Genome-wide association study and genomic prediction of tolerance to acute hypoxia in rainbow trout

M Prchal, Jonathan D'ambrosio, Henri Lagarde, Delphine Lallias, P Patrice, Y François, Charles Poncet, A Desgranges, P Haffray, Mathilde Dupont-Nivet, et al.

Genome-wide association study and genomic prediction of tolerance to acute hypoxia in rainbow trout

M. Prchal[1], J. D'Ambrosio[2], H. Lagarde[2], D. Lallias[2], P. Patrice[3], Y. François[3], C. Poncet[4], A. Desgranges[5], P. Haffray[3], M. Dupont-Nivet[2], F. Phocas[2]

[1] 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

[2] Université Paris-Saclay, INRAE, AgroParisTech, GABI, 78350 Jouy-en-Josas, France

[3] SYSAAF (French Poultry and Aquaculture Breeders Technical Centre), 35042 Rennes, France

[4] GDEC, INRAE, Université Clermont-Auvergne, 63039 Clermont-Ferrand, France

[5] SARL Milin Nevez, 29610 Plouigneau, France

* Corresponding author: Martin Prchal

Email: mprchal@frov.jcu.cz

Phone number: +420 377774753

Abstract

Hypoxia is one of the major threats to the aquaculture sector resulting in substantial economic losses to the fish farmers. Thus, tolerance to hypoxia is of high economic interest to be genetically improved by breeding programs. Rainbow trout (*Oncorhynchus mykiss*) is one of the most cultured salmonid species worldwide, with well-developed breeding programs. Still, studies of genetic potential to improve hypoxia tolerance in this species are rare. In the present study, 1,320 individuals of rainbow trout were used for a genome-wide association study of acute hypoxia tolerance based on imputed high-density genotypes to explore the genetic architecture and related candidate genes affecting hypoxia response. Three significant (Omy31_1, Omy31_2, Omy20) and two putative (Omy15, Omy28) quantitative trait loci (QTLs) were detected, but each of them only explained between 0.2% and 0.8% of the genetic variance of acute hypoxia tolerance. However, heritability was estimated at a moderate value of 0.24 – 0.28, that suggests a solid potential to improve hypoxia tolerance in the studied rainbow trout population by genetic selection. Moreover, it was shown that genomic prediction for hypoxia tolerance would lead to a relative increase of ~ 11% for genomic selection (GS) accuracy compared to the pedigree-based selection, considering a reference population of 1000 individuals. Finally, sixteen genes (*ids, fmr1, arx, lonrf3, commd5, map4k4, smu1, b4galt1, re1, abca1, noa1, igfbp7, noxo1, bcl2a, trim25, mylk3*) were proposed as potential functional candidates involved in hypoxia tolerance. Taking all proposed candidate genes within two main QTLs on Omy31 (12 out of 16 genes), we may hypothesize that the complex response to acute hypoxia in rainbow trout, i.e., the interplay between behavioural, morphological, and physiological responses, is primarily encoded by two supergenes. However, further functional validation of their effects may help to specify the biological mechanisms triggering a response to acute hypoxia in rainbow trout.

Key words: GWAS, Heritability, Hypoxia resistance, Hypoxia tolerance, *Oncorhynchus mykiss*

1. Introduction

A lack of oxygen referred to as hypoxia is one of the greatest challenges that most life can face. In terrestrial ecosystems, conditions of low oxygen are rare. In contrast, low concentration of dissolved oxygen (DO), is much more common in aquatic ecosystems (Diaz, 2001; Townhill et al., 2017). The DO is a primary limiting factor in aquaculture because fish have aerobic metabolism requiring DO at efficient levels (Abdel-Tawwab et al., 2019). Hypoxia is also a very complex condition caused by several interrelated factors that might be divided into three main groups: 1) substandard rearing conditions due to the technical failure of water aeration or oxygenation or too high stocking densities regarding post-prandial oxygen consumption (Abdel-Tawwab et al., 2019); 2) eutrophication associated with increased anthropogenic nutrient loading of lakes, rivers and coastal waters leading to blooms of algae and phytoplankton, the dissimilation or death of which subsequently leads massive depletion of dissolved oxygen (Friedrich et al., 2014); 3) global warming as the main aspect of climate change leading to increased microbial respiration rates and reduced oxygen solubility with increasing water temperatures (McBryan et al., 2013; Sae-Lim et al., 2017; Reid et al., 2019).

Hypoxia is one of the major threats for aquaculture sector (Farrell and Richards, 2009; Abdel-Tawwab et al., 2019) affecting seriously health and welfare of fish including growth, reproduction, behaviour, immunity, and other energy-demanding activities (Gallage et al., 2016; Abdel-Tawwab et al., 2019; Reid et al., 2019). Consequently, hypoxia often leads to severe losses to fish farmers. Thus, tolerance to hypoxia is of high economic interest to be genetically improved by breeding programs (Sae-Lim et al., 2017).

Of the total European aquaculture production, 80 – 83% originated from at least 37 different breeding programs that are primarily focused on production traits like growth or processing yields (Janssen et al., 2017). Moreover, improving of disease resistance to several pathogens in both freshwater and marine fishes is under strong interest (Yáñez et al., 2014; Gjedrem and Rye, 2016; Houston et al., 2020). However, improving resilience to environmental variation like e.g., tolerance to temperature, hypoxia or salinity is starting to be an interesting challenge for aquaculture breeding programs (Allal and Nguyen, 2022).

Through studies using mammalian model organisms, three hypotheses for oxygen-sensing and downstream response have been proposed: a "membrane hypothesis," a "mitochondrial/metabolic hypothesis," and a more recent "gas transmitter hypothesis". These hypotheses are in close parallel to fish physiological response to hypoxia conditions (Gattuso et al., 2018).

Many molecular and physiological responses to hypoxia are regulated by transcription factors named hypoxia-inducible factors (HIFs), heterodimeric proteins with an oxygen-sensitive α-subunit, HIF-α and an oxygen-insensitive β-subunit, HIF-β (Nikinmaa and Rees, 2005; Pelster and Egg, 2018; Dzhalilova and Makarova, 2020). HIF-α is a key regulator of many hypoxia-related proteins and signalling pathways that facilitate adaptation of organisms to hypoxic environments e.g.: insulin-like growth factor binding protein (*igfbp*, a growth inhibitory protein), vascular endothelial growth factor (VEGF), mitogen-activated protein kinases (MAPK), reactive oxygen species (ROS), reactive nitrogen species (RNS), oxidative phosphorylation (OXPHOS), NADPH oxidase or nitric oxide synthase (NOS, a RNS regulating enzyme) (Fu et al., 2000; Zhu et al., 2013; Fago and Jensen, 2015; Sahoo et al., 2016; Gattuso et al., 2018; Thomas et al., 2019; Dzhalilova and Makarova, 2020).

The acute response of fish to hypoxia mainly combines physiological changes in order to maintain normal oxygen supply to tissues (hyperventilation, bradycardia, increased blood O2-carrying capacity and redistribution of blood flow) with metabolic changes to provide adequate amounts of ATP (adenosine triphosphate) with less oxygen through stimulation of anaerobic glycolysis and gluconeogenesis pathways (Farrell and Richards, 2009; Richards, 2011; Genz et al., 2013; Gattuso et al., 2018). Due to the use of carbohydrates to produce ATP in the absence of oxygen, glycogen and glucose levels immediately rise and further resulting in lactate accumulation and circulation (Omlin and Weber, 2010; Li et al., 2018; Abdel-Tawwab et al., 2019; Léger et al., 2021). Under chronic hypoxia, lipolysis replaces glycolysis, and lipids are the main energy source of fish (Li et al., 2018). Besides, both acute and chronic hypoxia stress promotes cell apoptosis and oxidative stress leading to DNA damage (Poon et al., 2007; Mahfouz et al., 2015; Mustafa et al., 2015; Birnie-Gauvin et al., 2017; Chowdhury and Saikia, 2020), destroys the innate immune system, and makes fish more susceptible to pathogens (Giomi et al., 2016; Esteve et al., 2017; Sae-Lim et al., 2017; Abdel-Tawwab et al., 2019). Furthermore, fishes can show various behavioural responses such as rising to the surface to breathe the uppermost layer of water in contact with air, increasing activity to escape the hypoxic area or decreasing activity to reduce oxygen demand (Domenici et al., 2007; Domenici et al., 2013; Bowyer et al., 2014).

Rainbow trout (*Oncorhynchus mykiss*) is one of the most cultured salmonid species worldwide (FAO, 2020), with well-developed breeding programs resulting in significant selection responses in growth, slaughter yields and disease resistance (Gjedrem and Rye, 2016; Chavanne et al., 2016; Boudry et al., 2021). In France, four commercial breeding programs are carried out. Besides, French rainbow trout production is mostly developed with all-female diploid or triploid stocks (Piferrer et al., 2009) reared in a land-based facility in a

concrete race-way with oxygen enrichment of the water by liquid oxygen injected in the water or mechanical aeration. Precise continuous monitoring of oxygen level is done with electronic probes and adapted software according to oxygen concentration fluctuations due to feeding delivery and consumption. However, an increase in trout robustness is demanded to adapt fishes to the rapid daily changes of oxygen content associated in summer with the increased temperature variation of the river's waters. Both acute and long-term exposure to low dissolved oxygen levels that are less than 4 mg/L (while optimum values are in the range from 8 to 10 mg/L) can lead to massive mortality and growth depression caused by reduced growth hormone-I (GH-I) and insulin like growth factor I-II (IGF I-II) levels in rainbow trout (Hou et al., 2020; Aksakal and Ekinci, 2021; Royer et al., 2021). Although hypoxia is a common threat in rainbow trout farming, studies of genetic potential to hypoxia tolerance are still rare (Scott et al., 2015; Borey et al., 2018; Strowbridge et al., 2021; Lagarde et al., Submitted). Thus, understanding the genetic architecture of hypoxia tolerance could help to improve the resilience of rainbow trout stocks by genetic selection.

To select for genetically environmental-tolerant individuals, enough genetic variability must exist within the species. Although hypoxia tolerance is an interesting trait for a trout breeding program, heritability estimates for this trait are still missing in salmonids. As far as we know, there were only two estimates in the literature to date showing high heritability for acute hypoxia tolerance (0.50 and 0.61, respectively) in common carp (*Cyprinus carpio*) (Nagy et al., 1980) and large yellow croaker (*Larimichthys crocea*) (Ding et al., 2022).

Numerous genome-wide association studies (GWAS) have been run in the last decade to identify genomic regions (Quantitative trait loci – QTLs) and functional candidate genes associated with traits of interest in different aquaculture species (Robledo et al., 2017; Houston et al., 2020). However, only a few GWAS for hypoxia tolerance have been conducted

so far, and on limited number of fish species other than salmonids: Nile tilapia (Li et al., 2017; Yu et al., 2021) (*Oreochromis niloticus*), two catfish species (Wang et al., 2017; Zhong et al., 2017) (*Ictalurus punctatus* and *Ictalurus furcatus*), golden pompano (San et al., 2021) (*Trachinotus ovatus*) and large yellow croaker (Ding et al., 2022). These studies revealed multiple QTLs and potential candidate genes that might be responsible for response to hypoxia.

The aims of this study were i) to estimate the variance components of hypoxia tolerance; ii) to predict the genomic selection (GS) accuracy compared to pedigree-based BLUP selection for hypoxia tolerance in rainbow trout; iii) to perform GWAS to investigate the genetic architecture of hypoxia tolerance and to propose potential candidate genes for the main QTLs. To our knowledge, this is the first GWAS evaluating the genetic architecture and accuracy of genomic prediction of acute hypoxia tolerance in rainbow trout.

2. Material and Methods

2.1 Ethics Statement

This study used fin clips collected by the breeding company “Milin Nevez” (Bretagne Truite Group, Plouigneau, France) as part of their commercial breeding programs. All challenges were carried out in accordance with the European guidelines (Directive 2010–63-EU) and the corresponding French legislation. Animal experiment procedures were approved by the ethics committee on animal experimentation COMETH ANSES/ENVA/UPC No. 16 and were authorized by the French Ministry of Higher Education, Research, and Innovation under number APAFIS#24441-2020022417122193 v3, project N°20-032.

2.2 Establishment and rearing of challenged fish

166 The experimental stock was established (6.11. 2019) from 190 dams and 98 sex-
167 reversed neomales of a commercial selected all-female line of Milin Nevez breeding company
168 using ten independent full-factorial mating designs. The eggs were split by the dam (meaning
169 190 separated batches, one for each dam), but only the 90 best spawnings were conserved
170 for mixing at the larval stage. So, in the end, the experimental stock came from 90 dams and
171 98 neomales. From each of the parents used in the mating, a fin clip was collected and stored
172 in 98% ethanol for later DNA extraction and genotyping.
173 The progeny stock was then reared under common commercial conditions until 9
174 months. 1,508 individuals were PIT tagged by Bretagne Truite and transferred to the SYSAAF-
175 ANSES Fortior Genetics platform (ANSES, Plouzané, France) and acclimatized for four days
176 before acute hypoxia challenge. Out of 1,508 fish, 1,351 individuals were challenged to
177 hypoxia and fin-clipped for DNA and finally only 1,320 DNA samples were sent for genotyping.
178
179 **2.3 Hypoxia challenge**
180 The challenge to hypoxia was sub-divided into seven batches (one per day starting
181 from 17.8. 2020), and in each batch, a random sample of fish was challenged (Table 1.) At the
182 beginning of each trial, the initial oxygen level was recorded. The gradual decline of oxygen
183 was conducted by bubbling nitrogen and monitored every five minutes until the end of the
184 trial of a given day (Supplementary Fig. S1). Water temperature was constantly monitored
185 during the trial and ranged from 15.2°C to 17.3°C. Hypoxia tolerance phenotype was defined
186 as the time to loss of equilibrium (TLE) in minutes with a value set to 0 for the first fish losing
187 its equilibrium. When fish lost their equilibrium, they were removed from the tank, identified
188 (PIT-tag reading), weighed and euthanized in a concentrated bath of Eugenol (180mg/L). The
189 corresponding TLE and oxygenation levels (mg/l^{-1}) were recorded for each individual. The

challenge ended when the last fish lost its equilibrium and was removed from the tank. The parents were not challenged and were only used as a reference population for the imputation of their offspring genotypes.

2.4 Genotyping and imputation

Fin samples from hypoxia-challenged fish (1,320 ind.) and their parents (188 ind.) were sent to the INRAE genotyping platform Gentyane (Clermont-Ferrand, France) for DNA extraction and genotyping. The challenged fish were genotyped for 57,501 SNPs using the medium-density (MD) Rainbow Trout Axiom® 57K SNP array from Thermo Fisher (Palti et al., 2015). Parents were genotyped for 664,531 SNPs using a newly constructed high-density (HD) Rainbow Trout Axiom® 665K SNP array (Bernard et al., 2022).

The first round of quality control was done by Thermo Fisher software Axiom Analysis Suite™ with threshold values of 97% for SNP call rate and 95% for sample call rate for both MD and HD SNPs. All parents (288) passed the preliminary quality control, but twenty-three challenged fish samples did not pass quality control, and thus 1,297 progeny samples remained for the following step of quality control. Then, SNPs with probe polymorphism and multiple locations on the Arlee genome reference assembly (accession number: USDA_OmykA_1.1.; Gao et al., 2021) were discarded as described in Bernard et al. (2022).

Finally, PLINK v1.9 software (Chang et al., 2015) was used for keeping only SNPs with deviation from Hardy-Weinberg equilibrium with a p-value > 0.0001 and a minor allele frequency (MAF> 0.05). On the HD genotypes, 418,925 SNPs remained for the parents, while 29,091 SNPs remained on the MD genotypes of the challenged progeny. Parentage assignment was done using 1,000 randomly sampled markers with the R package APIS (Griot et al., 2020) with a positive assignment error rate set to 2%.

