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HAL Authorization

1 **Cold temperature during embryonic development and its influence on responses to**
2 **acute confinement and hyperthermia challenges in juvenile rainbow trout**

3

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16 **Abstract**

17 To expand the availability of marketable eyed eggs, a common practice in rainbow trout
18 aquaculture is to store eyed eggs at low temperatures (2-4°C) for periods of up to 2-3 weeks.
19 Early exposure to environmental stimuli such as temperature can impact fish physiology,
20 growth, metabolism, and nutrition at mid- or long-term questioning about the potential effects
21 of such breeder practice. This study aimed to test the impact of incubating eyed eggs at low
22 temperatures (3°C instead of 12°C) for 15 days on resistance to later stresses using two
23 experimental rainbow trout lines divergent for fat content. Plasma parameters (*i.e.* cortisol,
24 glucose, lactate, and chloride ions) have been measured after an acute confinement challenge.
25 For the hyperthermia challenge, time at the loss of equilibrium was compared between lines
26 and incubation temperatures. Our results showed that deformities rate and early survival were
27 not affected by cold temperature storage. Furthermore, plasma parameters measured after the
28 confinement were not impacted by temperature. The two lines differently responded to the
29 hyperthermia challenges with the fat line showing significantly higher resistance to
30 temperature elevation. These findings suggest that the incubation temperature did not impair
31 the responses to stress at the juvenile stage, indicating that the practice of cold storage of eyed
32 eggs does not appear to be detrimental to subsequent rearing.

33 **Keywords:** Cold; Incubation; Eyed-eggs; Salmonids; Stress

34

35 **1. Introduction**

36 Rainbow trout (*Oncorhynchus mykiss*) is the predominant species in French aquaculture and
37 globally significant in salmonid production, contributing to 953,000 tonnes in 2021, valued at
38 approximately 4.37 billion US dollars (FAO, 2023). Rainbow trout reproduction is governed
39 by photoperiodic cues (Whitehead and Bromage, 1978; Bromage et al., 1982), allowing for
40 the manipulation of spawning times in aquaculture. Protocols enable the advancement or
41 delay of spawning by up to 6 months (Bromage et al., 1993), facilitating year-round spawning
42 for selective breeding. Additionally, breeding companies commonly store eyed eggs at cold
43 temperatures to extend their market availability (Maddock, 1974; Richardson et al., 2002).
44 However, while cold temperature manipulation slows embryonic development and delays
45 hatching (Piper et al., 1982), its long-term consequences remain poorly investigated.

46 Chilled storage effects on unfertilised rainbow trout eggs have been extensively studied
47 (Niksirat et al., 2007; Ubilla et al., 2016), establishing storage conditions that preserve their
48 fertilising capacity (Komrakova and Holtz, 2009). However, the consequences of chilling
49 fertilised eggs are less understood. Studies have shown that early incubation at low
50 temperatures post-fertilisation affects embryonic development and reduces survival until the
51 eyed stage (Stonecypher et al., 1994; Babiak and Dabrowski, 2003). Rainbow trout embryos'
52 tolerance to chilling increases as they develop (Leveroni Calvi and Maise, 1998). Richardson
53 et al. (2002) extended the storage time of chilled rainbow trout eyed eggs by 35 days at 1 °C
54 using perfluorochemicals to facilitate oxygenation. While most studies focus on short-term
55 consequences such as hatching percentage, malformations, and survival of young stages, the
56 long-term effects on later stages (*i.e.* juveniles and adults) remain largely unknown.

57 This study aimed to assess the impact of cold temperature storage of rainbow trout eyed eggs
58 on their response to two acute challenges during the juvenile stage: confinement and
59 hyperthermia. Two experimental lines of rainbow trout, selectively bred for either low or high

60 muscle fat content, were used (Quillet et al., 2005). Given the observed differences in energy
61 utilisation and intermediate metabolism between these lines (Kolditz et al., 2008a, 2008b,
62 2010; Kamalam et al., 2012), we hypothesised that their responses to cold temperatures
63 during incubation would differ.

64 **2. Materials and methods**

65 2.1. Ethics statement

66 All the experiments were conducted at the INRAE experimental facilities (PEIMA, INRAE,
67 2021, Fish Farming systems Experimental Facility, doi: 10.15454/1.5572329612068406E12,
68 Sizun, France) authorised for animal experimentation under the French regulation C29-277-
69 02. The experiment was carried out according to the European guidelines; the protocols were
70 evaluated and approved by the ethical committee CEFEA No 74 and authorised by the French
71 Ministry of Higher Education and Research (APAFIS #31861-2021060117041206 v4).

72 2.2. Fish and rearing conditions

73 The study was conducted with two INRAE experimental lines of rainbow trout
74 (*Oncorhynchus mykiss*), designated as Fat line (FL) and Lean line (LL), obtained after seven
75 generations of divergent selection for respectively high or low muscle fat content using a non-
76 destructive method (Distell Fish Fat Meter ®) as detailed by Quillet et al. (2007). Muscle fat
77 content was found to be more than 3 times higher in the FL line (8.0%) than in the LL line
78 (2.3%) in 200 g-trout after five generations of selection (Jin et al., 2014).

79 For each of the two lines, clutches from 20 females among those available on the same day
80 were selected (*i.e.* homogeneity of the size of the oocytes between clutches, absence of blood,
81 fatty deposits, and degraded oocytes). To obtain approximately 500 eggs per female, we
82 estimated the weight needed from each clutch based on the average weight of an egg. The
83 eggs from the 20 females have been pooled and coelomic liquid was added to homogenise the

84 pool. The eggs were then drained and redistributed into 20 cups for fertilisation with sperm
85 from 20 males. All fertilisations were carried out on the same day. Fertilised eggs from each
86 line were then incubated in hatching trays supplied with spring water at 12.0°C.

87 At 17 days post fertilisation (dpf), eyed eggs from the two experimental lines were distributed
88 into small incubators installed in two separate 200-L tanks supplied with natural spring water
89 at either 12.0°C (control) or chilled at 3.0°C (cold storage condition) for 15 days to mimic
90 selective breeders' practices. For each line, three incubators containing 500 eyed eggs were
91 used for each incubation temperature condition (2 lines x 2 incubation temperatures x 3
92 incubators). For the cold storage condition, the water temperature was gradually lowered at a
93 rate of 3.0°C h⁻¹ and then maintained at 3.0 ± 0.5°C using a water chiller (Cooling Plus
94 Energy system, Hitema). After 15 days of cold storage, water temperature was increased at a
95 rate of 3.0°C h⁻¹, by allowing a controlled stream of water at the targeted temperature to flow,
96 reaching a final temperature of 12.0°C. The temperature was recorded continuously while the
97 water flow was kept at 20 L h⁻¹.

98 Fry were kept in incubators and individuals with deformities were counted and removed
99 regularly. Before the first feeding only viable individuals were transferred into 0.3 m³ indoor
100 tanks supplied with natural spring water at 12.0°C and lit by artificial neon lights from 8:00
101 AM to 8:00 PM, without mixing between lines and incubation temperature conditions (n = 3
102 tanks per condition and ~270 individuals per tank). At the age of 78 dpf (average weight: 0.6-
103 1.0 g), the fry were transferred first into covered 0.3 m³ tanks under a greenhouse and
104 supplied with lake dam water and then, at 155 dpf, into covered 1.8-m³ outdoor fiberglass
105 tanks. From 78 dpf and up to the challenges, fish were subjected to normal seasonal
106 temperature variations throughout the concerned period (daily mean temperature: 8.0 to
107 18.0°C) and lit by natural light. Until the beginning of the experiment, the fish were fed daily
108 to satiation using automatic feeders with a commercial diet from Le Guessant's company.

