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*)

Running title: Challenge and growth test correlations

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Acknowledgments

This research was funded by the Vietnamese Ministry of Agriculture and Rural Development and the Norwegian Quota scheme. Thanks go to all RIA2-staff members, producing the full-sib families and recording the data.

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 was to carry out a joint genetic analysis of survival and harvest body weight, recorded in growth performance test in Mekong striped catfish (*Pangasianodon hypophthalmus*), and susceptibility to bacillary necrosis (caused by *Edwarsiella ictaluri*), recorded in challenge tests. Data was 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 was 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 of indirectly improving field survival through selection should continue.

Keywords: *Edwardsiella ictaluri*; Challenge test; Growth performance test; Heritability; Common environmental effect

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 program. 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, Klemetsdal, Ødegård & Gjøen 2012). Thus, selection has been carried out for increased body weight since the start of the striped

catfish breeding program in Vietnam in 2001, resulting in a 9.3% selection response per generation (Vu, Sang, Phuc, Vuong & Nguyen 2019). However, the 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 analysed by Pham, Sang, Ødegård, Gjøen & Klemetsdal (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, dependent on frequency in the endpoint (Pham, Ødegård, Sang, Gjøen & Klemetsdal 2020). 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 program 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, Klemetsdal, Ødegård & Gjøen 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, i.e., one male mated to two females. In 2010, batches of families were

produced from July 29th - September 10th, while in 2011 this occurred from June 9th - July 7th. Spawning was induced by hormone treatment (HCG – Human Chorionic Gonadotropin). First, males were stripped, and the 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 using tannic acid, and eggs from one female was moved to a family air-supplied net-jar in one cement tank for hatching. Fertilized eggs hatched from 18 - 24 hours after fertilization. The total number of families produced in G3-2003 were 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-fiberglass 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 December 16th, 2010, to January 9th, 2011 (G3-2003) and from December 15th - 21st, 2011 (G4-2001). Fish for the challenge test 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 February 14th - March 18th, 2011, and in G4-2001 from November 15th, 2011, to January 4th, 2012. The tagged fish allocated for the growth test 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, i.e., 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 were 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 x 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, 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).

2.5 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: 1st - 14th November 2011, after 250 days in the grow-out pond (Table 1), and through 7th - 20th January 2012. During the first period, 3,777 fish were randomly sampled, and body weight (± 0.1g) 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, Ødegård, Sang, Gjøen & Klemetsdal (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, Klemetsdal, Ødegård & Gjøen (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, Klemetsdal, Ødegård & Gjøen (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, Ødegård, Sang, Gjøen & Klemetsdal 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:

$$\begin{bmatrix} \boldsymbol{y_1} \\ \boldsymbol{y_2} \\ \boldsymbol{y_3} \end{bmatrix} = \begin{bmatrix} \boldsymbol{X_1} & \boldsymbol{0} & \boldsymbol{0} \\ \boldsymbol{0} & \boldsymbol{X_2} & \boldsymbol{0} \\ \boldsymbol{0} & \boldsymbol{0} & \boldsymbol{X_3} \end{bmatrix} \begin{bmatrix} \boldsymbol{b_1} \\ \boldsymbol{b_2} \\ \boldsymbol{b_3} \end{bmatrix} + \begin{bmatrix} \boldsymbol{Z_1} & \boldsymbol{0} & \boldsymbol{0} \\ \boldsymbol{0} & \boldsymbol{Z_2} & \boldsymbol{0} \\ \boldsymbol{0} & \boldsymbol{0} & \boldsymbol{Z_3} \end{bmatrix} \begin{bmatrix} \boldsymbol{a_1} \\ \boldsymbol{a_2} \\ \boldsymbol{a_3} \end{bmatrix} + \begin{bmatrix} \boldsymbol{W_1} & \boldsymbol{0} & \boldsymbol{0} \\ \boldsymbol{0} & \boldsymbol{W_2} & \boldsymbol{0} \\ \boldsymbol{0} & \boldsymbol{0} & \boldsymbol{W_3} \end{bmatrix} \begin{bmatrix} \boldsymbol{c_1} \\ \boldsymbol{c_2} \\ \boldsymbol{c_3} \end{bmatrix} + \begin{bmatrix} \boldsymbol{e_1} \\ \boldsymbol{e_2} \\ \boldsymbol{e_3} \end{bmatrix}$$

