Selection effects on early life history traits and thermal resistance in brook charr *Salvelinus fontinalis*

Clémence Gourtay[1], Marine Rivolet[1], Léopold Ghinter[1], Louis Bernatchez[2], Dany Garant[3], Céline Audet[1]

[1] ISMER (Institut des Sciences de la MER de Rimouski), Université du Québec à Rimouski, QC, Canada

[2] IBIS (Institut de Biologie Intégrative et des Systèmes), Université Laval, Québec, QC, Canada

[3]Département de Biologie, Université de Sherbrooke, Sherbrooke, QC, Canada

ORCID numbers

C. Audet: 0000-0003-3366-210X

L. Bernatchez: 0000-0002-8085-9709

L. Ghinter: 0000-0002-7162-1774

D. Garant: 0000-0002-8091-1044

C. Gourtay: 0000-0002-0347-0847

Abstract

In the context of climate change, it is crucial to understand whether animals that have been domesticated and/or selected maintain their abilities to adapt to changes in their thermal environment. Here, we tested how selection for absence of early sexual maturation combined with better growth performance may have impacted thermal resistance and gene expression response in the presence of thermal stress in brook charr *Salvelinus fontinalis* (Mitchill, 1814). We performed temperature challenge tests on brook charr 0+ juveniles and studied the expression of genes involved in the response to oxidative stress, in synthesis of heat shock proteins, or involved in regulation of apoptosis, in heart and liver tissues. Juveniles from the selected lineage had a higher thermal resistance than controls and a loss of equilibrium occurred on average 1°C above what was observed for the controls. The relative expressions of *catalase* and *HSP70* were significantly higher in juveniles from the selection program. Overall, thermally sensitive fish were characterized by low mass and length and lower relative expressions of genes associated with stress response. Our results indicate that selection for traits of interests may be indirectly related to the significant lineage effect on growth in early stages of development.

Keywords: maternal effect, paternal effect, selection, temperature, challenge tests

Introduction

In animal production, it is essential to ensure that environmental rearing conditions are adequate for fish growth, welfare, and profitability. Factors such as rearing physical units, environmental conditions, season, species, genetic background, developmental stage, and exposure to multiple stressors should be considered because they can affect all levels of the organisms' growth responses (Ashley 2007; Alfonso et al. 2020; Islam et al. 2021). Except for recirculating aquaculture systems, where environmental conditions can be tightly controlled, several fish farming areas are already facing environmental changes such as increased water temperature (Ahmed et al. 2019). In flow-through or open production systems, where environmental conditions are difficult or impossible to control, fish are exposed to seasonal variations in water temperature, and these variations can differ from one location to another.

Changes in environmental conditions, such as temperature, hypoxia, and algal blooms, are increasing in frequency and duration, and are often unpredictable (Rabalais et al. 2010; Frölicher and Laufkötter 2018; Collins et al. 2019; Rodgers 2021). Fishes are especially vulnerable to these environmental changes because their physiology—as ectotherms—is determined by thermodynamic effects of the surrounding water temperature, which sets their body temperature (Currie and Schulte 2014; Fry 1971; Schulte et al. 2011). This context also affects stocking activities where released fish must cope with a changing environment. Resistance (in this paper, considered as a weak reaction to a sudden and intense change) and tolerance (adaptation to lasting changes) to temperature are therefore of interest for fish farm management and productivity.

Domestication has been practiced for centuries but applied to relatively few terrestrial crops and animals (Giuffra et al. 2000; Diamond 2002; Burt 2005; Pozzi and Salamini 2007; Kovach

et al. 2007). Phenotypic changes caused by artificial selection have been abundantly documented in several domesticated species and can be interpreted as rapid human-induced evolutionary changes (Duarte et al. 2007). In the last decades, selective breeding of fishes has considerably increased production performances of farmed species (Janssen et al. 2017; Wiens et al. 2018). Yet, it has been suggested that fish selected for faster growth may suffer from an immune-response deficiency and reduced adaptive potential to pathogen exposure (Glover et al. 2006a, 2006b). Therefore, the goals of breeding programs should be redefined to include not only production traits, but also economic traits, such as veterinary costs (e.g., resulting from higher rates of disease occurrences), as well as the welfare of animals and their response to environmental changes. Understanding how changes in relevant environmental parameters affect physiological performance is vital in selection processes.

Fish experiencing temperature variations may experience alterations in their biochemical, molecular, and physiological processes related to the maintenance of homeostasis (Birnie-Gauvin et al. 2017; Corey et al. 2017; Cheng et al. 2018; Vargas-Chacoff et al. 2018). Thermal stress can lead to oxidative stress in organisms through the production of reactive oxygen species (ROS) and the organism's inability to detoxify the ROS role in the maintenance of cellular activity, including inter- and intracellular active ROS or injury repair (Birnie-Gauvin et al. 2017). ROS are naturally synthesized by cells and play a major signaling role (ability of a cell to receive, process, and transmit signals) (Halliwell and Gutteridge 2015). Nevertheless, when organisms are exposed to environmental stress, ROS can increase extensively and cause cell injury. These active species are known to increase heat shock factors (Lesser 2011) and the expression of genes coding for heat shock proteins (HSP) (Kregel 2002; Heise et al. 2006). In fish, HSPs function as molecular chaperones to prevent protein aggregation and denaturation, and to maintain protein homeostasis during periods of thermal stress (Iwama et al. 1999).

HSP70 and HSP90 are two highly conserved proteins from the heat shock response (Lindquist and Craig 1988). The expressions of genes coding for HSP in response to a thermal challenge has been documented for salmonids species (Narum et al. 2010; Stitt et al. 2014; Corey et al. 2017). For example, the temperature of HSP70 induction in brook charr *Salvelinus fontinalis* (Mitchill, 1814) was correlated with the thermal ecological limits of this species in the wild (Chadwick Jr. et al. 2015), and levels of *HSP70* expression were shown to covary with differences in thermal tolerance among populations (Stitt et al. 2014). Variations among populations in the magnitude of HSP and the capacity for acclimation may play a significant role in determining the ecological response of species to climate change (Tomanek 2008, 2010; Somero 2010). Fishes have also developed antioxidant defense mechanisms to scavenge ROS and consequently control the oxidative damage they induce, including antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathion peroxidase (GPx; Martínez-Álvarez et al. 2005). In addition, oxidative stress can directly or indirectly damage DNA and cause cellular apoptosis (Chandra et al. 2000). Apoptosis is a process of programmed cell death that plays a vital role in cellular development and the immune system as an indispensable component of various cellular processes capable of mediating the phagocytic removal of dying or infected cells (AnvariFar et al. 2017). Caspase is a family of cysteine proteases that play essential roles in the process of apoptosis. Caspase-9, the initiator of caspases, can activate downstream caspases (Wang and Lenardo 2000). Caspase-3 is the major executioner of caspases; it is responsible for the proteolytic cleavage of many critical cellular proteins (Elmore 2007). Finally, the control and regulation of these apoptotic mitochondrial events occur through members of the BCL-2 (B-cell lymphoma) family of proteins (Cory and Adams 2002), which are anti-apoptotic. These proteins have special significance since they can determine if the cell undergoes apoptosis or aborts the process (Elmore 2007).

Brook charr supports ecologically, socioeconomically, and culturally important fisheries in North America, South America, Europe, and parts of Asia and Australia (MacCrimmon 1971; Budy et al. 2013). In Québec, brook charr is an important species for recreational fishing, and fish farming production is strongly linked to restocking (MAPAQ 2019). From 1948 to 2016, the annual average air temperature of Canada has increased by 1.7°C, about twice as fast as the world average (Bush and Lemmen 2019). Brook charr is particularly vulnerable to climate change because of its dependence on cold, clean water (Wenger et al. 2011). Genetic variation may not be sufficient to allow rapid adaptation to new selection pressures induced by global warming (Møller and Merilä 2004). Phenotypic plasticity could represent a rapid response mechanism to adapt to these changes (Merilä and Hendry 2014).

Two lineages, one control (C) and one under selection (S), of the Laval brook charr strain were used to produce and raise families under identical conditions. The selected lineage originated from a breeding program aiming to optimize growth and minimize early sexual maturation, while the control lineage was from random breeding (Sauvage et al. 2010; Houle et al. 2023). In this study, we tested how selection for the absence of early sexual maturation combined with better growth performance may have impacted thermal resistance and gene expression response in the presence of thermal stress in brook charr. We used an F5 generation from both the C and S lines and tested for the presence of parental effects and estimated heritability of early growth rates. At about seven months of age, we investigated the thermal resistance of 0+ brook charr juveniles and tested how selection may have impacted thermal resistance and the gene expression response in the presence of thermal stress by examining the expression of genes involved in the response to oxidative stress (*CAT*, *SOD*, *GPX*), heat shock (*HSP70*, *HSP90*), and apoptosis (*CASP3*, *CASP9*, *BCL*) in heart and liver tissue. These two tissues were chosen because of their high rate of aerobic metabolism (heart) and their role in detoxification (liver).

Using transcriptomics, Sauvage et al. (2010) showed that, after three generations of selection, there was an over-representation of genes related to growth (protein metabolism, different coenzymes) in the S line of the Laval strain when compared to individuals from the C lineage, but an under-representation of genes related to immunity, indicating potential negative effects of the selection process. Based on this, we hypothesized that control fish would perform better than selected fish in response to thermal stress.

Materials and methods

Breeding, animal husbandry, and thermal challenges were done according to Canadian Council of Animal Protection recommendations and protocols have been approved by the UQAR Animal Care Committee.

Animals and general rearing conditions

Juvenile brook charr were obtained from fifth-generation Laval strain breeders reared in captivity at the Station aquicole (ISMER/UQAR, Rimouski, QC, Canada). The Laval strain originates from a wild population of anadromous brook charr from the Laval River (48°44′N; 69°05′W) on the north shore of the St. Lawrence estuary (QC, Canada; Crespel et al. 2011, 2013). Two lineages were used: the control lineage was obtained from random crosses at each generation, while the selected lineage was issued from a breeding program aiming to optimize growth and minimize early sexual maturation (Sauvage et al. 2010). Fish were reared under natural temperature, salinity, and photoperiod conditions, according to their life history stage.

Breeders from both lineages, control (C) and selected (S), were reared in water with a salinity of 20‰ until mid-September, when gradual freshwater (FW) transition was completed within one week. Ovulation began in mid-November and lasted through mid-December within each

lineage. Crosses were made separately within each lineage. The eggs of each female were split into two batches, each fertilized by two different males, and each male was used to fertilize the eggs of two different females (Fig. 1).

From egg incubation (December 2018) to exogenous feeding (June 2019), half-sib families (9 for the C lineage; 14 for the S lineage) were kept separate in recirculating freshwater and reared in three troughs, each trough being divided into 11. Eggs were incubated in a flow-through system in darkness until hatching, and temperature followed the normal winter decrease to 4°C. Water temperature was maintained at 4°C until two weeks post hatching and then gradually increased to 8°C (1°C per week) with a 12:12 photoperiod to reach the optimal temperature for the first feeding stage. Temperature was recorded every day, and dead eggs or dead fry were counted each morning. Surviving fry were counted once first feeding started, which allowed us to determine the total number of eggs present for each family at the start of the experiment. Development times (100% hatch, 100% yolk-sac resorption) were calculated in degree days (sum of temperatures measured each morning; DD). At hatching, the time of development was calculated from fertilization to 100% hatching. At the yolk-sac resorption stage, the time of development was calculated from fertilization until resorption. When the seasonal water temperature reached 8°C, juveniles were exposed to natural seasonal temperature and photoperiod conditions (46°45′ N) and were fed according to commercial charts (% of food per body mass according to fish length and temperature conditions). They were marked according to paternal identity by fin clippings, and an average of 92 juveniles issued from each male were transferred to 0.2 m^3 tanks, combining progenies from eight different males per tank. A total of 14 paternal progenies were obtained, seven control and seven selected.