The imputation of the MD genotypes into HD genotypes for the 1,297 challenged offspring was run using FIMPUTE3 software (Sargolzaei et al., 2014) utilizing quality-filtered genotypes and pedigree information from 188 parents. The correctness of imputation was checked by mendelian testing and by observation of changes in original MD genotypes that varied from 1 (0.004%) to 106 SNPs (0.37%) per individual. The final dataset contained 1,297 phenotyped and genotyped (418,925 imputed SNPs) progeny, which were subjected to genomic analysis.

2.5 Estimation of breeding values and variance components

The following statistical model was derived to describe the hypoxia response:

$$TLE_{ijk} = \mu + trial_i + dam_j + anim_k + e_{ijkl}$$

where TLE_{ijk} is the TLE performance for animal k, produced by dam j and tested day i, μ the overall mean for hypoxia tolerance trait in minutes, *trial* is the fixed effect of challenge trial (i = 1 – 7); *dam* is the random maternal effect (j = 1 – 90); *anim* is the random genetic effect of the animal (k = 1 – 1,297) and e_{ijkl} is the random residual. In total, 49,402 animals were related through the pedigree relationship matrix (A) for BLUP evaluation, tracing back eight generations of ancestors of the 1,320 phenotyped animals.

For genomic-based evaluations, the pedigree matrix A was replaced by a genomic relationship matrix G (VanRaden, 2008), considering either MD (29,091 SNPs) or HD (418,925 SNPs) genotypes.

Variance components were estimated using the restricted maximum likelihood method and AIREML algorithm in BLUPF90 software (Misztal et al., 2002).

The selection accuracies of a pedigree-based selection approach (BLUP) and a genomic selection approach (GBLUP) were assessed from estimated breeding values (EBV and GEBV,

respectively) that were derived through consideration of either the pedigree relationship matrix (A) or the genomic matrix (G) using the software package BLUPf90 (Misztal et al., 2014)

To evaluate the accuracy of (G)EBVs, 100 replicates of Monte Carlo 'leave-one-group-out' cross-validation tests were run considering the same procedure as described in D'Ambrosio et al. (2020). For each replicate, 297 fish were randomly chosen for the validation set, and 1,000 fish were chosen for the training set. The phenotypes recorded in the validation population were then hidden and breeding values were estimated using BLUP and GBLUP models. The accuracy (*r*) for each replicate was computed as:

$$r = \text{cor}((G)EBV, y^*)/h$$

where cor ((G)EBV, y) is the correlation between the (G)EBV and the phenotypes y* adjusted for batch and maternal effects of the 297 individuals belonging to the validation population, and h is the square root of the pedigree-based heritability. Moreover, the degree of bias in BLUP and GBLUP estimations was evaluated by the regression coefficient of the adjusted phenotypes on the (G)EBVs. In the absence of selection bias, this coefficient is expected to be equal to 1; in the case of EBV over-dispersion (inflation), the coefficient is below 1, and in the case of EBV under-dispersion the value is above 1. The selection accuracies and the inflation coefficients of different tested models (BLUP, GBLUP – MD and GBLUP – HD) were presented as average values over all 100 replicates.

2.6 Bayesian-based GWAS

A Bayesian variable selection model with a Bayes Cπ approach (Habier et al., 2011) was used to perform GWAS to locate QTL regions and estimate the proportions of genetic variance explained by the identified QTLs. In this model, only a certain proportion of SNPs (π) are assumed to have a non-zero effect on the phenotype. The marker effects are estimated

through the Markov Chain Monte Carlo (MCMC) algorithm that considers a mixture of markers, of which proportion π has effects that follow a normal distribution $N(0, \sigma_a^2)$ and proportion 1 – π have zero effect. The following general model was used:

$$TLE_{ijl} = \mu + trial_i + dam_j + \sum_{k=1}^{n} \delta_{lm} a_k g_{kl} + \varepsilon_{ijlm}$$

where TLE_{ijl} is the time to loss of equilibrium of individual *l*, μ is the overall mean for hypoxia tolerance trait in minutes, *trial* is the fixed effect of challenge trial (*i* = 1 – 7), *dam* is the random maternal effect (*j* = 1 – 90), *n* is the total number of SNPs used in the analysis, a_k is the additive effect of the reference allele for the k^{th} SNP, with genotype g_{kl} (coded as 0, 1, or 2) for individual *l*, and ε_{ijlm} is the residual term for the *l*th individual in the m^{th} cycle of the MCMC algorithm.

At each cycle *m*, the decision to include SNP *k* in the model depended on the indicator variable δ_{lm}. This indicator variable was sampled from a binomial distribution with a probability π that δ_{lm} was equal to 1 (i.e., the SNP has a non-zero effect a_k) and a probability (1 – π) that δ_{lm} was equal to 0 (i.e., the SNP k is thus not accounted for in the model fit at cycle *m*). The proportion (1 – π) was sampled from a beta distribution, B(α, β), in which parameter α was set as the total number of markers (*n* = 418,925 – HD; *n* = 29,091 – MD) and β was set at 400, corresponding to a model retaining about 400 SNPs with non-zero effects at each cycle.

The BESSiE software (version 1.0) was used to compute this model (Boerner and Tier, 2016). A total of 410,000 cycles of Gibbs sampling were performed with a burn-in of 10,000 iterations using HD imputed genotypes. One Gibbs sample was saved every 40 iterations for further analysis. For MD analysis, a total of 210,000 cycles of Gibbs sampling were performed with a burn-in of 5,000 iterations. One Gibbs sample was saved every 20 iterations for further analysis. To check the convergence, the MCMC algorithm was initiated three times with three

different seeds for the random number generator. In addition to checking the consistency of peak SNPs identified across seeds, convergence was assessed by visual inspection of plots of the posterior density of genetic, maternal and residual variances and by high correlations *(r* >0.99) between the GEBVs estimated from the different seeds of the MCMC algorithm.

The evidence for association between the phenotype and each SNP was assessed by the Bayes Factor (BF):

$$\mathrm{BF} = \frac{P_i/(1 - P_i)}{\pi/(1 - \pi)}$$

where P_i is the probability that the i^{th} SNP has a non-zero effect in the model.

The existence of QTL was evaluated considering the values (hereafter named logBF) of twice the natural logarithm of BF for a peak SNP above the threshold value of 6 as proposed by Michenet et al. (2016). All SNPs close to the peak SNP and having a logBF greater than 2.5 were considered in the QTL region. The algorithm started at the peak SNP for each chromosome, followed by a sliding window of 150 kb on both sides of the peak SNP. A sliding window was applied until no SNP had a logBF greater than 2.5 in the current window, defining the borders of our QTL region, i.e., the credibility interval for the location of the causal mutation. In addition, we considered the putative existence of a QTL if 5 ≤ logBF < 6 for a peak SNP and the corresponding QTL region explained at least 0.1% of the total genetic variance.

The genetic variation explained by a QTL region was calculated by the explained variance of each SNP in QTL region as:

$$\sigma^2_{QTL} = \sum_i 2p_i(1 - p_i)a_i^2$$

With p_i the MAF of the SNP$_i$ and a_i the effect of the SNP located in the QTL region.

All annotated genes within each QTL region are listed in Supplementary Table S1 based on the NCBI annotation released for *Oncorhynchus mykiss* Arlee genome reference assembly

USDA_OmykA_1.1. (GCA_013265735.3) (Gao et al., 2021). The potential candidate genes that were meaningfully associated with acute hypoxia were subjected to further discussion.

2.7 Comparison of TLE performances across different genotypes

Homozygote standardized TLE difference was derived as the performance difference between the two homozygous genotypes AA = genotype coded as 0, and BB = genotype coded as 2) expressed in % of genetic standard deviation (σ_a): (BB – AA) / σ_a. Standardized TLE dominance effect was calculated as the difference between the performance of the heterozygote genotype (AB = genotype coded as 1) and the average performance of the two homozygous genotypes expressed in % of genetic standard deviation: [(AB-(AA + BB)/2)] / σ_a.

3. Results

3.1 Hypoxia challenge

The mean weight of 1,320 challenged fish was 50.8 ± 11.8 g, and the average time to loss of equilibrium (TLE) was 98.9 ± 71.8 min. (Table 1). The cumulative time to loss of equilibrium within each trial is shown in Figure 1. The initial level of dissolved oxygen (DO) started from 8.7 to 9.2 mg/L^{-1} and was gradually reduced to 2 – 3 mg/L^{-1} two hours after the beginning of the challenge (Table 1, Supplementary Fig. S1). Then, after 3 hours of challenge, the DO was kept constant (1 – 2 mg/L^{-1}) until the end of a given trial. The average level (across all trials) of DO when the first fish lost its equilibrium was 3.5 mg/L^{-1}, 50% of fish at 1.7 mg/L^{-1} and the last fish at 1.3 mg/L-1 (Table 1).

3.2 Parentage assignment

Of 1,320 genotyped fish, 1,314 (99.5%) were uniquely assigned to a single parental pair using pedigrees. The number of progeny per sire varied from 3 to 27, with an average of 13,

and from 1 to 32 per dam, with an average of 14. Assigned fish (single parental pair) belonged to 646 full-sib families.

3.3 Variance components

The estimates of variance components for TLE using different models and density of genotypes are shown in Table 2. The highest heritability estimate was based on pedigree (0.28). Genomic heritability estimates (0.22 – 0.24) were slightly lower than the pedigree estimate. Maternal effects explained a modest but significant part of phenotypic variance across all studied models (0.09 – 0.10).

3.4 Efficiency of genomic selection

The criteria for assessing the efficiency of the selection (pedigree or genomic) are summarized in Table 3 (accuracy (r) and bias (b)).

Inflation coefficients were statistically not indistinguishable from 1, while slightly lower for GBLUP than for BLUP EBVs, so (G)EBVs were not biased. On average, GBLUP accuracy was similar for MD and HD genotypes (0.65) and was 11% higher than BLUP accuracy (0.59). However, of the 100 replicates of the validation and training populations, a gain in accuracy between GBLUP and BLUP was observed for only 82 replicates (Figure 2).

3.5 QTLs for acute hypoxia tolerance

In summary**,** GWAS detected three significant and two putative QTLs using HD imputed genotypes (Figure 3) with the statistical characteristics described in Table 4. However, none of them explained over 1% of genetic variation. Hence, the genetic architecture of hypoxia tolerance seems to be highly polygenic.

The main QTL was named Omy31_1. It explained 0.8% of the genetic variance and spanned the region from 19.93 to 21.56 Mb on the chromosome Omy31. This was the only significant QTL that could be identified using MD genotypes (results not presented). All genes annotated within the QTL Omy31_1 are graphically visualized in Figure 4. The peak SNP for this QTL was located at 20.876 Mb in the intergenic region between *aff2* (AF4/FMR2 family member 2) and *ids* (iduronate 2-sulfatase isoform X1 – X2) genes. The Bayesian analysis identified the same peak SNP position of the QTL using two different seeds.

A second QTL region was found on the same chromosome (Omy31_2), spanning from 33.43 to 33.97 Mb and explaining only 0.3% of the genetic variance. The peak SNP for this QTL was located at 33.812 Mb, in the *smu1* gene (WD40 repeat-containing protein SMU1).

The third significant QTL explained only 0.2% of the genetic variance and spanned the region from 22.62 to 22.99 Mb on Omy20. Its SNP was located on 22.880 Mb in the gene *noxo1* (NADPH oxidase organizer 1).

Two other putative QTLs were also identified for hypoxia tolerance. The first one was on Omy15, spanned the region from 17.58 to 18.32 Mb, and explained 0.2% of the genetic variance. The peak SNP for this QTL was located at 17.668 Mb in the *bcl2a* (apoptosis regulator Bcl-2) gene. The second putative QTL was located on Omy28 between 14.92 and 15.09 Mb and also explained 0.2% of the genetic variance. The peak SNP for this QTL was located close to the *mylk3* (myosin light chain kinase 3) gene.

3.6 Comparison of TLE performances across different genotypes

Homozygote standardized TLE difference and standardized TLE dominance effect were calculated for the peak SNPs of all significant and putative QTLs (Table 4). Statistical

differences ($p < 0.05$) in the TLE performance for genotypes at all identified QTLs are listed in Supplementary Table S2.

The performance difference between the two homozygous genotypes ranged from 56.5% to 118.5% of genetic standard deviation in favour of the homozygotes BB (coded 2 in the GWAS). The TLE performance comparisons for genotypes at QTLs on Omy31_1 and Omy28 are illustrated in Figure 5. For Affx-569844518 (peak SNP of QTL Omy31_1), the TLE performance of the homozygote (GG) was better (128.6 ± 91.4 min.) in comparison to the homozygote (AA) (89.6 ± 65.7 min.) and heterozygote (112.3 ± 77.3 min.) (Figure 5A). For Affx-1237683396 (peak SNP of QTL Omy28), the TLE performance of the homozygote (CC) was higher (120.9 ± 81.3 min.) compared to the homozygote (AA) (93.5 ± 69.5 min.) and heterozygote (91.2 ± 65.2 min.) (Figure 5B). A complete dominance effect of the unfavourable allele (A) was observed at Affx-1237683396 (QTL on Omy28) as the performances of the heterozygous individuals were similar to those of the (AA) homozygotes (Supplementary Table S2). A full additive QTL with codominance effects of the two alleles was observed on Omy20 (the dominance effect was estimated close to zero, 1.7%).

4. Discussion

Besides major diseases such as flavobacteriosis, IPN or SHV (Yáñez et al., 2014; Fraslin et al., 2020b), hypoxia is one of the most critical threats to rainbow trout aquaculture production (Van Raaij et al., 1996; Hou et al., 2020). Yet, a limited number of studies dealing with the genetic basis of hypoxia tolerance have been conducted on salmonid species (Anttila et al., 2013; Scott et al., 2015; Borey et al., 2018; Lagarde et al., Submitted), and genetic determinism of this trait is still unknown. Hence, we aimed to estimate the genetic

400 architecture of acute hypoxia tolerance by estimating this heritability of this trait and its
401 potential for genomic selection and by proposing potential candidate genes for the main QTLs.
402 A new high-density (HD) 665K SNP array (Bernard et al., 2022) was used for the first
403 time for GWAS on a rainbow trout population. This HD chip was used for the imputation step
404 using HD genotypes of parents to get imputed HD genotypes of challenged offspring.
405 Genotype imputation of challenged fish from MD (medium-density) to HD showed a high
406 potential for identifying new QTLs and refining positions of QTL identified on MD genotypes.
407
408 **4.1 Estimation of the variance components of hypoxia tolerance: potential for selective**
409 **breeding**
410 We found a maternal effect explaining a significant part of the phenotypic variance of
411 hypoxia tolerance (Table 2.), mostly typical for growth at early stages in salmonids (Blanc,
412 2002; Fishback et al., 2002; Haffray et al., 2012). One explanation might be the
413 transgenerational epigenetics effect of dams (Wolf and Wade, 2009). Accordingly, Ho and
414 Burggren (2012) demonstrated that parental hypoxic exposure in adult zebrafish (*Danio rerio*)
415 has profound epigenetic effects on the morphological and physiological phenotype of their
416 offspring. However, as the females and their previous generations were reared in the same
417 conditions, additional investigations are needed to identify the rearing factors that may have
418 indirectly induced this potential epigenetic effect. Another hypothesis could be based on
419 different hypoxia-tolerant mitochondrial genomes as was reported in mitochondria
420 differences of cerebral cortex cells of tolerant- and susceptible-to-hypoxia rats (Dudchenko et
421 al., 1993; Luk'yanova et al., 1995). However, in fish, no data are to date available concerning
422 the involvement of mitochondria in O2 sensing, and thus this hypothesis could be of interest
423 for further research investigation (Gattuso et al., 2018).