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:

$$\boldsymbol{a} = \begin{bmatrix} \boldsymbol{a_1} \\ \boldsymbol{a_2} \\ \boldsymbol{a_3} \end{bmatrix} \sim \boldsymbol{N}(\boldsymbol{0}, \boldsymbol{A} \otimes \boldsymbol{G_0}),$$

$$\boldsymbol{c} = \begin{bmatrix} \boldsymbol{c_1} \\ \boldsymbol{c_2} \\ \boldsymbol{c_3} \end{bmatrix} \sim \boldsymbol{N}(\boldsymbol{0}, \boldsymbol{I_c} \otimes \boldsymbol{C_0}),$$

and

$$\boldsymbol{e} = \begin{bmatrix} \boldsymbol{e_1} \\ \boldsymbol{e_2} \\ \boldsymbol{e_3} \end{bmatrix} \sim \boldsymbol{N}(\boldsymbol{0}, \boldsymbol{I_N} \otimes \boldsymbol{R_0})$$

where $\boldsymbol{A}$ is the additive genetic relationship matrix among the animals (including ancestors back to the base), and $\boldsymbol{I}$ denote 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, i.e. $\boldsymbol{R_0}$ 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, Gogel, Cullis, Welham & Thompson 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:

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

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 were calculated for each trait as:

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

Likelihood-ratio testing was carried out to test for significance ($P < 0.05$) 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:

208 $$h_i^2 = \frac{4\sigma^2{}_{G_i}}{2\sigma^2_{G_i}+\sigma^2_{R_i}}$$

209 Also, with model 2, likelihood-ratio testing was done to test whether the family co-variances
210 were significant ($P < 0.05$). The full model contained a multivariate specification of $\boldsymbol{G_0}$, while
211 the reduced model constrained in sequence each of the three co-variance components to zero.

212 **3. Results**

213 In G3-2003, overall survival at the end of the challenge test in tanks 1 and 2 were 16.0% and
214 16.9%, respectively, whereas the overall growth survival was 73% at harvest (Table 1). In G4-
215 2001 the corresponding challenge-test survival were 12.9% and 12.3%, and the growth survival
216 was 72%. The mean weight in G3-2003 was 808 g and 835 g in G4-2001 (Table 1). Among
217 families, average growth survival varied from 9% to 100% (Figure 1).

218 Estimated variances and heritabilities for the three traits, using model 1, are given in Table 2.
219 Heritability for susceptibility to BN was low, 0.09 and 0.06 in G3-2003 and G4-2001, respectively.
220 Heritability of growth survival was moderate in G3-2003 (0.19), but higher in G4-2001 (0.28).
221 Harvest body weight had high heritabilities, 0.50 in G3-2003 and 0.27 in G4-2001. Results from
222 the likelihood-ratio testing showed that all the estimates of sire-dam variance components (except
223 growth survival in G3-2003) were larger than zero ($P < 0.05$). The common environmental effect
224 accounted for only a small amount of the total phenotypic variance for susceptibility to BN, 0.9%
225 and 4.2% in G3-2003 and G4-2001, respectively. These effects were larger for growth survival,
226 with 14.1% and 6.3%, and for body weight, with 10.7% and 18.6%, respectively.

227 With model 2, omitting the common environmental effects from model 1, the size of the estimated
228 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 in with sizes of 0.33 (± 0.16) and 0.40 (± 0.16).

Genetic correlations between susceptibility to BN, growth survival in 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 (χ^2 = 0.02, relative to zero) in G3-2003 to positive and significant 0.26 (χ^2 = 7.00) in G4-2001. The corresponding correlations between body weight and growth survival were positive and significant, ranging from 0.38 (χ^2 = 25.62) to 0.26 (χ^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 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, Sang, Phuc, Vuong & Nguyen (2019) ($h^2 = 0.27$), who utilized our data in addition to data from two other year-classes (experiments) in the same breeding program and assumed growth survival in to be the same trait across populations and year-classes. In the literature, highly variable heritability estimates of growth survival in have been reported, both when the cause of mortality was known ($h^2 = 0.38$, Gjøen, Refstie, Ulla & Gjerde 1997; $h^2 = 0.17$, Bangera, Ødegård, Mikkelsen, Nielsen, Seppola, Puvanendran, Gjøen, Hansen & Mortensen 2014; $h^2 = 0.10$, Wetten, Aasmundstad, Kjøglum & Storset 2007; $h^2 = 0.40 - 0.49$, Taylor, Kube, Muller & Elliott 2009) and unknown ($h^2 = 0.04 - 0.71$, Vehviläinen, Kause, Quinton, Koskinen & Paananen 2008; $h^2 = 0.04 - 0.09$, Rye, Lillevik & Gjerde 1990; $h^2 = 0.07$, Liu, Lai, Fu, Wu, Bao, Hu & Lai 2015; $h^2 = 0.14$, Gjerde, Boison, Aslam, Løvoll, Bakke, Rey & Lillehammer 2019; $h^2 = 0.34$, Nielsen, Ødegård, Olesen, Gjerde, Ardo, Jeney & Jeney 2010; $h^2 = 0.34$, Ødegård, Olesen, Dixon, Jeney, Nielsen, Way, Joiner, Jeney, Ardó, Rónyai & Gjerde 2010). Vehviläinen, Kause, Quinton, Koskinen & Paananen (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 in 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, χ^2 = 7.00) only in G4-2001 (Table 5). Others have found high genetic correlations when the same bacteria was present in both environments (Gjøen, Refstie, Ulla & Gjerde 1997; Ødegård, Olesen, Gjerde & Klemetsdal 2006; Wetten, Aasmundstad, Kjøglum & Storset 2007), whereas a low genetic correlation has been reported when this was not the case (Ødegård, Olesen, Dixon, Jeney, Nielsen, Way, Joiner, Jeney, Ardó, Rónyai & Gjerde 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 in 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, Ødegård, Nguyen, Gjøen & Klemetsdal 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 maximised (Pham, Ødegård, Sang, Gjøen & Klemetsdal 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 Pham, Ødegård, Sang, Gjøen & Klemetsdal (2020), and 39% in the last experiment referred to in Vu, Sang, Trong, Duy, Dang & Nguyen (2019). In both these experiments the heritability became much increased relative to our estimates, 0.18 and 0.19, respectively. This