Survival and development monitoring

189 At 100% hatching, 50 individuals per family were sampled, and measurements of
190 embryonic length, yolk-sac length (YSL), and yolk-sac diameter (mm) were made using a
191 caliper. The standard cylindrical relationship of yolk-sac volume ($YSV = \pi \times YSL \times r^2$) was
192 used to estimate yolk-sac volume (mm^3), where r represents the yolk-sac radius (Perry et al.
193 2004). In some families with very low survival percentages, measurements were made only on
194 25 individuals. At yolk-sac resorption, 50 individuals (25 in families with low survival) per
195 family were measured.
196
197 **Preliminary thermal resistance experiment**
198 To optimize conditions of the temperature challenge tests, i.e. to determine the inflexion
199 point at which temperature is raised at a slower rate to better discriminate thermal resistance of
200 tested fish, critical thermal maximum (CTmax) (McKenzie et al. 2021) experiments were
201 conducted prior to challenge tests. These trials were conducted on the same day on 0+ juveniles
202 in a 0.2 m^3 circular test tank equipped with two 1800 W heaters (EHEIM). A submersible pump
203 (10 L min^{-1}; 10 W; 60 Hz, EHEIM) was used to generate a slow circular current within the tank.
204 The water O_2 level, monitored with a FireSting GO_2 oximeter (PyrosScience GmbH, Aachen,
205 Germany), was maintained > 90% O_2 air saturation using gentle bubbling. At the beginning of
206 the trial, the tank was filled with the same fresh, aerated, dechlorinated, municipal tap water
207 supplying the rearing tanks at 12.6°C. A total of four CTmax trials were conducted. Fish were
208 starved 24 h prior to CTmax trials. Fifteen juveniles (different fish for each trial) were randomly
209 collected from rearing tanks and gently transferred to the trial tank. After a 20-min acclimation
210 period, the two heaters were turned on and the CTmax trial began. Water was heated at a
211 constant rate of 0.2°C min^{-1}, and temperature was recorded every 30 s during the trial (Optical
212 Oxygen and Temperature meter FireSting-O_2, Pyroscience, Aachen, Germany). The loss of
213 equilibrium (LOE) was considered as the CTmax endpoint (Ziegeweid et al. 2008). As soon as

214 a loss of equilibrium was noted, juveniles were removed from the tank and put back in rearing
215 tanks for recuperation. Once each reached this endpoint, the final temperature was recorded.
216 The CTmax trial ended when the last fish lost equilibrium. The mean CTmax was about 28.47
217 ± 0.21°C (mean ± S.D.).
218
219 **Thermal resistance trials**
220 Two thermal resistance trials (S and C juveniles) were conducted with the same set up
221 used for the CTmax trials (190 L conical tank, submersible pump 10 L min^{-1}, bubbling air,
222 oximeter, and heaters, O_2 > 90%). We needed to test both lines separately to avoid familial
223 marking overlaps A maximum of 350 fish (non-previously used for the CTmax tests) were
224 tested at once per challenge. Fish were randomly collected from their respective rearing tanks
225 and gently transferred to the trial tank. Identification of markings prior to the transfer would
226 have induced important stress. As initial numbers or juveniles per paternal progeny that have
227 been marked and maintained in rearing tanks were similar, we expected all male progenies
228 being represented in the challenge tests. After a 20-min acclimation period, heaters were turned
229 on and temperature was incrementally increased as described in Claireaux et al. (2013) and
230 Mauduit et al. (2016, 2019). During the first period, temperature increased by 0.1°C min^{-1}
231 (1800 W) until reaching 26°C after which a slower increase rate (0.02°C min^{-1}) was applied
232 (300 W). The inflection point of the curve was determined from the results obtained for the
233 CTmax trial (~ 2°C before CTmax was reached). Temperature was recorded every 30 s during
234 the trial. The thermal resistance endpoint was considered to be the LOE (Ziegeweid et al. 2008).
235 The thermal resistance trial ended when the last fish lost equilibrium. Every fish that was
236 removed from the experimental tank was identified to male breeder (fin clipping marks). LOE
237 temperature was expressed in heat accumulation (°C) over time (min), hereafter referred as
238 degree-minutes, and calculated as follows:

Cumulated degree-minutes at time t = $\sum_{t=0}^{t=n}(T_t - T_0)$

T_t: temperature (°C) at t time

T_0: initial temperature, here corresponding to the beginning of the experiment

n: duration of the experiment in minutes

Cumulated degree-minutes (CDM) were used instead of temperature or exposure time (taken separately) to combine the magnitude of temperature change experienced by fish with an exposure time "x". Using cumulated degree-minutes leads to an accurate comparison of experiments, allowing an integration of inter-experiment temperature variations (Fig. S1).

Tissue samplings

There were three sampling periods during each trial: 1) The first ten fish reaching LOE (classified as sensitive), a group of ten fish which reached LOE in the middle of the trial (the 170th to 180th fish removed from the experimental tank, classified as median), and the last 10 fish reaching LOE (340th–350th, classified as resistant). When fish lost equilibrium, they were anaesthetized with MS-222 (0.08 g L^{-1}; Sigma-Aldrich Co., Missouri, USA), weighed, measured (standard length [SL]), the spinal cord severed, and liver and heart were dissected out and stored at –80°C.

mRNA expression

Total liver RNA (n = 8 per treatment) was extracted from 30 mg of tissue using the RNeasy Plus Universal Mini Kit (ref: 73404, Qiagen Inc., Mississauga, ON, Canada) according to the manufacturer's instructions. Heart mass was < 30 mg, so pools of three hearts (same lineage, same level of sensitivity) were used to obtain 30 mg of tissue and extracted using

RNeasy Fibrous Tissue Mini Kit (ref: 74704, Qiagen Inc.). Total RNA purity and concentration were controlled using the 260/280 nm absorbance ratio measured with a NanoDrop instrument (NanoDrop ND-1000 spectrophotometer version 3.3.0; NanoDrop Technologies, Inc., Wilmington, Delaware, USA). RNA purity was also assessed by SYBR safe staining of 28S and 18S ribosomal RNA bands separated by electrophoresis on a 1.2% agarose gel. cDNA was obtained by reverse transcription (in duplicate) on 200 ng μl^{-1} of total RNA from each sample using a Quantitect Reverse Transcription kit® (ref: 205313, Qiagen Inc.) with integrated removal of genomic DNA contamination. cDNA concentrations were estimated using a NanoDrop spectrophotometer. Duplicate cDNAs were pooled for each sample and stored at −20°C until analyses. qPCR was performed for each sample on pooled cDNA using the iCycler iQ™ (Bio-Rad Laboratories Inc., Ontario, Canada) with TaqMan™ Fast Advanced Master Mix (ref: 4444964, Life Technologies, USA) and Taqman primers and probes (ref: 4331348, Life Technologies, USA).

Taqman mRNA primers were designed using Primer Express software version 3.0 (Applied Biosystems, Waltham, MA, USA; Table 1). For the three housekeeping genes, *β-actine* (KF783182.1), *18S* (FJ710889.1), *EF1 α* (KF783203.1), and for *HSP70* (KF783199.1), and *HSP90* (KF783201.1), mRNA sequences from brook charr were available on the GenBank database (Sayers et al. 2019). *CAT*, *SOD*, *CASP3*, and *CASP9* sequences from *S. fontinalis* transcriptome were obtained from L. Bernatchez's lab (Université Laval, QC, Canada) and used to design primers. The *GPx* sequence was obtained from K. Jeffries' lab (University of Manitoba, Canada). Finally, the *BCL* sequence for brook charr was not available, so we used the primer designing tool of NCBI (https://www.ncbi.nlm.nih.gov/tools/primer-blast/) for Atlantic salmon (*Salmo salar*) sequence (NM_001141086.1) to obtain primers and do classical PCR (AmpliTaq Gold 360 Master Mix, ref: 4398881, Applied Biosystems, USA) to obtain

products that were sequenced. Once a specific sequence of brook charr was obtained, TaqMan mRNA primers were designed using Primer Express.

TaqMan™ Fast Advanced Master mix (Thermo Fisher Scientific, Inc.) was used to prepare all qPCR reaction mixtures. The cycle parameters were as follows: UNG incubation at 50°C for 2 min, polymerase activation at 95°C for 20 s, denaturation at 95°C for 1 s, and then annealing and extension at 60°C for 20 s; 45 cycles were done with QuantStudio 3 Real Time PCR System (ThermoFisher, USA). All RT-qPCR reactions were performed in triplicate.

The comparative Cycle Threshold (CT) method (also known as the $2^{-\Delta\Delta CT}$ method) from Livak and Schmittgen (2001) was used to calculate the relative amount of transcripts in all groups. Before applying this method, several assumptions were verified: the efficiency of the PCR was close to 1 and the PCR efficiency of the target gene was similar to the internal control gene (Livak and Schmittgen 2001). To determine which gene transcripts were up- or down-regulated according to lineages and thermal resistance, $2^{-\Delta\Delta CT}$ was calculated as follows: $\Delta\Delta$Ct is the ΔCt for the unknown minus ΔCt for the calibrator sample (control – sensitive), and Ct is the difference between the Ct for the target gene and the mean of reference genes. A score was used to identify the most stable reference gene in samples (Vandesompele et al. 2002). For heart, *18S*, *β-actine*, and *EF1 α* were used as reference genes, while *β actine* and *EF1 α* genes were used for liver. Ct of *CASP3* was too high (mRNA level expression too low) to have reliable results for most samples, so we decided not to consider it for the rest of the study.

Statistical analyses

Arcsin transformation was applied to survival at hatching data to achieve normality. Generalized Linear Mixed Models (GLMM) and Linear Mixed Models (LMM) were built for

survival data and for SL and YSV, respectively (lineage: fixed effect, dam and sire identity: random effects; package lme4). Models were simplified by a backward elimination procedure, where the least significant term, based on p-value, was sequentially removed, until all remaining variables were significant (i.e., $p \leq 0.05$, confirmed by a Likelihood Ratio Test). Marginal and conditional R^2 were obtained via the r-squared function from the piecewiseSEM R package (Lefcheck 2016). Linear models were used for degree-days (DD) because random effects were not significant for this variable (see Table 2 for selected models).

Animal models were built to decompose the phenotypic variance using ASReml-R, version 4. The total phenotypic variance (V_P) was decomposed into the additive genetic variance (V_A), the variance associated to dam (V_D), and the variance attributed to sire (V_S). We also included fixed effects deemed significant by analyses described above. Since V_S was always negligible, only four models were retained: Null, V_D, V_A, V_A+V_D. Model selection was made following a comparison of Akaike information criteria (AIC). The ΔAIC values (difference between the AIC of one model and the lowest AIC obtained) were calculated. The models that were retained were those with the highest AIC (as in Vega-Trejo et al. 2018), and the weight (wi) of each model was calculated. The proportions of phenotypic variance and heritability h^2 (= V_A/V_P) were calculated with $V_P = \Sigma$ of variance components and V_D/V_P being the maternal variance.

A Cox proportional hazards regression with mixed effects was used to model LOE temperature and to examine the effects of lineage (C~~L~~, S~~L~~) on thermal resistance (Cox 1972; Therneau 2018). The model included lineage (C, S) as fixed effect and male spawner as random effect. A key assumption of Cox proportional hazards regression is that the effect of a given predictor variable is consistent over the period of interest. To test this assumption, a model including only fixed effects was fitted using the coxph() function in the survival package (Therneau 2015;

Therneau and Grambsch 2000). The proportional hazards assumption was tested by assessing the correlation of Schoenfeld residuals using the cox.zph() function (survival package). This approach revealed no violation of the proportional hazard assumption for lineage (Schoenfeld individual test: lineage $p = 0.37$, global $p = 0.37$). The effects of thermal resistance and lineage were tested using two-way ANOVAs for mass and length data collected on sampled fish.

To examine variations in the relative quantification of gene expression ($2^{-\Delta\Delta CT}$) for the seven candidate genes for lineage and thermal resistance, visual inspections of boxplots and QQ-plots were used to identify outlier values (total of eight individual liver data values; Fig. S2). We then performed principal component analysis (PCA) for both heart and liver data. For each tissue, two-way permutational multivariate analysis of variance (PERMANOVA) based on a Bray-Curtis distance was conducted. The homogeneity of multivariate dispersions was evaluated for each factor using the permutation analysis of multivariate dispersion routine before each PERMANOVA (Anderson 2001). Post-hoc tests were carried out using multiple pairwise comparisons with Bonferroni correction to identify differences among factors (Martinez Arbizu 2017). SIMPER analysis was performed to determine the contribution of the gene responsible for dissimilarities between treatments. Finally, gene expression of liver and heart were analyzed using two-way ANOVAs. Residuals were tested for normality using the Shapiro–Wilk test and homogeneity of variances was tested using a Levene test. Heart *GPx*, liver *CASP9,* and liver *GPx* $2^{-\Delta\Delta CT}$ were $\log_{10}$-transformed to meet normality. Tukey mean comparison tests were done because homoscedasticity was respected.

