Accuracies of genomic predictions for disease resistance of striped catfish to *Edwardsiella ictaluri*

using artificial intelligence algorithms

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Abstract

Assessments of genomic prediction accuracies using machine and deep learning methods are currently

not available or very limited in aquaculture species. The principal aim of this study was to examine the

predictive performance of these new methods for disease resistance to *Edwardsiella ictaluri* in a

population of striped catfish *Pangasianodon hypophthalmus* and to make comparisons with four common

methods, i.e., pedigree-based best linear unbiased prediction (PBLUP), genomic-based best linear

unbiased prediction (GBLUP), single-step GBLUP (ssGBLUP) and a non-linear Bayesian approach (notably

BayesR). Our analyses using machine learning (i.e., KAML) and deep learning (i.e., DeepGP) together with

the four common methods (PBLUP, GBLUP, ssGBLUP and BayesR) were conducted for two main disease

resistance traits (i.e., survival status coded as 0 and 1 and survival time, i.e., days that the animals were

still alive after the challenge test) in a pedigree consisting of 560 individual animals (490 offspring and 70

parents) genotyped for 14,154 Single Nucleotide Polymorphism (SNPs). The results showed that KAML

outperformed GBLUP and ssGBLUP, with the increases in the prediction accuracies for both traits by 5.1

– 47.7%. However, the prediction accuracies obtained from KAML were comparable to those estimated

using BayesR. Imputation of missing genotypes using AlphaFamImpute increased the prediction

accuracies by 0.2 – 33.2% in all the methods used. On the other hand, there were no significant increases

in the prediction accuracies for both survival status and survival time when multivariate models were used

in comparison to univariate analyses. Interestingly, the genomic prediction accuracies based on only

highly significant SNPs (P < 0.00001) were not largely different from those obtained from the whole set of

14,154 SNPs. In all our analyses, the accuracies of genomic prediction were somewhat higher for survival

time than survival status (0/1 data). It is concluded that there are prospects for the application of genomic

selection to increase disease resistance to *Edwardsiella ictaluri* in striped catfish breeding programs.

1. Introduction

Genomic selection has been increasingly practiced in genetic improvement programs for farmed animals

and plants, using a range of different statistical methods from genomic-based best linear unbiased

prediction (GBLUP) and its extension known as single-step GBLUP (ssGBLUP) to non-linear Bayesian

approaches (BayesA, BayesB, BayesC, BayesC-π and notably BayesR) (Lourenco et al. 2020; Moser et al.

2015; VanRaden 2008; Daetwyler et al. 2013; Al-Tobasei et al. 2021; Vallejo et al. 2020). Recently, there

has been a growing interest in using Artificial Intelligent (AI) algorithms (i.e., Machine Learning or Deep

Learning) to choose optimal genome-wide models without prior consumptions to determine genomic

prediction accuracies for quantitative complex traits, especially for disease resistance (tolerance or

resilience). Examples using simulated and real animal and plant data showed that the prediction

accuracies (r) using machine learning were 10% greater than linear (i.e. GBLUP) and 1.3% greater than

non-linear methods (i.e. BayesR), for instance, $r = 0.732 - 0.758$ for binary and continuous traits using

GBLUP vs $0.801 - 0.832$ (Yin et al. 2020). To date, however, no (or very limited) studies have used AI

methods to estimate genomic breeding values for aquaculture species.

Almost all studies in aquatic animal species have employed GBLUP, ssGBLUP or Bayesian methods to

examine genomic prediction accuracies for two main groups of traits, i.e., body weight and disease

resistance. A synthesis of the published information shows that the genomic prediction accuracy ranged

from 0.38 to 0.89 for growth-related traits (Houston et al. 2020). On the other hand, the predictions of

genomic breeding values for disease resistance were varied with studies, pathogens, populations and

methods used, with the estimates ranging from 0 (Silva et al. 2019) to 0.8 or greater (Barría et al. 2018).

For both growth and disease traits, the majority of the studies demonstrated that either linear (GBLUP,

ssGBLUP) or non-linear Bayesian methods increased the prediction accuracies of animal breeding values

relative to Pedigreed-based BLUP (PBLUP) by 22 - 24% (Houston et al. 2020). In addition to growth and

disease traits, three recent studies performed genomic predictions for meat quality in banana shrimp

(Nguyen et al. 2020) or Portuguese Oyster (*Crassostrea angulata*) (Vu et al. 2021) as well as behavior traits

(i.e., cannibalism) in Asian seabass (Nguyen and Khang 2021).

Despite the economic importance of the striped catfish in aquaculture, e.g., valuing 2.36 billion USD that

accounts for 1% GDP of Vietnam, there has been a paucity of knowledge in using genomic information in

breeding programs for high growth (Vu et al. 2019b) or increased disease resistance, especially Bacillary

Necrosis of Pangasius (Vu et al. 2019a). The Bacillary Necrosis of Pangasius (BNP) due to *Edwardsiella*

ictaluri has been the main contributor to $56 - 92\%$ mortality during larval and fingerling rearing in this

species (Vũ et al. 2017). Our earlier studies showed that there is heritable genetic component for BNP

resistance, but the heritability for this trait was low, around 0.10 across statistical methods used (Vu et al.

2019a; Pham et al. 2021; Dinh Pham et al. 2021). While these results suggests there are prospects for

genetic improvement of BNP resistance using conventional selective breeding, the pathogen test has

posed substantial challenges in terms of biosecurity issues, times, costs and environmental impacts (Pham

et al. 2020; Nguyen 2014). Due to these limitations, genomic selection has emerged as an alternative

option to increase resistance of striped catfish to BNP, one of the most severe diseases that has caused

significant economic loss for the sector world-wide.

Therefore, the principal of this study was to assess genomic prediction accuracies of machine and deep

learning methods for two main disease resistance traits (i.e., survival status and survival time) and to make

comparisons with four common methods (PBLUP, GBLUP, ssGBLUP and BayesR). Additionally, we

explored if imputation of missing genotypes, multiple traits analyses and significant genome-wide markers

can improve the prediction accuracies for the disease resistant traits. Our results open new opportunities

for genome-based selection to increase the animal resistance to *E. ictaluri* in striped catfish as well as

other aquaculture species infected by this highly infectious pathogen.

2. Materials and Methods

2.1. Fish and challenge test

The animal samples used in this study originated from a selective breeding program for improved disease

resistance of striped catfish to *Edwardsiella ictaluri* (Vu et al. 2019a). In 2020, the first generation was

produced based on a nested mating design with a ratio of one male to two females. A total of 166 families

(32 full- and 134 half-sib families) were successfully produced following the breeding protocol as detailed

in Van Sang et al. (2012) and Vu et al. (2019b). Fry of each family were kept in separate fiber glass tanks

up to 3 weeks before they were transferred to stock in net hapa installed in earthen pond. When the

fingerlings reached an average body weight of 15 – 20 g, a random sample of 100 fish per family was

individually identified using PIT (Passive Integrated Transponder) tag. After tagging, a half size of each

family was sent to ponds for performance testing and another half was used in pathogen challenge tests

for *E. ictaluri* resistance.

The challenge test involved a total of 5328 individuals from 166 families (averaging 32 individuals per

family). The experimental fish were initially acclimatized in cement tanks for about two weeks. Then the

same number of fish from each family was randomly allocated to six cement tanks (10 m^3) for the

challenge test using cohabitant method (Vu et al. 2019a). The cohabitant fish (16.7 ± 6.1 g) were firstly

inoculated with the bacteria *E. ictaluri* pathogen (10^6 CFU/0.2 ml per fish). Two days after the injection,

they were released into the cement tanks to rear with the experimental fish with a ratio of 1 to 3 (or

roughly 30% cohabitant fish in each tank). The experiment was conducted over a period of 23 days. During

this period, the feeding rate was reduced to 1.5% of the total biomass in tank. Mortality was highest in

day 5 and dead fish were sampled for laboratory PCR test to verify that their death symptoms (white spots

in spleen, liver and kidney) were due to *E. ictaluri* pathogen. At the conclusion of the experiment, all alive

fish were biosecure-buried, following the regulations of the national veterinary authority (Department of

Animal Health, Vietnam).