109 The growth was followed regularly by weighing a random subsample of 50 fish from each
110 tank while the fish individual body weight was measured at 190 dpf (n = 353-459 per
111 condition). Mortality has been checked daily throughout the experiment. Over the rearing
112 period, O₂ concentrations in the outlet water ranged from 8.5 to 9.9 mg L⁻¹ (*i.e.* 85-99 % of
113 saturation level) while NH₄⁺ and NO₂⁻ concentrations ranged from 0.2 to 0.7 mg L⁻¹ and <0.01
114 to 0.8 mg L⁻¹, respectively (assessed on a bi-weekly basis on average).

115 The course of the different rearing stages and challenges performed is shown in Figure 1.

116 2.3. Confinement challenge

117 Acute confinement challenges were performed over three days at 217-219 dpf. About one
118 month before the challenges, fish from each line and condition were transferred into 0.3 m³
119 tanks still supplied with lake dam water and kept separate with 50 fish in each tank (n = 3
120 tanks per condition). Before the start of the confinement challenge, all the fish were starved
121 for 24h and eight of them were euthanised by an overdose of anaesthetic (Tricaine MS-222,
122 100 mg L⁻¹), weighed using digital scales (\pm 0.1 g), and immediately sampled for blood.
123 Blood was taken from the caudal vein using heparinised syringes (heparin lithium, Sigma-
124 Aldrich, USA). Blood samples were kept on ice until plasma was separated from whole blood
125 by centrifugation at 2,500 g for 10 min at 4°C. Plasma samples were stored at -20°C before
126 analysis. These fish constituted the time-zero control group before the confinement challenge.
127 Then, 8 fish from each tank were transferred into a bucket filled with water according to the
128 average weight per tank to reach a fish density of 200 kg m⁻³ and kept for 4 minutes with no
129 water renewal or oxygen supplementation. Challenged fish were transferred under flow-
130 through water conditions into a 0.3 m³ recovery tank for 1h and then euthanised as described
131 above, weighed, and sampled for blood according to the same procedure as for fish before
132 confinement.

133 Glucose and lactate were measured in plasma (5 μ L per measurement) using a portable digital
134 blood glucose meter ACCU-CHEK $\text{\textcircled{R}}$ Active (Roche Diagnostic Systems, Herts, UK) and a
135 THE EDGE $\text{\textcircled{R}}$ blood lactate analyser (APEXBIO, Taiwan), respectively.

136 Plasma cortisol was extracted by adding 1 mL diethyl ether to 100 μ L of plasma in a 5 mL
137 glass tube. The sample and solvent were vortex-mixed and frozen at -20°C for at least 1h to
138 allow the phases to separate. The supernatant was then transferred into a 2 mL glass vial and
139 evaporated at room temperature under a stream of nitrogen. The residue was dissolved in 100
140 μ L of extraction buffer provided in the commercial ELISA kit (Cortisol ELISA KIT; Neogen
141 $\text{\textcircled{R}}$ Corporation) used for plasma cortisol measurements and performed according to the
142 manufacturer's instructions.

143 Chloride ions were measured in plasma using a commercial kit (Kit Chlorures, Biolabo $\text{\textcircled{R}}$).
144 Plasma (3 μ L), diluted at $\frac{1}{2}$ in deionised water, reacted with 300 μ L of Hg(II) thiocyanate for
145 5 min at room temperature, and then absorbance was read at 500 nm. For both cortisol and
146 chloride ions, measurements have been performed in triplicates, and values were calculated
147 from reference standard curves.

148 2.4. Hyperthermia challenges

149 Acute hyperthermia challenges were performed at 224-226 dpf. At 191 dpf, fish were
150 individually PIT-tagged (Biolog-id $\text{\textcircled{R}}$) and grouped into 3 separate 0.3 m^3 tanks containing
151 each 200 fish, *i.e.* 50 fish per line and incubation temperature. PIT tags (pit-tag length: 12
152 mm, diameter: 2 mm, weight: 0.092 g) were injected horizontally into the dorsal muscle just
153 behind the head. Animals were starved for three days before challenges according to Lagarde
154 et al. (2023b). The evening before each challenge, fish were transferred into the challenge
155 tank (0.3 m^3), supplied with the same lake dam water as the one used in the rearing tanks, and
156 left alone for the night for acclimation. Temperature was gradually increased from 8.5°C to
157 27.5°C by renewing challenge tank water with heated water from a buffer tank. Temperature

158 was first quickly increased at a rate of 0.7°C every 10 minutes until 22°C. This period was
159 followed by a slower temperature increase at a rate of 0.1°C every 15 minutes until all fish
160 have lost equilibrium. This slower temperature increase was intended to increase the between-
161 fish variability of acute hyperthermia resistance phenotypes (Lagarde et al., 2023b). Water
162 was oxygenated to keep oxygen levels near saturation (Figure S1). Temperature and O₂
163 concentration and saturation were recorded every 5 min during challenges using electronic
164 probes (HQ30d, Hach Company, Loveland, CO, USA), while NH₄⁺ concentration was
165 checked twice, *i.e.* at peak of loss of equilibrium - determined as the sudden increase in the
166 number of fish losing equilibrium - and at the end of the challenge, using a commercial
167 colorimetric kit (LCK 304, Hach Company, USA). CO₂ concentration was measured
168 continuously with a CO₂ analyzer (Oxyguard, Denmark). The maximum concentrations
169 measured were 2.6 mg L⁻¹ and 9 mg L⁻¹ for NH₄⁺ and CO₂, respectively.

170 As the temperature increased, fish were gradually losing equilibrium. When a fish lost
171 equilibrium, it was removed from the tank, its origin was identified using the PIT tag and the
172 exact time of loss of equilibrium was recorded (Time_{loss}) as the phenotype of interest (see
173 Section 4 for details). Fish were then softly anesthetised (Tricaine MS-222, 50 mg L⁻¹),
174 weighed using digital scales (\pm 0.1 g), and euthanised by an overdose of anaesthetic (Tricaine
175 MS-222, 100 mg L⁻¹). Challenges ended when the last fish lost its equilibrium.

176 2.5. Data analysis

177 For the deformity rate and survival rates, the assumptions of normality and homoscedasticity
178 were not met. Hence, non-parametric Kruskal-Wallis tests followed by Dunn's tests with
179 Bonferroni adjustment for p-values were used to analyse the differences between
180 experimental conditions. Body weight differences among experimental conditions at 190 dpf,
181 *i.e.* before the confinement and acute hyperthermia challenges, were analysed by analysis of
182 variance (ANOVA) and Tukey's test for multiple pairwise comparisons. ANOVA

183 assumptions of normality and homoscedasticity were verified by visual inspection of residual-
184 fit plots.

185 Concerning the confinement challenges, the plasma parameters were measured for four out of
186 the eight sampled fish (*i.e.*, 12 fish per experimental condition and sampling time). A linear
187 mixed model was computed with “line”, “temperature” and “sampling time” (before or after
188 the confinement challenge) as fixed effects and “replicates” as a random effect.

189 Acute hyperthermia resistance was quantified as the time at the loss of equilibrium ($\text{Time}_{\text{loss}}$;
190 expressed in min). Differences in $\text{Time}_{\text{loss}}$ were compared between lines and incubation
191 temperatures using individuals as the experimental unit. A linear mixed model was computed
192 with “line”, “temperature” and “weight” - given the differences in size between the two lines
193 for this challenge (see Section 3) - as fixed effects, and “replicates” as a random effect.

194 Models were fitted using the *lme4* and *nlme* packages, and contrasts were analysed using the
195 *emmeans* package. The interaction terms were not significant and not included in the final
196 models. The best models for fixed effects were chosen with Akaike information criteria (AIC)
197 and F-tests using the *lmerTest* package. The marginal r^2 (r^2_{m}) and the conditional r^2 (r^2_{c}) were
198 calculated using the *MuMin* package (Barton, 2020). r^2_{m} is the proportion of variance
199 explained by fixed factors and r^2_{c} is the proportion of variance explained by both fixed and
200 random factors (Nakagawa and Schielzeth, 2013). Assumptions of normality and
201 homoscedasticity were checked by visual inspections of residual-fit plots and log and square-
202 root transformations were operated on lactate and cortisol data, respectively.

