Fish and Shellfish Immunology 92 (2019) 209–215

Contents lists available at ScienceDirect

Fish and Shellfish Immunology

journal homepage: www.elsevier.com/locate/fsi

Full length article

# Heritability of immunity traits and disease resistance of bighead catfish, *Clarias macrocephalus* Günther, 1864

Prapansak Srisapoome[a], Satid Chatchaiphan[a], Anurak Bunnoy[b], Skorn Koonawootrittriron[c], Uthairat Na-Nakorn[a,*]

[a] Department of Aquaculture, Faculty of Fisheries, Kasetsart University, Jatujak, Bangkok, 10900, Thailand
[b] Doctoral Program in Aquaculture, Graduate School of Kasetsart University, Kasetsart University, Jatujak, Bangkok, 10900, Thailand
[c] Department of Animal Science, Faculty of Agriculture, Kasetsart University, Jatujak, Bangkok, 10900, Thailand

ARTICLE INFO

*Keywords:*
Walking catfish
*Aeromonas hydrophila*
Genetic parameter
Additive genetic variance
Immunity
Disease resistance

ABSTRACT

Disease outbreak is a major obstruction for intensive aquaculture worldwide. One of the promising solutions is genetic improvement by selective breeding, providing that a sufficient proportion of additive genetic variance (measured by heritability-$h^2$) of disease resistance traits exists. In addition, immunity traits are of interest as potential indirect targeted traits for disease resistance. In this study, the genetic parameters of resistance to *Aeromonas hydrophila* were reported for the first time in the bighead catfish, *Clarias macrocephalus* Günther, 1864 which is an important parental species for the production of the commercially important hybrid *C. macrocephalus* × *C. gariepinus*. The analyses were performed on 736 data records obtained from 74 full-sib families (31 half-sib families) produced by factorial mating design. The results showed that the heritability of survival rate after disease (*Aeromonas hydrophila*) challenge (intraperitoneal injection with 0.1 ml containing $1 \times 10^6$ CFU/ml of *A. hydrophila*) was low to moderate (0.05 ± 0.02–0.27 ± 0.15). The immune traits (bactericidal activity-BA, lysozyme activity-LA, and alternative complement activity-$ACH_{50}$) had low to moderate heritability ($h^2_{BA} = 0.05 \pm 0.02$; $h^2_{LA} = 0.16 \pm 0.04$; $h^2_{ACH50} = 0.31 \pm 0.06$) while heritability of hematocrit (Hct) was also low ($h^2_{Hct} = 0.17 \pm 0.04$). The results suggested the possibility to improve resistance to *A. hydrophila* by selection, while the possibility to use immunity traits as indirect selection criteria for disease resistance is still unclear.

## 1. Introduction

Diseases have become a major obstruction of the intensive aquaculture system practiced worldwide [1]. Among several approaches to cope with this problem, genetic improvement for disease resistant strains is a promising strategy [reviewed by Refs. [2–5]]. Disease resistance has generally been measured by directly challenging fish with the pathogen of concern. Genetic variation of disease resistance as measured by heritability has been found to be generally low (review by Ref. [6]). Although its determination is laborious and time consuming, disease resistance (based on challenge experiments) has been used in a majority of the genetic improvement programs in fishes and shellfishes [6,7].

Alternatively, immunity parameters, especially innate immunity have received much interest as indirect traits reflecting disease resistance [8,9]. However, to use these traits as selection criteria, it is crucial to have information on additive genetic variation (measured by heritability, $h^2$) of these traits as well as the correlation of these traits to survival rate. Lysozyme activity plays an important role in defending against invading pathogens [10]. A number of studies showed remarkable additive genetic variation of this trait [8,11,12], while negative correlation with survival rates was shown [8,11–14]. Alternative complement activity ($ACH_{50}$), which is an important component of the complement immunity of fishes [15] associated with genetic variation [16]. Bactericidal activity is normally involved in the presence of protective proteins in fish blood serum to inactivate invading pathogens including several highly effective molecules such as lysozymes, antimicrobial peptides, complements etc. This mechanism is found to play many crucial roles in the innate immune system of fish [17].

The bighead catfish, *Clarias macrocephalus* Günther, 1864 is an important freshwater fish in Southeast Asia in the sense that it receives high preference among consumers, but due to slow growth and susceptibility to disease it has been completely replaced by the hybrid (*C. macrocephalus* × the introduced *C. gariepinus*) [18]. Despite the lack of

* Corresponding author.
*E-mail address:* ffisurn@ku.ac.th (U. Na-Nakorn).

https://doi.org/10.1016/j.fsi.2019.05.060
Received 19 February 2019; Received in revised form 25 May 2019; Accepted 27 May 2019
Available online 07 June 2019

official records, the demand for the female bighead catfish is roughly estimated as ≈1,500 tonnes/year based on the annual production of hybrid catfish (e.g. 112,418 tonnes in 2016) [18].

A major disease that caused tremendous loss of production of *Clarias* catfishes, especially bighead catfish, was associated with the bacteria, *Aeromonas hydrophila* [19–21]. Resistance to this pathogen relies on a genetic basis [22–24] and thus suggests feasibility of genetic improvement programs. At present, a genetic improvement program has been established at Faculty of Fisheries, Kasetsart University, Bangkok, Thailand aiming to genetically improve bighead catfish for growth rate and disease resistance.

The present study provided the first report on heritability of disease resistance (based on its genetic component) and immunity traits of the genus *Clarias*. The information obtained would be of great benefit as a guideline for planning the genetic improvement of the bighead catfish and it may be applied to other *Clarias* catfish cultured worldwide.

## 2. Materials and methods

### 2.1. Fish population

The bighead catfish population used in this study was established from a crossing among two hatchery populations (Pan Panpla Farm, Amphur Muang, Nakornpathom province and Si Roy Farm, Amphur Viset Chaichan, Angthong province) and a wild population collected from Amphur Muang, Uthaithani province in Thailand. All possible crosses were made, followed by mass selection for five generations with low selection intensity. The cumulative selection response for body weight across five generations was approximately 20% (U. Na-Nakorn, unpublished data). The population has been maintained without further selection since 2008. A growth trial in 2015 showed superior growth of this population compared with a wild population [25]. The genetic variability based on six microsatellite loci (data not shown) was moderate (average number of alleles per locus = 8.62 ± 1.47; observed, and expected heterozygosity = 0.66 ± 0.38 and 0.71 ± 0.17, respectively).

