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Selective breeding for juvenile survival in Chinese tongue sole (*Cynoglossus semilaevis*): Heritability and selection response

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ABSTRACT

Chinese tongue sole (*Cynoglossus semilaevis*) is an economically important marine flatfish which is now severely threatened by various bacterial pathogens (especially at juvenile stage) in China. As we all known, it is of great importance to increase the natural disease resistance of farmed fish. So, the aim of this study was to verify the exitance of genetic variance of natural disease resistance and to detect the selection response by using juvenile natural survival data (involving four year-classes and three generations with 221 full-sib families, 195,589 individuals). Survival was defined as binary trait (dead/alive) fitted in two cross-sectional models (i.e. cross-sectional linear sire-dam model (CLM) and cross-sectional threshold (logit) sire-dam model (CTM)). Heritabilities of survival were estimated with each generation dataset and with complete dataset. Heritability estimates varied among generations regardless of model used, i.e., 0.01–0.17 and 0.03–0.25 for CLM and CTM respectively. On the observed (CLM) and underlying (CTM) scale with complete dataset, the heritabilities were 0.09 ± 0.04 and 0.13 ± 0.06 respectively. Both models performed nearly identical and very high selection accuracy (> 0.99), the accuracy of selection obtained from CLM (0.993) was slightly higher than CTM (0.991). By cross-validation, the prediction accuracy of CLM is 21% higher than CTM, which was 0.885 and 0.730 for CLM and CTM respectively. The average of predicted genetic gain for each generation was 14.89%, and the average of realized genetic gain was 8.10% per generation for juvenile survival. These results confirmed the existence of genetic variation for juvenile natural survival and highlighted the enormous potential for improving natural survival by selective breeding in tongue sole.

1. Introduction

Chinese tongue sole (*Cynoglossus semilaevis*) is an important indigenous marine flatfish species which is widely distributed in China's north coastal areas (Guan et al., 2018). Together with flounder (*Bothus*) and pomfret (*Monodactylus*), tongue sole is considered as the most precious marine seafood with high nutritive and economic values in China. After decades of overfishing, the wild resource of tongue sole had depleted science 1990s. Indoor farming of tongue sole began in 2003, and with the development of intensive aquaculture, infestations caused by bacteria pathogens are a major concern to the tongue sole farming industry. Actually, diseases are quite common and frequent outbreaks reduce profitability substantially in fish farms worldwide. For improving animal health in fish production systems, disease control and prevention can be achieved through either therapeutics or immunization. However, these approaches are usually only temporary and with high costs. From a long-term perspective, another alternative approach, more in accordance with the ultimate goal of sustainable animal production in intensive fish aquaculture systems, is genetic selection for disease resistance, which is not only economically but also environmentally important.

In aquaculture, as well as in animal production in general, maintaining a high survival rate is significant to economy, animal welfare and sustainability of the industry (Ødegård et al., 2011). Previous studies on field survival (during rearing period or after natural disease outbreaks) in Atlantic salmon and Atlantic cod showed significant

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phenotypic and genetic variances and underlying heritabilities were in low to moderate magnitude across studies (ranging from 0.10 to 0.38) (Gjerde et al., 2019; Bangera et al., 2014; Gjøen et al., 1997; Standal and Gjerde, 1987). However, for selective breeding of disease resistance traits, in most cases, survival data were obtained by means of challenge test using specific pathogens (Ødegård et al., 2011). In some studies, the genetic correlations between challenge test and natural outbreak survival were very high or challenge test survival traits fitted in appropriate models showed good predictive ability for the prediction of field survival (Gjerde et al., 2019; Bangera et al., 2014; Wetten et al., 2007; Ødegård et al., 2006; Gjøen et al., 1997). Nevertheless, there is a general caveat that whether the resistance and infection mechanisms involved in challenge tests especially those by injections (bypassing the first defense mechanisms (mucus and skin) of the animal) are similar to natural infection.

