GENETIC PARAMETERS AND GENOME-WIDE ASSOCIATION STUDY OF RESISTANCE TO ACUTE HYPERTHERMIA IN RAINBOW TROUT

H. Lagarde[1*], D. Lallias[1], P. Patrice[2], M. Prchal[3], Y. François[2], J. D'Ambrosio[2], A. Acin-Perez[4], F. Cachelou[4], P. Haffray[2], M. Dupont-Nivet[1], F. Phocas[1]

[1] Université Paris-Saclay, INRAE, AgroParisTech, GABI, 78350 Jouy-en-Josas, France; [2] SYSAAF, French Poultry and Aquaculture Breeders Association, 35042 Rennes, France; [3] University of South Bohemia in České Budějovice, Faculty of Fisheries and Protection of Waters, South Bohemian Research Center of Aquaculture and Biodiversity of Hydrocenoses, Zátiší 728/II, 389 25 Vodňany, Czech Republic; [4] Viviers de Sarrance, Pisciculture Labedan, 64490 Sarrance, France.
[*]henri.lagarde@inrae.fr

Abstract
Selective breeding is a promising solution to reduce fish farms vulnerability to heat peaks. Objectives of this study were to give a new insight on the genetic architecture of resistance to acute hyperthermia stress in rainbow trout. At 275 days post-fertilization, 1,384 fish were phenotyped for acute hyperthermia resistance and body weight. Challenged fish were genotyped for 57K SNP and their genotypes were imputed at high-density thanks to their parents being genotyped on a 665K SNP array. Heritability estimate of resistance to acute hyperthermia was 0.32 ± 0.04. This trait was genetically negatively correlated with body weight (-0.58 ± 0.17). The genome-wide association study revealed that resistance to acute hyperthermia is highly polygenic as altogether the 5 detected QTLs explained less than 5% of the genetic variance. The main QTL region explained 3% of the genetic variance and contained two candidate genes previously described to be associated with temperature resistance.

Introduction
Rainbow trout (*Oncorhynchus mykiss*), a cultured salmonid of major economic importance, thrives in a narrow cold thermal range (Bear et al., 2007). Exposition to high temperatures leads to the impairing of production efficiency with growth reduction, increase of disease and mortality. Extreme temperature events such as heat waves are expected to increase in intensity and frequency with global warming. The reduction of their impact represents a major challenge for the aquaculture industry. Among existing solutions, genetic improvement by selective breeding is an interesting approach: progress is cumulative from a generation to another and genetically improved animals could be exported to any farm without any prior equipment required. In rainbow trout, genetic determinism of resistance to acute temperature stress has already been studied in a Canadian commercial population (Perry et al. 2005) and QTLs were detected (Jackson et al., 1998; Perry et al., 2001). However, these studies concern a single population, distant from our study population, and recent advances in genomics may provide more accurate results for QTL detection.
Objectives of the present study were to complement existing knowledge for introducing acute hyperthermia resistance trait in rainbow trout breeding programs by i) assessing genetic parameters for acute hyperthermia resistance in a new commercial population, ii) detecting with a powerful tool (new high-density SNP array) QTLs associated with acute hyperthermia resistance and (iii) identifying candidate genes.

Materials & Methods

The experiment was carried out according to the European guidelines; the protocols were evaluated and approved by the ethic committee ANSES/ENVA/UPC No 16 and authorized by the French ministry of higher education and research (APAFIS#24441-2020022417122193).

Study population.

