Metabolic and transcriptomic response of two juvenile anadromous brook charr (*Salvelinus fontinalis*) genetic lines towards a chronic thermal stress

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Abstract

Many salmonid species are particularly susceptible to chronic and acute temperature changes caused by global warming. We aimed to study the differences in metabolic and transcriptomic responses of a chronic heat stress on a control and selected (absence of early sexual maturation and growth) line of brook charr *Salvelinus fontinalis* (Mitchill, 1814). We exposed individuals to different temperatures for 35 days (15, 17 and 19 °C). High temperature reduced the growth rate (in length) and the Fulton condition factor. Both maximal metabolic rate and the aerobic scope were higher in fish reared at 17 °C, while they decreased in fish maintained at 19 °C. The relative gene expression of cytochrome c oxidase was lower at 19 °C than at 15 °C. The relative gene expressions of both liver and gill *hsp90* was higher at the highest temperature. The standard metabolic rate, while not affected by temperature, was higher for the control line over the selected line. Only in the control line, the relative expression of *catalase* and of receptor of insulin-like growth factor-1 increased at 19 °C. Our results showed that the selected line was able to cope more effectively with the oxidative stress caused by the rise in temperature.

Key words: *Salvelinus fontinalis*, brook charr, chronic thermic stress, gene expression, respirometry, growth, genetic line

Introduction

Recent climate change repercussions have been shown to affect several biomes and species worldwide (Williams et al. 2008; Crozier and Hutchings 2014; Stitt et al. 2014). These observed changes can mostly be attributed to anthropogenic activities, which can act as stress factors for the impacted ecosystems (Häder and Barnes 2019). In aquatic environments, many abiotic factors such as temperature, dissolved oxygen, salinity, nutrient availability, and pH can be affected in response to climate change (Nunn et al 2007; Crozier and Hutchings 2014), but temperature fluctuations are considered to be the most impactful with regards to physiology of aquatic ectotherms (Brett 1971).

Fishes in particular are extremely sensitive to temperature fluctuations, especially when critical thresholds are met within the environments where they have evolved. For example, it has been predicted that salmonid distribution, reproduction, and survival will be negatively impacted by temperature increases in temperate water bodies (Williams et al. 2015). While often unable to acclimatize physiologically to these rapid temperature increases, target species have been shown to avoid at risk areas and to migrate towards more favourable environments, a phenomenon observed for several marine species originating from temperate regions (Munday et al. 2009).

High increase in temperature can lead to a disequilibrium in fish oxygen transport system (Norin et al. 2014) because oxygen solubility diminishes with temperature and can thus become limited at higher temperatures. In some species (e.g., rainbow trout *Oncorynchus mykiss* (Walbaum, 1792)) high temperatures lead to a reduction of haemoglobin affinity with oxygen (Jensen et al. 1993). When oxygen requirements increase, organisms are often constrained to increase their standard metabolic rate (SMR),

defined as the metabolic rate needed to sustain tissue oxygen requirements at rest, without food digestions, and at a given temperature (Glencross and Bermudes 2010). Organisms thus boost their energetic needs because of this increase in SMR, which can translate into greater food consumption. This increase in food consumption is even more important given that the energy conversion efficiency can be negatively influenced by rising temperatures (Khan et al. 2014). This temperature effect can also be observed at more active physiological states, such as when fish experience their maximum metabolic rate (MMR), thus affecting the energy available for activities such as migration, predator escape, food search, and epitomized through the aerobic scope.

When facing gradual temperature changes, fish can acclimate to preserve homeostasis (Pandley et al. 2021). This allows them to compensate for the temperature effects on given physiological functions by demonstrating reversible physiological traits (Crozier and Hutchings 2014; Schreck and Tort 2016). For example, a reduced growth rate can allow a reallocation of energy towards protein synthesis necessary for the achievement of given physiological functions under new thermal conditions. The synthesis of heat shock proteins (hsp) is a widely studied case occurring when temperatures become stressful for fishes (Lindquist and Craig 1988; Feder and Hofmann 1999; Basu et al. 2002; Ritcher et al. 2010, Deane and Woo, 2011).

Transcriptomics approaches are useful tools to inform about factors regulating growth rates and physiological responses to environmental changes. Genes coding for synthesis of insulin-like growth factor-1 (IGF-1) and receptors specific to IGF-1 (igf-1r) and GH (gh-1r*)* in liver and white muscle were shown to be of interest to evaluate growth performance in brook charr *Salvelinus fontinalis* (Mitchill, 1814) (Côté et al. 2007;

Martinez Silva et al. 2023). The survey of the expression of genes coding for proteins involved in secondary stress responses (heat shock proteins (hsp70 and hsp90), gill Na^+-K^+-ATPase, caspases and bcl-2), regulating aerobic (cytochrome oxidase, citrate synthase), anaerobic (lactate dehydrogenase, pyruvate kinase) metabolism, or the response to oxidative stress (superoxide dismutase, glutathione peroxidase, catalase) can also be informative about responsiveness to environmental changes (Migdal and Serres, 2011; Rebl et al. 2013; Scarso and Strukul, 2013; Akbarzadeh et al. 2018; Cheng et al. 2018; Antonopoulo et al. 2020; Kregel 2022). Transcriptome responses that indicate potential increases in oxidative stress and apoptosis have been suggested to occur at temperatures that lead to reduced physiological performance in fishes (Jeffries et al. 2018). Studying the differential expression of the genes that encode these proteins could potentially provide us with an understanding of the mechanisms driving the change in metabolic rate observed with increasing temperature.

Brook charr is a recreationally important species within its native range found in Eastern Canada and United-States (MFFP 2020). Optimal temperatures for this species have been found to fluctuate between 11-16 °C depending on local adaptations for the different populations (Baldwin 1957; Raleigh 1982; Hokanson et al. 1973; Chadwick and McCormick 2017). Laboratory studies have shown reduced ability to acclimate and an increase in expression of genes involved in a thermal stress response at temperatures >20°C for this species (Morrison et al. 2020; Mackey et al. 2021). Two different ecotypes can be observed for brook charr, one that relies entirely on freshwater habitats, while the other is anadromous (MFFP 2020). Within the province of Quebec, Canada, brook charr represent the most popular fish caught recreationally (MFFP 2020) and is also the species for which

aquaculture production, intended for consumption and stocking, is the most important (MAPAQ 2019). Most aquaculture facilities rely on open water circulation systems where the water source is natural, which could increase by 1 to 2.2 °C before the end of the century (Reid et al. 2019), thus leading to suboptimal rearing conditions for this species at the higher temperatures (Ficke et al. 2007). It can also be anticipated that mortality rates increase following the stocking of these fish within natural lakes. From a bioenergetics perspective, these higher temperatures will lead to an increased metabolic rate but if the food source is not limiting, could in fact lead to higher growth rates occurring during a longer growing season.

The objectives of this study were to analyse how temperature conditions, a serious environmental issue related to climate change, affects the growth, metabolism, and stress response of juvenile anadromous brook charr and to test whether selection for aquaculture traits of interest would modify this response. We hypothesized that high temperature conditions will affect juvenile brook charr metabolism resulting in growth impairment, and that fish issued from a selection program will be less able to perform in response to increases in temperature conditions than fish not submitted to selection. To test this, we exposed juveniles to different temperature conditions for a period of five weeks: ambient temperature conditions under our latitude during the experimental period and to temperature treatments that were 2 and 4 °C higher, respectively. Specific growth rate, aerobic scope and the relative expression of genes of interest in the gills, liver, and muscle were measured in either juveniles issued from a selection process or in control fish. The brook trout strains used in our study were also used in a prior study that determined which families within each strain (line) were thermally sensitive or resistant (Gourtay et al., pers. comm.). This allowed

us to compare the species' response to temperature changes is critical for the industry. Even though genetic selection aims to produce fish with specific traits of interests, co-selection for undesirable traits (Melo and Marroig, 2015), or appearance of non-expected physiological response as increased stress response may occur in selected animals (Glover et al. 2006a; 2006b).

Materials and methods

Animal husbandry, growth survey, and respirometry measurements were done according to Canadian Council of Animal Protection recommendations and protocols have been approved by the UQAR Animal Care Committee (CPA-81-20-222).

Animal husbandry

Juvenile fish (1+ year old) that were used for this experiment originated from a population of anadromous brook charr from the Laval River on the north shore of the Saint Lawrence River (Martin et al. 1997; Boula et al. 2002) held at the Institut des sciences de la mer's aquaculture facility. Over the course of seven generations, brook charr at the facility have been reared under two genetic lines: 1. the control line where broodstock crossings were made randomly while avoiding crossing siblings, and 2. the selected line where the biggest fish that are 1+ year old and lack sexual maturity traits thus selecting for fish that prioritize growth over reproduction are kept (Bastien et al. 2011; Sauvage et al. 2010). This selected line exists for research on aquaculture related questions. Within these two lines, different family groups were used representing offspring of broodstock that were subjected to an acute temperature treatment in a previous experiment (Gourtay, pers.

comm.). In this same experiment, 0+ fish were subjected to a thermal challenge trial (i.e., gradual temperature increase until loss of equilibrium) and performance was related back towards the temperature treatments experienced by the male broodstock. Crossings for this experiment followed a factorial design where each male was crossed with two females resulting in offspring being either full sibling or half sibling within the same family group (Houle et al. 2023). For both lines, two family groups were chosen for the current experiment: one family group resistant to a temperature challenge test (resistant group) and a sensitive one (sensitive group) (Fig. S1).

Growth experiment

At the beginning of the experiment, 60 fish from each family group were randomly taken before being anesthetized in a 0.018% solution of MS-222 (Tricaine methanesulfonate), weighed, measured, and marked in the dorsal muscle with a 12 mm passive integrated transponder (PIT; Avid, California). The location where the PIT was placed was subsequentially disinfected with methylene blue before placing the fish in one of twelve 50 L experimental tanks. Each tank was supplied with flowthrough dechlorinated city water with a 1 L min^{-1} flow rate. All tanks contained an air stone for aeration and a 300 W submersible heater to regulate the temperature. Temperature treatments were set to 15, 17, and 19 °C, and were controlled using an Apex system (Neptune, Morgan Hill, CA, United States) that was programmed to turn the heaters on and off when the temperature recorded departed from the treatment temperature by +/- 0.5 °C. Each temperature treatment tank was replicated four times, with 20 fish in each tank, comprising of five fish from each of the four family groups. Control and selected lines were kept in separate tanks

as food consumption was quantified and would have been impossible to attribute to the two different lines if kept together. The 15 °C treatment was considered the control treatment as it was the closest to the ambient water temperature coming into the tanks before the experiment begun. Once placed in the tanks, water temperature for the 17 °C and 19 °C treatments was achieved in two and four days, respectively, using a warming rate of 1 °C day^{-1}.