Differences were considered significant at $\alpha = 0.05$. Results are presented as mean ± SD. All data were analyzed using R (ver. 4.0.3; R Development Core Team) with the following packages: 'survival' (Therneau 2022), 'coxme' (Therneau 2020), 'factoMineR' (Lê et al. 2008),

364 'vegan' (Oksanen et al. 2020), 'ggplot2' (Wickham et al. 2016), 'ade4' (Thioulouse et al. 2018),
365 and 'lme4' (Bates et al. 2015).
366
367 # Results
368 ## Survival and development
369 The selection process did not affect survival at early life stages (hatching and yolk-sac
370 resorption stages; Tables 2, 3), with a 56.6% (± 26.4) overall survival once exogenous feeding
371 was established. Most mortality occurred at hatching. At both development stages, parental
372 effects played significant roles, explaining 13.5% of the variance (Table 2), but heritability was
373 null or low (Table 4). The main portion of the variance explained by dam identity at hatching
374 was reduced at the yolk resorption stage, but it still represented more than 50% of the total
375 variance (Table 4).
376
377 There was no lineage effect on DD at either hatching or yolk-sac resorption, and there were no
378 significant parental effects (Tables 2, 3). Similarly, there was no significant effect of lineage on
379 YSV, but in this case parental effects were strong (Table 2), and heritability was high (Table
380 4).
381
382 Lineage had a significant effect on SL at hatching and a marginally non-significant effect on
383 the yolk-sac resorption stage (Tables 2, 3). Parental effects were also strong at both stages
384 (Table 2). At hatching, dam identity significantly explained half of the variance: while the
385 heritability value for fry issued from the lineage under selection was low, dam identity in the
386 control lineage explained 28% of the variation, but with a stronger, yet non-meaningful,
387 heritability value (Table 4). At the yolk-sac resorption stage, the proportion of variance
388 explained by dam identity was lower and heritability was higher (Table 4).

389

390 **Thermal resistance trials**

391 LOE occurred significantly later in fish originating from the selected lineage, indicating
392 its greater thermal tolerance relative to the control line (Table 5, Figure 2A). When survival
393 probability reached 50%, LOE in CDM was about 2061.9 and 2113.6°C.min^{-1} (correspondence
394 in time: 3 h 55 min and 3 h 59 min; in degrees Celsius: 26.67 and 27.77), respectively, for the
395 control and selected lineages. Significant effects of mass and SL were observed on thermal
396 resistance: sensitive fish had a 0.3% lower mass (Fig.2B) and 1% lower length (Fig. 2C)
397 compared to other groups (median and resistant fish; ANOVA $p < 0.01$).

398

399 **mRNA expression**

400 PCA dimensions 1 and 2 explained 58.5% and 67.4% of total variability of gene
401 expression in the heart (Fig. 3A) and liver (Fig. 3B), respectively. In heart, the contributions of
402 *HSP70*, *HSP90*, *BCL,* and *CAT* to dimensions 1 and 2 were greater than the mean expected
403 contribution (1/7 = 14.3%). *BCL* and *HSP90* were strongly positively correlated but
404 independent of *CAT* expression. In liver, the contributions of *BCL*, *SOD*, *HSP70,* and *CAT* to
405 dimensions 1 and 2 were greater than 14.3%. *HSP70* and *BCL* were strongly positively
406 correlated but independent of *CAT* and *SOD* expressions. For both heart and liver, sensitive fish
407 stood out from median and resistant fish, which overlap almost entirely (Fig. 3).

408

409 Gene expression was significantly affected by thermal resistance in heart and liver, but not by
410 breeder lineage (PERMANOVA, Table 6). Pairwise tests revealed that sensitive fish had
411 significantly different gene expression in the heart compared to other groups (pairwise test,
412 sensitive–median $F = 3.29$, $p = 0.009$; sensitive–resistant $F = 3.93$, $p = 0.021$; median–resistant
413 $F = 2.60$, $p = 0.168$). In liver, all groups showed significantly different patterns of gene

414 expression (pairwise test, sensitive–median F = 10.80, p = 0.003; sensitive–resistant F = 6.80,
415 p = 0.003; median–resistant F = 4.23, p = 0.012). More than half (52%) of the dissimilarity
416 between sensitive fish and the other groups in heart gene expression was explained by *HSP70*
417 (21%), *BCL* (16%), and *CAT* (15%; SIMPER test). In liver, the main dissimilarities in gene
418 expression among all groups were explained by *HSP70* (sensitive–median: 18%, sensitive–
419 resistant: 25%, median–resistant: 17%). Dissimilarities between sensitive–resistant (18%) and
420 resistant–median (29%) were explained by *CAT*. More specifically, *HSP90* gene expression
421 explained 13% of the dissimilarities between sensitive–resistant fish, *CASP9* explained 13%
422 between resistant–median, and finally BCL made up 19% and *GPx* 17% of the dissimilarities
423 between sensitive–median.
424
425 No significant difference in gene expression was observed for the heart *CASP9*, *SOD*, and GPx
426 genes (two-way ANOVA; Table S1A). Lineage and interaction factors (thermal resistance ×
427 lineage) did not significantly influence expression of the other genes, only thermal resistance
428 significantly influenced gene expression (Fig. 4). *BCL* (Fig. 4A) and *HSP70* (Fig. 4C) gene
429 expressions were the lowest in sensitive fish ($P < 0.01$); *CAT* (Fig.4B) gene expression was
430 lowest in median fish and highest in resistant fish (sensitive fish had significant intermediate
431 *CAT* gene expression; $P < 0.05$). Sensitive fish had significantly lower *HSP90* (Fig. 4H) gene
432 expression compared to resistant fish ($P < 0.05$).
433
434 No significant difference in relative gene expression was observed for liver *CASP9* or *SOD*
435 (Table S1B). The lineage factor did not significantly influence expression of any genes.
436 Thermal resistance significantly explained the difference in relative expression observed for
437 *BCL*, *HSP90*, and *GPx* (Fig. 4E, 4H, 4I). *BCL* and *HSP90* gene expressions were the lowest in
438 sensitive fish, while *GPx* gene expression was the highest in median fish. The lineage × thermal

resistance interaction was significant for *HSP70* gene expression, with sensitive fish having the lowest gene expression and resistant selected fish having the highest (Fig. 4G).

Discussion

Despite the growth differences observed between lineages in previous generations (Bastien et al. 2011; Martinez-Silva et al. 2023), we found limited lineage effects. Indeed, we only found a significant difference for SL at hatch, a marginally non-significant difference at yolk-sac resorption, and no size difference in six-month-old juveniles. However, juveniles from the selected lineage had a higher thermal resistance than control juveniles, and higher relative gene expression was detected for liver *CAT* and *HSP70* genes in juveniles from the selection program. Irrespective of the lineage, sensitive fish were characterized by a lower mass and length, and usually by lower relative gene expression compared to median and resistant fish.

Lineage effects

After five generations of selection, we only detected a line effect at hatching (S greater than C); it was no longer present at the yolk-sac resorption stage. As a reminder, the selection process for growth was only applied to individuals that showed no sexual maturation at age 1+. From hatching to yolk-sac resorption, growth is highly dependent on yolk quality since the yolk sac provides all elements that embryos need to support development and embryonic growth (Brooks et al. 1997).

~~S~~elective breeding did not have a detrimental effect on thermal resistance in juvenile brook charr, although this trait was not involved in the selection process. Indeed, selected fish had better thermal resistance, with LOE occurring on average 1°C after control fish. While the selected lineage was developed for faster growth (Sauvage et al. 2010), no significant difference

in mass or length was observed between lineages during the thermal trial. This means that the thermal resistance difference between lineages cannot be explained by fish mass.

By investigating patterns of gene expression involved in physiological processes related to the maintenance of homeostasis, we wanted to know more about underlying mechanisms that can explain thermal resistance between lineages and fish resistance. Regarding the effect of selection on thermal resistance, previous work on these lineages has shown that substantial changes occurred in the regulation of gene transcription between selected and control lineages after just four generations (Sauvage et al. 2010). These authors demonstrated that differences in gene expression between selected and control lineages were low for immune and defense functions (including the activator of 90 kDa heat shock protein ATPase homolog 1 gene), and they proposed two hypotheses to explain such low differences: the relaxation on expression regulation because of controlled environmental conditions or a weakness of the selective breeding process. Here, our results revealed that a lineage effect was present for thermal resistance, and it can be partly explained by the expression of two stress-related genes. Selected fish had up-regulated expressions of liver *CAT* and heart *HSP70*. The increased expressions of *CAT* and *HSP70* induced by thermal stress contribute to the regulation of organismal metabolism, and the higher expression was correlated with higher resistance. These data suggest that the S lineage had a higher capacity to resist thermal stress partly because of better antioxidant defense. Within the framework of our study, it seems that selection did not hinder the capacity for thermal resistance.

Parental effects

Strong parental effects were present in both lineages on traits measured on early stages of development. Dam effects on different traits are almost always present in animal models,

488 while sire effects are generally negligeable. Few studies have been performed pertaining to
489 paternal effects on progeny in fishes. In the European seabass *Dicentrarchus labrax* (Linnaeus,
490 1758), Saillant et al. (2001) demonstrated the presence of paternal effects but noted that they
491 were less important than maternal effects on early life history characteristics. The presence of
492 early sexual maturation also impacted Atlantic salmon fry growth between hatching and yolk-
493 sac resorption (Garant et al. 2002) and sire identity in utthroat *Oncorhynchus clarki clarki*
494 (Richardson, 1836) and rainbow trout *Oncorhynchus mykiss* (Walbaum, 1792) has been
495 associated with varying abilities of fry to convert yolk reserves to body mass (Hawkins and
496 Foote 1998).
497
498
499 **Inter individual variability during thermal trials**
500 The thermal challenge revealed notable inter-individual variability. A difference of 2h30
501 to 2h50 was found in the time it took the least tolerant and the most tolerant individuals to lose
502 their ability to maintain equilibrium. Smallest fish were the least resistant. Even though
503 obtained on much larger fish, smaller fish (mass and length) were observed as being the least
504 resistant in a study by Clark et al. (2008) in chinook salmon *Oncorhynchus tshawytscha*
505 (Walbaum, 1792) body mass range = 2.2–5.4 kg). Also obtained on larger fish, a positive
506 relationship between thermal resistance and body mass was also observed by Zhang and Kieffer
507 (2014) in shortnose sturgeon *Acipenser brevirostrum* (Lesueur, 1818) weighing from few grams
508 to 300 g. Chen et al. (2013) found that thermal resistance (CTmax) at 90 days post-hatch from
509 four populations of sockeye salmon *Oncorhynchus nerka* (Walbaun, 1792) was positively
510 related to their mass, which corroborates the results of our study. However, there is also
511 evidence that CTmax in some species declines with fish size or mass, and no other relationship
512 was found (see McKenzie et al. 2021 for a review). Direct comparisons between studies
513 conducted on different species and life stages remains difficult. Nevertheless, our study again

514 shows that body size is a potential source of inter-individual variability in aquaculture
515 adaptation performance, particularly thermal resistance.
516
517 Our results on gene expression also confirm that sensitive fish stand out from other groups. For
518 heart, differences in sensitive fish were explained by *HSP70*, *BCL,* and *CAT*, while liver
519 differences were explained by *HSPs*, *CASP9*, *BCL*, *GPX*, and *CAT*. In general, sensitive fish
520 were characterized by lower gene expressions compared to other groups. The stress-related
521 gene expression induced by thermal stress supports the results observed during the thermal
522 resistance trial and suggests a possible role for these genes in whole-organism thermal
523 resistance, and this might contribute to the regulation of organismal metabolism.
524
525 Not surprisingly, relative *HSP* expressions were upregulated in resistant fish. *HSP70* proteins
526 are one of the most highly conserved groups of heat shock proteins (Beere and Green 2001).
527 They ensure the coordinated regulation of protein translocation processes, limiting cellular
528 damage (Iwama et al., 1999). Similar results have been frequently documented in fish facing
529 heat stress (Fangue et al. 2006; Liu et al. 2013; Barat et al. 2016; Jeffries et al. 2016). Exposure
530 to thermal stress can induce the generation of reactive oxygen species, which can damage
531 tissues (Almroth et al. 2015; Madeira et al. 2016; Maulvault et al. 2017). To counteract this,
532 fish produce genes that encode proteins with antioxidant activities, such as *CAT*. *CAT*
533 expression in fishes is upregulated with heat-shock exposure (Clotfelter et al. 2013; Madeira et
534 al. 2016), and this is corroborated by our results. Median and resistant fish had higher
535 expression levels of *BCL,* which is an anti-apoptotic protein (Cory and Adams 2002). It has
536 been demonstrated in fish that genes in the *BCL* family act by reducing cell apoptosis under
537 stressful conditions (Yuan et al. 2016), suggesting that induction of cell death may be better
538 regulated for median and resistant fish.

In our study, gene expression highlighted an important source of inter-individual variability that could indicate a threshold. Median and resistant fish had upregulated stress-related gene expression, which fits with a later LOE than sensitive fish. One hypothesis could be that response differences may indicate greater sensitivity to thermal stress, since the decrease in expression may be due to a widespread inhibition of gene transcription accompanying extensive cellular damage. However, linking the environment with phenotypic changes through modulation of gene expression is difficult. As reviewed in Rivera et al. (2021), if gene expression contributes to emergent stress responses such as thermal resistance, it would be of interest to know more about transcription profiles. Nevertheless, showing distinct transcription profiles, revealing the dynamic nature of gene expression, and interpreting gene expression results in a way that elucidates the functional connection between gene expression and the observed stress response remain challenging.