2.2. Phenotype data

During the challenge test, death fish were collected every 3 hours and their clinical symptoms were also

recorded. The data were used to calculate two measures of *E. ictaluri* resistance, i.e., survival status and

survival time. Survival status was expressed as a binary trait in which dead fish were designated as zero

(0) and alive animals at the end of the challenge test were assigned a number 1. Furthermore, survival

time was defined as the continuous trait from the start of test until the animal death. Both survival status

and survival time were analyzed using linear mixed model to estimate breeding values (EBVs) for all

individuals and families in the pedigree. Based on the EBVs ranking, 20 highest resistance families and 20

lowest resistance families were chosen from 166 families. Only families with the number of offspring

greater than 9 were selected to ensure that the EBVs were estimated with a high level of reliability. Next,

we randomly collected fin tissue samples of 12 – 15 fish per family per disease resistance group for

genome sequencing using Diversity Arrays Technology (DArTseq^{TM}).

2.3. Genotype data

A total of 564 DNA samples (from 564 individual fish) were sent to a commercial service provider in

Canberra, Australia for genotyping by sequencing using DArTseq^{TM} technology. The DArTseq^{TM} was based

on the genome complexity reduction method in combination with high throughput next generation

sequencing using Illumina platform. The sequencing protocol including choice of restricted enzymes and

library preparation was optimized for striped catfish (Pangasianodon DarTseq 1.0) using 96 independent

DNA samples of striped catfish in our earlier study (Vu et al. 2020). Briefly, the PstI-SphI method (Kilian

et al. 2012) was used and the compatible adaptors were designed to include Illumina flow-cell attachment

sequence, sequencing primer sequence and capturing variant length of barcode regions (Elshire et al.

2011). Then, only mixed fragments (PstI-SphI) were amplified in 30 rounds of PCR, followed by sequencing

on Illumina Hiseq2500 (77 cycles per single read). Next, sequences generated in each lane of 96-well

microliter were processed by proprietary DArT analysis protocol. The genotype calling were described in

detail in our previous studies (Nguyen et al. 2020; Nguyen et al. 2018). The average variant call-rate was

99% and the sample calling rate was 92%. Given with our strict quality control, four out of 564 samples

were discarded, and 560 samples (490 offspring and 70 parents) remained for downstream analyses.

2.4. Genotype imputation

The missing genotypes were about 10.0% in this study. They were imputed using AlphaFamImpute

(Whalen et al. 2020). The imputation was based on offspring-parent (i.e., pedigree) information.

Specifically, this method used parental genotypes to impute the missing values of offspring genotypes and

then, the offspring genotypes were used to fulfill the missing values of their parents (Whalen et al. 2020).

The pedigree used in our analysis was traced back to the base population.

2.5. Estimation of heritability using genotype data

Variances components of the two studied traits (survival status and survival time) were estimated using

linear mixed model under the Best Linear Unbiased Prediction (BLUP) framework in AIREMLf90 sub-

program of the BLUPF90 family package (Misztal et al. 2002). AIREMLf90 uses Average Information-

Restricted Maximum Likelihood method that can provide less computational resources and higher

accuracy of variance estimates (Masuda et al. 2014). The models included the fixed effects of spawning

batch (4 levels, 1 – 2 weeks interval between successive spawning batches) and pathogen challenge test

tanks (2 levels) and the random effect of additive genetics of the individual fish in the pedigree. Age from

birth to tagging (124 – 167 days) was also fitted as a linear covariate. The SNP heritability (h^2) was

calculated as a ratio of the additive genetic variance (σ_a^2) to total phenotypic variance (σ_p^2), where

$\sigma_p^2 = \sigma_a^2 + \sigma_e^2$ (σ_e^2 = environmental variance).

2.6. Statistical methods

Accuracies of genomic prediction for the disease resistance traits (survival status and survival time) were

estimated using BLUP-family methods (PBLUP, GBLUP, ssBLUP), BayesR, machine learning (i.e., KAML) and

deep learning (i.e., DeepGP).

2.6.1. PBLUP, GBLUP and ssGBLUP (using BLUPF90 program)

The mixed model used in PBLUP, GBLUP and ssGBLUP is written in a matrix notation as follows:

$$y = Xb + Za + e \qquad \text{(equation 1)}$$

Where y is the vector of phenotypic values (survival status or survival time), b is the vector of fixed effects

(i.e., spawning batches and experimental tanks and a linear covariate of age from birth to tagging), a is

the vector of the random term (i.e., the additive genetic effect of individual fish in the pedigree). *X* and *Z*

are the incidence matrices related to the fixed and random effects. The letter *e* refers to residual variance

or error of the estimates corresponded to each phenotypic value.

The main difference among BLUP-family methods (PBLUP, GBLUP and ssGBLUP) relates to relationship

matrices (**A**, **G** or **H**) used to solve the mixed model equation 1 above.

PBLUP: In PBLUP, *a* is the additive genetic variance of each individual fish with its corresponding matrix **Z**

following a normal distribution ~ N(0, $\mathbf{A}\sigma_a^2$), with **A** is the numerator relationship matrix calculated from

the pedigree records and σ_a^2 is the additive genetic variance.

GBLUP: In GBLUP, *a* is the random additive genetic effect underlying polygenic effects of SNPs with a

normal distribution ~ N(0, $\mathbf{G}\sigma_g^2$) where genomic relationship matrix **G** was generated from 14,154 SNPs

and polygenic genetic variance σ_g^2. The GBLUP method assumes that each SNP contributes little to

phenotypic variance of the traits studied.

ssGBLUP: In ssGBLUP, **A** and **G** matrices are blended to produce realized matrix **H** (Aguilar et al. 2010).

The inverse of H matrix is expressed as below:

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$$H^{-1} = A^{-1} + \begin{bmatrix} \mathbf{0} & \mathbf{0} \\ \mathbf{0} & G^{-1} - A_{22}^{-1} \end{bmatrix} \qquad \text{(equation 2)}$$

Where **A**$^{-1}$ and **G**$^{-1}$ are the inverse of matrices **A** and **G** as described above. **A**$^{-1}_{22}$ is the inverse of matrix of

genotyped individuals after combining of pedigree and genomic information.

The three BLUP methods (PBLUP, GBLUP and ssGBLUP) were implemented in BLUPF90 package (Misztal

et al. 2002). For survival status, we used generalized (threshold) mixed model in THRGIBBF1f90 (Tsuruta

and Misztal 2006) known as threshold Gibb Sampling method, whereas survival time was analyzed using

linear mixed models in AIREMLf90 (Masuda et al. 2014). In the Gibbs sampling, we used 200,000 iterations

with 20,000 iterations as burn-in for univariate analysis. The convergence of the parameters estimates

was checked using POSTGIBBSf90 program. In each sub-program, we performed 3 methods: traditional

pedigree BLUP (PBLUP) which utilizes only individual relationship records (pedigree information only)

(Henderson 1985), Genomic BLUP (GBLUP) which uptakes only genomic information (Meuwissen et al.

2001), and single step GBLUP (ssGBLUP) (Lourenco et al. 2020) which blended pedigree and genomic

information into a realized H matrix (Misztal et al. 2009; Lourenco et al. 2020).

