± 0.29	0.39	1.16
9	13.75 ± 0.08	8	43.9 ± 3.48	38	49	0.77 ± 0.19	0.47	1.08
10	13.35 ± 0.05	8	41.9 ± 2.85	38	45	0.65 ± 0.14	0.47	0.82
11	13.33 ± 0.17	8	41.8 ± 4.13	33	46	0.66 ± 0.19	0.29	0.88

Note: Water temperatures (mean ± 1 standard deviation [SD]) are given for each sample run.

$\dot{M}_{O_2} = \Delta O_2 \times (V_{chamber} - V_b)/\Delta t \times M_b$, where ΔO_2 is the change of oxygen concentration (mg/L) in the respirometer, $V_{chamber}$ is the respirometer chamber volume (mL), V_b is the volume of the fish body (assumes density of 1 kg/L), Δt is the change in time (h), and M_b is the fish mass (g; Clark et al. 2013). The mean fish to chamber ratio in this study was 1:69. The respirometry chambers were gently cleaned daily with wooden stick cotton swab applicators to reduce or eliminate any bacterial growth in the chambers. No cleaning solvents (e.g., bleach) were used to clean chamber walls, as done in many respiration studies, because (R. D. Durtsche, *personal observation*) cleaners can be potentially toxic for fish through adsorption on glass and later release to the water, and also because cleaners may damage the luminescent sensor coatings of the oxygen spot sensor in each chamber. Instead, oxygen consumption was measured in each chamber before each fish was added and after the fish was removed as a measure of background microbial respiration that might exist in the system in the absence of fish.

In addition to respiration rates, the number of opercular movements, referred to as ventilation rate, was counted for each trout while in its respiration chamber. Each fish was filmed (Canon XA10 camera, Canon Inc., Tokyo, Japan) for approximately 30–60 s toward the end of each SMR trial. The films were analyzed to obtain ventilation rates expressed as opercular movements per minute for comparison with corresponding oxygen consumption values from the final measurement of SMR. Two videos were not useable because the camera battery died early in the recording and were excluded from the analyses.

To obtain estimates of oxygen consumption, we calculated linear regression coefficients (slopes) for the relationship between oxygen concentration in the fish chamber and time, based on the measurements of oxygen concentration taken during static flow (9–10 measurement periods). Linear regression coefficients values were generated from the respR aquatic respiratory analysis package (Harianto et al. 2019) in R (software version 3.5.2; R Core Team 2018) using RStudio version 1.2.5033 (RStudio Team 2019). The auto-rate function was used in respR to perform rolling regressions on the data frame to determine the most linear rates of slopes for respiration bouts represented in SMR periods and to detect the maximum slope from the MMR measurement period for each fish. The two measures of oxygen consumption in the absence of fish (before each fish was added and after fish it was removed) were used in the respR program to estimate background respiration over the course of each trial. The respR program uses these initial and final rates to derive a linear relationship based on time for the background rate, and these values based on time of each respiratory bout were subtracted from overall respiration per bout when the fish were present in the chambers to calculate each fish's SMR and for the MMR calculation. Using the lowest 10% of all respiratory measurements for

each fish to calculate SMR would have meant using only one oxygen consumption slope for this estimate of SMR. To avoid an underestimation of SMR, we instead programmed respR to use the mean of the lowest 30% of the slopes (three slopes per fish) in this calculation as done in Nyboer and Chapman (2017). We made these calculations for 80 fish from this study (eight were eliminated from analysis based on oxygen levels below 80% throughout the measurement trials). The regression coefficients were standardized for body mass using the total length to mass equation. We ran separate two-way ANOVAs on SMR, MMR, and AS with both incubation temperature (cold, warm) and breeding cross as independent variables. We also ran a two-way ANOVA on ventilation rates (opercular movements·min^{-1}·body mass (g)$^{-1}$) with both incubation temperature and breeding cross as independent variables. Data not normally distributed were $\log_{10}$ transformed to meet assumptions of normality based on Kolmogorov–Smirnov normality tests. All data fulfilled the assumption of homoscedasticity as tested with Levene's test, and all analyses except the regression analyses of oxygen consumption, which were run on respR, were conducted with the IBM SPSS statistics package, version 26 (IBM Corp. 2019). Effect size on ANOVAs was calculated as Omega squared (ω^2) statistics (Olejnik and Algina 2003, Field 2014). Alpha (α) for all tests was set at 0.05.

