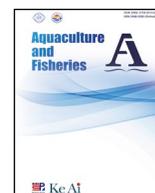




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Estimation of heritabilities of disease resistance to *Edwardsiella tarda* and genetic correlations between resistance and growth traits in Chinese tongue sole (*Cynoglossus semilaevis*)

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ABSTRACT

Chinese tongue sole (*Cynoglossus semilaevis*) is an important marine flatfish which is widely cultured in coastal areas in China. Infectious bacterial diseases such as *Edwardsiella tarda* imposed serious threats to this species. A potential strategy to prevent this disease is to select resistance strains. The aim of the present work was to estimate the genetic variation of disease resistance to *E. tarda* and to evaluate the genetic correlations between resistance traits and growth traits. Three types of models were fitted by using different trait definitions (binary, continuous and categorical). After a 9-day challenge test, the overall survival was 75.4% (ranging from 6.4% to 100% in families), and at test day 6, the overall survival was 50.7%. We set test day 6 and 9 as cut-off point times respectively. The heritabilities of survival traits were ranging from 0.10 to 0.36. Considerably higher heritability values were obtained at day 6 than at day 9, regardless of which model or trait definition used (except Trait 2 in LIN). The genetic correlations between disease resistance traits and growth traits (i.e. body weight and total length) were low and not significant from zero (−0.12–0.24). There is a substantial re-ranking of families when defined resistance as categorical and continuous traits compared to binary trait. These results confirm the existence of genetic variation for resistance against *E. tarda* and weak genetic correlations indicate that joint genetic improvement of *E. tarda* resistance and growth is scarcely available.

1. Introduction

Edwardsiella tarda is a Gram-negative bacteria and the etiological agent of edwardsiellosis which inhabits a wide range of animals, such as fish, amphibians, reptiles, birds and mammals including humans (Abbott & Janda, 2006). In aquaculture, *E. tarda* as a leading pathogen with a systemic hemorrhagic septicemia symptom caused serious loss in both freshwater and marine fish worldwide (Xu & Zhang, 2014). Currently, the common method to treat edwardsiellosis is by antibiotics and antimicrobials which results in environmental pollution, microbial resistance and drug residues (Liu, Tang, Sheng, Xing, & Zhan, 2017). Another alternative approach to prevent this disease is by using vaccines, which is safety and environmental friendly (Yamasaki et al., 2015; Zeng et al., 2018). However, vaccination provides only a temporary protection due to low immune competence in some aquaculture species and means an additional cost for fish farmers (Antonello et al., 2009).

Chinese tongue sole (*Cynoglossus semilaevis*) is a commercially important marine flatfish which is widely cultured in coastal areas in China due to delicacy and rarity (Hu et al., 2014; Sha, Wang, Liu, & Chen, 2012). However, the culture of Chinese tongue sole has been severely threatened by bacterial pathogens, such as *E. tarda*, *Vibrio harveyi* and *Vibrio anguillarum* (Gao et al., 2016; Tang, Zhou, & Zhan, 2008). In China, these bacterial diseases pose a major threat to tongue sole aquaculture, but neither systematic eradication programs nor efficient vaccines are yet available to minimize the occurrence of diseases. More seriously, studies about selective breeding for disease resistance traits in this species are still very limited.

From a long-term perspective, genetic improvement of disease resistance through selective breeding is a complementary and potentially strategy for disease prevention. In practical fish breeding programs, disease resistance is commonly assessed as survival by challenge test experiment with specific pathogens isolated from diseased fish

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(Ødegård, Baranski, Gjerde, & Gjedrem, 2011). In most aquaculture breeding programs, growth is the most important trait which has often relatively higher heritability, however, disease resistance trait has generally low heritability and it is difficult to measure or estimate (Antonello et al., 2009). Nevertheless, the prospect for significant genetic improvements in disease resistance have been confirmed in numerous recent studies by challenge test, such as Atlantic salmon (Doan et al., 2017; Taylor, Kube, Muller, & Elliott, 2009; Yáñez, Banger, Lhorente, Oyarzún, & Neira, 2013; Yáñez et al., 2014), sea bream (Antonello et al., 2009), Nile tilapia (Shoemaker et al., 2017), common carp (Jeney, Árdó, Ronyai, Bercsenyi, & Jeney, 2011; Ødegård et al., 2010), Atlantic cod (Ødegård, Sommer, & Prabel, 2010; Banger, Ødegård, Prabel, Mortensen, & Nielsen, 2011; Banger et al., 2014) and blunt snout bream (Xiong et al., 2017). Thus, substantial evidence shows that resistance to infectious diseases in aquaculture has significant genetic components.

