Norwegian University of Life Sciences
Faculty of Biosciences
Department of Animal and Aquacultural Sciences

Philosophiae Doctor (PhD)
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Breeding for disease resistance against *Edwardsiella ictaluri* in Mekong striped catfish (*Pangasianodon hypophthalmus*)

Avl for sykdomsresistens mot *Edwardsiella ictaluri* i Pangasius (*Pangasianodon hypophthalmus*)

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Khoi Dinh Pham
Ås, 9 November 2020

TABLE OF CONTENTS

SUMMARY

Bacillary necrosis (BN) is a disease caused by *Edwardsiella ictaluri* (*E. ictaluri*) that results in severe economic losses in farming of Mekong striped catfish (*Pangasianodon hypophthalmus*) in Vietnam. Genetic selection against the disease is a preventive measure that relies on a well-tested and stable challenge test, that the challenge tested survival shows genetic variance (heritability) and that the genetic correlation between the survival in the challenge and in the field is high. To accomplish these aims, a series of challenge tests for resistance against BN were carried out with a family material of Mekong striped catfish.

The Mekong striped catfish breeding program in Vietnam was initiated in 1999. Wild stocks that had gone through domestication in three hatcheries in Mekong delta were mated to establish three subpopulations or year-classes; 2001, 2002 and 2003 (generation 1). Fish used in this study, in the four challenge-test experiments, were from the third generations of year-class 2001 (experiment 1, n = 2,155), third generations of year-class 2002 (experiment 2, n = 1,988), third generations of year-class 2003 (experiment 3, n = 5,689), and the fourth generation of year-class 2001 (experiment 4, n = 6,177). Experiment 1 was carried out in a single tank, whereas the last three experiments were carried out in two replicated tanks. In the last two year-classes (experiments 3 and 4), harvest body weight and survival were available from a growth test for siblings of the BN tested families (n = 13,322 and n = 13,847, respectively).

Paper I drew inference from experiments 1 – 4 to propose a challenge test, based on three days acclimatization of test fish prior to the challenge, halving the water level in the test, with a temperature of 26^0 C, and the cohabitant shedders (fish injected

with *E. ictaluri*) released directly into the test tank, making up around ⅓ of the fish. The results indicated that the bacteria should be added directly to water and that additional experimentations are needed to clarify density and timing. Finally, genetic analyses of dead/alive at the end of the challenge in the two test tanks in the same experiment were considered two traits and their genetic correlation was estimated high by use of a bivariate linear sire-dam model. This means that the same trait is tested in the two replicated tanks and that data can be analysed across tanks.

In Paper II, the data were first analysed per experiment, across tanks by use of three statistical models; dead/alive at the end of the test and at 50% mortality, either with a linear sire-dam model or a comparable model that accounts for the variable being categoric, i.e. with a threshold sire-dam model. Moreover, a linear survival sire-dam model was used which analysed dead/alive per day of testing and where a fish was assigned a final phenotype of 0 the day it died. For the survival model and at the end of the test the family effects in one tank (sum of sire, dam and common environmental effects) were correlated to phenotypic survival for the same families in the other tank. The same was done for the two other models both at the end of the test and at 50% mortality, for cross-validation. The results showed that variance and heritability were highest at 50% mortality ($h^2 = 0.22$ with the threshold model and $h^2 = 0.135$ with the comparable linear model). Since the family effect at the end of the test and at 50% mortality was estimated with a relatively low rank correlation (≤ 0.72), it indicates that the trait at 50% mortality is a mixture of susceptibility to the disease and whether the fish is able to survive with the disease if it has been infected (endurance), while mortality at the end of the test, if it ceases, rather tests susceptibility to the disease. This led us to the following; that the survival test should cease at a mortality of 50%. Moreover, the cross-validation showed that the

breeding values could be estimated by use of a simple, linear sire-dam model, although this model did not cross-validate better than the corresponding linear survival model.

Paper III utilized data from experiments 3 and 4 in addition to harvest body weight and survival in the growth tests. The data for the three traits were analysed with a linear sire-dam model within experiment, without modelling of common environment, to reduce standard error of estimates. Low, non-significant (relative to zero) genetic correlations were estimated between BN and harvest body weight, which means that both traits can be improved in the same breeding program. Additionally, harvest body weight had positive genetic correlations with survival in the growth tests, which proposes that selection for growth will genetically improve survival in the growth test. Estimated genetic correlation between BN and survival in the growth test varied across experiments, from -0.02 ± 0.11 in experiment 3 to 0.26 ± 0.09 in experiment 4. The latter, weak genetic correlations led us to conclude that a stronger genetic relationship between BN and survival in the field needs to be established to defend continued challenge testing of Mekong striped catfish against *E. ictaluri.* This can be done in a new field test (in addition to the standard growth test) with siblings of the same families, where antibiotic treatment is not carried out and the cause of death is continuously monitored. Meanwhile, it is proposed to continue the routine challenge testing with the aim of indirectly improving field survival through selection.

SAMMENDRAG

Bakteriell nekrose (BN) er en sykdom forårsaket av *Edwardsiella ictaluri* (*E. ictaluri*) som resulterer i alvorlige økonomiske tap i oppdrettet av Pangasius (*Pangasianodon hypophthalmus*) i Vietnam. Avlsarbeid mot sykdommen er et preventivt tiltak som er avhengig av at det blir etablert en velfungerende og stabil test av overlevelse og at denne viser tilstrekkelig genetisk variasjon (arvegrad). Videre er en avhengig av høy genetiske korrelasjonen mellom test-overlevelse og overlevelse i felt. For å nå disse målene, ble en serie av tester av resistens mot BN gjennomført med et familiemateriale i Pangasius.

Avlsprogrammet med Pangasius i Vietnam startet opp i 1999. Avlsmaterialet som hadde gjennomgått domestisering i tre oppdrettsanlegg i Mekong ble paret for å danne tre subpopulasjoner eller årsklasser; 2001, 2002 og 2003 (førstegenerasjon). Fisken brukt i denne studien basert på fire forsøk fra tredje generasjon og årsklasse 2001 (forsøk 1, n = 2155), tredje generasjon og årsklasse 2002 (forsøk 2, n = 1988), tredje generasjon og årsklasse 2003 (forsøk 3, n = 5689) og fjerde generasjon og årsklasse 2001 (forsøk 4, n = 6177). Forsøk 1 ble gjennomført i en tank, mens de tre siste forsøkene ble utført i repliserte tanker. I de to siste årsklassene (forsøkene 3 og 4) var slaktevekt og overlevelse tilgjengelig fra en tilveksttest med søsken av de BN testede familiene (n =13322 og 13847).

Paper I trakk informasjon ut av forsøk 1 – 4 for å foreslå en overlevelsestest, basert på tre dagers akklimatisering, halvering av vann nivået i testen, med en vanntemperatur på 26 grader og at kohabitanter (fisk injisert den sykdomsfremkallende bakterien) sluppet direkte inn i testtankene utgjorde ca. 1/3 av fisken. Resultatene pekte også på at bakterier trengs å adderes til vann, men at

ytterligere forsøk trengtes for å avklare tetthet og tidspunkt. Til slutt ble genetiske analyser av død/levende ved avslutning av testen i de to testtankene i samme forsøk betraktet som to egenskaper og den genetiske korrelasjonen estimert som høg ved hjelp av en to-variabel lineær far-mor modell, som betyr at den samme egenskapen testes i de to tankene og at data kan analyseres på tvers av tanker.

I Paper II ble data først analysert per forsøk, over tank, ved hjelp av tre statistiske modeller; død/levende i slutt av testen og ved 50 % død, enten ved bruk av en lineær far-mor modell eller en tilsvarende modell som hensyntar at variabelen er kategorisk, en såkalt threshold far-mor modell. Videre ble det brukt en lineær levetids far-mor modell som analyserer død/levende hver dag i testen og hvor fisken tilegnes en endelig fenotype på 0 den dagen den dør. For levetidsmodellen og ved slutten av testen ble familieeffekten i en tank (sum av far, mor og felles miljøeffekter) korrelert til fenotypisk overlevelse for samme familie i den andre tanken, og det samme ble gjort for de to andre modellene, både ved slutten av testen og ved 50 % dødelighet, for kryssvalidering. Resultatene viste at varians og arvegrad var størst ved 50 % dødelighet (0.22 med threshold modellen og 0.135 med tilsvarende lineær modell) og siden familieeffektene ved slutten av testen og ved 50 % dødelighet ble estimert til å ha en relativt lav rang korrelasjon (≤ 0.72), indikerer dette at egenskapene ved 50% dødelighet er en blanding av både mottagelighet for sykdommen og om fisken klarer å overleve med sykdommen om den er infisert, mens dødelighet ved slutten av testen, om den flater ut, heller tester mottagelighet for sykdommen. Dette fører fram til følgende anbefaling; at overlevelsestesten bør flate ut ved en dødelighet på 50 %. Videre viste kryss-valideringen at avlsverdier gjerne kan bli estimert ved hjelp av en enkel lineær modell, selv om denne ikke kryssvaliderte signifikant bedre enn den lineære levetidsmodellen.

Paper III utnyttet data fra forsøkene 3 og 4 samt data for vekt og dødelighet i en tilveksttest. Data for de tre egenskapene ble analysert med en lineær far-mor modell innen hvert forsøk, uten modellering av felles miljø, for å redusere standardfeil på estimatene. Lave, ikke signifikante (relativt til null) genetiske korrelasjoner ble estimert mellom BN og slaktevekt, som betyr at begge egenskaper kan forbedres i ett og samme avlsprogram. I tillegg viste slaktevekt en positiv genetisk korrelasjon med overlevelse i tilveksttesten, som foreslår at seleksjon for vekst vil genetisk sett forbedre overlevelse i tilveksttesten. Estimert genetisk korrelasjon mellom BN og overlevelse i tilveksttesten varierte mellom forsøk, fra -0.002 ± 0.11 i forsøk 3 til 0.26 ± 0.09 i forsøk 4. De siste, svake korrelasjonene førte fram til følgende konklusjon: at en sterkere genetisk sammenheng trengs å etableres for å kunne forsvare fortsatt testing av Pangasius for *E. ictaluri*, i en ny felttest (i tillegg til tilveksttesten), basert på full-søsken av de samme familiene, hvor en ikke bruker antibiotika og hvor en kontinuerlig overvåker årsaken til død. I mellomtiden foreslås det å fortsette testingen med mål om å bedre feltoverlevelsen gjennom avl.

LIST OF ABBREVIATIONS

BLUP:	Best linear unbiased prediction
BN:	Bacillary necrosis
E. ictaliri:	*Edwardsiella ictaluri*
Exp:	Experiment
FCI:	Freshwater Columnaris Infection
GDP:	Gross Domestic Product
GS:	Genomic selection
LM:	Cross-sectional linear model
LSM:	Linear survival model
NABRECSOFA:	National Breeding Centre for Southern Freshwater Aquaculture
RIA2:	Research Institute for Aquaculture No. 2
SNP:	Single nucleotide polymorphism
TM:	Cross-sectional threshold model

LIST OF PAPERS

This thesis is based on the following three papers. The reference to these papers is given by their roman numbers throughout the thesis.

Khoi Dinh Pham, Sang Van Nguyen, Jørgen Ødegård, Hans Magnus Gjøen, Gunnar Klemetsdal. 2020. Case study development of a challenge test against *Edwardsiella ictaluri* in Mekong striped catfish (*Pangasianodon hypophthalmus*), for use in breeding: Estimates of the genetic correlation between susceptibility in replicated tanks. *Journal of Fish Diseases.* DOI: 10.1111/jfd.13292.

Khoi Dinh Pham, Jørgen Ødegård, Sang Van Nguyen, Hans Magnus Gjøen, Gunnar Klemetsdal. 2020. Genetic analysis of resistance in Mekong striped catfish (*Pangasianodon hypophthalmus*) to bacillary necrosis caused by *Edwardsiella ictaluri*. Journal of Fish Diseases. DOI: 10.1111/jfd.13279.

Khoi Dinh Pham, Jørgen Ødegård, Sang Van Nguyen, Hans Magnus Gjøen, Gunnar Klemetsdal. 2020. Genetic correlations between challenge tested susceptibility to bacillary necrosis, caused by *Edwardsiella ictaluri*, and growth performance tested survival and harvest body weight in Mekong striped catfish (*Pangasianodon hypophthalmus*). Journal of Fish Diseases. DOI: 10.1111/jfd.13277.

1. GENERAL INTRODUCTION

1.1 Aquaculture in Vietnam

Vietnam, with an area of about 3,448,000 km^2, has a coastline of 3,260 km. With its lakes, estuaries, canals and islands, about 1.7 million hectares of water surface are available for inland aquaculture (Tran, 2010). In the last decades, the aquaculture production has increased 10-fold: The annual production in 1995 was 415 tons, while it was 4,153 tons in 2018 (Figure 1). Corresponding numbers for the wild-caught production were 929 and 3,590 tons, respectively (VASEP, 2019). The export value of the aquaculture production increased from US $ 2.4 billion in 2004 to US $ 8.8 billion in 2018 (VASEP, 2019).

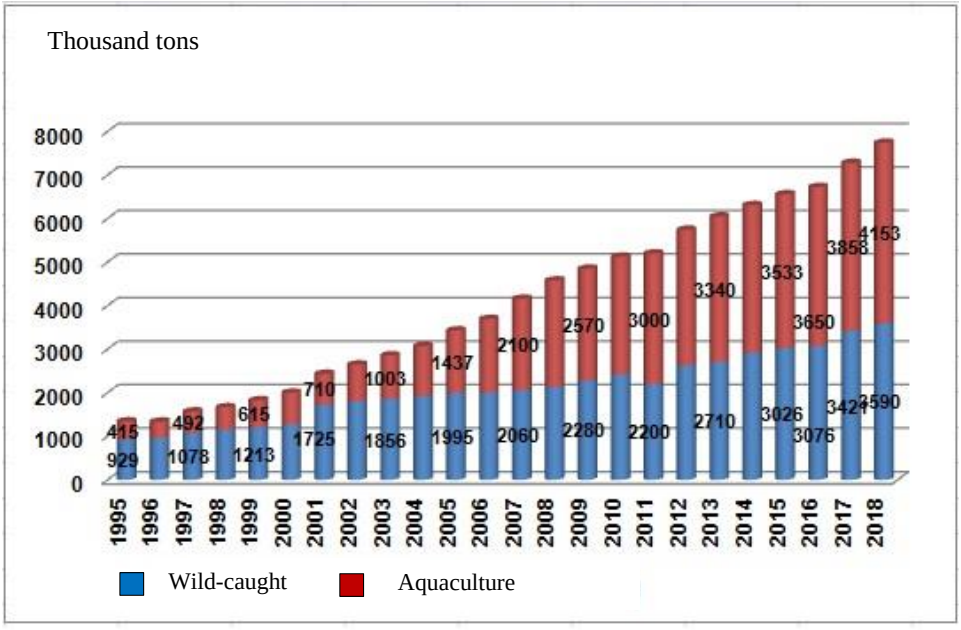

Figure 1. Tons of wild-caught fish and that from aquaculture over time in Vietnam (VASEP 2019).

1.2 Aquaculture of Mekong striped catfish in Vietnam

The Mekong striped catfish (Figure 2) is indigenous to Vietnam and Cambodia. During the monsoon season, in May to August, adult fish migrate upstream to spawn at specific breeding grounds in the Mekong River, all the way from Kratie town in Cambodia up to the Khone falls on the Cambodia/Laos border. Larvae and fry drift down to the floodplains of central Cambodia where they start-feed at newly flooded areas, before they tend to move to deeper areas downstream, such as the Tonle Sap Great Lake and the Mekong Delta (van Zalinge et al., 2002).

Figure 2. Mekong striped catfish. Photo: Research Institute for Aquaculture #2 (RIA2).

Initially, Mekong striped catfish was mainly farmed small-scale, i.e. in small ponds and cages using fries and fingerlings from the wild. In 1978, artificial propagation was first done, and in 1996 induced spawning and nursing of the fries had been well documented and described (Khanh, 1996). A shift to use of only artificially spawned seed occurred rapidly because catching of fry from the river was banned in 2000 (Trong et al., 2002).

The Mekong striped catfish is currently the most important freshwater fish species in Vietnam. In 2018, the production in Vietnam was 1.42 million tons. Mekong striped catfish is exported to over 136 countries worldwide, with an estimated export income of US $2.36 billion in 2018, making up about 1% of the Vietnam Gross Domestic Product or GDP (VASEP, 2019). The sector employs over 180,000 people, with a majority of women in the processing factories (De Silva and Phuong, 2011).

Production is carried out in three main systems: Open-river pond (80%), internal-field pond (15%), and river-net fence (5%) (Sang et al., 2012). Mekong striped catfish is omnivorous and capable of air breathing which allows exceptionally high stocking

density (18 - 125 fish/m^2), and the harvest can reach up to 850 tons/ha/crop (mean ~ 400 tons/ha/6 - 9 months) (Phan et al., 2009).

1.3 The Mekong striped catfish breeding program

Fecundity of farmed river catfish ranges from 80,000 - 190,000 egg/kg (Bui et al., 2010), and thus as few as three 7 - kg females can sufficiently provide seed for a one-hectare grow-out pond. Sang (2010) reported that 57% of the hatcheries received their brood fish from commercial grow-out ponds, while 31% was based on wild fish, and 11% from the national breeding program or from provincial hatcheries. To protect the wild population, use of wild fish as breeders may be become banned in the future. This promotes the use of improved seed from selective breeding programs. Although it is costly to run an advanced breeding program, the return on the investment is high. The benefit:cost ratio of a breeding program for Atlantic salmon in Norway has been estimated to 15 (Gjedrem, 2000), while the estimates for Nile tilapia range from 8.5 to 60 (Ponzoni et al., 2007).

In 1999, the Mekong striped catfish breeding program was initiated at the Research Institute for Aquaculture No. 2 (RIA2) by collecting stocks from four hatcheries in the Mekong delta. The stocks were from grow-out farms that reared wild fingerlings caught during several seasons at various locations in the Mekong river (Sang et al., 2012). The breeding program up to year 2011 was as shown in Figure 3. In brief, wild stocks that had gone through domestication in three hatcheries were mated to establish three year-classes: 2001, 2002, and 2003 (generation 1), funded by Support to Freshwater Aquaculture (SUFA, DANIDA) program. The traits considered in this project were body weight (year-classes 2001 and 2002) and fillet yield (year-class 2003). During 2006-2008, another project, funded by the Ministry of Agriculture and Rural Development, was carried out, focusing on fillet yield, fillet fat and fillet colour. Thus, in generation 2 the considered traits were body weight, fillet yield, fillet weight, fillet fat and fillet colour. From 2009 to 2012, a third project funded by the Ministry of Agriculture and Rural Development continued the breeding program. This project focused firstly on body weight and fillet yield traits in the third and fourth generation of the program. However, at the same time, bacillary necrosis (BN), caused by *Edwardsiella ictaluri* (*E. ictaliri*), had become a severe problem in Mekong striped catfish farming in the Mekong Delta. Hence,

development of a family-based test for BN resistance was included in the project as well. This thesis utilises data from the first four challenge tests done on the third generation of year-classes: 2001 (G3-2001), 2002 (G3-2002) and 2003 (G3-2003), and the fourth generation of year-class 2001 (G4-2001).

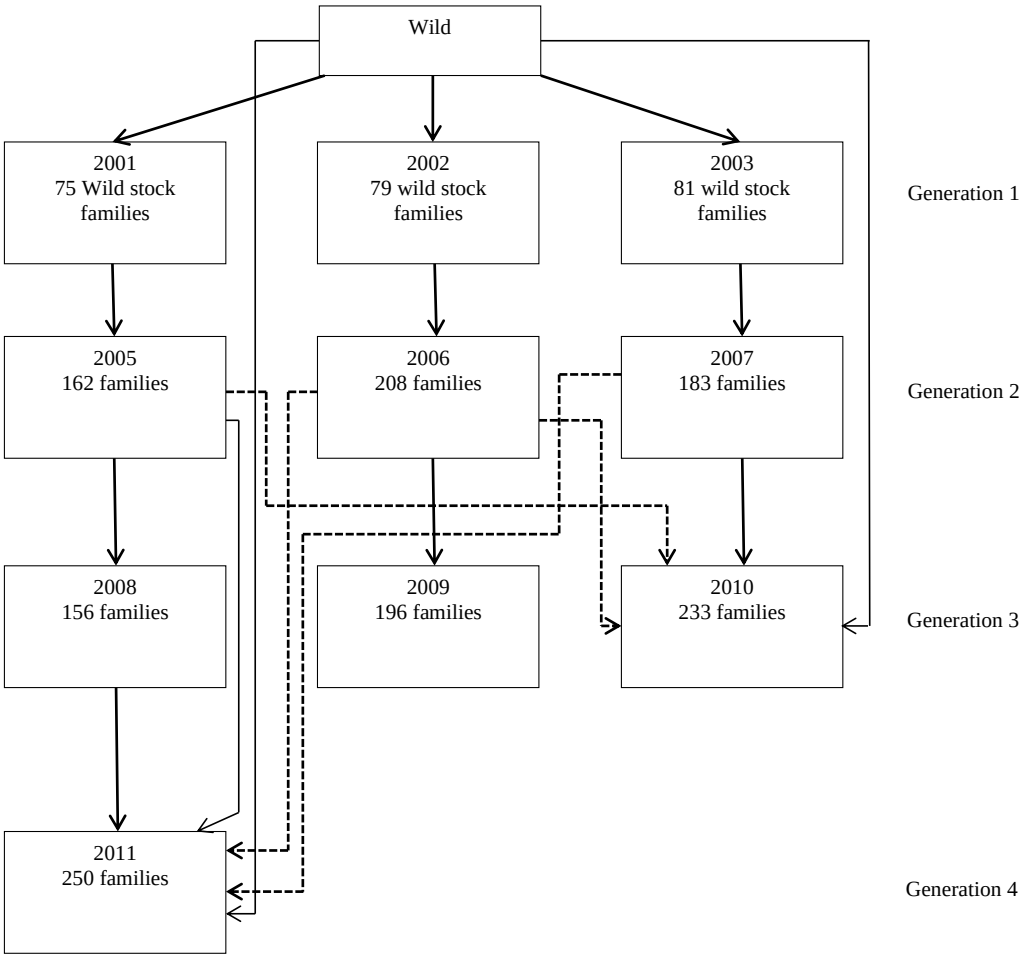

Figure 3. Schematic representation of the breeding program with Mekong striped catfish in Vietnam until 2011; year-classes/sub-populations and generations are indicated. Dashed arrows indicate broodstock usage across year-classes.

A fourth project was run in the period from 2013-2016, focusing on the application of quantitative and molecular genetic techniques in growth improvement of Mekong

striped catfish. It was funded by the Ministry of Agriculture and Rural Development (Trong Quoc Trinh, pers. com.). Moreover, a fifth project was carried out from 2013-2015, also funded by the Ministry of Agriculture and Rural Development. In this project, brood fish of G3-2002, G3-2003 and G4-2001 were crossed to wild fish, from the An Giang and Dong Thap provinces of Vietnam and Kratie, Cambodia, respectively. The project aimed to establish the base for a second breeding program in Vietnam selecting for improved disease resistance (Trong Quoc Trinh, pers. com.). Finally, funding was received in 2019 for a project aiming to implement recent genomic tools in selective breeding of Mekong striped catfish to BN, for instance to establish genetic markers for BN resistance (Phuc Huu Tran, pers. com.). Therefore, at current there exist two parallel selective breeding programs for Mekong striped catfish in Vietnam, one aiming for growth improvement and one for resistance to BN, respectively (Phuc Huu Tran, pers. com.).

1.4 Disease resistance and genetic improvement

Under farming conditions, factors like handling, sorting, transport, confinement, water quality, high stocking density etc. are considered as potential stress factors (van Muiswinkel et al., 1999). These factors might disturb the natural ability of the fish to fight the pathogen and can thus lead to outbreak of a disease. The typical BN disease symptoms are numerous white spots of different sizes in swelled liver, kidney and spleen (Figure 4), first observed in 1999 and identified as BN in 2001 (Ferguson et al., 2001). BN is considered the most problematic and devastating disease in farming of Mekong striped catfish in Vietnam, causing significant mortality and economic losses (Dung et al., 2004).

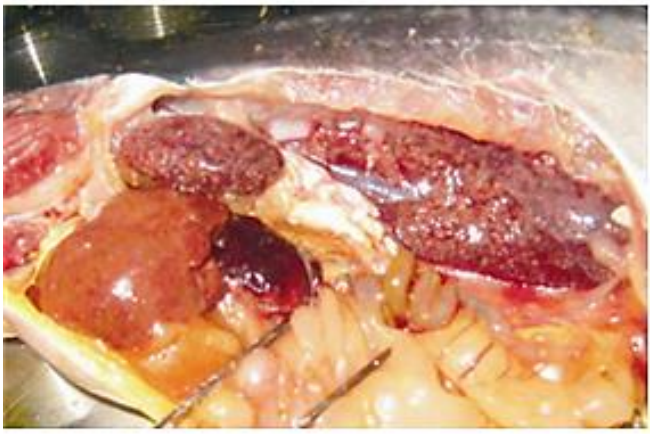

Figure 4. Internal clinical signs of BN. Photo: Khoi Dinh Pham.

In 2010, the first BN vaccine trial was conducted by Pharmaq Ltd. Vietnam (Thanh and Berntsen, 2012). The trial resulted in significant lower mortality of vaccinated than non-vaccinated groups, and the Alpha Ject® Panga 1 vaccine was licensed in 2013 (https://www.pharmaq.no/sfiles/8/66/4/file/2013_08-cty-pharmaq-vn_thuy-san-nam-14-so-164.pdf). However, improved resistance to BN using vaccination is relatively costly and time consuming since it must be administered to each fish produced. Therefore, BN is in main treated by use of antibiotics, but this could in turn cause problems in terms of drug resistance and contamination of the final product (Nordmo et al., 1998). Moreover, reducing mortality by drug application is costly and does not solve the problem on a long-term basis (van Muiswinkel et al., 1999).