In tongue sole aquaculture, *Vibrio* spp. such as *V. harveyi*, *V. vulnificus*, *V. anguillarum*, *V. alginolyticus* and *V. parahaemolyticus*, are the predominant causative agents (Hu et al., 2019; Wang et al., 2019; Gao et al., 2015; Xu et al., 2015a; Zhang et al., 2015; Tang et al., 2008) which have cost the aquaculture industry hundreds of million dollars in the past decade. Besides, other highly pathogenic species such as *Edwardsiella tarda* (Liu et al., 2016; Li et al., 2020b), *Photobacterium damselae* subsp. (Shao et al., 2019), *Streptococcus iniae* (Xu et al., 2015b), *Aeromonas salmonicida* (Zhou et al., 2017) and *Pseudomonas fluorescens* (Gao et al., 2016) have been reported recently. Worse, the major pathogenic agents of tongue sole varied over time (yearly and seasonally) and even differed between farms. The epidemic situation of these bacterial diseases showed sporadic or massive outbreaks at juvenile stage (5–20 cm) in summer (water temperature 23 ± 3 °C). In these cases, the selective breeding for disease resistance seems more difficult in tongue sole. For this situation, we have developed specific response programs for disease resistance breeding against *V. harveyi* (Li et al., 2019), *V. anguillarum* (Chen et al., 2010; Liu et al., 2016), *E. tarda* (Liu et al., 2016; Li et al., 2020a), and several disease resistant strains (i.e., vibriosis and edwardsienosis resistant strains) have been selected. However, in the field tests under commercial aquaculture conditions, it is not effective enough, more specifically, these vibriosis and edwardsienosis resistant strains are effective for corresponding bacterial pathogens, but not for other kinds of bacterial pathogens.

So, we readjusted our disease resistance breeding program of tongue sole, taking juvenile survival as a desirable breeding goal in dealing with resistance, though this trait was influenced by various pathogens. Therefore, the main objective of this study was to estimate variance components and heritability for juvenile survival in tongue sole, using data involving 195,589 individuals from 221 full-sib families (four year-classes, three generations). Survival was defined as binary survival trait (dead / alive) fitted in cross-sectional linear and threshold models for obtaining observed and underlying heritabilities respectively. Further, the predicted and realized selection response was also calculated.

2. Materials and methods

2.1. Broodstocks selection

Experiments were performed in Flatfish Breeding Centre, Yantai, China. The base population of broodstocks used for this selection program was from the wild-caught population (2011), cultured stocks (2011) from different farms (by mass selection) and 21 full-sib families established in 2012. Due to the sexual dimorphism (female far larger than male) and the effect that females mature later than males (about one year later), the broodstocks of females and males used in each year-class (YC) were not from the same year (Table 1). Females of 2013 were mainly selected from 15 full-sib families (total 28 full-sib families) with top 20% family estimated breeding values (EBVs) estimated based on *E. tarda* and *V. anguillarum* challenge test survival data and natural survival data (Table 6 by Liu et al., 2016), totally, 780 heathy individuals with good body shape were selected as broodstocks for further cultivation. Families produced in 2012 and 2013 originated from 2008 and 2010 YC families (Table 1). The Females and males of 2014 were selected from population of 78 full-sib families, with a natural survival rate of 31.4% at juvenile stage during the summer of 2014 (this study), then these fish were transferred to concrete tanks and reared under commercial production environment with a 19% finial survival rate. And combined with *V. harveyi* challenge test (Li et al., 2019) and natural disease outbreak test results (Hu et al., 2020), 900 individuals (300 females and 600 males) from 21 families with top 20% family EBVs of natural survival and/or challenge test survival were selected as broodstock. Females and males of 2015 were selected from 31 full-sib families, with a natural survival of 48.2% in juvenile stage (this study) and then after another commercial rearing test (tagged by family using visible implant elastomer, stocking in common tanks) to harvest stage, final survival was 38.7% (stocking no. 8111, harvest no. 3135). Then combined with *V. harveyi* challenge test results, 800 individuals (250 females and 550 males) from 12 families with top 20% of family EBVs of harvest survival and/or challenge test survival. Some of the selected families in each YC are multi-resistant. According to the above survival information, we assumed that the selection proportion (p) of broodstocks was approximate 20%. So, the selection intensity was 1.40, i.e., $i \approx 1.40$ (Table 11–3 by Falconer and Mackay, 1996). Pseudo-males were identified by using sex specific SSR markers and eliminated according to methods as described by Chen et al. (2012). All the selected broodstocks were individually tagged with Passive Integrated Transponders (PIT) (Qingdao Starfish Instruments Co., Ltd., China).