### 2.2. Mating design, induced breeding, and fertilization

The factorial mating design was followed by mating a male with 2–3 females. A breeding protocol generally used in hatcheries was applied. In brief, 10-month-old brooders were selected for reproductive readiness, namely swollen and pinkish urogenital papillae of both males and females, and swollen and soft bellies in females. The injected hormones were 30 or 20 μg/kg of LH-RH analogue (Buserellin acetate, Suprefact®) for females and males, respectively, plus 5 mg/kg Motilium® (Domperidone). Approximately nine hours after injection, the injected females were stripped, while the males were sacrificed, testes removed and minced. Then the eggs were mixed with sperm solution obtained from the minced testes followed by water activation. The fertilized eggs were then spread onto a fine mesh net submersed in a 50 × 50 $cm^2$ hapa with 50 cm water depth. Induced breeding was done twice, on 3 November and 25 November, 2016, and resulted in a total of 74 full-sib and 31 half-sib families.

### 2.3. Larval rearing

Fry hatched out within 24 h and started feeding after three days. Each family was stocked as two replicates into fine-mesh hapas (50 × 50 × 50 $cm^3$), each fixed in a 5-m diameter polyethylene (PE) circular tank equipped with recirculating water. The stocking density was approximately 1,200 larvae/hapa. At about 15 days after hatching (DAH) they were transferred to 70 × 50 × 50 $cm^3$ hapas with a larger mesh-size and the stocking density was reduced to 400 fry/hapa. The feeding regime was as follows: 4–14 DAH: live *Moina* spp.; 12–30 DAH: 45% protein power feed for shrimp (Betagro Public Company Ltd., Nakorn Pathom, Thailand) with 3 days weaning period; 31–60 DAH: 40% protein commercial floating pellets for walking catfish (Betagro Public Company Ltd., Lop Buri, Thailand); 61 DAH and onward: 30% protein commercial floating pellets for walking catfish (Betagro Public Company Ltd., Lop Buri, Thailand). Feeding was done until satiation.

### 2.4. Tagging

At 120 DAH, the fish were tagged by families with visible implant elastomer tags (Northwest Marine Technology, Washington, USA). Then a sample of 30 fish/family was measured and communally reared as two replicates in 5-m-diameter-PE ponds at about 57 fish/$m^3$ at Department of Aquaculture, Faculty of Fisheries, Kasetsart University, Bangkok for growth monitoring. The results on growth performance will be presented elsewhere.

### 2.5. Data collection for immunological parameters

The remaining fish from the growth trial were acclimatized wherein a random sample of 30 fish/family were equally distributed among six 5,000-l fiberglass tanks containing 3,000-l fresh water with full aeration. They were fed twice a day with 30% protein commercial floating pellets for walking catfish (Betagro Public Company Ltd., Nakorn Pathom, Thailand) at 5% body weight. To maintain water quality, 20% of water was changed weekly. After a week of acclimatization, all fish samples were taken for measurement of immune parameters.

During 10–16 May, 2017, a 1-ml blood sample was taken from the caudal vein of each individual fish and then separated into two portions. The first portion (20 μl) was used for the hematocrit study (Hct). The remaining portion was centrifuged at 400 × *g* for 30 min. Then, the serum was taken and used for measuring lysozyme activity (LA), bactericidal activity and alternative complement activity ($ACH_{50}$) as described below.

Hct was measured by transferring the blood samples into heparinized glass capillary tubes (Modulohm A/S, Roedovre, Denmark). At least 80% of the height of the capillary tube was filled with blood, and then the tube was sealed with modeling clay and centrifuged at 12,000 × *g* for 2 min in a micro-hematocrit centrifuge (Suranaree Medical Equipment, Nakhon Ratchasima, Thailand). The percent hematocrit in each blood sample was then measured in an automated hematocrit reader (Suranaree Medical Equipment, Nakhon Ratchasima, Thailand).

Lysozyme activity of fish serum was determined using the original method described by Parry et al. [26] and the modified microtitre assay [27]. Twenty-five microliters of each fish serum was put in triplicate in a 96-well plate, and 175 μl of a suspension of *Micrococcus lysodeikticus* (0.2 mg/ml in 0.1 M phosphate buffer, pH 6.2) was added and mixed. After flash shaking, the absorbance of the suspension was read using a microplate reader (iMark™ Microplate Absorbance Reader, Bio-Rad) at 30 s and 5 min. The decrease in absorbance at 540 nm was recorded for 5 min. The concentrations of lysozyme in units/ml enzyme were calculated by the following formula: Lysozyme activity (unit/ml) = [$A_{540}$/min sample - $A_{540}$/min blank]/[(0.001)(0.1)].

For bactericidal activity, *Aeromonas hydrophila* strain AQHAH001 was obtained from the Laboratory of Aquatic Animal Health Management (LAAHM), Department of Aquaculture, Faculty of Fisheries, Kasetsart University, and was cultured in trypticase soy broth (TSB) (Himedia Laboratories, India), at 30 °C, for 24 h. Then, the bacterial suspension was centrifuged and washed using sterile phosphate buffer solution (PBS) pH 7.4 at 3000 × *g* for 10 min, three times until complete TSB removal. Bacterial suspension was adjusted in sterile PBS to reach an absorbance of 0.5 at optical density of 600 nm, which equaled to the bacterial concentration of 1 × $10^8$ colony forming units (CFU)/ml. This concentration was further used as an original stock for serial dilution with PBS (pH 7.4) to get a final target concentration of 1 × $10^5$ CFU/ml.

To investigate bactericidal activity, 50 µl of serum from each fish was gently mixed together with 50 µl of $1 \times 10^5$ CFU/ml bacterial suspension in a 1.5 ml Eppendorf tube. The control tube contained 50 µl of PBS (instead of fish serum) mixed with bacterial suspension with the same conditions as previously described. Each suspension was incubated at 30 °C for 2 h and then 100 µl suspension was dropped and thoroughly spread on trypticase soy agar (TSA) plate (Himedia Laboratories, India). Inoculated plates were incubated at 32 °C for 24 h and surviving bacterial colonies were carefully counted. Bactericidal activity of fish serum was calculated as a percentage using the following formula: bactericidal activity (%) = (number of bacterial colonies of sample/number of bacterial colonies of control) x 100.