Conclusion

This study revealed that the better physiological thermal resistance in selected fish was correlated with an upregulation of liver *CAT* and heart *HSP70*. However, gene regulation can depend on many factors, so these findings alone cannot indicate a direct absolute link between thermal resistance and gene upregulation. Nevertheless, they do provide putative support for adaptive differences between selected and control lines of brook charr in their potential for gene expression-mediated phenotypic plasticity. In environments undergoing not just gradual changes but also an increase in the frequency or magnitude of extreme events, gene expression plasticity—the capacity of genes to change their expression levels under changing conditions—may be of particular importance, especially because gene expression plasticity can evolve rapidly and be heritable by genetic or epigenetic means.

Acknowledgements

The authors are grateful to all colleagues who provided technical and scientific assistance in the laboratory. Special thanks to Nathalie Morin, Émile Vadboncoeur, and Tamara Provencher (Institut des sciences de la mer de Rimouski) who contributed to brook charr breeding and rearing activities.

Competing interests

The authors declare there are no competing interests.

Funding

This work was supported by a NSERC strategic grant to L.B., D.G. and C.A. (grant no. STPGP 521227-18), Ouranos Inc., and Ressources Aquatiques Québec (Regroupement stratégique, Fonds de recherche du Québec – Nature et Technologies).

Author contribution statement

C.G., M.R., D.G., L.B., and C.A. contributed to the conceptualization and experimental design of the work. C.G., M.R., L.G., and C.A. performed all data collection. C.G., M.R., and D.G performed statistical analysis. C.G. and M.R. drafted the manuscript and all authors contributed to the final version. Supervision was provided by D.G., L.B., and C.A.

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.

References

590

591 Ahmed, N., Thompson, S., and Glaser, M. 2019. Global aquaculture productivity,
592 environmental sustainability, and climate change adaptability. Environ. Manage.
593 **63**(2): 159–172. doi: 10.1007/s00267-018-1117-3

594 Alfonso, S., Sadoul, B., Cousin, S., and Bégout, M.-L. 2020. Spatial distribution and activity
595 patterns as welfare indicators in response to water quality changes in European sea bass,
596 *Dicentrarchus labrax.* Appl. Anim. Behav. Sci. **226**(May): 104974. doi:
597 10.1016/j.applanim.2020.104974

598 Almroth, B.C., Asker, N., Wassmur, B., Rosengren, M., Jutfelt, F., Gräns, A., Sundell, K.,
599 Axelsson, M., and Sturve, J. 2015. Warmer water temperature results in oxidative damage
600 in an Antarctic fish, the bald notothen. J. Exp. Mar. Biol. Ecol. **468**(2015): 130–137.
601 doi:10.1016/j.jembe.2015.02.018

602 Anderson, M.J. 2001. Permutation tests for univariate or multivariate analysis of variance and
603 regression. Can. J. Fish. Aquat. Sci. **58**(3): 626–639. doi:10.1139/cjfas-58-3-626

604 AnvariFar, H., Amirkolaie, A.K., Miandare, H.K., Ouraji, H., Jalali, M.A., and Üçüncü, S.İ.
605 2017. Apoptosis in fish: environmental factors and programmed cell death. Cell Tiss. Res.
606 **368**(3): 425–439. doi:10.1007/s00441-016-2548-x

607 Ashley, P.J. 2007. Fish welfare: Current issues in aquaculture. Appl. Anim. Behav. Sci. 104
608 (3–4): 199–235. doi:10.1016/j.applanim.2006.09.001

609 Barat, A., Sahoo, P.K., Kumar, R. Goel, C., and Singh, A.K. 2016. Transcriptional response
610 to heat shock in liver of snow trout (*Schizothorax richardsonii*)—a vulnerable
611 Himalayan cyprinid fish. Funct. Integr. Genomics, **16**(2): 203–213.
612 doi:10.1007/s10142-016-0477-0
613 Bastien, A., Perry, G.M.L., Savaria, J.-Y., Bernatchez, L., and Audet, C. 2011. Genetic gain
614 for growth and delayed sexual maturation using a feral strain of anadromous brook trout.
615 North Am. J. Aquacult. **73**(1): 24–33. doi:10.1080/15222055.2011.544609
616 Bates, D., Maechler, M., Bolker, B., and Walker, S. 2015. lme4: linear mixed-effects models
617 using Eigen and S4. R package version 1.1–7. 2014.
618 Beere, H.M., and Green, D.R. 2001. Stress management – heat shock protein-70 and the
619 regulation of apoptosis, Trends Cell Biol. **11**(1): 6–10. doi:10.1016/S0962-
620 8924(00)01874-2
621 Brooks, S., Tyler, C.R., and Sumpter, J.P. 1997. Egg quality in fish: what makes a good egg?
622 Rev. Fish Biol. Fish. **7**(4), 387–416. doi:10.1023/A:1018400130692
623 Budy, P., Thiede, G.P., Lobón-Cerviá, J., Fernandez, G.G., McHugh, P., McIntosh, A.,
624 Vøllestad, E.B., and Jellyman, P. 2013. Limitation and facilitation of one of the world's
625 most invasive fish: an intercontinental comparison. Ecology **94**(2): 356–367.
626 doi:org/10.1890/12-0628.1
627 Burt, D.W. 2005. Chicken genome: current status and future opportunities. Genome Res.
628 15(12): 1692–1698. doi: 10.1101/gr.4141805
629 Bush, E., and Lemmen, D.S. 2019. Canada's changing climate report. Government of Canada.
630 Ottawa, ON, Canada, 444 pp.
631 Chadwick Jr., J.G., Nislow, K.H., and McCormick, S.D. 2015. Thermal onset of cellular and
632 endocrine stress responses correspond to ecological limits in brook trout, an iconic cold-
633 water fish. Cons. Physiol. **3**(1): cov017. doi:10.1093/conphys/cov017

634 Chandra, J., Samali, A., and Orrenius, S. 2000. Triggering and modulation of apoptosis by
635 oxidative stress, Free Radical Biol. Med. **29**(3–4): 323–333. doi:10.1016/S0891-
636 5849(00)00302-6
637 Chen, Z., Anttila, K., Wu, J., Whitney, C.K., Hinch, S.G., and Farrell A.P. 2013. Optimum and
638 maximum temperatures of sockeye salmon (*Oncorhynchus nerka*) populations hatched at
639 different temperatures. Can. J. Zool. **91**(5): 265–274. doi:10.1139/cjz-2012-0300
640 Cheng, C.H., Guo, Z.X., and Wang A.L. 2018. The protective effects of taurine on oxidative
641 stress, cytoplasmic free-Ca2+ and apoptosis of pufferfish (*Takifugu obscurus*) under low
642 temperature stress. Fish Shellfish Immunol. **77**(2018): 457–464.
643 doi:10.1016/j.fsi.2018.04.02.
644 Claireaux, G., Théron, M., Prineau, M., Dussauze, M., Merlin F.-X., and Le Floch, S. 2013.
645 Effects of oil exposure and dispersant use upon environmental adaptation performance and
646 fitness in the European sea bass, *Dicentrarchus labrax*, Aquat Toxicol, **130–131**(2013):
647 160–170. doi:10.1016/j.aquatox.2013.01.004
648 Clark, T.D., Sandblom, E., Cox, G.K., Hinch, S.G., and Farrell, A.P. 2008. Circulatory limits
649 to oxygen supply during an acute temperature increase in the Chinook salmon
650 (*Oncorhynchus tshawytscha*). Am. J. Physiol.-Reg., Integr. Comp. Physiol., **295**(5):
651 R1631-R1639. doi: 10.1152/ajpregu.90461.2008
652 Clotfelter, E.D., Lapidus, S.J.H., and Brown, A.C. 2013. The effects of temperature and
653 dissolved oxygen on antioxidant defences and oxidative damage in the fathead minnow
654 *Pimephales promelas*. J. Fish Biol. **82**(3): 1086–1092. doi: /10.1111/jfb.12050
655 Collins, M., Sutherland, M., Bouwer, L., Cheong, S.-M., Frolicher, T., DesCombes, H.J., Roxy,
656 M.K, Losada, I., McInnes, K., Ratter, B., Rivera-Arriga, E., Susanto, R.D., Swingedouw,
657 D., Tibig, L., Bakker, P., Eakin, C.M., Emanuel, K., Grose, M., Hemer, M., Jackson, L.,
658 Kaab, A., Kajtar, J., Knutson, T., Laufkotter, C., Noy, I., Payne, M., Ranasinghe, R.,

659 Sgubin, G., and Timmermans, M.-L. 2019. Extremes, abrupt changes and managing risk,
660 IPCC Special Report on the ocean and cryosphere in a changing climate, The
661 Intergovernmental Panel on Climate Change. Portner, H.-O., Roberts, D.C., Masson-
662 Delmotte, V., Zhai, P., Tignor, M., Poloczanska E., and Mintenbeck, K. (eds), United
663 Nations, pp. 589–655. https://www.ipcc.ch/srocc/chapter/chapter-6/
664 Corey, E., Linnansaari, T., Cunjak, R.A., and Currie, S. 2017. Physiological effects of
665 environmentally relevant, multi-day thermal stress on wild juvenile Atlantic salmon (*Salmo*
666 *salar*), Conserv. Physiol. **5**(1): cox014, doi: 10.1093/conphys/cox014
667 Cory, S., and Adams, J. 2002. The Bcl2 family: regulators of the cellular life-or-death switch.
668 Nat. Rev. Cancer **2**(2002): 647–656. doi:org/10.1038/nrc883
669 Crespel, A., Bernatchez, L., Garant, D., and Audet, C. 2011. Quantitative genetic analysis of
670 the physiological stress response in three strains of brook charr *Salvelinus fontinalis* and
671 their hybrids. J. Fish Biol., **79**(7): 2019–2033. doi:10.1111/j.1095-8649.2011.03149.x
672 Crespel, A., Bernatchez, L., Audet, C., and Garant, D. 2013. Strain specific
673 genotype−environment interactions and evolutionary potential for body mass in Brook
674 charr (*Salvelinus fontinalis*). G3: Genes, Genomes, Genetics, **3**(3): 379–386.
675 doi:10.1534/g3.112.005017
676 Cox, D.R. 1972. Regression models and life-tables. J.R. Stat. Soc.: Ser. B (Methodological),
677 **34**(2): 187–202. doi:org/10.1111/j.2517-6161.1972.tb00899.x
678 Currie, S., and Schulte, P.M. 2014. Thermal stress. In The physiology of fishes, 4th edn, Evans,
679 D.H., Claiborne, J., Currie S., Eds, pp. 257–279. Boca Raton, CRC Press
680 Diamond, J. 2002. Evolution, consequences and future of plant and animal domestication.
681 Nature **418**(6898): 700–707. doi:10.1038/nature01019
682 Duarte, C.M., Marbá, N., and Holmer, M. 2007. Rapid domestication of marine species.
683 Science, **316**(5823): 382–383. doi:10.1126/science.1138042

684 Elmore, S. 2007. Apoptosis: a review of programmed cell death. Toxicol. Pathol. **35**(4), 495–
685 516. doi:10.1080/01926230701320337
686 Fangue, N.A., Hofmeister, M., and Schulte, P.M. 2006. Intraspecific variation in thermal
687 tolerance and heat shock protein gene expression in common killifish, *Fundulus*
688 *heteroclitus*. J. Exp. Biol. **209**(15), 2859–2872. doi:10.1242/jeb.02260
689 Frölicher, T.L., and Laufkötter, C. 2018. Emerging risks from marine heat waves. Nat.
690 Commun. **9**(1): 650. doi:10.1038/s41467-018-03163-6
691 Fry, F.E.J.. 1971. The effect of environmental factors on the physiology of fish. Fish Physiolol.
692 **6**(C): 1–98. In Environmental Relations and Behavior, Hoar W.S., and Randall D.J., Eds,
693 doi:10.1016/S1546-5098(08)60146-6
694 Garant, D., Fontaine, P.M., Good, S.P., Dodson, J.J., and Bernatchez, L. 2002. The influence
695 of male parental identity on growth and survival of offspring in Atlantic salmon (*Salmo*
696 *salar*). Evol. Ecol. Res. **4**(4) 537-549. doi:10.1.1.614.5000
697 Giuffra, E.J.M., Kijas, J.M.H., Amarger, V., Carlborg, Ö., Jeon, J.T., and Andersson, L. 2000.
698 The origin of the domestic pig: independent domestication and subsequent introgression.
699 Genetics, **154**(4): 1785–1791. doi:10.1093/genetics/154.4.1785
700 Glover, K.A, Bergh, Ø., Rudra, H., and Skaala, Ø. 2006. Juvenile growth and susceptibility to
701 *Aeromonas salmonicida* subsp. *salmonicida* in Atlantic salmon (*Salmo salar* L.) of farmed,
702 hybrid and wild parentage. Aquaculture, **254**(1–4): 72–81. doi:
703 10.1016/j.aquaculture.2005.10.040
704 Glover, K.A., Skår, C., Christie, K.E., Glette, J., Rudra, H., and Skaala, Ø. 2006. Size-
705 dependent susceptibility to infectious salmon anemia virus (ISAV) in Atlantic salmon
706 (*Salmo salar* L.) of farm, hybrid and wild parentage. Aquaculture, **254**(1-4): 82–91. doi:
707 10.1016/j.aquaculture.2005.10.041

Halliwell, B., and Gutteridge, J.M. 2015. Free radicals in biology and medicine. Oxford University Press, USA.