Table 1
The year-class (YC) of broodstock population, selected traits of broodstock, selected family no., total family no. and generation of each YC families.

YC of family	YC of broodstock		Selected traits of broodstock	Selected family no. / Total family no.	Generation
	Dam	Sire			
2012	2008	2010	CTS-Va, NS-H	10/21	G0
2013	2010	2010	CTS-Et, CTS-Va, NS-H	15/28	
2014	2011	2012	NOS, NS-J, NS-H, CTS-Vh	24/78	G1
2015	2013	2014	NOS, NS-H, CTS-Vh	12/31	G2
2016	2014	2014	–	–	
2018	2015	2015	–	–	G3

CTS-Vh: challenge test survival by *V. harveyi*; CTS-Va: challenge test survival by *V. anguillarum*; CTS-Et: challenge test survival by *E. tarda*; NOS: natural outbreak survival; NS-J: natural survival at juvenile stage; NS-H: natural survival at harvest stage.

2.2. Family production and rearing conditions

Methods of family production and maintenance were mainly referenced from Li et al. (2019). Briefly, in the spring of each year, families were produced by stripping and artificial fertilization procedures. Dams were mated with one or two sires, and one sire mated with one dam. Inbreeding was strictly prohibited according to the pedigree information. In some cases, substantial matings were unsuccessful due to the poor quality of eggs or milt. Each family was reared in a separate tank ($2.5m^3$). About 90 days post hatch, 600–1000 offspring of each family were randomly sampled and reared in each separate tank for monitoring survival rate (families in each year with similar initial stocking number). During rearing period, fish were fed with commercial pellet feed (crude protein ~55%, crude lipid ~8% and crude fibre ~3%) (Santong Bio-engineering (Weifang) Co., Ltd., China) with approximate 1% feeding ratio. Water flow through rate was 400–500% per day, salinity was 28–32‰, and dissolved oxygen was 6–8 mg / L. After

Table 2
Descriptive statistics of dataset used.

Item	2014-G1	2015-G2	2016-G2	2018-G3	Total
Fish with data	76,440	18,580	45,709	54,860	195,589
No. of full-sib family	78	31	46	66	221
No. of sire	77	31	46	66	220
No. of dam	78	19	46	66	209
Average sample size	980	599	994	831	–
Temperature range (°C)	18.6–24.8	14.2–24.5	16.5–25.0	15.2–25.5	–
Final survival (%)	31.40	48.19	50.21	79.64	–
Variance of family survival (%)	0.8	5.0	4.1	1.0	–
Family survival range (%)	13.1–53.4	20.3–90.8	8.6–85.5	56.0–96.0	–
Average initial length (cm)	7.3	7.1	7.8	7.5	–
Average final length (cm)	14.2	12.2	13.8	13.6	–
Age at stocking (days)	85	86	91	96	–
Duration (days)	102	111	104	95	–

about a 100-day rearing test in each year, survivors of each family were counted and recorded. Dead fish were removed daily and no escape, predator and toxicity in our farming conditions. The management regimes were very similar across year-classes. In most cases, the classical symptoms of dead individuals across years were surface ulcer and tail-rot which should be mainly ascribed to *Vibrio* spp. according to the 16 s rDNA sequencing results. And in rare cases, pathogenic bacteria were identified as *Photobacterium* spp., *Pseudomonas* spp. and *Aeromonas* spp. It was almost certainly that all the mortalities were caused by bacterial diseases. No parasitic or viral diseases were observed. The causes of death could be associated to several factors, for example, the ubiquitous and widespread opportunistic pathogenic bacteria in marine farming environment. And we cannot rule out the other possible causal factors, including temperature oscillations and social interactions. No antibiotic drug was used throughout the experiments. Finally, 221 full-sib families from 4 year-classes (involving three generations, i.e., 2014-G1, 2015 and 2016-G2, 2018-G3) were used in this study. More details were showed in Table 2.