203 The significance level for statistical analyses was set to $\alpha = 0.05$. All statistics were performed
204 using R freeware version 4.2.2 (R Development Core Team, 2022). Throughout the
205 manuscript, values are given as mean \pm standard deviation.

206 **3. Results**

207 The incubation temperature exhibited no discernible impact on the fry's deformity rate from
208 hatching to the first feeding, with an average value of 6% across all experimental conditions
209 ($H_{(3)} = 7.21$, $P = 0.07$). Similarly, the survival rate during this phase remained unaffected by
210 the incubation temperature ($H_{(3)} = 2.13$, $P = 0.55$), with an average of 90% observed across all
211 conditions during the indoor rearing phase (Table 1). In the outdoor rearing phase, survival
212 rates slightly differed according to experimental conditions ($H_{(3)} = 8.44$, $P = 0.04$) with
213 survival rates significantly lower in LL kept at 3°C (80.8 ± 3.3 %) than FL under control
214 temperature (94.4 ± 1.2 %). Interestingly, within the same line, we did not find significant
215 effects of incubation temperature on survival rate (Table 1). As anticipated, the average
216 individual weight, measured at 190 dpf one month before the confinement and hyperthermia
217 challenges, was affected by both line ($F_{(1)} = 103.76$, $P < 0.001$) and incubation temperature
218 ($F_{(1)} = 107.23$, $P < 0.001$). Fish from the LL line surpassed those from the FL line in size
219 (40.4 ± 11.5 g vs 35.3 ± 11.1 g in normal temperature conditions), and those subjected to cold
220 storage during incubation exhibited smaller sizes (35.2 ± 12.0 g and 29.2 ± 9.8 g for LL and
221 FL, respectively) (Table 1). Basal blood parameters (i.e. chloride ions, cortisol, glucose, and
222 lactate) were similar between both lines (Figure 2).

223 Following the confinement challenge, there was a significant increase in plasma cortisol
224 levels ($F_{(1)} = 925.47$, $P < 0.001$) with values ranging from 2.6 ± 2.8 to 80.0 ± 24.0 ng mL⁻¹,
225 before and after the challenge respectively. On the other hand, neither the genetic lines nor
226 incubation temperature at the eyed eggs stage affected the cortisol levels in both control and
227 challenged fish (Figure 2). The same trend was observed for glucose ($F_{(1)} = 184.86$, $P <$
228 0.001) and lactate concentrations ($F_{(1)} = 327.42$, $P < 0.001$) with significant increases in
229 concentrations after the confinement challenge for both lines but no significant difference
230 between lines and incubation temperatures. Chloride ion concentrations were similar between

231 the experimental conditions and remained constant over the challenge (143 ± 10 mM; Figure
232 2).

233 The kinetics of loss of equilibrium over the acute hyperthermia challenge are presented in
234 Figure 3. Even when accounting for the significant effect of the weight ($F_{(1)} = 34.75$, $P <$
235 0.001 ; Table 2), we found significant differences between both experimental lines ($F_{(1)} =$
236 22.79 , $P < 0.001$) with LL being less resistant ($\text{Time}_{\text{loss}}: 547 \pm 59$ min) than FL ($\text{Time}_{\text{loss}}: 581$
237 ± 64 min). The incubation temperature did not significantly affect the resistance to
238 hyperthermia ($F_{(1)} = 0.23$, $P = 0.628$).

239 **4. Discussion**

240 To our knowledge, this is the first study to investigate how cold temperature storage of
241 rainbow trout eyed eggs affects the response to subsequent juvenile challenges. Previous
242 research suggests that early environmental stimuli, such as temperature, can have medium to
243 long-term effects on fish physiology (Auperin and Geslin, 2008; Scott and Johnston, 2012;
244 Mateus et al., 2017). Considering the potential impact of cold storage on early-stage
245 zootechnical performances (Stonecypher et al., 1994; Richardson et al., 2002; Babiak and
246 Dabrowski, 2003), we examined its effects on deformities and early survival before first
247 feeding. Our findings indicate that cold storage of eyed eggs did not significantly affect these
248 parameters, consistent with Richardson et al.'s observations (2002).

249 In subsequent rearing phases, the growth disparities observed at 190 dpf were attributed to the
250 earlier hatching of animals kept at 12°C during incubation (see Figure 1). This aligns with
251 expectations, as cold storage slows down embryonic development and delays hatching
252 (Stonecypher et al., 1994; Babiak and Dabrowski, 2003). However, to accurately assess the
253 impact of cold storage on growth compared to control condition, mean body weight should
254 have been measured at a constant number of days post-hatching, which was not done in this
255 study. The effects of low incubation temperature were further evaluated through blood

256 parameter assessments in 7-month-old fish. Measurements of chloride ions, cortisol, glucose,
257 and lactate obtained before the challenges showed no significant differences between the
258 incubation temperatures. To further investigate the effects of cold storage, the fish were
259 subjected to challenges involving two acute stressors.

260 Stress responses in teleost fishes involve three phases: primary, secondary, and tertiary. The
261 primary response includes the release of catecholamines and cortisol, triggering secondary
262 responses such as increased plasma glucose, lactate, and heart rate, as well as decreased
263 plasma chloride, sodium, potassium, liver glycogen, and muscle protein (Pickering, 1981;
264 Mommsen et al., 1999; Barton, 2002). These secondary responses can lead to tertiary
265 responses, including reduced growth rate, metabolic scope, disease resistance, reproductive
266 capacity, and altered behaviour and survivability (Wedemeyer et al., 1990; Barton and Iwama,
267 1991; Mommsen et al., 1999). Given the intensity and duration of the stressor, the challenges
268 in this study can be considered as acute (Schreck and Tort, 2016). The hyperthermia challenge
269 induced a loss of equilibrium, indicating a phenotype of resistance (Fry, 1971). In contrast,
270 the confinement challenge focused on primary stress responses, measuring cortisol synthesis,
271 and secondary responses through investigations on energy metabolism (glucose and lactate)
272 and hydromineral balance (chloride ions).

273 Several studies have investigated the effects of confinement on physiological stress indicators
274 in salmonids. Cortisol levels typically increase in response to confinement stress lasting
275 between 2 minutes and 4 hours (Pickering et al., 1991; Jentoft et al., 2005; Sadoul et al., 2015;
276 Magnoni et al., 2019). For example, Magnoni et al. (2019) observed a cortisol peak 1 hour
277 after subjecting trout to confinement stress for 2 minutes at 200 kg m⁻³ before returning them
278 to tanks. Gesto et al. (2015) made similar observations after a 3 min-disturbance stress.

279 Although we did not observe an effect of genetic background on the post-confinement
280 increase in cortisol in this study, it is interesting to note that Pottinger and Carrick (1999)

281 demonstrated the feasibility of divergent selection on the intensity of the response to 3h-
282 confinement stress repeated at monthly intervals for 5 months based on plasma cortisol
283 measurements in rainbow trout performed right after the challenge. The same authors
284 estimated a relatively high heritability for this trait ($h^2 \approx 0.4$) which suggests a strong genetic
285 component in the cortisol responsiveness to stress in this species even if the underlying
286 mechanisms remained unclear (Trenzado et al., 2003).

287 In our experiment, we found that acute stress led to a comparable release of plasma cortisol in
288 juvenile fish, regardless of whether they were exposed to cold temperature storage at the eyed
289 eggs stage. This pattern was consistent for both FL and LL fish lines, suggesting a uniform
290 regulation of cortisol synthesis and release in both lines. Our confinement protocol, which
291 involves a shorter stress duration than the protocol used by Pottinger and Carrick (1999) and a
292 1-hour recovery period, has proven effective in revealing genetic influences on cortisol
293 response, as shown in preliminary investigations with other rainbow trout genetic lines
294 (unpublished data). This suggests that, even after seven generations of divergent selection for
295 fat content, the two experimental lines still exhibit a similar stress response after 1h of
296 recovery.