Results

The warm trout hatched and started feeding endogenously ~5–6 weeks earlier than cold trout (Fig. 1). With this earlier feeding start, warm-incubated fish were larger ($\overline{X}_{\text{warm}}$ = 44.8 ± 0.7 mm, range 33–54 mm) than cold-incubated fish ($\overline{X}_{\text{cold}}$ = 39.9 ± 0.5 mm, range 33–45 mm) (Fig. 2; Table 2). There were no length differences among breeding crosses, nor was there an interaction between cross and incubation temperature.

Average SMR of warm-incubated *S. trutta* (back-transformed = 0.05 mg O_2·h^{-1}·g^{-1}) was 2.56 times lower than the average for cold-incubated fish (back-transformed = 0.13 mg O_2·h^{-1}·g^{-1}; Fig. 3A and Table 2). A two-way ANOVA on the $\log_{10}$-transformed SMR showed a difference between fish incubated at different temperatures. There was no difference among breeding crosses in SMR, nor was there an interaction effect between incubation temperature and breeding crossing.

A similar pattern was observed for MMR, with warm trout having 1.38 times lower average maximum oxygen consumption rate (1.133 mg O_2·h^{-1}·g^{-1}) than the cold trout (1.566 mg O_2·h^{-1}·g^{-1}; Fig. 3B and Table 2). The two-way ANOVA verified a difference in MMR between fish incubated at warm and cold temperatures; however, no differences were found among breeding crosses or the interaction effect between incubation temperature and breeding crossing. The AS likewise had a similar pattern (Fig. 3C), with warm trout having a 1.33 times lower average scope (1.046 mg O_2·h^{-1}·g^{-1}) than the cold trout (1.389 mg O_2·h^{-1}·g^{-1}), but there was no difference found among breeding crosses and there was no interaction effect. However, visual inspection of Fig. 3B, C suggests that AA responded differently than the other three crosses. Therefore, we conducted a series of simple effects one-way ANOVAs with False Detection Rate (FDR) corrections, recognizing that we have less power with n = 20 rather than n = 80. Here, we find for MMR and AS that none of the

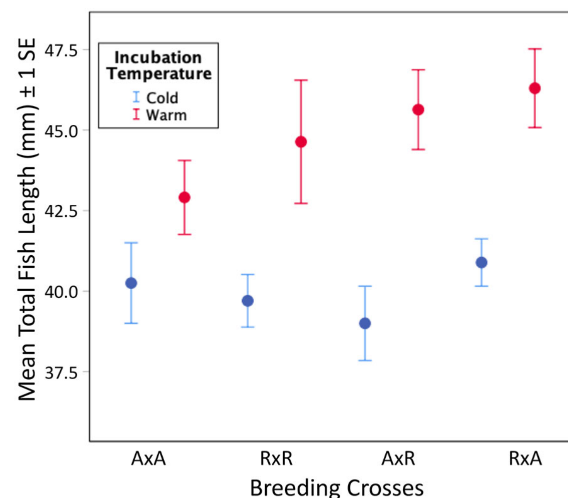

Fig. 2. Total body length (mm) ± 1 standard error (SE) of brown trout *Salmo trutta* from the four anadromous-resident crosses incubated in ambient (cold) and elevated (warm, +3°C) temperatures taken at the time of respiration measurements.