2.3. Data analysis

The disease resistance was analyzed as binary natural survival, based on whether the individuals were alive (score = 1) or dead (score = 0) at the end of the rearing period. Survived fish were assumed to be more resistant than those died. The datasets for each year-class and complete dataset were analyzed using two cross-sectional sire-dam models with complete pedigree information. Models were defined as follows:

(1) Cross-sectional linear sire-dam model (CLM):

$$y_{ijk} = \mu + f_i + s_j + d_k + c_{jk} + e_{ijk}$$

where y_{ijk} is the observed survival (0 = dead, 1 = alive) of animal i from full-sib family jk; μ is the overall mean; f_i is the fixed effect, i.e. year; s_j and d_k is the random genetic effect of sire j and dam k respectively; c_{jk} is the common full-sib environmental effect; e_{jk} is the residual effect.

(2) Cross-sectional threshold (logit) sire-dam model (CTM):

$$\Pr(y_{ijk} = 1) = \frac{\exp(\mu + f_i + s_j + d_k + c_{ijk})}{1 + (\mu + f_i + s_j + d_k + c_{ijk})}$$

where all the parameters are as described above.

Variance components and EBVs were estimated based on best linear unbiased prediction (BLUP) by using ASReml-R4 software package (Butler et al., 2017). The significance of fixed effect was tested using Wald-F statistics (within ASReml-R). For both models, the additive genetic sire and dam effects were assumed ~N (0, $A\sigma_{sd}^2$), where $\sigma_{sd}^2 = \sigma_s^2 = \sigma_d^2 = 1/4\sigma_a^2$; common full-sib environmental effects were assumed ~N (0, $I\sigma_c^2$), and residuals (if included in the model) were assumed ~N (0, $I\sigma_e^2$); where A is the additive genetic relationship matrix, I is the identity matrix, and σ_a^2, σ_s^2, σ_d^2, σ_{sd}^2, σ_c^2, σ_e^2 is additive genetic variance, additive genetic sire variance, additive genetic dam variance, additive genetic sire-dam variance, common full-sib environmental variance and residual variance ($\pi^2/3$ for CTM) respectively. The phenotypic variance was the sum of $2\sigma_{sd}^2$, σ_c^2 and σ_e^2 ($\sigma_P^2 = 2\sigma_{sd}^2 + \sigma_c^2 + \sigma_e^2$). For both models, heritability (h^2) was calculated as the ratio between $4\sigma_{sd}^2$ and σ_P^2 ($h^2 = 4\sigma_{sd}^2/\sigma_P^2$), common full-sib environmental effect (c^2) was calculated as the ratio between σ_c^2 and σ_P^2 ($c^2 = \sigma_c^2/\sigma_P^2$).

2.4. Model comparison

Model comparison was assessed based on the selection accuracy and prediction accuracy through the method of Gitterle et al. (2006) and cross-validation separately. Firstly, the selection accuracies (r_τ) of the both models were predicted based on the Pearson correlation coefficients (r_{EBV}) between the full-sib family EBVs ($1/2[s_j + d_k]$), where $r_{EBV} \approx r_\tau^2$ (Gitterle et al., 2006). Full-sib family EBVs were independently predicted from two random sampled and equally sized subsets of the complete dataset by using the both models respectively. Specifically, complete dataset (total 195,589 individual records) was randomly divided into two subsets (97,795 and 97,794 individual records respectively), then they were used to predict full-sib family EBVs for calculating Pearson correlation coefficients. This procedure was tripled to obtain the average values of Pearson correlation coefficients.

