Impact of early cold temperature on response to acute confinement and temperature challenges in juvenile Rainbow trout divergent lines for muscle fat content

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IMPACT OF EARLY COLD TEMPERATURE ON RESPONSE TO ACUTE CONFINEMENT AND TEMPERATURE CHALLENGES IN JUVENILE RAINBOW TROUT DIVERGENT LINES FOR MUSCLE FAT CONTENT.

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Introduction

To expand the availability of marketable eyed-eggs, a common practice in rainbow trout aquaculture is to store eyed-eggs at low temperature (2-4°C) for periods of up to 2-3 weeks. Recent studies have shown that early exposure to environmental stimuli (such as hypoxia or temperature) could impact fish physiology, growth, metabolism and nutrition at mid-or long-term questioning about the potential effects of such breeder practice. This study aimed to test the impact of incubating rainbow trout eyed-eggs at low temperature (3°C instead of 11°C) for 15 days on resistance to later stresses (acute temperature and confinement). Two experimental lines divergent for muscle lipid content (Quillet et al. 2005) that have been shown to utilise feed differently and to possess a well-differentiated intermediary and energetic metabolism (Kolditz et al., 2008) have been used. Our hypothesis is that they will react differently to the cold exposure during incubation.

Materials and methods

At 17 days post fertilization (dpf), eyed-eggs from two experimental lines selected for high or low muscle lipid content, fat line (FL) and lean line (LL), were either incubated at standard temperature (11°C) or incubated at 3°C for 15 days in 12 tanks (2 lines x 2 incubation temperatures x 3 tanks). From hatching to the beginning of the experiments, consisting of confinement and temperature challenges, fish were kept at 11°C.
Confinement challenges were performed at 217-219 dpf (12 tests in total, one per tank), by increasing fish density to 200 kg/m^3 for 4 minutes with no water renewal or oxygen supplementation. Four fish per tank were sampled for blood before the confinement stress (control condition) and 4 other fish per tank 1h after the confinement stress (stressed condition). Blood was taken from the caudal vein using heparinized syringes and then centrifuged. Plasma samples were stored at -20°C prior to analysis. Plasma cortisol was determined using the Neogen cortisol in saliva ELISA kit. Plasma glucose and lactate levels were assessed using Accu-Chek® Mobile (Roche) and The Edge™ Analyzer (Apexbio) systems, respectively. Chloride ions levels were assayed using a colorimetric method (Biolabo SAS). Statistical analyses were performed with linear mixed models using nlme package in R, with treatment (control or stressed), line and temperature as fixed effects and weight as a covariate when it significantly affected the response variable while tank was included as a random effect.
Acute temperature challenges were performed at 224-226 dpf. About one month before the challenges, fish were individually PIT-tagged and grouped into 3 separate tanks containing each 200 fish, i.e. 50 fish per line and per incubation temperature. The three temperature challenges (one per tank) were performed on three successive days. Fish were transferred into the challenge tank the day before the challenge for acclimation. Water temperature was increased progressively, about 0.7°C every 10 minutes until 22°C, then 0.1°C every 15 minutes. Water was oxygenated to keep oxygen levels near saturation. Fish were removed from the tank after the loss of equilibrium, weighed and their tag recorded alongside the time at loss of equilibrium. Kaplan-Meier curves and survival analysis were performed using the survival package in R, while differences

between lines and temperature were assessed using linear mixed models fitted using nlme package, with line and temperature as fixed effects and weight as a covariate while tank was included as a random effect.

Results

As expected, there was a significant increase of plasma cortisol levels after confinement challenges ($p<0.001$); however, there was no significant effect of the genetic line or incubation temperature (Figure 1A). For plasma glucose and lactate levels, there was a significant effect of the confinement challenge ($p<0.001$); the genetic line had a significant effect only for glucose ($p=0.015$). There were no significant differences in chloride ion levels.
For acute temperature challenges, Kaplan-Meier curves are shown in Fig. 1B. There was a significant effect of the genetic line ($p<0.001$) and incubation temperature ($p= 0.036$), with FL line and fish incubated at 3°C resisting longer.

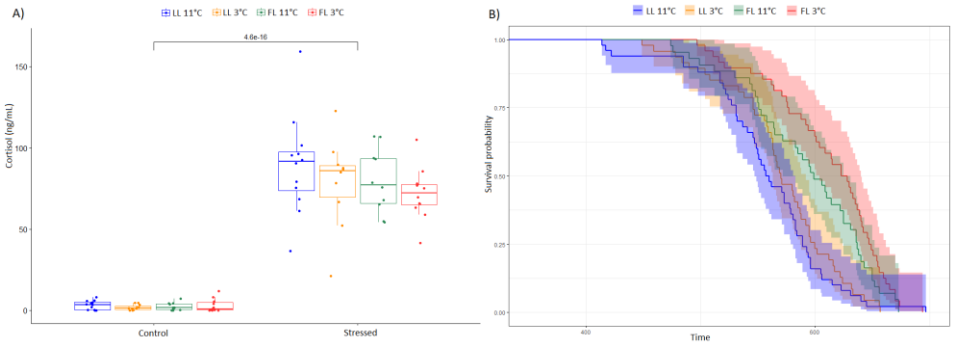

Figure 1. A) Plasma cortisol concentrations before (control) and 1H after (stressed) confinement challenges. B) Kaplan-Meir curves for acute temperature challenges.

Discussion

Using two divergent lines for muscle fat content allowed for testing the impact of the genetic background on the observed responses. As expected, the two lines differently responded to the confinement and temperature challenges. Preliminary results suggest that the incubation temperature did not impair the responses of the two genetic lines to confinement and temperature challenges performed on 7-month-old juveniles. Based on this information, the practice of cold storage of eyed eggs does not appear to be detrimental to subsequent rearing. This study will contribute to the use of early programming as a lever to improve the long-term performances of animals.

Acknowledgements

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