Therefore, the present experiment was conducted to estimate heritability of resistance against *E. tarda* in Chinese tongue sole using challenge test survival data by using three kinds of trait definitions based on different types of models. Besides, genetic correlations between resistance and growth traits (i.e. body weight and total length) are also investigated. This information will be beneficial for planning breeding programs for controlling this disease by genetic improvement.

2. Materials and methods

2.1. Production of families and fish rearing

All the brood stocks were collected and maintained at Laizhou Mingbo Aquatic Co., Ltd., and individually tagged with Passive Integrated Transponders (PIT) (Qingdao Starfish Instruments Co., Ltd.). Methods of full-sib family production and larvae and juvenile fish rearing were similar to previous studies (Chen et al., 2010; Li et al., 2019). Finally, due to the limitation of facilities only 39 families were used in this study.

2.2. Challenge test

When fish reached an average body weight of about 10 g, 600 individuals from each family were transferred to our experimental station in Weifang Shandong Province for challenge test. *E. tarda* strain used for challenge test in this study was isolated from diseased fish with typical symptoms in 2013. After a 2-week accommodation, challenge test was carried out by intraperitoneal (IP) injection. Briefly, the median lethal dose (LD₅₀) was determined using four different dilutions (1:100, 1:1000, 1:2000, 1:5000) from the original inoculum in a preliminary IP challenge (0.05 ml per 5 g fish body weight), which was carried out in a random sample of 780 fish (195 fish per dilution) from all the families (20 fish per family). Finally, the LD₅₀ was determined as 1:1500, and a 75% lethal dose (LD₇₅ 1:1200) was used for IP injection to obtain an approximate 75% overall mortality. About 100 individuals (with few exceptions) were sampled randomly from each family for challenge test and the inoculated fish were kept at separate tanks (2.5 m³) by family. There was no replicate due to the limitation of facilities.

The tank environments were standardized through a flowing water exchange, thus they were assumed to be in the same common environment. The day of challenge was defined as day 0, and we terminated the experiment at day 9. Dead or moribund individuals were removed from the tanks three times daily and the time of removal was recorded. Body weight (BW) and total length (TL) were measured and the sex was verified at the time of death for each dead fish or at the end of the test for survivors. During the challenge test period, fish were fed twice daily according to their appetite, the water temperatures were maintained at 22 ± 1 °C. Salinity was 28‰, water exchange rate was 400% per day and dissolved oxygen was 7 ± 1 mg/L. Before injection,

Table 1
Descriptive statistics of the dataset used.

Item	
Fish with data	3727
No. of full-sib family	39
Average sample size	96
No. of sire	39
No. of dam ^a	34
Final mortality (%)	75.4
Range of mortality of each family (%)	6.4–100
Duration (day)	9
Average body weight (g)	10.3
Average total length (cm)	12.2

^a There were 5 dams mated with two sires respectively.

fish were anaesthetized with MS-222 (3-aminobenzoic acid ethyl ester methanesulfonate) to avoid handling stress. Finally, 3727 individuals were used in the challenge test. More details of the experimental data about the two tests are given in Table 1.

2.3. Trait definition

In this study, the challenge test survival was defined using three different trait definitions.

Trait 1: Binary test survival (BTS), which was scored as 0 if the fish died before the cut-off point (test day) and scored 1 elsewhere. For the later analyses, day 6 and 9 was set as the cut-off points respectively, where days after cut-off point times, individuals were regarded as survival.

Trait 2: Day of death (DD), with one record per fish died at the specific test day. The observation was scored n ($n = 1-9$) if the fish died on the n th test day, and the survivals were censored. This definition was similar to the paper of Yáñez et al. (2013). This definition takes the time of death into account and thus potentially reveals information that is masked in Trait 1. It is assumed that resistance is associated with survival time.

Trait 3: Binary test day survival score (BSS), in which the survival time (days) was transformed to binary survival records with one record per day the fish stayed in the test and the number of records per fish equals the number of days until death or censoring. The observation was scored 0 if the fish died on the actual test day and scored 1 if the fish was alive on the actual test day, e.g., a fish died at day 5 had survival scores of [1 1 1 1 0] (Banger et al., 2014; Gitterle, Ødegård, Gjerde, Rye, & Salte, 2006; Ødegård et al., 2010). For the later analyses, day 6 and 9 was set as the cut-off points (censoring points) respectively, where days after cut-off point times, individuals were regarded as censoring.