2.6.2. Non-linear Bayesian method (BayesR)

BayesR assumes that SNP effects β_i follow a four-component normal mixture and the effect of SNP *i* is

assumed to be distributed as:

$\beta_i \sim \pi_1(\beta_i = 0) + \pi_2 N(0.0001\sigma_g^2) + \pi_3 N(0, 0.001\sigma_g^2) + \pi_4 N(0, 0.01\sigma_g^2)$ (equation 3)

Where σ_g^2 as defined above, σ_g^2 represents the total additive genetic variance (i.e., the cumulative

variance of all SNP effects) and $\pi = (\pi_1, \pi_2, \pi_3, \pi_4)$ the mixing proportions such that $\sum_{i=1}^{4} \pi_i = 1$. The

mixing proportions π are assumed to follow a Dirichlet prior, $\pi \sim Dirichlet\ (\alpha + \beta)$, with α representing

a vector of pseudo counts and β the cardinality of each component. In this work, we used a flat Dirchlet

distribution, with $\alpha = (1,1,1,1)$ for the prior. As suggested by Moser et al. (2015), σ_g^2 is assumed to be a

random variable following an Inverse—χ2 distribution.

The analysis of BayesR consisted of three mains steps. First, numeric coding genotypes of 560 animals

(including 490 offspring and 70 parents) obtained from DArT sequencing were converted to ATGC format

(i.e., biallelic variant). Second, the trimmed sequences of each variant were blasted to nucleotide non-

redundant striped catfish database using Blast2GO (Conesa et al. 2005), named GENO_Phyp_1.0 (Kim et

al. 2018). This analysis matched 13,766 SNPs to 30 autosomal chromosomes. The remaining SNPs (388

SNPs) were randomly assigned to 30 chromosomes ($12 - 13$ SNPs/chromosome). Third, PLINK v1.9 (Purcell

et al. 2007) that utilizes genotype and mapping files was used to generate a full dataset for BayesR

(https://github.com/syntheke/BayesR). The default running cycles of BayesR included 50,000 steps with

20,000 steps discarded as burn-in. We used predicted phenotypes (computed in AIREMLf90 using

equation 1), as this package does not allow fitting covariates in the model.

2.6.3. Machine learning and deep learning methods

KAML (Kinship Adjusted Multiple Loci)

KAML is a machine learning-based method that incorporates cross-validation, multiple regression, grid

search and bisection algorithm to improve genomic prediction accuracy for complex quantitative traits

(Yin et al. 2020). KAML extends linear mixed model (equation 1) by incorporating quantitative trait

nucleotides - QTNs (e.g., traits controlled by genes with large and moderate effects) as covariates and a

SNP-weighted trait-specific kinship matrix as the (co)-variance assumption corresponding to the random

effect. The selections of QTNs and SNP weights are optimized by machine learning. In a matrix notation,

equation 1 becomes:

$\boldsymbol{Y = Xb + Qq + Za* + e}$ with $\boldsymbol{a}* \sim N(0, K_w\sigma_g^2)$, $\boldsymbol{e} \sim N(0, I\sigma_e^2)$ (equation 4)

Where σ_g^2 and σ_e^2 are as described above; $\boldsymbol{Q}$ is covariates matrix of QTNs detected throughout multiple

regression analyses; $\boldsymbol{q}$ is a vector of random effect of each QTN corresponding to $\boldsymbol{Q}$ and K_w is SNP-weighted

Kinship that is optimized by grid search and bisection procedure. The implementation of KAML included

two main steps, i.e., define $\boldsymbol{Q}$ and K_w and then predicting genomic breeding values of the validation set. In

the training step, KAML optimized parameters for determining QTNs and SNPs weights which used all

testing subset data provided throughout cross-validation. Next, in the prediction step, the optimized

parameters were used to predict genomic breeding values of each individual in validation set. A detailed

description of the step-wise procedures and the underlying algorithms of KAML is given in Yin et al. (2020).

In our analyses, we used PLINK (Purcell et al. 2007) to convert the genotype data into the right format to

be analyzed in KAML (https://github.com/YinLiLin/KAML) in R environment (R Core Team 2015). KAML

utilized adjusted phenotypes (i.e., phenotypic y-hats) for testing sets and masked (i.e., 'NA' values)

phenotypes of validation sets. To define the best parameters (i.e., pseudo QTNs, the optimal SNPs

weights, the optimal Log value and the optimized kinship matrix) for mixed models of each testing set, we

used five-fold cross-validation with 100 replications.

Deep learning – Multilayer Perceptron (DL-MLP)

For deep learning analysis, we used DeepGP package (https://github.com/lauzingaretti/deepGP/). A

detailed description of the theoretical framework is given in Zingaretti et al. (2020) and Pérez-Enciso and

Zingaretti (2019). In our analyses, we used two different algorithms of deep learning: Multilayer

perceptron and convolutional neural network.

DL-MLP is fully connected networks consisting of an input layer, one or several hidden layers, and an

output layer. In the context of genomic prediction, input layer receives SNPs data (i.e., 14,154 SNPs). Each

of SNP information in the input layer is transferred its value to the first neuron of the first hidden layer by

the Z function:

$Z^{(k)} = b_{k-1} + W^{(k-1)}f^{(k-1)}(x)$ (equation 5)

Where W is weighted SNP information, $f(x)$ is non-linear function and b is bias (i.e., constant). In the case

of genomic prediction, the input layer is SNPs genotype (i.e., input variables = number of SNPs) of

individual fully connected to the first hidden layers. As a result, the value in the first neuron of the first

hidden layer is the sum effect of Z function over 14,154 SNPs ($\sum_{i=1}^{14154} Z$). Likewise, information in the first

hidden layer is the input for transferring its value to the second hidden layers and finally to the output

layers. The significance step in obtaining best DL-MLP model is to define MLP structure network (i.e,

number of hidden layer and neuron of each layer) and hyperparameters which related to equation 5.

These parameters and their values in our study include learning rate (--lr 0.025 0.01), type of drop out (--

dr 0 0.01), type of regularization (--reg 0.0001), activation function (--act linear tanh), number of hidden

layer (--h 1 5), optimizer function (--optimizer Adam) or number of epochs (--epochs 50). These scripts

provided in DeepGP (https://github.com/lauzingaretti/DeepGP) were used to obtain hyperparameters in

our DL-MLP analyses.

Deep learning – Convolutional Neural Network (DL-CNN)

Convolutional neural network comprises of 3 types of layers: (1) convolutional layers, (2) fully connected

layers and (3) input layers. In the context of genomic prediction, first convolutional layer receives data of

14154 SNPs and write it to the first layer using kernel (i.e., a definite number of SNPs is grouped or

combined). Then, the pooling step is responsible to reduce spatial size of SNPs data of the convolutional

layer into the second convolutional layer. There are two types of pooling such as max pooling and average

pooling corresponding to maximum values and mean values of SNPs portion covered by the kernel. The

process was repeated to make update value of the last convolutional layer. At the last convolutional layer,

data were flattened (i.e., convert data of pooled feature of the last convolutional layer into single column

feeding to fully connected layers). The fully connected layer works similarly the MLP. The

hyperparameters used in our study are size of kernel (--ks 3), learning rate (--lr 0.025 0.01), dropout in the

first layer (-dr1 0 0.01), dropout in the hidden layer (--dr2 0 0.1 0.2), number of pooling size (--ps 1),

number of convolutional layers (--ncov 1 3), number of strides (--ns 1), number of convolutional

operations (--nfilters 8). These hyperparameters are fine-tuned under DeepGP with grid search provided

in DeepGP package (Zingaretti et al. 2020). As DeepGP cannot handle missing values, we used imputed

genotype for all analyses.