During the 35-day experiment, fish were observed on a daily basis for abnormal behaviours before being fed *ad libitum* in two servings (1 mm salmonid feed, Corey, Fredericton, Canada). Extra attention was given to avoid over feeding the fish so a reliable estimate of food consumption could be made for the duration of the experiment. On the last day of the experiment, all fish were either sacrificed for tissue sampling or used for respirometry trials.

Respirometry

At the end of the experiment, two fish from each family group from each tank (96 total individuals) were randomly selected for the respirometry trials (see details in Table S1 following recommendations by Killen et al. 2021). Selected fish were not fed for 24 h prior to the trial and were transferred from the experimental room to the respirometry room (less than 5 m distance) using a dipnet before being placed in a 15 L container where they were chased to exhaustion for 5 min using the same dipnet, thus eliciting MMR (i.e., fish became unresponsive to the stimuli within the 5-min period). Following this procedure, fish were placed in 800 mL cylinder respirometers connected with an optode oxygen system (PreSens Precision Sensing Gmbh, Regensburg, Germany). Readings were

recorded using the AutoResp program (Loligo Systems, Toldboden, Denmark) and dissolved oxygen within each respirometer was never allowed to decline below 80% saturation (Rosewarne et al. 2016; Svendsen et al. 2016). Two 120V pumps (Eheim, Deizisau, Germany) connected to timers allowed to keep the water in movement within the respirometer as well as to flush the water every two minutes. A total of eight respirometers were used simultaneously and were submerged in two 100 L water baths supplied with air stones to keep the water well oxygenated. Heaters were used to maintain temperatures in both water baths similar to those used in the experiment. Respirometry trials each lasted between 21 and 23 hours.

Metabolic rate data were estimated from the slopes of decreasing oxygen concentrations calculated from the raw data files generated from AutoResp and using the RespR package (v. 2.0.2, Harianto et al. 2019) in RStudio (v. 4.2.0, R Core Team 2022). Three variables were calculated from these files: 1. The bacterial oxygen demand (BOD), 2. SMR, and 3. MMR, each considering water temperature, fish volume (for SMR and MMR), and respirometer volume (including tubes). BOD was applied throughout the respirometry trial by creating a linear regression between slopes calculated before the fish was placed in the respirometer and after the fish was taken out. MMR was calculated from the slope of the first cycle once the fish had been placed in the respirometer. SMR on the other hand was calculated based on the ensuing slopes that were calculated during the 21-23h period. To do so, slopes with a $r^2 \geq 90$ and within the 10^{th} percentile were used to calculate SMR. Slopes for BOD, MMR, and SMR were calculated using the kernel density estimation method with at least 20% of the datapoints within each slope taken into

consideration. Each respirometry cycle lasted 240 s, with a 10 s wait period, a 110 s measurement period, and a 120 s flushing period.

Total RNA extraction and cDNA synthesis

At the end of the experimental period, two fish per family group per tank (n = 24 per familial group; total n = 96) were anesthetized (MS 222, 0.018 %), and then euthanized by spinal cord severing. These fish were different than the ones used for the respirometry trials. Gill filaments from the second left branchial arch, liver and epaxial muscle were sampled and immersed in RNAlater (ThermoFisher, Montréal, Canada). After 24h at 4 °C, samples were transferred to -80 ˚C until RNA extraction could be performed. For each sampled fish, total RNA was extracted from 15 mg of liver and gill tissues (RNeasy Plus Mini Kits, Qiagen, Inc., Mississauga, ON, Canada) and from 50 mg of muscle samples (Universal Mini Kit, Qiagen Inc., Mississauga, ON, Canada). Extracted RNA was diluted to a final concentration of 200 ng μL^{-1}. Total RNA purity, quality, and concentration were evaluated by SYBRSafe DNA gel stain 2% agarose gel electrophoresis (Alpha Imager HP system, Alpha-Innotech, Alpha Software, Invitrogen, Inc., Carlsbad, CA, USA) and on a spectrophotometer with the absorbance ratio of 260/280 (NanoVue Plus Spectrophotometer, GE Healthcare, Pittsburg, PA, USA). Reverse transcription of RNA into complementary DNA (cDNA) was made in duplicate for each sample and then pooled using the SuperScript IV Vilo Master Mix Kit (Life Technologies, Carlsbad, CA, USA). The cDNA samples were diluted and aliquoted to a final concentration of 200 ng μL^{-1} and kept frozen at -20 °C until further analysis. The cDNA integrity and concentrations were verified using a NanoVue Plus spectrophotometer. The efficiency of reverse transcription

for individual primers was verified by quantitative polymerase chain reaction (qPCR) using 10-fold serial dilutions of a representative pool of cDNA samples and compared to the ideal slope of -3.3.

Primer design for target genes

To evaluate growth, metabolism and stress effects of our experimental treatments, the expression of selected genes was quantified in triplicates in liver (genes coding for insulin-like growth factor-1 *IGF1*, growth hormone receptor [*GHr*], cytochrome c oxidase [*COX*], citrate synthase [*CS*], catalase [*CAT*], superoxide dismutase [*SOD*], glutathione peroxidase [*GPx*], B cell lymphoma 2 [*BCL*], *hsp70, hsp90*]), in gills (genes coding for Na^+-K^+-*APase* subunits α*1a* and α*1b*, *hsp70*, *hsp90*, and *caspase 9*) and in muscle (genes coding for IGF1 receptors [*IGF1r*], *myogenin, myosin*, lactate dehydrogenase [*LDH*] and pyruvate kinase [*PK*]).

Measurement of gene expression by qPCR

Gene expression was measured by qPCR. Primers and TaqMan probes specific to brook charr were obtained from previous work conducted in Audet (Martinez-Silva et al. 2023) and Jeffries labs. Sequences are provided in Table 1.

For all samples, qPCR gene expression was performed in triplicate using a QuantStudio 3 Real Time PCR System (Applied Biosystems). Each reaction contained 5µl of TaqMAN Fast Advanced Master Mix, 0.5 µl of Custom TaqMan Gene Expression Assay, 2.5 µl of sterile water, and 2 µl of diluted cDNA, for a total volume of 10 µL. The

relative quantification of gene expression was calculated using the $2^{-\Delta\Delta CT}$ method of Livak and Schmittgen (2001), with C_T being a threshold cycle:

$$2^{-\Delta\Delta\text{^}CT} = 2\text{^}\text{-}(\Delta CTe - \Delta CTc) \quad (1)$$

where C_{Te} = C_T of the target gene - C_T of the reference genes for sample x, and C_{Tc} = C_T of the target gene - C_T of the reference genes for the calibrator. In this study, the calibrator was the familial temperature sensitive control group M8CC maintained at 15 ˚C. The stability of reference gene expressions between groups was verified with Expression Suite version 1.0, where the score was calculated according to Vandesompele *et al.* (2002). *18S*, *β-actin,* and *ef1α* were used as reference genes and the best score combination obtained with the QuantStudio Analysis software was kept for each tissue. According to these results, the combination of the three reference genes was used for the muscle tissue calculations, the combination of *β-actin,* and *ef1α* was used as the reference gene for the liver and gill tissues. For accurate averaging of the reference genes, we used the geometric mean instead of the arithmetical mean because the former better controls for possible outlier values and abundance differences between the different genes (Vandesompele *et al.*, 2002).

Statistical analyses

Initial and final masses were used on individual fish to calculate growth rates as follows:

$$G = \left(X_f - X_i/X_i\right)/35 \times 100 \quad (2)$$

where G represents the growth rate, and X_i and X_f represent initial and final mass or length

before and after the 35 day experimental period. The Fulton's condition factor (*K*) was calculated as:

$$K = W/L^3 \cdot 100$$

(3) where *W* represents the mass (g) of the fish and *L* represents the fork length (cm). A variability coefficient (ΔK) was calculated using *K* values at the beginning (K_i) and at the end (K_f) of the experiment:

$$\Delta K = K_f - K_i \quad (4)$$

In addition, gonado-(GSI) and hepato-somatic indices (HSI) were calculated at the end of the experiment as follows:

$$GSI\ (or\ HSI) = G_f\big(or\ H_f\big)/X_f \times 100 \quad (5)$$

where G_f and H_f represent the wet mass of the gonads and liver (both in g), respectively, at the end of the experiment.

Linear mixed models were used to differentiate treatment effects using the lme4 package (v. 1.1-10; Bates et al. 2015) in RStudio (R Core Team 2020). The general model formulation was described as follows:

$$R = T + S/F + (1|tank\ or\ resp) \quad (6)$$

where *R* represents either SMR, MMR, or AS (in mg O_2 g^{-1} h^{-1}), G (in g or cm), ΔK, GSI, HSI, or C (in %). Fixed variable *T* stands for the temperature treatment while *F* represents the different family groups nested within each genetic line *S*. Finally, we used the experimental tanks (*tank*) or individual respirometers (*resp*) as random variables. When normality of residuals was not met, logarithmic or box-cox transformations were employed

on the response variables. Factor significance was assessed using the maximum-likelihood method with significance assumed to occur with p-values < 0.05.

Relative gene expressions were compared using nested ANOVAs (factors: temperature, line; temperature sensitivity nested in line). When no significant nested effect was found, *a posteriori* two-way ANOVAs testing for interactions were conducted. In presence of significant effects, *a posteriori* HSD Tukey tests were used for mean comparisons. Normality and homoscedasticity have been verified using Kolmogorov-Smirnov and Levene tests respectively. Statistics were run using Statistica software (Tibco, CA, USA).

Results

Temperature condition exposure for the 35-day experimental period had a significant effect on length growth rates and on the condition factor variability coefficient, but not on the mass growth rate (Table 2). Length growth rates were significantly lower for individuals reared in the 19 °C treatment compared to 15 and 17 °C and significantly lower for individuals issued from the sensitive group compared to the resistant group (Table 3). Similarly, mass growth rates were significantly lower for sensitive individuals but only for those from the selected strain (Table 3). At 19 °C, the variation in the Fulton's condition factor was significantly greater than at 15 or 17 °C, with this coefficient being higher for the selected line as opposed to the controls (Table 3).

At the end of the experiment, female GSI was significantly greater for the sensitive-control line compared to the resistant-control line, regardless of temperature (Table 3). Females from the control line also displayed significantly higher GSI than those from the

selected line (Table 3). There were no temperature, line, or family group effects on male GSI.

Finally, genetic line had a significant effect on SMR, with the control line displaying a 6.7% higher SMR than the selected line (Fig. 1). However, no temperature effects were observed for SMR, which was not the case for MMR and AS (Table 2 and Fig. 1). Compared to the 15 °C treatment, MMR was 4.8% higher but 8% lower at 17 °C and 19 °C, respectively, while AS values increased by 6.2% and decreased by 11.3% at the same temperatures.