297 Concerning the other physiological stress indicators measured in plasma, the increase in
298 glucose and lactate observed indicates an establishment of the secondary response to
299 confinement stress. Jentoft et al. (2005) observed that the glucose peak occurred 3 hours post-
300 confinement. Here, we measured glucose and lactate at 1-hour post-stress, which agrees with
301 previous studies performing stress of similar duration. Indeed, in a 3-min disturbance test,
302 Gesto et al. (2015) demonstrated in rainbow trout that plasma glucose and lactate reached
303 their maximum levels 3 min after the disturbance challenge while these levels were
304 maintained for at least 45 min. It is interesting to note that this secondary stress response was
305 similar regardless of cold stress carried out at the eyed eggs stage and genetic background. In

306 addition, the osmoregulatory ability of fish can also be disrupted by stress, inducing a change
307 in blood osmolarity and ion contents (see review of Seibel et al. (2021)). In our experiment,
308 however, plasma chloride ion concentrations were similar 1 hour after acute stress in all
309 conditions. This result suggests that all fish, regardless of their life history or genetic
310 background, could cope with acute stress.

311 The hyperthermia challenges conducted in this study over three days were highly repeatable,
312 with consistent temperature rises and oxygen saturation levels across all three tests (Figure
313 S1). Due to the consistent starting temperature (8.5°C) and the high repeatability of
314 temperature rise profiles, we did not calculate cumulative thermal exposure (CTE) in degree-
315 minutes, as defined by Perry et al. (2005) and used by Lagarde et al. (2023a). Currently, there
316 is no consensus on the mechanisms behind the loss of equilibrium in acute hyperthermia
317 conditions in fish, with differences observed between species (Lefevre et al., 2021; Desforges
318 et al., 2023; Ern et al., 2023). Factors such as life stage, body size, phenotypic plasticity, and
319 genetic background influence intraspecific fish hyperthermia resistance (McKenzie et al.,
320 2021). This resistance phenotype, as measured here, has been demonstrated to be heritable (h^2
321 $\approx 0.3-0.4$; Perry et al., 2005; Lagarde et al., 2023a), with strong variability observed between
322 rainbow trout isogenic lines (Lagarde et al., 2023b). These results indicate a significant
323 genetic component in hyperthermia stress resistance, explaining the differing resistance
324 observed between the two experimental lines in this study.

325 Explaining the higher resistance of the FL compared to the LL to hyperthermia is challenging
326 due to the diversity of mechanisms involved. The major phenotypic difference between these
327 lines is their contrasting adiposity. Despite observing significant differences in average weight
328 at the time of hyperthermia challenges, we accounted for this effect in our statistical model.
329 Subcutaneous adiposity can act as an insulating layer, protecting organisms from temperature
330 fluctuations and this ancient mechanism can be exploited by fish to thrive in cold oceanic

331 environments (Alexander et al., 2015). This could explain why the FL showed greater
332 resistance to hyperthermia in our experiment. However, such mechanisms primarily protect
333 organisms in low-temperature conditions rather than warm environments. Fish acclimated to
334 high temperatures tend to have reduced lipid content in different body compartments, as
335 observed in rainbow trout (Ingemansson et al., 1993) and Atlantic salmon (Jobling and
336 Bendiksen, 2003). Additionally, no genetic correlation was found between resistance to acute
337 hyperthermia and fat meter measurements in a commercial population of rainbow trout
338 (Lagarde et al., 2023a). These findings suggest that increased resistance to hyperthermia in
339 the FL due to physical insulation is unlikely.

340 The LL and FL lines also differ in their energy metabolism. Several studies showed that the
341 FL had a higher capability to use glucose than the LL, linked to the enhancement of hepatic
342 glycolysis, glycogen storage, and lipogenesis (Kolditz et al., 2008a; Skiba-Cassy et al., 2009;
343 Kamalam et al., 2012; Jin et al., 2014). It is, therefore, possible that this ability to rapidly
344 mobilise energy improves the resistance of this line to acute hyperthermia. However,
345 additional studies that monitor plasma parameters during hyperthermia stress are necessary to
346 test this hypothesis.

347 **4. Conclusion**

348 The cold storage of rainbow trout eyed eggs for 15 days did not result in drastic changes in
349 early zootechnical performance. The responses to the acute confinement challenge after a 1-
350 hour recovery period and to the hyperthermia challenge were not significantly influenced by
351 the incubation temperature. Additionally, we demonstrated that the two lines exhibited
352 differential responses to hyperthermia challenges. Based on this information, the practice of
353 cold storage of eyed eggs does not appear to be detrimental to subsequent rearing, at least up
354 to the juvenile stage.

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358 **References**

- 359 Alexander, C.M., Kasza, I., Yen, C.L.E., Reeder, S.B., Hernando, D., Gallo, R.L., Jahoda,
360 C.A.B., Horsley, V., MacDougald, O.A., 2015. Dermal white adipose tissue: A new
361 component of the thermogenic response. *J. Lipid Res.* 56, 2061–2069.
362 <https://doi.org/10.1194/jlr.R062893>
- 363 Auperin, B., Geslin, M., 2008. Plasma cortisol response to stress in juvenile rainbow trout is
364 influenced by their life history during early development and by egg cortisol content.
365 *Gen. Comp. Endocrinol.* 158(3), 234–239. <https://doi.org/10.1016/j.ygcen.2008.07.002>
- 366 Babiak, I., Dabrowski, K., 2003. Refrigeration of rainbow trout gametes and embryos. *J. Exp.*
367 *Zool. Part A Comp. Exp. Biol.* 300, 140–151. <https://doi.org/10.1002/jez.a.10319>
- 368 Barton, K., 2020. MuMIn: Multi-model inference. version 1.43.17. [https://CRAN.R-](https://CRAN.R-project.org/package=MuMIn)
369 [project.org/package=MuMIn](https://CRAN.R-project.org/package=MuMIn)
- 370 Barton, B.A., 2002. Stress in fishes: A diversity of responses with particular reference to
371 changes in circulating corticosteroids. *Integr. Comp. Biol.* 42, 517–525.
372 <https://doi.org/10.1093/icb/42.3.517>
- 373 Barton, B.A., Iwama, G.K., 1991. Physiological changes in fish from stress in aquaculture
374 with emphasis on the response and effects of corticosteroids. *Annu. Rev. Fish Dis.* 1, 3–
375 26. [https://doi.org/10.1016/0959-8030\(91\)90019-G](https://doi.org/10.1016/0959-8030(91)90019-G)
- 376 Bromage, N., Randall, C., Davies, B., Thrush, M., Duston, J., Carillo, M., Zanuy, S., 1993.
377 Photoperiodism and the control of reproduction and development in farmed fish, in:
378 Lahlou, B., Vitiello, P. (Eds.), *Aquaculture: Fundamental and Applied Research*.
379 American Geophysical Union, Washington, pp. 81–102.
- 380 Bromage, N., Whitehead, C., Elliott, J., Breton, B., Matty, A., 1982. Investigations in the
381 importance of daylength on the photoperiodic control of reproduction in the female
382 rainbow trout, in: Richter, C.J.J., Goos, H.J.T. (Eds.), *Proceedings of the International*
383 *Symposium on Reproductive Physiology of Fish*. Centre for Agricultural Publishing and
384 Documentation, Wageningen, pp. 233–236.
- 385 Desforges, J.E., Birnie-Gauvin, K., Jutfelt, F., Gilmour, K.M., Eliason, E.J., Dressler, T.L.,
386 McKenzie, D.J., Bates, A.E., Lawrence, M.J., Fangue, N., Cooke, S.J., 2023. The
387 ecological relevance of critical thermal maxima methodology for fishes. *J. Fish Biol.*
388 1000–1016. <https://doi.org/10.1111/jfb.15368>
- 389 Ern, R., Andreassen, A.H., Jutfelt, F., 2023. Physiological mechanisms of acute upper thermal
390 tolerance in fish. *Physiology* 38, 141–158. <https://doi.org/10.1152/physiol.00027.2022>
- 391 FAO, 2023. FishstatJ - Software for Fishery and Aquaculture Statistical Time Series. Food
392 and Agriculture Organization, Roma
- 393 Fry, F.E.J., 1971. The effect of environmental factors on the physiology of fish, in: Hoar,
394 W.S., Randall, D.J., Brett, J.R. (Eds.), *Fish Physiology*. Academic Press, New-York, pp.
395 1–98.
- 396 Gesto, M., López-Patiño, M.A., Hernández, J., Soengas, J.L., Míguez, J.M., 2015. Gradation
397 of the stress response in rainbow trout exposed to stressors of different severity: The role
398 of brain serotonergic and dopaminergic systems. *J. Neuroendocrinol.* 27, 131–141.
399 <https://doi.org/10.1111/jne.12248>