On the other hand, due to the fact that the same trait definition was used in CLM and CTM, the prediction accuracies of the both models were performed by 5-fold cross-validation with 10 replicates. Before analysis, complete data subset was divided into 5 equal parts randomly. In each analysis, one of subsets was chosen as testing set, and the rest were used as training sets. Each subset was only used once as testing set in five verifications. The predictive ability of the both models was estimated as correlation coefficient between the predicted EBVs and the phenotypes. The accuracy of prediction for each model was estimated by dividing the correlation coefficient by the square root of the estimated heritability. The calculating formula of predictive accuracy by cross-validation is $A_{cv} = r_{(EBV,y)}/h$, where $r_{(EBV,y)}$ is the Pearson correlation between EBVs and phenotypes of validation set; h is the square root of the estimated heritability.

2.5. Selection response

2.5.1. Predicted genetic gain

In this breeding program, the selection of broodstocks was based on EBVs. So the genetic gain (ΔG) was predicted based on the accuracy of EBVs, r_{IH}, also known as the correlation between true and predicted breeding values (random effects). Genetic gain was calculated as $\Delta G = ir_{IH}\sigma_a$, where $r_{IH} = \sqrt{1 - \frac{PEV}{\sigma_a^2}}$; i is selection intensity which was 1.40 in this study; r_{IH} is accuracy of EBVs; PEV is predictor error variance, which corresponds to the square of the standard error (SE) of the EBV, i.e. $PEV = SE^2$; ${\sigma_a}^2$ is additive genetic variance (${\sigma_a}^2 = 4\,{\sigma_{sd}}^2$). Note that, PEV values closer to 1.0 are an indication of very good quality of a given additive effect estimate. And we also should aware that the involved genetic evaluations are based on linear mixed models, namely, in this study, the ${\sigma_a}^2$ was estimated from CLM, rather than CTM. More methodologies can be referred from case studies of calculating accuracy and reliability of random effects with ASReml-R (including codes) by VSNi, website: https://www.vsni.co.uk/case-studies/reliability. The observed gains (ΔG_O) were calculated from least-squares means (LSMs) between adjacent generations. The LSMs were the best linear unbiased estimates of the marginal means, and CLM (with dataset from each generation) was used to calculate LSM which is a fixed value.

2.5.2. Realized genetic gain in BLUP breeding values

In this study, G1 was set as the base population. Mean EBVs for juvenile survival in each generation were calculated to obtain a genetic trend estimate in next generation. The realized genetic gain (ΔG_R) for juvenile survival was evaluated as the difference between means of EBVs between generations. Then the realized genetic gain was expressed as a percentage of the LSMs of the base population in next generations.

3. Results

3.1. Descriptive statistics

Descriptive statistics for tongue sole juvenile natural survival of each year class were shown in Table 2. Overall survival rate across four year-classes was 50.92%. Family survival rates over year-classes ranged from 8.6% to 96.0%. Survival rates and EBVs (obtained by CLM) of each full-sib family from four year-classes were presented in Fig. 1. Box-plots of survival rates of each generation were shown in Fig. 2. Large variations of family survival were observed, especially for G2 (YC 2015 and 2016) which with approximate 50% overall mortalities. The Spearman ranking correlation between family survival rates and family EBVs was 0.67, indicating substantial re-ranking effect which also revealed the importance of selection based on EBV rather than phenotype.

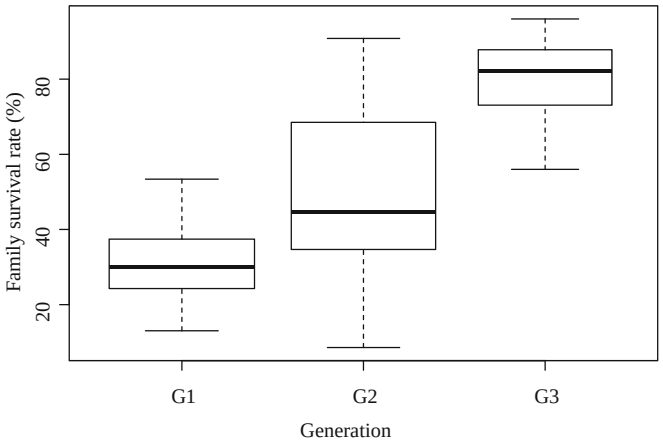

Fig. 2. Box-plots of family juvenile survival rates of tongue sole in each generation.