2.4. Statistical models

The variance components and estimated breeding values (EBVs) for resistance to *E. tarda* were analyzed with different types of models based on restricted maximum likelihood (REML) algorithm by using ASReml-R3.0 package (Butler, Cullis, Gilmour, & Gogel, 2009). The models were defined below:

(1) Cross-sectional linear animal model (CSL) for Trait 1 and linear animal model (LIN) for Trait 2:

$$y_{ik} = \mu + f + a_i + c_k + e_{ik}$$

where y_{ik} was the phenotypic observation for the traits; μ is the overall mean; f was the fixed effect, which was sex; a_i was the random additive effect for the i th individual; c_k was the random effects of common environment to full-sib family k (including tank effect); e_{ik} was random residual.

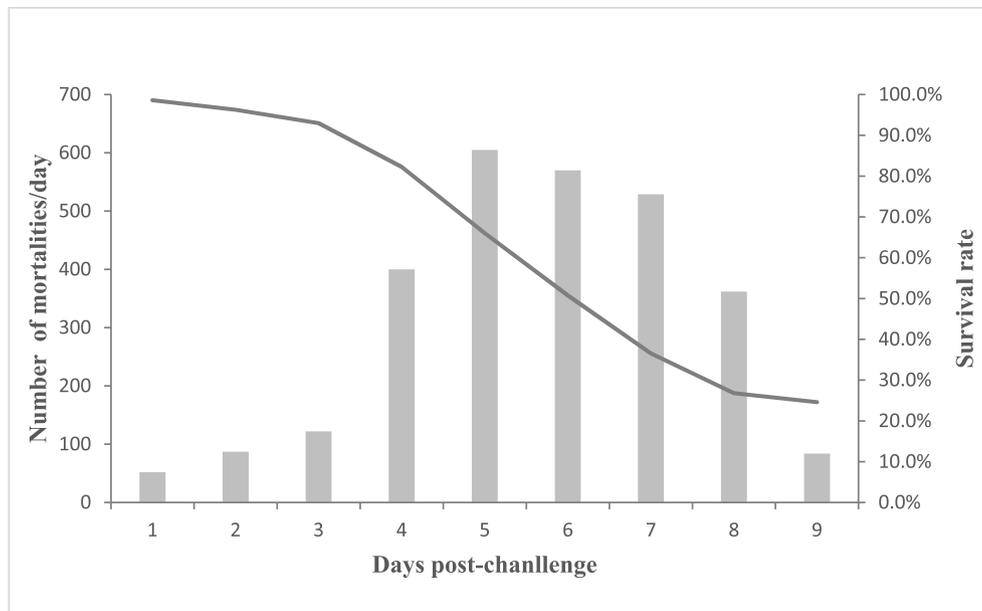


Fig. 1. The observed accumulated daily mortality bar charts and survival curve for all the full-sib families in the *E. tarda* challenge test.

(2) Cross-sectional threshold (logit) animal model (CST) for Trait 1:

$$Pr(Y_{ik} = 1) = \frac{\exp(\mu + f + a_i + c_k)}{1 + \exp(\mu + f + a_i + c_k)}$$

where all the parameters were as described in CSL and LIN.

(3) Linear repeatability model (LRM) for Trait 3:

$$Pr(Y_{ik} = 1) = \frac{\exp\left(\sum_{p=0}^2 b_p Z_p(t) + f + a_i + c_k\right)}{1 + \exp\left(\sum_{p=0}^2 b_p Z_p(t) + f + a_i + c_k\right)}$$

where $Z_p(t)$ is a p th order orthogonal polynomial of t (time at recording in days); b_p is the p th order fixed regression coefficient; and the other parameters are as described above.

For all models, the individual effects were equal to the total additive genetic effects, and were assumed $\sim N(0, A\sigma_a^2)$, where A is a matrix of additive genetic relationships, σ_a^2 additive genetic variance. Common environmental to full-sib effects were assumed $\sim N(0, I\sigma_c^2)$, and residuals were assumed $\sim N(0, I\sigma_e^2)$, where I is the identity matrix and σ_c^2 and σ_e^2 are the common environment to full-sib family variance and residual variance respectively. The significance of fixed effects and random effects was tested using Wald-F statistics (in ASReml-R3.0 package) and log likelihood ratio tests, respectively. Variance components and EBVs for two resistance traits were estimated using univariate analysis in all the models, whereas the genetic correlations between resistance and growth traits were obtained using bivariate analysis based on LIN.

