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## Selective breeding of saline-tolerant striped catfish (*Pangasianodon hypophthalmus*) for sustainable catfish farming in climate vulnerable Mekong Delta, Vietnam

Hai Dao Minh<sup>a,b</sup>, Yen Duong Thuy<sup>b</sup>, Liem Pham Thanh<sup>b</sup>, Tam Bui Minh<sup>b</sup>, Son Vo Nam<sup>b</sup>,  
Huong Do Thi Thanh<sup>b</sup>, Hang Bui Thi Bich<sup>b</sup>, Tran Nguyen Thi Ngoc<sup>b</sup>, Hieu Dang Quang<sup>c</sup>,  
Patrick Kestemont<sup>c</sup>, Phuong Nguyen Thanh<sup>b</sup>, Frédéric Farnir<sup>a,\*</sup>

<sup>a</sup> FARAH/Sustainable Animal Production, Faculty of Veterinary Medicine, University of Liege (B43), 4000 Liege, Belgium

<sup>b</sup> College of Aquaculture and Fisheries, Can Tho University, Viet Nam

<sup>c</sup> Research Unit in Environmental and Evolutionary Biology, Institute of Life, Earth & Environment, University of Namur, rue de Bruxelles 61, B-5000 Namur, Belgium

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### ABSTRACT

Striped catfish (*Pangasianodon hypophthalmus*), a freshwater species cultured mainly in the Mekong Delta region in Southern Vietnam, is facing a significant challenge due to salinity intrusion as a result of climatic changes. Given these evolving environmental conditions, selecting new strains with a higher salinity tolerance could make production of striped catfish economically feasible in brackish environments. In this study, we carried out a selection program aimed at developing a striped catfish strain able to survive and grow fast in a saline environment. To implement the selection program, we first collected males and females from different provinces in the Mekong delta. We next performed a factorial cross of these breeders to produce half- and full-sib families. When fish reached fry stage (47 dph), we put them in a saline environment (10 ppt) and subsequently kept 50 % of the fastest-growing fish after 143 days post hatching (dph). We repeated this mass selection procedure after 237 dph and 340 dph. We maintained in parallel a randomly selected group in saline conditions and a group of fish reared in freshwater to serve as controls. After crossing the selected individuals, we performed several tests on the next generation of fish to evaluate the effectiveness of selection after one generation in saline conditions. Average direct responses to selection were 18.0 % for growth and 11.4 % for survival rate after one generation of selection. We estimated a moderate realized heritability (0.29) for body weight. The genetic gains obtained in our study for body weight and survival rate after one generation of selection under saline conditions suggest that selection can be effective to improve ability of striped catfish to cope with saline stress. We conclude that our selection program has succeeded in developing a productive strain of striped catfish with better tolerance to salinity.

### 1. Introduction

Striped catfish (*Pangasianodon hypophthalmus*) has become one of the most important farmed freshwater indigenous species in the aquaculture sector in the Mekong Delta since artificial breeding was developed successfully in the mid-1990s (Nguyen et al., 2013). By the year 2018, catfish production reached nearly 1.42 million tons, (for a total production area of approximately 5400 ha) and the export value was 2.26 billion USD (contributing about 1 % GDP of Vietnam), with exports to more than 140 countries and territories (VASEP, 2019). De Silva and

Nguyen (2011) estimated that this sector employs more than 180,000 Vietnamese, primarily women, in the processing sector.

However, the Mekong Delta is projected to be heavily affected by salinity intrusion as a result of climate change impacts (IPCC, 2007). This region is one of three extensive low-lying regions in Vietnam, with a maximum elevation of less than 4.0 m above mean sea level, and current models predict a 1 m rise in sea level in this century (IPCC, 2007; Thi et al., 2015). If these predictions are realized, approximately 1000 km<sup>2</sup> of cultivated land and farming area in Vietnam will become salt marshland. In total, seawater is likely to inundate 15,000–20,000 km<sup>2</sup> of

\* Corresponding author.

E-mail address: [f.farnir@uliege.be](mailto:f.farnir@uliege.be) (F. Farnir).

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the Mekong Delta, with a loss of 76 % of arable land (De Silva and Soto, 2009).

Striped catfish culture is largely dependent on the availability of an abundant supply of fresh water from the Mekong river (Nguyen and Dang, 2010). Therefore, the increasing salinization of freshwater areas will negatively impact striped catfish culture (Sebesvari et al., 2011). The Mekong Delta has already faced serious drought and saline water intrusion into inland areas (up to 55–100 km from the coastline) in 2016 and 2020, causing damage to aquaculture, rice and fruit production and important economic losses (Hai et al., 2020; VAWR, 2017). These major environmental changes have a significant direct impact the growth rate of catfish (Hossain et al., 2021). Selectively breeding a salinity-tolerant strain of striped catfish is a promising approach to maintaining sustainable catfish aquaculture in the region in the current changing environmental context.