2.6.4.   Analysis of SNP subsets, highly significant SNPs and multi-trait mixed model

SNP subsets

In addition to the analysis of the whole genotype data (14,154 SNPs), we also analyzed two different SNP

subsets. The first genotype subset (8,481 SNPs) was obtained through the default quality control (i.e.,

excluding genotypes with call rate <90% and minor allele frequency >0.05) in the ssGBLUP of blup90

package. The second subset of 834 SNPs remained after strict filtering applied (i.e., excluding genotypes

with call rate <90%, minor allele frequency >0.05 and markers that show significant deviations from Hardy-

Weinberg Equilibrium).

ssGWAS to select significant SNPs for prediction

Single step genome-wide association analysis (ssGWAS) was accomplished in BLUPF90 suite using full

phenotype data for disease resistance traits. We used preGSf90 and postGSf90 together with BLUPF90

program to compute SNP effect and its P-values. A panel of 402 SNPs were selected based on the P-value

< 0.0001. This genotype subset was used to test the predictive performance of five genomic prediction

methods used in our study.

Multi-trait mixed model

Multi-trait analyses are only available in BLUPF90. In this study, two studied traits were jointly analyzed

using PBLUP, GBLUP and ssGBLUP models in THRGIBBS1F90 (Tsuruta and Misztal 2006). This involves using

Gibbs Sampling with MCMC updates including 1,000,000 iterations with 100,000 burn-in steps.

2.7. Five-fold cross validation

The predictive performances of all five methods (PBLUP, GBLUP, ssGBLUP, BayesR, KAML and DeepGP)

used in this study were evaluated using repeated 5-fold cross validation. The procedure involved a random

partitioning of the full data into 5 subsets (each subset has 98 individuals including representatives of all

the families). The phenotypes of one subset were masked and GEBV/EBV (Genomic/Estimated Breeding

Value) of this set were predicted using the information from the other four subsets. This means one

phenotype was used once in the validation process of one repetition. Each analysis was repeated 5 times,

giving 25 validation sets in total and the correlation coefficient was averaged over 25 values. To compute

evaluation metric in each validation set, the GEBV/EBVs of the predicted phenotypes were correlated to

the actual phenotype. We chose Pearson correlation coefficient (Benesty et al. 2009) for continuous trait

(survival time) and Mathew correlation coefficient, MCC (Matthews 1975) for binary trait (survival status).

MCC is proved to be less bias amongst common metrics and giving more meaningful interpretation for

binary classification evaluation (Chicco and Jurman 2020). Specifically, we used *PresenceAbsence* R

package (Freeman and Moisen 2008) to produce 4x4 confusion matrix which assigned GEBV/EBV values

against the actual phenotypes for computing MCC (Supplementary file S1). To obtain the consistency and

comparable results among tested software/packages, we used the same subsets of testing/validation data

in BLUPF90 family program, BayesR, ML-KAML, DL-MLP and DL-CNN.

The genomic prediction accuracy for survival status and survival time was calculated as $Accuracy = \frac{r_{y,\hat{y}}}{\sqrt{h^2}}$;

where $r_{y,\hat{y}}$ is the correlation between the predicted breeding values $(\hat{y})$ and actual phenotypes(y).

Data availability

307 Data are available via Figshare portal provided by the journal.

3. Results

3.1. Data, variance component and heritability

The average survival rate and survival time after the pathogen challenge test were 32.2% and 6.83 days,

respectively (Table 1). When exposed to the *Edwardsiella ictaluri* pathogen, mortality occurred after 2

days/or 32.5 hours and the highest death rate was observed after 5 days/or 97.3 hours (Figure 1). There

were significant differences (P < 0.0001, Figure 1) in both survival rate (60.00% vs. 4.49%) and survival

time (8.66 vs. 5.00 days) between 20 highest and 20 lowest resistant families (Table 1). The variations in

the disease resistant traits among families were also significant (Supplementary Figures S1 & S2).

The heritabilities estimated for survival status and survival time using traditional PBLUP (0.65 – 0.71) were

greater than those obtained from GBLUP (0.55 – 0.66) and ssGBLUP (0.45 – 0.46) but they were lower

than those estimated by BayesR (0.61 – 0.86) (Table 2). Across statistical models (methods) used, the

estimates of heritability for survival status were slightly higher than those obtained for survival time (Table

2).

3.2. Accuracy of genomic prediction using original genotype (un-imputed) data

Figure 2 presents the accuracies of genomic breeding values for survival status and survival time using

Machine learning (ML-KAML) and Deep Learning (DL-MLP and DL-CNN) together with four other methods

(PBLUP, GBLUP, ssGBLUP and BayesR). When the original genotype data (i.e., un-imputed data) were used,

the prediction accuracies for both survival status and survival time obtained from KAML was higher than

those calculated from GBLUP and ssGBLUP, ranging from 8.3 to 9.5% and 48.8 to 64.5%, respectively (also

see Supplementary Table S1). Additionally, KAML outperformed BayesR but the difference in the

prediction accuracy was small (0.574 vs 0.557 for survival status and 0.715 vs. 0.699 for survival time). For

both traits, ssGBLUP was slightly better GBLUP. Interestingly, both GBLUP and ssGBLUP did not improve

the prediction accuracies for both survival status and survival time as compared with conventional PBLUP

method.

3.3. Accuracy of genomic prediction using imputed genotype data

Imputation of missing genotypes increased the accuracies of genomic prediction for both survival status

and survival time across the five methods used (Figure 3). The improvements for survival status were from

8.9% (GBLUP) to 14.3% (BayesR). For survival time, the increase in the prediction accuracy when the

imputed genotypes were used as compared with original data was largest for GBLUP (33.2%), followed by

BayesR (9.9%) and KAML (5.0%). Deep learning (DL) using multilayer perceptron (DL-MLP) and

convolutional neural network (DL-CNN) had lower prediction accuracies for survival time (0.596 – 0.602)

than other methods but the reverse trend was observed for survival status (Supplementary Table S1).

3.4. Univariate vs. multi-variate analysis

Our multivariate analyses used PBLUP, GBLUP and ssGBLUP, and the prediction accuracies for both

survival status and survival time are shown in Table 3. There was only an increase in the prediction

accuracy when PBLUP was used to estimate genomic breeding values (GEBV) for survival status (0.627 vs

0.597) while other multivariate analyses slightly decreased the accuracy of GEBV relative to univariate

model (3.4 to 15.0%).

3.5. Accuracy using different subsets of genotype

Table 4 presents the accuracies of GEBV for both survival status and survival time using different subsets

of genotype data (or different level of quality control in BLUPF90 program). Either when 8,481k SNPs (after

a standard quality control) or 834 SNPs (deep filtering) was used, the prediction accuracies were similar

to those estimated using whole genotype dataset (14,154 SNPs). In both traits, the predictive abilities with

machine learning (KAML) and/or deep learning methods (DL-MLP and DL-CNN) were slightly better than

those of other methods, 0.544 – 0.774 vs 0.513 – 0.646 respectively.

3.6. Genomic prediction in combination with GWAS

The prediction accuracies for the disease resistance traits that incorporated only significant SNPs

identified from genome-wide association analysis (GWAS) are given in Table 5. When un-imputed

genotypes were used, the prediction accuracies were generally greater to those of the full set of 14,154

SNPs. One exception was that GBLUP increased the accuracy by 54.3% for survival time. Furthermore,

there were also no differences in the prediction accuracies for both traits between the subset of 402

significant SNPs and the full dataset, when imputed genotypes were analyzed. In all analyses irrespective

of using un-imputed or imputed genotypes, ML-KAML and BayesR outperformed other methods by 11.5

– 29.1% for survival time (PBLUP, GBLUP and ssGBLUP).