The alternative pathway of complement activity ($ACH_{50}$) was determined according to the modified procedure of Yano (1992) [28], using rabbit red blood cells (RRBCs) (Clinical Diagnotics, Thailand). Briefly, the RRBCs were adjusted to $2 \times 10^8$ cell/ml in 0.01 M ethylene glycol tetraacetic acid-magnesium-gelatin veronal buffer (EGTA–Mg–GVB). A ten microliter sample of the target fish serum was two-fold serially diluted with PBS to get dilutions ranging from 1:2 to 1:512 in a 96-well microtiter plate. One hundred microliters of $2 \times 10^8$ cell/ml of RRBCs was added into each diluted fish serum. The 100% and 0% lysis values were provided by lysing RRBCs with 100 µl distilled water and neutralizing RRBCs with 100 µl of PBS (pH 7.4), respectively. After incubation at 30 °C for 90 min with occasional shaking, the 96-well plate was centrifuged at 1600 × *g* for 10 min at 25 °C. The supernatant in each tube was transferred to a new well plate, and then absorbance of the supernatant was measured at 540 nm. A lysis curve was obtained by log-log plotting the percentage of haemolysis against the volume of serum added. The volume yielding 50% haemolysis was determined, and in turn used for assaying the complement activity of the alternative pathway of the sample ($ACH_{50}$ value = units/ml).

### 2.6. Disease challenge

Experimental infection was carried out with permission from the Ethical Committee for Animal Experiments, Kasetsart University (Permission ID: ACKU 61-FIS-004). All experimental fish (average 24.28 ± 2.16 cm and 113.92 ± 45.05 g) in each family were transferred to the nuclease challenge unit of the LAAHM, where 10 fish/family were randomly put in each of three replicate 5000-l fiberglass tanks of a cohabitat condition and acclimatized for seven days as described above. At day 8, all fish in each tank were intraperitoneally injected with 0.1 ml containing $1 \times 10^6$ CFU/ml of *Aeromonas hydrophila* AQHAH001, which was prepared with the same protocol as above. The fish were anaesthetized with 80 mg/l of clove oil (Hong Huat Co., Ltd, Thailand) before injection. All fish were monitored closely post-exposure until day 15. Moribund and dead fish were collected daily and their elastomer dye-tag codes were read. Moribund animals were sacrificed with an overdose of clove oil, and *A. hydrophila* was isolated from liver tissue of fish on trypticase soy agar plates, which were further incubated at 32 °C for 24 h. Target bacterial identity was verified by a previously described PCR method [29].

### 2.7. Data analyses

The animal model used considered tank as fixed contemporary group effect, and animal and residual as random effects. Initially, family was included in the model as common environmental effect. Unfortunately, our data structure did not allow for the analysis. We then assume no variation among environments we provided to the individual families in our study. The used model can be described in matrix notation as follows:

$$y \text{ (or } \lambda) = Xb + Za + e,$$

where Y is the vector of the trait(s) and $\lambda$ is the vector of the binary trait (alive-dead), b is the vector of the fixed effect (tank), a is the vector of the random additive genetic effect (animal), and X and Z are incidence matrices that relate the observations to the respective effects. Four-traits analysis was applied for hematocrit, lysozyme activity, bactericidal activity and $ACH_{50}$. Phenotypic correlation (Pearson's correlation) coefficients were estimated between the least-square means of the four traits in parallel samples from the set of families.

The mean survival rate of each family and hours to death showed normal distribution and thus they were analyzed using single trait analysis without prior transformation. Survival, which was considered as binary data, was analyzed using the liability threshold model [30]. Variance components were estimated based on the animal models using the average information - restricted maximum likelihood procedure in ASReml software [31]. Then, the variance component estimates were used to calculate heritability and genetic correlations among the immunological traits. It should be noted that genetic correlation between the survival traits could not be determined because they were from the same data set. Likewise, genetic correlation of survival traits and immunological traits could not be estimated because the experimental fish were raised in different sets of environmental conditions.

## 3. Results

### 3.1. Descriptive statistics

#### 3.1.1. Immunity traits

The average hematocrit values and bactericidal activity were different among families ($P = 0.0001$, 0.0018, respectively) (Table 1). Large variation in bactericidal activity was observed both within (as shown by SD) and among families (e.g. the lowest value was 13 times lower than the top family). Lysozyme activity and alternative complement activity ($ACH_{50}$) also showed significant difference among families ($P = 0.0001$) with the overall mean of 141.06 ± 32.42 unit/ml enzyme and 308.53 ± 50.62 unit/ml serum, respectively. The top family was only 4 and 3 times greater than the lowest family for lysozyme activity and $ACH_{50}$, respectively. Distribution of family means for the immunity traits is shown in Fig. 1.

#### 3.1.2. Survival after disease challenge

The highest mortality rates of challenged fish were observed from hour 12 to 36 after injection. All the dead fish showed clinical signs caused by *A. hydrophila,* such as weakness, slower movement, swimming near the surface, dermal and fin lesions with haemorrhagic

**Table 1**
Descriptive statistics (mean ± SD, range, and significant difference among families (P-value)) for hematocrit, bactericidal activity, lysozyme activity, alternative complement activity and mean family survival (%) of bighead catfish challenged with *Aeromonas hydrophila*.

| Trait | Mean ± SD | Range | P-value |
|---|---|---|---|
| Hematocrit (%) | 34.55 ± 1.56 | 30.85 ± 3.66–38.36 ± 4.37 | 0.0001 |
| Bactericidal activity (colonies) | 603.25 ± 217.17 | 82.04 ± 106.43-1,096.25 ± 1,127.35 | 0.0018 |
| Lysozyme activity (unit/ml enzyme) | 141.06 ± 32.42 | 52.10 ± 43.53–220 ± 231.35 | 0.0001 |
| Alternative complement activity (unit/ml serum) | 308.53 ± 50.62 | 156.89 ± 60.98–532 ± 456.88 | 0.0001 |
| Survival (%) | 39.10 ± 11.97 | 15.28 ± 9.69–60.74 ± 5.98 | 0.038 |