In aquaculture, as in all animal production, improved disease resistance and survival are crucial to the economy, animal welfare and sustainability of the industry. Therefore, besides the development of applicable vaccines, improvement of resistance to BN through selection is considered an important tool for sustainable disease control. Disease resistance in farmed fish is generally assessed in terms of survival after infection with a specific pathogen, normally in a controlled challenge test of full-sib family groups in a breeding nucleus (Ødegård et al., 2011a). Gjedrem et al. (1991) utilized this approach and reported a substantial genetic variation in mortality after challenging Atlantic salmon with the furunculosis bacteria: This work was considered as founding disease resistance studies in aquaculture species. In essence, several challenge systems

have been used for aquatic animals: Bath (Bangera et al., 2011; Gjerde et al., 2019; Glover et al., 2017; Henryon et al., 2002; Henryon et al., 2005; Lillehammer et al., 2019; Nordmo et al., 1997; Perry et al., 2004; Wetten et al., 2007), intraperitoneal injection (Barría et al., 2019; Bassini et al., 2019; Doan Q. et al., 2017; Li et al., 2019; Shoemaker et al., 2017; Srisapoome et al., 2019; Sukhavachana et al., 2019; Xiong et al., 2017; Yáñez et al., 2016), feeding (Gitterle et al., 2006; Ødegård et al., 2011c), cohabitation (Gjøen et al., 1997; Mahapatra et al., 2008; Nordmo et al., 1997), or a combination of the methods above (Flores-Mara et al., 2017; LaFrentz et al., 2016; Yáñez et al., 2014). The major objective of testing is to rank individuals/families with respect to disease resistance, and thus it was necessary to develop a challenge test applicable in the breeding program with Mekong striped catfish. It is a prerequisite that the trait recorded in the challenge test shows genetic variance, is heritable, and improves growth survival. In this regard, knowledge about the genetic correlation between disease resistance (*in vitro*) and actual growth survival rate (*in vivo*), as well as the genetic correlation to production trait are needed, to evaluate the necessity of conducting a challenge test for a specific disease.

Vehviläinen et al. (2008) reported highly variable heritabilities of survival in different generations and stations for rainbow trout, with inconsistent size of genetic correlations of survival between generations and between stations, which illustrates that field survival might be considered a mixture of traits: Survival towards specific diseases and mortality from other causes. Hence, field survival alone without taking the real cause of death into account has limited value in genetic selection (Ødegård et al., 2011a). Moreover, Henryon et al. (2002) concluded that additive genetic variation for field survival may well exist within the farmed population but will only be expressed under conditions where specific causative mortality exists. Therefore, selective breeding for improved resistance against a specific disease is in need of a challenge test with a specific pathogen.

2. AIMS OF THE STUDY

The objectives of this study were:

1. To establish an applicable challenge test against *E. ictaluri* in Mekong striped catfish, for use in breeding, and to test whether resistance to bacillary necrosis tested in replicated tanks in the same experiment is the same genetic trait (Paper I).
2. To estimate genetic variance and heritability for resistance against bacillary necrosis using three statistical models (two cross-sectional models linear and threshold using data either in the endpoint or at 50% mortality and a survival model utilizing time until death), and to cross-validate the models in replicated tanks (Paper II).
3. To estimate the genetic correlations between BN, body weight and growth survival in Mekong striped catfish (Paper III).

3. MATERIALS AND METHODS

A series of four cohabitation challenge-test experiments against *E. ictaluri* was carried out, using family materials from G3-2001 (year-class 2008, experiment 1), G3-2002 (year-class 2009, experiment 2), G3-2003 (year-class 2010, experiment 3) and G4-2001 (year-class 2011, experiment 4). Experimental factors that were varied across the experiments related to acclimatization time, water temperature and level, cohabitant shedder density, dose and placement, dead cohabitant removal, and timing and amount of addition of bacteria to water. Experiment 1 was carried out in a single tank, whereas the last three experiments were carried out in two replicated tanks (Table 1). In the last two experiments, siblings of the challenged fish were additionally tested in growth performance tests (Table 1).

Table 1. Number of families and test fish in four challenge-test experiments (Exp) of Mekong striped catfish with *E. ictaluri* and the corresponding for two growth performance tests

	Exp 1	Exp 2	Exp 3	Exp 4
Challenge test				
No. replicated tanks	1	2	2	2
No. test fish	2,155	1,988	5,689	6,177
No. fullsib families	81	64	187	233
Length of test (days)	22	23	18	19
Growth test				
No. test fish	-	-	13,322	13,847
No. fullsib families	-	-	216	250
Length of test (days)	-	-	250	269

In Paper I, a case study approach was applied, i.e. the experiences gained in one experiment were taken over to the next, aiming for a final overall mortality of 50%. Moreover, in order to test whether resistance to BN was genetically the same trait across replicated test tanks, a bivariate cross-sectional linear sire-dam model was used for dead/alive (= 0/1) at the end of the tests in the two replicated tanks, defining traits 1 and 2, respectively. For the latter, data utilised were from experiments 3 and 4 (Table 1).

In Paper II, a binary survival trait (dead = 0/alive = 1) was defined at the end of the test (endpoint) and at the day the truncated mortality was closest to 50% (50% mortality). The variables were genetically analysed with univariate cross-sectional sire-dam models (linear and threshold) across tanks, per experiment. Moreover, binary test-day survival was defined as 1/0 if the fish was alive/dead on test-day t, where 0 implied that there would be no further record for that fish, and a linear sire-dam survival model was run across tanks, per experiment. The models were cross-validated by calculating the Pearson correlation between the predicted family effects (sum of sire, dam and common environmental effects) in each of two tanks (at the two stages or only at the endpoint) and the observed family survival in the other tank. Data for the genetic analysis were from experiments 1, 3 and 4, whereas data used for cross-validation were from experiments 3 and 4.

In Paper III and for survival, binary traits (dead = 0/alive = 1) were defined at the end of the challenge test at the end of the growth performance test, respectively, whereas harvest body weight was recorded in gram. A tri-variate linear cross-sectional sire-dam model was used both with and without inclusion of the common environmental effect. Data used were from experiments 3 and 4.

4. RESULTS

Paper I gives detailed information for mortality of cohabitants and test fish as well as overall cumulative mortality in each of the experiments. For the latter, the overall cumulative mortality approached 25% in experiment 1. In experiment 2, cumulative mortality was only 3.0% and 5.7% for tanks 1 and 2, respectively (because of too low mortality, data from this experiment was excluded for analysing genetic parameters). In experiment 3, cumulative mortality was considerable in both tanks: 84.0% and 83.1%, while in the fourth experiment, cumulative mortality was 87.1% and 87.7% in the two tanks. Moreover, the genetic correlations of susceptibility to BN between test tanks (defined as traits) were estimated as high (close to unity in experiment 3 and 0.95 in experiment 4).

In Paper II, the heritability estimates of resistance to *E. ictaluri* infection were found ≤ 0.012 with the survival model, and up to 0.135 - 0.220 (50% survival) and 0.085 and 0.174 (endpoint survival) with the cross-sectional linear and threshold models, respectively. Regarding cross-validation by use of full-sib family effects, the threshold binary model predicted less precise than either of the linear models (cross-sectional and survival), the two latter could not be discriminated.

Paper III shows that the estimated heritabilities varied from 0.27 to 0.50 for harvest body weight, 0.06 to 0.09 for challenge-test susceptibility to BN and 0.19 to 0.28 for growth survival. When accounting for the common environmental component, the genetic correlation between growth survival and susceptibility to BN in the challenge test in one year-class was found high (0.58 ± 0.20). However, the genetic correlations were estimated with large standard errors. Omitting the common environmental component from the model, reduced the estimated standard errors and genetic correlations that were significantly different from zero were estimated between susceptibility to BN and growth survival in experiment 4 (0.26 ± 0.09), and between harvest body weight and growth survival in experiments 3 and 4 (0.38 ± 0.07 and 0.26 ± 0.07).

5. GENERAL DISCUSSION

4.1 Development of a challenge test for use in breeding

The first aim of this thesis was to establish a challenge test for infection with *E. ictaluri* applicable to breeding of Mekong striped catfish. To accomplish this, a series of cohabitation challenge experiments were carried out, as described and discussed in Paper I. A fundament is the choice of an appropriate cohabitation method, that depends on the species and the nature of the specific pathogen. Several challenge methods have been applied in disease resistance studies of aquatic species, as mentioned in the General introduction: Intraperitoneal injection, bath, cohabitation, feeding or even a combination of these methods. In general, intraperitoneal injection has been most widely used since it is easy to standardize. However, it does not test for resistance factors that are located to the surface of the fish (Nordmo et al., 1997). These defence mechanisms (mucus, skin, gills, etc.) are likely important under an infection but are artificially bypassed when the pathogen is introduced by injection. The immersion, or bath method, which exposes the fish to a causative pathogen environment for a predefined time period, before the fish is taken out and put to an observation unit, is also easy to conduct and standardize. With both of these methods, the disease infection will not only be from the original inoculation but also from the water-borne infection set off by the first dead fish. Therefore, Nordmo et al. (1997) suggested that cohabitation should be used when applicable since it best mimics a natural infection even though it is not easy to standardize. In Paper I, we thus chose to use the cohabitation challenge in combination with additional pathogen supply and stress factors. In Paper I, a natural reference of 50% mortality at the end of the test was aimed at, because it maximizes the phenotypic variance for a binary trait (Gjøen et al., 1997). Establishing such a testing protocol has implication not only for genetic analysis, but also when examining effect of treatments; for example, effects of vaccines (Drangsholt et al., 2012; Fredriksen et al., 2013) or feed ingredients (Ward et al., 2016). Paper I proposes three days acclimatization of test fish prior to the challenge, with restricted water level in the test, keeping a temperature of 26^0 C. In the challenge, cohabitant shedders should be released directly into the test tank and make up around ⅓ of the fish, and bacteria should be added directly to water. The last two experiments, with the highest mortality, suggest

that any factor involving the dead cohabitants should be removed, and that additional experimentation should focus on bacteria (density) and timing for addition of bacteria to water. These suggestions were instrumental to Vu et al. (2019) when carrying out another, fifth, experiment in 2015. In this experiment, the factors involving the dead cohabitants were totally removed, time for cohabitants release after injection was set to two days, virulence of bacteria as well as doses for injection and for addition of bacteria to water were determined *a priori* to the experiment, and pathogen was added to water four days after cohabitant injection. Now the average final mortality across the two test tanks became 39%, close to the desired frequency of 50%.

4.2 Genetic variance, traits and models in the challenge test

Another objective of this thesis was to estimate genetic variance and heritability of BN resistance in Mekong striped catfish using two trait definitions; time until death and dead/alive at both 50% mortality and at the end of test. The latter defines disease resistance as a single record i.e. whether a fish is still alive/dead at a fixed time point, normally chosen when the cumulative mortality is approximately 50%, to maximise the phenotypic variance, or when the mortality is naturally levelling off. When analysed with a linear model (LM), the binary observations are treated as if they are normally distributed, and the model does not account for the binary nature of the data (Ødegård et al., 2011a). A threshold model (TM) is thus an alternative to better account for the categorical nature of the phenotype by modelling an underlying normal distribution with thresholds that determine the categories into which the observed phenotype fall (Gianola and Foulley, 1983). However, neither of the mentioned models utilize information embedded in observed time until death, which to some extent is assumed to reflect resistance to the disease, i.e. the least resistance fish is the first to die. To account for time until death, a linear survival model (LSM) can be used by for example assigning a categorical phenotype per day, 0 = dead/1 = alive, until death of the fish or termination of the test (longitudinal survival scores) (Ødegård et al., 2011a). Survival models are assumed to make the best use of information regarding the entire life span of an animal during the test (e.g., Gitterle, Ødegård, Gjerde, Rye & Salte 2006). Other linear model approaches exists to survival analysis, for example did Vu et al. (2019) considered number of days until death as the relevant trait in Mekong striped catfish.

In Paper I, dead/alive at the end of the test was found to be the same trait across the two replicated tanks in the same experiment. Thus, in Paper II, the data were analysed on a per experiment basis with the models mentioned above. In the LM and in the TM, a binary trait (dead = 0/alive = 1) was defined at two stages: At the end of the test (endpoint) and at the day the truncated mortality was closest to 50% (50% mortality); for the LSM a binary variable per test day across the test period was defined as 1/0 if the fish was alive/dead on test day t, where 0 implied that there would be no further record for that fish. In summary, relatively low heritability estimates were obtained for BN, irrespective of the experiment, the statistical model used or trait definition, either endpoint mortality or 50% truncated mortality (Paper II). The most expressed heritability estimates were obtained at 50% mortality with both TM and LM likely because the this frequency maximizes the phenotypic variance of the disease (binary) trait (e.g., by Gjøen et al., 1997). The heritability estimated for daily survival (with LSM) was low (~ 0.01). The low heritability estimates corresponded with the heritability estimates reported by Vu et al. (2019)(0.10 on the linear scale and 0.16 on the underlying liability scale, with TM). With inclusion of the fifth experiment, Vu et al. (2019) could estimate resistance to BN across year-classes (experiments), which was not considered possible with the present data due to limited genetic ties (Paper II). In addition, the Spearman rank correlation values between family effects obtained with LM and TM at 50% mortality and in the endpoint were low (≤ 0.72, Table 4, Paper II), implying substantial re-ranking of family effects. The low correlation can be considered the consequence of the back-truncated mortality (at 50%) being a mixture of both susceptibility and endurance. Susceptibility is whether or not the animal is at risk of dying (non-cured or cured, respectively), while endurance is the individual's ability to live for some time given that it is susceptible (e.g., Kause and Ødegård, 2012). However, if mortality ceases, those fish that survive should be non-susceptible, and there does not any longer exist a mixture of the two traits. Therefore, endpoint mortality can be considered a measure of susceptibility, while the other measures of resistance as survival time and back truncated mortality to 50 % will reflect the mixture of the two traits. This dependence between the two traits can be eliminated by carrying out a genetic analysis with a cure model (Ødegård et al., 2011b; Ødegård et al., 2011c). An alternative is to only consider susceptibility, which requires that a natural endpoint mortality is reached (at the point where mortality naturally ceases). This leads to the

conclusion that, the challenge test should aim for an average mortality naturally ceasing at 50% (Paper II) that should increase heritability, and that back – truncated data analysis to 50% mortality (when the actual final mortality is much higher in the challenge test) is not to be recommended. Recently, host infectivity (i.e., the host's ability to infect an average individual upon contact) has been proposed as a trait (Anacleto et al., 2019; Tsairidou et al., 2019). However, with selection for susceptibility and when fish becomes more genetically resistant, host infectivity might become less important because the fish is then less likely to spread the pathogen (Paper II). Thus, one can argue that the focus should be on selection for BN resistance in the initial stages of the breeding program.

4.3 Testing in replicated tanks for cross-validation of genetic evaluation models

Testing in replicated tanks not only reduces the risk for accidents, but also allows to test the stability of the test procedures (Paper I) and to cross-validate the statistical models used to analyse BN resistance aimed at in Paper II. The models were compared by calculating the Pearson correlation coefficients between the family effects (sum of sire, dam and common environmental effects) in one tank to the observed family survival in the other tank, per experiment. In most cases, TM predicted least precise, compared to LSM and LM. However, in this study, it was not possible to discriminate between the LM and the LSM when comparison was done within experiment (Paper II). Thus, if the average, ceased, mortality is not deviating too much from 50% (mortality naturally levelled off), the LM should be used to estimate breeding values due to its simplicity and since this frequency maximises both the phenotypic and genetic variance (Paper II).

4.4 Genetic correlation between susceptibility to BN and growth survival

In Paper III, the estimated genetic correlation between BN and growth survival when accounting for the permanent environmental effect was not consistent in size across experiments (-0.01 ± 0.30 in experiment 3, and 0.58 ± 0.20 in experiment 4). Due to limited genetic ties in our study, estimation of the genetic parameters of growth survival had to be carried out on a year-class basis, as mentioned, while Vu et al. (2019) utilized the increased amount of genetic ties (from the test in 2015) and assumed growth

survival across year-classes to be the same traits, resulting in an estimate of 0.52 ± 0.10. Vehviläinen et al. (2008) suggested that treating field survival as one trait over time may not reveal its true genetic architecture because individuals from different year-classes might not be exposed to the same factor causing the mortality. In addition, the causative factors to growth survival is largely unknown in most growth performance test data. When the causative agent is the same in the challenge test as in the field, Gjøen et al. (1997) and Ødegård et al. (2006) have demonstrated very high positive genetic correlations between challenge test and field test survival (caused by *Areomonas salmonicida* in Atlantic salmon: 0.71 - 0.95, dependent on statistical model). Furthermore in Atlantic salmon, the same high genetic correlations (0.78 – 0.83) were reported by Wetten et al. (2007), between the resistance against infectious Pancreatic necrosis in a challenge test and survival from the same cause in a field-test. In our study, highly variable size of the estimated genetic correlations between susceptibility to BN and growth survival were found (experiments 3 and 4, Paper III). This is likely due to the growth performance test being carried out with the breeding population, meaning that antibiotic treatment will be applied, if necessary. This again suggests that growth survival should not be considered the same trait across year-classes. However, the referred genetic correlations were estimated with large standard errors which led us to reanalyse the data with a simplified statistical model without the common environmental effect (Paper III). The resulting genetic correlations between susceptibility to BN and growth survival became moderate in experiment 4 (0.26 ± 0.09), but only -0.02 ± 0.11 in experiment 3. In Paper III, it is concluded that reaching a conclusive genetic relationship between challenge and field survival would require a real field test for survival (a new test in addition to the standard growth performance test). In this test, siblings from the same families as in the challenge and the growth test are to be used, treatment is not to be carried out and the cause of death needs to be continuously monitored (for natural outbreak of BN or not). Meanwhile, it is proposed to continue the routine challenge testing with the aim of indirectly improving field survival.

4.5 Potential genetic gain for BN resistance in the breeding population

The results in Paper II show that a large scope exists for selective breeding against BN with Mekong striped catfish: By the breeders equation (Falconer and Mackay, 1996), the genetic gain in the breeding population can be calculated to ~10% per generation (experiment 3, LM, resulting in an estimated genetic standard deviation of 10.8%, and further assuming a standardized selection intensity of ~ 1.3 and a limiting accuracy on the estimated breeding value of 0.71). This compares well with the genetic gain calculated initially in the breeding population selected solely for BN resistance, of 8.3% per generation (Trong Quoc Trinh. per. com).

The low heritability of BN resistance suggests to test large family groups to obtain an accuracy of selection that is not too far away from the accuracy for body weight. The developed challenge test proposed the water level being halved, and an alternative approach would then be to double the density in each tank. This would allow up to 30 fish/family tested per tank. With two tanks to reduce the risk of testing, the number of fish tested per family would become 60, much higher than the minimum number of 20 fish per family proposed by Nielsen et al. (2009). Another option would be to select the survivors as breeding candidates. This approach has been shown very efficient in a simulation study carried out by Sonesson et al. (2011). However, this is a risky approach because the disease might then spread to the next generation.

4.6 Potential genetic gain for BN resistance in the field

The indirect selection response for survival in the field can be calculated as the product of the selection intensity, the accuracy of selection for BN resistance (both mentioned above in 4.5), the additive genetic standard deviation of field survival and the genetic correlation between BN resistance and field survival. This would require a new field test for survival, as proposed.

4.7 Genetic ties across sub-populations

Vu et al. (2019) assumed resistance to BN across year-classes (experiments) to be the same trait, which is not what was assumed herein (Papers II and III), where the analyses

were carried out experiment by experiment. However, prior to the fifth experiment carried out in 2015, stronger genetic ties might have become established such that all experiments (except experiment 1 that was not included in the analysis of Vu et al. (2019)) could be included in one joint genetic analysis. In the short-term, attempts to create genetic ties as described in Paper II could obviously increase genetic gain through both increased selection accuracy and intensity, the latter because Mekong striped catfish spawn repeatedly. However, in the longer run, additional selection response should be obtained by adopting optimum contribution selection (Meuwissen, 1997), known to maximize the genetic gain at a predefined rate of inbreeding.

4.8 Multi-trait selection

Growth-related traits, typically recorded as harvest body weight, is considered the most important trait in a fish breeding program (Gjedrem, 2005). The magnitude of the genetic correlation between growth and other important trait, such as disease resistance, is therefore of major importance when optimising selection, to control for possible adverse correlated genetic responses. A number of earlier studies in various fish species and diseases have demonstrated different degrees and nature of genetic associations between growth and disease resistance, from weak to favourable (Bangera et al., 2011; Henryon et al., 2005; Perry et al., 2004) or unfavourable (Drangsholt et al., 2012; Gitterle et al., 2005; Henryon et al., 2002; Yáñez et al., 2014; Yáñez et al., 2016). In this study, low, non-significant relative to zero, genetic correlations were estimated between BN and growth (Paper III); thus, there is no indication that selection for an increase growth rate would genetically reduce BN resistance in Mekong striped catfish. Similarly, the genetic correlation between body weight and growth survival has been estimated in several studies, reporting either favourable correlations (Gitterle et al., 2005; Liu et al., 2015) or low, positive correlations, that have not been significantly different from zero (Krishna et al., 2011; Nielsen et al., 2010). In this study, growth was found with moderate favourable genetic correlation to growth survival (Paper III). This suggests that selection for growth will likely genetically improve growth survival.

At current there are two Mekong striped catfish breeding programs established in Vietnam that are run in parallel, one aiming for growth improvement and the other for improved BN resistance (Paper III). The top-ranked individuals (on estimated breeding

values) from the two selection lines can then be utilized through crossbreeding to exploit heterosis. To our knowledge, heterosis effects have not been estimated for these traits in Mekong striped catfish, and since the breeding programs have not been carried out for long, and also utilizes fish from the wild (Paper II), both the inbreeding level and heterosis effects are expected to be minor (Falconer and Mackay, 1996). In addition, as discussed above, the genetic correlation between growth and BN resistance is likely toward positive which supports the possibility to improve both traits simultaneously by mean of index selection in a single breeding program, which could be more efficient in term of cost and labour.

4.9 Genomic selection

Breeding for disease resistance using traditional challenge tests often is limited by: 1) the testing capacity and costs and other practical limitations, 2) the family selection practice which does not allow to distinguish between untested individuals, and thus does not utilize the within family genetic variation; this limits selection intensity since it forces the breeder to choose random candidates from the high-ranking families, rather than the highest ranking candidates across families (Ødegård et al., 2011a). If survivors from the challenge test had been selected, one would partly have overcome these two limitations. However, this is normally not allowed, even though the pathogen seems to be present everywhere in the environment since the water supply during the hapa and grow-out periods are from the Mekong river.

In order to enhance the selection intensity and selection accuracy, a genomic selection (GS) program for BN resistance could have been carried out with Mekong striped catfish. In the original set-up of such a program, the effects of dense genetic markers are first estimated in a test population with large number of individuals which have both genotype and phenotype information (reference population) to optimize the prediction equations. With the optimised prediction equations, it will be applied to the selection candidates based on the summed effects of marker effects over the whole genome, allowing to utilise the whole genetic variance (Nielsen et al., 2009; Sonesson and Meuwissen, 2009; Toro et al., 2017; Vallejo et al., 2017). The limitation of this method is the high cost of genotyping, as well as the availability of a SNP (Single Nucleotide Polymorphism) array for Mekong striped catfish. Kim et al. (2018) have reported a draft genome in Mekong striped catfish. Another option would be genotyping by sequencing

(Vo et al., 2018), reporting 11.009 potential SNP loci in striped catfish across the populations in Vietnam, Cambodia, Thailand and Bangladesh. As mentioned, the main motivation for establishing a program that utilise genomic selection in Mekong striped catfish is the increased selection intensity, but also the possibility to increase the accuracy of selection beyond the traditional value of 0.71 when utilizing full-sib data (Haffray et al., 2018).

As mentioned, the cost for such an advanced program is high. An alternative is thus to carry out a combined scheme in which one pre-select potential families using BLUP (Best Linear Unbiased Prediction), and then genotype the relevant families to carry out within-family selection on genomic breeding values estimated using markers of low density (Lillehammer et al., 2013). At low marker density (10 SNP/chromosome), Dagnachew and Meuwissen (2019) have suggested that genotyping (using SNPs) of 60 individuals/family is optimal.

4.10 On the need to reduce the common environmental effect

The common environmental effects were considerable, especially for growth survival and harvest body weight (Paper III). This could be due to use of the nested mating design (one male mated to two females) and problem with separation of genetic and common environmental effects. Hence, the family effect (sum of sire, dam, and common environmental effects) was used for cross-validation of the statistical models in Paper II. Moreover, in fish breeding, the partial factorial mating design has advantageous over the nested design regarding separation of these effects (Berg and Henryon, 1998). The environmental effect can also be large from families being reared separately for a long time in a hapa in addition to possible maternal and/or dominance effect. Reduction of the effect of common environment could be sought by reducing the length of spawning and tagging time, reducing the variation in nursed time between families and the impact of common environment. This will require upgrading of the nursing and spawning capacity and a large work force. A third option to reduce the common environmental effect would be early communal rearing in a cement tank (at NABRECSOFA, National Breeding Centre For Southern Freshwater Aquaculture, several cement nursing tanks can be supplied with pathogen-free water) of newly-hatched fry, or 20-day-old

fingerlings after tank nursing, together with parental assignment using genetic marker (Haffray et al., 2018; Ninh et al., 2013; Premachandra et al., 2019).

4.11 On other diseases

The Mekong striped catfish farming in Vietnam is currently facing a new disease: Freshwater Columnaris Infection (FCI), caused by *Flavobacterium columnare.* This is considered the second most devastating disease of Mekong striped catfish, next to BN. Recently, mortality as large as 100% among fingerlings has been reported in commercial hatcheries. Infected fish are commonly presented with tail erosion, whitish spots on the body and greyish gills (Tien et al., 2012). An injection challenge has been carried out using *Flavobacterium columnare,* isolated from hatcheries with disease outbreaks, resulting in mortalities ranging 50% - 100%, dependent on the dose (Tien et al., 2012). Interestingly, Dong et al. (2015) reported co-infection of *F. columnare* and *E. ictaluri* in naturally diseased Thai striped catfish. In practice, one would like fish to be resistant to all diseases, but this seems to be unrealistic since diseases differ in their aetiologies, with each requiring a different mechanism of immunity to prevent infection. Thus, it can be relevant to also test for resistance towards FCI in future breeding programs of Mekong striped catfish in Vietnam.