3.2. Heritability and model comparison

Variance components, estimates of heritability (h^2) and proportion of common full-sib environmental effect (c^2) for two models are presented in Table 3. Heritability estimates across all generations with complete dataset in CLM and CTM were 0.09 ± 0.04 and 0.13 ± 0.06 respectively. For both models, heritabilities were highly variable in different generations, which should be ascribed to different overall mortalities and different degrees of family survival variation in each generation. Overall, heritability estimates on underlying scale (CTM) were higher than observed scale (CLM). The magnitudes of common to full-sib environmental effects obtained from the both models were low (0.00–0.10).

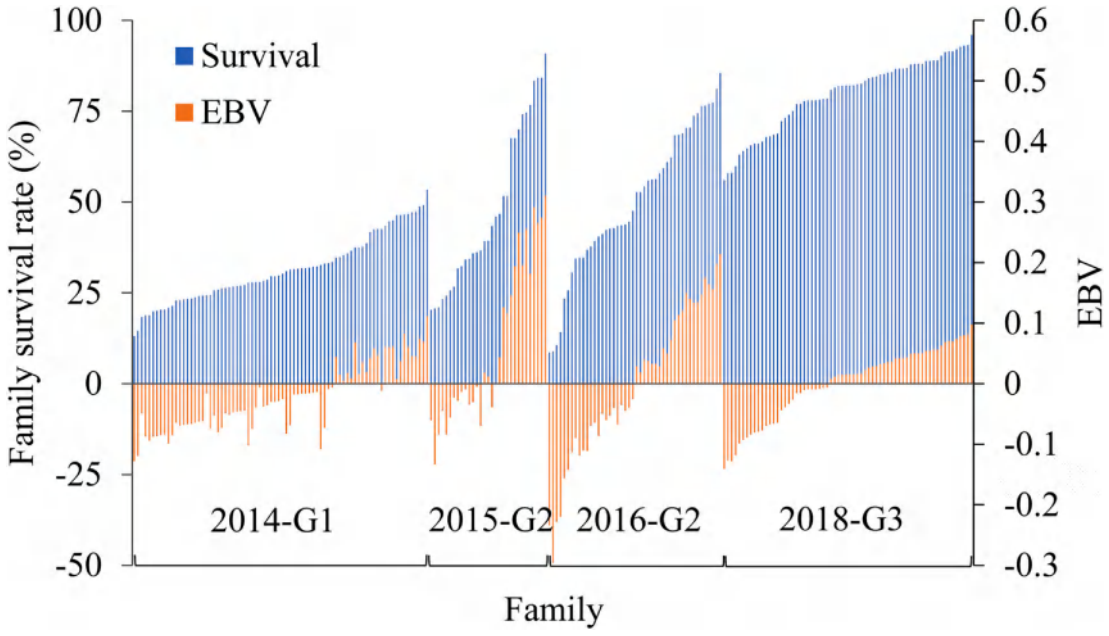

Fig. 1. Family juvenile survival rates and estimated breeding values (EBVs) from four year-classes (three generations) in tongue sole.

Table 3
Generation Estimates of additive genetic sire-dam variance (σ_{sd}^2), common to full-sib environmental variance (σ_c^2), residual variance (σ_e^2), heritability ($h^2 \pm$ SE) and common to full-sib environmental effect ($c^2 \pm$ SE) for juvenile tongue sole survival for two models in each generation.