4. Discussion

Development of disease resistance lines is essential for the aquaculture sector due to the combined

effects of aquaculture intensifications and environmental changes. Our study demonstrated that genomic

selection can be practiced improving resistance to *Edwardsiella ictaluri* disease in striped catfish

populations, using a range of different statistical methods especially machine and deep learning, and

BayesR. Salient findings from our study are discussed as follows.

First, the genomic prediction accuracies for the disease resistance traits (survival status and survival time)

were substantially improved when machine (i.e., KAML) and deep learning (DeepGP) methods were used

relative to BLUP-family methods (GBLUP, ssGBLUP). They also outperformed traditional pedigree-based

method (PBLUP) in this study. Hence, genomic selection to improve BNP disease is expected to achieve

greater genetic gain than the conventional selective breeding approach using pedigree and phenotype

information. The genomic selection can also shorten the breeding cycle of striped catfish from 3-4 to 1.5-

2 years as expected to reduce at least a half of generation interval in dairy cattle (Hayes et al. 2009). Our

results are consistent with those reported in farmed animal (Baker et al. 2020), livestock (Li et al. 2018;

González-Recio and Forni 2011) and plants (Montesinos-López et al. 2019; Montesinos-López et al. 2018;

Zingaretti et al. 2020). Despite the superiority of the machine learning methods (i.e., KAML) to PBLUP,

GBLUP and ssGBLUP, they had similar predictive power to BayesR for both traits in our study. Reports in

farmed animals concluded that BayesR is the best amongst Bayesian and GBLUP methods (Erbe et al.

2012) but no published information is available in aquaculture species to compare with our study. To date,

only two studies, one in shrimp (Palaiokostas 2021) that used extreme gradient boost method (one of the

machine learning approaches) and another in seabream that used support vector machines and linear

bagging classification (Bargelloni et al. 2021) and reported that these methods improved the prediction

accuracy for survival traits by 1-4% and 20 − 70% relative to GBLUP and Bayesian methods. Therefore,

machine or deep learning should be used for genomic evaluation of disease resistance traits, at least in

this population of striped catfish. The predictive powers of these methods using AI algorithms are

expected to be greater when big data (e.g., hundreds of thousands or million animals sequenced) are

analyzed for this population in the future.

Second, imputation of missing genotypes (using offspring and parent information available in the pedigree

of striped catfish) increased the predictive ability of all the five methods used, especially GBLUP by 9.9 to

33.2% relative to when un-imputed data were used. Also note that the imputation of missing genotypes

in our study differed from others that used software to compute missing genotypes without considering

parental relationship. With the use of offspring-parent information, the imputation likely increased

accuracy of G and H matrices used in GBLUP and single-step methods (i.e., ssGBLUP). Published

information across species also showed that imputation from low to high density SNP panels or from

commercial SNP arrays to whole genome sequence increased the genomic prediction accuracies for

resistance to parasite (i.e. sea lice count) and body traits in Atlantic salmon (Tsai et al. 2017), and

agronomic traits in maize (Crossa et al. 2013). However, some other studies also showed that imputed

genotype did not improve prediction accuracies in livestock animal breeding (Heidaritabar et al. 2016;

Chen et al. 2014; Van Binsbergen et al. 2015). Imputation has several benefits, firstly it can be made after

sequencing with no extra cost. Second, we can reduce sequence coverage to genotype a larger number

of individuals and eventually imputation can increase selection intensity (and selection accuracy), and this

can lead to increases in genetic progress made in selected populations. Thus, imputation of low-density

sequence can support our future incorporation of genomic selection for BNP disease in this striped catfish

population.

Third, we found that multivariate analysis improved the predictive ability for the two studied traits only

when PBLUP was used. However, both GBLUP and ssGBLUP did not increase the prediction accuracies for

both survival time and survival status. These results are expected due to the high and positive genetic

correlations (r_g) between the two traits (r_g = 0.81). A bivariate analysis of harvest body weight and fillet

yield in Nile tilapia also showed no improvement in the prediction accuracies for these traits (Joshi et al.

2020). Multi-trait analyses generally improve accuracy of genetic parameter estimates and animal

breeding values for traits whose genetic architectures are different (e.g., low vs high heritable) or

genetically antagonistic associated (i.e., unfavorably correlated). Examples of multivariate genomic

prediction models involving multi-trait in cassava and wheat plants gave better prediction accuracy over

univariate models (Okeke et al. 2017; Sun et al. 2017). To utilize the benefits of multi-trait analysis, we

continue collecting other traits of economic importance in striped catfish, namely disease tolerance,

disease resilience as well as immunological parameters to increase overall resistant capacity of the

animals in this catfish population.

Fourth, we also suggest using the full set of SNPs to obtain a reasonable level of prediction accuracy for

genomic selection to improve disease resistance traits in striped catfish. It has been well documented that

the prediction accuracy increased with number of markers and sequencing depth or genome coverage.

Due to characteristics of RAD-sequencing methods, there are still limited number of markers in our study

as compared with those reported in farmed animals or plants (Robledo et al. 2018; Davey and Blaxter

2010). It is necessary to examine prediction ability of whole genome data when sequencing costs are

reduced to an affordable level to sequence thousands (or hundreds of thousands) of animals in a near

future. However, there are studies in farmed animals reporting that whole genome sequence data

improved the predictive powers for complex traits by only 0.4 – 5.0% (Al Kalaldeh et al. 2019; VanRaden

et al. 2017).

Finally, the utilization of highly significant SNPs from ssGWAS can improve the prediction accuracies for

the two traits studied, for instance with un-imputed genotype data. On the one hand, these SNPs can be

used to develop a small SNP panel to genotype a large number of animals in both the training and

validation populations. On the other hand, the costs of genotyping animals for 402 SNPs may not be a lot

cheaper than DArT sequencing as used in this study. The use of significant SNPs can be beneficial when

their biological functions (pathways) are known. In species where reference genome or high quality

genome assembly is available, the incorporation of SNPs with biological functions (i.e. multi-omics)

improved the prediction accuracy, for instance, about 5 - 19% for fertility traits in dairy cattle (Abdollahi-

Arpanahi et al. 2017; Nani et al. 2019), or 27.4 – 60.7% for traits in inbred lines of Drosophila (Ye et al.

2020). Unfortunately, a good genome assembly is currently not available for striped catfish (Kim et al.

2018), this area thus deserves future studies to enable the efficient utilization of genomic information in

the genetic selection program for this economically important species in the aquaculture sector.

5. Concluding remarks

The genomic prediction accuracies for disease resistance traits of striped catfish were moderate to high,

suggesting possibilities for the application of genomic selection to improve resilience to BNP (Bacillary

Necrosis of Pangasius) disease caused by *Edwardsiella ictaluri* in this population. Machine learning

methods outperformed GBLUP-family in all our analyses, regardless of the original or imputed genotype,

full or subset data or only significant SNPs used. However, the prediction accuracies using machine

learning for both survival status and survival time were almost similar to those obtained from BayesR.

There were no clear advantages of deep learning to conventional methods (PBLUP, GBLUP and ssGBLUP).

Therefore, either machine learning or BayesR could be used for genomic evaluation of the disease

resistance traits in this striped catfish population. Furthermore, breeding to improve resistance to *E.*

ictaluri can use survival status or survival time as alternative selection criterion as there was no significant

difference in the prediction accuracies across the five different methods used in our study.

References

Abdollahi-Arpanahi, R., G. Morota, and F. Peñagaricano, 2017 Predicting bull fertility using genomic data and biological information. *Journal of Dairy Science* 100 (12):9656-9666.

Aguilar, I., I. Misztal, D. Johnson, A. Legarra, S. Tsuruta *et al.*, 2010 Hot topic: A unified approach to utilize phenotypic, full pedigree, and genomic information for genetic evaluation of Holstein final score. *Journal of Dairy Science* 93 (2):743-752.