6. CONCLUSIONS

- Environmental factors in a challenge test against *E. ictaluri,* for use in breeding in Mekong striped catfish were concluded through four experiments.
- The concluded environmental factors were the following: Three days acclimatization of test fish prior to the challenge, with restricted water level in the test, keeping a temperature of 26^0 C. In the challenge, cohabitant shedders should be released directly into the test tank and make up around ⅓ of the fish, and bacteria should be added directly to water. Any factor involving the dead cohabitants should be removed, and additional experimentation should focus on bacteria (density) and timing for addition of bacteria to water.
- Rather low heritability estimates (≤ 0.22 with the cross-sectional threshold model and 0.135 with the corresponding linear model) were obtained for resistance to *E. ictaluri.*
- In challenge testing, one should aim for an average endpoint mortality that naturally ceases around 50%.
- At the aimed endpoint mortality, the breeding values could preferably be calculated for susceptibility to BN by use of a cross-sectional linear model.
- The magnitude of the estimated genetic correlations between susceptibility to BN and harvest body weight was slightly positive, but not significantly different from zero, that should allow the two traits all to be improved simultaneously in one joint breeding program by means of selective breeding.
- The genetic correlation between harvest body weight and growth survival was slightly favourable (positive), implying that selection for growth will genetically improve growth survival.
- The genetic correlation between susceptibility to BN and growth survival was estimated as positive, but highly variable between experiments. To reach an indirect genetic response in field survival and to defend the advised, continued challenge testing, the program is in need of verifying a consistent, considerable and significant genetic correlation between BN and field survival.
- The former can be achieved by establishing a new field test (in addition to growth performance test) where antibiotic treatment is not carried out and the cause of death is continuously monitored (for outbreaks of BN).

7. IMPLICATIONS AND FUTURE PERFECTIVES

This thesis identifies the following:

- To realise the potential for considerable genetic gain in susceptibility to BN in the current breeding program, one should consider to increase the family sizes in the test to obtain higher accuracy on the estimated breeding values.
- In the breeding programs established, there is a need to control the increase of population inbreeding which can be done by implementing optimal contribution selection. Optimal contribution selection can be based on pedigree, but in future initiatives should be taken to incorporate genomic inbreeding.
- There is a need to scientifically and economically evaluate whether two breeding programs should be run in parallel in Vietnam.
- The genomic selection program that is underway has the potential to increase both selection intensity and selection accuracy. It needs to be developed towards practical implementation.
- Actions should be taken to reduce the impact of the common environmental effect on precision of the genetic parameters by use of the partial factorial mating design for family production as well as to shorten the time period used to produce families.
- To reduce the possibility for infection with *E. ictaluri* prior to challenge testing, one should consider to raise the fish in pathogen free water that can be supplied to cement tanks at NABRECSOFA. Combined with communal early rearing and parentage assignment by genotyping, it can be considered an alternative approach to reduce the common environmental effect.
- A challenge test against *Flavobacterium columnare* causing Freshwater Columnaris Infection (FCI) should be established. The genetic relationship between BN and FCI should be estimated.
- The Mekong Delta, Vietnam, is considered one of the most affected area by global climate change since the sea level might increase. Thus, testing of new economical important traits, such as salt tolerance, should be considered.

REFERENCES

Anacleto, O., Cabaleiro, S., Villanueva, B., Saura, M., Houston, R.D., Woolliams, J.A., Doeschl-Wilson, A.B., 2019. Genetic differences in host infectivity affect disease spread and survival in epidemics. Scientific Reports. 9, 4924.

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.

Barría, A., Doeschl-Wilson, A.B., Lhorente, J.P., Houston, R.D., Yáñez, J.M., 2019. Novel insights into the genetic relationship between growth and disease resistance in an aquaculture strain of Coho salmon (*Oncorhynchus kisutch*). Aquaculture. 511, 734207.

Bassini, L.N., Lhorente, J.P., Oyarzún, M., Bangera, R., Yáñez, J.M., Neira, R., 2019. Genetic parameters for *Piscirickettsia salmonis* resistance, sea lice (*Caligus rogercresseyi*) susceptibility and harvest weight in rainbow trout (*Oncorhynchus mykiss*). Aquaculture. 510, 276-282.

Berg, P., Henryon, M., 1998. A comparison of mating designs for inference on genetic parameters in fish. Proc. 6th WCGALP. 27, 115-118.

Bui, T.M., Phan, L.T., Ingram, B.A., Nguyen, T.T.T., Gooley, G.J., Nguyen, H.V., Nguyen, P.T., De Silva, S.S., 2010. Seed production practices of striped catfish, *Pangasianodon hypophthalmus* in the Mekong Delta region, Vietnam. Aquaculture. 306, 92-100.

Dagnachew, B., Meuwissen, T., 2019. Accuracy of within-family multi-trait genomic selection models in a sib-based aquaculture breeding scheme. Aquaculture. 505, 27-33.

De Silva, S.S., Phuong, N.T., 2011. Striped catfish farming in the Mekong Delta, Vietnam: a tumultuous path to a global success. Reviews in Aquaculture. 3, 45-73.

Doan Q., K., Vandeputte, M., Chatain, B., Haffray, P., Vergnet, A., Breuil, G., Allal, F., 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.

Dong, H.T., Nguyen, V.V., Phiwsaiya, K., Gangnonngiw, W., Withyachumnarnkul, B., Rodkhum, C., Senapin, S., 2015. Concurrent infections of *Flavobacterium columnare* and *Edwardsiella ictaluri* in striped catfish, *Pangasianodon hypophthalmus* in Thailand. Aquaculture. 448, 142-150.

Drangsholt, T.M.K., Gjerde, B., Ødegård, J., Finne-Fridell, F., Evensen, Ø., Bentsen, H.B., 2012. Genetic correlations between disease resistance, vaccine-induced side effects and harvest body weight in Atlantic salmon (*Salmo salar*). Aquaculture. 324–325, 312-314.

Dung, T.T., Crumlish, M., Ngoc, N., Thinh, N., Thy, D., 2004. Investigate the disease caused by the genus *Edwardsiella* from Tra catfish (*Pangasianodon hypophthalmus*). Journal of Science, Can Tho University. 1, 23-31.

Falconer, D.S., Mackay, T.F.C., 1996. Introduction to quantitative genetics (4th ed). Harlow, Essex, England: Longman: Pro-ed.

Ferguson, H., Turnbull, J., Shinn, A., Thompson, K., Dung, T.T., Crumlish, M., 2001. Bacillary necrosis in farmed *Pangasius hypophthalmus* (Sauvage) from the Mekong Delta, Vietnam. Journal of Fish Diseases. 24, 509-513.

Flores-Mara, R., Rodríguez, F.H., Bangera, R., Lhorente, J.P., Neira, R., Newman, S., Yáñez, J.M., 2017. Resistance against infectious pancreatic necrosis exhibits

significant genetic variation and is not genetically correlated with harvest weight in rainbow trout (*Oncorhynchus mykiss*). Aquaculture. 479, 155-160.
Fredriksen, B.N., Olsen, R.H., Furevik, A., Souhoka, R.A., Gauthier, D., Brudeseth, B., 2013. Efficacy of a divalent and a multivalent water-in-oil formulated vaccine against a highly virulent strain of *Flavobacterium psychrophilum* after intramuscular challenge of rainbow trout (*Oncorhynchus mykiss*). Vaccine. 31, 1994-1998.
Gianola, D., Foulley, J.L., 1983. Sire evaluation for ordered categorical data with a threshold model. Genetics, Selection, Evolution. 15, 201-224.
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, 210-218.
Gitterle, T., Rye, M., Salte, R., Cock, J., Johansen, H., Lozano, C., Arturo Suárez, J., Gjerde, B., 2005. Genetic (co)variation in harvest body weight and survival in *Penaeus (Litopenaeus) vannamei* under standard commercial conditions. Aquaculture. 243, 83-92.
Gjedrem, T., 2000. Genetic improvement of cold-water fish species. Aquaculture Research. 31, 25-33.
Gjedrem, T., 2005. Breeding plans. In T. Gjedrem (Ed.), Selection and breeding programs in aquaculture (pp. 251 - 277). Dordrecht: Springer.
Gjedrem, T., Salte, R., Gjøen, H.M., 1991. Genetic variation in susceptibility of Atlantic salmon to furunculosis. Aquaculture. 97, 1-6.
Gjerde, B., Boison, S.A., Aslam, M.L., Løvoll, M., Bakke, H., Rey, S., Lillehammer, M., 2019. Estimates of genetic correlations between susceptibility of Atlantic salmon to amoebic gill disease in a bath challenge test and a field test. Aquaculture. 511, 734265.
Gjøen, H.M., Refstie, T., Ulla, O., Gjerde, B., 1997. Genetic correlations between survival of Atlantic salmon in challenge and field tests. Aquaculture. 158, 277-288.
Glover, K.A., Hansen, T., Besnier, F., Solberg, M.F., Fjelldal, P.G., Eide, A.G.S., Dalvin, S., Nilsen, F., 2017. Cloned and outbred Atlantic salmon display equal parasite dispersion when infected with the salmon louse. Aquaculture. 480, 83-88.
Haffray, P., Enez, F., Bugeon, J., Chapuis, H., Dupont-Nivet, M., Chatain, B., Vandeputte, M., 2018. Accuracy of BLUP breeding values in a factorial mating design with mixed families and marker-based parentage assignment in rainbow trout *Oncorhynchus mykiss*. Aquaculture. 490, 350-354.
Henryon, M., Jokumsen, A., Berg, P., Lund, I., Pedersen, P.B., Olesen, N.J., Slierendrecht, W.J., 2002. Genetic variation for growth rate, feed conversion efficiency, and disease resistance exists within a farmed population of rainbow trout. Aquaculture. 209, 59-76.
Henryon, M., Berg, P., Olesen, N.J., Kjær, T.E., Slierendrecht, W.J., Jokumsen, A., Lund, I., 2005. Selective breeding provides an approach to increase resistance of rainbow trout (*Onchorhynchus mykiss*) to the diseases, enteric redmouth disease, rainbow trout fry syndrome, and viral haemorrhagic septicaemia. Aquaculture. 250, 621-636.
Kause, A., Ødegård, J., 2012. The genetic analysis of tolerance to infections: a review. Frontiers in Genetics. 3, 262.
Khanh, V.P., 1996. Sinh sản nhân tạo cá Tra σ ĐBSCL. PhD thesis, University of Fisheries, Nha Trang, Vietnam.
Kim, O.T.P., Nguyen, P.T., Shoguchi, E., Hisata, K., Vo, T.T.B., Inoue, J., Shinzato, C., Le, B.T.N., Nishitsuji, K., Kanda, M., Nguyen, V.H., Nong, H.V., Sattou, N., 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, 733.
Krishna, G., Gopikrishna, G., Gopal, C., Jahageerdar, S., Ravichandran, P., Kannappan, S., Pillai, S.M., Paulpandi, S., Kiran, R.P., Saraswati, R., 2011. Genetic parameters for growth and survival in *Penaeus monodon* cultured in India. Aquaculture. 318, 74-78.
LaFrentz, B.R., Lozano, C.A., Shoemaker, C.A., García, J.C., Xu, D.H., Løvoll, M., Rye, M., 2016. Controlled challenge experiment demonstrates substantial additive genetic variation in resistance of Nile tilapia (*Oreochromis niloticus*) to *Streptococcus iniae*. Aquaculture. 458, 134-139.
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.
Lillehammer, M., Meuwissen, T.H.E., Sonesson, A.K., 2013. A low-marker density implementation of genomic selection in aquaculture using within-family genomic breeding values. Genetics Selection Evolution. 45, 39.
Lillehammer, M., Boison, S.A., Norris, A., Løvoll, M., Bakke, H., Gjerde, B., 2019. Genetic parameters of resistance to amoebic gill disease in two Norwegian Atlantic salmon populations. Aquaculture. 508, 83-89.
Liu, J., Lai, Z., Fu, X., Wu, Y., Bao, X., Hu, Z., Lai, M., 2015. Genetic parameters and selection responses for growth and survival of the small abalone *Haliotis diversicolor* after four generations of successive selection. Aquaculture. 436, 58-64.
Mahapatra, K.D., Gjerde, B., Sahoo, P., Saha, J., Barat, A., Sahoo, M., Mohanty, B.R., Ødegård, J., Rye, M., Salte, R., 2008. Genetic variations in survival of rohu carp (*Labeo rohita*, Hamilton) after *Aeromonas hydrophila* infection in challenge tests. Aquaculture. 279, 29-34.
Meuwissen, T.H.E., 1997. Maximizing the response of selection with a predefined rate of inbreeding. J. of Animal Science. 75, 934-940.
Nielsen, H.M., Sonesson, A.K., Yazdi, H., Meuwissen, T.H.E., 2009. Comparison of accuracy of genome-wide and BLUP breeding value estimates in sib based aquaculture breeding schemes. Aquaculture. 289, 259-264.
Nielsen, H.M., Ødegård, J., Olesen, I., Gjerde, B., Ardo, L., Jeney, G., Jeney, Z., 2010. Genetic analysis of common carp (*Cyprinus carpio*) strains: I: Genetic parameters and heterosis for growth traits and survival. Aquaculture. 304, 14-21.
Ninh, N.H., Ponzoni, R.W., Nguyen, N.H., Woolliams, J.A., Taggart, J.B., McAndrew, B.J., Penman, D.J., 2013. A comparison of communal and separate rearing of families in selective breeding of common carp (*Cyprinus carpio*): Responses to selection. Aquaculture. 408–409, 152-159.
Nordmo, R., Sevatdal, S., Ramstad, A., 1997. Experimental infection with *Vibrio salmonicida* in Atlantic salmon (*Salmo salar* L.): an evaluation of three different challenge methods. Aquaculture. 158, 23-32.
Nordmo, R., Ramstad, A., Holth Riseth, J.M., 1998. Induction of experimental furunculosis in heterogenous test populations of Atlantic salmon (*Salmo salar* L.) by use of a cohabitation method. Aquaculture. 162, 11-21.
Perry, G.M.L., Tarte, P., Croisetiere, S., Belhumeur, P., Bernatchez, L., 2004. Genetic variance and covariance for 0+ brook charr (*Salvelinus fontinalis*) weight and survival time of furunculosis (*Aeromonas salmonicida*) exposure. Aquaculture. 235, 263-271.
Phan, L.T., Bui, T.M., Nguyen, T.T.T., Gooley, G.J., Ingram, B.A., Nguyen, H.V., Nguyen, P.T., De Silva, S.S., 2009. Current status of farming practices of striped catfish,

Pangasianodon hypophthalmus in the Mekong Delta, Vietnam. Aquaculture. 296, 227-236.

Ponzoni, R.W., Nguyen, N.H., Khaw, H.L., 2007. Breeding program requirements in developing countries may differ depending on circumstances. In A. J. van der Zijpp, J.A.J. Verreth. Le Quang Tri, M.E.F. van Meensvort, R.H. Bosma & M.C.M Beveridge (Eds.). Fishponds in farming systems (pp. 59 - 67). Wageningen: Wageningen Academic Publishers.

Premachandra, H.K.A., Nguyen, N.H., Knibb, W., 2019. Effectiveness of SNPs for parentage and sibship assessment in polygamous yellowtail kingfish *Seriola lalandi*. Aquaculture. 499, 24-31.

Sang, N.V., Klemetsdal, G., Ødegård, J., Gjøen, H.M., 2012. Genetic parameters of economically important traits recorded at a given age in striped catfish (*Pangasianodon hypophthalmus*). Aquaculture. 344–349, 82-89.

Sang, V.N., 2010. Genetic studies on improvement of striped catfish (*Pangasianodon hypophthalmus*) for economically important traits. Doctoral thesis. Norwegian University of Life Sciences, Ås. Retrieved from https://nmbu.brage.unit.no/nmbu-xmlui/handle/11250/2431968.

Shoemaker, C.A., Lozano, C.A., LaFrentz, B.R., García, J.C., Soto, E., Xu, D.H., Beck, B.H., Rye, M., 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.

Sonesson, A., Meuwissen, T., 2009. Testing strategies for genomic selection in aquaculture breeding programs. Genetics, Selection, Evolution. 41, 37.

Sonesson, A.K., Gjerde, B., Robinson, N., 2011. A simple selection scheme to improve disease resistance and growth. Aquaculture. 319, 337-341.

Srisapoome, P., Chatchaiphan, S., Bunnoy, A., Koonawootrittriron, S., Na-Nakorn, U., 2019. Heritability of immunity traits and disease resistance of bighead catfish, *Clarias macrocephalus* Günther, 1864. Fish & Shellfish Immunology. 92, 209-215.

Sukhavachana, S., Poompuang, S., Onming, S., Luengnaruemitchai, A., 2019. Heritability estimates and selection response for resistance to *Streptococcus agalactiae* in red tilapia *Oreochromis spp.* Aquaculture. 502, 384-390.

Thanh, P.C., Berntsen, J.O., 2012. Towards sustainability: Vaccinated *pangasius* in Vietnam. AQUA Culture Asia Pacific Magazine. May/June, 36-37.

Tien, N.T., Dung, T.T., Tuan, N.T., Crumlish, M., 2012. First identification of *Flavobacterium columnare* infection in farmed freshwater striped catfish *Pangasianodon hypophthalmus*. Diseases of Aquatic Organisms. 100, 83-88.

Toro, M.A., Saura, M., Fernandez, J., Villanueva, B., 2017. Accuracy of genomic within-family selection in aquaculture breeding programmes. J. of Animal Breeding and Genetics. 134, 256-263.

Tran, L.D., 2010. Genetic studies of Nile tilipia (*Oreochromis niloticus*) for farming in northern Vietnam: growth, survival and cold tolerance in different farm environments. PhD thesis. Norwegian University of Life Sciences, Ås. Retrieved from https://nmbu.brage.unit.no/nmbu-xmlui/handle/11250/2430611.

Trong, T.Q., Hao, N.V., Griffiths, D., 2002. Annex IV. Status of *Pangasiid* aquaculture in Viet Nam. Report prepared by MRC Fisheries Program on request of the "Technical Advisory Body of Fisheries Management in the Lower Mekong Basin". Retrieved from https://www.researchgate.net/publication/285076136_Status_of_Pangasiid_aquaculture_in_Viet_Nam.

Tsairidou, S., Anacleto, O., Woolliams, J.A., Doeschl-Wilson, A., 2019. Enhancing genetic disease control by selecting for lower host infectivity and susceptibility. Heredity. 122, 742-758.

Vallejo, R.L., Leeds, T.D., Gao, G., Parsons, J.E., Martin, K.E., Evenhuis, J.P., Fragomeni, B.O., Wiens, G.D., Palti, Y., 2017. Genomic selection models double the accuracy of predicted breeding values for bacterial cold water disease resistance compared to a traditional pedigree-based model in rainbow trout aquaculture. Genetics Selection Evolution. 49, 17.

van Muiswinkel, W.B., Wiegertjes, G.F., Stet, R.J.M., 1999. The influence of environmental and genetic factors on the disease resistance of fish. Aquaculture. 172, 103-110.

van Zalinge, N., Lieng, S., Ngor, P.B., Heng, K., Valbo-Jørgensen, J., 2002. Status of the Mekong *Pangasianodon hypophthalmus* resources, with special reference to the stock shared between Cambodia and Viet Nam. Report prepared by MRC Fisheries Program on request of the "Technical Advisory Body of Fisheries Management in the Lower Mekong Basin". Retrieved from https://www.researchgate.net/publication/285076136_Status_of_Pangasiid_aquaculture_in_Viet_Nam.

VASEP, 2019. Vietnam Association of Seafood Exporters and Producers. Retrieved from http://vasep.com.vn.

Vehviläinen, H., Kause, A., Quinton, C., Koskinen, H., Paananen, T., 2008. Survival of the currently fittest: Genetics of rainbow trout survival across time and space. Genetics. 180, 507-516.

Vo, T.T.B., Nguyen, H.D., Nguyen, T.T.H., Tran, H.T.T., 2018. Identification and analysis of snps in population of Vietnamese catfish (*Pangasianodon hypophthalmus*), using next generation sequencing and snp validation. MOJ Current Research & Reviews. 1, 12-19.

Vu, N.T., Sang, N.V., Trong, T.Q., Duy, N.H., Dang, N.T., Nguyen, N.H., 2019. Breeding for improved resistance to *Edwardsiella ictaluri* in striped catfish (*Pangasianodon hypophthalmus*): Quantitative genetic parameters. Journal of Fish Diseases. 42, 1409-1417.

Ward, D., Bengtson, D.A., Lee, C.M., Gomez-Chiarri, M., 2016. Incorporation of soybean products in summer flounder (*Paralichthys dentatus*) feeds: Effects on growth and survival to bacterial challenge. Aquaculture. 452, 395-401.

Wetten, M., Aasmundstad, T., Kjøglum, S., Storset, A., 2007. Genetic analysis of resistance to infectious pancreatic necrosis in Atlantic salmon (*Salmo salar* L.). Aquaculture. 272, 111-117.

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.

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.

Yáñez, J.M., Bangera, R., Lhorente, J.P., Barría, A., Oyarzún, M., Neira, R., Newman, S., 2016. Negative genetic correlation between resistance against *Piscirickettsia salmonis* and harvest weight in coho salmon (*Oncorhynchus kisutch*). Aquaculture. 459, 8-13.

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., Baranski, M., Gjerde, B., Gjedrem, T., 2011a. Methodology for genetic evaluation of disease resistance in aquaculture species: challenges and future prospects. Aquaculture Research. 42, 103-114.

Ødegård, J., Madsen, P., Labouriau, R., Gjerde, B., Meuwissen, T.H.E., 2011b. A sequential threshold cure model for genetic analysis of time-to-event data. J. of Animal Science. 89, 943-950.

Ødegård, J., Gitterle, T., Madsen, P., Meuwissen, T.H.E., Yazdi, M.H., Gjerde, B., Pulgarin, C., Rye, M., 2011c. Quantitative genetics of taura syndrome resistance in pacific white shrimp (*penaeus vannamei*): a cure model approach. Genetics Selection Evolution. 43, 14.

PAPER I

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Paper I

Case study development of a challenge test against *Edwardsiella ictaluri* in Mekong striped catfish (*Pangasianodon hypophthalmus*), for use in breeding: Estimates of the genetic correlation between susceptibility in replicated tanks

Running title: Challenge testing striped catfish

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Abstract

Bacillary necrosis is a problematic disease in farming of Mekong striped catfish (*Pangasianodon hypophthalmus*). The pathogenic bacterium is *Edwardsiella ictaluri*, causing

numerous white spots in swelled liver, kidney and spleen. An alternative to antibiotic treatment and vaccine is to select for improved genetic resistance to the disease that require to establish a proper challenge test. Here, four challenge tests of Mekong striped catfish against *E. ictaluri* are reported proposing three days acclimatization of test fish prior to the challenge, with restricted water level in the test, keeping a temperature of 26^0 C. In the challenge, cohabitant shedders should be released directly into the test tank and make up around ⅓ of the fish, and bacteria should be added directly to water. The last two experiments, with the highest mortality, suggest that any factor involving the dead cohabitants should be removed, and that additional experimentation should focus on bacteria (density) and timing for addition of bacteria to water. Genetic analyses revealed that resistance to bacillary necrosis tested in replicated tanks in the same experiment can be considered the same genetic trait.

Keywords: Bacillary necrosis; test environment.

1. Introduction

The Mekong striped catfish (*Pangasianodon hypophthalmus*) farming in Vietnam has faced increasing disease problems, particularly bacillary necrosis (BN). Clinical signs are internal numerous white spots of different sizes in swelled liver, kidney and spleen (Crumlish, Dung, Turnbull, Ngoc & Ferguson 2002). These authors described the histopathological lesions as acute to sub-acute multifocal areas of necrosis and pyogranulomatous inflammation, with several species of parasites associating with these lesions. Specifically, a variable number of large bacilli are usually seen at the margins of lesions. BN belongs to Edwardsiellosis and is caused by the *Edwardsiella ictaluri* (*E. ictaluri*) bacteria, first observed in 1998, with its cause identified in 2002 (Crumlish, Dung, Turnbull, Ngoc & Ferguson 2002). The disease has been found in the whole Mekong Delta, in all life stages of catfish, but is especially frequent in the

fingerling period. Unless antibiotic treatment is timely applied, fish mortality may rise as high as 90% (Dong & Hoa 2008). In 2010, the first BN vaccine trial was conducted by Pharmaq Ltd. Vietnam (Thanh & Berntsen 2012). The trial was successful with significant lower mortality of vaccinated than non-vaccinated groups. The Alpha Ject® Panga 1 vaccine was licensed in 2013 (https://www.pharmaq.no/sfiles/8/66/4/file/2013_08-cty-pharmaq-vn_thuy-san-nam-14-so-164.pdf). However, improved resistance to BN using vaccination is often costly and impractical since it must be repeated for each fish, and in each generation. At current, to treat BN, antibiotics is used which may lead to resistance and potential contamination of the final product (Chuah, Effarizah, Goni & Rusul 2016). Moreover, reduction of mortality through drug application is costly and does not solve the problem permanently (van Muiswinkel, Wiegertjes & Stet 1999).

Besides development of vaccines, improvement of resistance to BN through selective breeding can be used as a tool for sustainable disease control. This is aimed at in the Mekong striped catfish breeding program initiated by the Research Institute for Aquaculture No. 2 (RIA2), Vietnam. Initially in this program, three base sub-populations were created from fish being domesticated in three hatcheries, making up year-classes 2001, 2002 and 2003, respectively (Sang, Klemetsdal, Ødegård & Gjøen 2012). So far, selection has been carried out for final body weight and fillet yield. Heritability estimates have been found moderate for body weight (0.21 - 0.34), while low estimates have been obtained for fillet yield (0.03 - 0.05) (Sang, Klemetsdal, Ødegård & Gjøen 2012). Recently, Vu, Sang, Phuc, Vuong & Nguyen (2019) reported on the genetic response achieved in the program.