Regardless of the evaluation model applied, we estimated moderate heritability of TLE, with similar values estimated through MD or HD genomic relationship matrices, as previously observed in rainbow trout by Fraslin et al. (2020a). To our knowledge, there were only two estimates in the literature to date showing high heritability for hypoxia tolerance (0.50 and 0.61, respectively) in common carp (Nagy et al., 1980) and large yellow croaker (Ding et al., 2022). Furthermore, previous studies dealing with acute hypoxia challenges showed significant inter-family differences in Atlantic salmon (Anttila et al., 2013) or catfish (Wang et al., 2017; Zhong et al., 2017), as well as between-isogenic lines differences (Borey et al., 2018; Lagarde et al., Submitted) and between-strains differences (Scott et al., 2015; Strowbridge et al., 2021) in rainbow trout. So, there is substantial evidence for standing additive genetic variation in hypoxia tolerance in fishes, which might be exploited by genetic selection. However, genetic correlations between hypoxia tolerance and other production traits (e.g., harvest weight, slaughter yields, muscle fat etc.) need to be further assessed before integrating hypoxia tolerance in a future breeding program of rainbow trout.

4.2 Genomic prediction for hypoxia tolerance: increased accuracy compared to pedigree-based selection

Genomic selection (GS) is currently implemented in several aquaculture breeding programs (Boudry et al., 2021) and has been shown to improve selection accuracy, thus leading to higher genetic gains (Robledo et al., 2017). Besides, in fish species, GS is especially interesting for traits that cannot be measured directly on selection candidates (R2D2 Consortium et al., 2021), for example, disease resistance and meat quality, but also for traits associated with environmental changes like salinity, thermal or hypoxia tolerance (Gjedrem and Rye, 2016; Robledo et al., 2017; Houston et al., 2020).

Our results showed that genomic prediction for TLE would lead to a relative increase of ~ 11% for GS accuracy compared to the pedigree-based selection, considering a reference population of 1000 individuals. However, only a few studies investigated selection accuracy for environmental stress-related traits such as heat tolerance in Pacific abalone (*Haliotis discus hannai*) (Liu et al., 2022) and salinity tolerance in eastern oyster (*Crassostrea virginica*) (McCarty et al., 2022) or growth-related traits under chronic thermal stress in rainbow trout (Yoshida and Yáñez, 2022). Still, both genomic prediction accuracies and relative increase in accuracy in comparison to PBLUP were in a similar range as in this study. Genomic selection may thus significantly improve tolerance to hypoxia and other environmental stress-related traits, supported by the advantage of phenotyping only the reference population, but the relative expected gains need to be balanced with the operational costs of genotyping (Sonesson and Meuwissen, 2009).

Even though genotyping costs are gradually decrease yearly, dense SNP arrays are still expensive. Genotype imputation to generate high-density markers is thus a cost-effective method. In general, the accuracy of genomic prediction is highly associated with the genotype density used, which means that increasing marker densities should generate higher accuracies (Tsai et al., 2016; Correa et al., 2017; Yoshida et al., 2019). However, we observed no increase in prediction accuracy using HD-imputed genotypes compared to MD genotypes. It illustrated that high-density (imputed) markers might not be necessary for genomic prediction as previously reported in pigs (Song et al., 2019), cattle (Gunia et al., 2014; Van Binsbergen et al., 2015), and salmonids (Yoshida et al., 2018a; Yoshida et al., 2018b). Accordingly, recent genomic studies are widely focused on optimising low-density SNP panels to get strong predictive accuracy still outperforming BLUP (Vallejo et al., 2017; Vallejo et al., 2018; Kriaridou et al., 2020; Griot et al., 2021).

4.3 Genetic architecture of hypoxia tolerance: a highly polygenic trait and identification of potential candidate genes

As far as we know, this is the first study that identified significant QTLs associated with tolerance to hypoxia in rainbow trout. We found three significant QTLs (one on Omy20 and two on Omy31) and two putative QTLs (on Omy15 and Omy28), but they explained each only a limited proportion of genetic variation (0.2% – 0.8%). It suggests a highly polygenic nature of hypoxia tolerance controlled by many loci of small effect (Houston et al., 2020). In previous studies, multiple significant and suggestive SNPs associated with hypoxia tolerance explained between 4.22 – 12.44% (four linkage groups – LGs) of phenotypic variation in catfish strains and hybrids (Zhong et al., 2017; Wang et al., 2017), 6.6 – 14.7% (five LGs) in Nile tilapia (Li et al., 2017), up to 32% (four LGs) in golden pompano (San et al., 2021) and 5.5 – 18% (four LGs) in large yellow croaker (Ding et al., 2022). Thus, identified SNPs had a significantly stronger effect on hypoxia tolerance than in our study. However, those studies were focused on a limited number of tolerant/sensitive fish from a few number of different families used for GWAS, which may explain this difference – 208 fish (Zhong et al., 2017), 376 fish (Wang et al., 2017), 45 fish (Li et al. 2017), 100 fish (San et al., 2021) and 398 fish (Ding et al., 2022) in contrast to our study population – 1,320 fish from hundreds of families. Consequently, comparing these studies might be difficult and partially irrelevant. In any case, all studies reinforce the evidence of the polygenic architecture of hypoxia tolerance regardless of the studied species and populations, statistical models or marker densities. However, HD imputed genotypes significantly refined our QTL regions by filling the gaps of missing genotypes on the MD SNP panel. This is consistent with previous studies that also led to refining QTL and candidate genes associated with various performance traits of dairy cattle (Höglund et al.,

2014; Wu et al., 2015; Nayeri et al., 2016), pigs (Yan et al., 2017; Xu et al., 2019) and fishes (Palaiokostas et al., 2018; Fraslin et al., 2020a; Yoshida and Yáñez, 2021) using imputed HD genotypes or sequence data.

Although each QTL does not explain a large part of the genetic variance at the population level, significant phenotypic differences between individuals of different genotypes at peak SNPs across all significant and putative QTLs were observed. The association of favourable homozygous genotypes significantly prolonged TLE in the range of 17% to 30% (Supplementary Table S2). Thus, these SNPs might be used in marker-assisted selection for producing juveniles with tolerance for hypoxia in rainbow trout. Similarly, significant individual differences among different genotypes of the main SNPs associated with hypoxia tolerance were previously observed in golden pompano (San et al., 2021) and large yellow croaker (Ding et al., 2022).

Within the list of 113 genes annotated in our 5 identified QTL regions (see Supplementary Table S1), we proposed sixteen functional candidate genes with a meaningful biological association to hypoxia tolerance (*ids, fmr1, arx, lonrf3, commd5, map4k4, smu1, b4galt1, re1, abca1, noa1, igfbp7, noxo1, bcl2a, trim25, mylk3*). The potential candidate genes were firstly assessed with the database of Fish Hypoxia Responsive Genes (HRGFish) (Rashid et al., 2017). Furthermore, we also focused on the functional information given in the Mouse Genome Informatics (MGI) resource studied phenotypes in mutant mice (Eppig, 2017) or the zebrafish database (ZFIN) related to phenotypes in mutant zebrafish (Sprague et al., 2006).

The peak SNP of the main QTL (Omy31_1) was located in the intergenic region between *aff2* (AF4/FMR2 family member 2) and *ids* (iduronate 2-sulfatase) genes. Interestingly, a mutation of the *ids* gene in a zebrafish line ($ids^{ia200/ia200}$) led to a significantly larger liver (Bellesso et al., 2018). The liver is a significant source of glycogen which is the first mobilized

energy reserve during acute hypoxia leading to the glycogenolysis process and immediate increase of plasma glucose and glycogen, further increasing blood lactate concentration (Omlin and Weber, 2010; Li et al., 2018; Abdel-Tawwab et al., 2019; Léger et al., 2021). Therefore, liver size and glycogen level play a significant role in fish survival cope with acute hypoxia (Li et al., 2018), physiological stress response (Crespel et al., 2011) and energy mobilization during starvation (Crespel et al., 2013; Zhao et al., 2021). Similarly, male knockout mice (IdS-KO, ID MGI:259752) phenotypes for the *ids* gene exhibited significantly larger liver, spleen, and lungs and were hypoactive compared to wild mice (Garcia et al., 2007). This agrees with typical behaviour under hypoxia when some fish species remain static at the tank bottom to save their energy for facing the hypoxic condition (Abdel-Tawwab et al., 2019). Therefore, the *ids* gene might be suggested as a potential functional candidate. Besides, the *fmr1* gene (synaptic functional regulator FMR1) was annotated in the close vicinity of the peak SNP. The *fmr1* zebrafish mutant lines showed hyperactivity and abnormal swimming behaviour (Hu et al., 2020) and decreased startle reflex in case of loss of function of *fmr1* in mice (Chen and Toth, 2001; Pietropaolo et al., 2011; Rossignol et al., 2014). Abnormal behaviour under hypoxia conditions has been previously observed in fishes manifesting as rapid swimming in a circular motion with a wide mouth gape (Domenici et al., 2013; Bowyer et al., 2014) or as decreased startle reflex known as antipredator behaviour (Domenici et al., 2007). Moreover, *arx* gene (homeobox protein ARX-like) might also be proposed as an interesting functional candidate belonging to this QTL. Loss of function of this gene may cause hypoglycemia in mice (Collombat et al., 2003) and thus may block the natural response to acute hypoxia by upsetting the metabolism of carbohydrates (Polakof et al., 2012; Abdel-Tawwab et al., 2019). Let us also mention genes *lonrf3* (LON peptidase N-terminal domain and RING finger protein 3)*, commd5* (COMM Domain-Containing Protein 5), and *fam199x* (protein FAM199X), which are in our QTL

region as well as in the vicinity of the main SNP associated to hypoxia tolerance in golden pompano (San et al., 2021). Interestingly, mouse mutant genotype hm1 of *lonrf3* (Dickinson et al., 2016) and *commd5* gene in rats (Matsuda et al., 2014) are linked to calcium influx, which is strongly modified under hypoxia conditions and inhibits the expression of myoglobin (Kanatous et al., 2009). Myoglobin is an oxygen-binding hemoprotein that is widely thought to be expressed exclusively in oxidative skeletal and cardiac myocytes, where it plays a crucial role in coping with both acute and chronic hypoxia by the supply of oxygen to the fish heart (Rashid et al., 2017), as previously reported, for example, in the Tibetan Plateau fish (*Glyptosternum maculatum*) (Qi et al., 2018), Japanese medaka (*Oryzias latipes*) (Wawrowski et al., 2011) and zebrafish (Jaspers et al., 2014). Besides, MAPK (mitogen-activated protein kinases) signalling pathway have been shown to be involved in low oxygen tolerance in fishes (Wang et al., 2017; Tian et al., 2019; Yu et al., 2021). Thus, the candidate gene *map4k4* (mitogen-activated protein kinase 4) may also play an important role in response to hypoxia challenges.

The second significant QTL was also detected on chromosome 31 (Omy31_2) between 33.43 Mb and 33.97 Mb, with the peak SNP positioned within the *smu1* gene, a member of the WD40-repeat protein family. Loss of *smu1* function leads to multiple cellular defects, including chromosomal instability, aberrant DNA replication and alternative RNA splicing events. Accordingly, the DNA damage is also closely linked to hypoxia, as was previously described in common carp and Nile tilapia (Poon et al., 2007; Mahfouz et al., 2015; Mustafa et al., 2015). Besides, the *smu1* gene in zebrafish was upregulated under acute hypoxia (Ragsdale et al., 2020). Thus, the s*mu1* gene might be proposed as an interesting candidate gene. We also identified three other potential candidate genes linked to hypoxia: *b4galt1, re1* and *abca1*. *B4galt1* (beta-1,4-galactosyltransferase 1) and its exclusive function in the Golgi

compartment is a prerequisite for full catalytic activity. In contrast, a lack of this function is associated with cancers and intracellular hypoxia in human (Hassinen et al., 2019; Khoder-Agha et al., 2019). *Re1* was responsible for regulating approximately 20% of the hypoxia-repressed genes in human embryonic kidney cells. Hence, this gene was proposed as a key mediator of gene repression in hypoxia (Liang et al., 2014; Cavadas et al., 2016). *Abca1* (Phospholipid-Transporting ATPase ABCA1) is closely linked with hypoxia inducible factor α (HIF-α) which increases abca1 promoter activity. Thus, an expression of *abca1* might further stimulate the regulation of other hypoxia-inducible genes (Zhu et al., 2013; Pelster and Egg, 2018; Dzhalilova and Makarova, 2020). Moreover, not far from the peak SNP, an interesting gene *noa1* encodes nitric oxide-associated protein 1. It is involved in regulating mitochondrial protein translation and respiration, thereby controlling mitochondrial metabolism under acute and long-term hypoxia as previously described in goldfish (*Carassius auratus*) (Farhat et al., 2021), mice (Heidler et al., 2011) and humans (Tang et al., 2009). Mitochondria play a crucial role in coordinating responses to low oxygen because of their ability to detect physiological changes and supply the body with ATP through oxidative phosphorylation (OXPHOS) (Thomas et al., 2019). Moreover, the nitric oxide synthase (NOS) expression was observed to enhance the expression of HIF-1α that activates several pivotal genes (Zhu et al., 2013; Dzhalilova and Makarova, 2020) linked to hypoxia tolerance in goldfish and crucian carp (*Carassius carassius*). We thus hypothesize that *noa1* gene may have a complex role in rainbow trout response to hypoxia. In addition, the *igfbp7* gene (insulin-like growth factor-binding protein 7 precursor) might also be considered a potential candidate gene. Genes belonging to IGFs group are hypoxia-inducible genes involved in response to hypoxia and may have an important impact on fish growth during hypoxia conditions (Kajimura et al., 2006; Olsvik et al., 2013; Rashid et al., 2017; Hou et al., 2020; Aksakal and Ekinci, 2021). Moreover,

592 the *igfbp7* gene may inhibit the stimulatory effect of vascular endothelial growth factor (VEGF)
593 (Tamura et al., 2014; Ma et al., 2016), a key regulator of angiogenesis, hematopoiesis and
594 vascular density under low-oxygen conditions that activate genes involved in a series of
595 responses to hypoxia (Rehn et al., 2011; Dzhalilova and Makarova, 2020). In line with our
596 finding, Yu et al. (2021) annotated a candidate gene (*igf1ra)* belonging to the IGFs group in a
597 genome-wide association analysis of adaptation to long-term oxygen stress in Nile tilapia.
598 Within the two main QTLs on Omy31, seven and five genes are thus very convincing
599 functional candidates whose interplay may lead to behavioural, morphological, and
600 physiological responses to acute hypoxia stress. The response to acute hypoxia in rainbow
601 trout could then be explained through the supergene concept (Schwander et al., 2014).
602 Supergenes are tight clusters of multiple neighbouring genes inherited together because of
603 close genetic linkage, each affecting a different developmental, physiological or behavioural
604 characteristic. In combination, they provide integrated control of complex adaptive
605 phenotypes segregating within species (Schwander et al., 2014). Recently, a supergene was
606 identified in rainbow trout as a 55-Mb double-inversion region on Omy5 that mediates sex-
607 specific migratory tendency (Pearse et al., 2019). Here we suggest that two supergenes on
608 Omy31 may explain such enrichment of the QTL regions for involved hypoxia-responsive
609 genes.
610 On Omy20, the peak SNP was located in the intergenic region of the *noxo1* gene
611 (NADPH oxidase organizer 1), which is associated with NADPH oxidase — the enzyme
612 responsible for the production of reactive oxygen species (ROS) (Fu et al., 2000; Sahoo et al.,
613 2016). ROS can lead to oxidative stress during periods of environmental stress for fish
614 (Wischhusen et al., 2020). However, ROS are usually balanced by an antioxidant (redox)
615 defence and are considered a potent bactericide that actively destroys invading pathogens

(Birnie-Gauvin et al., 2017; Chowdhury and Saikia, 2020). But acute hypoxia commonly results in a disbalance between the production of oxidant and antioxidant components (oxidative stress) and thus significantly damages cell structures and DNA. In addition, hypoxia-tolerant fish appear to have an anticipatory response during low-oxygen availability by enhancing their ability to eliminate ROS production upon return to normoxia (Birnie-Gauvin et al., 2017; Qi et al., 2020). Hence, the *noxo*1 gene seems to be a promising functional candidate, playing an essential role in oxidative response pathways.