2.5. Heritability and genetic correlation

For all the models, heritability (h^2) and common to full-sib family effect (c^2) was calculated as follows:

$$h^2 = \frac{\sigma_a^2}{\sigma_a^2 + \sigma_c^2 + \sigma_e^2}, \quad c^2 = \frac{\sigma_c^2}{\sigma_a^2 + \sigma_c^2 + \sigma_e^2}$$

where σ_a^2 was additive genetic variance; σ_c^2 was environmental to full-sib family genetic variance; and σ_e^2 was the residual variance ($\pi^2/3$ for CST and LRM).

The genetic (r_g) and phenotypic (r_p) correlations of survival (i.e.

Trait 1 and Trait 2) between growth traits (i.e. BW and TL) were estimated using bivariate analyses (with a full dataset) based on LIN model. Trait 3 is a repeated trait which cannot well fitted in LIN for bivariate analyses to obtain genetic and phenotypic correlations. The correlations were calculated as follow (Falconer and Mackay, 1996):

$$r_{p/g(mn)} = \frac{\sigma_{p/g(mn)}}{\sqrt{\sigma_{p/g(m)}^2 \sigma_{p/g(n)}^2}}$$

where $\sigma_{p/g(mn)}$ was the phenotypic and genetic covariance between two traits (m and n), $\sigma_{p/g(m)}^2$ and $\sigma_{p/g(n)}^2$ was the phenotypic and genetic variance of trait m and n , respectively.

2.6. Rank correlation

In this study, rank (Spearman) correlation coefficients between mean family EBVs for each model (estimate in each model with respective full dataset) were obtained to assess the agreement between genetic predictions of different methods. Mean family EBVs were obtained $(u_s + u_d)/2$, where u_s and u_d represent the EBVs of the sire and dam of each family, respectively (Li et al., 2018).

3. Results

3.1. Challenge test

A total of 3727 individuals were experimentally challenged in this work. Fish inoculated with *E. tarda* showed typical clinical signs and pathological lesions of edwardsiellosis. The accumulated daily mortality bar charts and survival curves for all the full-sib families are showed in Fig. 1. Descriptive statistics of the challenge test was given in Table 1. As can be seen from Fig. 1, there was a sharp increase of mortalities at day 4, the peak mortality rate was observed at day 5 (daily mortality was 16.2%). Final survival rate at the end of the test was 24.6% (at day 9), and at day 6 the overall survival rate was 50.7%. In different full-sib families, survival rate ranged from 0 to 93.6%, indicating a substantial genetic variation in susceptibility for *E. tarda*.

3.2. Heritability and genetic correlation

Heritabilities of resistance traits were evaluated by using four models at day 6 (except LIN) and 9 (Table 2). For all the models,

Table 2
Variance components and heritabilities in different statistical models for *E. tarda* challenge test.

Model	Trait	Day	σ_a^2	σ_c^2	σ_e^2	$h^2 \pm SE$	$c^2 \pm SE$
LIN	DD	9	0.22	0.44	1.55	0.10 ± 0.48	0.20 ± 0.16
CSL	BTS	6	0.15	0.10	0.17	0.36 ± 0.11	0.24 ± 0.05
		9	0.034	0.021	0.089	0.24 ± 0.18	0.14 ± 0.10
CST	BTS	6	0.83	1.87	3.29	0.14 ± 0.03	0.31 ± 0.07
		9	0.64	2.30	3.29	0.10 ± 0.04	0.37 ± 0.08
LRM	BSS	6	0.40	0.26	3.29	0.09 ± 0.04	0.07 ± 0.04
		9	0.55	0.46	3.29	0.13 ± 0.03	0.11 ± 0.04

significant additive-genetic variation was observed with low to moderate heritability estimates ranging from 0.10 to 0.36. Estimated heritabilities for *E. tarda* resistance were slightly variable, depending on trait definitions, the models used and cut-off points. Relatively higher heritability values were obtained at day 6 than at day 9 in CSL and CST. However, there was an opposite trend for LRM. Besides, considerably higher values of heritability for resistance were obtained when the trait was assessed as the BTS, analyzed using CSL model at both corresponding cut-off points (0.24–0.36). It should be noted that the standard errors of heritability in CSL and LIN models were considerable. For all models, the variance component explained by the common full-sib family effects was substantial. The common to full-sib family effects accounted for a large proportion of the total phenotypic variance in all the models (ranging from 7% to 37%).