Development of a challenge-test for BN was initiated in 2009. The objective was to rank individuals with respect to disease resistance towards *E. ictaluri*. The developments were based on the cohabitation method, known *a priori* to be difficult to standardize (Nordmo, Sevatdal & Ramstad 1997) and even not always successful (Mahapatra, Gjerde, Sahoo, Saha, Barat, Sahoo,

71 Mohanty, Ødegård, Rye & Salte 2008). However, Gjøen, Refstie, Ulla & Gjerde (1997) have
72 shown that the cohabitation method mimics a natural infection well.

73 Here, findings in four experiments with family materials of Mekong striped catfish challenged
74 with *E. ictaluri* are summarized. The aim was to establish a challenge-test applicable in the
75 breeding program including to examine the genetic correlation of resistance to BN between two
76 replicated test-tanks within the same experiment.

77 **2. Materials and methods**

78 By granting the research, the Vietnamese Ministry of Agriculture and Rural Development pre-
79 approved the experiments carried out.

80 ***2.1 Inoculums***

81 A strain of *E. ictaluri* Gly09M (Southern Monitoring Centre for Aquaculture Environment &
82 Epidemic, RIA2, Ho Chi Minh City, Vietnam) was used in all experiments. The strain was
83 isolated from diseased fish sampled in commercial striped catfish farms in the Mekong Delta
84 in 2009. Each year, reference stocks were sub-cultured and stored in a solution of Brain Heart
85 Infusion Broth (BHIB) and 20% glycerine supplementation, at -20^0 C. Working seed was
86 cultured in Sheep Blood Agar (SBA) and incubated for 24 hours at 30^0 C. Bacterial biomass
87 was suspended into a flask containing 500 ml BHIB, incubated with shaking for 18 hours. The
88 liquid culture was transferred into a 5 litter BHIB medium contained in a 10 liter fermenter,
89 cultured for 18 - 20 hours, stirred at 150 rounds/minute, and air supplied 5 vvm (in 1 minute, 5
90 liters of air are passing through 1 liter of medium). The density of bacteria was measured at 550
91 nm (OD_{550}) to calibrate the bacterial doses.

92 ***2.2 Cohabitant shedders***

Cohabitant shedders were randomly sampled from the hapas with the highest survival among fish to be challenged, just prior to tagging of the latter fish. Cohabitants were not tagged while test fish were, for discrimination. Shedders were acclimated in a separate tank and were intraperitoneally injected at experimental day 0.

2.3 General test-environment: Tanks, water quality and feeding

Experiment 1 was conducted in one outdoor fiber-glass tank at the National Breeding Centre for Southern Freshwater Aquaculture (NABRECSOFA, in the Tien Giang province) in 2009 (Table 1). The tank was partly covered by net to prevent eutrophication. Experiments 2, 3 and 4 were carried out indoor in the Govap Experimental Centre (GEC), RIA2, in 2010, 2011 and 2012, respectively (Table 1). GEC is located 125 km away from NABRECSOFA, and the fish were transported there by a lorry. In these three latter experiments, two replicated tanks were used (Table 1). In all experiments, tagging was done when the fish were netted out of the original hapas. In the last three experiments an equal number of randomly tagged fish from each hapa were transferred to either of two tanks at NABRECSOFA, before transfer to GEC. In the first experiment, tagged fish were kept in separate hapas for seven days prior to transfer to the test tank.

The test tanks were of circular shape; diameter 4.0 m, depth 1.8 m, and water volume of 20 m^3. Water was not exchanged unless considered necessary. Daily, the water quality was monitored by the use of Sera Test Kits (Germany): Water oxygen levels were kept higher than 1.0 ppm, using aeration to avoid losing energy on air-breathing behaviour (Lefevre, Jensen, Huong, Wang, Phuong & Bayley 2011). Across experiments, water-pH levels varied from 6.5 to 7.5. Fish were daily fed a standard commercial fingerling feed, 1% of total biomass.

2.4 Specific test-environment: Acclimatization, water temperature and level

In experiment 1, fish to be challenged were transferred from the hapas to the test tank 22 days prior to the experiment (Table 1). In experiment 2, tagged fish in the two tanks at NABRECSOFA were transported separately to GEC in oxygenated containers and transferred to the two replicated test tanks 14 days prior to the experiment. In experiments 3 and 4, transportation was as in experiment 2, but just 3 days prior to challenge. Thus, in experiments 1 and 2 fish were more adapted to the new environment than in experiments 3 and 4 where, in fact, a shortened acclimation period was used as a stressor.

Since experiment 1 was conducted outdoor, water temperature was 29.5° C (standard deviation = 1.0° C), i.e., it followed the surrounding temperature (Table 1). In experiment 2, water temperature was partly controlled using air conditioner; 29.0° C during the first 10 days post-cohabitation and thereafter 26° C, until termination. In experiments 3 and 4, temperature was set at 26° C because this temperature has been widely used in Enteric Septicaemia of Catfish (ESC) studies with channel catfish (Camp, Wolters & Rice 2000; Lim & Klesius 1997; Patrie-Hanson & Jerald Ainsworth 1999).

In experiment 1, the water level was kept constant during acclimatization and the entire testing period (Table 1). In experiments 2, 3 and 4, water levels were halved 1 day prior to challenge and kept at this level throughout the entire experimental period. This resulted in increased density and posed additional stress to the fish.

2.5 Specific test-environment: Cohabitant shedder density, dose and placement, and addition of bacteria to water

In experiment 1, the ratio of cohabitant shedders to test fish (for number of cohabitants and test fish, see Tables 1 and 2, respectively) was 1:7, whereas in the other three experiments this ratio was approximately 1:3 (Table 1).

Injected doses are given in Table 1. Throughout the experiments, the doses for shedders were reduced from 2.5 x 10^6 to 1.0 x 10^5 (in experiment 2, two doses were used), with the intention to prolong the survival time and thus the time for pathogen dispersion.

In experiment 1, cohabitants were released into a 1 x 1 x 1 m^3 hapa located to the centre of the tank, whereas they were released directly into the tanks in the other experiments (Table 1). Release was done directly after injection.

In experiment 1, dead cohabitants were removed when they were observed lifeless on the bottom of the hapa (Table 1), while in experiment 2 only floating dead fish (note that all dead fish float for some time, before sinking and then floating again) were removed to mimic the practice used by the industry. Additionally, in experiments 3 and 4 dead and floating cohabitants were collected into plastic baskets, which were hung down into the water for another 2 days.

No pathogen was added directly to water in experiments 1 and 2. However, in experiment 3 external pathogen was added to the tanks after complete mortality of cohabitants, which occurred at day 6 post-challenge. The density aimed at was 2.5 x 10^6 bacteria/ml water (Table 1), which was kept for another 8 days. In experiment 4, pathogen addition to water was started earlier, at day 3 post-challenge and stopped at day 6. At day 3, cohabitant mortality was 30%, while it peaked at day 6.

2.6 Test fish

In all four experiments, spawning, incubation and nursing until tagging were done as described by Pham, Ødegård, Sang, Gjøen & Klemetsdal (2020).

Experiment 1: Challenge started April 30, 2009, with year-class 2008

Year-class 2008 was the third generation of the first sub-population in the breeding program. The fish were produced in June and July 2008 by use of a nested mating design, i.e. one male mated to two females. A total of 156 full-sib families were made. These were from 93 sires and 156 dams (Sang, Klemetsdal, Ødegård & Gjøen 2012). Due to high mortality in some families during nursing, only a total of 2,155 fish from 81 families could be included in the challenge test (Table 2). Mean weight of test-fish at the start of the experiment was 47.6 g.

Experiment 2: Challenge started February 23, 2010, with year-class 2009

Year-class 2009 was the third generation of the second sub-population. Using a nested mating design, as before, a total of 196 full-sib families (103 sires and 196 dams) were produced in July and August 2009. As in experiment 1, one hapa per family was used for nursing. High mortality after nursing resulted in only 64 full-sib families available for challenge (Table 2). All families were represented in tank 1 (1,019 fish), whereas only 60 families were represented in tank 2 (969 fish). Mean fish weight at the start of the experiment was 22.6 g in tank 1 and 22.4 g in tank 2.

Experiment 3: Challenge started January 14, 2011, with year-class 2010

Year-class 2010 was again the third generation, with parents mainly from the third sub-population. Families were produced from July to September 2010, by use of a nested mating design. A total of 233 families were made from 137 sires and 230 dams. Parents were mainly from year-class 2007 (88 sires and 151 dams), but parents from the two preceding year-classes were also used; 2005 (second generation of first sub-population: 21 sires and 32 dams) and 2006 (second generation of second sub-population: 12 sires and 18 dams) as well as some parents of wild type (16 sires and 29 dams). Each family was nursed in a separate hapa until tagging. A total of 187 full-sib families took part in the challenge (Table 2). The numbers of

fish were 2,944 and 2,745 in the two replicated tanks, respectively, and mean fish weight at the start of test was 20.4 and 19.5 g.

Experiment 4: Challenge started January 3, 2012, with year-class 2011

Year-class 2011 was the fourth generation of the first sub-population. Fish were produced in June and July 2011 with a nested mating design. Most parents came from year-class 2008 (93 sires and 154 dams), but parents from the other three preceding year-classes were also included: 2005 (3 sires), 2006 (16 sires and 37 dams), 2007 (20 sires and 43 dams) as well as parents of wild type (8 sires, 13 dams). The 250 families (from the 140 sires and 247 dams) produced were raised in a total of 269 hapas, meaning that some families were represented in more than one. Of these, 233 families were utilized in the challenge test (Table 2). A total of 3,246 and 2,931 fish were tested in the two replicated tanks, respectively. Mean fish weight at the start of test was 20.0 and 20.2 g, for tanks 1 and 2, respectively.

2.7 Recoding of data

Collection of dead fish was carried out twice daily, either at 8:00 or 14:00, throughout the test period. PIT-tag (Passive Integrated Transponder) identity was recorded for tested fish as well as weight, day and time of death. Moreover, time of death of cohabitants was recorded.

Mortality was observed for 22 - 23 days in experiments 1 and 2 and for 18 - 19 days in experiments 3 and 4, respectively. In the period with high daily mortality, random samples of dead fish were examined for presence of typical colonies of *E. ictaluri*, by having kidney samples grown on sheep blood agar plates, incubated at 30^0 C for 24 hours (Crumlish, Dung, Turnbull, Ngoc & Ferguson 2002). In experiment 2, with low daily mortality, one awaited it to increase implying that sampling was postponed, and in the end, it was not carried out. The sample sizes were 40, 100 and 100 in experiments 1, 3 and 4, respectively. After the challenge,

survivors were biosecure-buried, following the national veterinary regulations (Department of Animal Health, Vietnam).

2.8 Statistical analysis

In experiments 3 and 4, dead or alive (= 0/1) at the end of the test in the two replicated tanks defined traits 1 and 2, respectively. Bivariate analyses across tanks were performed by a cross-sectional linear sire-dam model.

In matrix notation, the bivariate model can be written:

$$\begin{bmatrix} \boldsymbol{y_1} \\ \boldsymbol{y_2} \end{bmatrix} = \begin{bmatrix} \boldsymbol{X_1} & \boldsymbol{0} \\ \boldsymbol{0} & \boldsymbol{X_2} \end{bmatrix} \begin{bmatrix} \boldsymbol{b_1} \\ \boldsymbol{b_2} \end{bmatrix} + \begin{bmatrix} \boldsymbol{Z_1} & \boldsymbol{0} \\ \boldsymbol{0} & \boldsymbol{Z_2} \end{bmatrix} \begin{bmatrix} \boldsymbol{a_1} \\ \boldsymbol{a_2} \end{bmatrix} + \begin{bmatrix} \boldsymbol{W_1} & \boldsymbol{0} \\ \boldsymbol{0} & \boldsymbol{W_2} \end{bmatrix} \begin{bmatrix} \boldsymbol{c_1} \\ \boldsymbol{c_2} \end{bmatrix} + \begin{bmatrix} \boldsymbol{e_1} \\ \boldsymbol{e_2} \end{bmatrix}$$

where $\boldsymbol{y_1}$ and $\boldsymbol{y_2}$ are vectors of the observed survival to BN in tanks 1 and 2, respectively; the vectors $\boldsymbol{b_i}$ both contain the intercept (b_0), fixed regression coefficient on number of days from spawning until tagging (b_1) and the fixed regression coefficient on number of days from tagging to start of experiment (b_2); $\boldsymbol{a_i}$ is a vector that for each trait contains the random additive genetic effect of sire l (s_l), and the random additive genetic effect of dam m (d_m); $\boldsymbol{c_i}$ is a vector that for a trait contains the random common environmental effect of full-sib family lm (c_{lm}), and $\boldsymbol{e_i}$ is a residual vector for a trait with the random error term for fish n (e_{lmn}) ~ N (0, $\mathbf{I}\sigma^2_e$), with σ^2_e being the error variance. This assumes that the residual covariances were zero which is appropriate since a fish could only be represented in one tank. The design matrices for $\boldsymbol{b_i}$, $\boldsymbol{a_i}$ and $\boldsymbol{c_i}$ are denoted $\boldsymbol{X_i}$, $\boldsymbol{Z_i}$ and $\boldsymbol{W_i}$, respectively. The $\boldsymbol{a_i}$, $\boldsymbol{c_i}$ and $\boldsymbol{e_i}$ effects were distributed as follows:

$$\begin{bmatrix} \boldsymbol{a_1} \\ \boldsymbol{a_2} \end{bmatrix} \sim \boldsymbol{N}\left(\boldsymbol{0}, \boldsymbol{A} \otimes \begin{bmatrix} \sigma^2_{sd_1} & \sigma_{sd_1,sd_2} \\ \sigma_{sd_1,sd_2} & \sigma^2_{sd_2} \end{bmatrix}\right),$$

$$\begin{bmatrix} \boldsymbol{c_1} \\ \boldsymbol{c_2} \end{bmatrix} \sim \boldsymbol{N}\left(\boldsymbol{0}, \boldsymbol{I} \otimes \begin{bmatrix} \sigma^2_{c_1} & \sigma_{c_1,c_2} \\ \sigma_{c_1,c_2} & \sigma^2_{c_2} \end{bmatrix}\right), \text{ and}$$

$$\begin{bmatrix} \boldsymbol{e_1} \\ \boldsymbol{e_2} \end{bmatrix} \sim \boldsymbol{N}\left(\boldsymbol{0}, \begin{bmatrix} \boldsymbol{I}\sigma^2{}_{e_1} & \boldsymbol{0} \\ \boldsymbol{0} & \boldsymbol{I}\sigma^2{}_{e_2} \end{bmatrix}\right),$$

where $\boldsymbol{A}$ is the additive genetic relationship matrix, and $\boldsymbol{I}$ are an identity matrices of appropriate sizes, while $\begin{bmatrix} \sigma^2{}_{sd_1} & \sigma_{sd_1,sd_2} \\ \sigma_{sd_1,sd_2} & \sigma^2{}_{sd_2} \end{bmatrix}$ and $\begin{bmatrix} \sigma^2{}_{c_1} & \sigma_{c_1,c_2} \\ \sigma_{c_1,c_2} & \sigma^2{}_{c_2} \end{bmatrix}$ are the sire-dam and common environmental (co)variance matrices, respectively, and $\sigma^2{}_{e_1}$ and $\sigma^2{}_{e_2}$ are the residual variances of the two traits. The relationships were traced back to the base. In experiments 3 and 4, $\boldsymbol{A}$ contained 6239 and 6896 animals, respectively.

The estimated heritability for each trait was calculated as:

$$h^2 = \frac{4\sigma^2{}_{sd}}{2\sigma^2_{sd} + \sigma^2_c + \sigma^2_e}$$

Additive genetic ($r_{a1,a2}$) and common environmental correlations ($r_{c1,c2}$) between traits were estimated as:

$$r_{a1,a2} = \frac{\sigma_{sd1,sd2}}{\sigma_{sd1}\sigma_{sd2}}$$

and

$$r_{c1,c2} = \frac{\sigma_{c1,c2}}{\sigma_{c1}\sigma_{c2}}$$

The ASREML software (Gilmour, Gogel, Cullis, Welham & Thompson 2015) was used to estimate (co)variance components and genetic parameters.

3. Results

In experiment 1, the overall cumulative mortality of test fish approached 25% (Figure 1). First mortality of cohabitants was recorded 2 days post-challenge and reached 100% on day 3 (Table

244 3). First mortality of test-fish was recorded on day 8, while daily mortality peaked at day 11, at
245 7.6% dead/day, and remained low until the end of challenge (Figure 2).

246 In experiment 2, cumulative mortality was only 3.0% and 5.7% for tanks 1 and 2, respectively
247 (Figure 1). In both tanks, mortality among cohabitants started 2 days post-challenge and reached
248 100% on day 8 (Table 3). In tanks 1 and 2, first mortality of fish was at days 11 and 9,
249 respectively, while daily peak mortality was reached on day 12, in both tanks (0.7% and 1.6%,
250 respectively) (Figure 2).

251 In experiment 3, cumulative mortality was considerable in both tanks: 84.0% and 83.1%,
252 respectively (Figure 1). Cohabitants in both tanks started to die 2 days post-challenge, and all
253 were dead (both tanks) on day 6 (Table 3). In both tanks, first mortality of fish was at day 3 but
254 with a much-delayed development of daily mortality in tank 2 compared to tank 1 (Figure 2).

255 In the fourth experiment, cumulative mortality was 87.1% and 87.7% in tanks 1 and 2,
256 respectively (Figure 1). In both tanks, first mortality of cohabitants occurred at day 2 and
257 reached 100% on day 8 (Table 3). Moreover, in both tanks, fish started to die at day 6, with
258 similar daily mortality patterns, peaking at days 12 and 11, respectively (Figure 2).

259 Test-fish that died during the challenge showed typical signs of BN; bugged-eyes, less reactive
260 to sound, and with jumping and spinning before death. Internal clinical signs were numerous
261 white spots of different sizes (0.3 - 3 mm) in swelled liver, kidney and spleen. The white spots
262 appeared primarily on the kidney, making them swollen, but could also in critical cases be found
263 in liver and spleen. In all samples, *E. ictaluri* was identified.

264 Estimated variance components and heritabilities of the traits (tank) as well as additive genetic
265 and common environmental correlations between the traits (tank), using the cross-sectional
266 linear sire-dam model, are presented in Table 4. In experiment 3, heritabilities were similar in

the two tanks (0.09 ± 0.05 and 0.08 ± 0.04, respectively), and the genetic correlation between tanks was close to unity (fixed at boundary). Moreover, the estimated correlation between common environmental effects in the two tanks was negative (-0.29), with a large standard error (0.78). In experiment 4, the estimated heritabilities were also low (0.08 ± 0.04 for tank 1 and 0.04 ± 0.03 for tank 2, Table 4). Again, the estimated additive genetic correlation was high (0.95 ± 0.19), while the common environmental correlation was fixed at boundary (0.99).

4. Discussion

In challenge testing of fish, a natural reference is a mortality of 50% because it maximizes the phenotypic variance for a binary trait (Gjøen, Refstie, Ulla & Gjerde 1997). This reasoning is as follows: If the infection load becomes too low/massive, all the fish will eventually live/die, and any phenotypic variance will not exist when analysing endpoint data (at least as dead/alive). More recently, Ødegård, Baranski, Gjerde & Gjedrem (2011) have argued that mortality should naturally cease in the endpoint because surviving fish then are more likely to be non-susceptible to the disease. Establishing such a testing practice will have implications not only for genetic analysis, but also when examining effects of treatments, for example effects of vaccines (Drangsholt, Gjerde, Ødegård, Finne-Fridell, Evensen & Bentsen 2012; Fredriksen, Olsen, Furevik, Souhoka, Gauthier & Brudeseth 2013) or effects of feed ingredients/feed additives (Ward, Bengtson, Lee & Gomez-Chiarri 2016).

In the first experiment, mortality was only 25%. To increase mortality in the second experiment, several of the environmental factors were made more extreme: Acclimatization shortened, temperature more stressful, water level reduced, direct contact between the test fish and the cohabitants, a larger fraction of cohabitants per test fish, and cohabitant shedders expected to contaminate over a longer time-period (half the cohabitants given a reduced dose) (Table 1).

However, the temperature was first changed from 29° C to 26° C, at day 10 of the experiment (Table 1), when realizing that mortality would be low, but this change probably occurred too late to have any effect since the cohabitants had already died out at day 8 (Table 3). The experience in the Mekong Delta is that outbreak of BN is highly affected by the temperature, since it is mostly found in the winter when water temperature is low and fluctuating. Moreover, in channel catfish, a temperature range of 22 - 28° C has been found to stimulate outbreaks of ESC, a disease also caused by *E. ictaluri* (Hawke, Durborow, Thune & Camus 1998; Patrie-Hanson & Jerald Ainsworth 1999; Thune, Fernandez, Benoit, Kelly-Smith, Rogge, Booth, Landry & Bologna 2007). Mortality in cohabitation challenge with *Aeromonas salmonicida* in Atlantic salmon can also to a large degree be adjusted by varying the water temperature (Nordmo & Ramstad 1999) and led us to choose a temperature of 26° C in the challenge test with Mekong striped catfish. Changes of the remaining environmental factors in experiment 2 were, however, decided *a priori* to carrying out the experiment. It was assumed that a shortened acclimatisation time, i.e., the time from arriving GEC (test station) to the release of the cohabitants (day 0), would pose stress to the fish. The same was assumed for halving the water level, despite Mekong striped catfish being an air breather. Regarding cohabitants, they were released directly into the test tanks with direct exposure of cohabitants to test fish, expected to increase the infectious pressure. So was the increased density of cohabitants (from 1:7 to 1:3) and the reduced dose applied for half the number of cohabitants, expected to increase the time for pathogen dispersion because those given the lowest dose should live longer. Despite all these environmental factors being more extreme in experiment 2 than in experiment 1, still one factor was less extreme, the earlier removal of the dead fish, and mortality became very low (< 5.7%).

The results of experiments 1 and 2 proposed that the practise around removal of the dead cohabitants affected mortality, meaning that the infectious load of the water could be instrumental to regulation of the disease frequency. Thus, in experiment 3 the load was increased by adding bacteria directly to water (from day 6 to day 13) but also from collecting dead cohabitants into plastic baskets that were hung down into the water for another 2 days. Additionally, smaller modifications were done to the experimental factors applied in experiment 2; the acclimatisation time was shortened from 14 to 3 days, and the lowest dose for the cohabitants was kept (10^5 bacteria). Now, the mortality increased considerably relative to the first two experiments, reaching 83 - 84% in the two test tanks at termination.

In order to reduce the high disease frequency obtained in experiment 3, addition of bacteria to water was done earlier in experiment 4 and for a shorter time (days 3 to 6), otherwise for the same environmental factors as in experiment 3. Despite the change, mortality was still high and approached 87% in the two test tanks at termination. However, mortality curves were more similar in experiment 4 than in experiment 3 suggesting that the bacteria need to be added to the water already in the initial phase of the challenge test.

An experiment 5 was reported by Vu, Sang, Trong, Duy, Dang & Nguyen (2019), also in replicated tanks with fish of size ~ 20 g. To reduce the pathogen load of experiments 3 and 4, the factor involving the dead cohabitants was removed from the experiment. Relative to the first four experiments, one awaited 2 days from cohabitant injection to release to the test tanks (released at day 0 in experiments 1 - 4). The bacterium used was now the most virulent (determined by lethal dose 50, LD_{50}, i.e. the dose needed for 50% of fish to die) of four serotypes (found by use of biochemical tests and polymerase chain reaction, PCR), as tested prior to the experiment. Cohabitant dose was 10^6 bacteria, and bacteria were added to water only once, 4 days after cohabitant injection (10^5 bacteria/ml). Otherwise the same

environmental factors were as established; acclimatisation time of 4 days, water temperature 26° C, restricted water level (2/3) and cohabitant density 1:3, with direct release into the test tanks. The reported average mortality across the two test tanks in experiment 5 became 39%, close to the desired frequency of 50%. This disease frequency is considered highly acceptable because the phenotypic variance is not much affected by frequencies deviating slightly from 50% (= 2*pq*, where *p* and *q* are the frequencies of dead and alive, respectively). In a longer time perspective, however, one should take into account that resistance due to selection will increase over time, meaning that a higher BN dose will likely be required to obtain the desired frequency.

A linear cross-sectional sire-dam model was applied to the data in experiment 3 and 4, and the estimates of the heritability were limited since they are frequency dependent (Gianola & Foulley 1983). Moreover, the estimated genetic correlations were consistently high, while the common environmental correlations were imprecisely estimated. The precision of the estimate is highly affected by the many parameters determined and from the nested mating design, known to weakly separate the common environmental and additive genetic effects (Berg & Henryon 1998). Genetic analyses revealed that resistance to bacillary necrosis tested in replicated tanks (in the same experiment) can be considered the same genetic trait.

In these experiments, no outbreak of disease was observed during the nursing period. However, note that the water supply during the hapa period was from the Mekong river, such that the fish might have been exposed to the pathogen *a priori* to the challenge.

5. Conclusions

The four challenge tests of Mekong striped catfish against *E. ictaluri* propose three days acclimatization of test fish prior to the challenge, with restricted water level in the test, keeping a temperature of 26^0 C. In the challenge, cohabitant shedders should be released directly into

the test tank and make up around ⅓ of the fish, and bacteria should be added directly to water. The last two experiments, with the highest mortality, suggest that any factor involving the dead cohabitants should be removed, and that additional experimentation should focus on bacteria (density) and timing for addition of bacteria to water. Genetic analyses revealed that resistance to bacillary necrosis tested in replicated tanks (in the same experiment) can be considered the same genetic trait.

Conflict of interest statement

There is no conflict of interest.

References

Berg, P. & Henryon, M. (1998) A comparison of mating designs for inference on genetic parameters in fish. *Proc. 6th WCGALP,* **27,** 115-118.

Camp, K.L., Wolters, W.R. & Rice, C.D. (2000) Survivability and immune responses after challenge with *Edwardsiella ictaluri* in susceptible and resistant families of channel catfish, *Ictalurus punctatus*. *Fish & Shellfish Immunology,* **10,** 475-487.