The peak SNP of the putative QTL on Omy15 was located within the gene *bcl2a* (apoptosis regulator Bcl-2). Bcl-2 proteins are a family of regulatory proteins that regulate cell apoptosis by either inducing or repressing cell death and are involved in several biological processes related to immune responses (Kratz et al., 2006). *Bcl-2a* gene was repressed after acute hypoxia stress in zebrafish (Cai et al., 2018) and channel catfish (Yuan et al., 2016), indicating that this gene is potentially involved in the hypoxia response by reducing cell apoptosis or autophagy, and could be considered a potential candidate gene. We may also propose *trim25* as a convincing candidate gene because the peak SNP is located just before this E3 ubiquitin/ISG15 ligase protein, with a major role in the initiation of intracellular antiviral response to herpesviruses (Gack et al., 2008). It is well known that hypoxia has substantial effects on fish's physiological and immune responses, which can depress their immune system leading to greater susceptibility to diseases (Giomi et al., 2016; Esteve et al., 2017; Sae-Lim et al., 2017; Abdel-Tawwab et al., 2019). Hence, loss of function of this gene may block an appropriate antiviral response as previously observed in common carp susceptible to koi herpesvirus (Palaiokostas et al., 2018).

Concerning the second putative QTL on Omy28, the candidate gene *mylk3* (myosin light chain kinase 3) was found close to the peak SNP. MGI mutant phenotype for the *mylk3*

gene was associated with several heart-related changes such as the increased response of the heart to induce stress, abnormal heart morphology, cardiac hypertrophy and necrosis (Ding et al., 2010; Bischof and Krishnan, 2016; Yuan et al., 2016) or influenced smooth blood pressure and angiogenesis in humans (Gordeuk et al., 2012). We thus hypothesise that acute hypoxia stress might lead to severe heart and blood changes causing limited oxygen transport to tissues and organs (Gattuso et al., 2018; Abdel-Tawwab et al., 2019).

5 Conclusions

In the present study, a newly designed HD chip was used to impute MD to HD markers in fish exposed to acute hypoxia. The GWAS showed clearly refined QTL regions and SNP peak position using HD-imputed markers but confirmed that hypoxia tolerance is of highly polygenic nature. Three significant and two putative QTLs explaining between 0.2% and 0.8% of the genetic variance, were identified for acute hypoxia tolerance, defined as TLE. However, moderate heritability suggests a solid potential to improve TLE in the studied rainbow trout population by genetic selection. As the variance explained by the five QTLs altogether is less than 5% of the total genetic variance, genomic selection should be promoted rather than marker-assisted selection to improve the selection efficiency of the broodstock population. However, marker-assisted selection may help to multiply broodstock producing offspring with high tolerance to hypoxia for production purposes. Finally, sixteen genes (*ids, fmr1, arx, lonrf3, commd5, map4k4, smu1, b4galt1, re1, abca1, noa1, igfbp7, noxo1, bcl2a, trim25, mylk3*) were proposed as potential functional candidates involved in hypoxia tolerance. Taking all proposed candidate genes within two main QTLs on Omy31 (12 out of 16 genes), we may hypothesize that the complex response to acute hypoxia in rainbow trout is encoded by two supergenes.

However, further functional validation of their effects may help to specify the biological mechanisms triggering a response to acute hypoxia in diploid rainbow trout.

Acknowledgements

This study was supported by the European Maritime and Fisheries Fund and France Agrimer (Hypotemp project, n° P FEA470019FA1000016) and by Ministry of Education, Youth and Sports of the Czech Republic – project CENAKVA (LM2018099).

References

Abdel-Tawwab, M., Monier, M.N., Hoseinifar, S.H., Faggio, C., 2019. Fish response to hypoxia stress: growth, physiological, and immunological biomarkers. Fish Physiol. Biochem. 45, 997-1013. http://dx.doi.org/10.1007/s10695-019-00614-9

Aksakal, E., Ekinci, D., 2021. Effects of hypoxia and hyperoxia on growth parameters and transcription levels of growth, immune system and stress related genes in rainbow trout. Comp. Biochem. Physiol. Part A Mol. Integr. Physiol. 262, 111060. http://dx.doi.org/10.1016/j.cbpa.2021.111060

Allal, F., Nguyen, N.H., 2022. Genomic Selection in Aquaculture Species. in: Ahmadi, N., Bartholomé, J. (Eds.), Genomic Prediction of Complex Traits: Methods and Protocols. Springer US, New York, NY, pp. 469-491. http://dx.doi.org/10.1007/978-1-0716-2205-6_17

Anttila, K., Dhillon, R.S., Boulding, E.G., Farrell, A.P., Glebe, B.D., Elliott, J.A., Wolters, W.R., Schulte, P.M., 2013. Variation in temperature tolerance among families of Atlantic salmon (*Salmo salar*) is associated with hypoxia tolerance, ventricle size and myoglobin level. J. Exp. Biol. 216, 1183-1190. http://dx.doi.org/10.1242/jeb.080556

Bellesso, S., Salvalaio, M., Lualdi, S., Tognon, E., Costa, R., Braghetta, P., Giraudo, C., Stramare, R., Rigon, L., Filocamo, M., 2018. FGF signaling deregulation is associated with early developmental skeletal defects in animal models for mucopolysaccharidosis type II (MPSII). Hum. Mol. Genet. 27, 2262-2275. http://dx.doi.org/10.1093/hmg/ddy131

Bernard, M., Dehaullon, A., Gao, G., Paul, K., Lagarde, H., Charles, M., Prchal, M., Danon, J., Jaffrelo, L., Poncet, C., Patrice, P., Haffray, P., Quillet, E., Dupont-Nivet, M., Palti, Y., Lallias, D., Phocas, F., 2022. Development of a High-Density 665 K SNP Array for Rainbow Trout Genome-Wide Genotyping. Front. Genet. 13, 941340. http://dx.doi.org/10.3389/fgene.2022.941340

Birnie-Gauvin, K., Costantini, D., Cooke, S.J., Willmore, W.G., 2017. A comparative and evolutionary approach to oxidative stress in fish: a review. Fish Fish. 18, 928-942. http://dx.doi.org/10.1111/faf.12215

Bischof, C., Krishnan, J., 2016. Exploiting the hypoxia sensitive non-coding genome for organ-specific physiologic reprogramming. Biochim Biophys Acta Mol Cell Res. 1863, 1782-1790. http://dx.doi.org/10.1016/j.bbamcr.2016.01.024

Blanc, J., 2002. Effects of egg size differences on juvenile weight between and within lots in rainbow trout *Oncorhynchus mykiss*. J. World Aquacult. Soc. 33, 278-286. http://dx.doi.org/10.1111/j.1749-7345.2002.tb00504.x

Boerner, V., Tier, B., 2016. BESSiE: a software for linear model BLUP and Bayesian MCMC analysis of large-scale genomic data. Genet. Sel. Evol. 48, 1-5. http://dx.doi.org/10.1186/s12711-016-0241-x

Borey, M., Paroissin, C., Quillet, E., Terrier, F., Maunas, P., Burel, C., Lauga, B., 2018. Acute hypoxia reveals diverse adaptation strategies to fully substituted plant-based diet in isogenic lines of the carnivorous rainbow trout. Aquaculture. 490, 288-296. http://dx.doi.org/10.1016/j.aquaculture.2018.02.005

Boudry, P., Allal, F., Aslam, M.L., Bargelloni, L., Bean, T.P., Brard-Fudulea, S., Brieuc, M.S., Calboli, F.C., Gilbey, J., Haffray, P., 2021. Current status and potential of genomic selection to improve selective breeding in the main aquaculture species of International Council for the Exploration of the Sea (ICES) member countries. Aquac. Rep. 20, 100700. http://dx.doi.org/10.1016/j.aqrep.2021.100700

Bowyer, J.N., Booth, M.A., Qin, J.G., D'Antignana, T., Thomson, M.J., Stone, D.A., 2014. Temperature and dissolved oxygen influence growth and digestive enzyme activities of yellowtail kingfish *Seriola lalandi* (Valenciennes, 1833). Aquacult. Res. 45, 2010-2020. https://doi.org/10.1111/are.12146

Cai, X., Zhang, D., Wang, J., Liu, X., Ouyang, G., Xiao, W., 2018. Deletion of the fih gene encoding an inhibitor of hypoxia-inducible factors increases hypoxia tolerance in zebrafish. J. Biol. Chem. 293, 15370-15380. http://dx.doi.org/10.1074/jbc.RA118.003004

Cavadas, M.A., Mesnieres, M., Crifo, B., Manresa, M.C., Selfridge, A.C., Keogh, C.E., Fabian, Z., Scholz, C.C., Nolan, K.A., Rocha, L., 2016. REST is a hypoxia-responsive transcriptional repressor. Sci. Rep. 6, 1-13. http://dx.doi.org/10.1038/srep31355

Collombat, P., Mansouri, A., Hecksher-Sørensen, J., Serup, P., Krull, J., Gradwohl, G., Gruss, P., 2003. Opposing actions of Arx and Pax4 in endocrine pancreas development. Genes Dev. 17, 2591-2603. http://dx.doi.org/10.1101/gad.269003

Correa, K., Bangera, R., Figueroa, R., Lhorente, J.P., Yáñez, J.M., 2017. The use of genomic information increases the accuracy of breeding value predictions for sea louse (*Caligus rogercresseyi*) resistance in Atlantic salmon (*Salmo salar*). Genet. Sel. Evol. 49, 1-5. http://dx.doi.org/10.1186/s12711-017-0291-8

747 Crespel, A., Bernatchez, L., Garant, D., Audet, C., 2011. Quantitative genetic analysis of the
748 physiological stress response in three strains of brook charr *Salvelinus fontinalis* and their hybrids.
749 J. Fish. Biol. 79, 2019-2033. https://doi.org/10.1111/j.1095-8649.2011.03149.x
750
751 Crespel, A., Bernatchez, L., Garant, D., Audet, C., 2013. Genetically based population divergence in
752 overwintering energy mobilization in brook charr (*Salvelinus fontinalis*). Genetica 141, 51-64.
753 https://doi.org/10.1007/s10709-013-9705-x
754
755 D'Ambrosio, J., Morvezen, R., Brard-Fudulea, S., Bestin, A., Perez, A.A., Guéméné, D., Poncet, C.,
756 Haffray, P., Dupont-Nivet, M., Phocas, F., 2020. Genetic architecture and genomic selection of
757 female reproduction traits in rainbow trout. BMC Genom. 21, 1-14.
758 http://dx.doi.org/10.1186/s12864-020-06955-7
759
760 Diaz, R.J., 2001. Overview of hypoxia around the world. J, Environ. Qual. 30, 275-281.
761 http://dx.doi.org/10.2134/jeq2001.302275x
762
763 Dickinson, M.E., Flenniken, A.M., Ji, X., Teboul, L., Wong, M.D., White, J.K., Meehan, T.F., Weninger,
764 W.J., Westerberg, H., Adissu, H., 2016. High-throughput discovery of novel developmental
765 phenotypes. Nature. 537, 508-514. http://dx.doi.org/10.1038/nature19356
766
767 Ding, J., Zhang, Y., Wang, J., Liu, C., Gao, X., Wu, Y., Wang, J., Wu, X., Zhu, J., Shen, W., 2022. Genome-
768 wide association study identified candidate SNPs and genes associated with hypoxia tolerance in
769 large yellow croaker (*Larimichthys crocea*). Aquaculture, 738472.
770 http://dx.doi.org/10.1016/j.aquaculture.2022.738472
771
772 Ding, P., Huang, J., Battiprolu, P.K., Hill, J.A., Kamm, K.E., Stull, J.T., 2010. Cardiac myosin light chain
773 kinase is necessary for myosin regulatory light chain phosphorylation and cardiac performance in
774 vivo. J. Biol. Chem. 285, 40819-40829. http://dx.doi.org/10.1074/jbc.M110.160499
775
776 Domenici, P., Lefrançois, C., Shingles, A., 2007. Hypoxia and the antipredator behaviours of fishes.
777 Philos. Trans. R. Soc. Lond., B, Biol. Sci. 362, 2105-2121. http://dx.doi.org/10.1098/rstb.2007.2103
778
779 Domenici, P., Herbert, N., Lefrançois, C., Steffensen, J., McKenzie, D., 2013. The effect of hypoxia on
780 fish swimming performance and behaviour, In: Palstra, A., Planas, J. (eds) Swimming Physiology of
781 Fish. Springer, Berlin, Heidelberg. pp. 129-159. https://doi.org/10.1007/978-3-642-31049-2_6
782
783 Dudchenko, A.M., Chernobaeva, G.N., Belousova, V.V., Vlasova, I.G., Luk'yanova, L.D., 1993.
784 Bioenergetic parameters of the brain in rats with different resistance to hypoxia. Bull. Exp. Bio.l
785 Med. 115, 263-267.
786 http://dx.doi.org/10.1007/BF00836406
787
788 Dzhalilova, D., Makarova, O., 2020. Differences in tolerance to hypoxia: physiological, biochemical, and
789 molecular- biological characteristics. Biomedicines. 8, 428. http://dx.doi.org/10.3390/biomedicine
790 s8100428
791
792 Eppig, J.T., 2017. Mouse genome informatics (MGI) resource: genetic, genomic, and biological
793 knowledgebase for the laboratory mouse. ILAR J. 58, 17-41. http://dx.doi.org/10.1093/ilar/ilx013
794