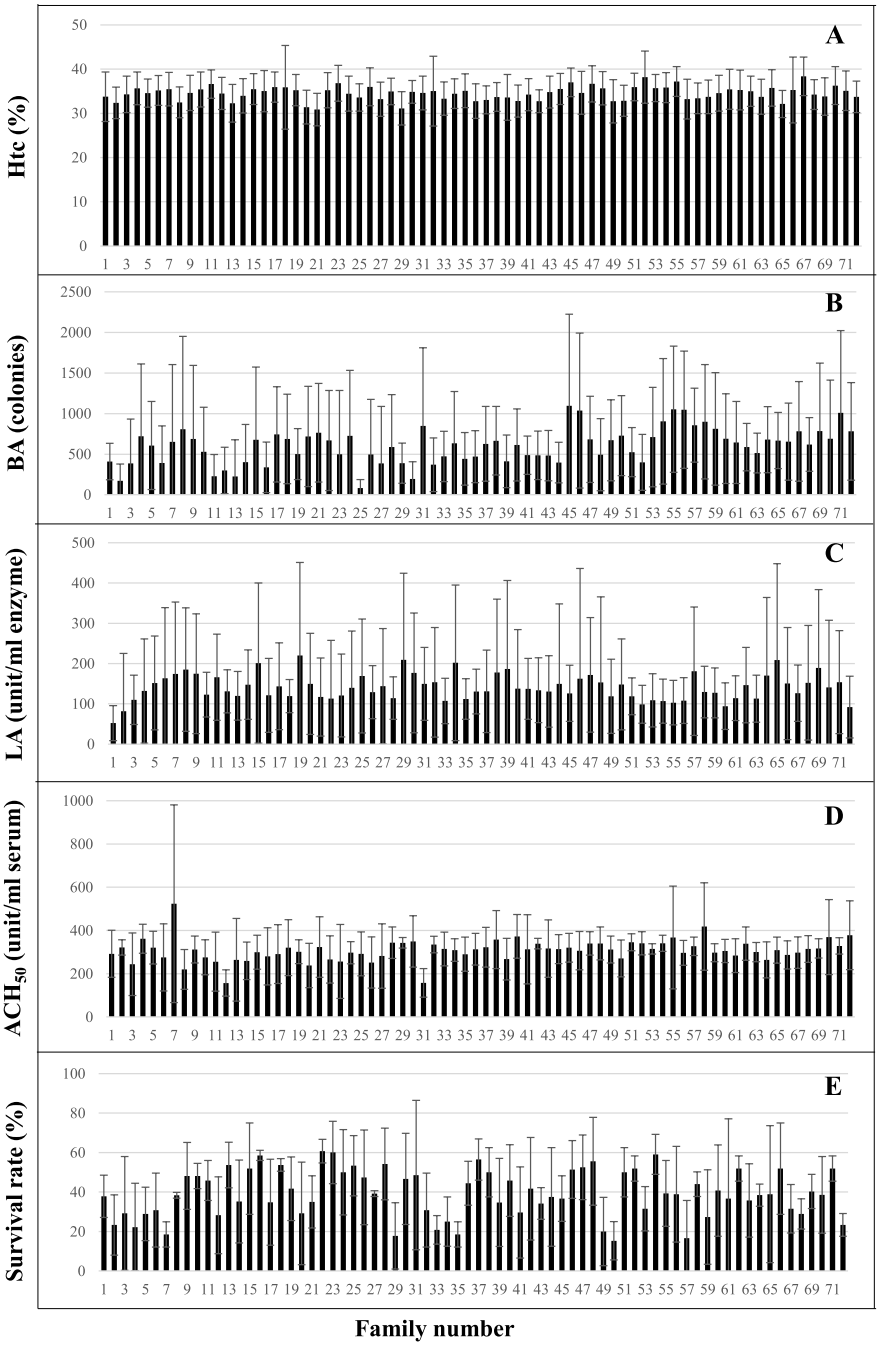


**Fig. 1.** Bar charts showing distribution of family mean of bighead catfish for immunity traits and survival rates: (A) hematocrit (Hct); (B) bactericidal activity (BA); (C) lysozyme activity (LA); (D) alternative complement activity ($ACH_{50}$); and (E) survival rate after challenge with *Aeromonas hydrophila.*

septicemia, and abdominal swelling. The family survival rates were statistically different ($P = 0.038$) with mean survival of 39.10 ± 11.97%. The survival rates were in a wide range with a minimum survival of 15.28 ± 9.69% and the highest survival of 60.74 ± 5.98% (Fig. 1).

### *3.2. Heritability, genetic correlation and phenotypic correlation of immunity traits*

The immunity traits showed a wide range of heritability; moderate for $ACH_{50}$ (0.31 ± 0.06), low for Hct and LA (0.17 ± 0.04 and 0.16 ± 0.04, respectively) and very low for BA (0.05 ± 0.02) (Table 2). Most of the heritability values (except for BA) associated with low SE. For most traits (Hct, BA and LA), the residual variance represented 84–95% of the phenotypic variance, while this proportion was low (6.8%) for $ACH_{50}$.

Genetic correlation ($g_r$) between traits, as well as phenotypic correlations ($p_r$) were all low ($g_r = -0.23 - 0.13$; $p_r = -0.01 - 0.02$) except for $g_r$ between BA and LA (0.75) (Table 3).

Among the three disease resistance traits, heritability was moderate for mean family survival rate after disease challenge (0.27 ± 0.15), but very low for hours to death and for survival as a binary trait (alive/dead) (0.05–0.06) (Table 4). All traits showed moderate SE. The residual variance associated with full-sib variation accounted for 73, 95, and 94% of the phenotypic variance of survival rate, hours to death and survival (alive/dead), respectively.

The genetic correlation between these traits could not be calculated because the data were not from the same individuals.

**Table 2**
Heritability, phenotypic variance, genetic variance and residual variance of immunity traits, hematocrit (Hct), bactericidal activity (BA), lysozyme activity (LA) and alternative complement activity ($ACH_{50}$).

| Trait | $h^2$ | phenotypic variance ($\sigma_p^2$) | genetic variance ($\sigma_a^2$) | residual variance ($\sigma_e^2$) |
|---|---|---|---|---|
| Hct | 0.17 ± 0.04 | 65.23 ± 2.20 | 6.11 ± 1.86 | 59.13 ± 2.33 |
| BA | 0.05 ± 0.02 | 148000 ± 48800 | 71330 ± 30613 | 140946 ± 51875 |
| LA | 0.16 ± 0.04 | 182000 ± 6488 | 29054 ± 7449 | 153009 ± 6916 |
| $ACH_{50}$ | 0.31 ± 0.06 | 184000 ± 790 | 5920.48 ± 1243.80 | 12478 ± 823 |