Chuah, L., Effarizah, M.E., Goni, A.M. & Rusul, G. (2016) Antibiotic application and emergence of multiple antibiotic resistance (MAR) in global catfish aquaculture. *Current Environmental Health Reports,* **3,** 118-127.

Crumlish, M., Dung, T.T., Turnbull, J.F., Ngoc, N.T.N. & Ferguson, H.W. (2002) Identification of *Edwardsiella ictaluri* from diseased freshwater catfish, *Pangasius hypophthalmus* (Sauvage), cultured in the Mekong Delta, Vietnam. *Journal of Fish Diseases,* **25,** 733-736.

Dong, H.T. & Hoa, T.D. (2008) *Identification the causative agent of "white patches in the internal organs disease" of Tra catfish in Ben Tre province. Retrieved from https://www.researchgate.net/publication/280932124_Identification_the_causative_agent_of_visceral_white_spot_disease_of_Tra_catfish_Pangasianodon_hypophthalmus*.

Drangsholt, T.M.K., Gjerde, B., Ødegård, J., Finne-Fridell, F., Evensen, Ø. & Bentsen, H.B. (2012) Genetic correlations between disease resistance, vaccine-induced side effects and harvest body weight in Atlantic salmon (*Salmo salar*). *Aquaculture,* **324–325,** 312-314.

Fredriksen, B.N., Olsen, R.H., Furevik, A., Souhoka, R.A., Gauthier, D. & Brudeseth, B. (2013) Efficacy of a divalent and a multivalent water-in-oil formulated vaccine against a highly virulent strain of *Flavobacterium psychrophilum* after intramuscular challenge of rainbow trout (*Oncorhynchus mykiss*). *Vaccine,* **31,** 1994-1998.

Gianola, D. & Foulley, J.L. (1983) Sire evaluation for ordered categorical data with a threshold model. *Genetics, Selection, Evolution,* **15,** 201-224.

Gilmour, A.R., Gogel, B.J., Cullis, B.R., Welham, S.J. & Thompson, R. (2015) ASReml User Guide Release 4.1 Structural Specification. Retrived from https://www.hpc.iastate.edu/sites/default/files/uploads/ASREML/UserGuideStructural.pdf.

Gjøen, H.M., Refstie, T., Ulla, O. & Gjerde, B. (1997) Genetic correlations between survival of Atlantic salmon in challenge and field tests. *Aquaculture,* **158,** 277-288.

Hawke, J.P., Durborow, R.M., Thune, R.L. & Camus, A.C. (1998) ESC- Enteric Septicemia of Catfish. *Southern Regional Aquaculture Center, SRAC Publication No. 477. Retrieved from https://appliedecology.cals.ncsu.edu/wp-content/uploads/477.pdf*.

Lefevre, S., Jensen, F.B., Huong, D.T.T., Wang, T., Phuong, N.T. & Bayley, M. (2011) Effects of nitrite exposure on functional haemoglobin levels, bimodal respiration, and swimming performance in the facultative air-breathing fish *Pangasianodon hypophthalmus*. *Aquatic Toxicology,* **104,** 86-93.

Lim, C. & Klesius, P.H. (1997) Responses of channel catfish (*Ictaluris punctatus*) fed iron-deficient and replete diets to *Edwardsiella ictaluri* challenge. *Aquaculture,* **157,** 83-93.

Mahapatra, K.D., Gjerde, B., Sahoo, P.K., Saha, J.N., Barat, A., Sahoo, M., Mohanty, B.R., Ødegård, J., Rye, M. & Salte, R. (2008) Genetic variations in survival of rohu carp (*Labeo rohita*, Hamilton) after *Aeromonas hydrophila* infection in challenge tests. *Aquaculture,* **279,** 29-34.

Nordmo, R. & Ramstad, A. (1999) Variables affecting the challenge pressure of *Aeromonas salmonicida* and *Vibrio salmonicida* in Atlantic salmon (*Salmo salar* L.). *Aquaculture,* **171,** 1-12.

Nordmo, R., Sevatdal, S. & Ramstad, A. (1997) Experimental infection with *Vibrio salmonicida* in Atlantic salmon (*Salmo salar* L.): an evaluation of three different challenge methods. *Aquaculture,* **158,** 23-32.

Patrie-Hanson, L. & Jerald Ainsworth, A. (1999) Humoral immune responses of channel catfish (*Ictalurus punctatus*) fry and fingerlings exposed to *Edwardsiella ictaluri*. *Fish & Shellfish Immunology,* **9,** 579-589.

Pham, K.D., Ødegård, J., Sang, V.N., Gjøen, H.M. & Klemetsdal, G. (2020) Genetic analysis of resistance in Mekong striped catfish (*Pangasianodon hypophthalmus*) to bacillary necrosis caused by *Edwardsiella ictaluri Journal of Fish Diseases, in press.*

Sang, N.V., Klemetsdal, G., Ødegård, J. & Gjøen, H.M. (2012) Genetic parameters of economically important traits recorded at a given age in striped catfish (*Pangasianodon hypophthalmus*). *Aquaculture,* **344–349,** 82-89.

Thanh, P.C. & Berntsen, J.O. (2012) Towards sustainability: Vaccinated *pangasius* in Vietnam. *AQUA Culture Asia Pacific Magazine,* **May/June,** 36-37.

Thune, R.L., Fernandez, D.H., Benoit, J.L., Kelly-Smith, M., Rogge, M.L., Booth, N.J., Landry, C.A. & Bologna, R.A. (2007) Signature-tagged mutagenesis of *Edwardsiella ictaluri* identifies virulence-related genes, including a Salmonella pathogenicity island 2 class of type III secretion systems. *Applied and Environmental Microbiology,* **73,** 7934-7946.

Van Muiswinkel, W.B., Wiegertjes, G.F. & Stet, R.J.M. (1999) The influence of environmental and genetic factors on the disease resistance of fish. *Aquaculture,* **172,** 103-110.

Vu, N.T., Sang, N.V., Phuc, T.H., Vuong, N.T. & Nguyen, N.H. (2019) Genetic evaluation of a 15-year selection program for high growth in striped catfish *Pangasianodon hypophthalmus*. *Aquaculture,* **509,** 221-226.

Vu, N.T., Sang, N.V., Trong, T.Q., Duy, N.H., Dang, N.T. & Nguyen, N.H. (2019) Breeding for improved resistance to *Edwardsiella ictaluri* in striped catfish (*Pangasianodon hypophthalmus*): Quantitative genetic parameters. *Journal of Fish Diseases,* **42,** 1409-1417.

Ward, D., Bengtson, D.A., Lee, C.M. & Gomez-Chiarri, M. (2016) Incorporation of soybean products in summer flounder (*Paralichthys dentatus*) feeds: Effects on growth and survival to bacterial challenge. *Aquaculture,* **452,** 395-401.

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.

Table 1. Environmental factors varied across the four challenge-test experiments (Exp) with Mekong striped catfish.

Factor	Exp1	Exp2	Exp3	Exp4
Year	2009	2010	2011	2012
Test-tank location	Outdoor at NABRECSOFA	Indoor at GEC	Indoor at GEC	Indoor at GEC
No. of replicated tanks	1	2	2	2
No. of days of acclimatization prior to experiment	22	14	3	3
Water temperature, °C	29.5	29 (the first 10 days post-cohabitation) and 26 (until termination)	26	26
Reduction of water level	No	Halved 1 day prior to test	Halved 1 day prior to test	Halved 1 day prior to test
No. of cohabitants	323	340 in tank 1 320 in tank 2	1,000 in tank 1 1,000 in tank 2	1,280 in tank 1 1,240 in tank 2
Ratio of cohabitant: test fish	~1:7	~1:3	~1:3	~1:3
No. of bacteria in cohabitant-dose	2.5×10^6	2.5×10^6 for half the cohabitants, and 2.5×10^5 for the rest	1×10^5	1×10^5
Placement of cohabitants	In hapa, located central in tank	Direct into tank	Direct into tank	Direct into tank
Dead cohabitant removal	When dead and sunk down to bottom of hapa	When dead and floating	Dead and floating fish collected into plastic baskets hung down into water for another 2 days	Dead and floating fish collected into plastic baskets hung down into water for another 2 days
Addition of pathogen to water	No	No	Post-exposure to cohabitants: From day 6 to day 13, to reach a density of 2.5×10^6 bacteria/ml	Post exposure to cohabitants: From day 3 to day 6, to reach a density of 2.5×10^6 bacteria/ml

Table 2. Numbers of families and fish per tank in the four challenge-test experiments (Exp) with *E. ictaluri* in Mekong striped catfish: Experiment 1 had one tank (t1), while the other experiments had two replicated tanks.

No. of families and fish	Tank	Exp1	Exp2	Exp3	Exp4
No. of families	t1	81	64	187	233
	t2	-	60	187	233
No. of fish	t1	2,155	1,019	2,944	3,246
	t2	-	969	2,745	2,931

Table 3. Number of days from injection (experimental day 0) to first and complete death of cohabitants in four challenge-test experiments with *E. ictaluri* in Mekong striped catfish: Experiment (Exp) 1 had one tank (t1), while the other experiments had two replicated tanks.

	No. of days to	
Experiment	First mortality of cohabitants	Complete mortality of cohabitants
Exp1-t1	2	3
Exp2-t1	2	8
Exp2-t2	2	8
Exp3-t1	2	6
Exp3-t2	2	6
Exp4-t1	2	8
Exp4-t2	2	8

Table 4. Estimates (linear model) of genetic sire-dam variance (σ^2_{sd}), common environmental variance (σ^2_c), heritability (h^2), and additive ($r_{a1,a2}$) and common environmental correlations ($r_{c1,c2}$) between survival (dead = 0/alive = 1) in the endpoint of two replicated tanks, defining traits 1 and 2, in two experiments (Exp) of Mekong striped catfish with *E. ictaluri*.

	Trait	Exp3	Exp4
σ^2_{sd}	Trait 1	0.0030	0.0020
	Trait 2	0.0029	0.0010
σ^2_c	Trait 1	0.0048	0.0037
	Trait 2	0.0020	0.0020
h^2[†)] ± SE	Trait 1	0.09 ± 0.05	0.08 ± 0.04
	Trait 2	0.08 ± 0.04	0.04 ± 0.03
$r_{a1,a2} \pm$ SE		0.99[‡)]	0.95 ± 0.19
$r_{c1,c2} \pm$ SE		-0.29 ± 0.78	0.99[‡)]

[†)] $h^2 = \frac{4\sigma^2_{sd}}{2\sigma^2_{sd}+\sigma^2_c+\sigma^2_e}$.

[‡)] Fixed at boundary.

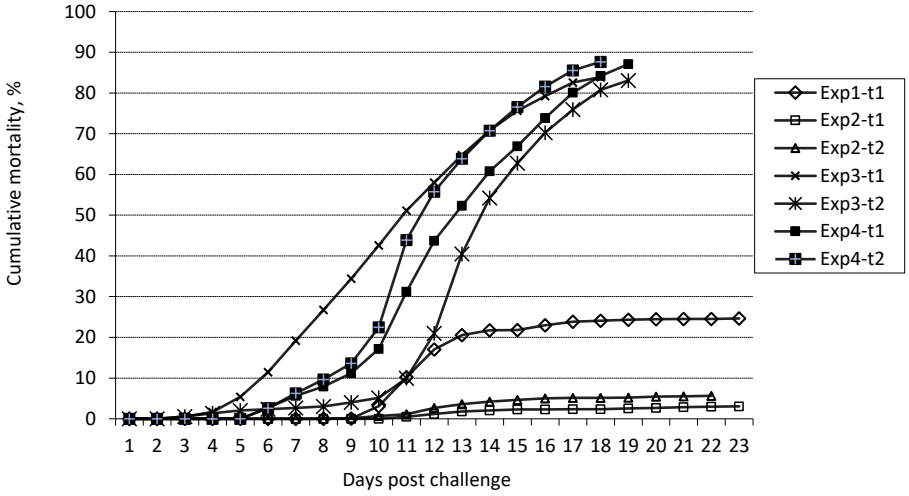

Figure 1. Cumulative mortality observed in four challenge-test experiments with *E. ictaluri* in Mekong striped catfish: Experiment (Exp) 1 had one tank (t1), while the other experiments had two replicated tanks.

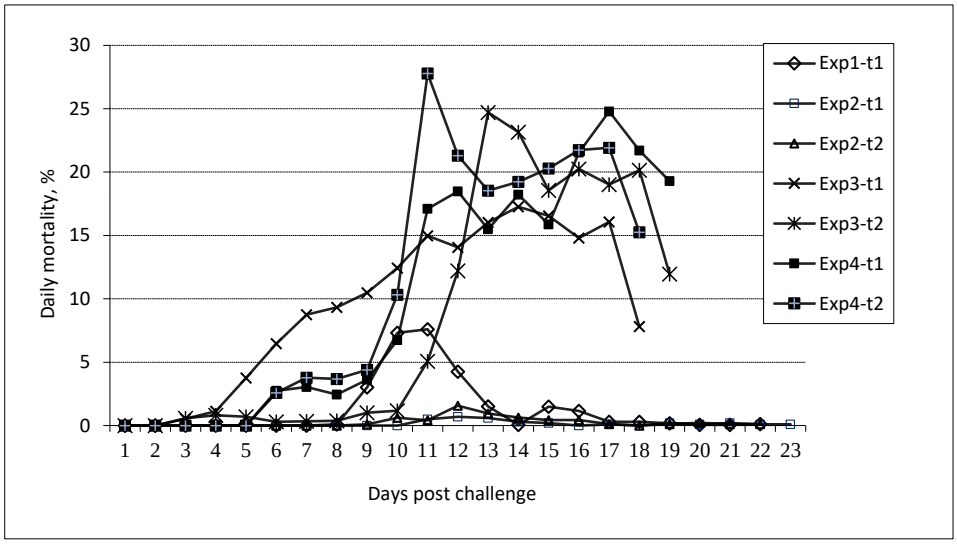

Figure 2. Daily mortality in four challenge-test experiments with *E. ictaluri* in Mekong striped catfish: Experiment (Exp) 1 had one tank (t1), while the other experiments had two replicated tanks.

PAPER II

Pham KD, Ødegård J, Nguyen SV, Gjøen HM, Klemetsdal G. (2020)

Genetic analysis of resistance in Mekong striped catfish (*Pangasianodon hypophthalmus*) to bacillary necrosis caused by *Edwardsiella ictaluri*.

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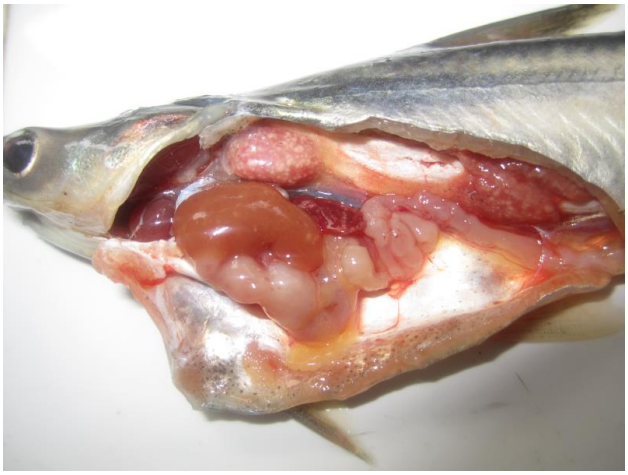

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Paper II

Genetic analysis of resistance in Mekong striped catfish (*Pangasianodon hypophthalmus*) to bacillary necrosis caused by *Edwardsiella ictaluri*

Running title: Genetic analysis of challenge-test data

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Data Availability Statement: The data that support the finding of this study can be made available on request, by the corresponding author.

Abstract

The aim of this study was to analyse four cohabitation challenge test experiments with Mekong striped catfish (*Pangasianodon hypophthalmus*) against the bacterium *Edwardsiella ictaluri*. The data was genetically analysed per experiment by three models; 1) a cross-sectional linear model, 2) a cross-sectional threshold model, and 3) a linear survival model; at both 50% mortality (for models 1 and 2) and at the end of the test (for all three models). In two of the experiments (3 and 4) that were carried out in two replicated tanks, the predicted family effects (sum of sire, dam and common environmental effects) in each tank were correlated to the family survival in the other replicated tank (cross-validation). The heritability estimates of resistance to *E. ictaluri* infection were ≤ 0.012 with the survival model, up to 0.135 - 0.220 (50% survival) and 0.085 and 0.174 (endpoint survival) for the cross-sectional linear and threshold models, respectively. The challenge test should aim for an endpoint survival that ceases naturally at 50%. Then, genetic analysis should be carried out for survival at the endpoint (reflecting susceptibility) with a simple cross-sectional linear model.

Keywords: Challenge test; Cross-sectional model; Heritability; Survival model

1. Introduction

In Vietnam, bacillary necrosis (BN) caused by the bacterium *Edwardsiella ictaluri* (*E. ictaluri*) has become a severe problem in farming of Mekong striped catfish (*Pangasianodon hypophthalmus*). Therefore, the first BN vaccine trial was conducted by Pharmaq Ltd. Vietnam in 2010 (Thanh & Berntsen 2012). The trial was successful with regard to significant lower mortality of vaccinated than non-vaccinated groups, observed in the field for 31 days. The Alpha Ject® Panga 1 vaccine was licensed in 2013 (https://www.pharmaq.no/sfiles/8/66/4/file/2013_08-cty-pharmaq-vn_thuy-

san-nam-14-so-164.pdf). However, improved resistance to BN using vaccination is costly and laborious since it must be administered to every fish produced. Therefore, the main method for treating BN at current is the use of antibiotics, but combating disease outbreaks by drug application is costly and a major concern to the environment and the consumer, and not a long-term solution to the problem (van Muiswinkel, Wiegertjes & Stet 1999). However, with fish, selective breeding for disease resistance has been proven to be an efficient strategy to prevent infectious diseases (Guy, Bishop, Woolliams & Brotherstone 2009; Lhorente, Gallardo, Villanueva, Araya, Torrealba, Toledo & Neira 2012; Taylor, Wynne, Kube & Elliott 2007). Controlled challenge testing is a widely used method for testing genetic variation in resistance (e.g., Gjedrem & Gjøen 1995). Then, individuals are typically infected by e.g. cohabitants, i.e., fish that have been injected with the causative agent (e.g., Gjøen, Refstie, Ulla & Gjerde 1997). Controlled challenge testing has been widely applied in Atlantic salmon (*Salmo salar*) breeding, demonstrating that resistance to diseases show substantial genetic variance and heritability (e.g., Gjerde, Boison, Aslam, Løvoll, Bakke, Rey & Lillehammer 2019; Yáñez, Lhorente, Bassini, Oyarzún, Neira & Newman 2014). The challenge method has also been applied in many other fish species, like common carp (*Cyprinus carpio*, Ødegård, Olesen, Dixon, Jeney, Nielsen, Way, Joiner, Jeney, Ardó, Rónyai & Gjerde 2010), Atlantic cod (*Gadus morhua L.*, Kettunen & Fjalestad 2006; Ødegård, Sommer & Præbel 2010), rainbow trout (*Oncorhynchus mykiss*, Bassini, Lhorente, Oyarzún, Bangera, Yáñez & Neira 2019), European sea bass (*Dicentrarchus labrax*, Doan Q., Vandeputte, Chatain, Haffray, Vergnet, Breuil & Allal 2017), coho salmon (*Oncorhynchus kisutch*, Barría, Doeschl-Wilson, Lhorente, Houston & Yáñez 2019), red tilapia (*Oreochromis spp.*, Sukhavachana, Poompuang, Onming & Luengnaruemitchai 2019), bighead catfish (*Clarias macrocephalus*, Srisapoome, Chatchaiphan, Bunnoy, Koonawootrittriron & Na-Nakorn 2019), Chinese tongue sole (*Cynoglossus semilaevis*,

Li, Wang, Yang, Li, Dai & Chen 2019), and Mekong striped catfish (*Pangasianodon hypophthalmus*, Vu, Sang, Trong, Duy, Dang & Nguyen 2019). The existence of genetic variation for a trait opens the possibility to improve the trait through selective breeding.

Selection of Mekong striped catfish in Vietnam has in main been carried out for increased growth rate (Vu, Sang, Phuc, Vuong & Nguyen 2019), and the authors calculated a selection response per generation of 9.3%. Moreover, Vu, Sang, Trong, Duy, Dang & Nguyen (2019) estimated genetic variance for resistance to BN across four challenge-test experiments carried out in 2010, 2011, 2012 and 2015. They assumed BN to be the same trait across experiments and found the heritability for dead/alive at maximum 29 days post-challenge to be 0.10 and 0.16, with a linear and threshold model, respectively. Here, the main objective was to analyse, experiment wise, three of the same experiments (2010 – 2012), but also an experiment carried out in 2009. In the genetic analyses, two different trait definitions were used; time until death or dead/alive, the latter measured at both 50% overall mortality and at end of the test, to assess genetic variance of disease resistance. The analysis was carried out by three genetic evaluation models; two cross-sectional models utilizing data either at the endpoint or at 50% mortality, and a survival model utilizing time until death. We evaluated these models by predicting the family survival (sum of sire, dam and common environmental effects) in one tank and correlated it to the family survival in another tank.

2. Materials and methods

By granting the research, the Vietnamese Ministry of Agriculture and Rural Development pre-approved the experiments carried out.

2.1 Data

The fish used in the four experiments were from different year-classes and sub-populations of the breeding program for Mekong striped catfish in Vietnam, illustrated in Figure 1. This study involved the year-classes produced in 2008, 2009, 2010, and 2011, being, respectively, the third generation of subpopulation 2001 (G3-2001), while the others were G3-2002, G3-2003 and G4-2001. In all experiments, the test-fish were the offspring from a nested mating design (one male mated to two females). In 2008, 2009, 2010, and 2011 families were produced from June 16^{th} - July 14^{th}, July 15^{th} - Aug 10^{th}, July 29^{th} - September 10^{th}, and from June 9^{th} - July 7^{th}, respectively. Spawning was done by hormone treatment (HCG - Human Chorionic Gonadotropin). First, males were stripped, milt was stored at 4^0 C, later it was split in two, mixed with samples of eggs from two females, before water was added for fertilization. The fertilized eggs were washed to remove sticky layers by use of tannic acid, and eggs from one female were moved to a family air-supplied net-jar in one cement tank for hatching, occurring from 18 - 24 hours after fertilization. The total number of families produced in 2008, 2009, 2010 and 2011 were 156, 196, 233, and 250.

Twenty-four hours post hatching, approximately 3,000 start-fed fry were randomly sampled from each family and reared in a 1 m^3 family-fiberglass tank for about 20 days. The tanks were air supplied, and about half the water was exchanged every three days. Fry were in sequence fed *ad libitum* with newly hatched Artemia sp., Moina sp. and bloodworm (*Limnodrilus hoffmeisteri*). After the 20 days, a random sample of about 300 small fingerlings from each full-sib family was moved to a family hapa located in an earthen pond. Here, fish were initially fed *ad libitum* by bloodworm, but within a week their diet was standard commercial pellet feed (V2-Feed, RIA2 – Research Institute for Aquaculture No. 2, Ho Chi Minh City, < 2.0 mm, 22 - 28% protein). Cleaning of hapas were done frequently. In 2011, 15 families were nursed in two replicated hapas.

Tagging was done when the fish were randomly netted out, hapa by hapa. Passive Integrated Transponder tags (PIT tags, Sokymat, Switzerland) were inserted from April 8th - 10th 2009 (year-class 2008 and experiment 1), January 13th - 25th, 2010 (year-class 2009 and experiment 2), December 16th, 2010, to January 9th, 2011 (year-class 2010 and experiment 3) and from December 15th - 21st, 2011 (year-class 2011 and experiment 4). Fish to be challenge tested were transferred to either of two tanks (in experiment 1, one tank was used) at the National Breeding Centre for Southern Freshwater Aquaculture (NABRECSOFA), before being transported to the Govap Experimental Center (Ho Chi Minh City), RIA2, for challenge testing. In experiment 1, the challenge test was carried out at NABRECSOFA.

The number of test-tanks in experiment 1 was one (20 m^3 each), as mentioned, whereas two were used in the last three experiments (Figure 1). Table 1 shows the number of families and test-fish in each tank of the four experiments, the latter making up a total of 2,155 (mean weight 48.8 g), 1,988 (mean weight 23.6 g), 5,689 (mean weight 20.0 g) and 6,177 (mean weight 20.0 g) fish, respectively

The cohabitation method was applied in four challenge-test experiments, started April 30th 2009, February 23rd 2010, January 14th 2011 and January 3rd 2012, respectively. Fish were transferred to the test units 22, 14, 3 and 3 days prior to challenge. Water temperature was 29.5^0 C, 29^0 C (26^0 C from day 11 until termination), 26° C and 26° C during the challenge, respectively. To get a sufficient response to the challenge, the fish in the last three experiments were stressed by halving the water level in the test tanks from one day prior to the test. The ratio of the number of cohabitants shedders to the number of test fish was ~1:7 in the first experiment and ~1:3 later. Cohabitants were infected by intraperitoneally injection (doses were: 2.5 x 10^6, 2.5 x 10^6 for half the cohabitants, and 2.5 x 10^5 for the rest, and 1 x 10^5 bacteria in the last two experiments) and released

directly into the test tanks (in the first experiment, cohabitants were located to a hapa, central in the tank). The bacteria were from a strain of *E. ictaluri* Gly09M (Southern Monitoring Center for Aquaculture Environment & Epidemic, RIA2, Ho Chi Minh City, Vietnam). In the first two experiments, dead cohabitants were removed (when sunk and floating, respectively), while in the last two experiments dead and floating cohabitants were collected into plastic baskets that were hung down into the water for another two days before removal. In experiment 3, external pathogen was added to the test tanks to reach a density of 2.5 x 10^6 bacteria/ml water, from day 6 post-challenge when the death of cohabitants had reached peak. This practice was continued for another 8 days. In experiment 4, addition of pathogen was started at day 3 post-challenge, and stopped at day 6, after the cohabitant mortality had reached peak. Throughout the tests, fish were daily fed with standard commercial pellets at a rate of 1% of total biomass. Random samples of dead fish were examined for presence of *E. ictaluri*, as typical colonies (Crumlish, Dung, Turnbull, Ngoc & Ferguson 2002). Kidney samples were grown on sheep blood agar plates and incubated at 30^0 C for 24 hours. In 100% of the samples, *E. ictaluri* was identified. Alive fish were biosecure-buried, following the national veterinary regulations (Department of Animal Health, Vietnam).