Table 2. Two-way ANOVA results of incubation temperature (T; cold, warm) and breeding cross (C; A × A, R × R, A × R, R × A) factors of brown trout (*Salmo trutta*) on total fish length, standard metabolic rate (SMR), maximum metabolic rate (MMR), aerobic scope (AS), and ventilation rate.

Variable and statistic	n	T	C	$T \times C$
Total length	80			
F		29.796	0.846	0.834
P		<0.001**	0.473	0.479
ω^2		0.26	0.001	0.001
SMR	80			
F		17.924	0.057	2.019
P		<0.001**	0.982	0.119
ω^2		0.17	0.009	0.01
MMR	80			
F		11.150	0.633	0.916
P		0.001**	0.596	0.438
ω^2		0.11	0.012	0.003
AS	80			
F		7.239	0.712	1.555
P		0.009**	0.548	0.208
ω^2		0.08	0.009	0.020
Ventilation	78			
F		24.955	1.157	0.774
P		<0.001**	0.332	0.512
ω^2		0.23	0.005	0.007

Notes: ANOVA statistics, significance values, and effect size results are given. Effect size was calculated as omega squared (ω^2).

** $P < 0.01$, *** $P < 0.001$.

crosses showed significant incubation temperature effects, but that the level of significance was near 0.05 for RR, AR and RA (MMR $0.06 < P < 0.084$; AS $0.086 < P < 0.16$), but not for AA (MMR $P = 0.83$; AS $P = 0.63$). Thus, there is a tendency for AA to behave differently than the other crosses.

Regression analysis revealed a positive relationship between ventilation rate and SMR ($F_{1,76} = 15.117$, $P < 0.001$, $R^2 = 0.166$; ventilation rate = 451.07 × O_2 consumption rate − 129.65). The ventilation rate of trout incubated under warm conditions averaged 181.0 ± 12.2 beats·min^{-1}·g^{-1}, range 75–456 beats·min^{-1}·g^{-1}, while the average rate for cold trout was 270.07 ± 12.2 beats·min^{-1}·g^{-1}, range 177–477 beats·min^{-1}·g^{-1} (Fig. 4; Table 2). Similar to SMR measures, a two-way ANOVA for ventilation rate data revealed a difference between warm and cold-incubated fish, but not among breeding crosses, nor for the interaction between incubation temperature × breeding cross.

Discussion

We found that the early thermal conditions experienced by brown trout during the egg stage affected energetic requirements of a later life stage, namely the juvenile stage. We hypothesized that SMR, MMR, AS, and ventilation rate were lower for juveniles originating from eggs incubated at elevated temperatures than for juveniles originating from eggs incubated at colder ambient temperatures, and our results were overall consistent with this hypothesis. We also hypothesized that under warmer conditions offspring of freshwater resident trout would have lower metabolic scopes compared to other crosses to compensate for presumed resource limitation in their freshwater environment. We reject this hypothesis, as there was no difference in the metabolic rates of warm fish from the different crosses. Thus, the effect of incubation temperature, in combination with the lack of trout-type crossing effect, implicates phenotypic plasticity and not local adaptation as an explanation for the metabolic response by brown trout.

Our results are consistent with the countergradient variation hypothesis (CGV; Levins 1969, Conover and Schultz 1995). The significant effect of incubation temperature corroborates previous findings of an inverse relationship between SMR for populations in relation to temperature differences caused by variation in latitude or altitude (Garland and Adolf 1991, Ayres and Scriber 1994, Sinnatamby et al. 2015). Mechanistic studies of the CGV have typically raised different populations in the same environment, eliminating environmental effects on phenotypes, so that any observable differences can be attributed to genetics. Here, we eliminated genetic differences and found that environmental differences at the embryonic stage alone could produce a pattern consistent with the CGV. Thus, the metabolic differences observed in brown trout raised under different thermal conditions are likely to be the result of phenotypic plasticity within the species. Moreover, this phenotypic plasticity may represent a response to the environmental condition change experienced in early development. Such plasticity could alter efficiency of the