795 Esteve, C., Merchán, R., Alcaide, E., 2017. An outbreak of Shewanella putrefaciens group in wild eels
796 Anguilla anguilla L. favoured by hypoxic aquatic environments. J. Fish. Dis. 40, 929-939.
797 https://doi.org/10.1111/jfd.12574
798
799 Fago, A., Jensen, F.B., 2015. Hypoxia tolerance, nitric oxide, and nitrite: lessons from extreme animals.
800 Physiology. 30, 116-126. http://dx.doi.org/10.1152/physiol.00051.2014
801
802 FAO, 2020. The State of World Fisheries and Aquaculture 2020 – Sustainability in Action. Rome
803 https://www.fao.org/3/ca9229en/ca9229en.pdf (accessed 14 February 2022).
804
805 Farhat, E., Cheng, H., Romestaing, C., Pamenter, M., Weber, J.-M., 2021. Goldfish Response to Chronic
806 Hypoxia: Mitochondrial Respiration, Fuel Preference and Energy Metabolism. Metabolites. 11, 187.
807 http://dx.doi.org/10.3390/metabo11030187
808
809 Farrell, A.P., Richards, J.G., 2009. Chapter 11 Defining hypoxia: an integrative synthesis of the
810 responses of fish to hypoxia, Fish Physiol. 27, 487-503. https://doi.org/10.1016/S1546-
811 5098(08)00011-3
812
813 Fishback, A.G., Danzmann, R.G., Ferguson, M.M., Gibson, J.P., 2002. Estimates of genetic parameters
814 and genotype by environment interactions for growth traits of rainbow trout (*Oncorhynchus*
815 *mykiss*) as inferred using molecular pedigrees. Aquaculture. 206, 137-150.
816 http://dx.doi.org/10.1016/S0044-8486(01)00707-4
817
818 Fraslin, C., Phocas, F., Bestin, A., Charles, M., Bernard, M., Krieg, F., Dechamp, N., Ciobotaru, C., Hozé,
819 C., Petitprez, F., 2020a. Genetic determinism of spontaneous masculinisation in XX female rainbow
820 trout: new insights using medium throughput genotyping and whole-genome sequencing. Sci. Rep.
821 10, 1-13. http://dx.doi.org/10.1038/s41598-020-74757-8
822
823 Fraslin, C., Quillet, E., Rochat, T., Dechamp, N., Bernardet, J.-F., Collet, B., Lallias, D., Boudinot, P.,
824 2020b. Combining multiple approaches and models to dissect the genetic architecture of resistance
825 to infections in fish. Front. Genet. 11, 677. http://dx.doi.org/10.3389/fgene.2020.00677
826
827 Friedrich, J., Janssen, F., Aleynik, D., Bange, H.W., Boltacheva, N., Çagatay, M., Dale, A.W., Etiope, G.,
828 Erdem, Z., Geraga, M., 2014. Investigating hypoxia in aquatic environments: diverse approaches to
829 addressing a complex phenomenon. Biogeosciences. 11, 1215-1259. http://dx.doi.org/10.5194/bg-
830 11-1215-2014
831
832 Fu, X.W., Wang, D., Nurse, C.A., Dinauer, M.C., Cutz, E., 2000. NADPH oxidase is an O2 sensor in airway
833 chemoreceptors: evidence from K+ current modulation in wild-type and oxidase-deficient mice.
834 Proc. Natl. Acad. Sci. 97, 4374-4379. https://doi.org/10.1073/pnas.97.8.4374
835
836 Gack, M.U., Kirchhofer, A., Shin, Y.C., Inn, K.S., Liang, C., Cui, S., Myong, S., Ha, T., Hopfner, K.P., Jung,
837 J.U., 2008. Roles of RIG-I N-terminal tandem CARD and splice variant in TRIM25-mediated antiviral
838 signal transduction. Proc. Natl. Acad. Sci. 105, 16743-16748.
839 http://dx.doi.org/10.1073/pnas.0804947105
840

841 Gallage, S., Katagiri, T., Endo, M., Futami, K., Endo, M., Maita, M., 2016. Influence of moderate hypoxia
842 on vaccine efficacy against Vibrio anguillarum in Oreochromis niloticus (Nile tilapia). Fish Shellfish
843 Immunol. 51, 271-281. http://dx.doi.org/10.1016/j.fsi.2016.02.024
844
845 Gao, G., Magadan, S., Waldbieser, G.C., Youngblood, R.C., Wheeler, P.A., Scheffler, B.E., Thorgaard,
846 G.H., Palti, Y., 2021. A long reads-based de-novo assembly of the genome of the Arlee homozygous
847 line reveals chromosomal rearrangements in rainbow trout. G3-Genes Genom. Genet. 11, jkab052.
848 http://dx.doi.org/10.1093/g3journal/jkab052
849
850 Garcia, A., Pan, J., Lamsa, J., Muenzer, J., 2007. The characterization of a murine model of
851 mucopolysaccharidosis II (Hunter syndrome). J. Inherited Metab. Dis. 30, 924-934.
852 http://dx.doi.org/10.1007/s10545-007-0641-8
853
854 Gattuso, A., Garofalo, F., Cerra, M.C., Imbrogno, S., 2018. Hypoxia tolerance in teleosts: implications
855 of cardiac nitrosative signals. Front. Physiol. 9, 366. http://dx.doi.org/10.3389/fphys.2018.00366
856
857 Genz, J., Jyde, M., Svendsen, J.C., Steffensen, J.F., Ramløv, H., 2013. Excess post-hypoxic oxygen
858 consumption is independent from lactate accumulation in two cyprinid fishes. Comp. Biochem.
859 Physiol. Part A Mol. Integr. Physiol. 165, 54-60. http://dx.doi.org/10.1016/j.cbpa.2013.02.002
860
861 Giomi, F., Rinaldi, A., Mandich, A., Fuentes, V., Mirto, S., Sara, G., Piraino, S., 2016. Concurrent
862 environmental stressors and jellyfish stings impair caged European sea bass (*Dicentrarchus labrax*)
863 physiological performances. https://doi.org/10.1038/srep27929
864
865 Gjedrem, T., Rye, M., 2016. Selection response in fish and shellfish: a review. Rev. Aquac. 0, 1–12.
866 https://doi.org/10.1111/raq.12154.
867
868 Gordeuk, V.R., Zhang, X., Zhang, W., Ma, S.-F., Sable, C., Miasniakova, G., Sergueeva, A., Ammosova,
869 T., Xu, M., Nekhai, S., 2012. Novel Putative Polymorphism in SERPINC1 Encoding Antithrombin III Is
870 Implicated in Elevated Estimated Systolic Pulmonary Pressure in Patients with Chuvash
871 Polycythemia. Blood. 120, 2869. http://dx.doi.org/10.1182/blood.V120.21.2869.2869
872
873 Griot, R., Allal, F., Brard-Fudulea, S., Morvezen, R., Haffray, P., Phocas, F., Vandeputte, M., 2020. APIS:
874 An auto-adaptive parentage inference software that tolerates missing parents. Mol. Ecol. Resour.
875 20, 579-590. http://dx.doi.org/10.1111/1755-0998.13103
876
877 Griot, R., Allal, F., Phocas, F., Brard-Fudulea, S., Morvezen, R., Haffray, P., François, Y., Morin, T., Bestin,
878 A., Bruant, J.-S., 2021. Optimization of Genomic Selection to Improve Disease Resistance in Two
879 Marine Fishes, the European Sea Bass (*Dicentrarchus labrax*) and the Gilthead Sea Bream (*Sparus*
880 *aurata*). Front. Genet, 1294. http://dx.doi.org/10.3389/fgene.2021.665920
881
882 Gunia, M., Saintilan, R., Venot, E., Hoze, C., Fouilloux, M.N., Phocas, F., 2014. Genomic prediction in
883 French Charolais beef cattle using high-density single nucleotide polymorphism markers. J. Anim.
884 Sci. 92, 3258-3269. https://doi.org/10.2527/jas.2013-7478
885
886 Habier, D., Fernando, R.L., Kizilkaya, K., Garrick, D.J., 2011. Extension of the Bayesian alphabet for
887 genomic selection. BMC Bioinform. 12, 1-12. http://dx.doi.org/10.1186/1471-2105-12-186
888

Haffray, P., Vandeputte, M., Petit, V., Pincent, C., Chatain, B., Chapuis, H., Mériaux, J.-C., Coudurier, B., Quillet, E., Dupont-Nivet, M., 2012. Minimizing maternal effect in salmonid families mixed since eyed stages and a posteriori DNA-pedigreed. Livest. Sci. 150, 170-178. http://dx.doi.org/10.1016/j.livsci.2012.08.017

Hassinen, A., Khoder-Agha, F., Khosrowabadi, E., Mennerich, D., Harrus, D., Noel, M., Dimova, E.Y., Glumoff, T., Harduin-Lepers, A., Kietzmann, T., 2019. A Golgi-associated redox switch regulates catalytic activation and cooperative functioning of ST6Gal-I with B4GalT-I. Redox Biol. 24, 101182. http://dx.doi.org/10.1016/j.redox.2019.101182

Heidler, J., Al-Furoukh, N., Kukat, C., Salwig, I., Ingelmann, M.-E., Seibel, P., Krüger, M., Holtz, J., Wittig, I., Braun, T., 2011. Nitric oxide-associated protein 1 (NOA1) is necessary for oxygen-dependent regulation of mitochondrial respiratory complexes. J. Biol. Chem. 286, 32086-32093. http://dx.doi.org/10.1074/jbc.M111.221986

Ho, D.H., Burggren, W.W., 2012. Parental hypoxic exposure confers offspring hypoxia resistance in zebrafish (*Danio rerio*). J. Exp. Biol. 215, 4208-4216. http://dx.doi.org/10.1242/jeb.074781

Höglund, J.K., Sahana, G., Brøndum, R.F., Guldbrandtsen, B., Buitenhuis, B., Lund, M.S., 2014. Fine mapping QTL for female fertility on BTA04 and BTA13 in dairy cattle using HD SNP and sequence data. BMC Genom. 15, 1-10. http://dx.doi.org/10.1186/1471-2164-15-790

Hou, Z.-S., Wen, H.-S., Li, J.-F., He, F., Li, Y., Qi, X., 2020. Environmental hypoxia causes growth retardation, osteoclast differentiation and calcium dyshomeostasis in juvenile rainbow trout (*Oncorhynchus mykiss*). Sci. Total Environ. 705, 135272. http://dx.doi.org/10.1016/j.scitotenv.2019.135272

Houston, R.D., Bean, T.P., Macqueen, D.J., Gundappa, M.K., Jin, Y.H., Jenkins, T.L., Selly, S.L.C., Martin, S.A., Stevens, J.R., Santos, E.M., 2020. Harnessing genomics to fast-track genetic improvement in aquaculture. Nat. Rev. Genet. 21, 389-409. http://dx.doi.org/10.1038/s41576-020-0227-y

Hu, J., Chen, L., Yin, J., Yin, H., Huang, Y., Tian, J., 2020. Hyperactivity, memory defects, and craniofacial abnormalities in zebrafish fmr1 mutant larvae. Behav. Genet., 1-9. http://dx.doi.org/10.1007/s10519-020-09995-7

Chang, C.C., Chow, C.C., Tellier, L.C., Vattikuti, S., Purcell, S.M., Lee, J.J., 2015. Second-generation PLINK: rising to the challenge of larger and richer datasets. Gigascience. 4, 7.http://dx.doi.org/10.1186/s13742-015-0047-8

Chavanne, H., Janssen, K., Hofherr, J., Contini, F., Haffray, P., Komen, H., Nielsen, E.E., Bargelloni, L., Consortium, A., 2016. A comprehensive survey on selective breeding programs and seed market in the European aquaculture fish industry. Aquacult. Int. 24, 1287-1307. http://dx.doi.org/10.1007/s10499-016-9985-0

Chen, L., Toth, M., 2001. Fragile X mice develop sensory hyperreactivity to auditory stimuli. Neuroscience. 103, 1043-1050. http://dx.doi.org/10.1016/S0306-4522(01)00036-7

936 Chowdhury, S., Saikia, S., 2020. Oxidative stress in fish: a review. J. Sci. Res. 12, 145-160.
937 http://dx.doi.org/10.3329/jsr.v12i1.41716
938
939 Janssen, K., Chavanne, H., Berentsen, P., Komen, H., 2017. Impact of selective breeding on European
940 aquaculture. Aquaculture. 472, 8-16. https://doi.org/10.1016/j.aquaculture.2016.03.012.
941
942 Jaspers, R.T., Testerink, J., Della Gaspera, B., Chanoine, C., Bagowski, C.P., van der Laarse, W.J., 2014.
943 Increased oxidative metabolism and myoglobin expression in zebrafish muscle during chronic
944 hypoxia. Biol. Open. 3, 718-727. http://dx.doi.org/10.1242/bio.20149167
945
946 Kajimura, S., Aida, K., Duan, C., 2006. Understanding hypoxia-induced gene expression in early
947 development: in vitro and in vivo analysis of hypoxia-inducible factor 1-regulated zebra fish insulin-
948 like growth factor binding protein 1 gene expression. Mol. Cell. Biol. 26, 1142-1155.
949 http://dx.doi.org/10.1128/MCB.26.3.1142-1155.2006
950
951 Kanatous, S.B., Mammen, P.P., Rosenberg, P.B., Martin, C.M., White, M.D., DiMaio, J.M., Huang, G.,
952 Muallem, S., Garry, D.J., 2009. Hypoxia reprograms calcium signaling and regulates myoglobin
953 expression. Am. J. Physiol. Cell Physiol. 296, 393-402. http://dx.doi.org/10.1152/ajpcell.00428.2008
954
955 Khoder-Agha, F., Harrus, D., Brysbaert, G., Lensink, M.F., Harduin-Lepers, A., Glumoff, T., Kellokumpu,
956 S., 2019. Assembly of B4GALT1/ST6GAL1 heteromers in the Golgi membranes involves lateral
957 interactions via highly charged surface domains. J. Biol. Chem. 294, 14383-14393.
958 http://dx.doi.org/10.1074/jbc.RA119.009539
959
960 Kratz, E., Eimon, P., Mukhyala, K., Stern, H., Zha, J., Strasser, A., Hart, R., Ashkenazi, A., 2006. Functional
961 characterization of the Bcl-2 gene family in the zebrafish. Cell Death Differ. 13, 1631-1640.
962 http://dx.doi.org/10.1038/sj.cdd.4402016
963
964 Kriaridou, C., Tsairidou, S., Houston, R.D., Robledo, D., 2020. Genomic prediction using low density
965 marker panels in aquaculture: performance across species, traits, and genotyping platforms. Front.
966 Genet. 11, 124. http://dx.doi.org/10.3389/fgene.2020.00124
967
968 Lagarde, H., Prchal, M., Pouil, S., Goardon, L., Bideau, M., Guyvarc'h, F., Labbé, L., Dechamp, N., Phocas,
969 D., Dupont-Nivet, M., Lallias, D., Submitted. Are resistances to acute hyperthermia or hypoxia stress
970 similar and consistent between early and late ages in rainbow trout using isogenic lines?
971
972 Léger, J.A., Athanasio, C.G., Zhera, A., Chauhan, M.F., Simmons, D.B., 2021. Hypoxic responses in
973 *Oncorhynchus mykiss* involve angiogenesis, lipid, and lactate metabolism, which may be triggered
974 by the cortisol stress response and epigenetic methylation. Comp. Biochem. Physiol. Part D
975 Genomics Proteomics. 39, 100860. http://dx.doi.org/10.1016/j.cbd.2021.100860
976
977 Li, H.L., Gu, X.H., Li, B.J., Chen, C.H., Lin, H.R., Xia, J.H., 2017. Genome-wide QTL analysis identified
978 significant associations between hypoxia tolerance and mutations in the GPR132 and ABCG4 genes
979 in Nile tilapia. Mar. Biotechnol. 19, 441-453. http://dx.doi.org/10.1007/s10126-017-9762-8
980
981 Li, M., Wang, X., Qi, C., Li, E., Du, Z., Qin, J.G., Chen, L., 2018. Metabolic response of Nile tilapia
982 (*Oreochromis niloticus*) to acute and chronic hypoxia stress. Aquaculture. 495, 187-195.
983 http://dx.doi.org/10.1016/j.aquaculture.2018.05.031

Liang, H., Studach, L., Hullinger, R.L., Xie, J., Andrisani, O.M., 2014. Down-regulation of RE-1 silencing transcription factor (REST) in advanced prostate cancer by hypoxia-induced miR-106b~ 25. Exp. Cell Res. 320, 188-199. http://dx.doi.org/10.1016/j.yexcr.2013.09.020