2.2 Statistical analyses

Initially, experiment- and tank-specific (Kaplan-Meier) survival curves were calculated. In addition, for each family survival (number of survivors to number of test fish at the start of the experiment) at the end of the tests were obtained for each tank. Because of the low mortality in experiment 2, the genetic analyses in this study had to be based on the three remaining experiments. Genetic analyses of these experiments were carried out with three different sire - dam models per experiment. In experiments 3 and 4, with replicated tanks, the models were validated by correlating

the predicted family effects in one tank to the corresponding observed family survival in the other tank.

Experiment-wise analyses were conducted since only four sires and two dams from year-class 2006 were used in both experiments 3 and 4, while these numbers in year-class 2007, used in experiment 4, were five and seven, respectively (Figure 1). This led us to conclude that the genetic ties were too few and that the analyses had to be carried out on a per experiment basis. In the linear model (LM) and in the threshold model (TM), a binary trait (dead = 0/alive = 1) was defined at two stages: At the end of the test (endpoint) and at the day the truncated mortality was closest to 50% (50% mortality), which was at days 11 and 14 in tanks 1 and 2 in experiment 3 and at days 13 and 12 in tanks 1 and 2 in experiment 4. Note that only endpoint mortality could be considered in experiment 1 since the mortality in this experiment was lower than 50%. In the linear survival model (LSM) a binary variable per test day across the test period was defined as 1/0 if the fish was alive/dead on test-day t, where 0 implied that there would be no further record for that fish.

First, for experiments 3 and 4 Kaplan-Meier trajectories of the survival curves of the two tanks were compared with a log-rank test (https://www.real-statistics.com/survival-analysis/kaplan-meier-procedure/log-rank-test/).

Then, the following cross-sectional LM was applied to the binary trait (dead/alive = 0/1, both at the endpoint and at 50% mortality):

$$Y_{klmn} = \mu + b_1X_1 + b_2X_2 + T_k + s_l + d_m + c_{lm} + e_{klmn}$$

where Y_{klmn} = alive or dead (0 = dead, 1 = alive) for fish n; μ = the overall mean; b_1 = fixed regression coefficient on number of days from spawning until tagging (X_1); b_2 = fixed regression coefficient on

number of days from tagging to start of experiment (day 0) (X_2); T_k = the fixed effect of tank k (k = 1, 2); s_l = random additive genetic effect of sire l; d_m = random additive genetic effect of dam m; c_{lm} = random common environmental effect pertaining to fullsib family lm; and e_{klmn} = random error term for fish n.

Above, the random additive genetic effects of sire and dam can be represented by a vector of sire and dam effect: $\begin{bmatrix} \boldsymbol{s} \\ \boldsymbol{d} \end{bmatrix}$. Further, E($\boldsymbol{s}$) = E($\boldsymbol{d}$) = E($\boldsymbol{c}$) = E($\boldsymbol{e}$) = **0;** Var($\boldsymbol{s}$) = Var($\boldsymbol{d}$) = $\mathbf{A}\sigma^2_{sd}$, where **A** is the additive genetic relationship matrix, and σ^2_{sd} is the common sire-dam variance component; Var($\boldsymbol{c}$) = $\mathbf{I}\sigma^2_c$, where **I** is an identity matrix, and σ^2_c is the common environmental variance (potentially including also maternal and dominance effects in addition to the environmental effect of hapa), and Var($\boldsymbol{e}$) = $\mathbf{I}\sigma^2_e$, with σ^2_e being the residual variance.

Additionally, the data was analysed with a cross-sectional (probit) TM, assuming a normal underlying liability variable l that determines the categorical outcome, such that $l_{klmn} \leq 0$ gives Y_{klmn} = 0, and $l_{klmn} > 0$ gives $Y_{klmn} = 1$. Restricting the residual variance on the underlying liability scale to $\sigma^2_e = 1$, the model can be written:

$$Pr\,(Y_{klmn} = 1) = Pr\,(l_{klmn} > 0) = \Phi(b_1X_1 + b_2X_2 + T_k + s_l + d_m + c_{lm})$$

where $\Phi(.)$ is the cumulative standard normal distribution function, and the other parameters are as described for the LM.

Finally, the linear survival model LSM was specified as:

$$Y_{klmnt} = \mu + b_1X_1 + b_2X_2 + T_k + \sum_{p=0}^{4} \beta_{pk} Z_p(t) + s_l + d_m + c_{lm} + e_{klmnt}$$

where Y_{klmnt} = fish *n* alive or not (dead/alive = 0/1) at test-day *t*; $Z_p(t)$= p^{th} order orthogonal polynomial of a specific day *t* (test day), with *p* = 0, 1, 2, 3 and 4; β_{pk} = p^{th} order fixed regression coefficient nested within tank *k*; e_{klmnt} = random error term for fish *n* at test-day *t* ~ $N(0, \mathbf{I}\sigma^2_e)$, and the remaining parameters as described above.

The relationships were traced back to the base, comprising a total of 2,389, 6,145 and 6,905 animals for experiments 1, 3 and 4, respectively. The data was analysed with ASReml, version 4.1 (Gilmour, Gogel, Cullis, Welham & Thompson 2015).

2.3 Heritability

Heritabilities were calculated as follows:

$$h^2 = \frac{4\sigma^2_{sd}}{2\sigma^2_{sd} + \sigma^2_c + \sigma^2_e}$$

where σ^2_{sd} is the common sire-dam variance component, σ^2_c is the common environmental variance, and σ^2_e is the residual variance.

2.4 Model comparison

To compare the predictive value of the three models, the Pearson correlation coefficient between the predicted family effects (sum of sire, dam and common environmental effects) in one tank and the mean family survival in the other tank was calculated. The validation data was always the endpoint survival. Training data was survival at the endpoint, at 50% overall survival or as time until death (endpoint for survivors). The test of the difference between the dependent correlations (the same data used by the three models) was calculated by the software of Lee & Preacher (2013).

Finally, Spearman rank correlation coefficients between predicted family effects across the two replicate tanks were calculated at both 50% mortality and at the endpoint, with the three different models.

3. Results

In three out of the four linear model analyses carried out across tanks in experiments 3 and 4 (both at 50% mortality and at the endpoint), increased number of days from spawning till tagging decreased survival ($P < 0.001$), while increased number of days from tagging to start of the experiment led to enhanced survival ($P < 0.001$) (results not shown).

Cumulative Kaplan-Meier survival curves in the challenge-test experiments are shown in Figure 2. Mortality was much lower in experiments 1 and 2 than in experiments 3 and 4. The development of mortality was also different among the experiments. In the first, where the overall cumulative mortality reached 25%, the earliest mortality of fish was recorded on day 8, reaching a maximum of 7.6% at day 11. In experiment 2, cumulative mortality was only 3.0% and 5.7% for tanks 1 and 2, respectively. In experiment 3, cumulative mortality was high; 84.0% and 83.1% for tanks 1 and 2. Moreover, the trajectories of the survival curves were seemingly different between the two tanks, with earlier initiation of mortality in tank 1 than in tank 2. In experiment 4, cumulative mortality was about 87% in both tanks, with more similar survival curves. In both experiment 3 and 4, a log-rank test showed that the survival curves of the two replicated tanks were significantly different (with test statistics of 123.37 and 40.09, respectively, with one degree of freedom).

Survival at the end of the tests are shown for each family per experiment in Figure 3. In experiment 1, all families had surviving members, and mean fraction of survivors across families was 75% (ranging from 29 to 100% among families). In experiment 2, mean survival across families in tanks

1 and 2 were 97% and 94% (range: 88 - 100% and 68 - 100%, respectively). In experiment 3, with two tanks, 140 (75% of all families) and 152 (81%) families had survivors at the end of the challenge test. Mean survival across families in tank 1 was 15% (range: 0 - 69%), while it was 16% (range: 0 - 56%) in tank 2. In experiment 4, mean survival across families were 12% in both tanks (range: 0 - 70% and 0 - 60%), and 152 and 146 families (65% and 63% of all families) had survivors at the end of the test.

Variance components and heritabilities obtained at the two stages, endpoint (all three models) and 50% overall mortality (with LM and TM), in experiments 1, 3 and 4 are presented in Table 2. Generally, as expected, the estimated heritabilities were lower for LM than for TM, because the latter estimates heritability on the underlying scale. Heritability estimates were also considerably lower with the LSM (test-day survival) than with the cross-sectional models. This is expected due to the fact that LSM models daily survival, while the LM and TM accumulates survival over the entire test period. With LSM, estimated heritability on the test-day level was ~1% in all the three experiments analysed. With the cross-sectional models, the largest heritabilities were obtained at 50% mortality, with 0.22 and 0.13 for TM and LM, respectively. At the endpoint, both genetic variance and heritability of the cross-sectional models were found to be reduced due to lower frequency of survivors. Moreover, the estimated common environmental variance for endpoint survival was somewhat enlarged in experiment 4, likely due to statistical uncertainty.

The predictive ability of the models, assessed as the Pearson correlation coefficient between the observed family survival in one tank and the predicted family survival in the other tank using survival at both 50% mortality and at the endpoint as training data, are presented in Table 3. In general, the linear models (LM and LSM) predicted family survival better than TM, while no

differences ($P < 0.05$) were obtained between LM and LSM.

Spearman rank correlations between family survival calculated across the two replicated tanks, with the three models at both 50% mortality and at the endpoint, in the same experiment are given in Table 4, showing that LM and TM correlated closely. However, with these two models, survival at 50% mortality and at the endpoint correlated moderately (< 0.72), indicating substantial reranking depending on the timing of the cut-off point. Moreover, LSM correlations with LM and TM ranged 0.81 - 0.92 at both stages.

4. Discussion

The average mortality varied much across the four challenge test experiments (5.7 – 87.7%), far away from a natural reference of 50% maximizing the phenotypic variance for a binary trait (Gjøen, Refstie, Ulla & Gjerde 1997). The four tests propose three days acclimatization of test fish prior to the challenge, with restricted water level, keeping a temperature of 26^0 C. In the challenge, cohabitant shedders should be released directly into the test tank and make up ~⅓ of the fish, and bacteria should be added directly to water. Experiments 3 and 4, with the highest mortality, suggest that any factor involving the dead cohabitants should be removed, and that additional experimentation should focus on bacteria (density) and timing for addition of bacteria to water.

The study shows that resistance to BN is heritable (Table 2). As expected, the largest estimate of heritability was obtained with the TM since these parameter estimates are on the underlying and unobserved liability scale. This heritability will only be realized given that one could observe liability directly, which is not possible in practice. One problem with the LM is that heritability estimates are frequency dependent (Gianola & Foulley 1983). Moreover, LSM estimates were even smaller than those obtained with the cross-sectional models (TM and LM) since the information

per animal is split onto several days. As expected, the heritability for survival from the cross-sectional models were higher at 50% overall mortality than at the endpoint. A main reason for this is that several families had no survivors at the endpoint (Figure 3), leaving less genetic variance to be detected. The stage of 50% mortality was chosen because it has been frequently used in challenge tests with fish (Gjøen, Refstie, Ulla & Gjerde 1997), likely because this frequency maximizes the phenotypic variance of the binary survival trait.

The two experiments 3 and 4, each with two replicated tanks, had the advantage of allowing comparison of the predictive ability of different statistical models and trait definitions, by performing a between-tank validation as also done by Gitterle, Ødegård, Gjerde, Rye & Salte (2006). Herein, the comparison was based on the use of full-sib family effects, while Gitterle, Ødegård, Gjerde, Rye & Salte (2006) based their comparison on estimated breeding values. The family effect was chosen over the additive genetic effect because of the weakness of the nested mating design in separation of the additive genetic, non-additive genetic and common environmental effects (Berg & Henryon 1998). From the results (Table 3), it can be inferred that the TM model predicts the family survival inferior to the linear models. With some families having no survivors, extreme category problems may affect the TM, which might be a reason for the inferior performance. The validation did not discriminate between the LM and the LSM (Tables 3). However, in experiment 3, with the largest difference in trajectories of survival curves between tanks and with the lower average mortality at the endpoint, the LSM was found to have the highest correlations to survival in the other tank (Table 3), which indicates that accounting for time until death may be useful as also reported by Gitterle, Ødegård, Gjerde, Rye & Salte (2006), Ødegård, Olesen, Gjerde & Klemetsdal (2006), and Ødegård, Olesen, Gjerde & Klemetsdal (2007).

The Spearman rank correlation values between family survival calculated across the two replicated tanks in the same experiment obtained at 50% mortality and at the endpoint of the challenge were moderate (≤ 0.72, Table 4), implying substantial re-ranking of family survival effects. Moreover, the corresponding correlation of family survival effects at 50% mortality to that obtained with LSM was higher (≥ 0.85), since back-truncating the test to 50% overall mortality is equivalent to a binary analysis of time-to-death, binary categorizing survival time as either long (1) or short (0). Observed survival during a challenge test may be a mixture of two underlying traits, called susceptibility and endurance (Ødegård, Madsen, Labouriau, Gjerde & Meuwissen 2011). Susceptibility is whether or not the animal is at risk of dying to the disease, while endurance is the ability of susceptible individuals to stay alive for some time (latency) during exposure (Kause & Ødegård 2012). Given that the exposure period is sufficiently long (i.e., continued until mortality ceases) the observed endpoint survival will approach the phenotypic susceptibility, while taken at earlier time-points the observed survival will be a mixture of susceptibility and endurance traits. This may explain the substantial re-ranking of families when correlating survival at 50% overall mortality with that at the endpoint. Another possibility would be to perform a more complex genetic analysis with a cure survival model (Ødegård, Gitterle, Madsen, Meuwissen, Yazdi, Gjerde, Pulgarin & Rye 2011; Ødegård, Madsen, Labouriau, Gjerde & Meuwissen 2011), attempting to separate endurance and susceptibility from survival time (mixture trait). An easier and more robust option is to only consider susceptibility at the endpoint, requiring that mortality has naturally ceased. This was not fully reached in experiments 3 and 4 where the tests ideally should have been prolonged.

In addition to susceptibility and endurance, host infectivity has received attention in genetic analysis of disease resistance data (Anacleto, Cabaleiro, Villanueva, Saura, Houston, Woolliams & Doeschl-Wilson 2019). These authors define the trait as the host's ability to infect an average

individual upon contact. However, it can be argued that if animals become non-susceptible, and this arises from the fish being resistant to the pathogen, these fish may also be less likely to spread the pathogen.

The present study has implications as to how the challenge test against BN should be carried out in Mekong striped catfish. If mortality is naturally ceasing, endpoint mortality is a measure of susceptibility and should have preference over the other measures of resistance. Preferably, this natural endpoint mortality should be attained at ~50% since this maximizes the phenotypic variance of susceptibility (Table 2) at which EBV's can be obtained by a simple cross-sectional linear model.

The models above do not allow to distinguish between individuals within a family and do not utilize the possibility to carry out within-family selection in a sib-population of untested breeding candidates. In practice, inbreeding considerations will force the breeder to select from a broader range of families, reducing the realized selection differential. Furthermore, selection accuracy will also become reduced as Mendelian sampling variation (within-family genetic variance) constitutes half the total genetic variance (likely more due to Bulmer effects in populations under selection), which is not considered through family selection (Ødegård, Baranski, Gjerde & Gjedrem 2011). In order to obtain both higher selection intensity and selection accuracy, a genomic selection program for BN resistance in Mekong striped catfish is advisable. Then, candidates can be selected based on the summed effects of markers spanning the whole genome of individual fish, allowing to utilise the whole genetic variance also when selecting among untested selection candidates. The limitation of this method is the cost of genotyping as well as the availability of a SNP array. There is work conducted to construct a high density SNP array in Mekong striped catfish, e.g. Vo, Nguyen, Nguyen & Tran (2018). Another advantage of genomic markers and genomic

relationships would be the possibility to perform a more efficient statistical correction for environmental effects common to fullsibs caused by the separate rearing of the families. Parental assignment through genetic markers could allow communal rearing of the families from a much early life stage to be used that would reduce the common environmental effect.

5. Conclusions

It is concluded that resistance to *E. ictaluri* causing BN in Mekong striped catfish is heritable. The challenge test should aim for an endpoint survival that ceases naturally at ~50%. Breeding values should preferably be calculated for endpoint survival, with a simple cross-sectional linear model. With the considerable genetic variance estimated in this study, susceptibility to BN has the potential to become considerably changed by selection over time.

Conflict of interest statement

There is no conflict of interest.

References

Anacleto, O., Cabaleiro, S., Villanueva, B., Saura, M., Houston, R.D., Woolliams, J.A. & Doeschl-Wilson, A.B. (2019) Genetic differences in host infectivity affect disease spread and survival in epidemics. *Scientific Reports,* **9,** 4924.

Barría, A., Doeschl-Wilson, A.B., Lhorente, J.P., Houston, R.D. & Yáñez, J.M. (2019) Novel insights into the genetic relationship between growth and disease resistance in an aquaculture strain of Coho salmon (*Oncorhynchus kisutch*). *Aquaculture,* **511,** 734207.

Bassini, L.N., Lhorente, J.P., Oyarzún, M., Bangera, R., Yáñez, J.M. & Neira, R. (2019) Genetic parameters for *Piscirickettsia salmonis* resistance, sea lice (*Caligus rogercresseyi*) susceptibility and harvest weight in rainbow trout (*Oncorhynchus mykiss*). *Aquaculture,* **510,** 276-282.

Berg, P. & Henryon, M. (1998) A comparison of mating designs for inference on genetic parameters in fish. *Proc. 6th WCGALP,* **27,** 115-118.

Crumlish, M., Dung, T.T., Turnbull, J.F., Ngoc, N.T.N. & Ferguson, H.W. (2002) Identification of *Edwardsiella ictaluri* from diseased freshwater catfish, *Pangasius hypophthalmus* (Sauvage), cultured in the Mekong Delta, Vietnam. *Journal of Fish Diseases,* **25,** 733-736.

Doan Q., K., Vandeputte, M., Chatain, B., Haffray, P., Vergnet, A., Breuil, G. & Allal, F. (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.

Gianola, D. & Foulley, J.L. (1983) Sire evaluation for ordered categorical data with a threshold model. *Genetics, Selection, Evolution,* **15,** 201-224.

Gilmour, A.R., Gogel, B.J., Cullis, B.R., Welham, S.J. & Thompson, R. (2015) ASReml User Guide Release 4.1 Structural Specification. Retrived from https://www.hpc.iastate.edu/sites/default/files/uploads/ASREML/UserGuideStructural.pdf.

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,** 210-218.

Gjedrem, T. & Gjøen, H.M. (1995) Genetic variation in susceptibility of Atlantic salmon, *Salmo salar L.*, to furunculosis, BKD and cold water vibriosis. *Aquaculture Research,* **26,** 129-134.

Gjerde, B., Boison, S.A., Aslam, M.L., Løvoll, M., Bakke, H., Rey, S. & Lillehammer, M. (2019) Estimates of genetic correlations between susceptibility of Atlantic salmon to amoebic gill disease in a bath challenge test and a field test. *Aquaculture,* **511,** 734265.

Gjøen, H.M., Refstie, T., Ulla, O. & Gjerde, B. (1997) Genetic correlations between survival of Atlantic salmon in challenge and field tests. *Aquaculture,* **158,** 277-288.

Guy, D.R., Bishop, S.C., Woolliams, J.A. & Brotherstone, S. (2009) Genetic parameters for resistance to Infectious Pancreatic Necrosis in pedigreed Atlantic salmon (*Salmo salar*) post-smolts using a Reduced Animal Model. *Aquaculture,* **290,** 229-235.

Kause, A. & Ødegård, J. (2012) The genetic analysis of tolerance to infections: a review. *Frontiers in Genetics,* **3,** 262.

Kettunen, A. & Fjalestad, K.T. (2006) Resistance to vibriosis in Atlantic cod (*Gadus morhua* L.): First challenge test results. *Aquaculture,* **258,** 263-269.

Lee, I.A. & Preacher, K.J. (2013) Calculation for the test of the difference between two dependent correlations with one variable in common [Computer software]. Retrived from http://quantpsy.org/corrtest/corrtest2. In.

Lhorente, J.P., Gallardo, J.A., Villanueva, B., Araya, A.M., Torrealba, D.A., Toledo, X.E. & Neira, R. (2012) Quantitative genetic basis for resistance to *Caligus rogercresseyi* sea lice in a breeding population of Atlantic salmon (*Salmo salar*). *Aquaculture,* **324–325,** 55-59.

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.

Srisapoome, P., Chatchaiphan, S., Bunnoy, A., Koonawootrittriron, S. & Na-Nakorn, U. (2019) Heritability of immunity traits and disease resistance of bighead catfish, *Clarias macrocephalus* Günther, 1864. *Fish & Shellfish Immunology,* **92,** 209-215.

Sukhavachana, S., Poompuang, S., Onming, S. & Luengnaruemitchai, A. (2019) Heritability estimates and selection response for resistance to *Streptococcus agalactiae* in red tilapia *Oreochromis spp. Aquaculture,* **502,** 384-390.

Taylor, R.S., Wynne, J.W., Kube, P.D. & Elliott, N.G. (2007) Genetic variation of resistance to amoebic gill disease in Atlantic salmon (*Salmo salar*) assessed in a challenge system. *Aquaculture,* **272,** S94-S99.

Thanh, P.C. & Berntsen, J.O. (2012) Towards sustainability: Vaccinated *pangasius* in Vietnam. *AQUA Culture Asia Pacific Magazine,* **May/June,** 36-37.

Van Muiswinkel, W.B., Wiegertjes, G.F. & Stet, R.J.M. (1999) The influence of environmental and genetic factors on the disease resistance of fish. *Aquaculture,* **172,** 103-110.

Vo, T.T.B., Nguyen, H.D., Nguyen, T.T.H. & Tran, H.T.T. (2018) Identification and analysis of snps in population of Vietnamese catfish (*Pangasianodon hypophthalmus*), using next generation sequencing and snp validation. *MOJ Current Research & Reviews,* **1,** 12-19.

Vu, N.T., Sang, N.V., Phuc, T.H., Vuong, N.T. & Nguyen, N.H. (2019) Genetic evaluation of a 15-year selection program for high growth in striped catfish *Pangasianodon hypophthalmus*. *Aquaculture,* **509,** 221-226.

Vu, N.T., Sang, N.V., Trong, T.Q., Duy, N.H., Dang, N.T. & Nguyen, N.H. (2019) Breeding for improved resistance to *Edwardsiella ictaluri* in striped catfish (*Pangasianodon hypophthalmus*): Quantitative genetic parameters. *Journal of Fish Diseases,* **42,** 1409-1417.

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.

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., Gitterle, T., Madsen, P., Meuwissen, T.H.E., Yazdi, M.H., Gjerde, B., Pulgarin, C. & Rye, M. (2011) Quantitative genetics of taura syndrome resistance in pacific white shrimp (*penaeus vannamei*): a cure model approach. *Genetics Selection Evolution,* **43,** 14.

Ødegård, J., Madsen, P., Labouriau, R., Gjerde, B. & Meuwissen, T.H.E. (2011) A sequential threshold cure model for genetic analysis of time-to-event data. *J. of Animal Science,* **89,** 943-950.

Ødegård, J., Olesen, I., Dixon, P., Jeney, Z., Nielsen, H.M., Way, K., Joiner, C., Jeney, G., Ardó, L., Rónyai, A. & Gjerde, B. (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) Positive genetic correlation between resistance to bacterial (furunculosis) and viral (infectious salmon anaemia) diseases in farmed Atlantic salmon (*Salmo salar*). *Aquaculture,* **271,** 173-177.

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.

453 Table 1. Number of families, sires, dams and fish in four challenge-test experiments of Mekong
454 striped catfish with *E. ictaluri*. Experiment (Exp) 1 was carried out in only one tank (t1), while
455 experiments 2, 3 and 4 had two replicated tanks.

Exp	Identity	No. of families	No. of sires	No. of dams	No. of fish
1	Exp1-t1	81	54	80	2,155
2	Exp2-t1	64	41	63	1,019
	Exp2-t2	60	40	59	969
3	Exp3-t1	187	118	183	2,944
	Exp3-t2	187	118	183	2,745
4	Exp4-t1	233	137	230	3,246
	Exp4-t2	233	137	230	2,931

456

Table 2. Estimates of additive genetic sire-dam variance (σ^2_{sd}), common environmental variance (σ^2_c) and heritability (h^2) of survival at the endpoint and at 50%-mortality (not for LSM) by use of three statistical models in three challenge-test experiments (Exp) of Mekong striped catfish with *E. ictaluri*.

		σ^2_{sd}		σ^2_c		h^2 (±SE)	
Exp	Model [†)]	Endpoint	50%	Endpoint	50%	Endpoint	50%
1 [‡)]	LM	4.6×10^{-3}	-	5.5×10^{-3}	-	0.100 (0.071)	-
	TM [§)]	5.2×10^{-2}	-	4.8×10^{-2}	-	0.180 (0.120)	-
	LSM	4.4×10^{-5}	-	7.9×10^{-5}	-	0.010 (0.008)	-
3	LM	2.9×10^{-3}	8.3×10^{-3}	1.2×10^{-3}	4.2×10^{-3}	0.085 (0.030)	0.135 (0.042)
	TM	4.9×10^{-2}	6.3×10^{-2}	2.0×10^{-2}	2.4×10^{-2}	0.174 (0.066)	0.220 (0.065)
	LSM	1.9×10^{-4}	-	1.2×10^{-4}	-	0.012 (0.004)	-
4	LM	1.1×10^{-3}	4.1×10^{-3}	4.5×10^{-3}	7.8×10^{-3}	0.044 (0.024)	0.084 (0.033)
	TM	2.4×10^{-2}	3.6×10^{-2}	1.2×10^{-1}	7.1×10^{-2}	0.083 (0.064)	0.125 (0.054)
	LSM	2.7×10^{-4}	-	4.5×10^{-4}	-	0.012 (0.004)	-

[†)] The models were: LM: Cross-sectional linear model; TM: Cross-sectional threshold model, and LSM: Linear survival model.