Genetic and phenotypic correlations between survival traits and growth traits were shown in Table 3. The genetic correlations between BW and two resistance traits were negative low and not significantly different from zero. The genetic correlations between TL and two resistance traits were at a positive low level but still not significantly different from zero. The phenotypic correlations between growth traits and resistance traits were all low and not significantly different from zero regardless of the sign. Hence, the weak and non-significant genetic correlation implies that there is little evidence that selection for improved growth would have an adverse or beneficial effect of resistance against *E. tarda* and vice versa.

3.3. Rank correlation

Table 4 shows the Spearman correlation coefficients between the predicted EBVs for full-sib families with different models at two cut-off points (i.e. day 6 and 9). The Spearman correlation coefficients between CSL and CST (for both cut-off test days), and between LIN and LRM (for day 9) were very high (ranging from 0.93 to 0.97). However, the correlation coefficients between cross-sectional models (i.e. CSL and CST) and the other two models were at a medium level, ranging from 0.49 to 0.78, which indicate a substantial re-ranking of families.

4. Discussion

In this study, challenge test survival was analyzed by using three kinds of trait definition with four statistical models at two cut-off points. Survival was analyzed as a binary trait (survival or dead), as an ordered categorical trait (binary survival scores) and as a continuous

Table 3
The genetic (above diagonal) and phenotypic (below diagonal) correlations between survival traits and growth traits.

Trait	BTS	DD	BW	TL
BTS				
DD			−0.09 ± 0.04	0.24 ± 0.05
BW	0.07 ± 0.04	0.09 ± 0.06		0.10 ± 0.38
TL	−0.04 ± 0.18	0.17 ± 0.07		

Table 4
The Spearman rank correlation coefficients of EBVs of full-sib families between different models.

Model	Day 6		Day 9		
	CSL	LRM	LIN	CSL	LRM
CSL	0.96	0.52	0.61	0.97	0.73
LIN				0.63	0.93
CST		0.49			0.78

trait (day of death). Cross-sectional models (i.e. CSL and CST), analyzing survival as a binary trait, early and late mortalities were grouped into the same class, and therefore do not utilize all available information. Whereas, longitudinal model (i.e. LRM in this study), taking time until death into account, may be a more appropriate statistical model (Ødegård et al., 2011). Similar conclusions have been achieved in genetic analysis of challenge test data in Atlantic salmon (Ødegård, Olesen, Gjerde, & Klemetsdal, 2006, 2007; Yáñez et al., 2013).

For analysis of binary trait data, threshold models and linear models are widely used in aquaculture species (Ødegård et al., 2011), and the two kinds of models usually show the similar predictive ability for predicting EBVs of full-sib families (Gitterle et al., 2006; Liang et al., 2017; Xiong et al., 2017). As also can be inferred from our results that the rank correlations between CSL and CST are very high (0.96–0.97) (Table 4). The rank correlations of full-sib families EBVs between two cross-sectional models and other two models were at a medium level (0.49–0.78), which should contribute to different trait definitions. However, the rank correlation between LIN and LRM is in a high level (0.93), in spite of that different trait definitions were used. This may be because that both of the definitions take time until death into account.

Here, genetic parameters of survival traits were estimated at day 6 (49.3% overall mortality) and 9 (75.4% overall mortality) respectively. Heritabilities estimated at day 6 was higher than at day 9 in cross-sectional and longitudinal models. This may be explained by the theory that when the cumulative mortality is 50% maximal response will be achieved (Fjalestad, Gjedrem, & Gjerde, 1993). The detection of significant genetic variation and the estimation of low to moderate heritability values using different trait definitions and statistical models for resistance to *E. tarda* in tongue sole are in accordance with other researches that also have found significant genetic variation and similar magnitude of heritability estimates for resistance against other bacterial diseases in other aquaculture species (Das Mahapatra et al., 2008; Ødegård et al., 2010; Kettunen, Serenius & Fjalestad, 2007; Yáñez et al., 2014; Bangera et al., 2011; Xiong et al., 2017; Bangera et al., 2014). However, in most of the studies, infectious agents, levels of mortality, infection modes, and implemented statistical models are extremely variable, hindering a proper comparative analysis of the obtained results. In our research, for binary trait, heritabilities estimated by CSL model were higher than CST at both specific test days (Table 2). This estimate is comparable to values reported in tongue sole (0.19–0.26) in previous similar study in terms of experimental setting (challenge test with *E. tarda*, mortality (65%), definition of disease resistance as a binary trait, and with cross-sectional linear and threshold models) (Liu et al., 2016). Latest researches revealed a similar underlying heritability (0.11–0.28) (estimated by animal and sire-dam models based on binary and categorical traits) for survival after challenge with *V. harveyi* in Chinese tongue sole (Li et al., 2019). Heritability estimated by LIN based on DD was closed to values obtained by CST and LRM at day 9, which may imply a similar predictive ability of these models. Besides, heritabilities estimated by LRM were substantially lower than values obtained by CSL and CST based on binary trait. This can be interpreted that, in LRM the test period is split into many days, and survival is scored for every period as a binary trait, where scores necessarily “dilute” the information per record (Bangera et al., 2014). This has also been shown in other studies (Bangera et al.,