Al-Tobasei, R., A. Ali, A.L. Garcia, D. Lourenco, T. Leeds *et al.*, 2021 Genomic predictions for fillet yield and firmness in rainbow trout using reduced-density SNP panels. *BMC genomics* 22 (1):1-11.

Al Kalaldeh, M., J. Gibson, N. Duijvesteijn, H.D. Daetwyler, I. MacLeod *et al.*, 2019 Using imputed whole-genome sequence data to improve the accuracy of genomic prediction for parasite resistance in Australian sheep. *Genetics Selection Evolution* 51 (1):1-13.

Baker, L.A., M. Momen, K. Chan, N. Bollig, F.B. Lopes *et al.*, 2020 Bayesian and Machine Learning Models for Genomic Prediction of Anterior Cruciate Ligament Rupture in the Canine Model. *G3: Genes, genomes, genetics* 10 (8):2619-2628.

Bargelloni, L., O. Tassiello, M. Babbucci, S. Ferraresso, R. Franch *et al.*, 2021 Data imputation and machine learning improve association analysis and genomic prediction for resistance to fish photobacteriosis in the gilthead sea bream. *Aquaculture Reports* 20:100661.

Barría, A., K.A. Christensen, G.M. Yoshida, K. Correa, A. Jedlicki *et al.*, 2018 Genomic predictions and genome-wide association study of resistance against Piscirickettsia salmonis in coho salmon (Oncorhynchus kisutch) using ddRAD sequencing. *G3: Genes, genomes, genetics* 8 (4):1183-1194.

Benesty, J., J. Chen, Y. Huang, and I. Cohen, 2009 Pearson correlation coefficient, pp. 1-4 in *Noise reduction in speech processing*. Springer.

Chen, L., C. Li, M. Sargolzaei, and F. Schenkel, 2014 Impact of genotype imputation on the performance of GBLUP and Bayesian methods for genomic prediction. *PloS one* 9 (7):e101544.

Chicco, D., and G. Jurman, 2020 The advantages of the Matthews correlation coefficient (MCC) over F1 score and accuracy in binary classification evaluation. *BMC genomics* 21 (1):1-13.

Conesa, A., S. Götz, J.M. García-Gómez, J. Terol, M. Talón *et al.*, 2005 Blast2GO: a universal tool for annotation, visualization and analysis in functional genomics research. *Bioinformatics* 21 (18):3674-3676.

Crossa, J., Y. Beyene, S. Kassa, P. Pérez, J.M. Hickey *et al.*, 2013 Genomic prediction in maize breeding populations with genotyping-by-sequencing. *G3: Genes, genomes, genetics* 3 (11):1903-1926.

Daetwyler, H.D., M.P. Calus, R. Pong-Wong, G. de Los Campos, and J.M. Hickey, 2013 Genomic prediction in animals and plants: simulation of data, validation, reporting, and benchmarking. *Genetics* 193 (2):347-365.

Davey, J.W., and M.L.J.B.i.f.g. Blaxter, 2010 RADSeq: next-generation population genetics. 9 (5-6):416-423.

Dinh Pham, K., J. Ødegård, S. Van Nguyen, H. Magnus Gjøen, and G. Klemetsdal, 2021 Genetic analysis of resistance in Mekong striped catfish (Pangasianodon hypophthalmus) to bacillary necrosis caused by Edwardsiella ictaluri. *Journal of fish diseases* 44 (2):201-210.

Elshire, R.J., J.C. Glaubitz, Q. Sun, J.A. Poland, K. Kawamoto *et al.*, 2011 A robust, simple genotyping-by-sequencing (GBS) approach for high diversity species. *PloS one* 6 (5):e19379.

Erbe, M., B. Hayes, L. Matukumalli, S. Goswami, P. Bowman *et al.*, 2012 Improving accuracy of genomic predictions within and between dairy cattle breeds with imputed high-density single nucleotide polymorphism panels. *Journal of Dairy Science* 95 (7):4114-4129.

Freeman, E.A., and G. Moisen, 2008 PresenceAbsence: An R package for presence absence analysis.
Journal of Statistical Software. 23 (11): 31 p.
González-Recio, O., and S. Forni, 2011 Genome-wide prediction of discrete traits using Bayesian
regressions and machine learning. *Genetics Selection Evolution* 43 (1):1-12.
Hayes, B.J., P.J. Bowman, A.J. Chamberlain, and M.E. Goddard, 2009 Invited review: Genomic selection
in dairy cattle: Progress and challenges. *Journal of Dairy Science* 92 (2):433-443.
Heidaritabar, M., M.P. Calus, H.J. Megens, A. Vereijken, M.A. Groenen *et al.*, 2016 Accuracy of genomic
prediction using imputed whole-genome sequence data in white layers. *Journal of Animal*
Breeding and Genetics 133 (3):167-179.
Henderson, C., 1985 Best linear unbiased prediction of nonadditive genetic merits in noninbred
populations. *Journal of Animal Science* 60 (1):111-117.
Houston, R.D., T.P. Bean, D.J. Macqueen, M.K. Gundappa, Y.H. Jin *et al.*, 2020 Harnessing genomics to
fast-track genetic improvement in aquaculture. *Nature Reviews Genetics* 21 (7):389-409.
Joshi, R., A. Skaarud, M. de Vera, A.T. Alvarez, and J. Ødegård, 2020 Genomic prediction for commercial
traits using univariate and multivariate approaches in Nile tilapia (Oreochromis niloticus).
Aquaculture 516:734641.
Kilian, A., P. Wenzl, E. Huttner, J. Carling, L. Xia *et al.*, 2012 Diversity arrays technology: a generic
genome profiling technology on open platforms, pp. 67-89 in *Data production and analysis in*
population genomics. Springer.
Kim, O.T.P., P.T. Nguyen, E. Shoguchi, K. Hisata, T.T.B. Vo *et al.*, 2018 A draft genome of the striped
catfish, Pangasianodon hypophthalmus, for comparative analysis of genes relevant to
development and a resource for aquaculture improvement. *BMC genomics* 19 (1):733.
Li, B., N. Zhang, Y.-G. Wang, A.W. George, A. Reverter *et al.*, 2018 Genomic prediction of breeding values
using a subset of SNPs identified by three machine learning methods. *Frontiers in genetics* 9:237.
Lourenco, D., A. Legarra, S. Tsuruta, Y. Masuda, I. Aguilar *et al.*, 2020 Single-step genomic evaluations
from theory to practice: using SNP chips and sequence data in BLUPF90. *Genes* 11 (7):790.
Masuda, Y., I. Aguilar, S. Tsuruta, and I. Misztal, 2014 Acceleration of Computations in AI REML for
Single-step GBLUP Models in *Proceedings of the 10th World Congress on Genetics Applied to*
Livestock Production.
Matthews, B.W., 1975 Comparison of the predicted and observed secondary structure of T4 phage
lysozyme. *Biochimica et Biophysica Acta (BBA)-Protein Structure* 405 (2):442-451.
Meuwissen, T.H., B.J. Hayes, and M.E. Goddard, 2001 Prediction of total genetic value using genome-
wide dense marker maps. *Genetics* 157 (4):1819-1829.
Misztal, I., A. Legarra, and I. Aguilar, 2009 Computing procedures for genetic evaluation including
phenotypic, full pedigree, and genomic information. *Journal of Dairy Science* 92 (9):4648-4655.
Misztal, I., S. Tsuruta, T. Strabel, B. Auvray, T. Druet *et al.*, 2002 BLUPF90 and related programs (BGF90),
pp. 743-744 in *Proceedings of the 7th world congress on genetics applied to livestock production*.
Montesinos-López, O.A., J. Martín-Vallejo, J. Crossa, D. Gianola, C.M. Hernández-Suárez *et al.*, 2019 New
deep learning genomic-based prediction model for multiple traits with binary, ordinal, and
continuous phenotypes. *G3: Genes, genomes, genetics* 9 (5):1545-1556.
Montesinos-López, O.A., A. Montesinos-López, J. Crossa, D. Gianola, C.M. Hernández-Suárez *et al.*, 2018
Multi-trait, multi-environment deep learning modeling for genomic-enabled prediction of plant
traits. *G3: Genes, genomes, genetics* 8 (12):3829-3840.
Moser, G., S.H. Lee, B.J. Hayes, M.E. Goddard, N.R. Wray *et al.*, 2015 Simultaneous discovery, estimation
and prediction analysis of complex traits using a Bayesian mixture model. *PLoS Genet* 11
(4):e1004969.
Nani, J.P., F.M. Rezende, and F. Peñagaricano, 2019 Predicting male fertility in dairy cattle using markers
with large effect and functional annotation data. *BMC genomics* 20 (1):258.