**Table 3**
Genetic correlation ($g_r$) between immunity traits, hematocrit (Hct), bactericidal activity (BA), lysozyme activity (LA) and alternative complement activity ($ACH_{50}$) (above diagonal) and phenotypic correlation between traits (below diagonal) of bighead catfish determined at 120 days after hatching.

| | Hct | BA | LA | $ACH_{50}$ |
|---|---|---|---|---|
| Hct | – | 0.13 ± 0.23 | −0.23 ± 0.17 | −0.02 ± 0.16 |
| BA | 0.01 ± 0.02 | – | 0.75 ± 0.19 | 0.12 ± 0.22 |
| LA | 0.01 ± 0.03 | −0.01 ± 0.02 | – | −0.06 ± 0.17 |
| $ACH_{50}$ | 0.02 ± 0.03 | −0.01 ± 0.02 | −0.01 ± 0.03 | – |

### 3.3. Correlation between immunity traits and survival

Pearson correlations showed non-significant correlation between immunity traits and survival (−0.150 – 0.091; $P > 0.05$). Notably low but significant correlation was observed between hematocrit and survival (r = 0.257 ± 0.031). Correlation between survival and growth was not significantly different from zero (−0.132 ± 0.290 for total length and −0.188 ± 0.130 for body weight).

## 4. Discussion

### 4.1. Immunity traits

Immunity traits have received much interest as potential selection criteria towards disease resistance. Several studies showed indirect evidence supporting genetic basis of the immune traits but without attention to genetic components of the traits [15,32–35]. In the present study we reported for the first time the heritability of three immunity traits and a blood parameter of the bighead catfish. Among the three traits studied, $ACH_{50}$ showed moderate heritability (0.31) while those of lysozyme activity and bactericidal activity were low (0.16 and 0.05, respectively). To our knowledge, heritability of $ACH_{50}$ has not been reported in fish. However, the value reported herein was comparable with the heritability of a related trait, spontaneous haemolytic activity ($h^2$ = 0.04 ± 0.09–0.23 ± 0.15, [11]; 0.03 ± 0.24, [8]), which is partially involved in the alternative complement pathway of the innate immune system. The moderate heritability suggested substantial improvement of $ACH_{50}$ in bighead catfish by selection. However, whether this trait can be used as a marker for disease resistance is beyond the capacity of our data, and relevant literature is lacking. The only available information was non-significant correlation between spontaneous haemolytic activity and disease resistance [8,11], while Wiegertjes et al. [16] reported no correlation between $ACH_{50}$ and haemolytic activity. This leaves opportunity for further studies.

Heritability of lysozyme activity, which represents effectiveness of serum in prevention of pathogen invasion by directly destroying bacterial cell walls [10], was low in our study and thus indicated low additive genetic variation of this trait in bighead catfish. Studies using other fish species reported a large range of heritability values of lysozyme activity, e.g. 0.08 ± 0.05 in Atlantic salmon, *Salmo salar* [11]; 0.27 ± 0.12 in rainbow trout, *Oncorhynchus mykiss* [12]; 0.28–0.69 in Nile tilapia, *Oreochromis niloticus* [8]. It is interesting that this trait showed negative correlation with disease resistance in many cases [8,11,13,14], whereas Sahoo et al. [9] and the present study, based on Pearson correlation, reported no correlation of lysozyme activity with resistance to *A. hydrophila*. This may suggest the possibility of using this trait as an indirect marker for disease resistance. However, Røad et al. [14] noted difficulty in using this trait as a selection criterion because lysozyme activity varied with measuring conditions (e.g. temperature, [8]; immune status of the fish at blood sampling [14]).

The low heritability of bactericidal activity was not surprising, despite the lack of previous information on this trait. It fell within the range of heritability for other immunity traits [8,9,11–13]. In regard to applying this trait as an indirect marker for resistance to *A. hydrophila*, more information is needed. However, Hollebecq et al. [7] suggested, based on a study in resistant and susceptible strains of rainbow trout, that bactericidal activity was more reliable as a marker for resistance to furunculosis than the alternative complement activity.

Hematocrit (Hct), which is a haematological indicator of basic fish health status, was within the normal range for mature *Clarias* catfish (36.0 ± 9.04 [36]) and thus reflected the good health of experimental fish, even when they were reared in experimental conditions for a long period of time. Heritability of Hct was low, and thus implied that the selection to improve this trait is difficult.

### 4.2. Genetic correlation between immune traits

We observed no genetic correlation among the three immune traits and hematocrit. This implied that these traits are controlled by different mechanisms associated with several effective molecules and components in the immune system. Our results also implied that selection on one trait would not affect the others.

### 4.3. Disease resistance

Despite the worldwide occurrence, the heritability of resistance to *A. hydrophila* has been reported in only a few fish species [22–24]. The present study showed low heritability of resistance to *A. hydrophila* based on the binary survival (alive/dead) trait and time to death, and moderate heritability for mean family survival. These were in line with realized heritability of this trait in bighead catfish ($h_R^2$ = 0.10, 0.17 in two populations [37]), rohu carp (*Labeo rohita*)

**Table 4**
Heritability of disease resistance traits in bighead catfish after being challenged with *Aeromonas hydrophila*.

| Trait | $h^2$ | phenotypic variance ($\sigma_p^2$) | genetic variance ($\sigma_a^2$) | residual variance ($\sigma_e^2$) |
|---|---|---|---|---|
| survival rate | 0.27 ± 0.15 | 336.79 ± 33.46 | 91.88 ± 52.80 | 244.91 ± 50.60 |
| hours to death | 0.05 ± 0.02 | 1279.20 ± 42.18 | 62.78 ± 26.72 | 1216.45 ± 44.87 |
| binary (alive/dead) | 0.06 ± 0.03 | 1.07 ± 0.03 | 0.07 ± 0.03 | 1.00 |

($h^2$ = 0.0165 ± 0.0042 to 0.114 ± 0.039 [22,23]) and blunt snout bream (*Megalobrama amblycephala*) ($h^2$ = 0–0.33) [24]. However, resistance to other diseases tended to show higher heritability than our estimates, e.g., 0.31 ± 0.06 to 0.45 ± 0.07 for $h^2$ of resistance against infectious pancreatic necrosis in Atlantic salmon (*Salmo salar*) [38–44]; 0.22 ± 0.07 to 0.38 ± 0.08 for resistance to *Edwardsiella tarda* in rohu (*Labeo rohita*) [45]; 0.79 ± 0.14 for resistance to koi herpes virus in common carp, *Cyprinus carpio* [23]; 0.39 ± 0.08 (day to death) for infectious pancreatic necrosis in rainbow trout [46]; 0.21 ± 0.08 to 0.36 ± 0.09 for resistance to a bacterium causing the withering syndrome disease in *Haliotis rufescens* abalone [47]. However, some exceptions were observed, e.g., 0.093 for resistance to IHNV in steelhead trout [48]; 0.15 ± 0.034 and 0.14 ± 0.045 for resistance to *Flavobacterium columnare* in Nile tilapia [49].