Effects of temperature and genetic selection on gene expression

Liver *COX*, *hspP90* and gill *hsp90* showed significant relative expression differences in response to temperature rearing conditions independently of the genetic line that was considered. The relative expression of *COX* in liver was significantly lower in individuals reared at 19 ˚C (Table 4, Fig. 2A), while the relative expression of *hsp90* both in liver (Fig. 2B) and in the gills (Fig. 2C) was significantly higher in fish reared at 17 ˚C and 19 ˚C compared to those maintained at 15˚C.

Some other genes also showed differential relative expression depending on temperature treatments, but the response varied between the two lines (Table 4). No effect of the relative expression of *IGF1r* was observed in the selected line but it was significantly more elevated at 19 ˚C than at the two lower temperatures in the control line (Fig. 3A). The relative expression of *Caspase 9* was significantly higher at 19 ˚C in control line compared to the selected one (Fig. 3B). However, in the selected line, the relative expression was

significantly higher at 15 ˚C than at 19 ˚C, while it was significantly lower in the control line.

The relative expression of *IGF1* did not differ among temperature treatments but was in average 35.3% more elevated in the selected line over the control (Table 4, Fig. 4A). Relative expressions of *Catalase* (Table 4, Fig. 4B), *LDH* (Table 4, Fig. 4C), and *Myogenin* (Table 4, Fig. 4D) did not differ according to temperature treatment but were significantly more elevated in the selected line. The relative expression of *SOD* response was characterized by multiple cross effects (Table 4, Fig. 5). Globally, the *SOD* relative expression was 13% and 24.6% lower at 17 ˚C and 19 ˚C respectively when compared to fish sampled at 15 ˚C. The temperature resistant fish within the selected line showed an upregulation compared to the other groups. In contrast, the temperature resistant fish within the same line showed a global downregulation of *SOD* with a relative expression 27.9% lower than the temperature sensitive selected fish at 15 ˚C group showing the highest relative expression. The relative expression of *PK* was significantly lower in the control line exposed to 15 ˚C (0.98 ± 0.31) than in those reared at 17 ˚C (1.26 ± 0.53) or 19 ˚C (1.20 ± 0.3). No line effect, neither temperature × line effects were present (Table 4). Differences relative to thermal sensitivity was detected by ANOVA, but *a posteriori* tests failed to highlight specific differences.

The thermal sensitive fish from the control line had a significantly lower relative expression of *NaK ATPase* α1b (Table 4, Fig. 6) than the ones observed in temperature-resistant control fish and temperature-sensitive selected individuals. Overall, no effect of experimental conditions were observed for the relative expression of the following genes:

GHr, *GPx*, *BCl*, *HSP70* in liver, *Na-K-ATPase α1a* and *HSP70* in gills, and *myosin* in muscle (Table 4).

Discussion

The general objective for this research project was to verify how environmental conditions could affect brook charr growth and to understand the underlying physiological mechanisms. We had hypothesized that the physiological response towards an increase in temperature would be more pronounced for the selected line, and we found that elevated temperatures led to a decrease in growth rates. Indeed, it is not the line that was shown to have an impact on the growth rates at different temperatures but rather the resistance to an acute thermal stress, which had been quantified in a previous experiment (Gourtay *et al*., submitted), that had an impact. In a previous study on juvenile brook charr, Chadwick & McCormick (2017) were also able to show that specific growth rates were significantly hindered at 24 °C compared to 16 °C.

Given that growth rates diminish past optimal temperatures, it can be hypothesized that this might be linked to an increase in standard metabolic rate and possibly to a reduced aerobic scope, but our results do not seem to support this. While the literature shows many cases where metabolic rate is positively correlated with temperature (Stitt et al. 2014, Mackey et al. 2021), our results only indicated a genetic line effect. In their study on juvenile brook charr metabolism, Mackey et al. (2020) used five exposure temperatures between 5 and 23 °C and observed proportional increases in SMR and MMR without observing any significant changes for the aerobic scope. Because our SMR was not significantly different between temperatures, but that MMR increased at 17 °C, we denoted

a significant increase in AS at that temperature. Oppositely, MMR and AS were both significantly reduced at 19 °C, which appears to agree with what has been shown in the literature. Durhack *et al*. (2021) observed similar results, with an increase in MMR and AS at 15 °C compared to 5 °C followed by a decrease for individuals subjected to 20 °C and 23 °C temperatures. The decrease in MMR and AS in our study corresponds well with the decrease in growth rates, which could be attributed to a reduction in oxygen solubility and (or) in hemoglobin affinity for oxygen at higher temperatures (Jensen *et al*. 1993, Norin *et al*. 2014).

Aerobic scope provides an estimation of energy available for activities other than those related to homeostasis, including growth, locomotion, feeding activities, and reproduction. Growth is controlled by a hormonal cascade including the growth hormone secreted in the hypophysis and IGF1 synthesized in liver. We have no indication that the decrease in growth observed at 19 °C was related to this regulation loop as no temperature effect was observed on relative expressions of *IGF1* or liver *GHr*. However, an increase in the relative expression of muscle *IGF1r* was present in the control line. The increase in relative expression of *IGF1r* could indicate compensatory mechanisms in the control line in response to negative temperature effects on growth. Interestingly, the relative expression of *COX* decreased inversely with temperature conditions in contrast to observations reported in other salmonids (*Oncorhynchus nerka* (Walbaum, 1792), Jeffries et al., 2012; *O. mykiss*, Garvin et al., 2015), while no change in *CS* expression was observed in the liver. If less COX proteins are synthesized, this could partially explain the decrease in MMR observed at 19 °C. No effect on *LDH* relative expression was observed in white muscle, a tissue fuelled through glycolysis, but relative expression of *PK* differed among familial

groups with a positive correlation with temperature. In tilapia *Oreochromis aureus* (Steindachner, 1864), submitted to temperatures of 18, 22, 26, 30, and 34 °C, the activity of white muscle pyruvate kinase was positively correlated with temperature (Younis, 2015), suggesting a rise in glycolytic potential. An increase in *PK* expression could also indicate an increase in the use of energy reserves in fish at high temperature.

We hypothesized that elevated temperature conditions may be stressful for juveniles. Relative expressions of *HSP90*, *Caspase 9*, and liver *SOD* not only increased with temperature conditions, but also showed distinct response relative to temperature sensitivity level of familial groups. Rise in liver and gill hsp90 is a very well-documented stress response (e.g., *Oncorhynchus tshawytscha* (Walbaum, 1792), Palmisano et al., 2000). Heat shock proteins are stabilizing and protecting protein structure to prevent denaturation caused by thermal stress. Similarly, Viant et al. (2003) showed that expression of *hsp63, hsp72, hsp78*, and *hsp89* rose when *O. mykiss* was submitted to a temperature of 20 °C, this being negatively correlated to metabolic condition. The increase of protein production, including hsps, may increase energetic costs to maintain homeostasis and then decreasing the amount of energy available for other functions including growth (Feder and Hofmann, 1999; Myrick and Cech, 2000; Pörtner and Peck, 2010). Moreover, the upregulation of production of such proteins may result in the decrease of the production rate of other proteins normally associated to homeostasis maintenance (e.g. Viant et al., 2003). The rise in expression of *Caspase 9* in the control line in response to the rise in temperature is similar to what was observed in the pupperfish *Takifugu obscurus* (Abe, 1949) (Cheng et al. 2018). Caspase 9 is an enzyme promoting cellular apoptosis. Indeed, the increase in ROS production in response to a thermal stress may provoke cell damages,

which will initiate an enzymatic cascade including *Caspase 9* (Sakamaki et Satou, 2009). A reduction in the number of gill cells may induce ischemia and reduce oxygen availability (Czabotar et al., 2014), and then having an effect on oxidative metabolism. Interestingly, in the selected line, the pattern was inverted. The decrease in relative expression of *Caspase* under high temperature conditions seems to indicate that individuals of this line can metabolize ROS more efficiently. On the other hand, the reduction of *SOD* expression is surprising. Indeed, many studies showed a rise in *SOD* expression and of SOD activity following a rise in temperature conditions, including studies on *O. mykiss* (Yang et al., 2021). However, a reduction in transcription does not mean that enzyme activity is decreased as mRNA transcription can be turned off through cellular feedback mechanisms if protein level if sufficient. A rise in ROS production would stimulate SOD, its antioxidative activity reducing cell damages (Liu et al., 2015). In zander *Sander lucioperca* (Linnaeus, 1758), Chen et al. (2021) showed a transient response of the gill antioxidant response following exposure to a chronic stress. The higher relative expression in the temperature resistant selected fish may indicate that the protection system against free radicals is better functioning in the presence of chronic thermal stress. There was also a rise in relative expression of gill *Na^+-K^+-ATPase* in response to thermal sensitivity. Osmotic disorder is a very well-known secondary level stress response (brook charr: Claireaux and Audet 2000; Crespel et al. 2011) and a rise in enzymatic activity may indicate a regulatory response to counteract osmo-ionic imbalance. These combined responses are indicative that high temperature was not optimal thermal environment for juvenile brook charr.

In the selected line, the resistant temperature familial group showed the highest growth rate, indicating that thermal stress had less impact on that group. Control females had a higher GSI than selected ones which correspond to the selection process applied on the selected line (absence of sexual maturation in 1+ fish). Such an energy investment in reproduction could have a negative impact on growth because of a decrease in energy resources. In rainbow trout, Kaushik and Médale (1994) showed that the energy investment allowed towards gonad development can reach 8 to 15% of total body energy. SMR was also found to be more elevated in the control line, indicating that metabolic costs associated with homoeostasis are also greater. In the selected line, relative expression of *myogenin,* a transcription factor regulating the expression of different genes involved in muscle fibers production (Koumans et Akster, 1995) was higher, which is compatible with enhanced growth in this line. The relative expression of white muscle *LDH* was also higher in the selected line (Table 4). The activity of this enzyme was shown to be correlated with growth in Atlantic cod, *Gadus morhua* (Linnaeus, 1758) (Pelletier et al. 1994)). The higher expression of *Catalase* in the liver of selected juveniles may indicate a greater ability to mitigate free radical effects.

In conclusion, high temperature affected both the growth rate in length and the Fulton's condition factor, which may be related to the reduction in AS, concomitant to a reduction of the relative expression of *Cox* indicating possible effects at the level of enzymatic regulation of aerobic metabolism. The increase in relative expression of both liver and gill *hsp90* at the highest temperature is indicative of a stress induced response. The control line showed a higher SMR while *IGF1*, *Caspase 9*, *LDH*, and *Myogenin* relative expression

were higher in the selected line. Globally, our results showed that the selection process did not impair temperature response in brook charr and that the selected line even showed that it could cope more effectively with the oxidative stress caused by the rise in temperature. These findings could have applications towards the salmonid aquaculture industry, where production often takes place in uncontrolled environments (e.g., sea pens, rearing ponds), and where temperature might become limiting.