Nguyen, N.H., and P.V. Khang, 2021 Genome-Wide Marker Analysis for Traits of Economic Importance in
548   Asian Seabass Lates calcarifer. *Journal of Marine Science and Engineering* 9 (3):282.

549   Nguyen, N.H., C. Phuthaworn, and W. Knibb, 2020 Genomic prediction for disease resistance to
550   Hepatopancreatic parvovirus and growth, carcass and quality traits in Banana shrimp
551   Fenneropenaeus merguiensis. *Genomics* 112 (2):2021-2027.

552   Nguyen, N.H., P. Rastas, H. Premachandra, and W. Knibb, 2018 First high-density linkage map and single
553   nucleotide polymorphisms significantly associated with traits of economic importance in
554   Yellowtail Kingfish Seriola lalandi. *Frontiers in genetics* 9:127.

555   Nguyen, N.P., 2014 Enviromental factors affecting the pathogenesis of Edwardsiella ictaluri in striped
556   catfish Pangasianodon hypophthalmus (Sauvage).

557   Okeke, U.G., D. Akdemir, I. Rabbi, P. Kulakow, and J.-L. Jannink, 2017 Accuracies of univariate and
558   multivariate genomic prediction models in African cassava. *Genetics Selection Evolution* 49 (1):1-
559   10.

560   Palaiokostas, C., 2021 Predicting for disease resistance in aquaculture species using machine learning
561   models. *Aquaculture Reports* 20:100660.

562   Pérez-Enciso, M., and L.M. Zingaretti, 2019 A guide on deep learning for complex trait genomic
563   prediction. *Genes* 10 (7):553.

564   Pham, K.D., S.V. Nguyen, J. Ødegård, H.M. Gjøen, and G. Klemetsdal, 2020 Case study development of a
565   challenge test against Edwardsiella ictaluri in Mekong striped catfish (Pangasianodon
566   hypophthalmus), for use in breeding: Estimates of the genetic correlation between
567   susceptibilities in replicated tanks. *Journal of fish diseases*.

568   Pham, K.D., J. Ødegård, S.V. Nguyen, H.M. Gjøen, and G. Klemetsdal, 2021 Genetic correlations between
569   challenge tested susceptibility to bacillary necrosis, caused by Edwardsiella ictaluri, and growth
570   performance tested survival and harvest body weight in Mekong striped catfish (Pangasianodon
571   hypophthalmus). *Journal of fish diseases* 44 (2):191-199.

572   Purcell, S., B. Neale, K. Todd-Brown, L. Thomas, M.A. Ferreira *et al.*, 2007 PLINK: a tool set for whole-
573   genome association and population-based linkage analyses. *The American Journal of Human
574   Genetics* 81 (3):559-575.

575   R Core Team, 2015 R: A Language and Environment for Statistical Computing. Vienna, Austria: R
576   Foundation for Statistical Computing; 2014. R Foundation for Statistical Computing. ISBN 3-
577   900051-07-0. http://www.R-project.org.

578   Robledo, D., C. Palaiokostas, L. Bargelloni, P. Martínez, and R. Houston, 2018 Applications of genotyping
579   by sequencing in aquaculture breeding and genetics. *Reviews in aquaculture* 10 (3):670-682.

580   Silva, R.M., J.P. Evenhuis, R.L. Vallejo, G. Gao, K.E. Martin *et al.*, 2019 Whole-genome mapping of
581   quantitative trait loci and accuracy of genomic predictions for resistance to columnaris disease
582   in two rainbow trout breeding populations. *Genetics Selection Evolution* 51 (1):42.

583   Sun, J., J.E. Rutkoski, J.A. Poland, J. Crossa, J.L. Jannink *et al.*, 2017 Multitrait, random regression, or
584   simple repeatability model in high-throughput phenotyping data improve genomic prediction
585   for wheat grain yield. *The plant genome* 10 (2):plantgenome2016.2011.0111.

586   Tsai, H.-Y., O. Matika, S.M. Edwards, R. Antolín–Sánchez, A. Hamilton *et al.*, 2017 Genotype imputation
587   to improve the cost-efficiency of genomic selection in farmed Atlantic salmon. *G3: Genes,
588   genomes, genetics* 7 (4):1377-1383.

589   Tsuruta, S., and I. Misztal, 2006 THRGIBBS1F90 for estimation of variance components with threshold
590   and linear models. *Threshold* 3 (4).

591   Vallejo, R.L., B.O. Fragomeni, H. Cheng, G. Gao, R.L. Long *et al.*, 2020 Assessing Accuracy of Genomic
592   Predictions for Resistance to Infectious Hematopoietic Necrosis Virus With Progeny Testing of
593   Selection Candidates in a Commercial Rainbow Trout Breeding Population. *Frontiers in
594   Veterinary Science* 7.

Van Binsbergen, R., M.P. Calus, M.C. Bink, F.A. van Eeuwijk, C. Schrooten *et al.*, 2015 Genomic prediction
using imputed whole-genome sequence data in Holstein Friesian cattle. *Genetics Selection*
Evolution 47 (1):1-13.
Van Sang, N., G. Klemetsdal, J. Ødegård, and H.M. Gjøen, 2012 Genetic parameters of economically
important traits recorded at a given age in striped catfish (Pangasianodon hypophthalmus).
Aquaculture 344:82-89.
VanRaden, P.M., 2008 Efficient methods to compute genomic predictions. *Journal of Dairy Science* 91
(11):4414-4423.
VanRaden, P.M., M.E. Tooker, J.R. O'connell, J.B. Cole, and D.M. Bickhart, 2017 Selecting sequence
variants to improve genomic predictions for dairy cattle. *Genetics Selection Evolution* 49 (1):1-
12.
Vu, N.T., T.T.T. Ha, V.T.B. Thuy, V.T. Trang, and N.H. Nguyen, 2020 Population Genomic Analyses of Wild
and Farmed Striped Catfish Pangasianodon Hypophthalmus in the Lower Mekong River. *Journal*
of Marine Science and Engineering 8 (6):471.
Vu, N.T., N.V. Sang, T.Q. Trong, N.H. Duy, N.T. Dang *et al.*, 2019a Breeding for improved resistance to
Edwardsiella ictaluri in striped catfish (Pangasianodon hypophthalmus): Quantitative genetic
parameters. *Journal of fish diseases* 42 (10):1409-1417.
Vũ, N.T., T.Q. Trọng, L.T. Đỉnh, H.T.N. Nga, and N.V. Hiệp, 2017 Draft report: Survey results from Project
"Investigation for develop national standard regulation: freshwater fish - striped catfish
broodtsock and fingerlings - quality requirements". *Draft report to Fishery Department by*
Research Institute for Aquacultute No 2:25.
Vu, N.T., N. Van Sang, T.H. Phuc, N.T. Vuong, and N.H. Nguyen, 2019b Genetic evaluation of a 15-year
selection program for high growth in striped catfish Pangasianodon hypophthalmus.
Aquaculture.
Vu, S.V., C. Gondro, N.T. Nguyen, A.R. Gilmour, R. Tearle *et al.*, 2021 Prediction Accuracies of Genomic
Selection for Nine Commercially Important Traits in the Portuguese Oyster (Crassostrea
angulata) Using DArT-Seq Technology. *Genes* 12 (2):210.
Whalen, A., G. Gorjanc, and J.M. Hickey, 2020 AlphaFamImpute: high-accuracy imputation in full-sib
families from genotype-by-sequencing data. *Bioinformatics* 36 (15):4369-4371.
Ye, S., J. Li, and Z. Zhang, 2020 Multi-omics-data-assisted genomic feature markers preselection
improves the accuracy of genomic prediction. *Journal of animal science and biotechnology* 11
(1):1-12.
Yin, L., H. Zhang, X. Zhou, X. Yuan, S. Zhao *et al.*, 2020 KAML: improving genomic prediction accuracy of
complex traits using machine learning determined parameters. *Genome Biology* 21 (1):1-22.
Zingaretti, L.M., S.A. Gezan, L.F.V. Ferrão, L.F. Osorio, A. Monfort *et al.*, 2020 Exploring deep learning for
complex trait genomic prediction in polyploid outcrossing species. *Frontiers in plant science*
11:25.