Hawkins, D.K., and Foote, C.J. 1998. Early survival and development of coastal cutthroat trout (*Oncorhynchus clarki clarki*), steelhead (*Oncorhynchus mykiss*), and reciprocal hybrids. Can. J. Fish. Aquat. Sci. **55**(9): 2097–2104. doi:10.1139/f98-099

Heise, K., Puntarulo, S., Nikinmaa, M., Abele, D., and Pörtner, H.O. 2006. Oxidative stress during stressful heat exposure and recovery in the North Sea eelpout *Zoarces viviparus* L. J. Exp. Biol. **209**(2): 353–363. doi:10.1242/jeb.01977

Houle, C., Gossieaux, P., Bernatchez, L., Audet, C., and Garant, D. 2023. Transgenerational effects on body size and survival in brook charr (*Salvelinus fontinalis*). Evol. Appl. **16**(5): 1061–1070. doi.org/10.1111/eva.13553

Islam, M.J., Slater, M.J., Thiele, R., and Kunzmann, A. 2021. Influence of extreme ambient cold stress on growth, hematological, antioxidants, and immune responses in European seabass, *Dicentrarchus labrax* acclimatized at different salinities. Ecol. Indic. **122**(2021)*:* 107280. doi:10.1016/j.ecolind.2020.107280

Iwama, G.K., Vijayan, M.M., Forsyth, R.B., and Ackerman, P.A. 1999. Heat shock proteins and physiological stress in fish. Am. Zool. **39**(6): 901–909. doi:10.1093/icb/39.6.901

Janssen, K., Chavanne, H., Berentsen, P., and Komen, H. 2017. Impact of selective breeding on European aquaculture. Aquaculture, **472**(2017): 8–16. doi:10.1016/j.aquaculture.2016.03.012

728 Jeffries, K.M., Connon, R.E, Davis, B.E., Komoroske, L.M., Britton, M.T., Sommer, T.,
729 Todgham, A.E., and Fangue, N.A. 2016. Effects of high temperatures on threatened
730 estuarine fishes during periods of extreme drought. J. Exp. Biol. **219**(11): 1705–1716.
731 doi:10.1242/jeb.134528.
732 Kovach, M.J., Sweeney, M.T., and McCouch, S.R. 2007. New insights into the history of rice
733 domestication. Trends Genet. **23**(11): 578–587. doi:10.1016/j.tig.2007.08.012
734 Kregel, K.C. 2002. Invited review: heat shock proteins: modifying factors in physiological
735 stress responses and acquired thermotolerance. J. Appl. Physiol. **92**(5): 2177–2186.
736 doi:10.1152/japplphysiol.01267.2001
737 Lê, S., Josse, J., and Husson, F. 2008. FactoMineR: An R package for multivariate analysis. J.
738 Stat. Soft. **25**(1): 1–18. doi:10.18637/jss.v025.i01
739 Lefcheck, J., Byrnes, J., & Grace, J. (2016). Package 'piecewiseSEM'. R package version, 1(1).
740 Lesser, M.P. 2011. Coral bleaching: causes and mechanisms. In: Coral reefs: an ecosystem in
741 transition, pp. 405–419. Springer, Dordrecht.
742 Lindquist, S., and Craig, E.A. 1988. The heat-shock proteins. Annu. Rev. Genet. **22(1)**:
743 631–677. doi:10.1146/annurev.ge.22.120188.003215
744 Liu, S., Wang, X., Sun, F., Zhang, J., Feng, J., Liu, H., Rajendran, K.V., Sun, L., Zhang, Y.,
745 Jiang, Y., Peatman, E., Kaltenboeck, L., Kucuktas, H., and Liu, Z. (2013). RNA-Seq
746 reveals expression signatures of genes involved in oxygen transport, protein synthesis,
747 folding, and degradation in response to heat stress in catfish. Physiol. Genom. **45**(12):
748 462–476. doi:10.1152/physiolgenomics.00026.2013
749 Livak, K.J., and Schmittgen, T.D. 2001.Analysis of relative gene expression data using real-
750 time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. Methods **25**(4): 402–408. doi:
751 10.1006/meth.2001.1262.

752 MacCrimmon, H.R. 1971. World distribution of rainbow trout (*Salmo gairdneri*). J. Fish. Res.
753 Board Can. **28**(5): 663–704. doi:org/10.1139/f71-098
754 Madeira, D., Costa, P.M., Vinagre, C., and Diniz, M.S. 2016. When warming hits harder:
755 survival, cellular stress and thermal limits of *Sparus aurata* larvae under global change.
756 Mar. Biol., **163**(4), 1–14. doi:10.1007/s00227-016-2856-4
757 MAPAQ. 2019. Portrait-diagnostic sectoriel sur l'aquaculture en eau douce au Québec.
758 Gouvernement du Québec. Dépôt légal: 2019. Bibliothèque et Archives nationales du
759 Québec. Bibliothèque et Archives Canada. ISBN 978-2-550-83813-5
760 Martínez-Álvarez, R.M., Morales, A.E., and Sanz, A. 2005. Antioxidant defenses in fish: biotic
761 and abiotic factors. Rev. Fish Biol. Fisheries, **15**(1): 75–88. doi:10.1007/s11160-005-7846-
762 4
763 Martinez Arbizu, P. (2017). pairwiseAdonis: Pairwise multilevel comparison using adonis. R
764 package version 0.0.1
765 Martinez-Silva, M.A., Dupont-Prinet A., Houle C., Vagner M., Garant D., Bernatchez L. and
766 Audet. C. 2023. Growth regulation of brook charr *Salvelinus fontinalis*. Gen. Comp.
767 Endocrionol. **331**: 114160. doi:10.1016/j.ygcen.2022.114160
768 Mauduit, F., Domenici, P., Farrell, A.P., Lacroix C., Le Floch, S., Lemaire, P., Nicolas-Kopec,
769 A., Whittington, M., Zambonino-Infante, J.L., and Claireaux, G. 2016. Assessing chronic
770 fish health: An application to a case of an acute exposure to chemically treated crude oil.
771 Aquat. Toxicol. **178**(2016): 197–208. doi:10.1016/j.aquatox.2016.07.019
772 Mauduit, F., Farrell, A.P., Domenici, P., Lacroix, C., Le Floch, S., Lemaire, P., Nicolas-Kopec,
773 A., Whittington, M., Le Bayon, N. Zambonino-Infante, J.-L., and Claireaux, G. 2019.
774 Assessing the long-term effect of exposure to dispersant-treated oil on fish health using
775 hypoxia tolerance and temperature susceptibility as ecologically relevant biomarkers.
776 Environ. Toxicol. Chem. **38**(1)**:** 210–221. doi:10.1002/etc.4271

777 Maulvault, A.L., Barbosa, V., Alves, R., Custódio, A., Anacleto, P., Repolho, T., Ferreira, P.P.,
778 Rosa, R., Marques, A., and Diniz, M. 2017. Ecophysiological responses of juvenile seabass
779 (*Dicentrarchus labrax*) exposed to increased temperature and dietary methylmercury. Sci.
780 Tot. Environ. **586**(2017): 551–558. doi:10.1016/j.scitotenv.2017.02.016
781 McKenzie, D.J., Zhang, Y., Eliason, E.J., Schulte, P.M., Claireaux, G., Blasco, F.R., Nati,
782 J.J.H., and Farrell, A.P. 2021. Intraspecific variation in tolerance of warming in fishes. J.
783 Fish Biol. **98**(6): 1536–1555. doi:10.1111/jfb.14620
784 Merilä, J., and Hendry, A.P. 2014. Climate change, adaptation, and phenotypic plasticity: the
785 problem and the evidence. Evol. Appl. 7(1): 1–14. doi: 10.1111/eva.12137
786 Møller, A.P., and Merilä J. 2004. Analysis and interpretation of long-term studies investigating
787 responses to climate change. Adv. Ecol. Res. **35**(2004): 111–130. doi: 10.1016/S0065-
788 2504(04)35006-3. doi:10.1098/rspb.2002.2224
789 Narum, S.R., Campbell, N.R., Kozfkay, C.C., and Meyer, K.A. 2010. Adaptation of redband
790 trout in desert and montane environments. Mol. Ecol. **19**(21): 4622–4637.
791 doi:10.1111/mec.12240
792 Oksanen, J., Blanchet, F.G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., ..., and
793 Wagner, H. 2020. vegan: Community ecology package. R package version 2.5-6. 2019.
794 Perry, G., Audet, C., Laplatte, B., and Bernatchez, L. 2004. Shifting patterns in genetic control
795 at the embryo-alevin boundary in brook charr. Evolution, **58**(9): 2002–2012. doi:
796 10.1111/j.0014-3820.2004.tb00485.x
797 Pozzi, C., Salamini, F. (2007). Genomics of wheat domestication. In: Varshney, R.K., and
798 Tuberosa, R. (eds) Genomics-Assisted Crop Improvement. Springer, Dordrecht.
799 doi:10.1007/978-1-4020-6297-1_17

Rabalais, N.N., Diaz, R.J., Levin, L.A., Turner, R.E., Gilbert, D., and Zhang, J. 2010. Dynamics and distribution of natural and human-caused hypoxia. Biogeosciences, **7**(2): 585–619. doi:10.5194/bg-7-585-2010

Rivera, H.E., Aichelman, H.E., Fifer, J.E., Kriefall, N.G., Wuitchik, D.M., Wuitchik, S.J., and Davies, S.W. 2021. A framework for understanding gene expression plasticity and its influence on stress tolerance. Mol. Ecol. **30**(6): 1381–1397. doi:10.1111/mec.15820

Rodgers, E.M. 2021 Adding climate change to the mix: responses of aquatic ectotherms to the combined effects of eutrophication and warming. Biol. Lett. **17**(10): 20210442. doi: 10.1098/rsbl.2021.0442

Saillant, E., Chatain, B., Fostier, A., Przybyla, C., and Fauvel, C. 2001. Parental influence on early development in the European sea bass. J. Fish Biol. **58**(6): 1585–1600. doi: 10.1111/j.1095-8649.2001.tb02314.x

Sauvage, C., Derôme, N., Normandeau, E., St.-Cyr, J., Audet, C., and Bernatchez, L. 2010. Fast transcriptional responses to domestication in the brook charr. Genetics, **185**(1): 105–112. doi:10.1534/genetics.110.115071

Sayers, E.W., Cavanaugh, M., Clark, K., Ostell, J., Pruitt, K.D., and Karsch-Mizrachi, I. 2019. GenBank. Nucleic Acids Res. 47(D1), D94–D99.

Schulte, P.M., Healy, T.M., and Fangue, N.A. 2011. Thermal performance curves, phenotypic plasticity, and the time scales of temperature exposure. Integr. Comp. Biol. **51**(5): 691–702. doi:10.1093/icb/icr097

Somero, G.N. 2010. The physiology of climate change: how potentials for acclimatization and genetic adaptation will determine 'winners' and 'losers'. J. Exp. Biol. **213**(6): 912–920. doi:10.1242/jeb.037473

Stitt, B.C., Burness, G., Burgomaster, K.A., Currie, S., McDermid, J.L., and Wilson, C.C. 2014. Intraspecific variation in thermal tolerance and acclimation capacity in brook trout

(*Salvelinus fontinalis*): physiological implications for climate change. Physiol. Biochem. Zool. **87**(1): 15–29. doi:10.1086/675259

Therneau, T. 2015. Mixed effects Cox models. CRAN repository.

Therneau, T. 2018. Total least squares: Deming, Theil-Sen, and Passing-Bablock Regression. R Package Vignette.

Therneau, T.M. 2020. coxme: Mixed effects Cox models. R package version 2.2-5. 2015.

Therneau, T. 2022. A package for survival analysis in R. R package version 3.2-12, 2021.