2014; Gitterle et al., 2006; Yáñez et al., 2013; Ødegård et al., 2006, 2007).

The genetic correlations between disease resistance to *E. tarda* and growth traits were weak and not significantly different from zero (-0.09 to -0.12 and 0.10 – 0.24 for BW and TL, respectively). Similar results have been shown in previous studies, such as in Atlantic salmon (*Salmo salar*), the genetic correlation between resistance to *Piscirickettsia salmonis* and body weight was -0.19 ± 0.12 (Yáñez et al., 2014), and in rainbow trout (*Oncorhynchus mykiss*), the genetic correlation between resistance to *Flavobacterium psychrophilum* and growth was very low and not different from zero (Silverstein et al., 2009). However, the genetic correlations between resistance to *V. harveyi* and growth traits (BW and TL) showed a moderately positive level (0.27 – 0.51) in Chinese tongue sole (Li et al., 2019). Likewise, results from other studies have demonstrated positive or negative genetic correlations of moderate to high magnitude between resistance to various bacterial diseases and growth-related traits (Antonello et al., 2009; Imsland et al., 2002; Yáñez et al., 2016). Therefore, genetic correlations between disease resistance and growth traits are probably specific to each fish species and infectious agents and could not be generalized. These relationships should be evaluated case by case. In this study, we found weak evidence that selection for BW and TL would have an impact on resistance to *E. tarda*, and vice versa.

In conclusion, analyses based on LIN with DD and LRM with BSS are recommended to use for selection for Chinese tongue sole. The presence of low to moderate heritabilities for *E. tarda* resistance indicates that selective breeding for these traits can be successfully applied in this species. However, the genetic correlations between disease resistance and growth traits were low and nonsignificant. Hence, genetic improvement of growth traits is unlikely to have any substantial effect on disease resistance to *E. tarda*.