296 demonstrates that it should be possible to enhance the value of the challenge testing primarily by
297 increasing the heritability of BN, from ensuring mortality to naturally cease around 50% (Pham,
298 Sang, Ødegård, Gjøen & Klemetsdal 2020).

299 Growth is considered the most important trait in striped catfish, as in most aquaculture species
300 subjected to selective breeding. Thus, selection has been carried out for increased body weight
301 since start of the breeding program in 2001. The moderate to high heritabilities, 0.27 - 0.50, found
302 for growth in this study with model 1 (Table 2) correspond well with the estimates of Sang,
303 Klemetsdal, Ødegård & Gjøen (2012) (h^2 = 0.21 - 0.34), obtained in the two preceding generations
304 to G4-2001. Moreover, they compare well with the estimates of Vu, Sang, Phuc, Vuong & Nguyen
305 (2019) (h^2 = 0.34), utilizing all growth data generated in the breeding program.

306 Challenge testing can be defended if considerable, consistent and significant genetic correlations
307 exist between BN and growth survival. This could, however, only be estimated in G4-2001, with
308 model 1 (0.58, Table 4). With model 2, the corresponding genetic correlation obtained in G4-2001
309 became much reduced (0.26, Table 5). Vu, Sang, Trong, Duy, Dang & Nguyen (2019) reported a
310 genetic correlation between the traits of 0.52 with a variant of model 1. As mentioned, they
311 assumed growth survival to be the same traits across experiments, and the same assumption was
312 made for BN. Their assumptions can be questioned from our results, with highly variable size of
313 the estimated genetic correlations between the two traits in the two experiments (Tables 4 and 5).
314 This is likely due to growth performance testing being carried out with the breeding population,
315 meaning that antibiotic treatment will be applied. Reaching a conclusive genetic relationship
316 between challenge and field survival would require to field test for survival (a new test in addition
317 to the growth performance test). In this test, siblings from the same families as in the challenge
318 and the growth test are to be used, treatment is not to be carried out and the cause of death is

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 in were positive and significant ($P < 0.05$, Table 5) meaning that selection for growth, as practised in the breeding program at current, will likely genetically improve growth survival.

Above, the genetic correlations ignoring common environmental effects were utilised to draw inference as to likely correlated responses in the breeding program. 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 were 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, Vandeputte, Haffray & Chevassus 2006), and the use of a partial-

342 factorial mating design has been advised, which also was used initially in this breeding program
343 (Sang, Klemetsdal, Ødegård & Gjøen 2012).

344 The common environmental effects were estimated as considerable, especially for growth survival
345 and harvest body weight (Table 2). This could be due to the problem of separation of genetic and
346 common environmental effects, but it could also be due to families being separately reared for a
347 long period, approximately 5 months in hapa, in addition to potential maternal and/or dominance
348 effects (Joshi, Woolliams, Meuwissen & Gjøen 2018). Reduction of the effect of common
349 environment should be sought by shortening the length of the spawning and tagging times, that
350 would reduce the variation in nursed time between families and thus the impact of common
351 environment. This may require upgrading of the hatchery capacity and a larger workforce. Yet
352 another alternative would be early communal rearing, but this would require application of genetic
353 markers to identify the parentage among all recorded fish.