Acknowledgements

The authors are grateful to all colleagues who provided technical and scientific assistance in the laboratory. Special thanks to Nathalie Morin and Émile Vadboncoeur (Institut des sciences de la mer de Rimouski) who contributed to brook charr rearing, their involvement was essential to this work especially during the COVID period.

Competing interests

The authors declare there are no competing interests.

FUNDING

This work was funded by the Government of Canada through Genome Canada and the Ontario Genomics Institute (OGI-184) This research was also supported by the Conseil de Recherche en Sciences Naturelles et en Génie du Canada (CRSNG, subvention à la découverte No RGPIN-2019-05739 to C. Audet), and by the Ressources Aquatiques Québec Research Network (Regroupement stratégique, Fonds de Recherche du Québec – Nature et Technologie).

Author contribution statement

All authors contributed to the conceptualization and experimental design of the work. C. J.-B. performed all data collection, and statistical analyses. C. J.-B. drafted the manuscript and all authors contributed to the final version. Supervision was provided by C.A. and D.D.

Data Availability

The data that supports the findings of this study are available from the corresponding author, CA, upon reasonable request. The raw data will be available on a public repository after manuscript publication.

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Figure Captions

Figure 1. Standard Metabolic Rate (SMR; black), Maximal Metabolic Rate (MMR; dark grey), and Aerobic Scope (AS; light grey) for brook charr *Salvelinus fontinalis* (N =96) exposed at three different temperature regimes for 35 days. Dark grey and light grey boxplot contour lines represent the control and selected lines, respectively. Boxplots describe the 25^{th} and 75^{th} quartiles while the whiskers represent the 10^{th} and 90^{th} quartiles, with the horizontal line within each boxplot indicating the median. Genetic line was shown to significantly affect SMR and MMR ($p < 0.05$), while temperature significantly affected MMR and AS ($p < 0.05$).

Figure 2. Relative expression of **(A)** *Cytochrome c oxidase* (*COX*) in liver (n = 95), **(B)** Heat shock protein 90 (*HSP90*) in liver (n = 95) and **(C)** in gills (n = 96) in brook charr *Salvelinus fontinalis* reared at different temperatures. Different letters indicate significant differences ($\alpha = 0.05$). The horizontal bars indicate relative expression measured in the calibrator group (temperature sensitive control fish at 15 °C). Mean ± SD.

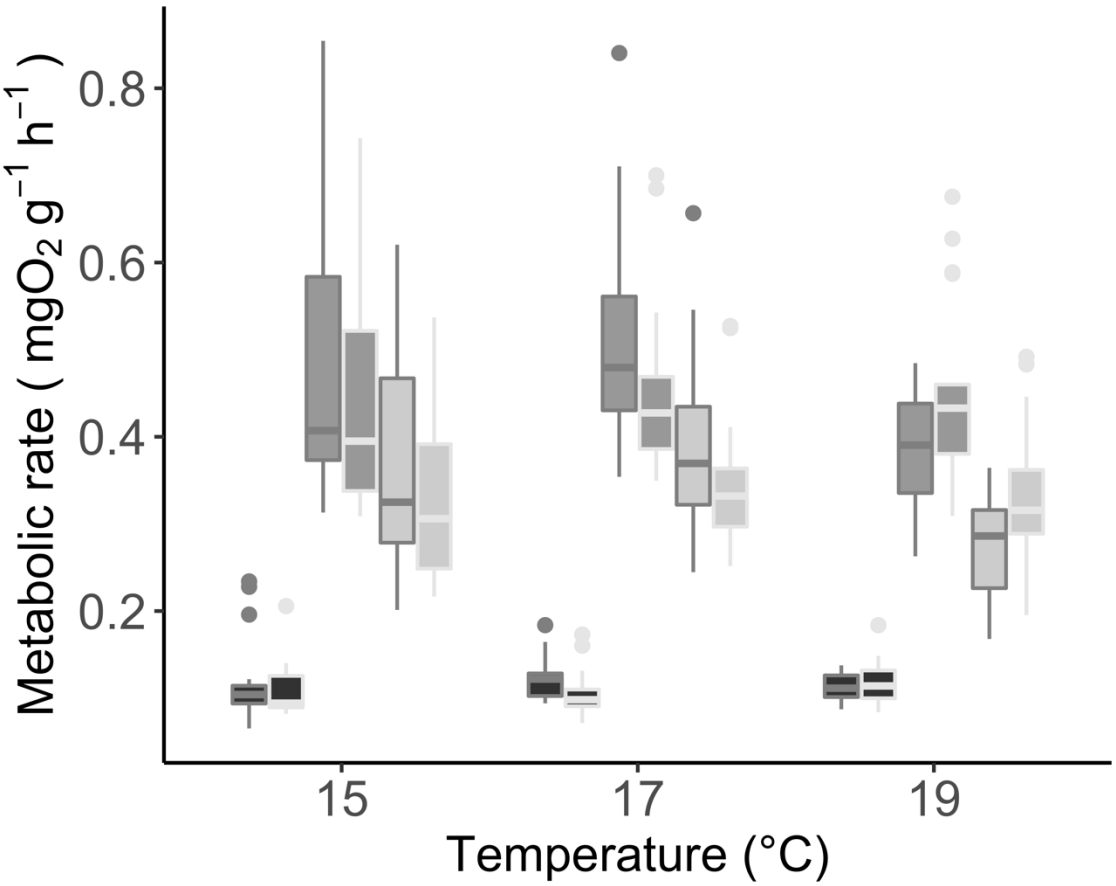
Figure 3. Relative expression of **(A)** Insulin-like growth factor receptor (*IGF1r*) in muscle (n = 92), and **(B)** *Caspase 9* in gills (n = 96) in control and selected brook charr *Salvelinus fontinalis* reared at different temperature conditions. Different letters indicate significant differences ($\alpha = 0.05$). The horizontal bars indicate relative expression measured in the calibrator group (control temperature sensitive fish at 15 °C). Mean ± SD.

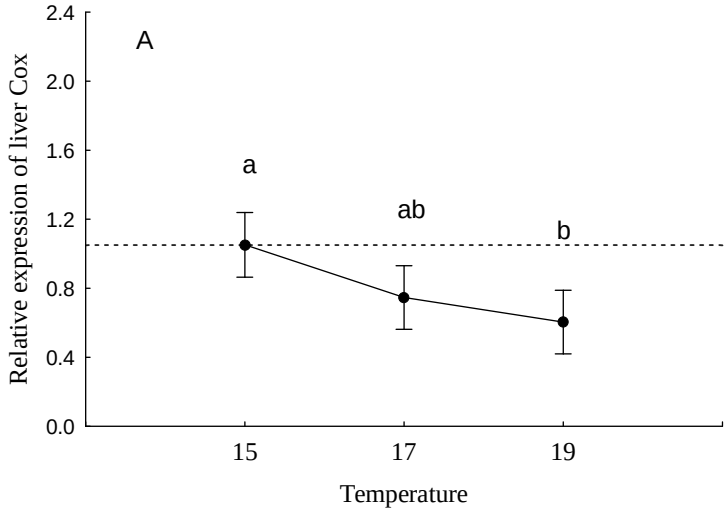
Figure 4. Relative expression of genes coding for **(A)** Insulin-like growth factor receptor (*IGF1*) in liver (n = 95), of **(B)** *Catalase* (*CAT*) in liver (n = 95), of **(C)** lactate

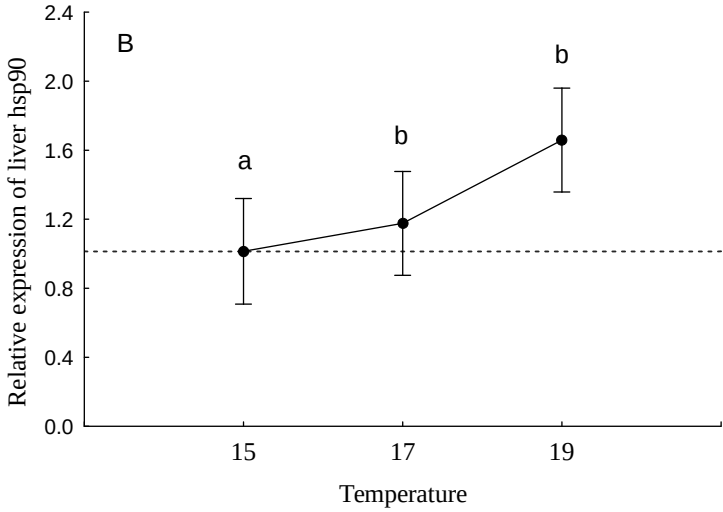
dehydrogenase (*LDH*) in muscle (n = 92), and **(D)** *Myogenin* (n = 92) relative to the line of brook charr *Salvelinus fontinalis* that was considered. Asterisks indicate significant differences ($\alpha = 0.05$). The horizontal bars indicate relative expression measured in the calibrator group (control temperature sensitive fish at 15 °C). Mean ± SD.

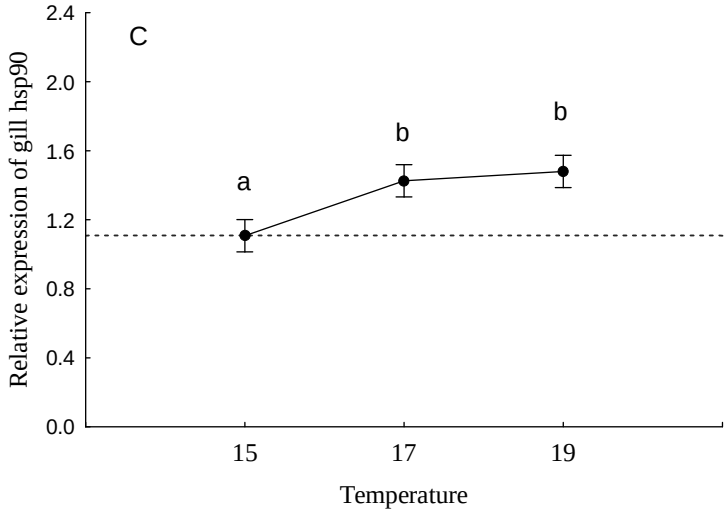
Figure 5. Relative expression of gene coding for superoxide dismutase (*SOD*) in brook charr *Salvelinus fontinalis* liver. Differences among temperature conditions are indicated with horizontal lines below the three temperatures indicated on the right, differences according to line and temperature sensitivity are indicated by letters with group identification on the left. Total n = 95. Different letters indicate significant differences. The horizontal bar in the graph indicates the relative expression measured in the calibrator group (control temperature sensitive fish at 15 °C). Mean ± SD.