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References

- Abbott, S. L., & Janda, J. M. (2006). The genus *Edwardsiella*. *Prokaryotes*, 6, 72–89.
- Antonello, J., Massault, C., Franch, R., Haley, C., Pellizzari, C., Bovo, G., et al. (2009). Estimates of heritability and genetic correlation for body length and resistance to fish pasteurellosis in the gilthead sea bream (*Sparus aurata* L.). *Aquaculture*, 298, 29–35.
- Bangera, R., Ødegård, J., Mikkelsen, N., Nielsen, H. M., Puvanendran, et al. (2014). Genetic analysis of francisellosis field outbreak in Atlantic cod (*Gadus morhua* L.) using an ordinal threshold model. *Aquaculture*, 420–421, S50–S56.
- Bangera, R., Ødegård, J., Præbel, A. K., Mortensen, A., & Nielsen, H. M. (2011). Genetic correlations between growth rate and resistance to vibriosis and viral nervous necrosis in Atlantic cod (*Gadus morhua* L.). *Aquaculture*, 317, 67–73.
- Butler, D. G., Cullis, B. R., Gilmour, A. R., & Gogel, B. J. (2009). *ASReml-R reference manual*. Brisbane: Queensland Department of Primary Industries and Fisheries, NSW Department of Primary Industries.
- Chen, S., Du, M., Yang, J., Hu, Q., Xu, Y., & Zhai, J. (2010). Development and characterization for growth rate and disease resistance of families in half-smooth tongue sole (*Cynoglossus semilaevis*). *Journal of Fisheries of China*, 34(12), 1789–1794.
- Das Mahapatra, K., Gjerde, B., Sahoo, P. K., Saha, J. N., Barat, A., Sahoo, M., et al. (2008). Genetic variations in survival of rohu carp (*Labeo rohita*, Hamilton) after *Aeromonas hydrophila* infection in challenge tests. *Aquaculture*, 279, 29–34.
- Doan, Q. K., Vandeputte, M., Chatain, B., Haffray, P., Vergnet, A., Breuil, G., et al. (2017). Genetic variation of resistance to Viral Nervous Necrosis and genetic correlations with production traits in wild populations of the European sea bass (*Dicentrarchus labrax*). *Aquaculture*, 478, 1–8.
- Fjalestad, K. T., Gjedrem, T., & Gjerde, B. (1993). Genetic improvement of disease resistance in fish: An overview. *Aquaculture*, 111, 65–74.
- Gao, X., Zhang, X., Sun, J., Du, X., Li, X., Zhang, L., et al. (2016). Passive protection effect of anti-*Vibrio anguillarum* IgY-encapsulated feed on half-smooth tongue sole (*Cynoglossus semilaevis*) against *V. anguillarum*. *Fish & Shellfish Immunology*, 56, 483–488.
- Gitterle, T., Ødegård, J., Gjerde, B., Rye, M., & Salte, R. (2006). Genetic parameters and accuracy of selection for resistance to White Spot Syndrome Virus (WSSV) in Penaeus (*litopenaeus*) vannamei using different statistical models. *Aquaculture*, 251(2–4), 210–218.
- Hu, Q., Chen, S. L., Gao, F., Li, Y., Liu, S., Liu, F., et al. (2014). Differences in sex reversion and growth between normal and neomale stock in half-smooth tongue sole, *Cynoglossus semilaevis*. *Aquaculture International*, 22(4), 1437–1449.
- Imsland, A. K., Jonassen, T. M., Langston, A., Hoare, R., Wergeland, H., FitzGerald, R., et al. (2002). The interrelation of growth and disease resistance of different populations of juvenile Atlantic halibut (*Hippoglossus hippoglossus* L.). *Aquaculture*, 204, 167–177.
- Jeney, G., Ardó, L., Ronyai, A., Bercsenyi, M., & Jeney, Z. (2011). Resistance of genetically different common carp, *Cyprinus carpio* L., families against experimental bacterial challenge with *Aeromonas hydrophila*. *Journal of Fish Diseases*, 34(1), 65–70.
- Kettunen, A., Serenius, T., & Fjalestad, K. T. (2007). Three statistical approaches for genetic analysis of disease resistance to vibriosis in Atlantic cod (*Gadus morhua* L.). *Journal of Animal Science*, 85, 305–313.
- Liang, B. B., Jiang, F. J., Zhang, S. J., Yue, X., Wang, H. X., & Liu, B. Z. (2017). Genetic variation in vibrio resistance in the clam *Meretrix petechialis* under the challenge of *Vibrio parahaemolyticus*. *Aquaculture*, 468, 458–463.
- Liu, F., Li, Y. Z., Wang, X. X., Liu, X. F., Xing, H. F., Wu, Y. H., ... Chen, S. L. (2016). Estimation of genetic parameters for disease-resistance traits in *Cynoglossus semilaevis* (Günther, 1873). *Journal of Applied Ichthyology*, 32, 643–651.
- Liu, F., Tang, X., Sheng, X., Xing, J., & Zhan, W. (2017). Comparative study of the vaccine potential of six outer membrane proteins of *Edwardsiella tarda* and the immune responses of flounder (*Paralichthys olivaceus*) after vaccination. *Veterinary Immunology and Immunopathology*, 185, 38–47.
- Li, Y., Wang, L., Yang, Y., Li, X., Dai, H., & Chen, S. (2019). Genetic analysis of disease resistance to *Vibrio harveyi* by challenge test in Chinese tongue sole (*Cynoglossus semilaevis*). *Aquaculture*, 503, 430–435.