[‡)] Experimental survival < 50%.

[§)] In TM, the residual variance = 1.

Table 3. Pearson correlation coefficients between the observed family survival (y) in one tank (t1 or t2) to the predicted family survival (sum of sire, dam and common environmental effects = y-hat) in the other tank, in experiments (Exp) 3 and 4 both at the endpoint and at 50% mortality, calculated with either a linear model (LM), a threshold model (TM), or a linear survival model (LSM, not at 50% mortality). P-values are given for the test of difference between pairs of correlations.

Exp	r	Model	Endpoint	50%	P-value		
					Endpoint		50%
					LM	TM	LM
3	$r_{y\text{-}t1,y\text{-}hat\text{-}t2}$	LM	0.278	0.279	-	0.130	-
		TM	0.257	0.263	-	-	0.177
		LSM	0.297	-	0.534	0.219	-
	$r_{y\text{-}t2,\ y\text{-}hat\text{-}t1}$	LM	0.330	0.350	-	0.812	-
		TM	0.334	0.351	-	-	0.879
		LSM	0.352	-	0.463	0.617	-
4	$r_{y\text{-}t1,y\text{-}hat\text{-}t2}$	LM	0.407	0.336	-	< 0.001	-
		TM	0.286	0.304	-	-	0.021
		LSM	0.388	-	0.577	0.018	-
	$r_{y\text{-}t2,\ y\text{-}hat\text{-}t1}$	LM	0.381	0.283	-	< 0.001	-
		TM	0.267	0.243	-	-	0.008
		LSM	0.353	-	0.407	0.036	-

Table 4. Spearman rank correlation coefficients between predicted family survival across the two tanks (sum of sire, dam and common environmental effects) in experiments (Exp) 3 and 4 both at the endpoint and at 50% mortality, calculated with either a cross-sectional linear model (LM), a threshold model (TM), or a linear survival model (LSM, not at 50% mortality).

Exp	Model	Endpoint		50%	Endpoint - 50%		
		TM	LSM	LM	LM	TM	LSM
3	LM	0.99	0.89	-	0.71	0.72	0.92
	TM	-	0.89	0.99	0.71	0.71	0.91
4	LM	0.92	0.81	-	0.59	0.59	0.87
	TM	-	0.83	0.98	0.56	0.60	0.85

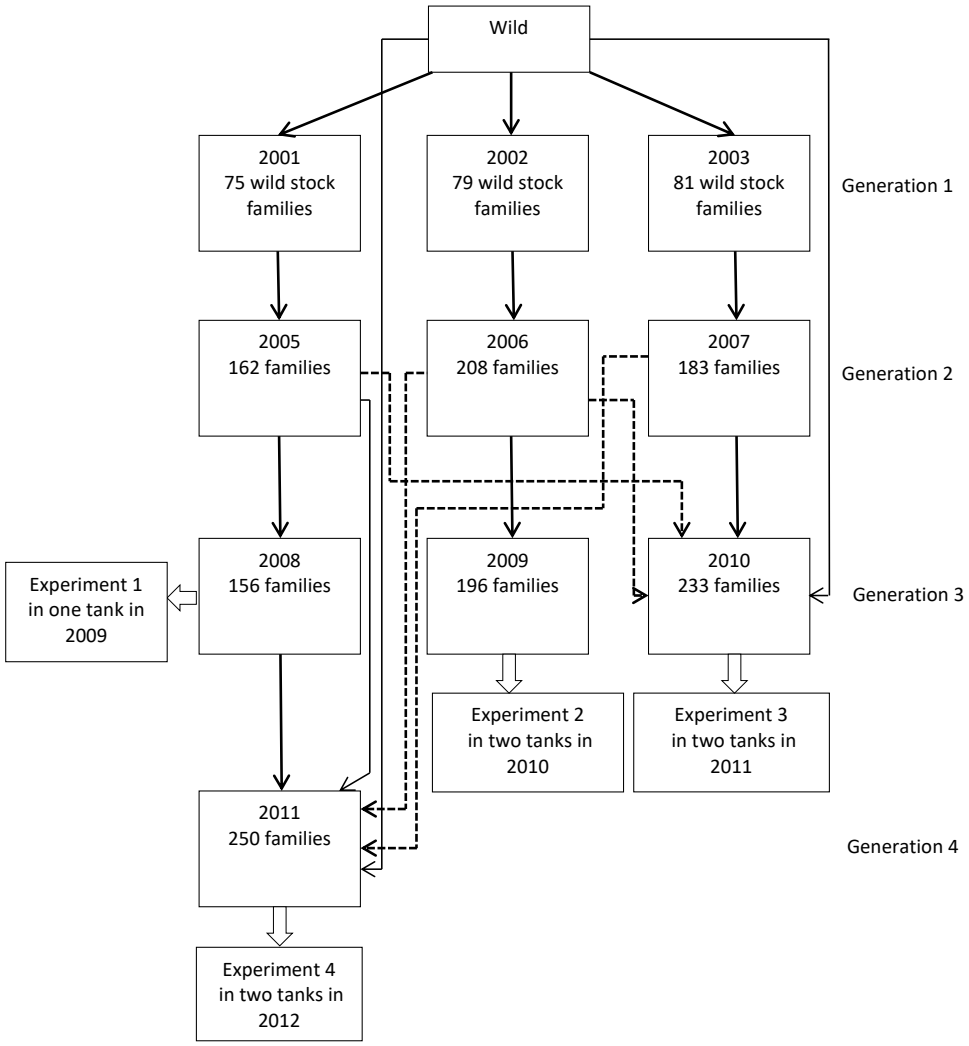

Figure 1. Schematic representation of the breeding program with Mekong striped catfish in Vietnam; year-classes, sub-populations and generations in which the challenge-test experiments were carried out is shown. Number of families per year-class is given. Dashed arrows indicate broodstock usage across year-classes.

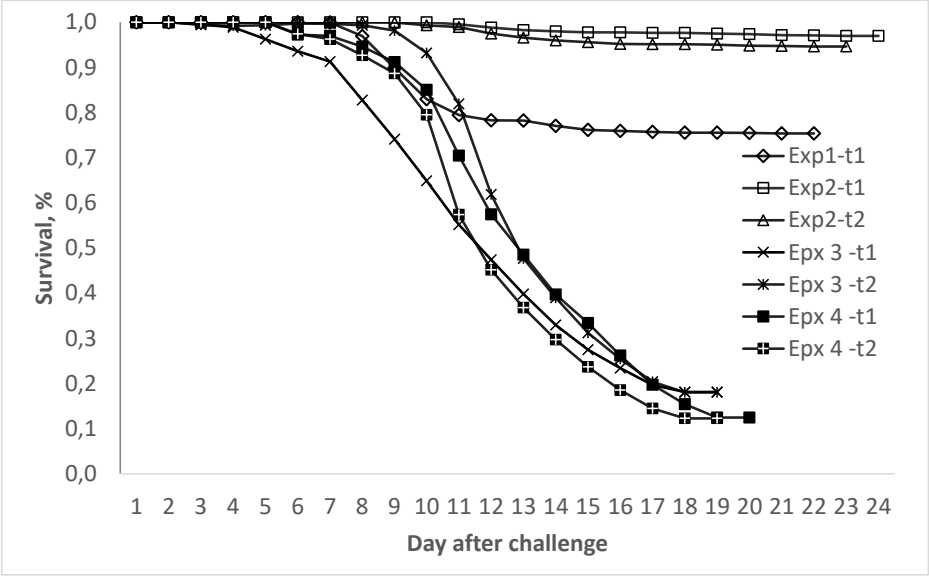

Figure 2. Cumulative Kaplan-Meier survival curves in four challenge-test experiments of Mekong striped catfish with *E. ictaluri*: Experiment (Exp) 1 was carried out in only one tank (t1), while experiments 2, 3 and 4 had two replicated tanks.

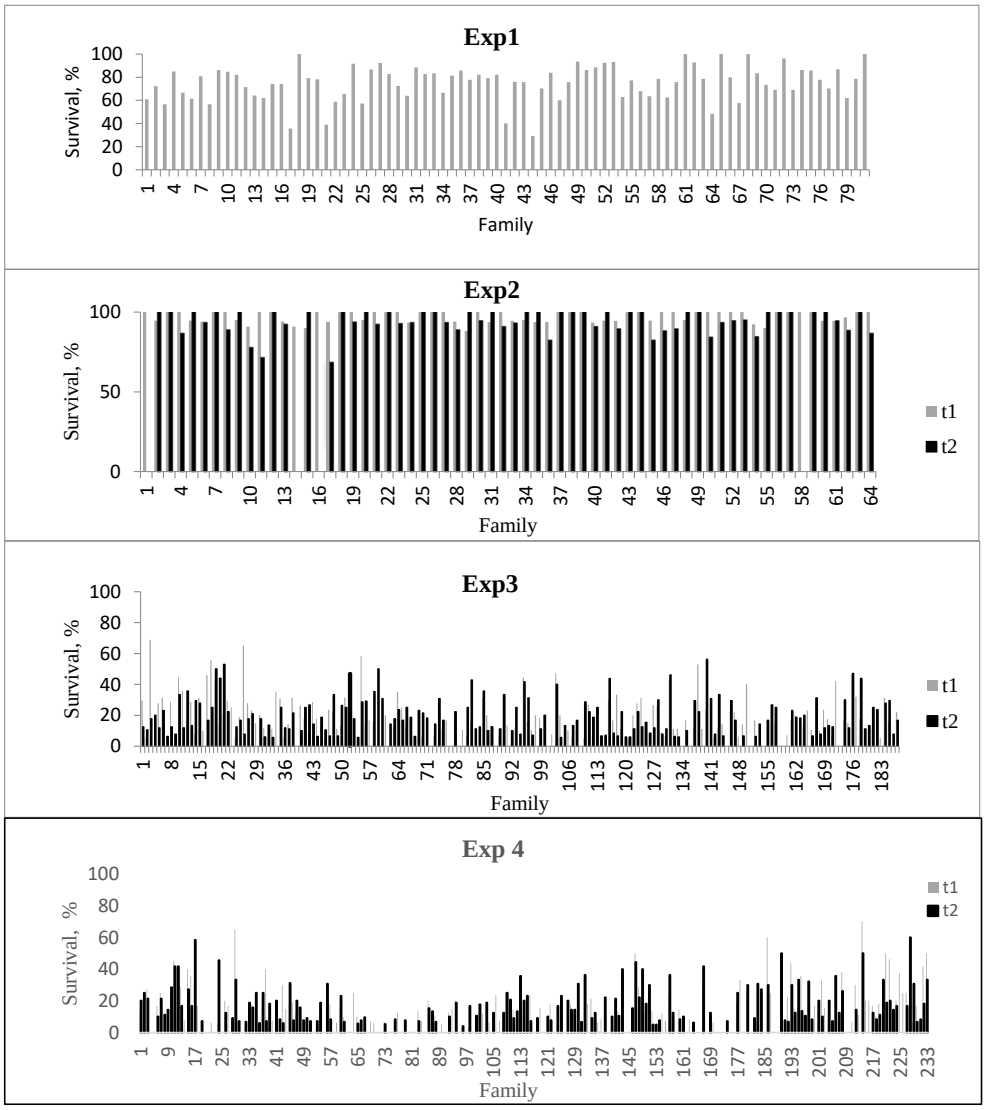

Figure 3. Terminal survival (number of survivors to number of test fish at the start of the tests) by family in four challenge-test experiments of Mekong striped catfish with *E. ictaluri*. Experiment (Exp) 1 was carried out in only one tank, while experiments 2, 3 and 4 had two replicated tanks (t1 and t2).

PAPER III

Pham KD**,** Ødegård J, Nguyen SV, Gjøen HM, Klemetsdal G. (2020)

Genetic correlations between challenge tested susceptibility to bacillary necrosis, caused by *Edwardsiella ictaluri*, and growth performance tested survival and harvest body weight in Mekong striped catfish (*Pangasianodon hypophthalmus*).

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Genetic correlations between challenge tested susceptibility to bacillary necrosis, caused by *Edwardsiella ictaluri*, and growth performance tested survival and harvest body weight in Mekong striped catfish (*Pangasianodon hypophthalmus*)

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Abstract

The aim was to carry out a joint genetic analysis of survival and harvest body weight, recorded in a growth performance test in Mekong striped catfish (*Pangasianodon hypophthalmus*), and susceptibility to bacillary necrosis (caused by *Edwardsiella ictaluri*), recorded in challenge tests. Data were from two challenge tested year-classes (~6,000 fish in both) that both had growth test data available for survival and body weight (~13,000 fish each year). Data were analysed with a linear tri-variate sire-dam model without the common environmental effect because otherwise genetic correlations were estimated with large standard errors. Susceptibility to bacillary necrosis was found weakly genetically correlated to both growth and survival in the growth test, while growth was found with moderate favourable genetic correlation to growth survival. To defend continued challenge testing of striped catfish in Vietnam, a strong genetic relationship needs to be established between bacillary necrosis and survival under a natural disease outbreak. This requires another field test (in addition to the growth test) with siblings, without antibiotic treatment and the cause of death continuously monitored. Meanwhile, the routine challenge testing with the aim to indirectly improve field survival through selection should continue.

KEYWORDS

challenge test, common environmental effect, *Edwardsiella ictaluri*, growth performance test, heritability

1 | INTRODUCTION

Growth, typically recorded as final body weight at a fixed time of slaughter, is usually the most important trait in the initial phase of a fish breeding programme. Genetic variation of growth has been shown in Mekong striped catfish (*Pangasianodon hypophthalmus*), with estimates of heritability for body weight recorded in a growth test as high as 0.34 (Sang et al., 2012). Thus, selection has been carried out for increased body weight since the start of the striped catfish breeding programme in Vietnam in 2001, resulting in a 9.3% selection response per generation (Vu et al., 2019). However, the Mekong striped catfish has experienced outbreaks of bacillary necrosis (BN) caused by *Edwardsiella ictaluri* (*E. ictaluri*), and the prospect of selection against susceptibility to BN has therefore been examined through four challenge-test experiments by Pham et al. (2020). Genetic analyses of three of these experiments, with a sufficient mortality, revealed that the heritability obtained with the preferred cross-sectional linear model for susceptibility when mortality naturally ceased was ≤0.18,

TABLE 1 Descriptive statistics relevant for two challenge-test experiments against susceptibility to bacillary necrosis and for two growth test experiments (both challenge and growth tests in G3-2003 and G4-2001, respectively), in which harvest body weight and growth survival were recorded, in Mekong striped catfish

Year-class	Experiment	Fish, no.	Full-sib families, no.	Sires, no.	Dams, no.	Average no. of fish per family	Average tagging age, days	Length of test, days	Average body weight, g	Average growth survival, %
G3-2003	Challenge, tank 1	2,944	187	118	183	15.7	144.2	19	-	16.0
	Challenge, tank 2	2,745	187	118	183	14.7	144.1	20	-	16.9
	Growth test	13,322	216	133	213	62.0	150.7	250[a]	808.1	72.9
G4-2001	Challenge, tank 1	3,246	233	137	230	13.9	180.9	20	-	12.9
	Challenge, tank 2	2,931	233	137	230	12.6	181.1	19	-	12.3
	Growth test	13,847	250	140	247	55.0	177.7	269[a]	834.9	71.8

[a]At date of first harvest.

dependent on frequency in the endpoint. The aim of the current study was to estimate genetic correlations between susceptibility to BN under challenge testing and, respectively, growth (harvest body weight) and survival, both recorded in a growth performance test.

2 | MATERIALS AND METHODS

By granting the research, the Vietnamese Ministry of Agriculture and Rural Development pre-approved the experiments carried out.

2.1 | Fish materials

At Research Institute for Aquaculture No. 2 (RIA 2) in Vietnam a selective breeding programme with striped catfish, originally with three separate subpopulations named 2001, 2002 and 2003, has been established from fish that had gone through domestication in three hatcheries (Sang et al., 2012). This study involved the year-class produced in 2010, being the third generation of subpopulation 2003 (G3-2003), and the year-class produced in 2011, which is fourth generation of subpopulation 2001 (G4-2001). In both year-classes, fish were produced by use of a nested mating design, that is one male mated to two females. In 2010, batches of families were produced from July 29 to September 10, while in 2011 this occurred from June 9 to July 7. Spawning was induced by hormone treatment (HCG—human chorionic gonadotropin). First, males were stripped, and the milt was stored at 4°C. Later, it was split in two, mixed with samples of eggs from two females, before water was added for fertilization. The fertilized eggs were washed to remove sticky layers using tannic acid, and eggs from one female were moved to a family air-supplied net-jar in one cement tank for hatching. Fertilized eggs hatched from 18 to 24 hr after fertilization. The total number of families produced in G3-2003 was 233, and of these, 187 families had offspring taking part in the successive challenge test, while offspring from 216 families were recorded for growth and survival in the growth test (Table 1). In G4-2001, offspring from 233 families were challenge tested, while 250 families (all families produced) had offspring in the growth test. Number of sires and dams in G3-2003 and G4-2001 are also given in Table 1.

2.2 | Nursing of fish

Twenty-four hours post-hatching, approximately three thousand start-fed fry were randomly sampled from each family to be reared in a 1-m^3 family-fibreglass tank for about 20 days. The tanks had air supply, and about half the water was exchanged every three days. There, fry was in sequence fed ad libitum with newly hatched *Artemia* sp., *Moina* sp. and bloodworm (*Limnodrilus hoffmeisteri*). After the 20 days, a random sample of about 300 small fingerlings from each full-sib family was transferred to a hapa located in one earthen pond. Here, fish was initially fed ad libitum by bloodworm,

but within a week they were transferred to standard commercial pellet feed (V2-Feed, RIA2, Ho Chi Minh City, <2.0 mm, 22%–28% protein). The net hapas were cleaned frequently to maintain good water circulation. In G3-2003, each full-sib family was raised in a separate hapa, while in G4-2001 some families were nursed in more than one.

2.3 | Tagging of fish

In both G3-2003 and G4-2001, tagging was done when the fish were randomly netted out, hapa by hapa. For the challenge tested fish, Passive Integrated Transponder tags (PIT tags, Sokymat, Switzerland) were inserted from 16 December 2010 to 9 January 2011 (G3-2003) and from 15 to 21 December 2011 (G4-2001). Fish for the challenge tests were transferred to either of two tanks at the National Breeding Centre for Southern Freshwater Aquaculture (NABRECSOFA), before being transported to the Govap Experimental Center (Ho Chi Minh City), RIA2, for challenge testing. Tagging for the growth test was in G3-2003 carried out from 14 February to 18 March 2011 and in G4-2001 from 15 November 2011 to 4 January 2012. The tagged fish allocated for the growth tests were transferred to hapas located within the grow-out pond. The average age at tagging ranged 144–151 days in G3-2003 and 177–181 days in G4-2001 (Table 1).

2.4 | Challenge test

The challenge experiments were conducted in two tanks, that is replica, with the same number of representative families in both tanks, 187 in G3-2003 and 233 in G4-2001 (Table 1). The total number of test fish was 5,689 in G3-2003 and 6,177 in G4-2001, corresponding to an average number per family ranging from 13 to 16 (Table 1).

The cohabitation method was applied in both challenge-test experiments, carried out in January–February 2011 (started January 14th) and during January 2012 (started January 3rd), respectively. Fish were transferred to the test units only three days prior to challenge. Water temperature was kept constant at 26°C during the challenge by regulating the room temperature through air conditioning. To get a sufficient response to the challenge, the fish were stressed by halving the water level in the test tanks from one day prior to the test. The ratio of cohabitants to test fish was ~1:3. Cohabitants were infected by intraperitoneally injection (dose = 1×10^5 bacteria) and released directly into the test tanks. The bacteria were from a strain of *E. ictaluri* Gly09M (Southern Monitoring Center for Aquaculture Environment & Epidemic, RIA2, Ho Chi Minh City, Vietnam). Dead and floating cohabitants were collected into plastic baskets that were hung down into the water for another two days before removal. In G3-2003, external pathogen was added to the test tanks to reach a density of 2.5 x 10^6 bacteria/ml water, from day 6 post-challenge when the death of cohabitants had reached peak. This practice was continued for another 8 days. In G4-2001, addition of pathogen was started at day 3 post-challenge and stopped at day 6, after the cohabitant mortality had reached peak. Throughout the tests, fish were daily fed with standard commercial pellets at a rate of 1% of total biomass. Random samples of dead fish were examined for presence of *E. ictaluri*, as typical colonies (Crumlish et al., 2002). Kidney samples were grown on sheep blood agar plates and incubated at 30^0 C for 24 hr. In 100% of the samples, *E. ictaluri* was identified. Alive fish were biosecure-buried, following the national veterinary regulations (Department of Animal Health, Vietnam).

2.5 | Growth test

After one week in the communal hapa, the siblings of the challenged families were released to the 2,000-m^2 pond at NABRECSOFA. A total of 13,322 fish were included in the growth test in G3-2003 and 13,847 in G4-2001, representing an average of 62 and 55 fish per family, respectively (Table 1). All growth-tested fish were fed ad libitum with standard commercial pellets.

2.6 | Data recording

2.6.1 | Challenge-test data

In both experiments, the challenge test was continued until mortality ceased. Dead fish were collected twice daily, at 8:00 and 14:00, throughout the test periods, and PIT tags and time of death were recorded. In one tank in each experiment, mortality was observed for 19 days and in the other tank for 20 days (Table 1).

2.6.2 | Growth test data

In G3-2003, harvest was carried out over two periods: 1–14 November 2011, after 250 days in the grow-out pond (Table 1), and through 7–20 January 2012. During the first period, 3,777 fish were randomly sampled, and body weight (±0.1 g) was recorded to calculate family breeding values. In the second period, when selection was carried out on the breeding values, body weight was recorded for the remaining 5,922 fish. In G4-2001, in September–October 2012 after approximately 270 days of culture (measured to the date of first harvest, Table 1), 10,235 fish were sampled and recorded for body weight over two periods as in G3-2003. In both year-classes, body weight was recorded by the same person for all fish. Fish were not fed during harvest.

2.7 | Data analysis

The variables analysed from the growth tests were body weight and survival, while test endpoint susceptibility was used from the challenge tests. The latter was chosen because Pham et al. (2020)

experienced endpoint survival to cross-validate well in genetic analysis of data from these challenge-test experiments.

Since fish were not fed during harvest, it was decided that the length of the grow-out period should be considered only until the first date of harvest, in accordance with Sang et al. (2012). Correction of body weight for fish age from spawning until first date of harvest was accomplished through definition of two variables: 1) number of days from spawning till tagging (nursed time, G3-2003 with range 113–159 days and G4-2001 with range 166–195 days) and 2) number of days in the pond from tagging till date of first harvest (growth time, varied between fish because tagging dates varied between families while first harvest date did not), following the nomenclature and modelling of Sang et al. (2012). These authors accounted for nursed time and growth time as fixed effects, when estimating breeding values in one generation, and found these breeding values (relative to breeding values from models with alternative fixed effects representation) to predict the offspring phenotypes the best.

A tri-variate analysis of the data was performed using a linear cross-sectional sire-dam model for each trait, both in G3-2003 and G4-2001. The linear model was chosen because a previous study showed it preferable over the threshold model in our challenge-test data (Pham et al., 2020). Susceptibility to BN (trait 1) in the challenge and survival in the growth test (trait 2) were defined according to whether the individual was alive (score = 1) or dead (score = 0) at the end of the trial, whereas body weight (trait 3, at the end of the growth test) was a continuous trait. In matrix notation, model 1 can be written:

$$\begin{bmatrix} \boldsymbol{y}_1 \\ \boldsymbol{y}_2 \\ \boldsymbol{y}_3 \end{bmatrix} = \begin{bmatrix} \boldsymbol{X}_1 & 0 & 0 \\ 0 & \boldsymbol{X}_2 & 0 \\ 0 & 0 & \boldsymbol{X}_3 \end{bmatrix} \begin{bmatrix} \boldsymbol{b}_1 \\ \boldsymbol{b}_2 \\ \boldsymbol{b}_3 \end{bmatrix} + \begin{bmatrix} \boldsymbol{Z}_1 & 0 & 0 \\ 0 & \boldsymbol{Z}_2 & 0 \\ 0 & 0 & \boldsymbol{Z}_3 \end{bmatrix} \begin{bmatrix} \boldsymbol{a}_1 \\ \boldsymbol{a}_2 \\ \boldsymbol{a}_3 \end{bmatrix} + \begin{bmatrix} \boldsymbol{W}_1 & 0 & 0 \\ 0 & \boldsymbol{W}_2 & 0 \\ 0 & 0 & \boldsymbol{W}_3 \end{bmatrix} \begin{bmatrix} \boldsymbol{c}_1 \\ \boldsymbol{c}_2 \\ \boldsymbol{c}_3 \end{bmatrix} + \begin{bmatrix} \boldsymbol{e}_1 \\ \boldsymbol{e}_2 \\ \boldsymbol{e}_3 \end{bmatrix}$$

where $\boldsymbol{y}_1$, $\boldsymbol{y}_2$ and $\boldsymbol{y}_3$ are vectors of the susceptibility to BN in challenge, and, respectively, survival and harvest body weight in the growth test; $\boldsymbol{b}_i$ are the vectors of fixed effects with corresponding design matrix $\boldsymbol{X}_i$: For all traits containing the overall mean and a fixed regression coefficient for number of days from spawning till tagging (nursed time), for trait 1 also with a fixed effect of the two test tanks and a fixed regression coefficient for number of days from tagging till first day of the challenge experiment, and for trait 3 with a fixed regression coefficient for number of days from tagging till date of first harvest (growth time); $\boldsymbol{a}_i$ is a vector that for each trait contains random additive genetic effect of sires and dams, with $\boldsymbol{Z}_i$ being the corresponding design matrix; $\boldsymbol{c}_i$ is a vector that for a trait contains the random common environmental effect of full-sib families, with design matrix $\boldsymbol{W}_i$, and finally $\boldsymbol{e}_i$ is a random residual vector for each trait. The common environmental effect included for all traits in model 1 accounts for the common environment of full-sibs due to separate rearing of the families until tagging as well as possible maternal and dominance effects.