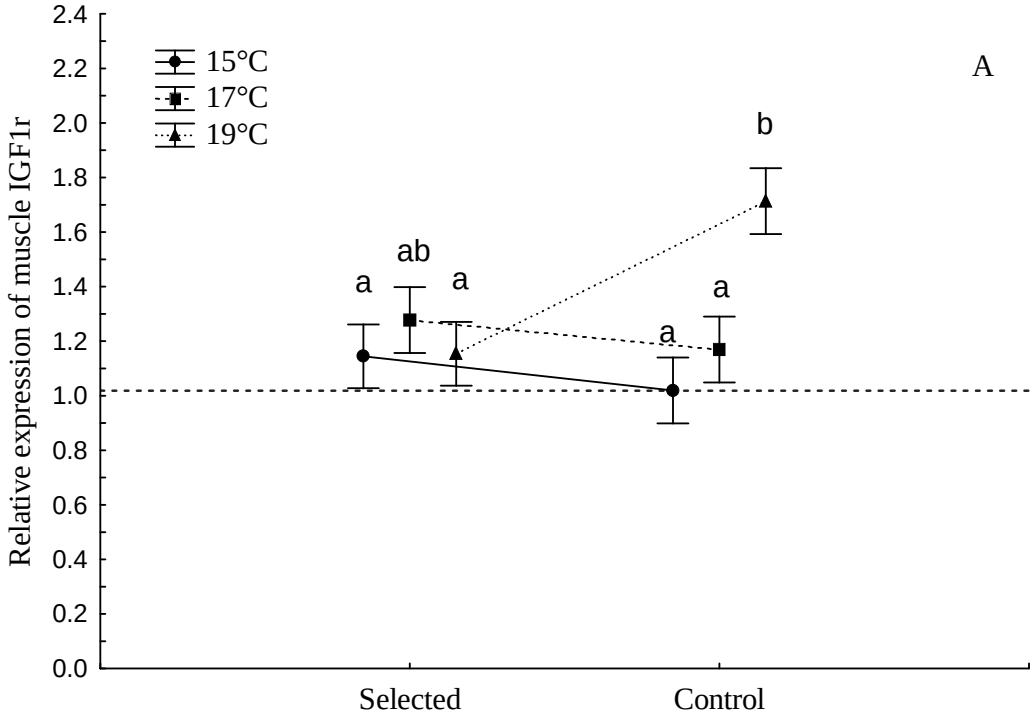
Figure 6. Relative expression of the gene coding for the sodium-potassium ATPase subunit α1b (*Na-K-ATPα1b*) in gills in resistant and sensitive fish within control and selected brook charr *Salvelinus fontinalis*. Different letters indicate significant differences. The horizontal bar represents the relative expression of the control temperature sensitive group at 15°C. Mean ± SD.

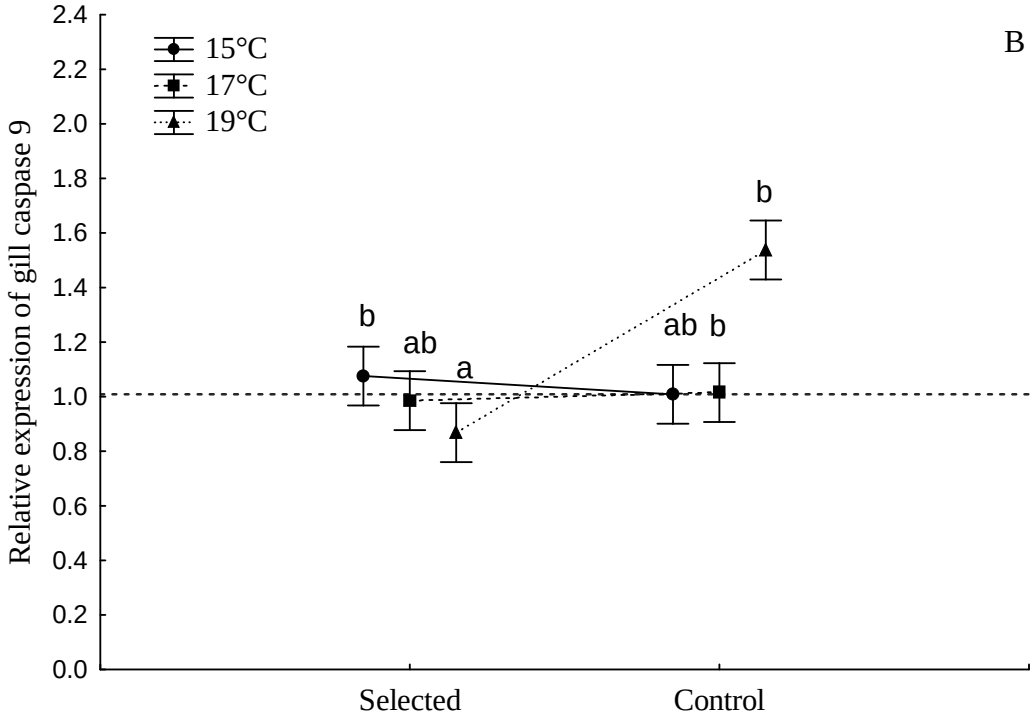

Metabolic rate (mgO2 g−1 h−1)
0.8
0.6
0.4
0.2
15
17
19
Temperature (°C)

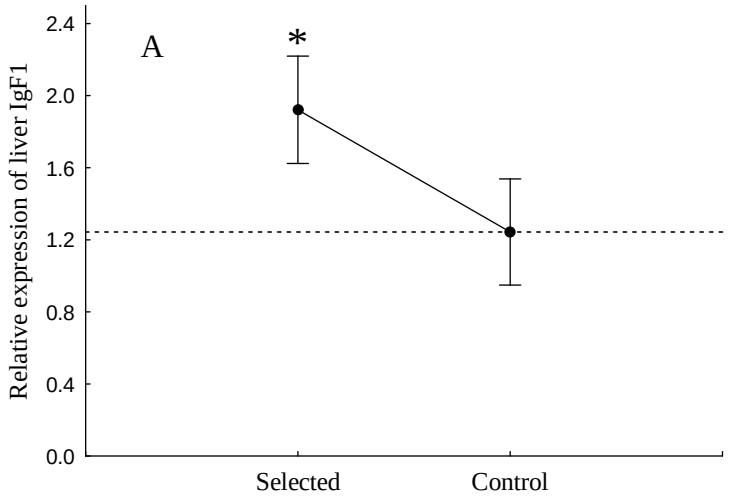

A
Relative expression of liver Cox
2.4
2.0
1.6
1.2
0.8
0.4
0.0
a
ab
b
15
17
19
Temperature

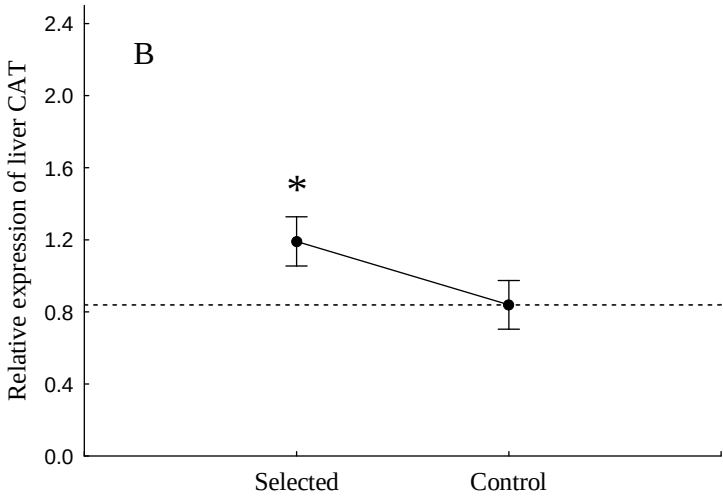

B
Relative expression of liver hsp90
2.4
2.0
1.6
1.2
0.8
0.4
0.0
a
b
b
15
17
19
Temperature

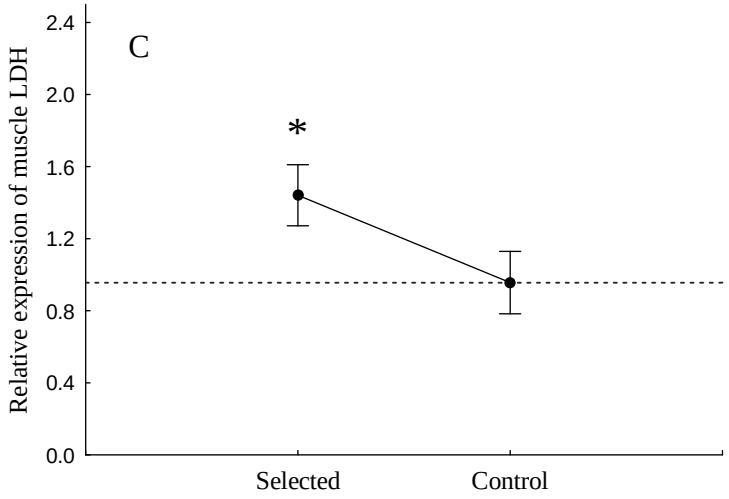

C
Relative expression of gill hsp90
2.4
2.0
1.6
1.2
0.8
0.4
0.0
a
b
b
15
17
19
Temperature

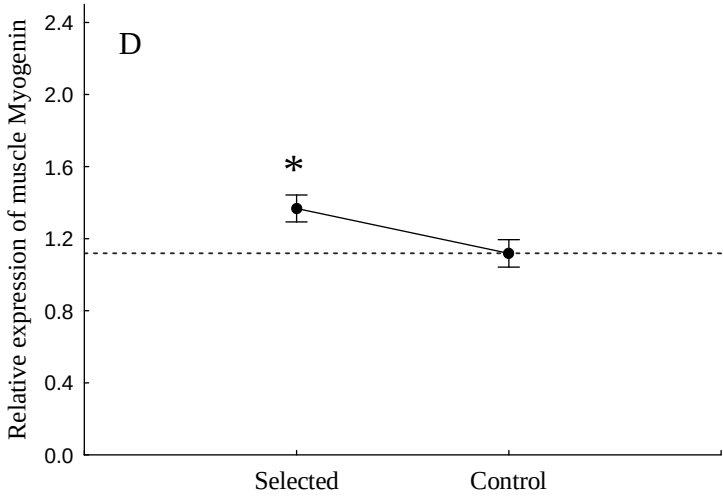

A
15°C
17°C
19°C
Relative expression of muscle IGF1r
2.4
2.2
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
a
ab
a
a
a
b
Selected
Control