- Li, Y., Zhang, B., Lu, S., Yang, Y., Tian, Y., & Chen, S. (2018). Genetic parameters estimates for growth performance traits at harvest in Japanese flounder (*Paralichthys olivaceus*). *Aquaculture*, 489, 56–61.
- Ødegård, J., Baranski, M., Gjerde, B., & Gjedrem, T. (2011). Methodology for genetic evaluation of disease resistance in aquaculture species: Challenges and future prospects. *Aquaculture Research*, 42, 103–114.
- Ødegård, J., Olesen, I., Dixon, P., Jeney, Z., Nielsen, H. M., Way, K., et al. (2010). Genetic analysis of common carp (*Cyprinus carpio*) strains. II: Resistance to koi herpesvirus and *Aeromonas hydrophila* and their relationship with pond survival. *Aquaculture*, 304, 7–13.
- Ødegård, J., Olesen, I., Gjerde, B., & Klemetsdal, G. (2006). Evaluation of statistical models for genetic analysis of challenge test data on furunculosis resistance in Atlantic salmon (*Salmo salar*): Prediction of field survival. *Aquaculture*, 259, 116–123.
- Ødegård, J., Olesen, I., Gjerde, B., & Klemetsdal, G. (2007). Evaluation of statistical models for genetic analysis of challenge-test data on ISA resistance in Atlantic salmon (*Salmo salar*): Prediction of progeny survival. *Aquaculture*, 266, 70–76.
- Ødegård, J., Sommer, A. I., & Præbel, A. K. (2010). Heritability of resistance to viral nervous necrosis in Atlantic cod (*Gadus morhua* L.). *Aquaculture*, 300, 59–64.
- Sha, Z. X., Wang, Q. L., Liu, Y., & Chen, S. L. (2012). Identification and expression analysis of goose-type lysozyme in half-smooth tongue sole (*Cynoglossus semilaevis*). *Fish & Shellfish Immunology*, 34, 914–921.
- Shoemaker, C. A., Lozano, C. A., LaFrentz, B. R., García, J. C., Soto, E., Xu, D. E., et al. (2017). Additive genetic variation in resistance of Nile tilapia (*Oreochromis niloticus*) to *Streptococcus iniae* and *S. agalactiae* capsular type Ib: Is genetic resistance correlated? *Aquaculture*, 468, 193–198.
- Silverstein, J. T., Vallejo, R. L., Palti, Y., Leeds, T. D., Rexroad, C. E., Welch, T. J., et al. (2009). Rainbow trout resistance to bacterial cold-water disease is moderately heritable and is not adversely correlated with growth. *Journal of Animal Science*, 87, 860–867.
- Tang, X. Q., Zhou, L., & Zhan, W. B. (2008). Isolation and characterization of pathogenic *Listonella anguillarum* of diseased half-smooth tongue sole (*Cynoglossus semilaevis* Günther). *Journal of Ocean University of China*, 7(3), 343–351.
- Taylor, R. S., Kube, P. D., Muller, W. J., & Elliott, N. G. (2009). Genetic variation of gross gill pathology and survival of Atlantic salmon (*Salmo salar* L.) during natural amoebic gill disease challenge. *Aquaculture*, 294, 172–179.
- Xiong, X. M., Chen, Y. L., Liu, L. F., Wang, W., Robinson, N. A., & Gao, Z. X. (2017). Estimation of genetic parameters for resistance to *Aeromonas hydrophila* in blunt snout bream (*Megalobrama amblycephala*). *Aquaculture*, 479, 768–773.
- Xu, T., & Zhang, X. H. (2014). *Edwardsiella tarda*: An intriguing problem in aquaculture. *Aquaculture*, 431, 129–135.
- Yamasaki, M., Araki, K., Maruyoshi, K., Matsumoto, M., Nakayasu, C., Moritomo, T., et al. (2015). Comparative analysis of adaptive immune response after vaccine trials using live attenuated and formalin-killed cells of *Edwardsiella tarda* in ginbuna crucian carp (*Carassius auratus langsdorffii*). *Fish & Shellfish Immunology*, 45, 437–442.
- Yáñez, J. M., Bangera, R., Lhorente, J. P., Barriá, A., Oyarzún, M., Neira, R., et al. (2016). Negative genetic correlation between resistance against *Piscirickettsia salmonis* and harvest weight in coho salmon (*Oncorhynchus kisutch*). *Aquaculture*, 459, 8–13.
- Yáñez, J. M., Bangera, R., Lhorente, J. P., Oyarzún, M., & Neira, R. (2013). Quantitative genetic variation of resistance against *Piscirickettsia salmonis* in Atlantic salmon (*Salmo salar*). *Aquaculture*, 414–415, 155–159.
- Yáñez, J. M., Lhorente, J. P., Bassini, L. N., Oyarzún, M., Neira, R., & Newman, S. (2014). Genetic co-variation between resistance against both *Caligus rogercresseyi* and *Piscirickettsia salmonis*, and body weight in Atlantic salmon (*Salmo salar*). *Aquaculture*, 433, 295–298.
- Zeng, C., Tang, X., Du, Y., Sheng, X., Xing, J., & Zhan, W. (2018). Dynamic distribution of formalin-inactivated *Edwardsiella tarda* in olive flounder (*Paralichthys olivaceus*) post intramuscular injection. *Veterinary Immunology and Immunopathology*, 199, 53–60.