Therneau, T.M., and Grambsch, P.M. 2000. The Cox model. In: Modeling survival data: Extending the Cox model. Statistics for biology and health. Springer, New York, NY. doi:10.1007/978-1-4757-3294-8_3

Thioulouse, J., Dray, S., Dufour, A.-B., Siberchicot, A., Jombart, T. and Pavoine, S. 2018. Multivariate analysis of ecological data with ade4.

Tomanek, L. 2008. The importance of physiological limits in determining biogeographical range shifts due to global climate change: the heat-shock response. Physiol. Biochem. Zool. **81**(6): 709–717. doi:10.1086/590163

Tomanek, L. 2010. Variation in the heat shock response and its implication for predicting the effect of global climate change on species' biogeographical distribution ranges and metabolic costs. J. Exp. Biol. **213**(6): 971–979. doi:10.1242/jeb.038034

Vandesompele, J., De Preter, K., Pattyn, F., Poppe, B., Van Roy, N., De Paepe, A., and Speleman, F. 2002. Accurate normalization of real-time RT-PCR data by geometric averaging of multiple internal control genes. Genome Biol. 3(7): 1–13. doi:10.1186/gb-2002-3-7-research0034

Vargas-Chacoff, L., Regish, A.M., Weinstock, A., and McCormick, S.D. 2018. Effects of elevated temperature on osmoregulation and stress responses in Atlantic salmon *Salmo*

849 *salar* smolts in fresh water and seawater. J. Fish Biol. **93**(3): 550–559. doi:
850 10.1111/jfb.13683
851 Vega-Trejo, R., Head, M.L., Jennions, M.D., and Kruuk, L.E. 2018. Maternal-by-environment
852 but not genotype-by-environment interactions in a fish without parental care. Heredity,
853 **120**(2): 154–167. doi:10.1038/s41437-017-0029-y
854 Wang, J., and Lenardo, M.J. 2000. Roles of caspases in apoptosis, development, and cytokine
855 maturation revealed by homozygous gene deficiencies. J. Cell Sci. **113**(5): 753–757. doi:
856 10.1242/jcs.113.5.753
857 Wenger, S.J., Isaak, D.J., Dunham, J.B., Fausch, K.D., Luce, C.H., Neville, H.M., Rieman,
858 B.E., Young, M.K., Nagel, D.E., Horan, D.L., and Chandler, G.L. 2011. Role of climate
859 and invasive species in structuring trout distributions in the interior Columbia River Basin,
860 USA. Can. J. Fish. Aquat. Sci. **68**(6): 988–1008. doi:10.1139/f2011-034
861 Wickham, H., Chang, W., and Wickham, M.H. 2016. Package 'ggplot2'. Create elegant data
862 visualisations using the grammar of graphics. Version **2**(1): 1–189.
863 Wiens, G.D., Palti, Y., and Leeds, T.D. 2018. Three generations of selective breeding improved
864 rainbow trout (*Oncorhynchus mykiss*) disease resistance against natural challenge with
865 *Flavobacterium psychrophilum* during early life-stage rearing. Aquaculture, **497**(2018):
866 414–421. doi:10.1016/j.aquaculture.2018.07.064
867 Yuan, Z., Liu, S., Yao, J., Zeng, Q., Tan, S., and Liu, Z. 2016. Expression of Bcl-2 genes in
868 channel catfish after bacterial infection and hypoxia stress. Dev. Comp. Immunol.
869 **65**(2016): 79–90. doi: 10.1016/j.dci.2016.06.018
870 Zhang, Y., and Kieffer, J.D. 2014. Critical thermal maximum (Ctmax) and hematology of
871 shortnose sturgeons (*Acipenser brevirostrum*) acclimated to three temperatures. Can. J.
872 Zool. **92**(3): 215–221. doi: 10.1139/cjz-2013-0223

873 Ziegeweid, J.R., Jennings, C.A., and Peterson, D.L. 2008. Thermal maxima for juvenile
874 shortnose sturgeon acclimated to different temperatures. Environ. Biol. Fish. **82**(3): 299–
875 307. doi:10.1007/s10641-007-9292-8

Tables

Table 1. Specific primers used for quantitative PCR with Genbank accession numbers and PCR amplicon sizes.

Gene	Primer (5′ – > 3′)	Sequence used for primer design	PCR amplicon size (bp)
β actine	F: CCAACTGGGACGACATGGA R: GAGCCACTCTCAGCTCGTTGT Probe: ATCTGGCATCACACCTT	*Salvelinus fontinalis* (KF783182.1)	63
18S	F: AGAAACGGCTACCACATCCAA R: CGAGTCGGGAGTGGGTAATTT Probe: AAGGCAGCAGGCGC	*Salvelinus fontinalis* (FJ710889.1)	60
EF1 α	F: TCGCCCCCGCTAATGTC R: AGGGTCTCGTGGTGCATCTC Probe: CCACTGAAGTCAAGTCT	*Salvelinus fontinalis* (KF783203.1)	58
CAT	F: GAAGGGAGCCCAAGTCTTCAT R: TCTGCATGCACAGCCATCA Probe: CAGAAACGCTGGGTTC	Transcriptome L. Bernatchez	63
SOD	F: CCCAGTAAGGGATTGTGTTTCTTT R: CGCCAGGCTTGTGGAGTTA Probe: CTGGGCAATGCCA	Transcriptome L. Bernatchez	58
HSP70	F: TGACGTGTCCATCCTGACCAT R: CCAGCCGTGGCCTTCA Probe: AGGATGGGATCTTTG	*Salvelinus fontinalis* (KF783199.1)	57
HSP90	F: GGCCAAGAAACACCTGGAGAT R: TGCCTCAGGGTCTCCACAA	*Salvelinus fontinalis* (KF783201.1)	57

	Probe: AACCCAGACCACCCC		
BCL	F: GCCTGGACGCAGTGAAAGAG	Sequencing	62
	R: GGCATAACGCAGCTCAAACTC		
	Probe: CATTGCGGGACTCTG		
GPx	F: TTCTCCTGATGTCCGAATTGATT	K. Jeffries laboratory	59
	R: ACCGACAAGGGTCTCGTGAT		
	Probe: CAGGGCACCCCCAG		
CASP9	F: ATGTCCTCCAGCAGTGACTCTCT	Transcriptome L. Bernatchez	66
	R: GGGTAGTGTGGCCTTTGCA		
	Probe: AGCACTCAGTCTGATGAG		
CASP3	F: CGGCACGCCTGTATGAAGA	Transcriptome L. Bernatchez	59
	R: GGAGACCGCTGCAAAACACT		
	Probe: CAGTTTGGGCTTTCC		

Table 2. Models output testing the effect of lineage for each variable at the two developmental stages. Generalized linear mixed models were used for survival data, linear mixed models were used for standard length and yolk sac volume, and linear models for development time. Estimates (reference level = control line), standard errors (SE) and associated p-values of lineage effects are provided, with the conditional (fixed + random effects) r^2 of each model (adjusted r^2 are provided for development time).

Developmental stage	**Variable**	**Estimate**	**SE**	***P*-value**	***r*²**
Hatching	Survival	0.05	0.51	0.92	0.135
	Yolk sac volume	-4.03	12.14	0.73	0.640
	Standard length	1.00	0.42	0.02	0.676
	Development time	16.10	9.67	0.11	0.075
Yolk sac resorption	Survival	-0.39	0.50	0.44	0.135
	Standard Length	0.76	0.41	0.06	0.483
	Development time	18.87	14.56	0.21	0.030

Table 3: A) Survival and development time measured at the familial level in each lineage; B) Yolk-sac volume, and standard length measured at the individual level in each lineage at the two developmental stages. Mean ± S.D.

Lineage		**Selected**	**Control**
A) Families		*n = 14*	*n = 9*
Survival (%)	Hatching	57.3 ± 28.2	64.3 ± 24.9
	Yolk-sac resorption	54.2 ± 27.8	60.5 ± 25.1
Development time (degree days)	Hatching	497.4 ± 24.3	481.3 ± 19.6
	Yolk-sac resorption	770.6 ± 30.2	751.8 ± 39.6
B) Fry		*n = 675*	*n = 450*
Yolk-sac volume (mm^3)	Hatching	60.1 ± 25.8	65.1 ± 23.6
Standard length (mm)	Hatching	17.3 ± 0.9	16.3 ± 0.8
	Yolk-sac resorption	23.3 ± 0.9	22.5 ± 1.2

Table 4: Variance components, estimated heritability and relative variance proportion for Dam effect (*m*). S: Selected; C: Control

	$V_A \pm SE$	$V_D \pm SE$	$V_R \pm SE$	h^2	*m*
Hatching					
Survival	0	0.074 ± 0.037	0.027 ± 0.011	0	0.73
Standard length (S)	0.157 ± 0.126	0.489 ± 0.321	0.296 ± 0.066	0.17	0.52
Standard length (C)	0.506 ± 0.379	0.230 ± 0.286	0.086 ± 0.207	0.62	0.28
Yolk sac volume	467.5 ± 88.1	223.4 ± 271.7	23.3 ± 116.9	0.66	0.31
Yolk sac resorption					
Survival	0.034 ± 0.308	0.090 ± 0.450	0.041 ± 0.032	0.21	0.54
Standard length	0.844 ± 0.441	0.162 ± 0.253	0.202 ± 0.238	0.70	0.13

V_A: additive genetic variance; V_D: the variance associated to dam; V_R: residual component; h^2: heritability.

Table 5: Statistical results of the random effects Cox proportional hazards model.

Effect	Estimate	S.E.	Z	p
Fixed effects	Lineage	0.1852824	-2.75	**0.0059**
				Variance
Random effect	Male identification			0.09741966

Table 6: Results of two-way permutational multivariate analysis of variance tests, PERMANOVA. Variable tested, sum of squares of the test (SS), pseudo-F of the statistic, and P(perm) for the test are presented. Bold characters indicate significant results.

Gene expression	Variable	Df	SS	Pseudo-F	P(perm)
Heart	**Thermal resistance**	**2**	**0.12569**	**3.2728**	**0.002**
	Lineage	1	0.01994	1.0383	0.424
	Thermal resistance × lineage	2	0.02788	0.7260	0.701
Liver	**Thermal resistance**	**2**	**0.47008**	**7.7448**	**0.0001**
	Lineage	1	0.07494	2.4693	0.051
	Thermal resistance × lineage	2	0.07901	1.3017	0.234