A total of 1,384 fish from 573 full-sib families was produced in a partial factorial mating design from 74 dams and 99 sires derived from the Viviers de Sarrance (Nouvelle-Aquitaine, France) breeding program. At 265-days post-fertilization (dpf), fish were tagged with ISO RFID tags and fin-clipped for later DNA extraction and genotyping. At 272 dpf, fish were transported to ANSES-SYSAAF Fortior Genetics platform (Brittany, France). The challenge to hyperthermia was spread over 7 days (between 275 dpf and 285 dpf) with 198 ± 12 fish per group. Around 9 a.m., fish from one of the 7 batches were transferred to the challenge tank, supplied with identical river water. Once the transfer completed, temperature was gradually increased from initial river water temperature (17.3 ± 0.7 °C) at a rate of 1.5 ± 0.2 °C/hour. Above 27°C, fish gradually began to lose equilibrium. Each fish losing equilibrium was removed from the tank and identified. The time was recorded and the fish was weighed and euthanized. The acute hyperthermia resistance phenotype was considered as time to loss of equilibrium (TLE). Body weight (BW) was measured to estimate phenotypic and genetic correlation between BW (mean BW 87 ± 12 grams) and TLE. Fish were genotyped with the 57K Axiom™ Trout Genotyping array (Palti et al., 2015) at the INRAE genotyping Platform Gentyane. Their parents were genotyped for 664,531 SNPs with a new high-density Axiom™ Trout Genotyping array. After quality control and parental assignation with APIS software (Griot et al., 2020), the final dataset contained 1,332 offspring genotyped for 30,379 SNPs (LD genotypes). The parent's final dataset contained 172 individuals genotyped for 420,079 SNPs (HD genotypes). The mean number of progenies per sire, per dam and per full-sibs family were respectively 14.0± 6.4, 18.7± 7.5 and 2.4± 2.5. Offspring's LD genotypes were imputed to HD thanks to the parental reference HD genotypes using FIMPUTE3 software (Sargolzaei et al., 2014).

Statistical Analysis.

Genetic (co)variance components of acute hyperthermia resistance and body weight at 275 dpf were estimated with AIREML algorithm in BLUPF90 software (Misztal et al., 2002) using the following animal model:

$$y_{ij} = \mu + day_i + a_j + \varepsilon_{ij} \quad (1)$$

where y_{ij} is the performance (TLE or BW at 275 days) of animal j, μ is the overall mean of the population, day_i is the fixed effect of day of challenge i, a_j is the additive effect of animal j and ε_{ij} is the random residual error. Both pedigree and genomic relationship matrices were built. The pedigree was constituted of 20,372 animals over ten generations. The genomic matrix was built with the HD genotypes.

GWAS was performed on temperature resistance trait using the Bayesian approach BayesCπ implemented in the BESSiE software with the following model:

$$TLE_{ij} = \mu + day_i + \sum_{k=1}^{n} \delta_{kl} z_{ijk} a_k + \varepsilon_{ijl} \quad (2)$$

With TLE_{ij}, the time before loss of equilibrium, the temperature resistance phenotype of animal j, μ the overall mean of the population, day_i the fixed effect of day of challenge i and n the total number of SNPs (420,079). δ_{kl} is an indicator variable: within a cycle l, δ_{kl}=1 if the effect of SNP k is estimated in this cycle or δ_{kl}=0 if not. In each cycle, δ_{kl} is sampled from a binomial distribution with a probability π that δ_{kl} was equal to 1. The proportion π was sampled from a beta distribution B(α,β) with α = 400 and β =420,079; which represents approximatively 0.1%

of SNPs selected at each cycle. z_{ijk} was the genotype on locus *k* for individual *j* of day *i* (coded as 0, 1, or 2), a_k the effect of the reference allele of SNP *k*, and ε_{ijl} the residual effect. The Markov chain Monte Carlo was run with 400,000 cycles and a burn-in period of 10,000 cycles. Results were saved every 40 cycles. The Bayes factor (BF) was calculated to quantify the degree of association between a SNP and the phenotype. Evidence for a QTL was provided by a value 2*ln(BF)≥ 6 at a peak SNP. A credibility interval for the QTL was computed around the peak SNP including all SNPs for which 2*ln(BF)≥3 in a sliding window of 0.2Mb. Genes in the QTL region were annotated with the NCBI *O. mykiss* genome assembly (GCA_013265735.3 USDA_OmykA_1.1) (Gao et al., 2021).

Results & Discussion

Genetic parameters of TLE and BW are presented in Table 1.

Table 1. Genetic parameters for resistance to acute hyperthermia in rainbow trout.