Model	Generation	σ_{sd}^2	σ_c^2	σ_e^2	σ_P^2	$h^2 \pm$ SE	$c^2 \pm$ SE
CLM	G1	0.004	0.000	0.207	0.211	0.07 ± 0.01	0.00 ± 0.00
	G2	0.011	0.022	0.208	0.241	0.17 ± 0.09	0.09 ± 0.08
	G3	0.0003	0.009	0.153	0.163	0.01 ± 0.05	0.06 ± 0.03
	Across	0.005	0.014	0.192	0.211	0.09 ± 0.04	0.07 ± 0.02
CTM	G1	0.089	0.000	3.290	3.379	0.10 ± 0.02	0.00 ± 0.00
	G2	0.263	0.444	3.290	4.997	0.25 ± 0.18	0.10 ± 0.10
	G3	0.026	0.363	3.290	4.173	0.03 ± 0.11	0.10 ± 0.05
	Across	0.127	0.324	3.290	3.741	0.13 ± 0.06	0.08 ± 0.03

Table 4
Selection accuracy (r_τ) and prediction accuracy (A_{cv}) of both models for juvenile survival of tongue sole.

Model	r_τ	A_{cv}
CLM	0.993	0.885
CTM	0.991	0.730

Selection accuracy (r_τ) and prediction accuracy (A_{cv}) of the both models were shown in Table 4. Based on the methodologies of Gitterle et al. (2006), both models performed very high and nearly identical selection accuracy, where the value obtained from CLM (0.993) was slightly higher than CTM (0.990). However, the prediction accuracy of CLM by cross-validation is 21% higher than CTM, which was 0.885 and 0.730 for CLM and CTM respectively.

3.3. Selection response

The predicted genetic gains (ΔG) of juvenile survival from G1 and G2 were 17.53% and 24.96% respectively which were similar to corresponding observed gains (ΔG_O), from G1 to G2 and G2 to G3 were 18.00% and 30.54% respectively (Table 5). However, there is a significant declining of ΔG from G3, which was 2.18%. The accuracies of prediction (r_{IH}) based on family EBVs from G1 and G2 were high, while this value sharply decreased to 0.45 in G3. Mean family EBVs and realized genetic gain (ΔG_R) for juvenile survival in each generation were shown in Table 6. Genetic trend analysis based on mean family BLUP EBVs across generations predicted an average realized genetic gain of 8.10% per generation for juvenile survival with a rising then decreasing trend.

4. Discussion

It is of great importance to increase the natural disease resistance of farmed fish for prevent outbreak of diseases (Fjalestad et al., 1993). In aquaculture, genetic improvement of disease resistance by challenge test under controlled environment has been proved an effective method (Ødegård et al., 2011). It has been shown that the results from challenge test experiments can be highly consistent with mortalities following natural disease outbreaks. For example, case studies in Atlantic salmon, the genetic correlation between *Areomonas salmonicida*

Table 5
Predictive accuracy (r_{IH}), predicted genetic gain (ΔG) from each generation and observed gain (ΔG_O) between adjacent generations for juvenile survival of tongue sole.

Generation	r_{IH}	ΔG (%)	ΔG_O (%)
G1 → G2	0.99	17.53	18.00
G2 → G3	0.85	24.96	30.54
G3 → G4	0.45	2.18	–
Average	–	14.89	–

Table 6
Mean family EBVs, realized genetic gain (ΔG_R) and least squares mean (LSM) in each generation for tongue sole juvenile survival.

Generation	Mean EBV	ΔG_R	LSM (%)	Percentage [a]
G1	−0.0370	–	31.23	–
G2	0.0378	0.0748	49.23	23.95
G3	−0.0004	−0.0382	79.77	−7.76
Cumulative	–	0.0366	–	16.19
Average	–	0.0183	–	8.10

[a] Percentage refers to actual units in relation to the LSMs of family survival of the parent generation.

challenge test survival and field outbreak survival was 0.95 (Gjøen et al., 1997), and the genetic correlations between infectious pancreatic necrosis challenge tests and field outbreaks were high (range 0.78–0.83) (Wetten et al., 2007). In tongue sole, however, the genetic correlation between *V. harveyi* challenge test and field outbreak vibriosis was very low, 0.06 (Hu et al., 2020), which may be ascribed to the co-infection of several *Vibrio* spp., though the main pathogen was verified as *V. harveyi*. As mentioned in the introduction section, tongue sole can be severely infected by a wide range of pathogenic bacteria at juvenile stage, we proposed an alternative disease resistant breeding program by using juvenile natural survival data.