In the present study the disease challenge was done by IP injection. Although this method has been criticized as not being a natural mechanism of infection because it bypasses the physical barriers (e.g. mucous, skin) of the animals [50], it has been used in many disease resistance studies [46]. This is because it is reproducible [51]. Moreover, the alternative method, a cohabitat challenge, generally showed low infectivity and hence resulted in too low a mortality rate, e.g. in rohu carp (*Labeo rohita*) [9] and rainbow trout [46]. Therefore, at present, the IP injection seems to be an acceptable method to measure resistance to *A. hydrophila*. This method has been used in a selection program for disease resistance in bighead catfish and resulted in realized heritability of 0.10–0.17 [37].

The present study demonstrates that there was additive genetic variation for resistance of bighead catfish to *Aeromonas hydrophila* and thus, selection to improve this trait is possible. However, according to the low heritability, high precision selection methods should be used [52]. Our result was supported by Na-Nakorn et al. [37] who failed to improve resistance to *A. hydrophila* in bighead catfish by mass selection.

However, it should be noted that the heritability of disease resistance not only varies with species and population [52], but also can occur due to the dose of pathogen used for challenge (e.g. $h^2$ = 0.05 and 0.50, respectively, for the dosages of $10^{2.55}$ and $10^{3.55}$ $TCID_{50}$/ml infectious hematopoietic necrosis virus-IHNV [53]) and the parameters used to define resistance, as in this study and others [22,54].

### 4.4. Correlation between disease resistance (survival after disease challenge) and immune traits or growth

Our results could not provide a clear answer of whether immune traits genetically correlate with survival after disease challenge. The Pearson correlation showed neither a correlation between mean survival rate after disease challenge and immune traits nor between mean survival rate and growth traits.

## 5. Conclusion and recommendation

The conclusions were reached that

1) The heritability of immunity traits was low for bactericidal activity and lysozyme activity (0.05 ± 0.02 and 0.16 ± 0.04, respectively) and moderate for $ACH_{50}$ (0.31 ± 0.06). No genetic correlation between these traits was observed except high genetic correlation between lysozyme activity and bactericidal activity. Heritability for hematocrit was low (0.17 ± 0.04) and showed no genetic correlation with the immunity traits.
2) The heritability of survival traits after challenge with *Aeromonas hydrophila* was moderate based on mean family survival (0.27 ± 0.15) but very low when measured as hours to death (0.05 ± 0.02) and as a binary trait (0.06 ± 0.03).
3) The moderate heritability of resistance to *A. hydrophila* (survival after challenge) implied that this population can be improved to increase disease resistance by selection method based on mean family survival. However, the possibility of using the immunity traits as selection criteria for disease resistance was inconclusive.

## CRediT authorship contribution statement

**Prapansak Srisapoome:** Conceptualization, Methodology, Data curation, Writing - review & editing. **Satid Chatchaiphan:** Methodology, Formal analysis. **Anurak Bunnoy:** Methodology, Data curation. **Skorn Koonawootrittriron:** Supervision, Validation, Writing - review & editing. **Uthairat Na-Nakorn:** Conceptualization, Writing - original draft, Writing - review & editing, Funding acquisition.

## Acknowledgements

Funding: This work was supported by Thailand Research Fund and Betagro Science Center Co. Ltd. (Contract No. DPG5980003; Distinguished Research Professor TRF FY 2016) awarded to UN. For their technical support, we thank staff and students of the Fish Genetics Laboratory, Laboratory of Aquatic Animal Health Management, Faculty of Fisheries, Kasetsart University, especially Dr. Pattanapon Kayansanruaj. Thanks also to Mr. David John Anderson who provided English editing of the manuscript.