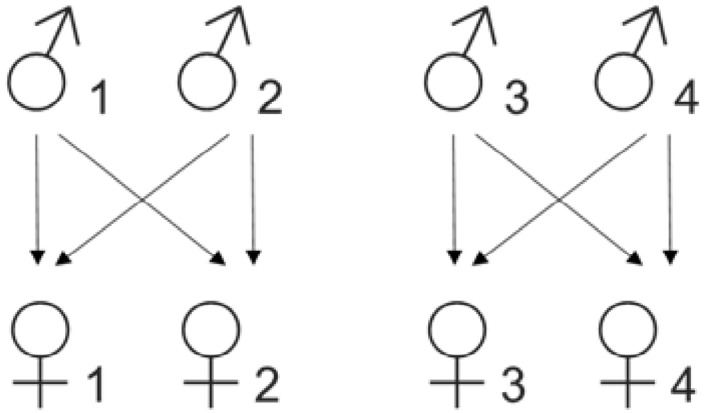
Figure Captions

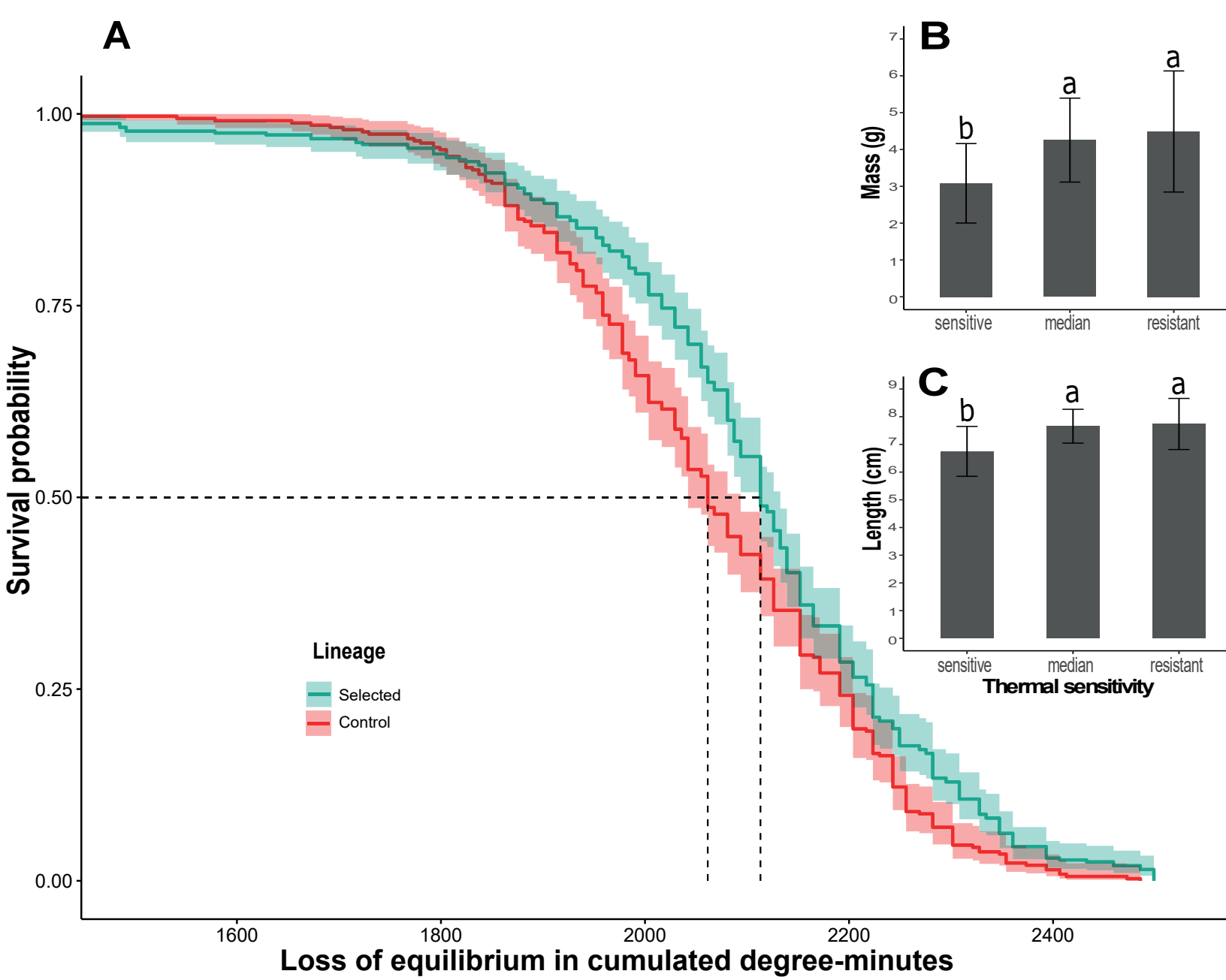
Figure 1. Breeding scheme. Each factorial breeding (partial 2 × 2) generated four full-brother families.

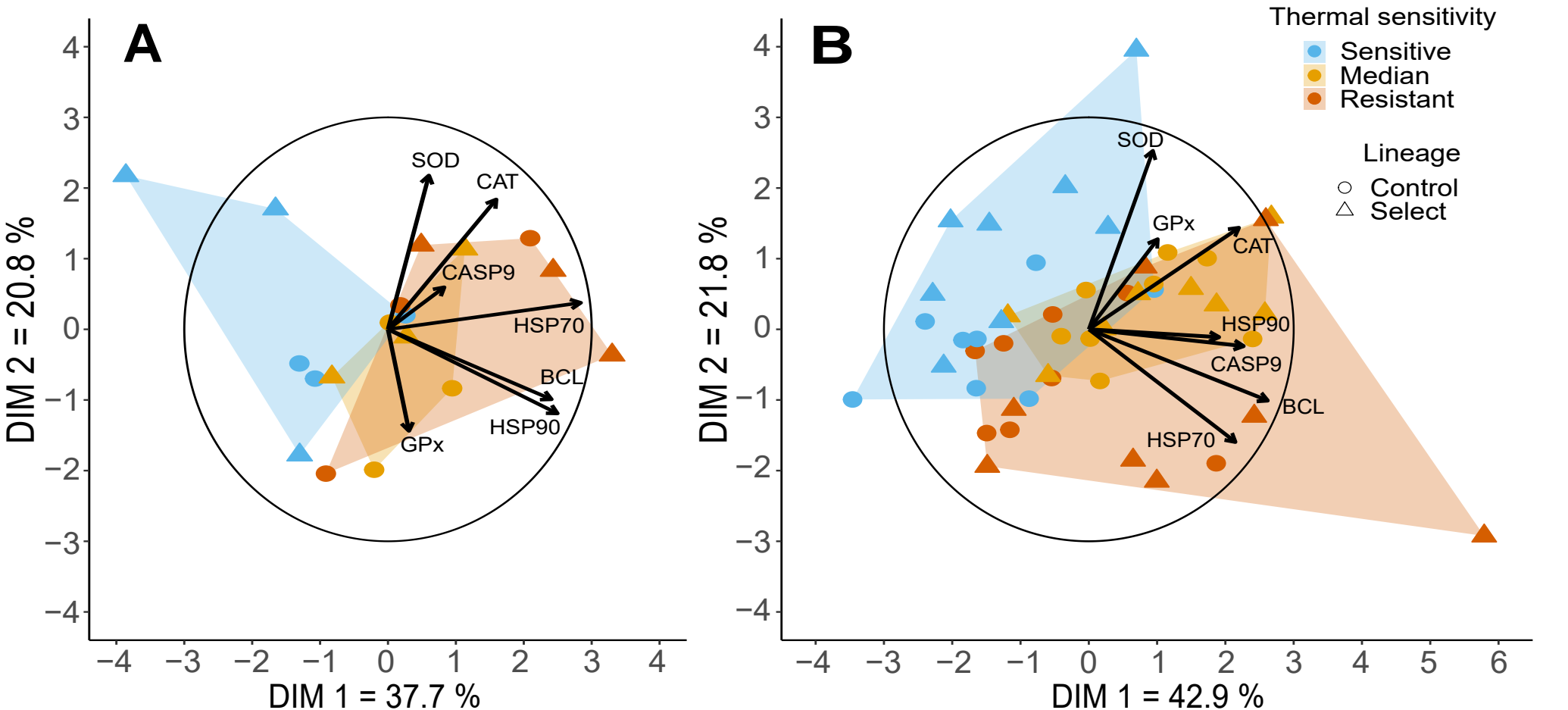
Figure 2. Survival curve of thermal resistance trials (A) and the influence of thermal resistance on mass (B) and length (C) data. Shaded areas represent 95% confidence intervals.

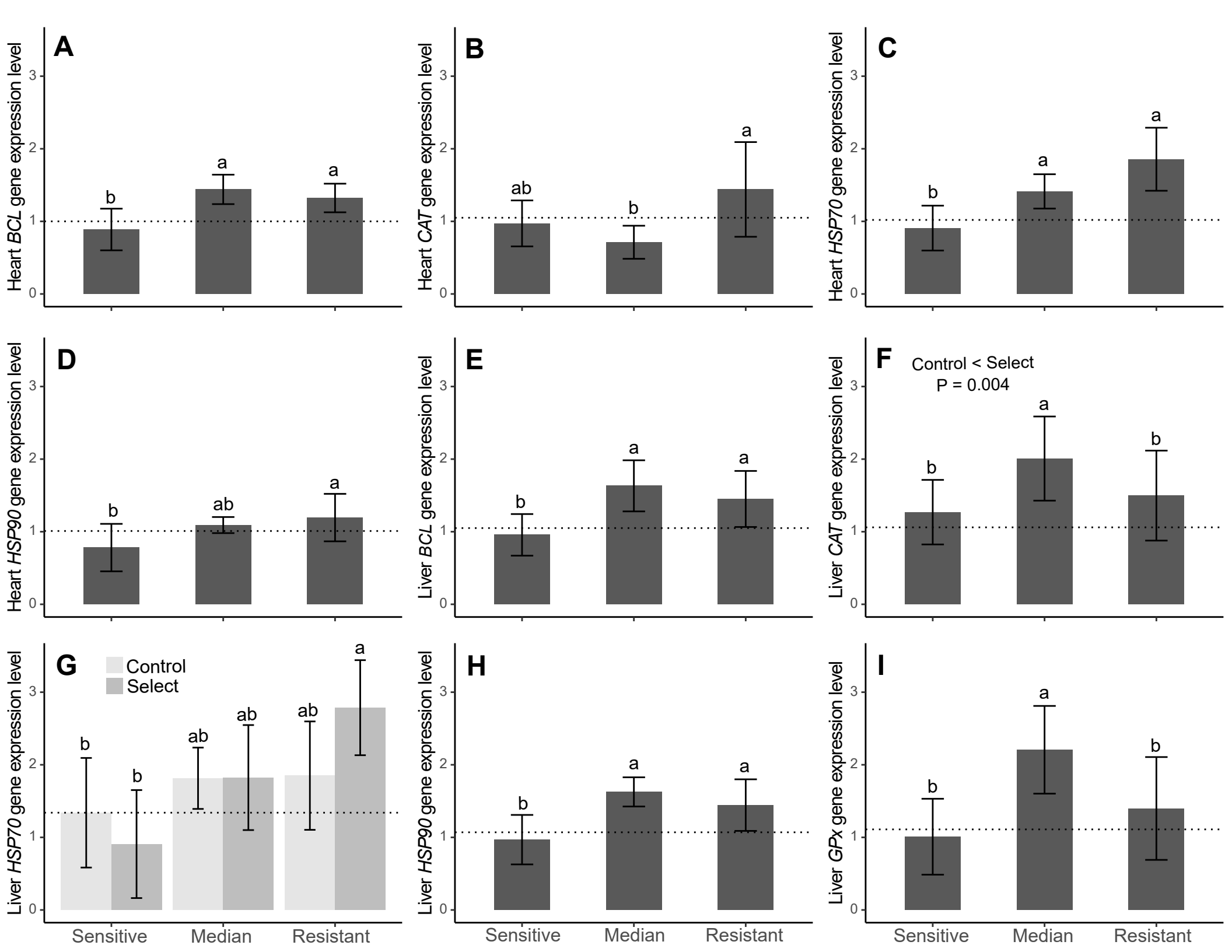
Figure 3: Principal component analysis (PCA) of challenged fish for (A) heart and (B) liver tissue. The first principal component (Dim 1, X axes) explains 37.7% (heart) and 42.9% (liver) of the total variance of the dataset, while the second principal component (Dim 2, Y axes) explains 20.8% (heart) and 21.8% (liver) of the total variance of the dataset. *CASP*: caspase, *BCL*: B-cell lymphoma, *CAT*: catalase, *SOD*: superoxide dismutase, *HSP*: heat shock protein, *GPx*: glutathion peroxidase.

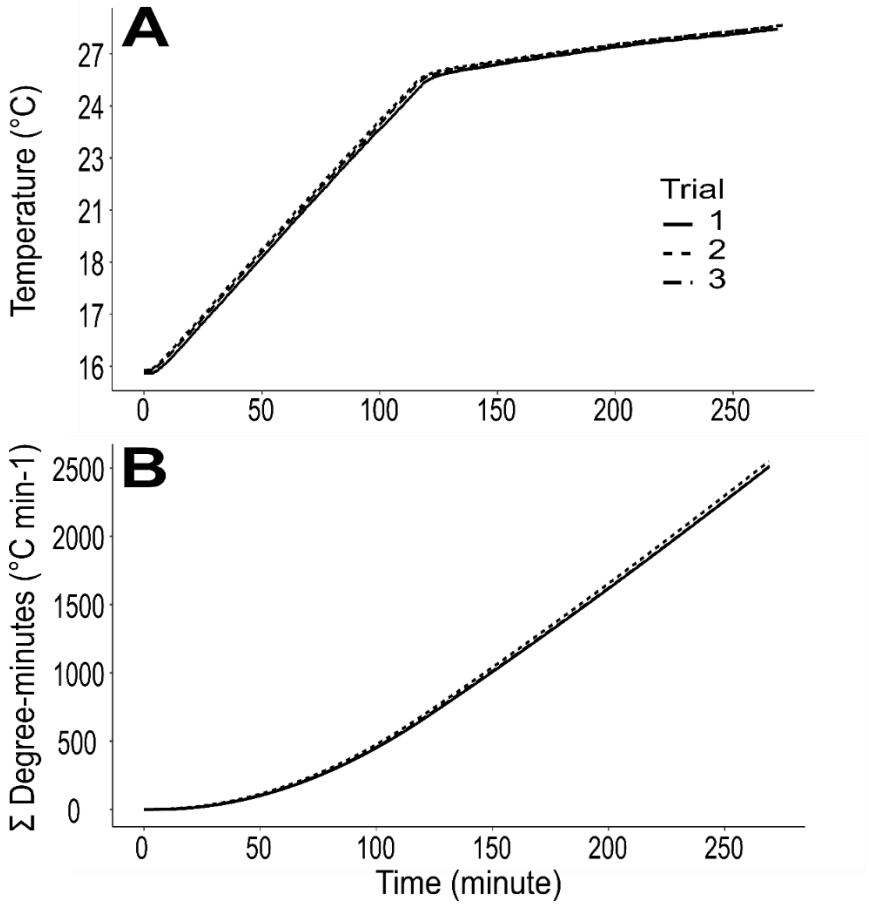
Figure 4: Effects of thermal resistance and lineage on mRNA expression in heart (A, B, C, and D) and liver (E, F, G. H, and I) tissue. Lower-cased letters indicate results of Tukey a posteriori tests.