Trait	Relationship matrix	σ^2_a	σ^2_e	σ^2_p	h^2(±se)
TLE	Pedigree	369	787	1156	0.32 (±0.06)
TLE	Genomic	324	819	1143	0.28 (±0.04)
BW	Pedigree	19	110	129	0.14 (±0.04)
BW	Genomic	26	103	129	0.20 (±0.04)

with σ^2_a: additive genetic variance, σ^2_e: environmental variance, σ^2_p: phenotypic variance and h^2(±se): heritability ± standard error

Pedigree heritability and genomic heritability of acute hyperthermia resistance were estimated at similar values of 0.32 ± 0.06 and 0.28 ± 0.04. Those values are lower than the previous heritability estimate 0.41± 0.06 (Perry et al. 2005), but still suggest a good potential for genetic improvement. Heritability of BW was estimated to 0.14 ± 0.04 with the pedigree relationship matrix and 0.20 ± 0.04 with the genomic relationship one, which is consistent with previous estimates (e.g. Leeds et al., 2016). Phenotypic correlation between acute hyperthermia resistance and BW was not statistically different from zero (-0.067 ± 0.039), but the genetic correlation was estimated significantly negative (-0.58 ± 0.17) while Perry et al. (2005) found it not significant (-0.032 ± 0.18). If our result is confirmed, it will be necessary to consider the trade-off between temperature resistance and fish growth in selective breeding programs.

With an evidence threshold of 2×ln(BF) ≥6, five QTLs were found, located on three chromosomes (Figure 1).

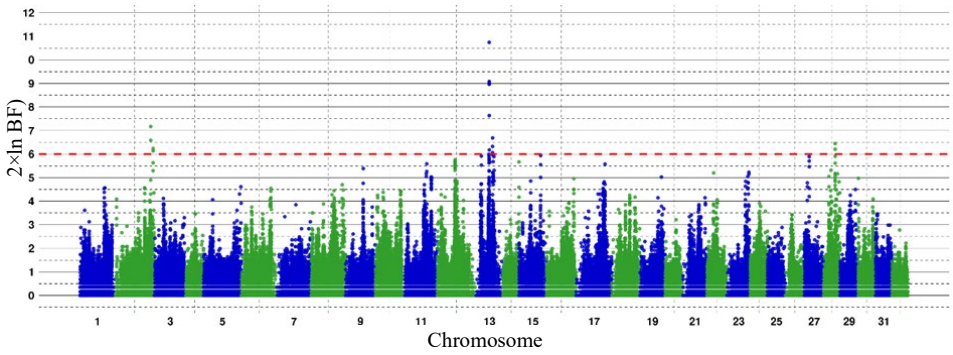

Figure 1. Manhattan plot of QTL detected for acute hyperthermia resistance trait. The red line corresponds to the QTL evidence threshold (2×ln(BF) ≥6).

Table 2. Effect and location of acute hyperthermia resistance QTL

Chr.	Peak SNP	% of variance explained by peak SNP	2×ln(BF)	Credibility interval (Mb)	% of variance explained by QTL	# genes in QTL range
2	Affx-1237560225	0.2%	7.2	[94.49-94.95]	0.2%	15
2	Affx-277325684	0.1%	6.2	[101.37-101.43]	0.1%	3
13	Affx-1237409861	1.7%	10.7	[34.33-36.93]	2.9%	81
13	Affx-1237414100	0.1%	6.7	[45.60-45.93]	0.2%	11
28	Affx-1248614594	0.1%	6.4	[30.21-30.40]	0.2%	8

One QTL, located on chromosome 13, had a very high evidence (2×ln(BF) > 10) and explained almost 3% of the genetic variance of acute hyperthermia resistance (Table 2). This result suggests that resistance to acute hyperthermia is a highly polygenic trait. In the credibility interval of this QTL, 81 genes were identified. In a window of 100Kb around the peak SNP of this QTL, we identified two genes, *HSP70* and *FKB10*, which are directly involved in proteins folding in generic response to stress exposure. Both genes have already been reported to be differentially expressed during temperature stress in salmonids (Akbarzadeh et al., 2018) and thus may be suggested as functional candidates' genes.

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