## References

[1] K.D. Lafferty, C.D. Harvell, J.M. Conrad, C.S. Friedman, M.L. Kent, A.M. Kuris, E.N. Powell, D. Rondeau, S.M. Saksida, Infectious diseases affect marine fisheries and aquaculture economics, Annu. Rev. Mar. Sci. 7 (2015) 471–496.
[2] A. Storset, C. Strand, M. Wetten, S. Kjøglum, A. Ramstad, Response to selection for resistance against infectious pancreatic necrosis in Atlantic salmon (*Salmo salar* L.), Aquaculture 272 (2007) 62–68.
[3] S. Kjøglum, M. Henryon, T. Aasmundstad, I. Korsgaard, Selective breeding can increase resistance of Atlantic salmon to furunculosis, infectious salmon anaemia and infectious pancreatic necrosis, Aquacult. Res. 39 (2008) 498–505.
[4] T.D. Leeds, J.T. Silverstein, G.M. Weber, R.L. Vallejo, Y. Palti, C.N. Rexroad III, J. Evenhuis, S. Hadidi, T.J. Welsh, G.D. Wiens, Response to selection for bacterial cold water disease resistance in rainbow trout, J. Anim. Sci. 88 (2010) 1936–1946.
[5] T. Gjedrem, Disease resistant fish and shellfish are within reach: a review, J. Mar. Sci. Eng. 3 (2015) 145–153.
[6] J. Ødegård, M. Baranski, B. Gjerde, T. Gjedrem, Methodology for genetic evaluation of disease resistance in aquaculture species: challenges and future prospects, Aquacult. Res. 42 (2011) 103–114.
[7] M.-G. Hollebecq, B. Faivre, C. Bourmaud, C. Michel, Spontaneous bactericidal and complement activities in serum of rainbow trout (*Oncorhynchus mykiss*) genetically selected for resistance or susceptibility to furunculosis, Fish Shellfish Immunol. 5 (1995) 407–426.
[8] J. Chiayvareesajja, K.H. Røed, A.E. Eknath, J.C. Danting, M.P. De Vera, H.B. Bentsen, Genetic variation in lytic activities of blood serum from Nile tilapia and genetic associations with survival and body weight, Aquaculture 175 (1999) 49–62.
[9] P.K. Sahoo, K. Das Mahapatra, J.N. Saha, A. Barat, M. Sahoo, B.R. Mohanty, B. Gjerde, J. Ødegård, M. Rye, R. Salte, Family association between immune parameters and resistance to *Aeromonas hydrophila* infection in the Indian major carp, *Labeo rohita*, Fish Shellfish Immunol. 25 (2008) 163–169.
[10] O. Lie, O. Evensen, A. Sorensen, E. Froysadal, Study on lysozyme activity in some fish species, Dis. Aquat. Org. 6 (1989) 1–5.
[11] K.H. Røed, K.T. Fjalestad, A. Stromsheim, Genetic variation in lysozyme activity and spontaneous haemolytic activity in Atlantic salmon (*Salmo salar*), Aquaculture 114 (1993) 19–31.
[12] K.H. Røed, H.J.S. Larsen, R.D. Linder, T. Refstie, Genetic variation in lysozyme activity in rainbow trout (*Oncorhynchus mykiss*), Aquaculture 109 (1993) 237–244.
[13] T. Lund, T. Gjedrem, H.B. Bentsen, D.M. Eide, H.J.S. Larsen, K.H. Røed, Genetic variation in immune parameters and associations to survival in Atlantic salmon, J. Fish Biol. 46 (1995) 748–758.
[14] K.H. Røed, S.E. Fevolden, K.T. Fjalestad, Disease resistance and immune characteristics in rainbow trout (*Oncorhynchus mykiss*) selected for lysozyme activity, Aquaculture 209 (2002) 91–101.
[15] S. Nayak, I. Portugal, D. Zilberg, Analyzing complement activity in the serum and body homogenates of different fish species, using rabbit and sheep red blood cells, Vet. Immunol. Immunopathol. 199 (2018) 39–42.
[16] G.F. Wiegertjes, T. Yano, W.B. van Muiswinkel, Estimation of the genetic variation in complement activity of common carp (*Cyprinus carpio* L.), Vet. Immunol. Immunopathol. 37 (1993) 309–319.
[17] J.D. Biller-Takahashi, L.S. Takahashi, F. Pilarski, F.A. Sebastião, E.C. Urbinati, Serum bactericidal activity as indicator of innate immunity in pacu *Piaractus mesopotamicus* (Holmberg, 1887), Arq. Bras. Med. Vet. Zootec. 65 (2013) 1745–1751.
[18] FAO, Fishery, Aquaculture Statistics, Global Aquaculture Production 1950-2016 (FishstatJ), FAO Fisheries and Aquaculture Department [online], 2018 Rome.