Liu, J., Peng, W., Yu, F., Shen, Y., Yu, W., Lu, Y., Lin, W., Zhou, M., Huang, Z., Luo, X., 2022. Genomic selection applications can improve the environmental performance of aquatics: a case study on the heat tolerance of abalone. Evol. Appl. 5, 992-1001 http://dx.doi.org/10.1111/eva.13388

Luk'yanova, L.D., Chernobaeva, G.N., Romanova, V.E., 1995. Effects of adaptation to intermittent hypoxia on oxidative phosphorylation in brain mitochondria of rats with different sensitivities toward oxygen deficiency. Bull. Exp. Biol. Med. 120, 1189-1192. http://dx.doi.org/10.1007/BF02445567

Ma, X., Dai, W., Kang, J., Yang, L., He, S., 2016. Comprehensive transcriptome analysis of six catfish species from an altitude gradient reveals adaptive evolution in Tibetan fishes. G3-Genes Genom. Genet. 6, 141-148. https://doi.org/10.1534/g3.115.024448

Mahfouz, M.E., Hegazi, M.M., El-Magd, M.A., Kasem, E.A., 2015. Metabolic and molecular responses in Nile tilapia, Oreochromis niloticus during short and prolonged hypoxia. Mar. Freshwat. Behav. Physiol. 48, 319-340. https://doi.org/10.1080/10236244.2015.1055915

Matsuda, H., Hamet, P., Tremblay, J., 2014. Hypertension-related, calcium-regulated gene (HCaRG/COMMD5) and kidney diseases: HCaRG accelerates tubular repair. J. Nephrol. 27, 351-360. http://dx.doi.org/10.1007/s40620-014-0054-3

McBryan, T., Anttila, K., Healy, T., Schulte, P., 2013. Responses to temperature and hypoxia as interacting stressors in fish: implications for adaptation to environmental change. Integr. Comp. Biol. 53, 648-659. http://dx.doi.org/10.1093/icb/ict066

McCarty, A.J., Allen Jr, S.K., Plough, L.V., 2022. Genome-wide analysis of acute low salinity tolerance in the eastern oyster Crassostrea virginica and potential of genomic selection for trait improvement. G3-Genes Genom. Genet. 12, jkab368. http://dx.doi.org/10.1093/g3journal/jkab368

Michenet, A., Barbat, M., Saintilan, R., Venot, E., Phocas, F., 2016. Detection of quantitative trait loci for maternal traits using high-density genotypes of Blonde d'Aquitaine beef cattle. BMC Genet. 17, 1-13. http://dx.doi.org/10.1186/s12863-016-0397-y

Misztal, I., Tsuruta, S., Strabel, T., Auvray, B., Druet, T., Lee, D., 2002. BLUPF90 and related programs (BGF90). 7th WCGALP 28, 743.

Misztal, I., Tsuruta, S., Lourenco, Masuda, Y., Aguilar, I., Legarra, A., Vitezica, Z., 2014. Manual for BLUPF90 family of programs., University of Georgia, Athens, USA. pp. 142. http://nce.ads.uga.edu /wiki/lib/exe/fetch.php?media=blupf90_all7.pdf (accessed 4 April 2021).

Mustafa, S.A., Karieb, S.S., Davies, S.J., Jha, A.N., 2015. Assessment of oxidative damage to DNA, transcriptional expression of key genes, lipid peroxidation and histopathological changes in carp *Cyprinus carpio* L. following exposure to chronic hypoxic and subsequent recovery in normoxic conditions. Mutagenesis. 30, 107-116. https://doi.org/10.1093/mutage/geu048

Nagy, A., Csanyi, V., Bakos, J., Horvath, L., 1980. Development of a short-term laboratory system for the evaluation of carp growth in ponds. Bamidgeh. 32, 6-15.

Nayeri, S., Sargolzaei, M., Abo-Ismail, M.K., May, N., Miller, S.P., Schenkel, F., Moore, S.S., Stothard, P., 2016. Genome-wide association for milk production and female fertility traits in Canadian dairy Holstein cattle. BMC Genet. 17, 1-11. https://doi.org/10.1186/s12863-016-0386-1

Nikinmaa, M., Rees, B.B., 2005. Oxygen-dependent gene expression in fishes. Am. J. Physiol. Regul. Integr. Comp. Physiol. 288, 1079-1090. http://dx.doi.org/10.1152/ajpregu.00626.2004

Olsvik, P.A., Vikeså, V., Lie, K.K., Hevrøy, E.M., 2013. Transcriptional responses to temperature and low oxygen stress in Atlantic salmon studied with next-generation sequencing technology. BMC Genom. 14, 1-21. https://doi.org/10.1186/1471-2164-14-817

Omlin, T., Weber, J.-M., 2010. Hypoxia stimulates lactate disposal in rainbow trout. J. Exp. Biol. 213, 3802-3809. http://dx.doi.org/10.1242/jeb.048512

Palaiokostas, C., Robledo, D., Vesely, T., Prchal, M., Pokorova, D., Piackova, V., Pojezdal, L., Kocour, M., Houston, R.D., 2018. Mapping and Sequencing of a Significant Quantitative Trait Locus Affecting Resistance to Koi Herpesvirus in Common Carp. G3-Genes Genom. Genet. 8, 3507-3513. http://dx.doi.org/10.1534/g3.118.200593

Palti, Y., Gao, G., Liu, S., Kent, M., Lien, S., Miller, M., Rexroad III, C., Moen, T., 2015. The development and characterization of a 57 K single nucleotide polymorphism array for rainbow trout. Mol. Ecol. Resour. 15, 662-672. https://doi.org/10.1111/1755-0998.12337

Pearse, D.E., Barson, N.J., Nome, T., Gao, G., Campbell, M.A., Abadía-Cardoso, A., Anderson, E.C., Rundio, D.E., Williams, T.H., Naish, K.A., 2019. Sex-dependent dominance maintains migration supergene in rainbow trout. Nat. Ecol. Evol. 3, 1731-1742. http://dx.doi.org/10.1038/s41559-019-1044-6

Pelster, B., Egg, M., 2018. Hypoxia-inducible transcription factors in fish: expression, function and interconnection with the circadian clock. J. Exp. Biol. 221, jeb163709. http://dx.doi.org/10.1371/journal.pone.0017073

Pietropaolo, S., Guilleminot, A., Martin, B., d'Amato, F.R., Crusio, W.E., 2011. Genetic-background modulation of core and variable autistic-like symptoms in Fmr1 knock-out mice. PLoS ONE. 6, e17073. http://dx.doi.org/10.1371/journal.pone.0017073

Piferrer, F., Beaumont, A., Falguière, J.-C., Flajšhans, M., Haffray, P., Colombo, L., 2009. Polyploid fish and shellfish: production, biology and applications to aquaculture for performance improvement and genetic containment. Aquaculture. 293, 125-156. http://dx.doi.org/10.1016/j.aquaculture.2009.04.036

Polakof, S., Skiba-Cassy, S., Kaushik, S., Seiliez, I., Soengas, J.L., Panserat, S., 2012. Glucose and lipid metabolism in the pancreas of rainbow trout is regulated at the molecular level by nutritional status and carbohydrate intake. J. Comp. Physiol. B. 182, 507-516. https://doi.org/10.1007/s00360-011-0636-5

Poon, W., Hung, C., Nakano, K., Randall, D., 2007. An in vivo study of common carp (Cyprinus carpio L.) liver during prolonged hypoxia. Comp. Biochem. Physiol. Part D Genomics Proteomics 2, 295-302. http://dx.doi.org/10.1016/j.cbd.2007.05.002

Qi, D., Chao, Y., Zhao, Y., Xia, M., Wu, R., 2018. Molecular evolution of myoglobin in the Tibetan Plateau endemic schizothoracine fish (Cyprinidae, Teleostei) and tissue-specific expression changes under hypoxia. Fish Physiol. Biochem. 44, 557-571. http://dx.doi.org/10.1007/s10695-017-0453-1

Qi, M., Wu, Q., Liu, T., Hou, Y., Miao, Y., Hu, M., Liu, Q., 2020. Hepatopancreas transcriptome profiling analysis reveals physiological responses to acute hypoxia and reoxygenation in juvenile Qingtian paddy field carp *Cyprinus carpio* var qingtianensis. Front. Physiol. 11. http://dx.doi.org/10.3389/fphys.2020.01110

R2D2 Consortium, Fugeray-Scarbel, A., Bastien, C., Dupont-Nivet, M., Lemarié, S., 2021. Why and how to switch to genomic selection: lessons from plant and animal breeding experience. Front Genet. 12, 629737. https://doi.org/10.3389/fgene.2021.629737

Ragsdale, A., Ortega-Recalde, O., Dutoit, L., Besson, A.A., Chia, J.H., King, T., Nakagawa, S., Hickey, A., Gemmell, N.J., Hore, T., 2020. Paternal hypoxia exposure primes offspring for increased hypoxia resistance. bioRxiv. https://doi.org/10.1101/2020.12.09.416727

Rashid, I., Nagpure, N.S., Srivastava, P., Kumar, R., Pathak, A.K., Singh, M., Kushwaha, B., 2017. HRGFish: A database of hypoxia responsive genes in fishes. Sci. Rep. 7, 1-9. http://dx.doi.org/10.1038/srep42346

Rehn, M., Olsson, A., Reckzeh, K., Diffner, E., Carmeliet, P., Landberg, G., Cammenga, J., 2011. Hypoxic induction of vascular endothelial growth factor regulates murine hematopoietic stem cell function in the low-oxygenic niche. Blood. 118, 1534-1543. https://doi.org/10.1182/blood-2011-01-332890

Reid, G.K., Gurney-Smith, H.J., Flaherty, M., Garber, A.F., Forster, I., Brewer-Dalton, K., Knowler, D., Marcogliese, D.J., Chopin, T., Moccia, R.D., 2019. Climate change and aquaculture: considering adaptation potential. Aquac. Environ. Interact. 11, 603-624. http://dx.doi.org/10.3354/aei00333

Richards, J.G., 2011. Physiological, behavioral and biochemical adaptations of intertidal fishes to hypoxia. J. Exp. Biol. 214, 191-199. http://dx.doi.org/10.1242/jeb.047951

Robledo, D., Palaiokostas, C., Bargelloni, L., Martínez, P., Houston, R., 2017. Applications of genotyping by sequencing in aquaculture breeding and genetics. Rev. Aquac. 0, 1-13. http://dx.doi.org/10.1111/raq.12193

Rossignol, R., Ranchon-Cole, I., Pâris, A., Herzine, A., Perche, A., Laurenceau, D., Bertrand, P., Cercy, C., Pichon, J., Mortaud, S., 2014. Visual sensorial impairments in neurodevelopmental disorders: evidence for a retinal phenotype in Fragile X Syndrome. PLoS ONE. 9, e105996. http://dx.doi.org/10.1371/journal.pone.0105996

Royer, E., Faccenda, F., Pastres, R., 2021. Estimating oxygen consumption of rainbow trout (*Oncorhynchus mykiss*) in a raceway: A Precision Fish Farming approach. Aquacult. Eng. 92, 102141. http://dx.doi.org/10.1016/j.aquaeng.2020.102141

Sae-Lim, P., Kause, A., Mulder, H., Olesen, I., 2017. Breeding and genetics symposium: Climate change and selective breeding in aquaculture. J. Anim. Sci. 95, 1801-1812. http://dx.doi.org/10.2527/jas2016.1066

Sahoo, S., Meijles, D.N., Pagano, P.J., 2016. NADPH oxidases: key modulators in aging and age-related cardiovascular diseases? Clin. Sci. 130, 317-335. http://dx.doi.org/10.1042/CS20150087

San, L.-Z., Liu, B.-S., Liu, B., Zhu, K.-C., Guo, L., Guo, H.-Y., Zhang, N., Jiang, S.-G., Zhang, D.-C., 2021. Genome-wide association study reveals multiple novel SNPs and putative candidate genes associated with low oxygen tolerance in golden pompano *Trachinotus ovatus* (Linnaeus 1758). Aquaculture. 544, 737098. https://doi.org/10.1016/j.aquaculture.2021.737098

Sargolzaei, M., Chesnais, J.P., Schenkel, F.S., 2014. A new approach for efficient genotype imputation using information from relatives. BMC Genom. 15, 1-12. http://dx.doi.org/10.1186/1471-2164-15-478

Scott, M.A., Dhillon, R.S., Schulte, P.M., Richards, J.G., 2015. Physiology and performance of wild and domestic strains of diploid and triploid rainbow trout (Oncorhynchus mykiss) in response to environmental challenges. Can. J. Fish. Aquat. Sci. 72, 125-134. http://dx.doi.org/10.1139/cjfas-2013-0450

Schwander, T., Libbrecht, R., Keller, L., 2014. Supergenes and complex phenotypes. Curr. Biol. 24, 288-294. http://dx.doi.org/10.1016/j.cub.2014.01.056

Sonesson, A.K., Meuwissen, T.H., 2009. Testing strategies for genomic selection in aquaculture breeding programs. Genet. Sel. Evol. 41, 1-9. http://dx.doi.org/10.1186/1297-9686-41-37

Song, H., Ye, S., Jiang, Y., Zhang, Z., Zhang, Q., Ding, X., 2019. Using imputation-based whole-genome sequencing data to improve the accuracy of genomic prediction for combined populations in pigs. Genet. Sel. Evol. 51, 1-13. http://dx.doi.org/10.1186/s12711-019-0500-8

Sprague, J., Bayraktaroglu, L., Clements, D., Conlin, T., Fashena, D., Frazer, K., Haendel, M., Howe, D.G., Mani, P., Ramachandran, S., 2006. The Zebrafish Information Network: the zebrafish model organism database. Nucleic Acids Res. 34, 581-585. http://dx.doi.org/10.1093/nar/gkj086

Strowbridge, N., Northrup, S.L., Earhart, M.L., Blanchard, T.S., Schulte, P.M., 2021. Acute measures of upper thermal and hypoxia tolerance are not reliable predictors of mortality following environmental challenges in rainbow trout (*Oncorhynchus mykiss*). Conserv. Physiol. 9, 1-16. https://doi.org/10.1093/conphys/coab095

Tamura, K., Yoshie, M., Hashimoto, K., Tachikawa, E., 2014. Inhibitory effect of insulin-like growth factor-binding protein-7 (IGFBP7) on in vitro angiogenesis of vascular endothelial cells in the rat corpus luteum. J. Reprod. Dev. 60, 447-453. https://doi.org/10.1262/jrd.2014-069

Tang, T., Zheng, B., Chen, S.-h., Murphy, A.N., Kudlicka, K., Zhou, H., Farquhar, M.G., 2009. hNOA1 interacts with complex I and DAP3 and regulates mitochondrial respiration and apoptosis. J. Biol. Chem. 284, 5414-5424. http://dx.doi.org/10.1074/jbc.M807797200

Thomas, Y., Flye-Sainte-Marie, J., Chabot, D., Aguirre-Velarde, A., Marques, G.M., Pecquerie, L., 2019. Effects of hypoxia on metabolic functions in marine organisms: Observed patterns and modelling assumptions within the context of Dynamic Energy Budget (DEB) theory. J. Sea Res. 143, 231-242. https://doi.org/10.1016/j.seares.2018.05.001

Tian, Y., Wen, H., Qi, X., Zhang, X., Li, Y., 2019. Identification of mapk gene family in Lateolabrax maculatus and their expression profiles in response to hypoxia and salinity challenges. Gene. 684, 20-29. http://dx.doi.org/10.1016/j.gene.2018.10.033

Townhill, B.L., van der Molen, J., Metcalfe, J.D., Simpson, S.D., Farcas, A., Pinnegar, J.K., 2017. Consequences of climate-induced low oxygen conditions for commercially important fish. Mar. Ecol. Prog. Ser. 580, 191-204. http://dx.doi.org/10.3354/meps12291