- 400 Ingemansson, T., Olsson, N.U., Kaufmann, P., 1993. Lipid composition of light and dark
401 muscle of rainbow trout (*Oncorhynchus mykiss*) after thermal acclimation: A multivariate
402 approach. *Aquaculture* 113, 153–165. [https://doi.org/10.1016/0044-8486\(93\)90348-3](https://doi.org/10.1016/0044-8486(93)90348-3)
- 403 Jentoft, S., Aastveit, A.H., Torjesen, P.A., Andersen, Ø., 2005. Effects of stress on growth,
404 cortisol and glucose levels in non-domesticated Eurasian perch (*Perca fluviatilis*) and
405 domesticated rainbow trout (*Oncorhynchus mykiss*). *Comp. Biochem. Physiol. - A Mol.*
406 *Integr. Physiol.* 141, 353–358. <https://doi.org/10.1016/j.cbpb.2005.06.006>
- 407 Jin, J., Panserat, S., Kamalam, B.S., Aguirre, P., Véron, V., Médale, F., 2014. Insulin
408 regulates lipid and glucose metabolism similarly in two lines of rainbow trout divergently
409 selected for muscle fat content. *Gen. Comp. Endocrinol.* 204, 49–59.
410 <https://doi.org/10.1016/j.ygcen.2014.04.027>
- 411 Jobling, M., Bendiksen, E.Å., 2003. Dietary lipids and temperature interact to influence tissue
412 fatty acid compositions of Atlantic salmon, *Salmo salar* L., parr. *Aquac. Res.* 34, 1423–
413 1441. <https://doi.org/10.1111/j.1365-2109.2003.00970.x>
- 414 Kamalam, B.S., Medale, F., Kaushik, S., Polakof, S., Skiba-Cassy, S., Panserat, S., 2012.
415 Regulation of metabolism by dietary carbohydrates in two lines of rainbow trout
416 divergently selected for muscle fat content. *J. Exp. Biol.* 215, 2567–2578.
417 <https://doi.org/10.1242/jeb.070581>
- 418 Kolditz, C.-I., Borthaire, M., Richard, N., Corraze, G., Panserat, S., Vachot, C., Lefèvre, F.,
419 Médale, F., 2008a. Liver and muscle metabolic changes induced by dietary energy
420 content and genetic selection in rainbow trout (*Oncorhynchus mykiss*). *Am. J. Physiol.*
421 *Integr. Comp. Physiol.* 294, 1154–1164. <https://doi.org/10.1152/ajpregu.00766.2007>
- 422 Kolditz, C.-I., Paboeuf, G., Borthaire, M., Esquerré, D., SanCristobal, M., Lefèvre, F.,
423 Médale, F., 2008b. Changes induced by dietary energy intake and divergent selection for
424 muscle fat content in rainbow trout (*Oncorhynchus mykiss*), assessed by transcriptome
425 and proteome analysis of the liver. *BMC Genomics* 9, 506. <https://doi.org/10.1186/1471-2164-9-506>
- 427 Kolditz, C.I., Plagnes-Juan, E., Quillet, E., Lefèvre, F., Médale, F., 2010. Changes in white
428 muscle transcriptome induced by dietary energy levels in two lines of rainbow trout
429 (*Oncorhynchus mykiss*) selected for muscle fat content. *Br. J. Nutr.* 103, 629–642.
430 <https://doi.org/10.1017/S0007114509992340>
- 431 Komrakova, M., Holtz, W., 2009. Factors responsible for successful chilled storage of
432 unfertilized rainbow trout (*Oncorhynchus mykiss*) eggs. *Aquaculture* 286, 156–163.
433 <https://doi.org/10.1016/j.aquaculture.2008.09.019>
- 434 Lagarde, H., Lallias, D., Patrice, P., Dehaullon, A., Prchal, M., François, Y., D'Ambrosio, J.,
435 Segret, E., Acin-Perez, A., Cachelou, F., Haffray, P., Dupont-Nivet, M., Phocas, F.,
436 2023a. Genetic architecture of acute hyperthermia resistance in juvenile rainbow trout
437 (*Oncorhynchus mykiss*) and genetic correlations with production traits. *Genet. Sel. Evol.*
438 55, 1–21. <https://doi.org/10.1186/s12711-023-00811-4>
- 439 Lagarde, H., Phocas, F., Pouil, S., Goardon, L., Bideau, M., Guyvarc'h, F., Labbé, L.,
440 Dechamp, N., Prchal, M., Dupont-Nivet, M., Lallias, D., 2023b. Are resistances to acute
441 hyperthermia or hypoxia stress similar and consistent between early and late ages in
442 rainbow trout using isogenic lines? *Aquaculture* 562, 738800.
443 <https://doi.org/10.1016/j.aquaculture.2022.738800>