Effects of sire and dam, common environment, and residual were assumed random with the following distributions:

$$\boldsymbol{a} = \begin{bmatrix} \boldsymbol{a}_1 \\ \boldsymbol{a}_2 \\ \boldsymbol{a}_3 \end{bmatrix} \sim \boldsymbol{N}\left(0, \boldsymbol{A} \otimes \boldsymbol{G}_0\right),$$

$$\boldsymbol{c} = \begin{bmatrix} \boldsymbol{c}_1 \\ \boldsymbol{c}_2 \\ \boldsymbol{c}_3 \end{bmatrix} \sim \boldsymbol{N}\left(0, \boldsymbol{I}_c \otimes \boldsymbol{C}_0\right),$$

and

$$\boldsymbol{e} = \begin{bmatrix} \boldsymbol{e}_1 \\ \boldsymbol{e}_2 \\ \boldsymbol{e}_3 \end{bmatrix} \sim \boldsymbol{N}(0, \boldsymbol{I}_N \otimes \boldsymbol{R}_0)$$

where $\boldsymbol{A}$ is the additive genetic relationship matrix among the animals (including ancestors back to the base), and $\boldsymbol{I}$ denotes identity matrices of appropriate sizes. Further, $\boldsymbol{G}_0$, $\boldsymbol{C}_0$ and $\boldsymbol{R}_0$ are, respectively, the 3x3 (co)variance matrices of the sire-dam additive genetic, common environmental and residual effects. The traits were partly recorded on different individuals (survival in challenge test vs. growth and survival in grow-out), and growth could only be recorded on survivors from growth testing. Hence, residual correlations among the traits could not be estimated. All residual covariances among traits were thus restricted to zero, that is $\boldsymbol{R}_0$ became:

$$\boldsymbol{R}_0 = \begin{bmatrix} \sigma^2_{e_1} & 0 & 0 \\ 0 & \sigma^2_{e_2} & 0 \\ 0 & 0 & \sigma^2_{e_3} \end{bmatrix}$$

The ASREML software (Gilmour et al., 2015) was used for estimation of (co)variance components and genetic parameters. For all traits and with model 1, the estimated heritability was calculated as:

$$h^2_i = \frac{4\sigma^2_{G_i}}{2\sigma^2_{G_i} + \sigma^2_{C_i} + \sigma^2_{R_i}}$$

where $\sigma^2_{G_i}$, $\sigma^2_{C_i}$ and $\sigma^2_{R_i}$ are the sire-dam, the common environmental and the residual variances, from the diagonals of $\boldsymbol{G}_0$, $\boldsymbol{C}_0$ and $\boldsymbol{R}_0$, for trait *i*. Correspondingly, the ratio of the common environmental variance to the total phenotypic variance was calculated for each trait as:

$$c^2_i = \frac{\sigma^2_{C_i}}{2\sigma^2_{G_i} + \sigma^2_{C_i} + \sigma^2_{R_i}}$$

Likelihood-ratio testing was carried out to test for significance ($p < .05$) of sire-dam variance components. The full model contained a univariate specification of $\boldsymbol{G}_0$ in model 1, while the reduced model

constrained in sequence each of the three variance components to zero.

In addition, the data were analysed with a model 2, removing the common environmental effects from model 1. Then, the heritability for trait *i* was estimated as follows:

$$h_i^2 = \frac{4\sigma_{G_i}^2}{2\sigma_{G_i}^2 + \sigma_{R_i}^2}$$

Also, with model 2, likelihood-ratio testing was done to test whether the family covariances were significant ($p < .05$). The full model contained a multivariate specification of $\mathbf{G_0}$, while the reduced model constrained in sequence each of the three covariance components to zero.

3 | RESULTS

In G3-2003, overall survival at the end of the challenge test in tanks 1 and 2 were 16.0% and 16.9%, respectively, whereas the overall growth survival was 73% at harvest (Table 1). In G4-2001, the corresponding challenge-test survival was 12.9% and 12.3%, and the growth survival was 72%. The mean weight in G3-2003 was 808 g and 835 g in G4-2001 (Table 1). Among families, average growth survival varied from 9% to 100% (Figure 1).

Estimated variances and heritabilities for the three traits, using model 1, are given in Table 2. Heritability for susceptibility to BN was low, 0.09 and 0.06 in G3-2003 and G4-2001, respectively. Heritability of growth survival was moderate in G3-2003 (0.19), but higher in G4-2001 (0.28). Harvest body weight had high heritabilities, 0.50 in G3-2003 and 0.27 in G4-2001. Results from the likelihood-ratio testing showed that all the estimates of sire-dam variance components (except growth survival in G3-2003) were larger than zero ($p < .05$). The common environmental effect accounted for only a small amount of the total phenotypic variance for susceptibility to BN, 0.9% and 4.2% in G3-2003 and G4-2001, respectively. These effects were larger for growth survival, with 14.1% and 6.3%, and for body weight, with 10.7% and 18.6%, respectively.

With model 2, omitting the common environmental effects from model 1, the size of the estimated heritabilities for body weight and growth survival were generally enlarged relative to those obtained with model 1, becoming 0.77 and 0.78 for body weight and 0.56 and 0.47 for growth survival, in the two successive year-classes (Table 3). Heritability of susceptibility to BN was numerically less affected, with estimates of 0.11 and 0.13 in G3-2003 and G4-2001, respectively.

Genetic and common environmental correlations between susceptibility to BN, growth survival and body weight, using model 1, are given in Table 4. The size of the genetic correlations varied across the two experiments, G3-2003 and G4-2001: Between susceptibility to BN and growth survival from −0.01 to 0.58 (± 0.20), between susceptibility to BN and body weight from 0.23 (± 0.23) to −0.20 (± 0.28), and between growth survival and body weight from 0.45 (± 0.23) to 0.09 (± 0.19). In G4-2001, numerical sizes of common environmental correlations were more pronounced than in G3-2003, especially between body weight and, respectively, susceptibility to BN and growth survival with sizes of 0.33 (± 0.16) and 0.40 (± 0.16).

Genetic correlations between susceptibility to BN, growth survival and body weight were also estimated with model 2, ignoring the common environmental effects (Table 5). Using this model, the estimated genetic correlations had considerably smaller standard errors than those estimated with model 1, shown in Table 4. The size of the genetic correlation between susceptibility to BN and growth survival varied across the two experiments, from negative and non-significant - 0.02 ($\chi^2 = 0.02$, relative to zero) in G3-2003 to positive and significant 0.26 ($\chi^2 = 7.00$) in G4-2001. The corresponding correlations between body weight and growth survival were positive and significant, ranging from 0.38 ($\chi^2 = 25.62$) to 0.26 ($\chi^2 = 12.55$). Estimated genetic correlations between susceptibility to BN and body weight were positive, albeit non-significant.

4 | DISCUSSION

The genetic variance of survival in the growth performance test was found substantial (Table 2), with heritability estimates of 0.19 and 0.28 in G3-2003 and G4-2001, respectively. These results correspond well with the estimate of Vu et al. (2019) ($h^2 = 0.27$), who utilized our data in addition to data from two other year-classes (experiments) in the same breeding programme and assumed growth survival to be the same trait across populations and year-classes. In the literature, highly variable heritability estimates of growth survival have been reported, both when the cause of mortality was known ($h^2 = 0.38$, Gjøen et al., 1997; $h^2 = 0.17$, Bangera et al., 2014; $h^2 = 0.10$, Wetten et al., 2007; $h^2 = 0.40$–0.49, Taylor et al., 2009) and unknown ($h^2 = 0.04$–0.71, Vehviläinen et al., 2008; $h^2 = 0.04$–0.09, Rye et al., 1990; $h^2 = 0.07$, Liu et al., 2015; $h^2 = 0.14$, Gjerde et al., 2019; $h^2 = 0.34$,

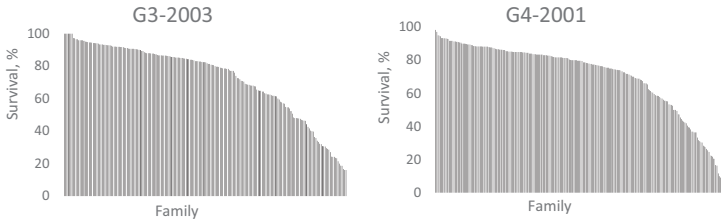

FIGURE 1 Average family survival in descending order for the two growth performance tests in the two year-classes, G3-2003 and G4-2001

TABLE 2 Estimates ± SE of additive genetic sire-dam variance (σ^2_{sd}), common environmental variance (σ^2_c), heritability (h^2), and fraction of variance explained by the common environmental effect (c^2), for susceptibility to bacillary necrosis (BN), survival in the growth performance test and harvest body weight in two year-classes (G3-2003 and G4-2001) of Mekong striped catfish, as obtained with model 1. The χ^2-test statistics for the additive genetic sire-dam effect being different from zero[a] is given

Trait	Year-class	σ^2_{sd}	χ^2	σ^2_c	h^2	c^2
BN	G3-2003	0.0030 ± 0.0010	7.76	0.0012 ± 0.0020	0.09 ± 0.03	0.009 ± 0.014
	G4-2001	0.0014 ± 0.0004	5.60	0.0027 ± 0.0012	0.06 ± 0.02	0.042 ± 0.012
Growth survival	G3-2003	0.0093 ± 0.0047	2.32	0.0280 ± 0.0083	0.19 ± 0.09	0.141 ± 0.042
	G4-2001	0.0140 ± 0.0031	20.80	0.0125 ± 0.0040	0.28 ± 0.06	0.063 ± 0.020
Body weight	G3-2003	6,935.2 ± 1,926.5	40.02	5,949.4 ± 2,793.1	0.50 ± 0.12	0.107 ± 0.051
	G4-2001	3,488.1 ± 1,190.5	14.43	9,576.3 ± 1,930.7	0.27 ± 0.09	0.186 ± 0.037

[a]Significant ($p < .05$) if $\chi^2 > 3.84$.

TABLE 3 Estimates ± SE of sire-dam variance (σ^2_{sd}) and heritability (h^2) for susceptibility to bacillary necrosis (BN), survival in the growth performance test, and harvest body weight in two year-classes (G3-2003 and G4-2001) of Mekong striped catfish, as obtained with model 2, not containing a common environmental effect

Trait	Year-class	σ^2_{sd}	h^2
BN	G3-2003	0.0037 ± 0.0007	0.11 ± 0.02
	G4-2001	0.0032 ± 0.0005	0.13 ± 0.02
Growth survival	G3-2003	0.0293 ± 0.0031	0.56 ± 0.04
	G4-2001	0.0247 ± 0.0025	0.47 ± 0.04
Body weight	G3–2003	11,155.3 ± 1,170.6	0.77 ± 0.05
	G4-2001	11,281.2 ± 1,115.9	0.78 ± 0.05

Nielsen et al., 2010; $h^2 = 0.34$, Ødegård et al., 2010). Vehviläinen et al. (2008) argued that treating growth survival as one trait over time may not reveal its true genetic architecture because individuals from different year-classes might not be exposed to the same factors causing the mortality. Therefore, growth survival of one year-class may not be the same trait as in another year-class that might be exposed to a different environment. In consequence, the genetic parameters may become unstable over time and space, which also can be inferred from the present results. Primarily, the genetic correlation between susceptibility to BN and growth survival was estimated inconsistent across the two year-classes with model 1: In G3-2003, this correlation was close to zero, while in G4-2001 the correlation was as high as 0.58 ± 0.20 (Table 4). The same picture was obtained with model 2, with a significant genetic correlation (0.26, $\chi^2 = 7.00$) only in G4-2001 (Table 5). Others have found high genetic correlations when the same bacteria were present in both environments (Gjøen et al., 1997; Ødegård et al., 2006; Wetten et al., 2007), whereas a low genetic correlation has been reported when this was not the case (Ødegård et al., 2010). This suggests that mortality in the growth performance test in G4-2001 was partly due to the same bacteria as in the challenge test, while this seems not to have been the case for G3-2003, albeit no bacterial identifications were carried out. Consequently, growth survival may not have been the same trait across the two year-classes.

The estimated heritabilities of susceptibility to BN with model 1 in the two successive year-classes were 0.09 and 0.06 (Table 2), respectively, which is in close agreement with the estimates previously obtained by analysing the same data with a univariate cross-sectional linear model ($h^2 = 0.085$ for G3-2003 and 0.044 for G4-2001, Pham et al., 2020). However, when analysing categorical data with a cross-sectional linear model, heritability estimates will be frequency dependent which explains the lower estimate obtained in G4-2001, having the highest average endpoint mortality of the two year-classes. This promotes a testing protocol where mortality naturally ceases at a lower frequency than obtained in this study, preferably at ~50% where the phenotypic variance is maximized (Pham et al., 2020). Actually, in two other challenge experiments carried out in Vietnam (other year-classes), the endpoint mortality was closer to 50% than in G3-2003 and G4-2001; 25% in G3-2001, analysed by

TABLE 4 Estimated genetic (r_g, above diagonal) and common environmental (r_c, below diagonal) correlations ± SE between susceptibility to bacillary necrosis (BN), survival in the growth performance test and harvest body weight in two year-classes (G3-2003 and G4-2001) of Mekong striped catfish, as obtained with model 1

	G3–2003			G4-2001		
Trait	BN	Growth survival	Body weight	BN	Growth survival	Body weight
BN		−0.01 ± 0.30	0.23 ± 0.23		0.58 ± 0.20	−0.20 ± 0.28
Growth survival	−0.05 ± 0.51		0.45 ± 0.23	−0.12 ± 0.20		0.09 ± 0.19
Body weight	0.01 ± 0.54	0.35 ± 0.24		0.33 ± 0.16	0.40 ± 0.16	

TABLE 5 Estimated genetic correlations ± SE between susceptibility to bacillary necrosis (BN), survival in the growth performance test and harvest body weight in two year-classes (G3-2003 and G4-2001) of Mekong striped catfish, as obtained with model 2, not containing a common environmental effect. The χ^2-test statistics for the genetic covariance being different from zero[a] is given in brackets

	G3-2003			G4-2001		
Trait	**BN**	**Growth survival**	**Body weight**	**BN**	**Growth survival**	**Body weight**
BN	–	−0.02 ± 0.11 (0.02)	0.19 ± 0.11 (2.72)		0.26 ± 0.09 (7.00)	0.16 ± 0.10 (2.40)
Growth survival	–	–	0.38 ± 0.07 (25.62)	–	–	0.26 ± 0.07 (12.55)
Body weight	–	–	–	–	–	–

[a]Significant ($p < .05$) if $\chi^2 > 3.84$.

Pham et al. (2020), and 39% in the last experiment referred to in Vu et al. (2019). In both these experiments, the heritability became much increased relative to our estimates, 0.18 and 0.19, respectively. This demonstrates that it should be possible to enhance the value of the challenge testing primarily by increasing the heritability of BN, from ensuring mortality to naturally cease around 50% (Pham et al., 2020).

Growth is considered the most important trait in Mekong striped catfish, as in most aquaculture species subjected to selective breeding. Thus, selection has been carried out for increased body weight since start of the breeding programme in 2001. The moderate-to-high heritabilities, 0.27–0.50, found for growth in this study with model 1 (Table 2) correspond well with the estimates of Sang et al. (2012) ($h^2 = 0.21–0.34$), obtained in the two preceding generations to G4-2001. Moreover, they compare well with the estimates of Vu et al. (2019) ($h^2 = 0.34$), utilizing all growth data generated in the breeding programme.

Challenge testing can be defended if considerable, consistent and significant genetic correlations exist between BN and growth survival. This could, however, only be estimated in G4-2001, with model 1 (0.58, Table 4). With model 2, the corresponding genetic correlation obtained in G4-2001 became much reduced (0.26, Table 5). Vu et al. (2019) reported a genetic correlation between the traits of 0.52 with a variant of model 1. As mentioned, they assumed growth survival to be the same traits across experiments, and the same assumption was made for BN. Their assumption can be questioned from our results, with highly variable size of the estimated genetic correlations between the two traits in the two experiments (Tables 4 and 5). This is likely due to growth performance testing being carried out with the breeding population, meaning that antibiotic treatment will be applied. Reaching a conclusive genetic relationship between challenge and field survival would require to field test for survival (a new test in addition to the growth performance test). In this test, siblings from the same families as in the challenge and the growth test are to be used, treatment is not to be carried out and the cause of death is to be continuously monitored (for natural outbreak of BN or not). A disadvantage of such a test would be possible infrequent outbreaks of BN and the extra costs. Thus, it is advised to be solely used until the genetic relationship between BN and survival in the field has become sufficiently established.

Low, non-significant (relative to zero) genetic correlations were estimated between BN and growth when ignoring common environmental effects (Table 5). This implies that both these traits can be simultaneously improved through selection. Moreover, the corresponding genetic correlations between growth and growth survival were positive and significant ($p < .05$, Table 5) meaning that selection for growth, as practised in the breeding programme at current, will likely genetically improve growth survival.

Above, the genetic correlations ignoring common environmental effects were utilized to draw inference as to likely correlated responses in the breeding programme. These genetic correlations (Table 5) were preferred because they were estimated much more precisely than the genetic correlations in Table 4. The reason for the low precision of the latter genetic correlations is to be found in the use of a nested mating design, in which most sires were mated to two dams, while each dam is mated with one sire only. Consequently, only sires with two offspring groups contribute with information to separation of common environmental and genetic effects. The number of sires with two offspring groups in the challenge and growth tests in G3-2003 was 65 and 79, respectively, while in G4-2001 the corresponding numbers were 87 and 100. These numbers were considerably lower than the numbers in Table 1 showing that a much reduced and limited number of sires contribute to estimation of (co) variance components. For long, the problem with separation of common environment and genetic effects have been researched (Berg & Henryon, 1998; Dupont-Nivet et al., 2006), and the use of a partial-factorial mating design has been advised, which also was used initially in this breeding programme (Sang et al., 2012).

The common environmental effects were estimated as considerable, especially for growth survival and harvest body weight (Table 2). This could be due to the problem of separation of genetic and common environmental effects, but it could also be due to families being separately reared for a long period, approximately 5 months in hapa, in addition to potential maternal and/or dominance effects (Joshi et al., 2018). Reduction of the effect of common environment should be sought by shortening the length of the spawning and tagging times that would reduce the variation in nursed time between families and thus the impact of common environment. This may require upgrading of the hatchery capacity and a larger workforce. Yet, another alternative would be early communal rearing, but

this would require application of genetic markers to identify the parentage among all recorded fish.

Currently, there are two striped catfish breeding programmes established in Vietnam that are run in parallel, one aiming for improved growth and the other for improved resistance to BN. The top-ranked individuals, based on estimated breeding values, from the two selected lines can be used in crossbreeding to exploit both additive genetic and possible heterosis effects. To our knowledge, heterosis effects have not been estimated for these traits in striped catfish, and since the breeding programmes have not been carried out for long and also utilizes fish from the wild (Pham et al., 2020), both the inbreeding level and heterosis effects are expected to be minor (Falconer & Mackay, 1996). Alternatively, one breeding programme could have been run selecting simultaneously for both resistance to BN and growth, but such a programme would also rely on a high genetic correlation being verified between BN in the challenge and survival under a natural disease outbreak of BN in the field.

5 | CONCLUSIONS

Continued challenge testing towards *E. ictaluri* causing BN in the Mekong striped catfish requires verification of a consistent, considerable and significant genetic correlation between susceptibility to BN and survival under natural disease outbreak of BN in the field. We conclude the current evidence to be weak. The programme is in need of a field test for survival (a new test in addition to the grow-out test). In this test, siblings from the same families as in the challenge and the grow-out are to be used, in a pond that is not practising antibiotic treatment and where the cause of death is continuously monitored (for natural outbreak of BN or not, i.e. field survival). Meanwhile, we propose to continue the routine challenge testing by ensuring mortality to naturally cease at around 50%, aiming at indirect improvement of field survival.

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CONFLICT OF INTEREST

There is no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the finding of this study can be made available on request, by the corresponding author.

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REFERENCES

Bangera, R., Ødegård, J., Mikkelsen, H., Nielsen, H. M., Seppola, M., Puvanendran, V., Gjøen, H. M., Hansen, Ø. J., & Mortensen, A. (2014). Genetic analysis of francisellosis field outbreak in Atlantic cod (*Gadus morhua* L.) using an ordinal threshold model. *Aquaculture*, *420–421*, S50–S56.

Berg, P., & Henryon, M. (1998). A comparison of mating designs for inference on genetic parameters in fish. *Proceeding of the 6th WCGALP*, *27*, 115–118.

Crumlish, M., Dung, T. T., Turnbull, J. F., Ngoc, N. T. N., & Ferguson, H. W. (2002). Identification of *Edwardsiella ictaluri* from diseased freshwater catfish, *Pangasius hypophthalmus* (Sauvage), cultured in the Mekong Delta, Vietnam. *Journal of Fish Diseases*, *25*, 733–736. https://doi.org/10.1046/j.1365-2761.2002.00412.x

Dupont-Nivet, M., Vandeputte, M., Haffray, P., & Chevassus, B. (2006). Effect of different mating designs on inbreeding, genetic variance and response to selection when applying individual selection in fish breeding programs. *Aquaculture*, *252*, 161–170. https://doi.org/10.1016/j.aquaculture.2005.07.005

Falconer, D. S., & Mackay, T. F. C. (1996). *Introduction to quantitative genetics*, 4th ed. Longman: Pro-ed.

Gilmour, A. R., Gogel, B. J., Cullis, B. R., Welham, S. J., & Thompson, R. (2015) *ASReml User Guide Release 4.1 Structural Specification*. Retrieved from https://www.hpc.iastate.edu/sites/default/files/uploads/ASREML/UserGuideStructural.pdf

Gjerde, B., Boison, S. A., Aslam, M. L., Løvoll, M., Bakke, H., Rey, S., & Lillehammer, M. (2019). Estimates of genetic correlations between susceptibility of Atlantic salmon to amoebic gill disease in a bath challenge test and a field test. *Aquaculture*, *511*, 734265. https://doi.org/10.1016/j.aquaculture.2019.734265

Gjøen, H. M., Refstie, T., Ulla, O., & Gjerde, B. (1997). Genetic correlations between survival of Atlantic salmon in challenge and field tests. *Aquaculture*, *158*, 277–288. https://doi.org/10.1016/S0044-8486(97)00203-2

Joshi, R., Woolliams, J. A., Meuwissen, T. H. E., & Gjøen, H. M. (2018). Maternal, dominance and additive genetic effects in Nile tilapia; influence on growth, fillet yield and body size traits. *Heredity*, *120*, 452–462. https://doi.org/10.1038/s41437-017-0046-x

Liu, J., Lai, Z., Fu, X., Wu, Y., Bao, X., Hu, Z., & Lai, M. (2015). Genetic parameters and selection responses for growth and survival of the small abalone *Haliotis diversicolor* after four generations of successive selection. *Aquaculture*, *436*, 58–64. https://doi.org/10.1016/j.aquaculture.2014.10.046

Nielsen, H. M., Ødegård, J., Olesen, I., Gjerde, B., Ardo, L., Jeney, G., & Jeney, Z. (2010). Genetic analysis of common carp (*Cyprinus carpio*) strains: I: Genetic parameters and heterosis for growth traits and survival. *Aquaculture*, *304*, 14–21. https://doi.org/10.1016/j.aquaculture.2010.03.016

Ødegård, J., Olesen, I., Dixon, P., Jeney, Z., Nielsen, H. M., Way, K., Joiner, C., Jeney, G., Ardó, L., Rónyai, A., & Gjerde, B. (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. https://doi.org/10.1016/j.aquaculture.2010.03.017

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. https://doi.org/10.1016/j.aquaculture.2006.05.034

Pham, K. D., Ødegård, J., Sang, V. N., Gjøen, H. M., & Klemetsdal, G. (2020) Genetic analysis of resistance in Mekong striped catfish (Pangasianodon hypophthalmus) to bacillary necrosis caused by Edwardsiella ictaluri. *Journal of Fish Diseases*.. https://doi.org/10.1111/jfd.13279

Rye, M., Lillevik, K. M., & Gjerde, B. (1990). Survival in early life of Atlantic salmon and rainbow trout: Estimates of heritabilities and genetic correlations. *Aquaculture*, *89*, 209–216. https://doi.org/10.1016/0044-8486(90)90126-8

Sang, N. V., Klemetsdal, G., Ødegård, J., & Gjøen, H. M. (2012). Genetic parameters of economically important traits recorded at a given age in striped catfish (*Pangasianodon hypophthalmus*). *Aquaculture*, *344–349*, 82–89. https://doi.org/10.1016/j.aquaculture.2012.03.013

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. https://doi.org/10.1016/j.aquaculture.2009.06.007

Vehviläinen, H., Kause, A., Quinton, C., Koskinen, H., & Paananen, T. (2008). Survival of the currently fittest: Genetics of rainbow trout survival across time and space. *Genetics*, *180*, 507–516. https://doi.org/10.1534/genetics.108.089896

Vu, N. T., Sang, N. V., Phuc, T. H., Vuong, N. T., & Nguyen, N. H. (2019). Genetic evaluation of a 15-year selection program for high growth in striped catfish *Pangasianodon hypophthalmus*. *Aquaculture*, *509*, 221–226. https://doi.org/10.1016/j.aquaculture.2019.05.034

Vu, N. T., Sang, N. V., Trong, T. Q., Duy, N. H., Dang, N. T., & Nguyen, N. H. (2019). Breeding for improved resistance to *Edwardsiella ictaluri* in striped catfish (*Pangasianodon hypophthalmus*): Quantitative genetic parameters. *Journal of Fish Diseases*, *42*, 1409–1417.

Wetten, M., Aasmundstad, T., Kjøglum, S., & Storset, A. (2007). Genetic analysis of resistance to infectious pancreatic necrosis in Atlantic salmon (*Salmo salar* L.). *Aquaculture*, *272*, 111–117. https://doi.org/10.1016/j.aquaculture.2007.08.046

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