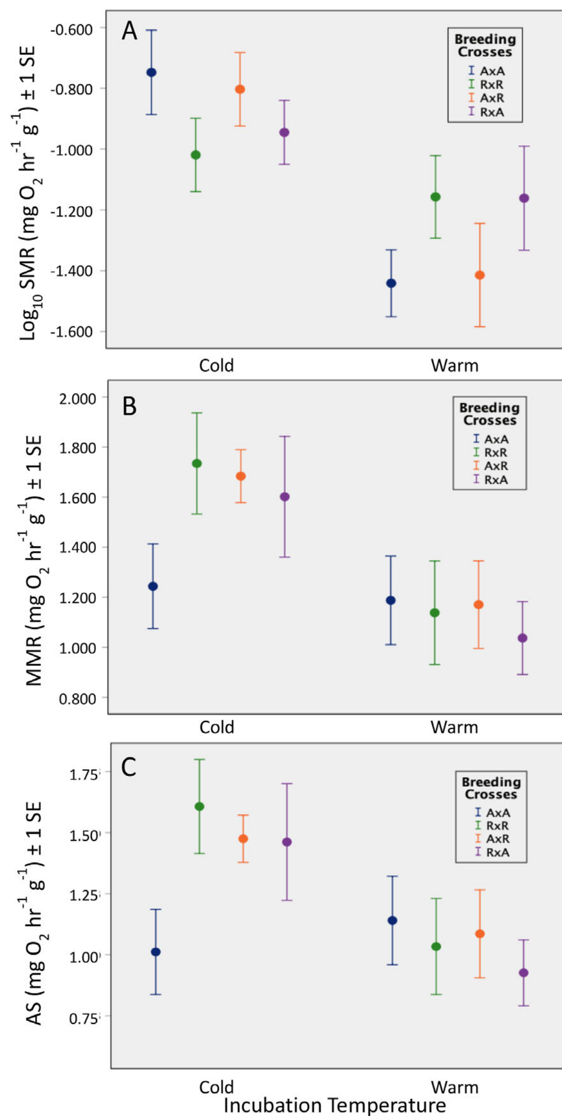

Fig. 3. Measures of (A) standard metabolic rates (SMR; mean ± 1 standard error [SE]), (B) maximum metabolic rates (MMR; mean ± 1 SE), and (C) absolute aerobic scope (AS; mean ± 1 SE) of brown trout *Salmo trutta* from the four anadromous-resident crosses incubated in ambient (cold) and elevated (warm, +3°C) temperatures.

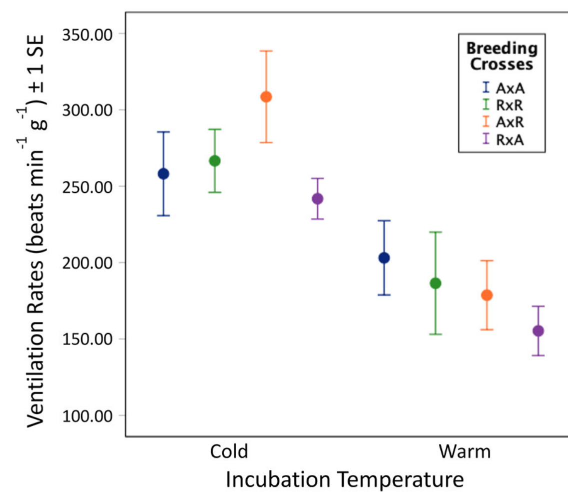

Fig. 4. Ventilation rates (beats/min; mean ± 1 standard error [SE]) of brown trout *Salmo trutta* from the four anadromous-resident crosses incubated in ambient (cold) and elevated (warm, +3°C) temperatures.