- 444 Lefevre, S., Wang, T., McKenzie, D.J., 2021. The role of mechanistic physiology in
445 investigating impacts of global warming on fishes. *J. Exp. Biol.* 224, 238840.
446 <https://doi.org/10.1242/jeb.238840>
- 447 Leveroni Calvi, S., Maise, G., 1998. Cryopreservation of rainbow trout (*Oncorhynchus*
448 *mykiss*) blastomeres: Influence of embryo stage on postthaw survival rate. *Cryobiology*
449 36, 255–262. <https://doi.org/10.1006/cryo.1998.2084>
- 450 Maddock, B., 1974. A technique to prolong the incubation period of brown trout ova.
451 *Progress. Fish-Culturist* 36, 219–222. [https://doi.org/10.1577/1548-](https://doi.org/10.1577/1548-8659(1974)36[219:ATTPTI]2.0.CO;2)
452 [8659\(1974\)36\[219:ATTPTI\]2.0.CO;2](https://doi.org/10.1577/1548-8659(1974)36[219:ATTPTI]2.0.CO;2)
- 453 Magnoni, L.J., Novais, S.C., Eding, E., Leguen, I., Lemos, M. F., Ozório, R.O., Geurden, I.,
454 Prunet, P., Schrama, J.W., 2019. Acute stress and an electrolyte-imbalanced diet, but not
455 chronic hypoxia, increase oxidative stress and hamper innate immune status in a rainbow
456 trout (*Oncorhynchus mykiss*) isogenic line. *Front. Physiol.* 10, 453.
457 <https://doi.org/10.3389/fphys.2019.00453>
- 458 Mateus, A.P., Costa, R.A., Cardoso, J.C.R., Andree, K.B., Estévez, A., Gisbert, E., Power,
459 D.M., 2017. Thermal imprinting modifies adult stress and innate immune responsiveness
460 in the teleost sea bream. *J. Endocrinol.* 233, 381–394. [https://doi.org/10.1530/JOE-16-](https://doi.org/10.1530/JOE-16-0610)
461 [0610](https://doi.org/10.1530/JOE-16-0610)
- 462 McKenzie, D.J., Zhang, Y., Eliason, E.J., Schulte, P.M., Claireaux, G., Blasco, F.R., Nati,
463 J.J.H., Farrell, A.P., 2021. Intraspecific variation in tolerance of warming in fishes. *J.*
464 *Fish Biol.* 98, 1536–1555. <https://doi.org/10.1111/jfb.14620>
- 465 Mommsen, T.P., Vijayan, M.M., Moon, T.W., 1999. Cortisol in teleosts: Dynamics,
466 mechanisms of action, and metabolic regulation. *Rev. Fish Biol. Fish.* 9, 211–268.
467 <https://doi.org/10.1023/A:1008924418720>
- 468 Nakagawa, S., Schielzeth, H., 2013. A general and simple method for obtaining R² from
469 generalized linear mixed-effects models. *Methods Ecol. Evol.* 4, 133–142.
470 <https://doi.org/10.1111/j.2041-210x.2012.00261.x>.
- 471 Niksirat, H., Sarvi, K., Amiri, B. M., & Hatef, A., 2007. Effects of storage duration and
472 storage media on initial and post-eyeing mortality of stored ova of rainbow trout
473 *Oncorhynchus mykiss*. *Aquaculture*, 262(2-4), 528–531.
474 <https://doi.org/10.1016/j.aquaculture.2006.10.031>
- 475 Perry, G.M.L., Martyniuk, C.M., Ferguson, M.M., Danzmann, R.G., 2005. Genetic
476 parameters for upper thermal tolerance and growth-related traits in rainbow trout
477 (*Oncorhynchus mykiss*). *Aquaculture* 250, 120–128.
478 <https://doi.org/10.1016/j.aquaculture.2005.04.042>
- 479 Pickering, A.D., 1981. *Stress and Fish*. Academic Press, New-York
- 480 Pickering, A.D., Pottinger, T.G., Sumpter, J.P., Carragher, J.F., Le Bail, P.Y., 1991. Effects of
481 acute and chronic stress on the levels of circulating growth hormone in the rainbow trout,
482 *Oncorhynchus mykiss*. *Gen. Comp. Endocrinol.* 83, 86–93. [https://doi.org/10.1016/0016-](https://doi.org/10.1016/0016-6480(91)90108-I)
483 [6480\(91\)90108-I](https://doi.org/10.1016/0016-6480(91)90108-I)
- 484 Piper, R.G., McElwain, I.B., Orme, L.E., McCraren, J.P., Fowler, L.G., Leonard, J.R., 1982.
485 *Fish Hatchery Management*, U.S. Department of the Interior, U.S. Fish and Wildlife
486 Service, Washington

- 487 Pottinger, T.G., Carrick, T.R., 1999. Modification of the plasma cortisol response to stress in
488 rainbow trout by selective breeding. *Gen. Comp. Endocrinol.* 116, 122–132.
489 <https://doi.org/10.1006/gcen.1999.7355>
- 490 Quillet, E., Le Guillou, S., Aubin, J., Fauconneau, B., 2005. Two-way selection for muscle
491 lipid content in pan-size rainbow trout (*Oncorhynchus mykiss*). *Aquaculture* 245, 49–61.
492 <https://doi.org/10.1016/j.aquaculture.2004.12.014>
- 493 Quillet, E., Le Guillou, S., Aubin, J., Labbé, L., Fauconneau, B., Médale, F., 2007. Response
494 of a lean muscle and a fat muscle rainbow trout (*Oncorhynchus mykiss*) line on growth,
495 nutrient utilization, body composition and carcass traits when fed two different diets.
496 *Aquaculture* 269, 220–231. <https://doi.org/10.1016/j.aquaculture.2007.02.047>
- 497 R Development Core Team, 2022. R: A Language and Environment for Statistical
498 Computing. The R Project for Statistical Computing, Vienna
- 499 Richardson, G.F., Gardiner, Y.T., McNiven, M.A., 2002. Preservation of rainbow trout
500 (*Oncorhynchus mykiss*) eyed eggs using a perfluorochemical as an oxygen carrier.
501 *Theriogenology* 58, 1283–1290. [https://doi.org/10.1016/s0093-691x\(02\)00955-x](https://doi.org/10.1016/s0093-691x(02)00955-x)
- 502 Sadoul, B., Leguen, I., Colson, V., Friggens, N.C., Prunet, P., 2015. A multivariate analysis
503 using physiology and behavior to characterize robustness in two isogenic lines of
504 rainbow trout exposed to a confinement stress. *Physiol. Behav.* 140, 139–147.
505 <https://doi.org/10.1016/j.physbeh.2014.12.006>
- 506 Schreck, C.B., Tort, L., 2016. The concept of stress in fish, in: Schreck, C.B., Tort, L., Farrell,
507 A.P., Brauner, C.J. (Eds.), *Biology of Stress in Fish*. Elsevier, Amsterdam, pp. 1–34.
- 508 Seibel, H., Baßmann, B., Rebl, A., 2021. Blood will tell: What hematological analyses can
509 reveal about fish welfare. *Front. Vet. Sci.* 8, 616955.
510 <https://doi.org/10.3389/fvets.2021.616955>
- 511 Scott, G.R., Johnston, I.A., 2012. Temperature during embryonic development has persistent
512 effects on thermal acclimation capacity in zebrafish. *Proc. Natl. Acad. Sci. U. S. A.* 109,
513 14247–14252. <https://doi.org/10.1073/pnas.1205012109>
- 514 Skiba-Cassy, S., Lansard, M., Panserat, S., Médale, F., 2009. Rainbow trout genetically
515 selected for greater muscle fat content display increased activation of liver TOR
516 signaling and lipogenic gene expression. *Am. J. Physiol. - Regul. Integr. Comp. Physiol.*
517 297, 1421–1429. <https://doi.org/10.1152/ajpregu.00312.2009>
- 518 Stonecypher, R.W., Hubert, H.A., Gern, W.A., 1994. Effect of reduced incubation
519 temperatures on survival of trout embryos. *Progress. Fish-Culturist* 56, 180–184.
520 [https://doi.org/10.1577/1548-8640\(1994\)056<0180:EORITO>2.3.CO;2](https://doi.org/10.1577/1548-8640(1994)056<0180:EORITO>2.3.CO;2)
- 521 Trenzado, C.E., Carrick, T.R., Pottinger, T.G., 2003. Divergence of endocrine and metabolic
522 responses to stress in two rainbow trout lines selected for differing cortisol
523 responsiveness to stress. *Gen. Comp. Endocrinol.* 133(3), 332–340.
524 [https://doi.org/10.1016/S0016-6480\(03\)00191-6](https://doi.org/10.1016/S0016-6480(03)00191-6)
- 525 Ubilla, A., Valdebenito, I., Árias, M.E., Risopatrón, J., 2016. Viability and DNA
526 fragmentation of rainbow trout embryos (*Oncorhynchus mykiss*) obtained from eggs
527 stored at 4 °C. *Theriogenology* 85, 1499–1506.
528 <http://doi.org/10.1016/j.theriogenology.2016.01.012>
- 529 Wedemeyer, G.A., Barton, B.A., McLeay, D.J., 1990. Stress and acclimation, in: Schreck,
530 C.B., Moyle, P.B. (Eds.), *Methods for Fish Biology*. Bethesda, Maryland, pp. 451–489.