354 Currently, there are two striped catfish breeding programs established in Vietnam that are run in
355 parallel, one aiming for improved growth and the other for improved resistance to BN. The top-
356 ranked individuals, based on estimated breeding values, from the two selected lines can be used in
357 crossbreeding to exploit both additive genetic and possible heterosis effects. To our knowledge,
358 heterosis effects have not been estimated for these traits in striped catfish, and since the breeding
359 program has not been carried out for long and also utilizes fish from the wild (Pham, Ødegård,
360 Sang, Gjøen & Klemetsdal 2020), both the inbreeding level and heterosis effects are expected to
361 be minor (Falconer & Mackay 1996). Alternatively, one breeding program could have been run
362 selecting simultaneously for both resistance to BN and growth, but which also would rely on a
363 high genetic correlation being verified between BN in the challenge and survival under a natural
364 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 program 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.

Conflict of interest statement

There is no conflict of interest.

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443 Table 1. Descriptive statistics relevant for two challenge-test experiments against susceptibility to bacillary necrosis and for two
444 growth-test experiments (both challenge and growth tests in G3-2003 and G4-2001, respectively), in which harvest body weight and
445 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[†]	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[1]	834.9	71.8

446 [†] At date of first harvest.

447 Table 2. Estimates ± SE of additive genetic sire-dam variance (σ^2_{sd}), common environmental

448 variance (σ^2_c), heritability (h^2), and fraction of variance explained by the common

449 environmental effect (c^2), for susceptibility to bacillary necrosis (BN), survival in the growth

450 performance test, and harvest body weight in two year-classes (G3-2003 and G4-2001) of

451 Mekong striped catfish, as obtained with model 1. The χ^2-test statistics for the additive genetic

452 sire-dam effect being different from zero† 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	6935.2 ± 1926.5	40.02	5949.4 ± 2793.1	0.50 ± 0.12	0.107 ± 0.051
	G4-2001	3488.1 ± 1190.5	14.43	9576.3 ± 1930.7	0.27 ± 0.09	0.186 ± 0.037

453 † Significant ($P < 0.05$) if $\chi^2 > 3.84$.

454 Table 3. Estimates ± SE of sire-dam variance (σ^2_{sd}) and heritability (h^2) for susceptibility to
455 bacillary necrosis (BN), survival in the growth performance test, and harvest body weight in two
456 year-classes (G3-2003 and G4-2001) of Mekong striped catfish, as obtained with model 2, not
457 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	11155.3 ± 1170.6	0.77 ± 0.05
	G4-2001	11281.2 ± 1115.9	0.78 ± 0.05

458

459 Table 4. Estimated genetic (r_g, above diagonal) and common environmental (r_c, below diagonal)

460 correlations ± SE between susceptibility to bacillary necrosis (BN), survival in the growth

461 performance test, and harvest body weight in two year-classes (G3-2003 and G4-2001) of

462 Mekong striped catfish, as obtained with model 1.

Trait	G3-2003			G4-2001		
	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	

463

464 Table 5. Estimated genetic correlations ± SE between susceptibility to bacillary necrosis (BN),
465 survival in the growth performance test, and harvest body weight in two year-classes (G3-2003
466 and G4-2001) of Mekong striped catfish, as obtained with model 2, not containing a common
467 environmental effect. The χ^2-test statistics for the genetic covariance being different from zero[†]
468 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	-	-	-	-	-	-

469 [†] Significant ($P < 0.05$) if $\chi^2 > 3.84$.

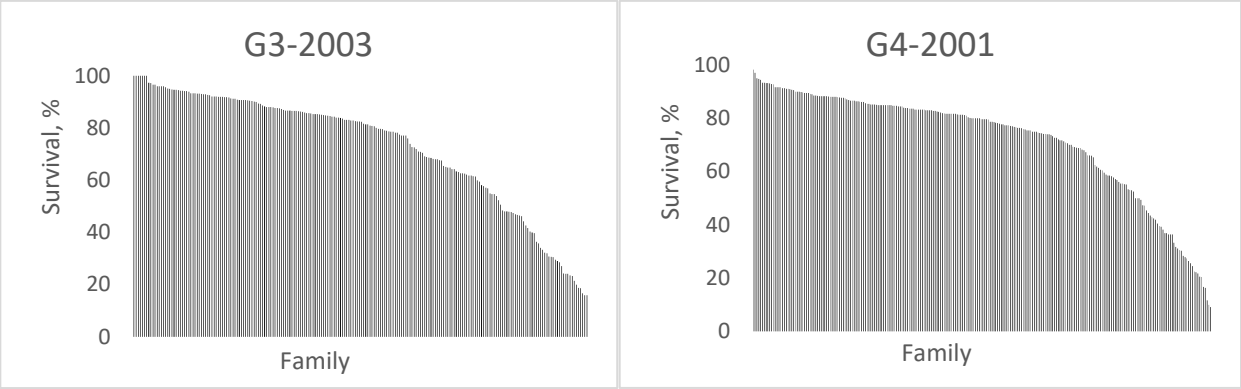

Figure 1. Average family survival in descending order for the two growth performance tests in the two year-classes, G3-2003 and G4-2001.