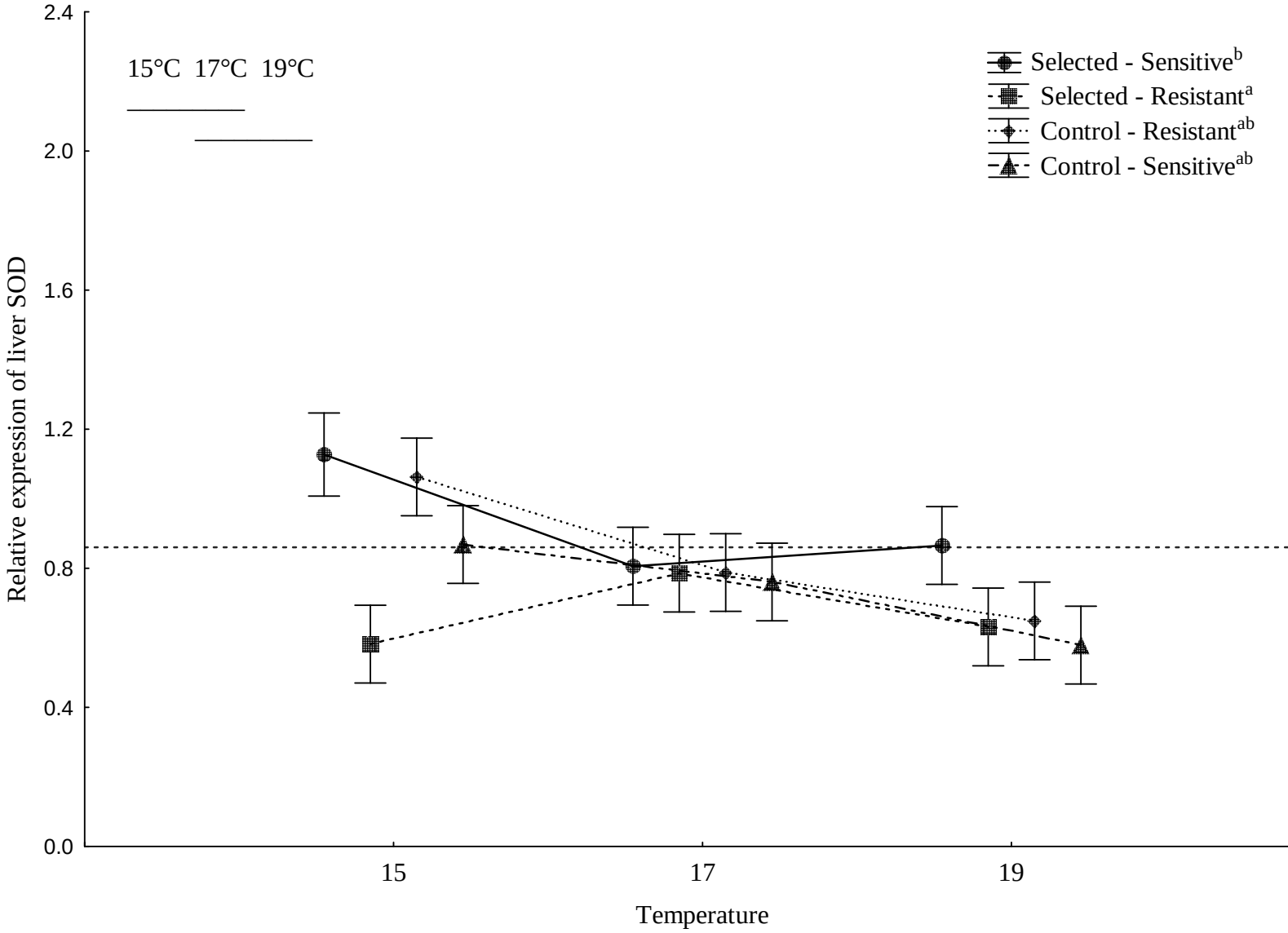

B
15°C
17°C
19°C
Relative expression of gill caspase 9
2.4
2.2
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
b
ab
a
ab
b
b
Selected
Control


A
*
Relative expression of liver IgF1
2.4
2.0
1.6
1.2
0.8
0.4
0.0
Selected
Control


B
*
Relative expression of liver CAT
2.4
2.0
1.6
1.2
0.8
0.4
0.0
Selected
Control


C
*
Relative expression of muscle LDH
2.4
2.0
1.6
1.2
0.8
0.4
0.0
Selected
Control


D
*
Relative expression of muscle Myogenin
2.4
2.0
1.6
1.2
0.8
0.4
0.0
Selected
Control


15°C 17°C 19°C
Selected - Sensitive[b]
Selected - Resistant[a]
Control - Resistant[ab]
Control - Sensitive[ab]
Relative expression of liver SOD
2.4
2.0
1.6
1.2
0.8
0.4
0.0
15
17
19
Temperature

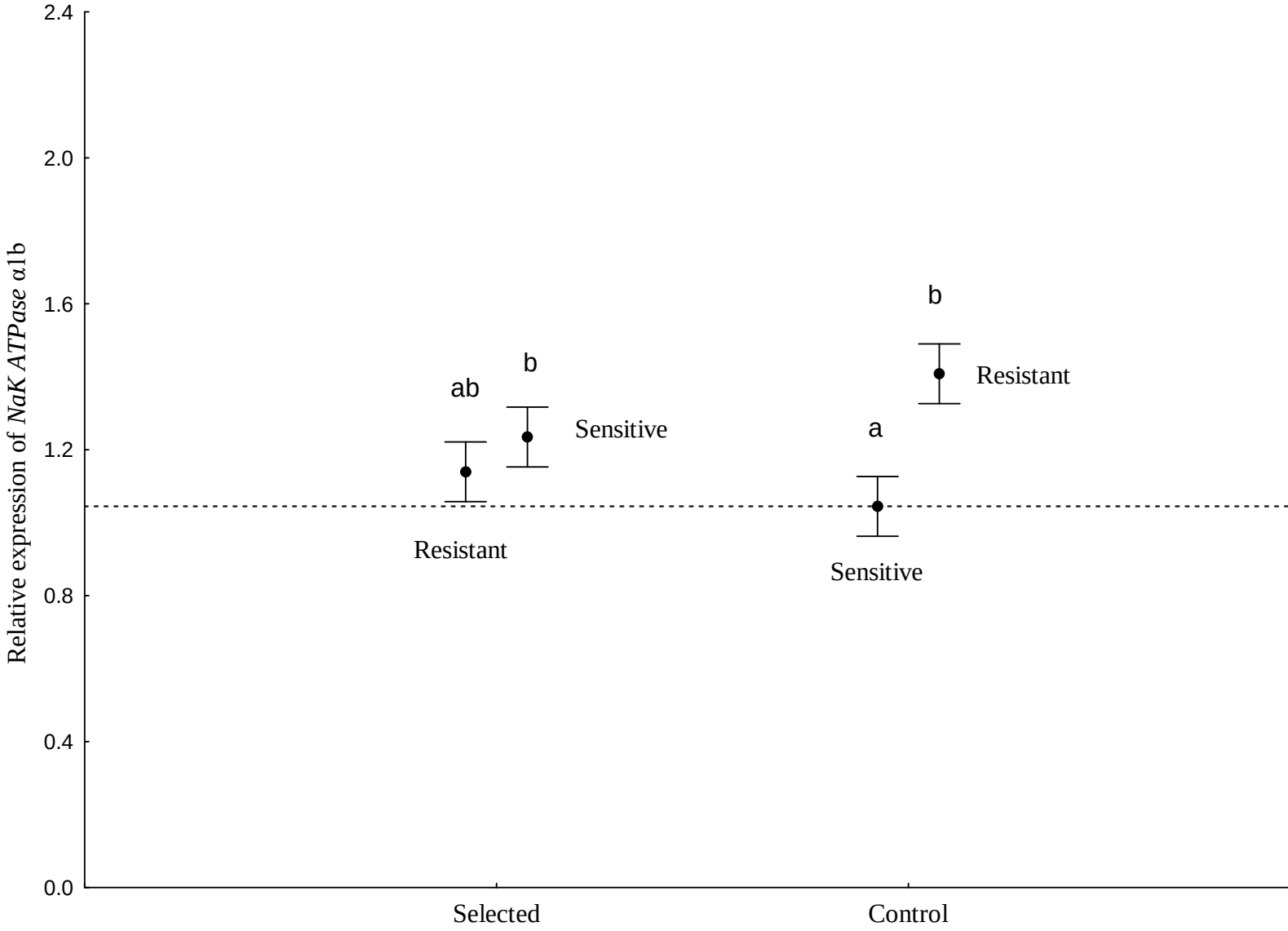

2.4
2.0
1.6
1.2
0.8
0.4
0.0
Relative expression of NaK ATPase α1b
ab
b
Sensitive
Resistant
a
b
Resistant
Sensitive
Selected
Control

Table 1

Information on sequences and primers used for *Salvelinus fontinalis*. For each studied gene, we present the qPCR TaqMan probe and primers

Gene		Sequences (5'-3')	Number of base pairs
β-actine	Probe	ATCTGGCATCACACCTT	17
	Forward	CCAACTGGGACGACATGGA	19
	Reverse	GAGCCACTCTCAGCTCGTTGT	21
EF1	Probe	CCACTGAAGTCAAGTCT	17
	Forward	TCGCCCCCGCTAATGTC	17
	Reverse	AGGGTCTCGTGGTGCATCTC	17
18S	Probe	AAGGCAGCAGGCGC	14
	Forward	AGAAACGGCTACCACATCCAA	21
	Reverse	CGAGTCGGGAGTGGGTAATTT	21
IGF1	Probe	CGAGTGCTGCTTCC	14
	Forward	CGGTCACATAACCGTGGTATTG	22
	Reverse	GCCGCAGCTCGCAACT	16
GHr-1	Probe	CTTCAGAAGGAGGCTGTTTTGC	22
	Forward	CCCACTGCCCCCTGTATCT	19
	Reverse	ACCATGGTGGAAGGAG	16
Cox sub-unit 6a	Probe	TTGCTGCGAATGCG	14
	Forward	CATCACCCCAAGGGAAACG	19
	Reverse	AGAGTTTGTCCCTTACAGCCATCT	24
CS	Probe	CGGATTGGCCGGACC	15
	Forward	TGTCCTTCAGCGCAGCTATG	20
	Reverse	TCCTGGTTAGCCAGTCCATGA	21
Catalase	Probe	CAGAAACGCTGGGTTC	16
	Forward	GAAGGGAGCCCAAGTCTTCAT	21