Figure 1. Survival trend in day post challenge of high and low resistance groups in genotyped individuals of striped catfish.

Figure 2. Accuracy of survival status and survival time traits using original genotype. Middle line of the box is mean accuracy; top and bottom lines of the box is accuracy ± one standard deviation. End points of vertical line represent min and max values. Note that PBLUP uses phenotype and pedigree information only.

Figure 3. Accuracy of survival status and survival time traits using imputed genotype. Middle line of the box is mean accuracy; top and bottom lines of the box is accuracy ± one standard deviation. End points of vertical line represent min and max values. Note that PBLUP uses phenotype and pedigree information only.

Table 1. Number of animals (n) and phenotypes in the disease resistant and susceptible groups used for genotyping.

Groups	20 Lowest survival family		20 Highest survival families		All 40 families	
Trait	Individuals	Mean ± SD	Ind/family	Mean ± SD	n	Mean ± SD
Survival status (%)	245	4.49 ± 20.75	245	60.00 ± 49.09	490	32.22 ± 46.79
Survival time (day)	245	5.00 ± 2.20	245	8.66 ± 4.33	490	6.83 ± 3.89

Table 2. Variance components and heritability for survival status and survival time using AIREMLf90 and THRGIBBS1f90 sub-program in BLUPF90 and BayesR

Trait	Models	Additive genetic variance	Phenotypic variance	heritability
Survival status	PBLUP	0.147	0.207	0.71
	GBLUP	0.128	0.194	0.66
	ssGBLUP	0.085	0.190	0.45
	BayesR	0.016	0.027	0.61
Survival time	PBLUP	9.579	14,736	0.65
	GBLUP	7.395	13,450	0.55
	ssGBLUP	6.408	13.893	0.46
	BayesR	10,470	12,175	0.86

Note that estimate of genetic variance was not provided by ML-KAML or DeepGP Including DL-MLP and DL-CNN

Table 3. Genomic prediction accuracy for survival status and survival time using uni- and multivariate models

Trait	Method	Univariate		Multivariate		Increasement (%)
		Accuracy	Standard deviation	Accuracy	Standard deviation	
Survival status	PBLUP	0.597	0.101	0.627	0.095	+ 5.0
	GBLUP	0.577	0.095	0.542	0.087	- 6.1
	ssGBLUP	0.602	0.089	0.556	0.090	- 7.6
Survival time	PBLUP	0.622	0.098	0.601	0.083	- 3.4
	GBLUP	0.645	0.089	0.548	0.094	- 15.0
	ssGBLUP	0.646	0.089	0.558	0.094	- 13.6

Note: multivariate analysis is not available in BayesR, KAML and DeepGP.

Table 4. Accuracy of genomic prediction using different subsets of imputed genotypes

Trait	Method	14,154 SNPs		8,481 SNPs		834 SNPs	
Survival status		Accuracy	Standard deviation	Accuracy	Standard deviation	Accuracy	Standard deviation
	GBLUP	0.577	0.090	0.603	0.136	0.513	0.106
	ssGBLUP	0.600	0.082	0.611	0.141	0.530	0.107
	BayesR	0.654	0.095	0.646	0.107	0.606	0.126
	ML-KAML	0.645	0.085	0.650	0.101	0.592	0.094
	DL-MLP	0.739	0.087	0.628	0.117	0.622	0.088
	DL-CNN	1.059	0.038	0.711	0.132	0.643	0.080
Survival time	GBLUP	0.645	0.072	0.649	0.088	0.555	0.104
	ssGBLUP	0.646	0.089	0.649	0.088	0.560	0.104
	BayesR	0.768	0.069	0.757	0.076	0.747	0.081
	ML-KAML	0.751	0.067	0.774	0.073	0.722	0.086
	DL-MLP	0.607	0.087	0.642	0.121	0.612	0.101
	DL-CNN	0.596	0.090	0.544	0.094	0.599	0.100

Whole genotype data = 14,154 SNPs. Subset 1 (8,481 SNPs) resulted from standard QC (i.e., filtering minor allele frequency (>0.05), call-rate for SNP and animal (>0.90) and removal of monomorphic SNPs). Subset 2 (834 SNPs) from deep QC (as default QC but also remove markers that showed significant deviation from Hardy Weinberg Equilibrium and offspring-parent conflicting genotypes)

Table 5. Accuracy of genomic prediction for survival status and survival using highly significant SNPs (P < 0.0001)

Trait	Method	Original (un-imputed) genotype			Imputed genotype		
		14K SNPs	402 SNPs	%	14K SNPs	402 SNPs	%
Survival status	PBLUP	0.597	-	-	-	-	-
	GBLUP	0.530	0.533 ± 0.083	+ 0.0	0.577	0.521 ± 0.080	- 9.7
	ssGBLUP	0.545	0.541 ± 0.092	- 0.0	0.600	0.530 ± 0.083	- 11.7
	BayesR	0.572	0.578 ± 0.125	+ 1.0	0.654	0.654 ± 0.095	+ 0.0
	ML-KAML	0.628	0.584 ± 0.112	- 7.0	0.645	0.652 ± 0.088	+ 1.1
	DeepGP-MLP*	-	-	-	0.739	0.615 ± 0.111	- 16.7
	DeepGP-CNN*	-	-	-	1.059	0.747 ± 0.077	- 29.5
Survival time	PBLUP	0.622	-	-	-	-	-
	GBLUP	0.484	0.747 ± 0.102	+ 54.3	0.645	0.595 ± 0.091	- 7.8
	ssGBLUP	0.645	0.744 ± 0.105	+ 13.7	0.646	0.601 ± 0.091	- 7.0
	BayesR	0.699	0.833 ± 0.103	+ 19.2	0.768	0.768 ± 0.069	+ 0.0
	ML-KAML	0.715	0.855 ± 0.106	+ 19.6	0.751	0.761 ± 0.089	+ 1.3
	DeepGP-MLP	n.e.	-	-	0.607	0.620 ± 0.152	+ 3.1
	DeepGP-CNN	n.e.	-	-	0.596	0.665 ± 0.106	+ 11.6

* Pearson correlation were used instead of Mathew's coefficient.