Proceedings, 10th World Congress of Genetics Applied to Livestock Production

Genetic analysis of resistance to acute or chronic temperature stress using isogenic lines of Rainbow trout (*Oncorhynchus mykiss*)

M. Dupont-Nivet[1], **M.Crusot**[1], **D. Rigaudeau**[2], **E. Quillet**[1]
[1]INRA, UMR 1313 GABI, Jouy en Josas, France, [2] INRA, UE 0907 IERP, Jouy en Josas, France.

ABSTRACT: Three chronic temperature conditions were applied to ten isogenic lines of Rainbow trout (aged ~6 months): 12°C (L), 20 °C (H) and a fluctuating one (F): 12°-20°C-12°C every day. Survival and growth were individually monitored during 7 weeks to assess acclimatization to chronic stress. Acute temperature stress were also applied at the end of the period. Survival was high in all groups while growth was significantly lower in F and H groups. Significant genetic variability was evidenced for growth *ie* acclimatization to chronic stress as well as significant genetic-temperature interactions. Significant genetic variability was found for resistance to acute stress with significant genetic-rearing temperature interactions. However some lines were found resistant or sensible whatever the rearing temperature. Finally, no significant correlation was found between responses to chronic stress and acute stress which will complicate introduction of such traits in breeding programs.
Keywords: Genetic variability; Genetic correlation; Thermal stress; Rainbow trout

Introduction

Global climatic change will have many impacts on aquaculture production. The most important ones are changes in water quality since fish live in tight relation with water. Fish are poïkilotherm and thus highly sensitive to temperature changes. But evolution of oxygen availability, pH, mud presence will also occur. However, to preserve water resources, it is expected that fish farms will move towards recirculated systems. In this kind of systems, water quality can be handled through technical ways. But temperature will still be difficult to monitor unless using solutions very costly in terms of energy. Rainbow trout is adapted to cold water but with high plasticity. In general trout seems to be able to adapt to temperatures in the range 0-22°C provided the fish are supplied with well oxygen saturated water (Ihsses, 1986). Different results concerning temperature optimum for growth are reported: 10-16° C or 16-18°C (Jobling, 1984; Aupérin and Boeuf, 2010) may be because of different origins of the population studied. Moreover, temperature may cause problems in trout aquaculture when it is too high but also in case of sudden changes. Thus adaptation to high or fluctuating temperatures is a key issue in aquaculture of Rainbow trout, (*Oncorhynchus mykiss*).

Genetics of thermotolerance has been seldom studied in rainbow trout. Molony et al. (2004) have shown that a domesticated line in Australia (thus used to relatively hot water) is more tolerant to acute high temperature stress than a naturalised population which suggests existence of genetic variability for heat tolerance. Genetic parameters of heat tolerance have been estimated in a US line of rainbow trout and a QTL was found (Perry et al, 2001; Perry et al, 2005). However this concerns only one line and especially only acute stress *ie* rapid response to quick and high temperature increase. Genetics of acclimatization to chronic high -but still allowing growth- temperature and/or fluctuating temperature is still needed.

The study reported here studied the genetic variability of acclimatization to high or fluctuating temperatures and of resistance to acute temperature stress. It focused also on the correlation between both the kinds of resistance. This will determine if selection, *ie* long term adaptation to temperatures changes, is possible in Rainbow trout.

Materials and Methods

Animal material. Ten isogenic lines of Rainbow trout were used in this experiment. Within each line, all fish have the same genome. They were produced through the matings of homozygous fish previously obtained after several generations of gynogenesis. These homozygous lines are stabilized at INRA and have been shown to exhibit large genetic variability (Quillet et al., 2007; Dupont-Nivet et al., 2009). The ten heterozygous isogenic lines were obtained by mating homozygous female from one isogenic line with ten individual homozygous males from ten other isogenic lines. Ova were collected from different females from the same isogenic line to produce enough fish. In doing so, only one maternal line (*ie* same genome) was used to avoid initial maternal effects associated with egg size and hatching time.

These lines were reared separately in duplicates until six months. Between 4 and 5 months, fish were individually tagged with pit tags.

Acclimatization to different temperatures. From six months old, fish were randomly dispatched in three different temperature regimes: two with constant temperature, 12 (L) or 20 °C (H) and one with a fluctuating temperature (F): temperature increasing from 12 to 20 °C and decreasing again to 12°C every day. For each temperature regime, three replicates were reared. Each replicate was composed of 55-60 fish from each of the ten lines. Fish were reared in recirculated system in 0.25 m^3 ewos and fed twice a day.

Fish were individually weighed at the beginning of the experiment, and after 5 and 7 weeks. As mean weight was different for the lines at the beginning of the experiment, mean weight of each line in H or F groups was also expressed as a ratio to mean weight of the same line in L group (WR in %)

Acute temperature stress. At the end of the growth study, each group was submitted to an acute temperature stress. Water temperature was raised from initial temperature to 22 °C rapidly (+ 0.6°/10 min) and then to 25 °C, less rapidly (+ 0.2°C/10 min). From 25 °C, the temperature was raised very slowly. Oxygen level was maintained over 4.5 mg/ml with bubblers. Fish were removed when they lost equilibrium for more than 5 seconds and replaced in current water. Time at equilibrium lost was noted down. To take into account differences between trials of temperature kinetics during the stress, upper thermal tolerance (UTT) was also calculated following Perry et al. (2001). UTT is cumulative exposure in degree.minutes and calculated by summing all differences between the rearing temperature and the experimental temperature at each minute until fish lost equilibrium.