Tsai, H.-Y., Hamilton, A., Tinch, A.E., Guy, D.R., Bron, J.E., Taggart, J.B., Gharbi, K., Stear, M., Matika, O., Pong-Wong, R., 2016. Genomic prediction of host resistance to sea lice in farmed Atlantic salmon populations. Genet. Sel. Evol. 48, 1-11. http://dx.doi.org/10.1186/s12711-016-0226-9

Vallejo, R.L., Leeds, T.D., Gao, G., Parsons, J.E., Martin, K.E., Evenhuis, J.P., Fragomeni, B.O., Wiens, G.D., Palti, Y., 2017. Genomic selection models double the accuracy of predicted breeding values for bacterial cold water disease resistance compared to a traditional pedigree-based model in rainbow trout aquaculture. Genet. Sel. Evol. 49, 1-13. http://dx.doi.org/10.1186/s12711-017-0293-6

Vallejo, R.L., Silva, R.M., Evenhuis, J.P., Gao, G., Liu, S., Parsons, J.E., Martin, K.E., Wiens, G.D., Lourenco, D.A., Leeds, T.D., 2018. Accurate genomic predictions for BCWD resistance in rainbow trout are achieved using low-density SNP panels: Evidence that long-range LD is a major contributing factor. J. Anim. Breed. Genet. 135, 263-274. http://dx.doi.org/10.1111/jbg.12335

Van Binsbergen, R., Calus, M.P., Bink, M.C., van Eeuwijk, F.A., Schrooten, C., Veerkamp, R.F., 2015. Genomic prediction using imputed whole-genome sequence data in Holstein Friesian cattle. Genet. Sel. Evol. 47, 1-13. http://dx.doi.org/10.1186/s12711-015-0149-x

Van Raaij, M., Van den Thillart, G., Vianen, G., Pit, D., Balm, P., Steffens, A., 1996. Substrate mobilization and hormonal changes in rainbow trout (*Oncorhynchus mykiss,* L.) and common carp (*Cyprinus carpio*, L.) during deep hypoxia and subsequent recovery. J. Comp. Physiol. B. 166, 443-452. https://doi.org/10.1007/BF02337889

VanRaden, P.M., 2008. Efficient methods to compute genomic predictions. J. Dairy Sci. 91, 4414-4423. http://dx.doi.org/10.3168/jds.2007-0980

Wang, X., Liu, S., Jiang, C., Geng, X., Zhou, T., Li, N., Bao, L., Li, Y., Yao, J., Yang, Y., 2017. Multiple across-strain and within-strain QTLs suggest highly complex genetic architecture for hypoxia tolerance in channel catfish. Mol. Genet. Genomics. 292, 63-76. http://dx.doi.org/10.1007/s00438-016-1256-2

Wawrowski, A., Gerlach, F., Hankeln, T., Burmester, T., 2011. Changes of globin expression in the Japanese medaka (*Oryzias latipes*) in response to acute and chronic hypoxia. J. Comp. Physiol. B. 181, 199-208. http://dx.doi.org/10.1007/s00360-010-0518-2

1223 Wischhusen, P., Larroquet, L., Durand, T., Oger, C., Galano, J.-M., Rocher, A., Vigor, C., Prabhu, P.A.J.,
1224 Véron, V., Briens, M., 2020. Oxidative stress and antioxidant response in rainbow trout fry exposed
1225 to acute hypoxia is affected by selenium nutrition of parents and during first exogenous feeding.
1226 Free Radical Biol. Med. 155, 99-113. http://dx.doi.org/10.1016/j.freeradbiomed.2020.05.006
1227
1228 Wolf, J.B., Wade, M.J., 2009. What are maternal effects (and what are they not)? Philosophical Philos.
1229 Trans. R. Soc. Lond., B, Biol. Sci. 364, 1107-1115. http://dx.doi.org/10.1098/rstb.2008.0238
1230
1231 Wu, X., Lund, M.S., Sahana, G., Guldbrandtsen, B., Sun, D., Zhang, Q., Su, G., 2015. Association analysis
1232 for udder health based on SNP-panel and sequence data in Danish Holsteins. Genet. Sel. Evol. 47,
1233 1-14. http://dx.doi.org/10.1186/s12711-015-0129-1
1234
1235 Xu, W., Chen, D., Yan, G., Xiao, S., Huang, T., Zhang, Z., Huang, L., 2019. Rediscover and refine QTLs for
1236 pig scrotal hernia by increasing a specially designed F3 population and using whole-genome
1237 sequence imputation technology. Front. Genet. 10, 890.
1238 http://dx.doi.org/10.3389/fgene.2019.00890
1239
1240 Yan, G., Qiao, R., Zhang, F., Xin, W., Xiao, S., Huang, T., Zhang, Z., Huang, L., 2017. Imputation-based
1241 whole-genome sequence association study rediscovered the missing QTL for lumbar number in
1242 Sutai pigs. Sci Rep. 7, 1-10. http://dx.doi.org/10.1038/s41598-017-00729-0
1243
1244 Yáñez, J.M., Houston, R.D., Newman, S., 2014. Genetics and genomics of disease resistance in salmonid
1245 species. Front. Genet. 5, 415. https://doi.org/10.3389/fgene.2014.00415
1246
1247 Yoshida, G.M., Bangera, R., Carvalheiro, R., Correa, K., Figueroa, R., Lhorente, J.P., Yáñez, J.M., 2018a.
1248 Genomic prediction accuracy for resistance against *Piscirickettsia salmonis* in farmed rainbow trout.
1249 G3-Genes Genom. Genet. 8, 719-726. http://dx.doi.org/10.1534/g3.117.300499
1250
1251 Yoshida, G.M., Carvalheiro, R., Lhorente, J.P., Correa, K., Figueroa, R., Houston, R.D., Yáñez, J.M.,
1252 2018b. Accuracy of genotype imputation and genomic predictions in a two-generation farmed
1253 Atlantic salmon population using high-density and low-density SNP panels. Aquaculture. 491, 147-
1254 154. http://dx.doi.org/10.1016/j.aquaculture.2018.03.004
1255
1256 Yoshida, G.M., Lhorente, J.P., Correa, K., Soto, J., Salas, D., Yáñez, J.M., 2019. Genome-wide association
1257 study and cost-efficient genomic predictions for growth and fillet yield in Nile tilapia (*Oreochromis*
1258 *niloticus*). G3-Genes Genom. Genet. 9, 2597-2607. http://dx.doi.org/10.1534/g3.119.400116
1259
1260 Yoshida, G.M., Yáñez, J.M., 2021. Multi-trait GWAS using imputed high-density genotypes from whole-
1261 genome sequencing identifies genes associated with body traits in Nile tilapia. BMC Genom. 22, 57.
1262 http://dx.doi.org/10.1186/s12864-020-07341-z
1263
1264 Yoshida, G.M., Yáñez, J.M., 2022. Increased accuracy of genomic predictions for growth under chronic
1265 thermal stress in rainbow trout by prioritizing variants from GWAS using imputed sequence data.
1266 Evol. Appl. 15, 537-552 http://dx.doi.org/10.1111/eva.13240
1267 Yu, X., Megens, H.-J., Mengistu, S.B., Bastiaansen, J.W., Mulder, H.A., Benzie, J.A., Groenen, M.A.,
1268 Komen, H., 2021. Genome-wide association analysis of adaptation to oxygen stress in Nile tilapia
1269 (*Oreochromis niloticus*). BMC Genom. 22, 1-13. http://dx.doi.org/10.1186/s12864-021-07486-5

Yuan, Z., Liu, S., Yao, J., Zeng, Q., Tan, S., Liu, Z., 2016. Expression of Bcl-2 genes in channel catfish after bacterial infection and hypoxia stress. Dev. Comp. Immunol. 65, 79-90. http://dx.doi.org/10.1016/j.dci.2016.06.018

Zhao, J., Prchal, M., Kause, A., Vandeputte, M., Gela, D., Kroupová, H.K., Piačková, V., Šauer, P., Steinbach, C., Allamellou, J.-M., 2021. The role of energy reserves in common carp performance inferred from phenotypic and genetic parameters. Aquaculture. 541, 736799. http://dx.doi.org/10.1016/j.aquaculture.2021.736799

Zhong, X., Wang, X., Zhou, T., Jin, Y., Tan, S., Jiang, C., Geng, X., Li, N., Shi, H., Zeng, Q., 2017. Genome-wide association study reveals multiple novel QTL associated with low oxygen tolerance in hybrid catfish. Mar. Biotechnol. 19, 379-390. http://dx.doi.org/10.1007/s10126-017-9757-5

Zhu, C.-D., Wang, Z.-H., Yan, B., 2013. Strategies for hypoxia adaptation in fish species: a review. J. Comp. Physiol. B. 183, 1005-1013. http://dx.doi.org/10.1007/s00360-013-0762-3

Table 1. Summary statistics of average body weight and hypoxia tolerance phenotypes and dissolved oxygen levels during hypoxia challenge trials

	Phenotypes			Dissolved oxygen level mg/L^{-1}			
Trial	***N*[a]**	**BW (g)[b]**	**TLE (min.)[c]**	**Initial**	**First fish**	**50%**	**Last fish**
1.	202	46.1±11.1	100.1±65.0	9.4	2.2	1.6	1.2
2.	205	47.3±10.6	65.3±60.1	9.6	2.1	1.5	1.3
3.	202	47.4±10.7	111.3±80.0	9.1	2.3	1.6	1.2
4.	203	52.9±11.8	115.7±64.4	9.3	4.4	1.7	1.2
5.	206	52.5±10.7	74.6±71.0	8.8	2.4	1.7	1.5
6.	217	55.3±11.5	129.3±75.2	8.7	5.9	1.9	1.3
7.	85	57.4±12.7	89.8±44.2	9.6	5.1	2.2	1.5
Mean	189	50.8±11.8	98.9±71.8	9.2	3.5	1.7	1.3

[a] number of fish

[b] body weight

[c] time to loss of equilibrium

Table 2. Variance components for acute hypoxia tolerance in rainbow trout estimated with three models (BLUP, GBLUP, Bayes Cπ) and two genotype densities (MD, HD)

Model	σ_a^2	σ_m^2	σ_e^2	h^2	m^2
BLUP	1332	416	2984	0.28 (0.09)	0.09 (0.04)
GBLUP_MD	1126	481	3081	0.24 (0.05)	0.10 (0.03)
GBLUP_HD	1118	471	3100	0.24 (0.05)	0.10 (0.03)
Bayes Cπ_MD	1054	502	3218	0.22 (0.04)	0.11 (0.03)
Bayes Cπ_HD	1084	484	3215	0.23 (0.04)	0.10 (0.03)

σ_a^2: additive genetic variance; σ_m^2: maternal variance; σ_e^2: residual variance; h^2: heritability (standard error); m^2**:** proportion of the phenotypic variance explained by maternal effect (standard error)

Table 3. Mean ± standard deviation over 100 replicates of the selection accuracy (r) and inflation coefficient (b) for BLUP and GBLUP evaluation for hypoxia tolerance using MD or HD genotypes

	r	b
BLUP	0.594 ± 0.079	1.456 ± 0.260
GBLUP_MD	0.656 ± 0.072	1.121 ± 0.174
GBLUP_HD	0.653 ± 0.071	1.202 ± 0.172

Table 4. Summary statistics for GWAS for acute hypoxia tolerance in rainbow trout using Bayes Cπ method

Chr.	Peak SNP	Peak position (pb)	MAF	logBF	QTL start (Mb)	QTL end (Mb)	Var (%)	Homozygote standardized TLE difference[A]	Standardized TLE dominance effect[B]
Omy15	Affx-1237442714	17668393	0.32	5.16	17.58	18.32	0.22	82.07	6.99
Omy20	Affx-569843766	22880000	0.42	6.14	22.62	22.99	0.19	59.88	-1.67
Omy28	Affx-1237683396	15091174	0.49	5.13	14.92	15.09	0.17	90.27	38.15
Omy31_1	Affx-569844518	20875730	0.22	7.37	19.93	21.56	0.78	118.54	-9.73
Omy31_2	Affx-1237632075	33812474	0.49	6.27	33.43	33.97	0.33	56.53	13.37

Chr.: Chromosome; **logBF:** Twice the natural logarithm of the Bayes Factor; **MAF:** Minor allele frequency; **Var(%):** The percentage of genetic variance explained by the QTL

[A] the performance difference between the two homozygous genotypes (BB-AA) expressed in % of genetic standard deviation

[B] difference between the performance of the heterozygote genotype (AB) and the average performance of the two homozygous genotypes (AA+BB)/2 expressed in % of genetic standard deviation

Figure Captions

Fig. 1. The cumulative loss of equilibrium in response to hypoxia challenge across seven trials. Time represents the time since the first fish lost its equilibrium.

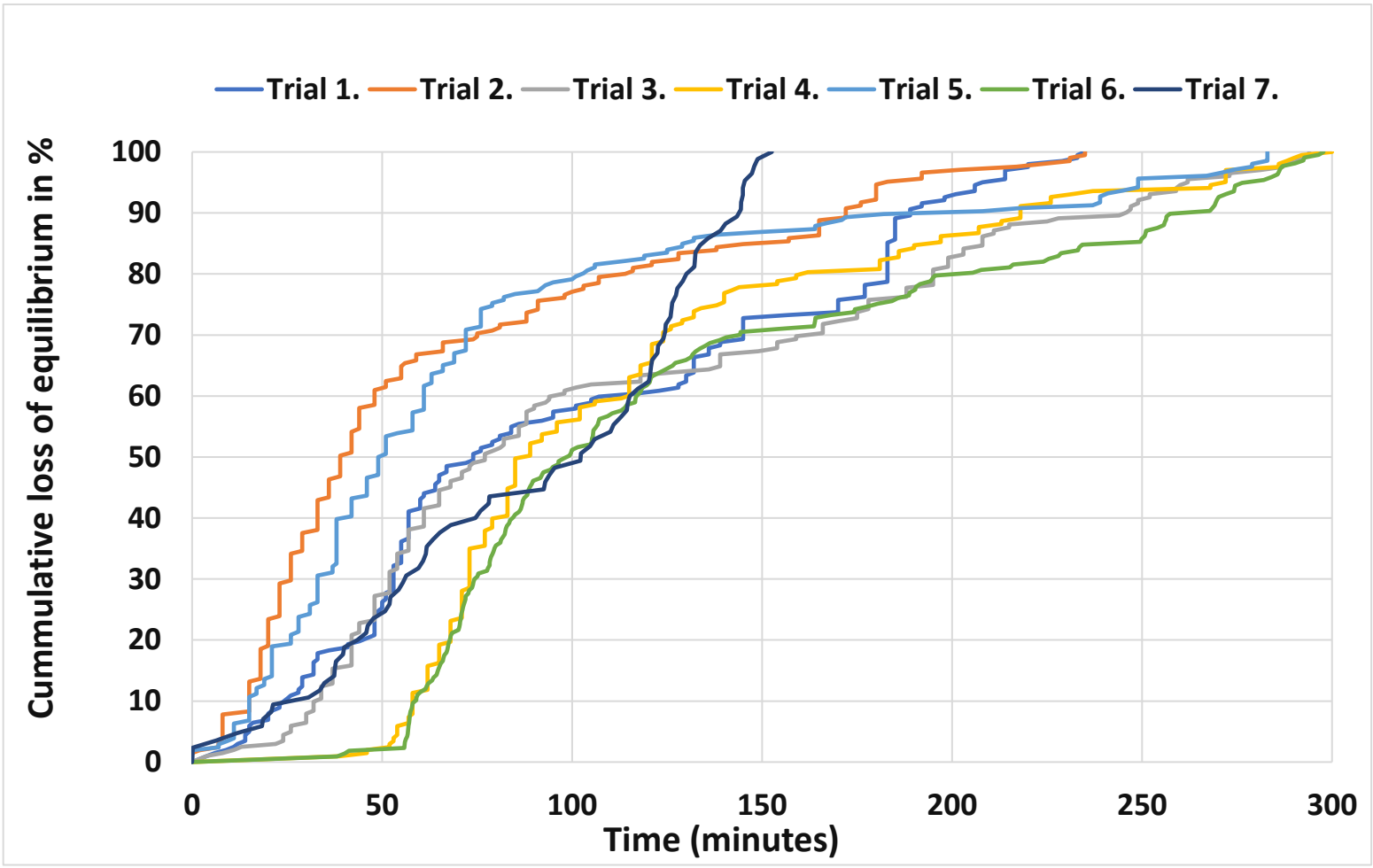
Fig. 2. Boxplot of accuracy difference of GBLUP versus BLUP of hypoxia tolerance for each of 100 replicates.