cardiovascular system or lead to changes in mitochondrial function, resulting in lower metabolic rates and physiological capacities under warmer incubation temperatures. For example, Schnurr et al. (2014) found that elevated embryonic temperature in zebrafish *Danio rerio* altered metabolic activity of adult fish via reduced activity of oxidative enzymes such as cytochrome *c* oxidase, citrate synthase, and pyruvate kinase in the swimming muscles. Metabolic rates (e.g., oxidative phosphorylation, Na^+/K^+ ATPase pumps) could also be altered by changes to the fluid cell membranes of the mitochondria from a homeoviscous adaptation (Hazel 1995) during embryogenesis. If fish developing under warm conditions produced fewer fluid cell membranes, and those membranes became less functional at colder temperatures then metabolic rates would be lower than cold developed fish. Additionally, elevated developmental temperatures have been shown to alter cardiac anatomy (increased ventricular roundness), lower aerobic exercise performance, and affect gene upregulation in zebrafish, conditions that persist into adulthood (Dimitriadi et al. 2018).

The AS was lower for trout raised at elevated incubation temperatures than for trout raised at natural (cold) incubation temperatures. Sandblom et al. (2016) documented that MMR has a ceiling that is consistent across a range of temperatures in fish. However, we did not see a uniformity in MMR when comparing warm and cold-incubated trout. It could be that the fish in our study did not experience high enough temperatures during testing so that they could reach their maximal metabolic ceiling. Nevertheless,

the experiment was performed at the optimal temperature for growth from this population (Forseth et al. 2009), and the lower overall aerobic scope in warm fish suggests that they may avoid or lose their capacity to deal with a range of conditions for various activities influenced by metabolic rates. An example of a reduction in MMR and AS is observed in some tropical fish species after long acclimation periods above their natural temperatures, and such a response has been explained as an increase in metabolic efficiency to survive these adverse conditions (Norin et al. 2014, Nyboer and Chapman 2017). The phenotypic plasticity responsible for a shift to reduced metabolism and a narrower AS in fish that experienced warmer temperatures through embryogenesis may be a result of genetic adjustments to environmental interactions.

Patterns of ventilation rates of fish corresponded to oxygen consumption and followed the same patterns as SMR. We thus conclude that opercular beating frequency during ventilation (ventilation rate) can be used as a proxy for metabolic rate in juvenile brown trout, similar to what was found for Atlantic salmon (Millidine et al. 2008) and other fish species (Frisk et al. 2012, Rosewarne et al. 2016). While ventilation rates are not as precise as respiration rates to measure oxygen consumption, and the relationship between metabolic rate and ventilation rate that we found was weak, with a low R^2 value, ventilation rate could be used as a proxy for metabolic rate for juvenile brown trout when precise estimates are not required.

We reject our second hypothesis that under warmer temperatures fish from resident crosses had lower metabolic rates than fish from anadromous crosses. Whether or not the brown trout originated from anadromous or resident parents, or some combination thereof, they responded metabolically in a similar fashion to altered embryonic temperature differences. There was a tendency for the AA cross to not show any metabolic plasticity and thus behave differently than the other three crosses when we evaluated each cross independently with one-way ANOVAs. However, the lack of significant effects and the reduced power of these tests does not allow us to draw any firm conclusions about this, but it does suggest that this may be an interesting avenue for further study. It is perhaps not surprising though that we did not find an effect of cross type as the two life-history forms have been reported from the same population (Jonsson 1985) and can even be offspring of the same parents (Nevoux et al. 2019). In our common garden study, it is important to realize that the trout were not raised under fully natural conditions where other environmental stressors could have influenced metabolic responses of fish, with repercussions for the decision to migrate. However, as a common garden experiment with similar conditions during embryogenesis (except temperature), the lack of an effect of trout type on metabolic rate under warmer conditions indicates that migratory behavior is phenotypically plastic. Giger et al. (2006) reported that they found no inherent genetic difference between resident and migratory brown trout, but instead they found differences in gene expression related to their mode of lifestyle. This was supported by Amstutz et al. (2006), who identified the transaldolase 1 gene (Taldo1) as being differently expressed in the liver between resident and migratory brown trout just before the onset of migration. The presence of two life-history forms is common in several salmonid fish species, and thus patterns observed in closely related species are likely relevant to brown trout. For example, Baerwald et al. (2016) identified 57 differentially methylated regions (DMRs) between smolts and freshwater resident juvenile rainbow trout *Oncorhynchus mykiss* that may influence their genetic expressions. Many of the DMRs encode for proteins relevant to migration-related transitions and smolting (e.g., circadian rhythm pathway, nervous system development, protein kinase activity). There is yet little information about possible causes of different DNA methylations, although low body temperature appears associated with higher DNA methylation levels (Kakutani 2002, Varriale and Bernardi 2006). Moreover, we already know that environments play large roles in brown trout's decision to migrate or remain resident (Olsson et al. 2006, Davidsen et al. 2014, Jones et al. 2015).