Selective breeding has been successful in improving the salinity tolerance of tilapias (euryhaline fish) (Jaspe and Caipang, 2011), and a few pioneer studies suggest that substantial additive genetic variance exists for growth rate and survival in saline environments (Tayamen et al., 2010; Tran et al., 2008). Cnaani and Hulata (2011) argued that the salinity tolerance of the fish is the overall fitness or productivity of fish in a saline environment resulting from a combination of quantitative traits including metabolism, growth, osmoregulation, immunocompetence and fecundity, each of which is influenced by multiple genes. Several selection programs on tilapias have all also evaluated salinity tolerance based on growth and survival data (Cnaani and Hulata, 2011; Tayamen et al., 2010).

Only one published study has described a selection program for striped catfish, and it focused growth in freshwater (Vu et al., 2019). To our knowledge, no such selection program has been carried out in saline conditions. On the other hand, several studies have investigated the effects of increased salinity on growth performance of striped catfish.

Although there is no published data demonstrating that salinity tolerance could be increased by selection on striped catfish, differential growth and survival within groups facing the same salinity conditions observed in previous studies (Huong and Quyen, 2012; Nguyen et al., 2014) might suggest the presence of genetic components involved in the salinity tolerance traits. To test this genetic hypothesis, we have conducted a selection experiment in saline conditions. Therefore, the main objectives of the present study were to assess the efficiency of selection on the salinity tolerance of striped catfish in terms of growth and survival after one generation of selection in 10 ppt brackish water. In addition, as a first step towards a better understanding of the involved genetic mechanisms, we have investigated physiological parameters differences between the selected and control strains.

## 2. Materials and methods

The fieldwork of the study was conducted in the College of Aquaculture and Fisheries (CAF), Can Tho University and three striped catfish hatcheries in Viet Nam from July/2017 to May/2021. The analysis of the genetic data was performed at Liege University, Belgium.

### 2.1. The base population (G0) and production of the first generation (G1)

We obtained striped catfish broodstock (base population G0) from three different hatcheries located in An Giang, Vinh Long, and Can Tho provinces (freshwater areas), with 10 unrelated males and 10 unrelated females from each hatchery (*i.e.* 60 broodstock total), weighing between 5 and 7 kg on average. Principally, the base population individuals had to be healthy without visible injury or deformities. We collected a piece of fin tissue (~1 cm<sup>2</sup>) from each fish and preserved these biopsies in 95 % ethanol to extract DNA for parentage assignment.

We produced the first generation (G1) using a full factorial cross of the G0 broodstock. each of 30 females (~3000 eggs of each female) being individually crossed with each of 30 males in 1:1 crosses to create

900 full-sib families. Spawning was induced by injection with human chorionic gonadotrophin with a total dose of 5500 UI/kg weight of female and 1000 UI/kg for male. A dry fertilization process was used, where eggs and milt were mixed gently. The fertilization solution (3 g urea and 4 g salt in 1 L of water) was added to the mixture of eggs and milt to trigger fertilization after 4 min. The fertilized eggs were then transferred into family-specific plastic boxes for incubation. The fertilized eggs started to hatch 24 h after fertilization. Fertilization rates varied from 83 % to 90 % and the hatching rates were 61–73 % (except for one female whose eggs did not hatch). From each family, we selected 2000 good quality larvae (no deformities, uniform size, swimming actively and responding to external stimuli quickly) for nursing. Larvae from all mixed families were transferred to two rearing earthen ponds within 15 h after hatching (leading to 1,740,000 larvae in total) under freshwater condition. After 47 days post-hatching (dph), 21,550 randomly chosen fry were transferred from the nursing ponds to CAF, Can Tho University. Twenty-one thousand fry were put into a recirculating aquaculture system (RAS - 700 m<sup>3</sup> water volume) to start the selection process under saline condition. Simultaneously, the remaining fish (550 fry) were transferred to another RAS system (50 m<sup>3</sup> water volume) to be reared in freshwater until maturation. This group (referred to as “freshwater group”) represents normal broodstock sources currently used in Mekong Delta (Fig. 1).