[19] S. Boonyaratpalin, Bacterial pathogens involved in the epizootic ulcerative syndrome of fish in Asia, Songklanakarin J. Sci. Technol. 9 (1987) 495–502.
[20] S.L. Angka, T.J. Lam, Y.M. Sin, Some virulence characteristics of *Aeromonas hydrophila* in walking catfish (*Clarias gariepinus*), Aquaculture 130 (1995) 103–112.
[21] T. Aoki, Motile aeromonads (*Aeromonas hydrophila*), in: P.T.K. Woo, D.W. Bruno (Eds.), Fish Diseases and Disorders, Viral, Bacterial and Fungal Infections, vol. 3, CAB International, Wallingford, 1999, pp. 427–453.
[22] K. Das Mahapatra, B. Gjerde, P.K. Sahoo, J.N. Saha, A. Barat, M. Sahoo, B.R. Mohantya, J. Ødegård, M. Rye, R. Salte, Genetic variations in survival of rohu carp (*Labeo rohita*, Hamilton) after *Aeromonas hydrophila* infection in challenge tests, Aquaculture 279 (2008) 29–34.
[23] J. Ødegård, I. Olesen, P. Dixon, Z. Jeney, H.-M. Nielsen, K. Way, C. Joiner, G. Jeney, L. Ardó, A. Rónyai, B. Gjerde, Genetic analysis of common carp (*Cyprinus carpio*) strains. II: resistance to koi herpesvirus and *Aeromonas hydrophila* and their relationship with pond survival, Aquaculture 304 (2010) 7–13.
[24] X.-M. Xiong, Y.-L. Chen, L.-F. Liu, W. Wang, N.A. Robinson, Z.-X. Gao, Estimation of genetic parameters for resistance to *Aeromonas hydrophila* in blunt snout bream (*Megalobrama amblycephala*), Aquaculture 479 (2017) 768–773.
[25] D.A. Muiocha, S. Onming, U. Na-Nakorn, Growth performance, genetic diversity and morphometric traits of an introduced wild- and a hatchery population of *Clarias macrocephalus* Günther, 1864, J. Fish. Envi. 41 (2) (2017) 1–19.
[26] R.M. Parry Jr., R.C. Chandan, K.M. Shahani, A rapid and sensitive assay of muramidase, Proc. Soc. Exp. Biol. Med. 119 (2) (1965) 384–386.
[27] N.E. Demers, C.J. Bayne, The immediate effects of stress on hormones and plasma lysozyme in rainbow trout, Dev. Comp. Immunol. 21 (4) (1997) 363–373.
[28] T. Yano, Assays of haemolytic complement activity, in: J.S. Stolen, T.C. Fletcher, D.P. Anderson, S.L. Kaatari, A.F. Rowley (Eds.), Techniques in Fish Immunology, vol. 2, SOS, USA, 1992, pp. 131–141.
[29] A. Sarkar, M. Saha, P. Roy, Identification and typing of *Aeromonas hydrophila* through 16S rDNA-PCR fingerprinting, Aquacult. Res. Dev. 3 (2012) 146.
[30] K.K. Holst, T.H. Scheike, J.B. Hjelmborg, The liability threshold model for censored twin data, Comput. Stat. Data Anal. 93 (2016) 324–335.
[31] A.R. Gilmour, B.J. Gogel, B.R. Cullis, S.J. Welham, R. Thompson, ASReml User Guide Release 1.0, VSN International Ltd, Hemel Hempstead, HP1 1ES, UK, 2002.
[32] M.J. Marsden, L.C. Freeman, D. Cox, C.J. Secombes, Non-specific immune responses in families of Atlantic salmon (*Salmo salar*) exhibiting differential resistance to furunculosis, Aquaculture 146 (1996) 1–16.
[33] S.K. Balfry, M. Shariff, G.K. Iwama, Strain differences in non-specific immunity of tilapia *Oreochromis niloticus* following challenge with *Vibrio parahaemolyticus*, Dis. Aquat. Org. 30 (1997) 77–80.
[34] C. Michel, M.G. Hollebecq, Independence of phagocytic activity and susceptibility to furunculosis in families of rainbow trout (*Oncorhynchus mykiss*) genetically selected for differential resistance, Fish Shellfish Immunol. 9 (1999) 81–93.
[35] M.R. Sarder, K.D. Thompson, D.J. Penman, B.J. McAndrew, Immune responses of Nile tilapia (*Oreochromis niloticus* L.) clones: I. Non-specific responses, Dev. Comp. Immunol. 25 (1) (2001) 37–46.
[36] N.O. Erhunmwunse, M.O. Ainerua, Characterization of some blood parameters of African catfish (*Clarias gariepinus*), Am.-Eurasian J. Toxicol. Sci. 5 (2013) 72–76.
[37] U. Na-Nakorn, S. Chantsawang, W. Tarnchalanukit, Response to mass selection for resistance to *Aeromonas hydrophila* in *Clarias microcephalus*, J. Appl. Aquac. 4 (4) (1994) 65–74.
[38] D.R. Guy, S.C. Bishop, S. Brotherstone, A. Hamilton, R.J. Roberts, B.J. McAndrew, J.A. Woolliams, Analysis of the incidence of infectious pancreatic necrosis mortality in pedigreed Atlantic salmon, *Salmo salar* L., populations, J. Fish Dis. 29 (11) (2006) 637–647.
[39] D.R. Guy, S.C. Bishop, J. Woolliams, S. Brotherstone, Genetic parameters for resistance to Infectious pancreatic necrosis in pedigreed Atlantic salmon (*Salmo salar*) post-smolts using a reduced animal model, Aquaculture 290 (2009) 229–235.
[40] M. Wetten, T. Aasmundstad, S. Kjøglum, A. Storset, Genetic analysis of resistance to infectious pancreatic necrosis in Atlantic salmon (*Salmo salar* L.), Aquaculture 272 (2007) 111–117.
[41] A. Storset, C. Strand, M. Wetten, S. Kjøglum, A. Ramstad, Response to selection for resistance against infectious pancreatic necrosis in Atlantic salmon (*Salmo salar* L.), Aquaculture 272S1 (2007) S62–S68.
[42] S. Kjøglum, M. Henryon, T. Aasmundstad, I. Korsgaard, Selective breeding can increase resistance of Atlantic salmon to furunculosis, infectious salmon anaemia and infectious pancreatic necrosis, 39 (5) (2008) 498–505.
[43] A.A. Gheyas, R.D. Houston, J.C. Mota-Velasco, D.R. Guy, A.E. Tinch, C.S. Haley, J.A. Woolliams, Segregation of infectious pancreatic necrosis resistance QTL in the early life cycle of Atlantic salmon (*Salmo salar*), Anim. Genet. 41 (5) (2010) 531–536.
[44] A.A. Gheyas, C.S. Haley, D.R. Guy, A. Hamilton, A.E. Tinch, J.C. Mota-Velasco, J.A. Woolliams, Effect of a major QTL affecting IPN resistance on production traits in Atlantic salmon, Anim. Genet. 41 (6) (2010) 666–668.
[45] B.R. Mohanty, P.K. Sahoo, K.D. Mahapatra, J.N. Saha, Differential resistance to edwardsiellosis in rohu (*Labeo rohita*) families selected previously for higher growth and/or aeromoniasis-resistance, J. Appl. Genet. 53 (1) (2012) 107–114.
[46] R. Flores-Mara, F.H. Rodríguez, R. Bangera, J.P. Lhorente, R. Neira, S. Newmand, J.M. Yáñez, Resistance against infectious pancreatic necrosis exhibits significant genetic variation and is not genetically correlated with harvest weight in rainbow trout (*Oncorhynchus mykiss*), Aquaculture 479 (2017) 155–160.
[47] K. Brokordt, R. González, W. Farías, F.E. Winkler, K.B. Lohrmann, First insight into the heritable variation of the resistance to infection with the bacteria causing the withering syndrome disease in *Haliotis rufescens* abalone, J. Invertebr. Pathol. 150 (2017) 15–20.
[48] M.S. Brieuc, M.K. Purcell, A.D. Palmer, K.A. Naish, Genetic variation underlying resistance to infectious hematopoietic necrosis virus in a steelhead trout (*Oncorhynchus mykiss*) population, Dis. Aquat. Org. 117 (1) (2015) 77–83.
[49] P. Wonmongkol, S. Sukhavachana, K. Ampolsak, P. Srisapoome, T. Suwanasopee, S. Poompuang, Genetic parameters for resistance against *Flavobacterium columnare* in nile tilapia *Oreochromis niloticus* (Linnaeus, 1758), J. Fish Dis. 41 (2) (2018) 321–328.
[50] D. Zhang, G.S.A. Moreira, C. Shoemaker, J.C. Newton, D.-H. Xu, Detection and quantification of virulent *Aeromonas hydrophila* in channel catfish tissues following waterborne challenge, FEMS Microbial. Lett. 363 (9) (2016).
[51] J.F. Sovenyi, M. Bercsenyi, J. Bakos, Comparative examination of susceptibility of two genotypes of carp (*Cyprinus carpio* L.) to infection with *Aeromonas salmonicida*, Aquaculture 70 (1988) 301–308.
[52] D.S. Falconer, T.F.C. Mackay, Introduction to Quantitative Genetics, fourth ed., Pearson Education Ltd., Essex, England, 1996, p. 464p.
[53] S. Yamamoto, I. Saniyo, R. Sato, M. Kohara, H. Tahara, Estimation of the heritability for resistance to infectious haematopoitic necrosis in rainbow trout, Nippon Suisan Gakk 57 (1991) 1519–1522.
[54] J. Antonello, C. Massault, R. Franch, C. Haley, C. Pellizzari, G. Bovo, T. Patarnello, D.-J. de Koning, L. Bargelloni, Estimates of heritability and genetic correlation for body length and resistance to fish pasteurellosis in the gilthead sea bream (*Sparus aurata* L.), Aquaculture 298 (2009) 29–35.