While the physiological basis of partially migratory fish is not well understood, brown trout do show a continuum of migratory strategies (Jonsson and Jonsson 1993). Boel et al. (2014) suggest that the migratory distance achieved by these fish depends on the nutritional condition or

status of the fish when it smolts and migrates. At the time of smolting, energy reserves of brown trout are low and at the same level as breeders after spawning (Jonsson and Jonsson 1998), and particularly low in short-distance migratory anadromous brown trout (Boel et al. 2014). With lower metabolic rates under climate change conditions, it is unclear whether or not these fish will migrate to the same extent as now, as it is expected that feeding opportunities will increase in a warmer climate in northern streams, making sea migration less favorable (Finstad and Hein 2012).

Using fish from the same source, with similar environmental backgrounds, and altering the temperature at which eggs are incubated, allowed us to explore how ectotherms respond to thermal changes such as those predicted by climate change models (IPCC 2013). Our results indicate that warmer winter temperatures during embryogenesis will produce fish that hatch earlier and are likely to have lower metabolic rates as juveniles (SMR, MMR) than fish produced from eggs subjected to colder winter temperatures. With the predicted increase in winter air temperatures for different scenarios averaging by as much as +6°C in Sweden over the next 60 yr (Swedish Meteorological and Hydrological Institute's Rossby Center, https://www.smhi.se/en/climate/future-climate/climate-scenarios/sweden/nation/rcp85/winter/temperature), aquatic ecosystems will most certainly be affected (Harvey et al. 2011, Vliet et al. 2011).

If resources are plentiful during these periods of climate change, trout should still grow rapidly (Finstad and Jonsson 2012), which in turn may result in a younger age at migration but a larger proportion could remain as freshwater residents because the advantage of migration will decrease (cf. Gross et al. 1988). This expectation is consistent with field-based observations showing that natural cohorts of juvenile salmon that undergo embryogenesis in warm winters tend to grow faster and move to sea at a younger age than cohorts developed from eggs incubated during colder winters (Jonsson et al. 2005). On the other hand, reduced metabolism and aerobic scope could also reduce fitness if the fish are unable to compete for food in an altered fish community, or escape from predators (Auer et al. 2016). Pintor et al. (2014) suggested that reduced plasticity could alter their performance as a top predator. For the related Atlantic salmon, juveniles were found to have higher metabolic rates (increased MMR) in low-food environments to maintain their high competitive ability in foraging (Auer et al. 2018). If however, elevated temperatures associated with future climate change occur during embryonic development, *S. trutta* may not have the capability to attain high enough metabolic rates to be effective foragers. If food also becomes scarce under these predicted environmental conditions, growth may be poorer and the trout may mature at an older age and potentially smaller size (Jonsson et al. 1984). Nutritional and energetic deficiencies (e.g., reduced lipids) and their lower aerobic scope could impact the decision of brown trout to migrate or partially migrate (Forseth et al. 1999), and thus alter their life-history decision in a future warmer climate (Jonsson and Jonsson 2009).

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Data Availability

The data that support the findings of this study are located in the Dryad Data Repository: https://doi.org/10.5061/dryad.fttdz08rb (Durtsche et al. 2020).