### 2.2. Selection rounds

In RAS (700 m<sup>3</sup> water volume), we progressively (1 ppt/day) raised the salinity to 10 ppt and maintained at this level during the whole selection experiment. Fish dying during the acclimation or later were removed from the protocol, meaning that survival was the first selected trait. When fish reached 148 dph, they were randomly divided into two groups of equal sizes. The fish in each group were distributed equally into three hapas (12 × 4 × 4 m). At this time, a first selection round was carried out. In the first group (referred to as the “selected group”), the fish were individually weighed and approximately 50 % of the heaviest fish were kept for the next rounds of selection. In parallel, the second group (referred to as “random group”) underwent a random selection of the fish until reaching the same biomass as in the selected group. These fish served as a control population for the selected group (to see the effect of selecting heaviest fish in saline conditions on the progeny) and for the freshwater group (to see the effect of the saline conditions on various traits). The fish not selected in either group were removed from

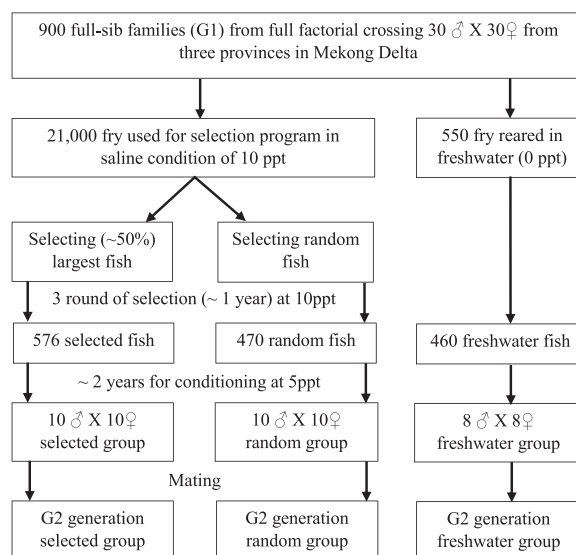


Fig. 1. Schematic representation of mating design for obtaining the experimental fish.

the RAS system. We monitored all fish from the selected and random groups to keep them in the same conditions (feed, biomass).

Two additional rounds of selection took place 3 months later (237 dph) and 6 months later (340 dph) along the same lines as for the first round (Fig. 1). The growth rate of striped catfish is fast during the first year of age, and then slows down. In practice, grow-out farmers often harvest striped catfish when they are around one-year old and reach a weight of around 1 kg (De Silva and Nguyen, 2011; Nguyen and Dang, 2010; Phan et al., 2009). Since growth profiles might vary among fish during the first year, and because we wanted to maintain biomass conditions compatible with the size of our RAS system, we have used this 3 stages selection based on biomass (Fig. 1). Similar protocols have been used in various other studies such as on brown trout (*Salmo trutta fario*) (Bernard et al., 2004) and on rainbow trout (*Oncorhynchus mykiss*) (Le Boucher et al., 2012). After the third selection round, all remaining fish were tagged using Passive Integrated Transponders (PIT) tags. These PIT-tags were injected into muscle of fish and corresponding DNA samples were collected.

In order to prevent any reduction in gamete quality potentially associated with the saline stress and differences in gamete quality between selected, random and freshwater breeders and growth performance of their offspring, we then decreased the salinity was to 5 ppt and maintained this level until the fish matured. After two years in these conditions, the three-year-old fish from each of the three groups had matured and could be used as parents for the next generation (G2).

### 2.3. Parentage assignment and estimation of heritability in G1 generation

We implemented and used a new algorithm allowing parentage assignment in the three groups based on very low sequencing depth (SD) from the offspring (SD between 0.2 and 0.5) and the 60 possible parents (SD = 1.0). Briefly, this method is based on a likelihood approach to identify the most suitable (*i.e.* likely) set of parents for each offspring: the method computes the likelihood for each possible parental couple, keeps the most likely one and checks whether this best couple is significantly more likely than any other possible couple. Significant couples are considered as the putative parents for this offspring. The parentage information was used to identify couples of unrelated individuals (to avoid inbreeding) and to mate them to produce the next generation (G2).

### 2.4. Production of the G2 generation

We wanted to assess the effects of the selection in the previous generation on the performances of the offspring in the next generation on one hand, and of the rearing in saline conditions on the other hand. To that end, we produced a G2 generation using random couples from each of the three G1 groups (*i.e.* from the selected group (10♂ X 10♀), the random group (10♂ X 10♀), and the freshwater group (8♂ X 8♀) (Fig. 1)). We pooled the same number of fertilized eggs per family within each group and incubated them together. We subsequently reared the three groups in identical freshwater environments under optimal conditions for the development of striped catfish. At each developmental stage, we subsampled a set of individuals from the three groups to challenge them with various salinity levels. Larvae hatched after 29–34 h and were transferred to three 2 m<sup>3</sup> outdoor composite tanks for nursing. The larvae from each group were nursed separately in composite tanks with the same density of 5 individuals/L. The larvae were fed Rotifer, *Artemia* nauplii, and *Moina* in the first 5 days. After that period, a high protein flake diet (40 % protein) was used in combination with *Moina* until 4–5 weeks of age. Subsequently, fries (0.6–0.8 g of body weight) from each group were transferred to 10 m<sup>3</sup> outdoor composite tanks, where they were raised until fingerling stage with an average body weight of 20 g. Rearing experiments were divided into four distinct periods according to each developmental stage of striped catfish: embryonic stage, larva to fry stage, fry to fingerling stage and

fingerling to adult stage, as described in the next sections.