	Reverse	TCTGCATGCACAGCCATCA	19
SOD	Probe	CTGGGCAATGCCA	13
	Forward	CCCAGTAAGGGATTGTGTTTCTTT	24
	Reverse	CGCCAGGCTTGTGGAGTTA	19
GPx	Probe	CAGGGCACCCCCAG	14
	Forward	TTCTCCTGATGTCCGAATTGATT	23
	Reverse	ACCGACAAGGGTCTCGTGAT	20
BCl	Probe	CATTGCGGGACTCTG	15
	Forward	GCCTGGACGCAGTGAAAGAG	20
	Reverse	GGCATAACGCAGCTCAAACTC	21
hsp70	Probe	AGGATGGGATCTTTG	15
	Forward	TGACGTGTCCATCCTGACCAT	21
	Reverse	CCAGCCGTGGCCTTCA	16
hsp90	Probe	AACCCAGACCACCCC	15
	Forward	GGCCAAGAAACACCTGGAGAT	21
	Reverse	TGCCTCAGGGTCTCCACAA	19
NaK α1a	Probe	CCCCCTGGAAACC	13
	Forward	GTCCCGATTTCTCCAATGACA	21
	Reverse	TGGTAGAGAAGAAGGCGATGTTC	23
NaK α1b	Probe	AGAATATACTTGCTCCTTATC	21
	Forward	GGACTCTCAATCCCAGTGGTTT	22
	Reverse	GTGTAACAGGTGGCGTTTCTCA	22
Caspase 9	Probe	AGCACTCAGTCTGATGAG	18
	Forward	ATGTCCTCCAGCAGTGACTCTCT	23
	Reverse	GGGTAGTGTGGCCTTTGCA	19
Igf1r	Probe	CCGCCGGACTATAG	14
	Forward	TCCTCAGTGGGACCCTTCTG	20
	Reverse	GGACCATGAAGCCCAGTAGGT	21
LDH	Probe	CTGATGAGCTGGCTCT	16
	Forward	AGCGTCCTCCTCAGGGACTT	20
	Reverse	AGCTTATCCTCCATCACGTCAAC	23

PK	Probe	ATGGCGGCCTCTG	13
	Forward	CCTCAAACATCTGCCTGTGGTA	22
	Reverse	CCGCACACAGCACAAGATTG	20
Myosine	Probe	CACCACACTAGAACTGT	17
	Forward	CAAACCACATTGAACACCATCAG	23
	Reverse	GGGTTAAGCTTTATTGATACAGGAAGTG	28
Myogenin	Probe	TGCAAACGCAAGACT	15
	Forward	CCTTGGGCCTGCAAGCT	17
	Reverse	CGCTTTTCGTCGGTCCAT	18

EFI: Elongation factor 1; *IGF1*: Insulin-like growth factor; *GHr*: Growth hormone receptor; *COX*: Cytochrome c oxidase; *CS*: Citrate synthase; *CAT*: Catalase; *SOD*: Superoxide dismutase; *GPx*: Glutathione peroxidase; *BCl*: B-cell lymphoma 2; *hsp70*: Heat shock protein 70; hsp*90*: Heat shock protein 90; *Na-K-ATPα1a*: Sodium-potassium ATPase, subunit α1a; *Na-K-ATPα1b* : Sodium-potassium ATPase, subunit α1b; *igf1r* : IGF1 receptor; *LDH* : Lactate dehydrogenase; *PK* : Pyruvate kinase.

Table 2. Linear mixed effect models retained for each response variable. χ^2 and p-values from significant factors were issued following maximum likelihood testing while those from non-significant factors were obtained from the base model.

Response variable	**Fixed effect factors**	**Coefficient**	**Standard error**	**χ2**	**Pr(>|t|)**
Specific growth rate (length) mm j^{-1}	Intercept	0,844	0,099	na	na
	Family group (Resistant)	0,043	0,019	9,162	**0,010**
	Line	-0,036	0,026	3,867	0,145
	Temperature	-0,031	0,006	28,942	**<0,001**
Specific growth rate (mass) g j^{-1}	Intercept	0,437	0,067	na	na
	Family group (control-resistant)	0,009	0,055	13,928	**<0,001**
	Family group (selected-resistant)	0,205	0,055	13,928	**<0,001**
	Line	0,018	0,055	15,038	**<0,001**
	Temperature	-0,019	0,012	3,305	0,191
Δ Fulton condition factor	Intercept	-0,108	0,048	na	na
	Family group (control-resistant)	0,010	0,013	2,866	0,239
	Family group (selected-resistant)	0,019	0,013	2,866	0,239
	Line	0,028	0,009	9,310	**0,002**
	Temperature	0,007	0,048	7,075	**0,008**
Female gonadosomatic index (GSI)	Intercept	3,652	1,135	na	na

	Family group (control-resistant)	-0,738	0,291	na	**0,015**
	Family group (selected-resistant)	-0,283	0,291	na	0,340
	Line	-1,776	0,301	na	**<0,001**
	Temperature	-0,062	0,066	na	0,353
Standard metabolic rates	Intercept	0,122	0,010	na	na
	Family group (control-resistant)	0,003	0,005	1,232	0,540
	Family group (selected-resistant)	-0,004	0,005	1,232	0,540
	Line	-0,013	0,004	11,935	**0,003**
	Temperature	0,003	0,001	0,089	0,766
Maximal metabolic rate (MMR)	Intercept	0,628	0,076	na	na
	Family group (control-resistant)	-0,008	0,018	0,247	0,884
	Family group (selected-resistant)	-0,005	0,018	0,247	0,884
	Line	-0,031	0,013	5,084	**0,024**
	Temperature	-0,009	0,004	5,839	**0,016**
Aerobic scope	Intercept	-0,670	0,199	na	na
	Family group (control-resistant)	-0,011	0,017	0,484	0,785
	Family group (selected-resistant)	0,000	0,016	0,484	0,785
	Line	-0,023	0,017	2,096	0,148
	Temperature	-0,027	0,011	7,499	**0,006**

Table 3. Length specific growth rates, mass specific growth rate, variation of the Fulton's condition factor, and female gonado-somatic index for brook trout *Salvelinus fontinalis* exposed at three different temperatures during a 35 day period. Mean ± SD. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. Bold characters indicate a significant difference between lines ($p < 0.001$).

	Length Specific Growth Rate ($mm\ day^{-1}$)	Mass Specific Growth Rate ($g\ day^{-1}$)	Δ Fulton	Female GSI
15°C	0.39 ± 0.13	0.53 ± 0.27	0.035 ± 0.053	
17°C	0.37 ± 0.14	0.53 ± 0.32	0.038 ± 0.061	
19°C	0.25 ± 0.14***	0.45 ± 0.38	0.044 ± 0.070**	
Control	0.34 ± 0.15		0.023 ± 0.049	2.22 ± 0.88
Selected	0.33 ± 0.15		0.055 ± 0.067**	0.66 ± 0.70***
Resistant	0.35 ± 0.16		0.034 ± 0.071	
Sensitive	0.31 ± 0.16*		0.020 ± 0.072	
Control-Resistant		0.45 ± 0.32		1.87 ± 0.81
Control-Sensitive		0.44 ± 0.28		2.61 ± 0.81*
Selected-Resistant		**0.66 ± 0.32***		0.54 ± 0.62
Selected-Sensitive		**0.46 ± 0.35**		0.78 ± 0.77

Table 4. Statistical nested ANOVA results on the relative expression of target genes under study.

Tissue	Candidate gene	Temperature	Line	Resistance (nested in Line)	Temperature x Line
Liver	*IGF1*	$F_{2,89} = 0.08$, p = 0.93	**$F_{2,89} = 10.36$, p = 0.002**		$F_{2,89} = 0.78$, p = 0.46
	GHr	$F_{2,89} = 1.03$, p = 0.36	$F_{1,89} = 1.96$, p = 0.16		$F_{2,89} = 2.59$, p = 0.08
	COX	**$F_{2,89} = 5.97$, p = 0.003**	$F_{1,89} = 0.87$, p = 0.35		$F_{2,89} = 0.27$, p = 0.76
	CS	$F_{2,89} = 2.16$, p = 0.12	$F_{1,89} = 0.27$, p = 0.61		$F_{2,89} = 0.23$, p = 0.79
	CAT	$F_{2,89} = 2.64$, p = 0.08	**$F_{2,89} = 13.29$, p < 0.001**		$F_{2,89} = 0.35$, p = 0.71
	SOD	**$F_{2,89} = 3.38$, p = 0.04**	$F_{2,89} = 0.02$, p = 0.90	**$F_{2,89} = 4.42$, p = 0.01**	
	GPx	$F_{2,89} = 1.48$, p = 0.23	$F_{1,89} = 0.23$, p = 0.64		$F_{2,89} = 0.39$, p = 0.68
	BCI	$F_{2,89} = 1.79$, p = 0.17	$F_{1,89} = 1.04$. p = 0.31		$F_{2,89} = 0.51$, p = 0.60
	HSP70	$F_{2,89} = 0.18$, p = 0.83	$F_{1,89} = 2.39$, p = 0.13		$F_{2,89} = 0.09$, p = 0.91
	HSP90	$F_{2,89}$ = **6.76, p = 0.002**	**$F_{1,89}$ =**1.82, p = 0.17		$F_{2,89}$ = 1.33, p = 0.25
Gills	*Na-K-ATPα1a*	$F_{2,90}$ = 1.38, p = 0.26	$F_{2,90}$ = 0.02, p = 0.90	**$F_{2,90}$ = 4.57, p = 0.01**	
	Na-K-ATPα1b	$F_{2,89} = 1.28$, p = 0.28	$F_{1,89} = 0.02$, p = 0.90		$F_{2,89} = 1.10$, p = 0.34
	HSP70	$F_{2,89} = 1.44$, p = 0.24	$F_{1,89} = 2.55$, p = 0.11		$F_{2,89} = 0.61$, p = 0.55
	HSP90	**$F_{2,90} = 4.64$, p = 0.01**	$F_{1,90}$ = 0.41, p = 0.54		$F_{2,90}$ = 0.88, p = 0.42
	Caspase 9	$F_{2,90}$ = 1.13, p = 0.33	**$F_{2,90} = 6.27$, p = 0.01**		**$F_{2,90} = 6.87$, p= 0.002**
Muscle	*IGF1r*	**$F_{2,86} = 4.41$, p= 0.02**	$F_{2,86}$ = 0.94, p = 0.33		**$F_{2,86} = 5.17$, p = 0.008**
	Myogenin	$F_{2,86}$ = 0.45, p = 0.64	**$F_{2,86} = 5.52$, p = 0.02**	$F_{2,86}$ = 1.13, p = 0.33	

Myosin	$F_{2,89} = 0.64$, $p = 0.53$	$F_{1,89} = 0.10$, $p = 0.75$		$F_{2,89} = 1.98$, $p = 0.14$
LDH	$F_{2,86} = 2.05$, $p = 0.13$	$F_{2,86} = 4.45$, $p = 0.04$		$F_{2,86} = 0.81$, $p = 0.45$
PK	**$F_{2,86} = 5.19$, $p = 0.007$**	$F_{2,86} = 1.09$, $p = 0.30$	**$F_{2,86} = 3.93$, $p = 0.02$**	

IGF1: Insulin-like growth factor; *GHr*: Growth hormone receptor; *COX*: Cytochrome c oxidase; *CS*: Citrate synthase; *CAT*: Catalase; *SOD*: Superoxide dismutase; *GPx*: Glutathione peroxidase; *BCl*: B-cell lymphoma 2; *hsp70*: Heat shock protein 70; hsp*90*: Heat shock protein 90; *Na-K-ATPα1a*: Sodium-potassium ATPase, subunit α1a; *Na-K-ATPα1b* : Sodium-potassium ATPase, subunit α1b; *IGF1r* : IGF1 receptor; *LDH* : Lactate dehydrogenase; *PK* : Pyruvate kinase. Significant effects are indicated in bold. In absence of significant nested effect, the temperature x line interaction was tested.