Statistical analyses. UTT was analysed using a mixed model including temperature, line and replicate effects. WR was analysed with the same model without replicate effects. Since temperature-line interactions were significant, separate analysis for each temperature were carried out with the same models without temperature effect. Pearson correlations for mean data for each line were calculated between temperature groups to quantify importance of the interaction temperature*line.

Pearson correlations were also estimated between the different traits.

Results and Discussion

Growth at different temperatures. Survival was higher than 90 % in all groups. Growth was significantly ($P< 0.05$) lower for H and F groups than for L group: mean weights at the end of the growth experiment were 9.36, 10.51 and 13. 41 g. respectively. Figure 1 shows the WR ratio for the different lines and the different temperatures at the end of the growth experiment. All lines exhibited decreased growth in H and F groups, except line A22. Statistical analysis showed significant temperature and line effects ($P< 0.05$) and significant temperature*line interaction. Correlation between mean WR of each line was 0.41 between L and H, 0.57 between L and F and 0.94 between H and F showing high differences of lines ranking between L group and others. Analysis within each temperature regime showed significant differences between lines *ie* the existence of genetic variability for acclimatization to temperature.

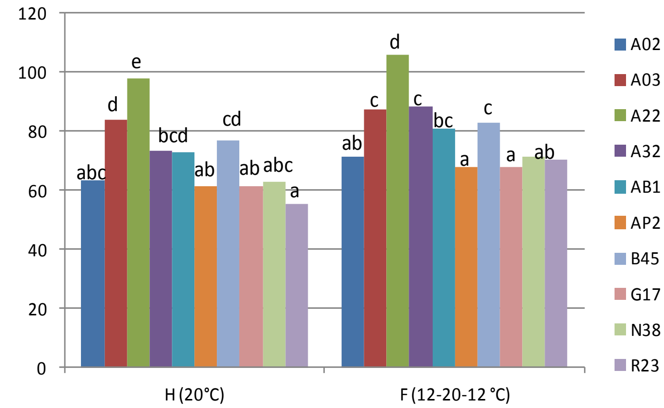

Figure 1. Weight ratio (R In % of the L group) for the different lines. Within each temperature, lines with different letters are significantly different (P<0.05)

Resistance to acute temperature stress. Mean UTT per line and per rearing temperature ranged from 9450 to 14355 °.min. Significant ($P<0.05$) effects were found for rearing temperature and line and for rearing temperature*line interaction. Separated analysis for each rearing temperature revealed highly significant ($P<0.0001$) effect for line *ie* existence of genetic variability for resistance to acute temperature stress. Low correlations for UTT between temperature groups were found for H and F groups with L group: 0.26 and 0.42, respectively, while correlation was very high between H and F groups : 0.95 ($P<0.0001$). Thus, according to rearing temperature, most sensitive or most resistance lines are not totally similar. High rankings changes were observed with some lines switching from very resistant to very sensitive (*ie* G17, A32) according to rearing temperature. However, some lines (*ie* A03, AB1) were consistently highly resistant other different rearing temperatures.

Finally the correlation between WR and UTT was very low (ranged from -0.08 to 0.08 according to rearing temperature group) showing that acclimatization to high or fluctuating temperature and resistance to acute temperature stress are two different traits.

Conclusion

Results confirmed that high temperature led to lower growth but did not influence mortality. At fluctuating temperatures, growth was quite similar to growth at high temperatures. Thus, fluctuating temperature does not seem to be more challenging for fish than high temperature. The study clearly showed that there was genetic variability for acclimatization to high or fluctuating temperature and also for resistance to acute stress. The high temperature * line interactions for growth reveals that genotypes ranking is different according to rearing temperature, thus a specific selection with fish reared at the targeted temperature should be carried out if one wants to improve thermotolerance. Moreover, the correlation between both kinds of adaptation (to chronic or acute stress) was very low and non significant. Thus resistance to acute stress cannot become a simple test to select for growth at high temperature.

Two of these lines, chosen for their contrasted response to temperature, were produced again and will be characterized in depth for physiological and behavior traits to understand how fish adapt or not to high temperature.

Acknowledgements

The project was funded by INRA Metaprogramme ACCAF. Animal material was produced by INRA, UE 0937 PEIMA, France.

Literature Cited

Aupérin, B., and Boeuf, G., (2010). Adaptation et stress. 83-100 in La truite arc-en-ciel, de la biologie à l'élevage. Ed. Quae, Paris, France.

Dupont-Nivet, M., Médale, F., Leonard, J. et al. (2009). Aquaculture 295:15-21

Ihsses, P.E., (1986). Aquaculture 57:370-377

Jobling, M., (1984). Chapman & Hall, London, 309 p.

Molony, B.W., Church, A.R., and Maguire, G.B., (2004). Aquaculture 241:655-665

Perry, G.M.L., Danzmann, R.G., Ferguson, M.M, et al. (2001). Heredity 86:333-341

Perry, G.M.L, Martyniuk, C.M., Ferguson, M.M et al. (2005). Aquaculture 250 :120-128

Quillet, E., Dorson, M., Le Guillou, S, et al. (2007). Fish & Shellfish Imm. 22:510-519