### 2.5. Evaluation of salinity tolerance of striped catfish after selection

To assess the salinity tolerance of striped catfish, the G2 generation from three groups were challenged under different levels of salinity for the various developmental stages of striped catfish.

#### 2.5.1. Experiment on the embryonic stage

In this stage, we measured salinity tolerance using gastrulation rate (*i.e.* percentage of eggs reaching the gastrula stage) and hatching rate (higher rates meaning higher tolerance), deformity rate (lower rate meaning higher tolerance), and hatching time (longer times corresponding to lower tolerance). To evaluate these traits, fertilized eggs from the three groups were transferred from freshwater to saline water and incubated in five different salinities (0, 2.5, 5, 7.5 and 10 ppt). Three hundred fertilized eggs from each group were randomly allocated to 60 plastic boxes (five salinity levels x three groups x four replicates). The salinity in all boxes was maintained using RAS systems where well-aerated water was supplied continuously for all boxes. Saline water was produced by mixing deep sea water (90 ppt seawater, from Bac Lieu province, a coastal province in Vietnam) with tap water in 1000 L composite tanks to correspond to the desired salinity treatments. Eight hours after incubation, transparent living eggs were separated from the opaque undeveloped ones and counted. The undeveloped eggs were removed immediately to avoid fungal infection. In each box, the gastrulation rate was calculated as the ratio of the number of fertilized eggs reaching the gastrula stage to the total number of eggs, multiplied by 100. The hatching rate was calculated as the ratio of the number of hatched larvae to the number of fertilized eggs, multiplied by 100. The deformity rate was calculated as the ratio of the number of deformed larvae to the total number of larvae, multiplied by 100. The hatching time was defined as the total time from the fertilization to the time when 50 % of larvae had hatched out.

We monitored water quality parameters such as dissolved oxygen (DO), pH, temperature, and salinity every five hours using a multiple-parameter water quality meter (DKK-TOA, WQC-24, Japan). During the incubating period, water temperatures were similar among RAS systems and fluctuated from 26.3 to 28.4 °C. Values of pH were stable in the range from 8.2 to 8.6. DO was 5.1–5.6 mg L<sup>-1</sup>.

#### 2.5.2. Experiment on larval to fry stages

In this and the subsequent experiments, we considered survival and growth as indirect indicators of the salinity tolerance, with higher values corresponding to higher tolerance. Two hundred larvae (2 dph) from each group were transferred from freshwater and distributed randomly into each of five tanks with different salinities (0, 5, 10, 15 and 20 ppt), and we made 4 replicates of each condition. This design led to 60 composite tanks (20 L water volume), each tank containing 200 larvae. To avoid potential biases linked to the relative positions of the tanks, we allocated these positions randomly. Tanks with saline water were brought to their targeted salinity by progressively replacing water in each tank with saline such that all treatments reached their target concentration 10 days after the start of the experiment. Aeration was provided in each tank by an air-stone. We fed the larvae in each tank on rotifers, nauplii of *Artemia*, and *Moina* during the first 5 days and only nauplii of *Artemia*, and *Moina* from day 6 to day 21. The larvae were manually fed to apparent satiation four times per day (at 06:00 h, 10:00 h, 14:00 h, 18:00 h). We siphoned off the waste accumulated in the bottoms of the tanks daily to avoid negative effects on larvae. After 21 days of experiment, all fish were harvested to evaluate the survival rate and growth performance.

The same water quality parameters as for the embryonic stage were monitored daily in this period. During the experiment, temperature varied over time from 27.2 °C to 28.7 °C, levels of pH ranged from 7.2 to 8.1 and DO from 4.8 to 5.5 mg L<sup>-1</sup>, although these parameters were

similar among tanks at any given time.

### 2.5.3. Experiment on fry to fingerling stage

The experimental design was similar to the one described for the larvae, with 200 fry (28 dph) from each group (initial body weights:  $0.8 \pm 0.1$  g for the selected group,  $0.7 \pm 0.1$  g for the random group and  $0.6 \pm 0.1$  g for the freshwater group) distributed randomly in 60 300 L composite tanks. Individuals in each tank were fed to apparent satiation twice daily at 08:00 h and 15:00 h using a commercial pelleted feed with protein content of 35 %. We maintained a constant salinity in all tanks using a RAS where well-aerated water was supplied continuously. Around 5 % of the culture water in each tank was exchanged every 3 days in addition to siphoning off the accumulated wastes each of the at tank bottoms. After a 70-day experimental period, we harvested all survivors for growth rate assessment, and analyses of osmoregulatory parameters. Tank water samples were also collected and stored at time of sampling to measure osmotic pressure.