In this study, the heritability estimates of survival on the underlying scale were larger than estimates on the observed scale, which are in agreements with the previous studies (Ødegård et al., 2007; Yáñez et al., 2013; Wonmongkol et al., 2018; Li et al., 2020b). Due to the differences in scaling of trait between models, it is difficult to compare their heritability estimates (Gitterle et al., 2006). The heritability estimates varied among generations which should be ascribed to the different magnitudes of phenotypic variance in each generation, where when cumulative mortality is approximate 50%, the heritability is expected to increase (Gjerde et al., 2009). Though each full-sib family was reared in separate tanks throughout the experiments, the common environmental effects were not high, which was 0.07 ± 0.02 and 0.08 ± 0.03 for CLM and CTM with complete dataset respectively.

The selection accuracies (r_τ) of both models were estimated based on the correlation between family (mid-parent) EBVs by using two randomly split halves of the complete dataset. The square root of the Pearson correlation coefficient was used as an indicator of the predictive ability of each model (Gitterle et al., 2006). Very high estimated r_τ values (CLM: $r_\tau = 0.993$, CTM: $r_\tau = 0.991$) indicated the estimated family breeding values are pretty close to the actual values. As far as we known, this was higher than all the previous similar studies. This may be ascribed to a large sample size (offspring ≥599) of each family in each year-class. Because of that r_τ can be improved by adding more offspring per family and better family structure (Bangera et al., 2014). The prediction accuracy of the both models by cross-validation revealed that CLM yielded 21% higher prediction accuracy than CTM. Model comparison through cross-validation method has been widely used in genomic selection studies in aquaculture species (Sonesson and

Meuwissen, 2009; Yoshida et al., 2019). However, in pedigree-based BLUP (PBLUP), this validation strategy for model predictive accuracy for disease resistance trait is still scarce which should be ascribed to the different trait definitions when estimated disease resistance traits (e.g., binary survival, test-day and binary test-day).

The selection response of juvenile survival in terms of predicted genetic gain was substantial and in accordance with the observed gain in G1 and G2 (Table 5). However, genetic gain predicted from G3 decreased significantly to 2.18%. This can be interpreted that the juvenile survival of G3 population is 79.64% which is in a high level, and there must be a low selection intensity and accuracy of broodstocks when generating the next generation. Thus, the genetic improvement of juvenile survival is expected to slow down. The realized genetic gain of juvenile survival was 8.10% units per generation. There is an increasing first then decreasing trend which may due to the 'homogeneous' effect when cross generation survival data was used to estimate breeding values. In aquatic species, the mean estimate of realized genetic gains in general survival (not challenge test survival) is very low (average of 4.9% per generation, range 1.1%–8.4%, $n = 5$) (reviewed by Gjedrem and Rye, 2018), which can be explained by the fact that this trait is influenced by numerous environmental factors (Gjedrem and Rye, 2018). Furthermore, we believe that the finite cap (survival rate top to 1) and unstable rearing environments across year-classes (generations) will inevitably slow down or even reverse the selection response during disease resistance selective breeding process in aquaculture.

In the present study, considerable selection response for survival at juvenile stage in tongue sole has been obtained, one major reason was that the selection intensity was strong enough due to low survival rate of parental populations, specifically, the survival rate of population in 2013, 2014 and 2015 was 45%, 19% and 19% respectively; on the other hand, parental fish were selected from families with top 20% family EBVs of different survival traits (i.e., challenge test survival and/or juvenile survival and/or harvest survival). It is widely acknowledged that selection based on EBVs was more accurate and efficient than based on phenotype in animal breeding. In hindsight, this was a combination of mass selection and family selection which showed its effectiveness. Anyway, these approaches facilitated our selection progress in tongue sole.

5. Conclusion

This is the first report of heritabilities and selection response for natural survival in juvenile tongue sole. Substantial variations of survival were observed and the survival was low to moderately heritable. Considerable genetic gain has been achieved. The results demonstrated the feasibility of selection for improvement of juvenile survival of tongue sole under commercial rearing environment. Our successful selective breeding program for juvenile survival in tongue sole would be beneficial for farmers.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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