1
2
3
4
1
2
3
4

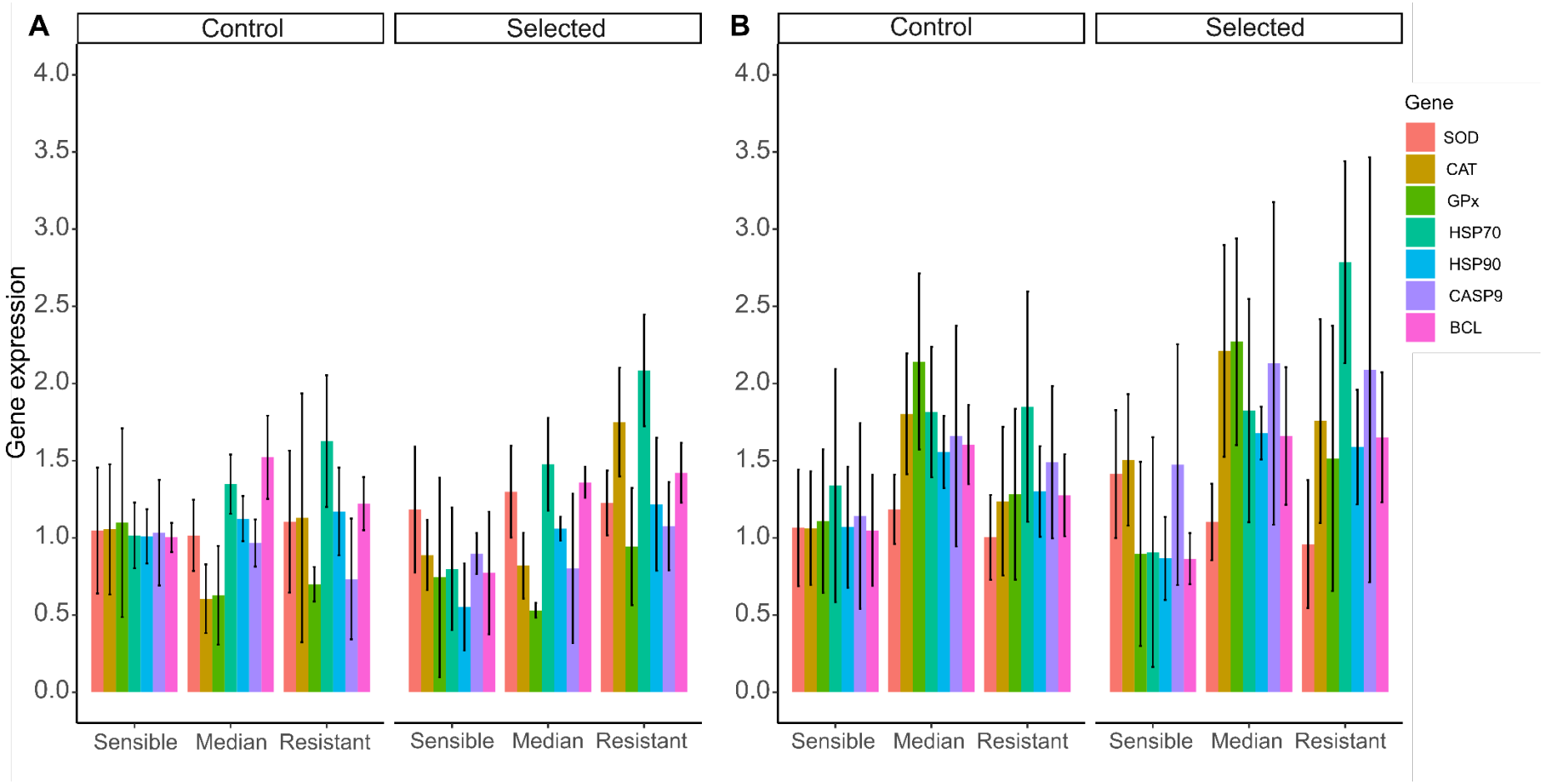

A
1.00
0.75
0.50
0.25
0.00
Survival probability
Lineage
Selected
Control
1600
1800
2000
2200
2400
Loss of equilibrium in cumulated degree-minutes
B
Mass (g)
b
a
a
sensitive
median
resistant
C
Length (cm)
b
a
a
sensitive
median
resistant
Thermal sensitivity


A
B
DIM 2 = 20.8 %
DIM 1 = 37.7 %
DIM 2 = 21.8 %
DIM 1 = 42.9 %
SOD
CAT
CASP9
HSP70
BCL
HSP90
GPx
Thermal sensitivity
Sensitive
Median
Resistant
Lineage
Control
Select


A
Heart BCL gene expression level
b
a
a
B
Heart CAT gene expression level
ab
b
a
C
Heart HSP70 gene expression level
b
a
a
D
Heart HSP90 gene expression level
b
ab
a
E
Liver BCL gene expression level
b
a
a
F
Control < Select
P = 0.004
Liver CAT gene expression level
b
a
b
G
Control
Select
Liver HSP70 gene expression level
b
b
ab
ab
ab
a
H
Liver HSP90 gene expression level
b
a
a
I
Liver GPx gene expression level
b
a
b
0
1
2
3
Sensitive
Median
Resistant



Supplementary Figure 1. Temperature in °C (A) and Σ Degree-minutes in °C min^{-1} (B) as function of time exposure in minutes during thermal trials.



Supplementary Figure 2. Thermal trial gene expression in juvenile (A) heart and (B) liver.

Supplementary Table 1: Statistical results of the two-way ANOVA for (A) heart tissue and (B) liver tissue. *CASP*: caspase, *BCL*: B-cell lymphoma, *CAT*: catalase, *SOD*: superoxide dismutase, *HSP*: heat shock protein, *GPx*: glutathion peroxidase. Bold characters indicate significant results.

A	Control			Selected					
Heart	sensitive	median	resistant	sensitive	median	resistant	Thermal Resistance	Lineage	Thermal S. × lineage
CASP9	1.03 ± 0.34 *(n=3)*	0.97 ± 0.15 *(n=3)*	0.73 ± 0.39 *(n=3)*	0.9 ± 0.13 *(n=3)*	0.8 ± 0.48 *(n=3)*	1.07 ± 0.29 *(n=3)*	P = 0.90 *DF =2, F= 0.106*	P = 0.93 *DF = 1, F= 0.008*	P = 0.35 *DF = 2, F= 1.161*
BCL	1 ± 0.09 *(n=3)*	1.52 ± 0.27 *(n=3)*	1.22 ± 0.17 *(n=3)*	0.77 ± 0.4 *(n=3)*	1.36 ± 0.1 *(n=3)*	1.42 ± 0.19 *(n=3)*	**P = 0.003 **** *DF =2, F= 9.625*	P = 0.56 *DF =1, F= 0.353*	P = 0.26 *DF = 2, F= 1.515*
CAT	1.05 ± 0.42 *(n=3)*	0.6 ± 0.22 *(n=3)*	1.13 ± 0.81 *(n=3)*	0.89 ± 0.23 *(n=3)*	0.82 ± 0.21 *(n=3)*	1.75 ± 0.35 *(n=3)*	**P = 0.036*** *DF =2, F= 4.453*	P = 0.29 *DF = 1, F= 1.222*	P = 0.32 *DF = 2, F= 1.266*
SOD	1.05 ± 0.41 *(n=3)*	1.02 ± 0.23 *(n=3)*	1.11 ± 0.46 *(n=3)*	1.18 ± 0.41 *(n=3)*	1.3 ± 0.3 *(n=3)*	1.23 ± 0.21 *(n=3)*	P = 0.97 *DF =2, F= 0.036*	P = 0.30 *DF = 1, F= 1.199*	P = 0.91 *DF = 2, F= 0.101*
HSP90	1.01 ± 0.18 *(n=3)*	1.12 ± 0.15 *(n=3)*	1.17 ± 0.28 *(n=3)*	0.55 ± 0.28 *(n=3)*	1.06 ± 0.08 *(n=3)*	1.22 ± 0.43 *(n=3)*	**P = 0.043 *** *DF =2, F= 4.125*	P = 0.22 *DF = 1, F= 1.680*	P = 0.25 *DF = 2, F= 1.556*
HSP70	1.02 ± 0.21 *(n=3)*	1.35 ± 0.19 *(n=3)*	1.63 ± 0.43 *(n=3)*	0.8 ± 0.4 *(n=3)*	1.48 ± 0.3 *(n=3)*	2.09 ± 0.36 *(n=3)*	**P = 0.001 **** *DF =2, F= 12.60*	P = 0.44 *DF = 1, F= 0.649*	P = 0.24 *DF = 2, F= 1.603*
GPx	1.1 ± 0.61 *(n=3)*	0.63 ± 0.32 *(n=3)*	0.7 ± 0.11 *(n=3)*	0.74 ± 0.65 *(n=3)*	0.53 ± 0.05 *(n=3)*	0.94 ± 0.38 *(n=3)*	P = 0.39 *DF =2, F= 1.022*	P = 0.610 *DF = 1, F= 0.274*	P = 0.40 *DF = 2, F= 1.000*

B	Control			Selected					
Liver	sensitive	median	resistant	sensitive	median	resistant	Thermal Resistance	Lineage	Thermal S. × Lineage
CASP9	1.14 ± 0.6 *(n=8)*	1.66 ± 0.71 *(n=8)*	1.49 ± 0.49 *(n=8)*	1.47 ± 0.78 *(n=8)*	2.13 ± 1.04 *(n=8)*	2.67 ± 2.07 *(n=8)*	P = 0.07 *DF =2, F = 2.810*	P = 0.07 *DF = 1, F = 3.40*	P = 0.95 *DF = 2, F = 0.05*
BCL	1.05 ± 0.36 *(n=8)*	1.6 ± 0.26 *(n=8)*	1.28 ± 0.27 *(n=8)*	0.87 ± 0.17 *(n=8)*	1.66 ± 0.45 *(n=8)*	1.65 ± 0.42 *(n=7)*	**P = 2.81e-06** *** *DF =2, F = 17.7*	P = 0.44 *DF = 1, F = 0.61*	P = 0.078 *DF = 2, F = 2.75*
CAT	1.06 ± 0.37 *(n=8)*	1.8 ± 0.39 *(n=8)*	1.24 ± 0.48 *(n=8)*	1.51 ± 0.43 *(n=7)*	2.21 ± 0.69 *(n=8)*	1.76 ± 0.66 *(n=8)*	**P = 0.001** *** *DF =2, F = 8.27*	**P = 0.004** ** *DF = 1, F= 9.09*	P = 0.95 *DF = 2, F= 0.05*
SOD	1.07 ± 0.38 *(n=8)*	1.19 ± 0.22 *(n=8)*	1 ± 0.28 *(n=8)*	1.41 ± 0.41 *(n=8)*	1.1 ± 0.25 *(n=8)*	0.96 ± 0.41 *(n=8)*	P = 0.10 *DF =2, F = 2.43*	P = 0.45 *DF = 1, F = 0.58*	P = 0.14 *DF = 2, F= 2.03*
HSP90	1.07 ± 0.39 *(n=8)*	2.11 ± 1.11 *(n=8)*	1.2 ± 0.29 *(n=8)*	0.87 ± 0.27 *(n=8)*	1.68 ± 0.17 *(n=8)*	1.59 ± 0.37 *(n=8)*	**P = 1.22e-06** *** *DF =2, F = 19.52*	P = 0.46 *DF = 1, F = 0.55*	P = 0.08 *DF = 2, F = 2.72*
HSP70	1.34 ± 0.75 *(n=8)*	1.81 ± 0.42 *(n=8)*	1.85 ± 0.75 *(n=7)*	0.91 ± 0.74 *(n=8)*	1.82 ± 0.72 *(n=8)*	2.79 ± 0.65 *(n=7)*	**P = 0.0001** *** *DF =2, F = 11.62*	P = 0.50 *DF = 1, F= 0.47*	**P = 0.03** * *DF = 2, F= 3.85*
GPx	1.11 ± 0.47 *(n=8)*	2.14 ± 0.57 *(n=8)*	1.28 ± 0.55 *(n=8)*	0.9 ± 0.6 *(n=7)*	2.27 ± 0.67 *(n=8)*	1.51 ± 0.86 *(n=8)*	**P = 2.31e-05** *** *DF =2, F = 14.0*	P = 0.68 *DF = 1, F= 0.17*	P = 0.37 *DF = 2, F= 1.02*