We monitored water quality daily using the same parameters as for the previous stages. During the experiment, temperatures ranged from 27.1 °C to 30.5 °C among RAS systems. The values of pH ranged from 7.1 to 8.0, and DO ranged from 5.0 to 6.5 mg L<sup>-1</sup>. Additionally, total ammonia nitrogen (TAN) and N-NO<sub>2</sub> were measured weekly using a Multiparameter Photometer (Hana Hi 83300). The concentrations of TAN and N-NO<sub>2</sub> were lower than 0.5 mg L<sup>-1</sup> and 0.3 mg L<sup>-1</sup>, respectively.

We estimated growth performance for each salinity treatment as weight gain (WG, g), daily weight gain (DWG, g day<sup>-1</sup>), specific growth rate (SGR, % day<sup>-1</sup>) and feed conversion ratio (FCR). The estimated values were based on the following standard formulae used routinely as performance indicators in aquaculture studies (Bandyopadhyay and Das Mohapatra, 2009; Fagbenro and Arowosoge, 1991):

$$\text{SGR}(\%/day) = (\ln W_f - \ln W_i)/T \times 100$$

$\text{DWG}(\text{g}/\text{day}) = (W_f - W_i)/T$ , where  $W_f$  and  $W_i$  refer to the mean final and initial weights, respectively, and  $T$  is the experimental period in days.

$\text{Feed conversion ratio (FCR)} = \text{total weight of feed consumed (g)}/\text{total fish weight gain (g)}$ .

We collected blood samples from 3 fish per tank with a heparinized syringe and transferred the individual blood samples to 1.5-ml labeled tubes. Blood samples were then centrifuged for 15 min at 4500 rpm at 4 °C. Next, we separated plasma, and stored it frozen at -20 °C for later analysis. Plasma osmolality was measured using Advanced Instrument Osmometer Model 3320. Na<sup>+</sup> and Cl<sup>-</sup> ions concentrations were measured using Flame Photometer 420 and MKII Chloride Analyzer 926s, respectively.

### 2.6. Estimation of realized heritability and the response to selection in G2 generation

In this experiment, the three fish groups were stocked together in a common garden design. The environment was identical for all groups to minimize the error caused by confounding between-tank variation in separate replicates and genetic effects (Dussault and Boulding, 2018; Liu et al., 2016). The fingerlings from the three groups were distinguished using visible implant elastomer (VIE) tags with three different colors: red for the selected, yellow for the random and green for the freshwater fish. The method for tagging VIE on fish was presented in detail in GEV (2020). It was reported that VIE tags in Zebrafish (*Danio rerio*) could be retained for one year and that tagging did not interfere with long-term growth and survival (Hohn and Petrie-Hanson, 2013).

We used a RAS system with four 10 m<sup>3</sup> composite culture tanks (each tank corresponding to one replicate) in this experiment. From each group, 130 fish (100 dph, initial body weight of selected fish:  $22.0 \pm 1.4$  g; random fish:  $20.2 \pm 0.7$  g, freshwater fish:  $19.3 \pm 2.6$  g) were

randomly allocated to a culture tank (total of 390 fish per tank). We then brought the tanks gradually to the targeted saline condition by progressively replacing water in each tank with saline water over 10 days (1 ppt per day) until reaching the desired salinity of 10 ppt (the same level as in the selection experiment). We fed each tank to apparent satiation twice daily at 09:00 h and 16:00 h using a commercial pelleted feed with protein content of 28 %. Approximately 3 % of the culture water in each tank was exchanged every 2 days in addition to siphoning off the accumulated waste at the tank bottom. The entire experiment lasted for 8 months when the fish reached commercial sizes and an age (11 months) similar to the age of the fish at the end of the selection experiment. At harvest, all survivors were weighted individually for growth rate assessment. The fish from the three groups were classified according to their VIE color.

In this stage, the same water quality parameters as above were monitored daily in the RAS system. During this experiment, temperatures ranged from 27.3 °C to 30.8 °C, the level of pH ranged from 7.1 to 8.5 and DO ranged from 3.9 to 5.2 mg L<sup>-1</sup>. The concentration of TAN and N-NO<sub>2</sub> was smaller than 1.5 mg L<sup>-1</sup> and 0.8 mg L<sup>-1</sup>, respectively. At this stage, the direct genetic response to selection of the growth in saline condition in the G2 generation was estimated as the relative weight superiority of the selected group on the random group, i.e.  $R = W_s - W_r$ , or  $R \% = 100 * [(W_s - W_r)/W_r]$ , where  $W_s$  and  $W_r$  were the mean weights at harvest of offspring from the selected and random groups, respectively, and assuming that the average  $W_r$  estimated the corresponding average in the preceding generation. The realized heritability for growth in saline condition was calculated as:  $h^2 = R/S$ , where  $S$  was the selection differential, computed as the average difference in trait values between the selected individuals and the overall population in the preceding generation. Coefficients of variation for body weight for each group were estimated as: (standard deviation/mean) \* 100.