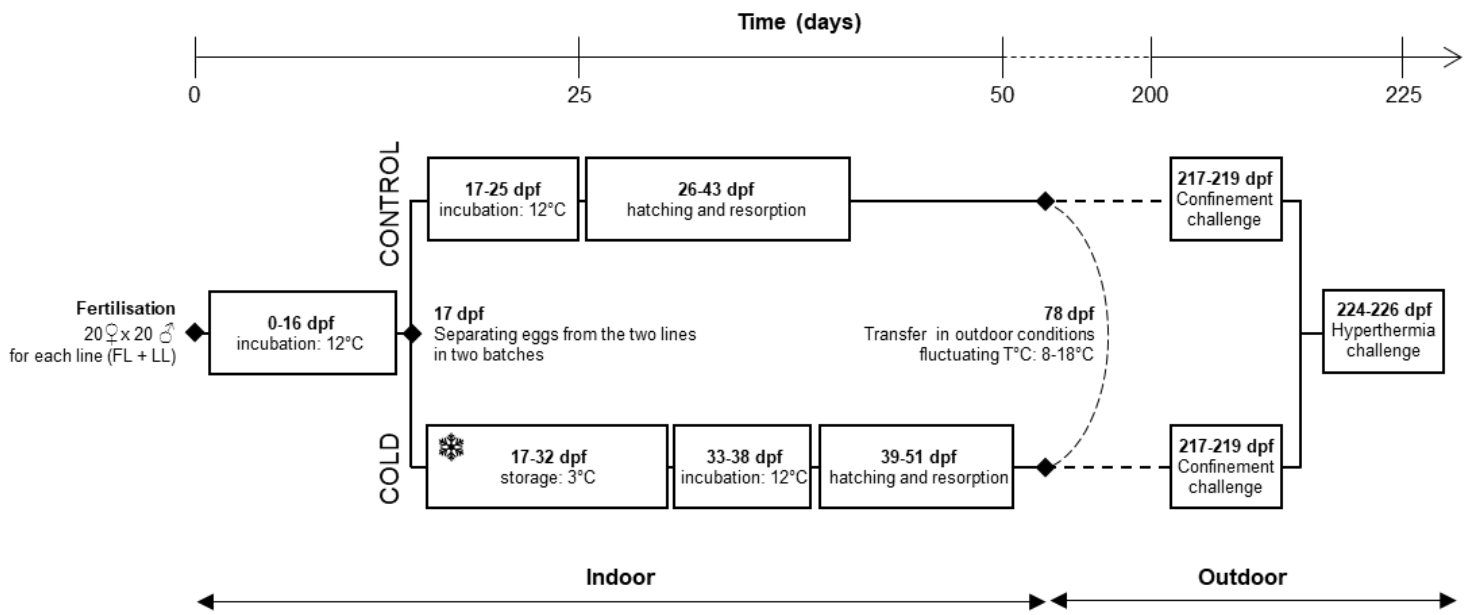
531 Whitehead, C., Bromage, N.R., 1978. The effects of alterations in photoperiod on ovarian
532 development and spawning time in the rainbow trout (*Salmo gairdneri*). Ann. Biol.
533 Anim. Biochim. Biophys. 18, 1035–1043. <https://doi.org/10.1051/rnd:19780543>
534

535 **Captions to figures**

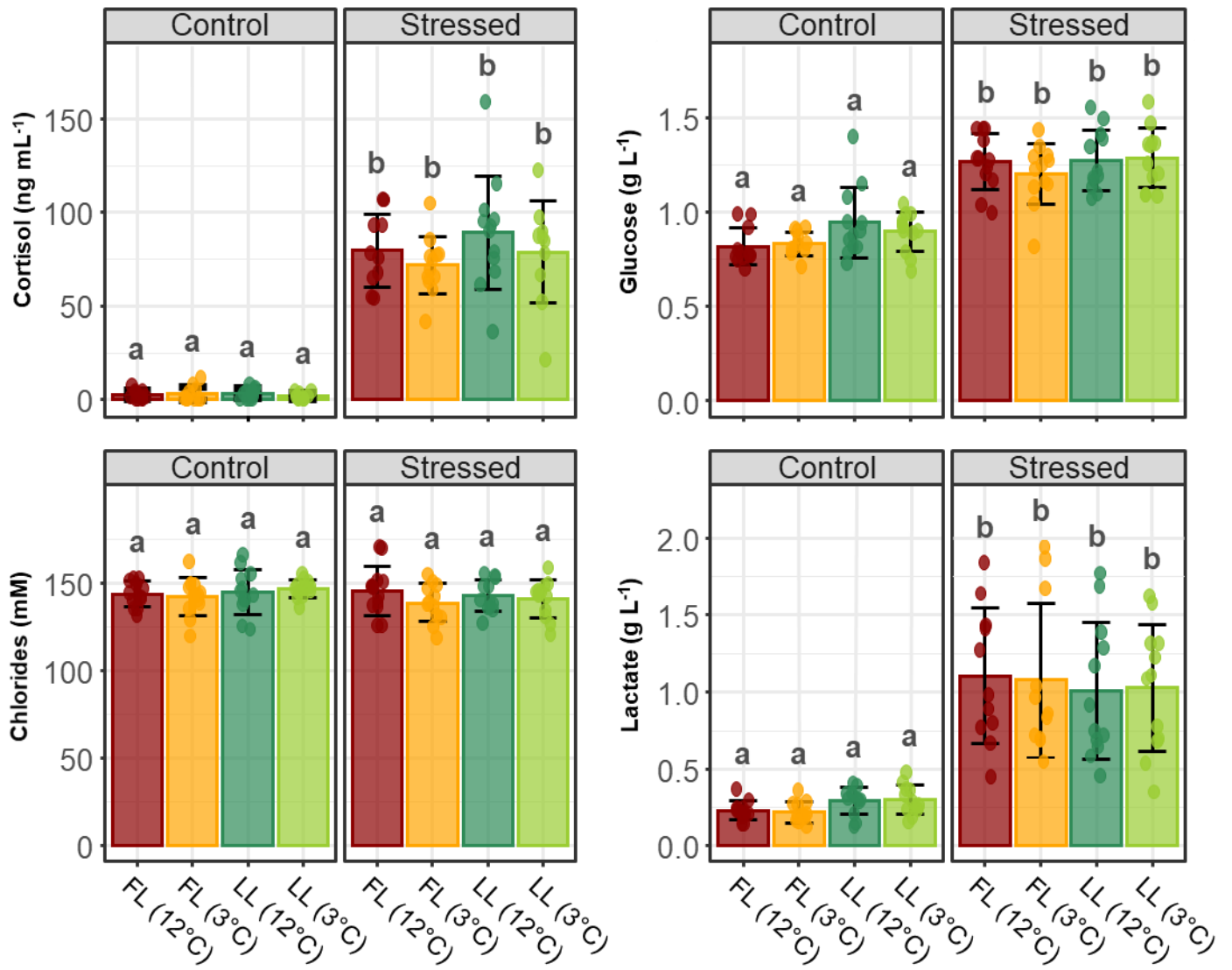
536 Figure 1. Schematic view of the experimental protocol (FL: fat line, LL: lean line,
537 CONTROL: eyed eggs kept at 12°C, COLD: eyed eggs stored at 3°C for 15 days, dpf: days
538 post fertilisation).

539 Figure 2. Cortisol, glucose, chloride ions, and lactate concentrations in plasma collected in
540 juvenile fish before (Control, n = 12) and 1h after the confinement challenge (Stressed, n =
541 12) from the two lines (FL: fat line and LL: lean line) kept at two incubation temperatures at
542 the eyed egg stage (3 or 12°C). Different letters denote significant differences ($P < 0.05$).