Fig. 3. Manhattan plots of QTL detected under Bayesian GWAS for hypoxia tolerance. The dashed red line corresponds to the threshold logBF >6 for defining evidence for a significant QTL and dashed blue line corresponds to the threshold logBF >5 for defining evidence for a putative QTL.

Fig. 4. Annotated genes within the main QTL region on Omy31_1. The red dots correspond to significant SNPs that have logBF >6 (dashed red line). The positions of the five genes located within the QTL region are figured by rectangles of a different colour: *slitrk4* (SLIT and NTRK-like protein 4), *slitrk2* (SLIT and NTRK-like protein 2), *fmr1 (*synaptic functional regulator FMR1), *aff2* (AF4/FMR2 family member 2), *ids* (iduronate 2-sulfatase), *cracdl* (CRACD-like protein isoform), *lonrf3* (LON peptidase N-terminal domain and RING finger protein 3), *commd5* (COMM domain-containing protein 5), *fam199x* (protein FAM199X), *arx* (homeobox protein ARX-like), *il1rapl2* (X-linked interleukin-1 receptor accessory protein-like 2), *map4k4 (mitogen-activated protein kinase 4).*

Fig. 5 Violin box plots of time to loss of equilibrium (TLE) with different genotypes of peak SNPs at QTLs located on Omy31_1 (A) and Omy28 (B). The vertical axis is the TLE of the individuals, and different colours represent different genotypes.

1333 **Fig 1.**



1334

1335 **Fig 2.**

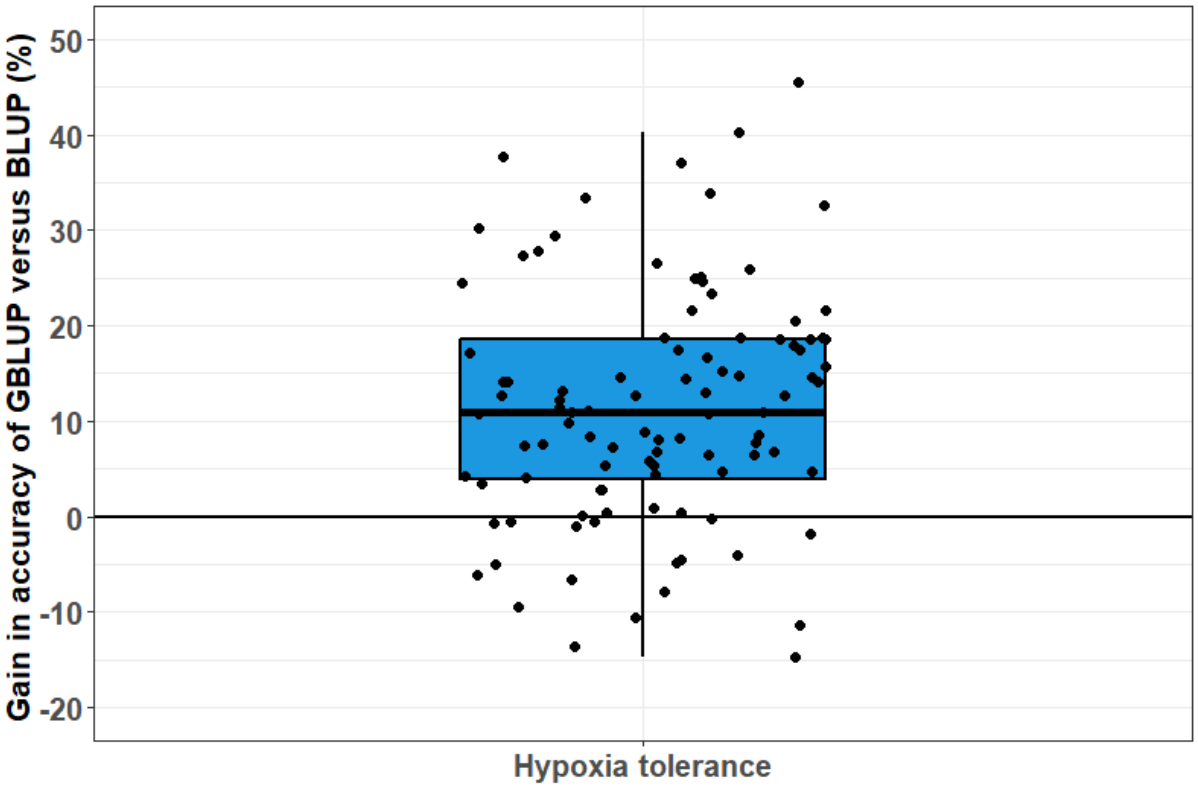
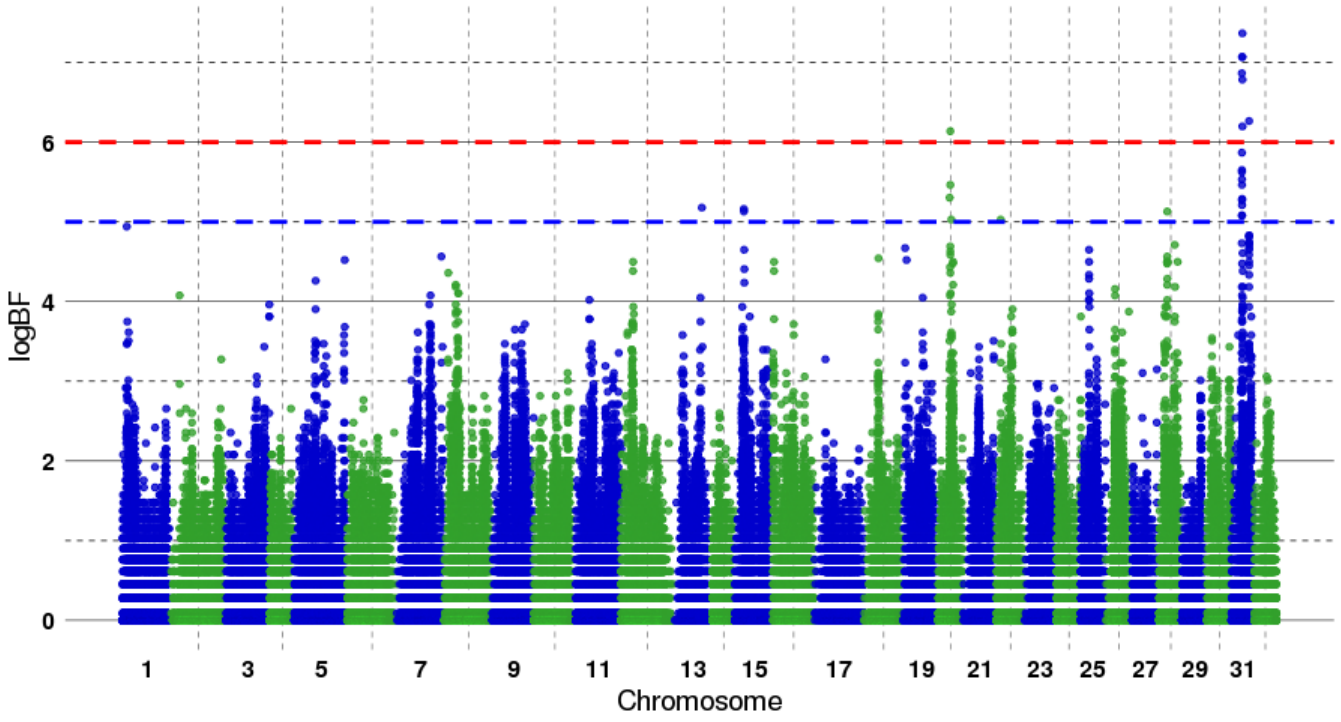
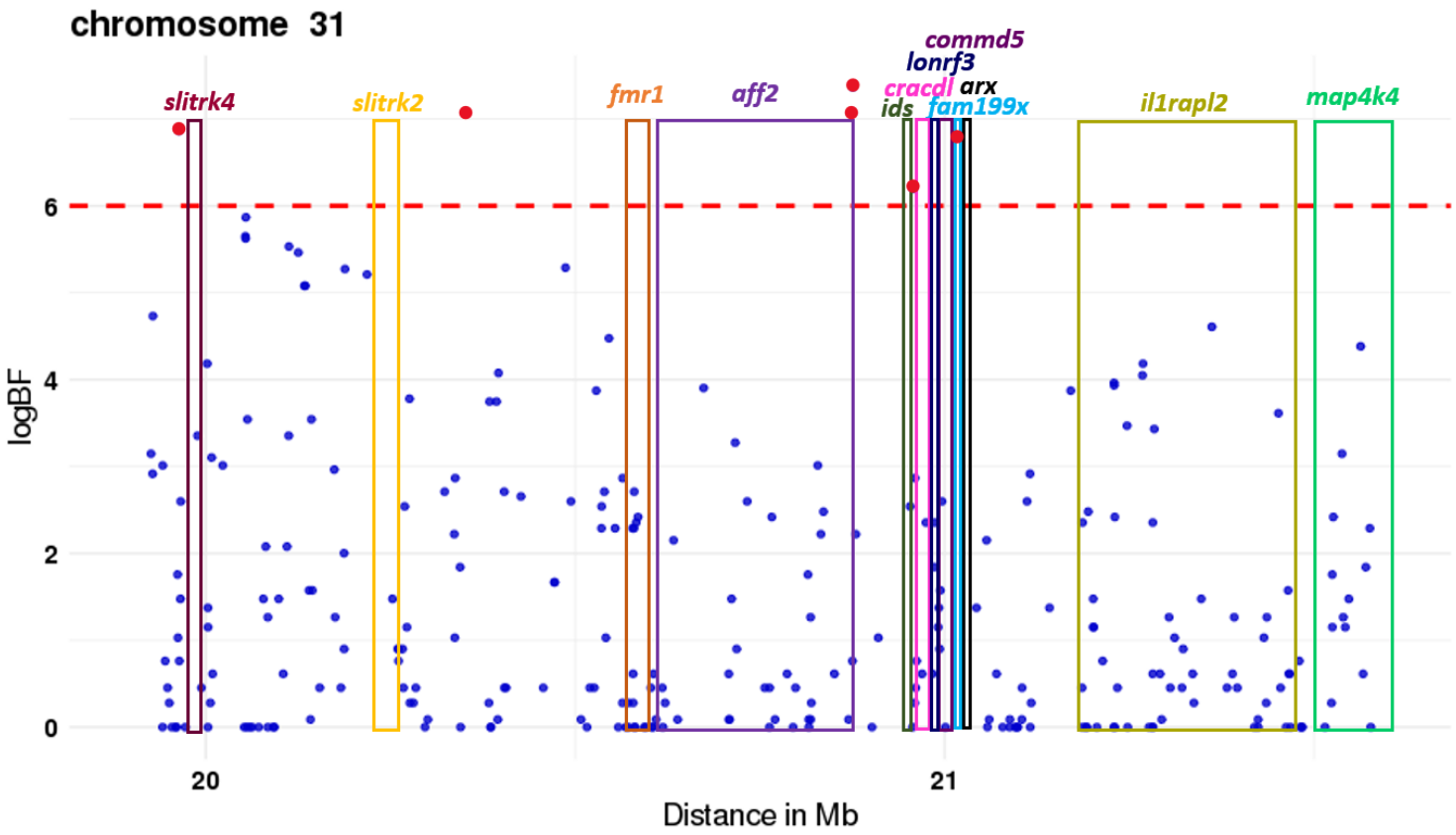

Gain in accuracy of GBLUP versus BLUP (%)
50
40
30
20
10
0
-10
-20
Hypoxia tolerance

Fig. 3



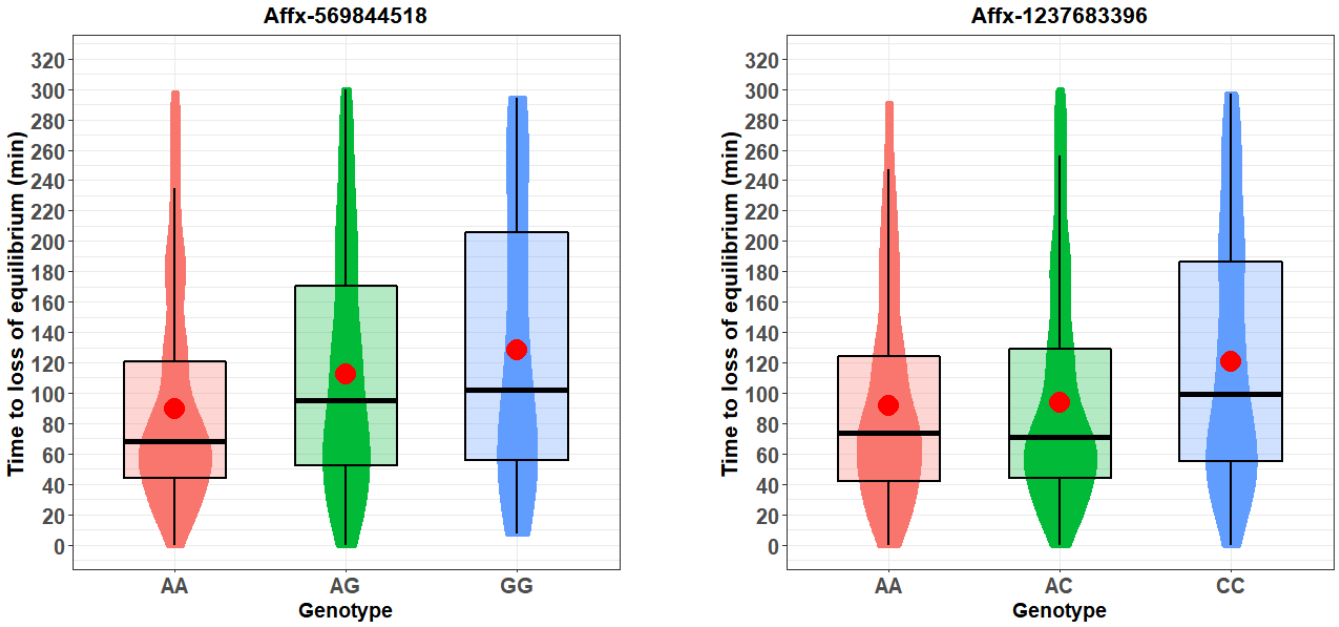
1339 **Fig.4**



1340

Fig. 5

A) **B)**


Affx-569844518
Time to loss of equilibrium (min)
0
20
40
60
80
100
120
140
160
180
200
220
240
260
280
300
320
AA
AG
GG
Genotype
Affx-1237683396
Time to loss of equilibrium (min)
AA
AC
CC
Genotype