### 2.7. Statistical analysis

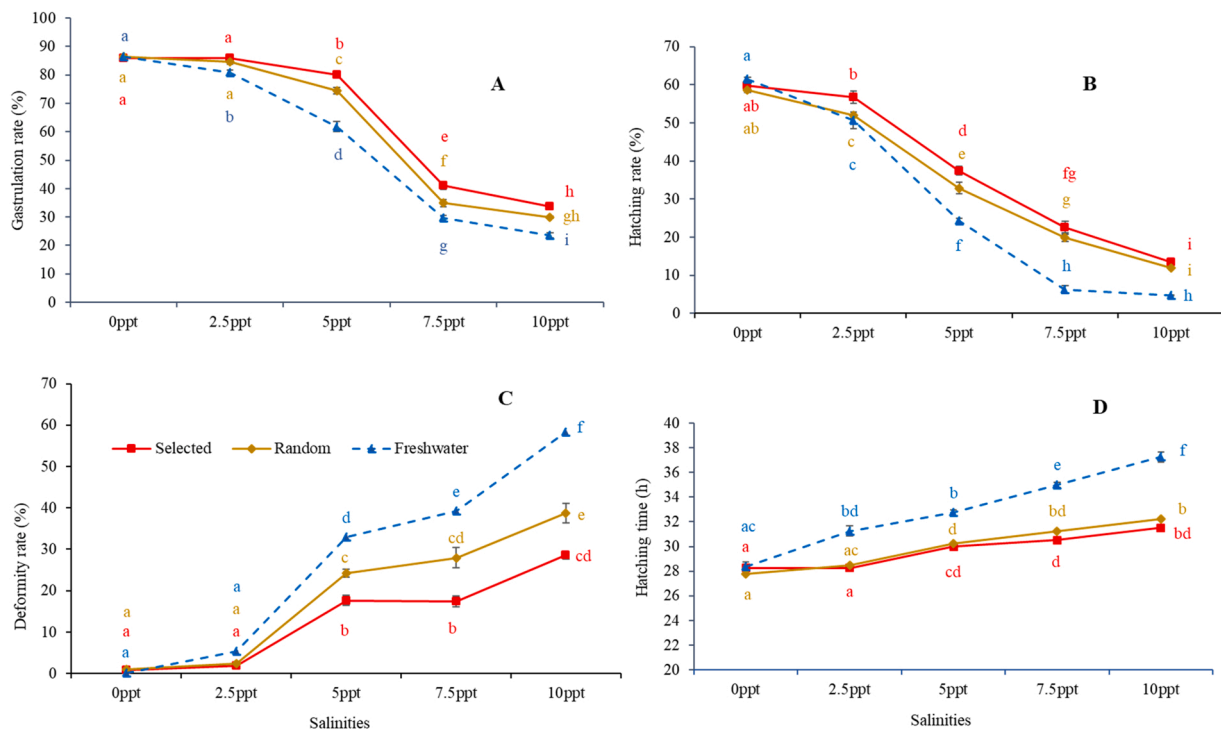
Since most investigated parameters were not normally distributed, we used permutations procedures (Manly, 2007) applied to our factorial designs in order to evaluate the effects of salinities, fish groups and their interaction. Differences between factor least square means were tested using Duncan's multiple-range tests. For all tests, the level of significance was set at  $P < 0.05$ . Statistical analyses were conducted using SAS 9.3 (SAS, 2009).