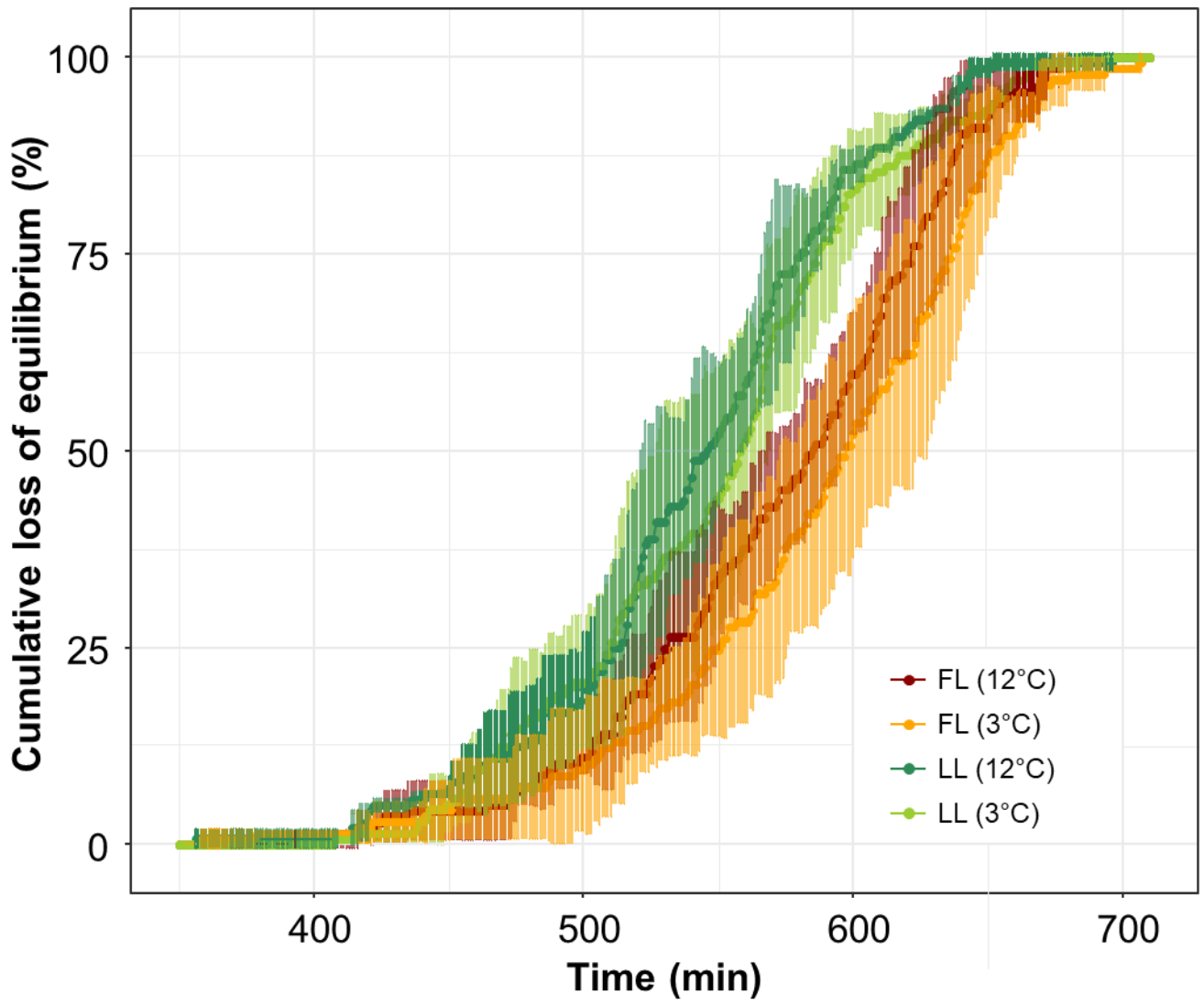
543 Figure 3. Kinetics of cumulative loss of equilibrium in juvenile fish from the two lines (FL:
544 fat line and LL: lean line) kept at two incubation temperatures at the eyed egg stage (3 or
545 12°C). Values are means \pm SD from the three replicates.



546 Figure 1



547 Figure 2



548 Figure 3

549 Table 1. Zootechnical performances of the fish from the different experimental conditions
 550 (lines and incubation temperatures). Data are expressed as means \pm SD. Letters denote
 551 significant differences.

Parameter	n per condition	Fat line (FL)		Lean Line (LL)	
		12°C	3°C	12°C	3°C
<i>Indoor rearing</i>					
Deformity rate (%)	3	5.3 \pm 2.1 ^A	4.5 \pm 0.8 ^A	5.5 \pm 1.1 ^A	9.6 \pm 2.6 ^A
Survival rate (%)	3	89.9 \pm 3.2 ^A	87.8 \pm 5.8 ^A	91.4 \pm 4.6 ^A	86.9 \pm 2.7 ^A
<i>Outdoor rearing</i>					
Survival rate (%)	3	94.4 \pm 1.2 ^A	89.8 \pm 3.6 ^{AB}	92.5 \pm 2.3 ^{AB}	80.8 \pm 3.3 ^B
Weight at 190 dpf (g)	353-459	35.3 \pm 11.1 ^A	29.2 \pm 9.8 ^B	40.4 \pm 11.5 ^C	35.2 \pm 12.0 ^A

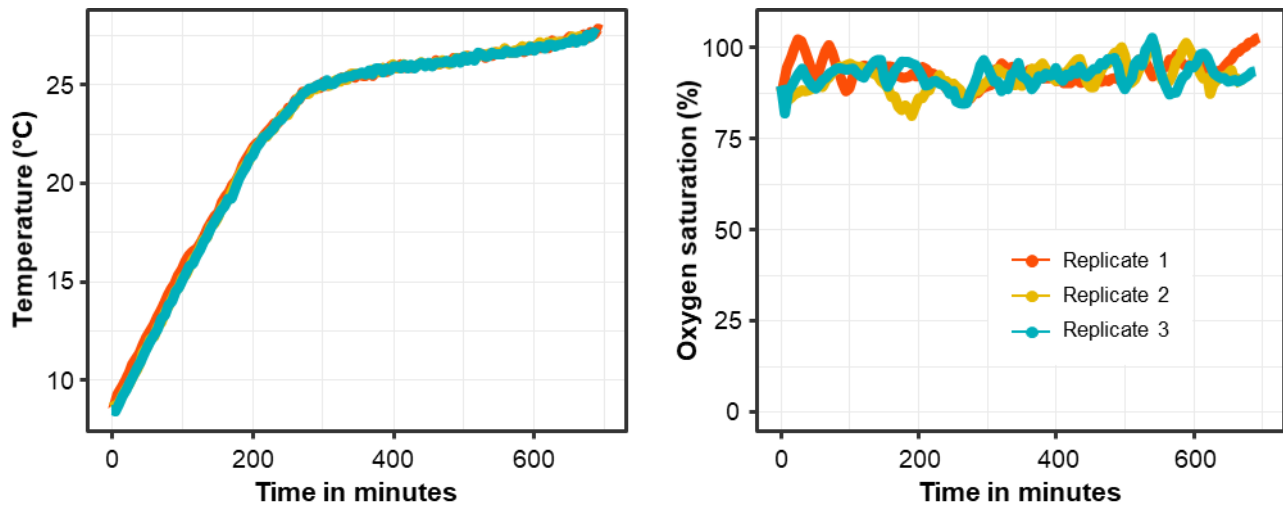
552

553 Table 2. Weights of the fish from the different experimental conditions (lines and incubation
 554 temperatures) sampled during the confinement and hyperthermia challenges. Data are

Challenge	Line	Incubation temperature	n	Weight (g)	555	556	557	558	559	560	561
Confinement	FL	12°C	24	57.3 ± 9.6 ^A							
	FL	3°C	24	57.2 ± 13.7 ^A							
	LL	12°C	24	62.1 ± 9.6 ^A							
	LL	3°C	24	54.5 ± 8.4 ^A							
Hyperthermia	FL	12°C	135	57.8 ± 13.4 ^A							
	FL	3°C	139	49.0 ± 12.0 ^B							
	LL	12°C	139	66.0 ± 16.9 ^C							
	LL	3°C	137	59.8 ± 15.0 ^A							

562 ers denote significant differences.

563 FL: fat line, LL: lean line



565 Figure S1. Temperature and O₂ saturation over the hyperthermia challenges for the three
566 replicates.