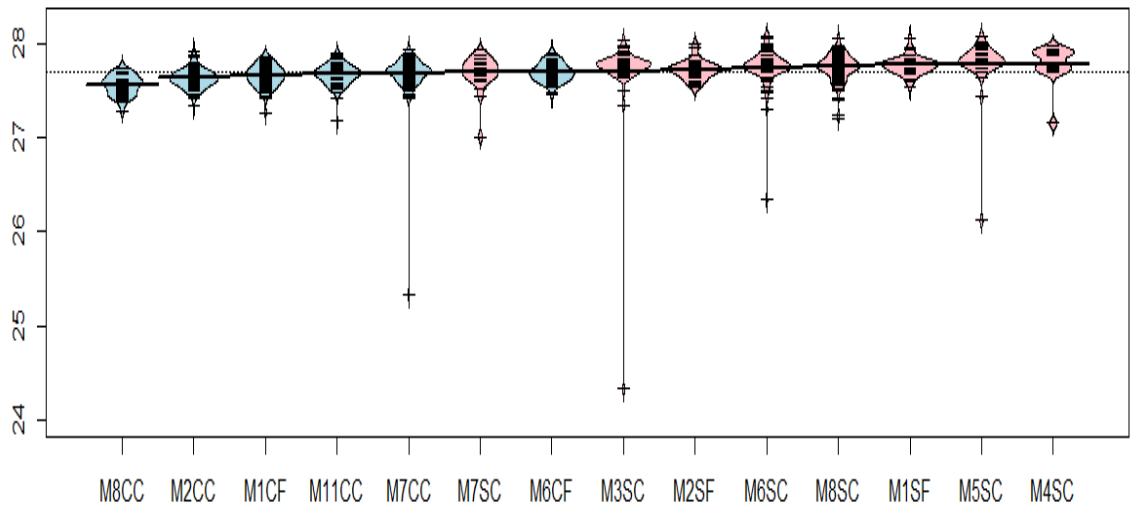


Figure S1. Temperature at which juveniles lost equilibrium during a thermal experimental challenge test (Gourtay, pers. comm.) for different families from the control (M8CC, M2CC, M1CF, M11CC, M7CC, M6CF) and from the selected line (M7SC, M3SC, M2SF, M1SF, M5SC, and M4SC). For the work presented in this article, we used juveniles issued from M8CC (temperature sensitive control), M11CC (temperature resistant control), M7SC (temperature sensitive selected), and M5SC (temperature resistant selected) families.

TABLE S1. Checklist of 53 essential criteria for the reporting of methods for aquatic intermittent-flow respirometry according to Killen et al. (2021).

Number	Criterion and Category	Response	Value (where required)	Units
	EQUIPMENT, MATERIALS, AND SETUP			
1	Body mass of animals at time of respirometry		15°C = 97.25 ± 6.17 17°C = 89.50 ± 4.99 19°C = 96.00 ± 5.63	Mean mass in g ± SE
2	Volume of empty respirometers		0.75 (Length = 250 mm; Inside diameter = 62 mm)	L
3	How chamber mixing was achieved	Mixing was achieved using a 120 V 300 L/min Eheim pump		
4	Ratio of net respirometer volume (plus any associated tubing in mixing circuit) to animal body mass		15°C = 1: 8.97 (0.87 L to 0.097 kg) 17°C = 1:9.67 (0.87 L to 0.0.090 kg) 19°C = 1: 9.06 (0.87 L to 0.096 kg)	
5	Material of tubing used in any mixing circuit	Silicon		
6	Volume of tubing in any mixing circuit		0.12	L
7	Confirm volume of tubing in any mixing circuit was included in calculations of oxygen uptake	Yes		
8	Material of respirometer (e.g. glass, acrylic, etc.)	Acrilyc		
9	Type of oxygen probe and data recording	Oxygen probe: optode oxygen probe by PreSens Data recording: AutoResp by Loligo Systems		
10	Sampling frequency of water dissolved oxygen		1	s
11	Describe placement of oxygen probe (in mixing circuit or directly in chamber)	In mixing circuit		
12	Flow rate during flushing and recirculation, or confirm that chamber returned to normoxia during flushing		300	L/h
13	Timing of flush/closed cycles		120 flush/110 closed	s

14	Wait (delay) time excluded from closed measurement cycles		10	s
15	Frequency and method of probe calibration (for both 0 and 100% calibrations)	Probes were calibrated (0 and 100%) before every new temperature treatment		
16	State whether software temperature compensation was used during recording of water oxygen concentration	Yes, temperature compensation was used during water oxygen recording		
	MEASUREMENT CONDITIONS			
17	Temperature during respirometry		15 ± 0.5 17 ± 0.5 19 ± 0.5	°C
18	How temperature was controlled	Temperature was controlled by two 300 W submersible heaters placed in each water bath		
19	Photoperiod during respirometry	Photoperiod was set to mimic that of Point-au-père, QC, Canada, during the experimental period (August-September 2020)		
20	If (and how) ambient water bath was cleaned and aerated during measurement of oxygen uptake (e.g. filtration, periodic or continuous water changes)	The supply of the water bath was set on a continuous water exchange with two aerators being placed at opposite corners		
21	Total volume of ambient water bath and any associated reservoirs		100	L
22	Minimum water oxygen dissolved oxygen reached during closed phases		80	%
23	State whether chambers were visually shielded from external disturbance	Yes, a black curtain was placed in front of the water baths to avoid any external disturbances		
24	How many animals were measured during a given respirometry trial (i.e. how many animals were in the same water bath)	A total of 8 animals were measured at any given time, with four animals per water bath		
25	If multiple animals were measured simultaneously, state whether they were able to see each other during measurements	Yes, fish within the same water bath were able to see each other during measurements		
26	Duration of animal fasting before placement in respirometer		24	h
27	Duration of all trials combined (number of days to measure all animals in the study)		12 (12-23 of September 2020)	d
28	Acclimation time to the laboratory (or time since capture for field studies) before respirometry measurements	Fish were hatched in the laboratory so they had been acclimated to		

		laboratory conditions for their whole life.		
	BACKGROUND RESPIRATION			
29	State whether background microbial respiration was measured and accounted for, and if so, method used (e.g. parallel measures with empty respirometry chamber, measurements before and after for all chambers while empty, both)	Yes background respiration was accounted for using measurements before and after fish were placed and removed from each respirometer		
30	State if background respiration was measured at beginning and/or end, state how many slopes and for what duration	Background respiration was measured at the beginning and end for one full cycle (1 slope		
31	State how changes in background respiration were modelled over time (e.g. linear, exponential, parallel measures)	Background respiration were modelled linearly		
32	Level of background respiration (e.g. as a percentage of SMR)		<10	%
33	Method and frequency of system cleaning (e.g. system bleached between each trial, UV lamp)	While each individual chambers were not cleaned after each trial, a large water exchange was provided within the water bath after every trial		
	STANDARD OR ROUTINE METABOLIC RATE			
34	Acclimation time after transfer to chamber, or alternatively, time to reach beginning of metabolic rate measurements after introduction to chamber	Metabolic rate measurements (MMR) began immediately following fish being placed in the respirometers		
35	Time period, within a trial, over which oxygen uptake was measured (e.g. number of hours)		21-23	h
36	Value taken as SMR/RMR (e.g. quantile, mean of lowest 10 percent, mean of all values)	10^{th} percentile		
37	Total number of slopes measured and used to derive metabolic rate (e.g. how much data were used to calculate quantiles)		~300	slopes
38	Whether any time periods were removed from calculations of SMR/RMR (e.g. data during acclimation, periods of high activity [e.g. daytime])	Only the first slope was not used to calculate SMR as it was used to calculate MMR		
39	r^2 threshold for slopes used for SMR/RMR (or mean)		0.9	
40	Proportion of data removed due to being outliers below r-squared threshold	No outliers were removed		

	MAXIMUM METABOLIC RATE			
41	When MMR was measured in relation to SMR (i.e. before or after)	Before		
42	Method used (e.g. critical swimming speed respirometry, swim to exhaustion in swim tunnel, or chase to exhaustion)	Chase		
43	Value taken as MMR (e.g. the highest rate of oxygen uptake value after transfer, average of highest values)	The first slope following fish being placed in the respirometer		
44	If MMR measured post-exhaustion, length of activity challenge or chase (e.g. 2 min, until exhaustion, etc.)		5	min
45	If MMR measured post-exhaustion, state whether further air-exposure was added after exercise	Further air-exposure was not added other than for transferring the fish from the chase tank to the respirometer		
46	If MMR measured post-exhaustion, time until transfer to chamber after exhaustion or time to start of oxygen uptake recording		< 1	min
47	Duration of slopes used to calculate MMR (e.g. 1 min, 5 min, etc.)		110	s
48	Slope estimation method for MMR (e.g. rolling regression, sequential discrete time frames)	Rolling regression		
49	How absolute aerobic scope and/or factorial aerobic scope is calculated (i.e. using raw SMR and MMR, allometrically mass-adjusted SMR and MMR, or allometrically mass-adjusting aerobic scope itself)	Raw SMR and MMR		
	DATA HANDLING AND STATISTICS			
50	Sample size	**8**		
51	How oxygen uptake rates were calculated (software or script, equation, units, etc.)	RespR package		
52	Confirm that volume (mass) of animal was subtracted from respirometer volume when calculating oxygen uptake rates	Confirmed		
53	State whether analyses accounted for variation in body mass and describe any allometric mass-corrections or adjustments	Yes, specific metabolic rates were compared between treatments		