## 3. Results

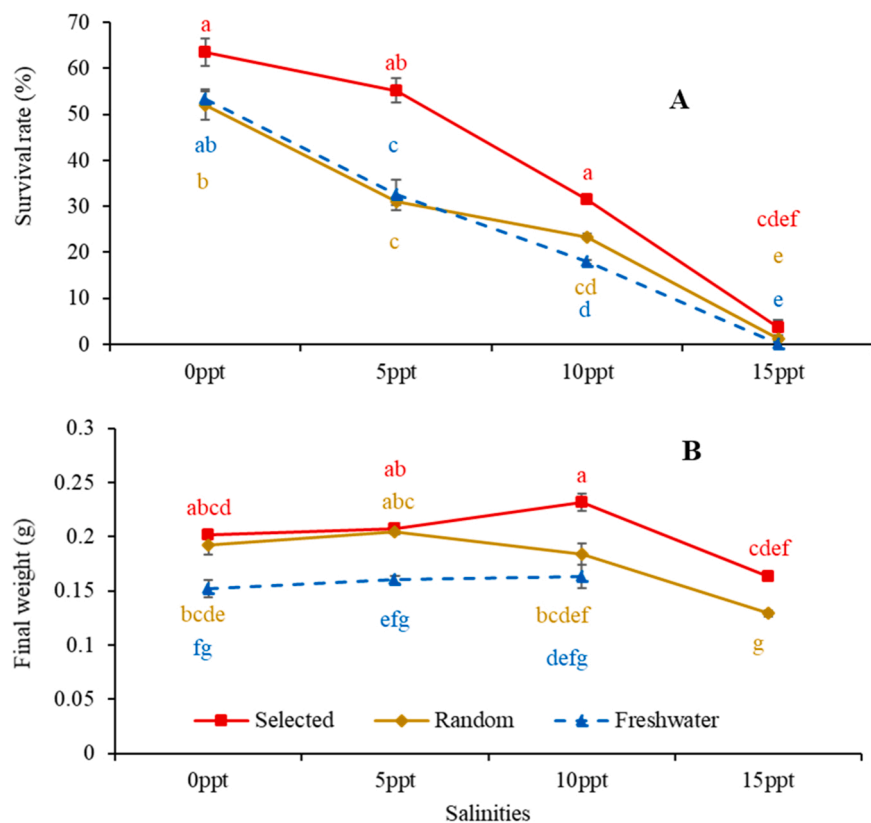
After 3 selection stages (11 months of age), the average body weight and SEM of the fish in the selected group and the random group in the G1 population were  $1380 \pm 7.3$  g (576 fish) and  $793 \pm 10.6$  g (470 fish), respectively. As described above, these G1 fish were the parents of the measured G2 fish (Fig. 1).

### 3.1. Embryonic development of fish under saline conditions

Increased salinity resulted in a decrease in gastrulation and hatching rates and an increase in deformity rate and hatching time. The statistical analyses showed that salinity, fish group and their interaction had significant effects on gastrula, hatching, and deformity rates ( $P < 0.05$ ) (Fig. 2). For salinity level set to 0 ppt, these parameters were similar for the three groups of fish ( $P > 0.05$ ). The parameters started to differ significantly between the three groups above 2.5 ppt: the gastrulation and hatching rates of the selected and random groups were significantly higher and their hatching time and deformity rate were significantly lower than for the freshwater group. Although not always significantly, the values from the selected group were consistently better than those from the random group.



**Fig. 2.** Effects of salinity, fish group on embryonic development of three fish groups under saline conditions; Gastrulation rate (A), Hatching rate (B), Deformity rate (C) and Hatching time (D). Values are given least square means (LSM) ± SEM. Different letters indicate significant difference (P < 0.05).



**Fig. 3.** Growth and survival of three fish groups under saline conditions on larvae to fry stage; Survival rate (A) and Final weight (B). Values are given least-square means (LSM) ± SEM. Different letters indicate significant difference (P < 0.05).

3.2. Growth and survival of fish under saline conditions on larvae to fry stage

Results showed that the survival rates and the final weights tended to decrease with increasing salinities. Salinity levels and fish group had significant effects ( $P < 0.05$ ) on the survival rates and final weights, with no indication of significant interactions (Fig. 3). No fish survived when the water salinity was 20 ppt. At 15 ppt, only few fish from the selected and random groups survived. Although the survival rates were systematically higher in the selected group, the differences were not significant at 0 ppt, but became significant for 5 and 10 ppt. Similarly, the final weights in the selected group tended to be higher than in the random and freshwater groups, with significant differences from 10 ppt ( $P < 0.05$ ).

3.3. Growth performance and changes in osmoregulatory parameters of fish under saline conditions on fry to fingerling stage

3.3.1. Fish survival, growth rate, and FCR

For all tested salinity levels, the fish from the selected group grew faster, followed by those from the random, and the freshwater group (Fig. 4). Fish growth rates (WG, DWG, and SGR) were highest in 5 ppt ( $P < 0.05$ ) and did not differ between 0 and 10 ppt ( $P < 0.05$ ). In terms of feed utilization efficiency, FCR values were higher for the freshwater group than for the random group, and higher for the random group than

for the selected group, with the differences tending to become significant as the salinity increased (Fig. 4). The survival rates were similar for the selected and the random groups from 0 ppt to 15 ppt, and lower, although not significantly, in the freshwater group. At 20 ppt, the survival rates dropped significantly, with a more marked decrease for the random and the freshwater groups than for the selected group (Fig. 4).

3.3.2. Osmolality and ionic concentration ( $Na^+$  and  $Cl^-$ )

Generally, raising salinity led to an increase in these parameters (Fig. 5). Plasma osmolality values from the three groups were similar up to salinity of 15ppt. The differences became significant at 20ppt, with osmolalities in the selected ( $417.3 \pm 17.9$  mOsm/kg) and random ( $414.9 \pm 8.2$  mOsm/kg) groups significantly higher than in the freshwater group ( $384.5 \pm 10.1$  mOsm/kg). At 10 ppt, there were no significant difference between external osmotic pressure ( $288.8 \pm 3.2$  mOsm/kg) and plasma osmotic pressure of fish in the three groups. At this level, fish were in conditions that were essentially isosmotic to their internal environment. Similar trends were observed for  $Na^+$  and  $Cl^-$  concentrations in our experiment.

3.4. Heritability and selection response

After one generation of mass selection under saline condition, the realized heritability for the body weight of striped catfish was 0.29 (Table 1). Interestingly, our selection procedure led to increases in both

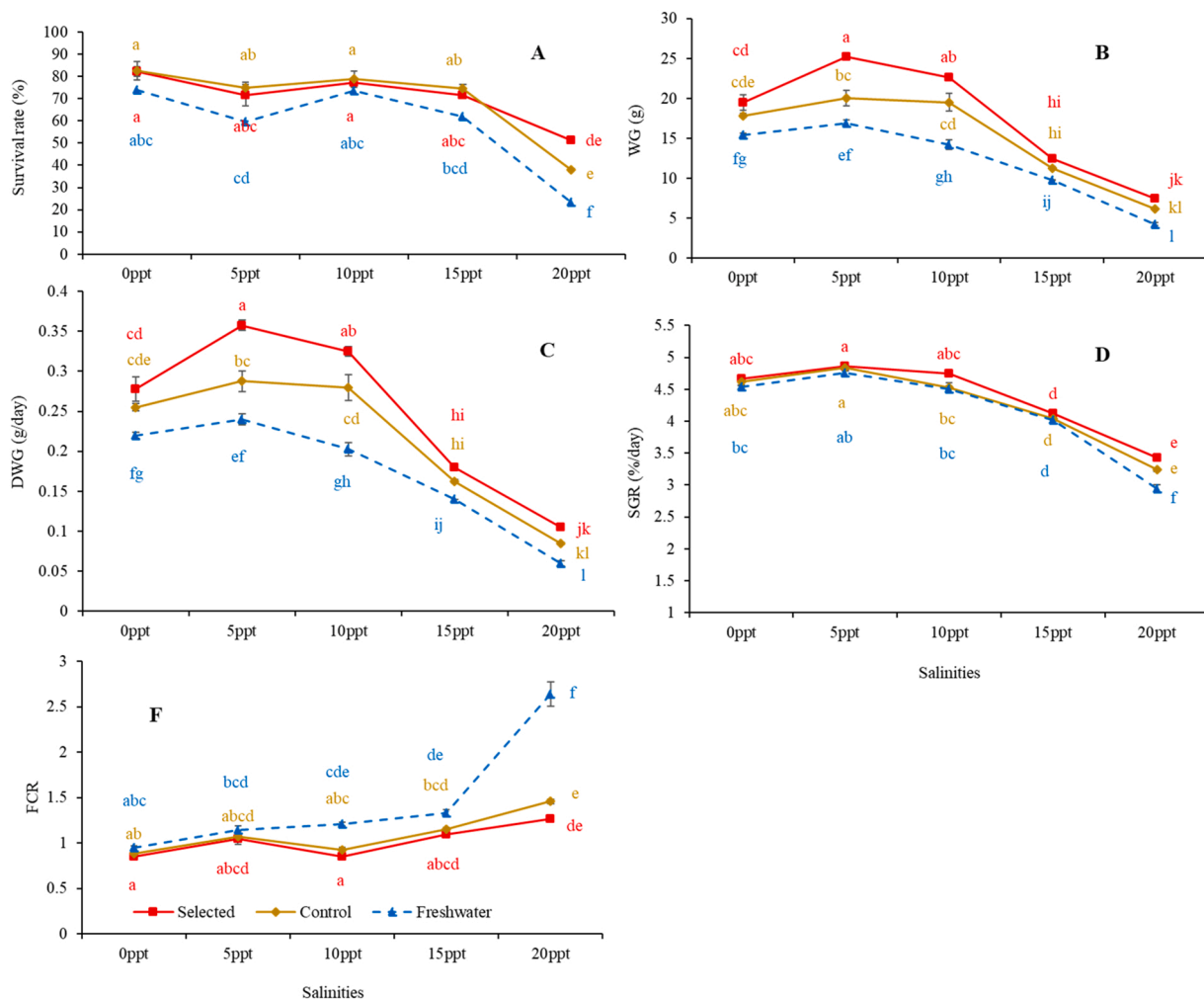
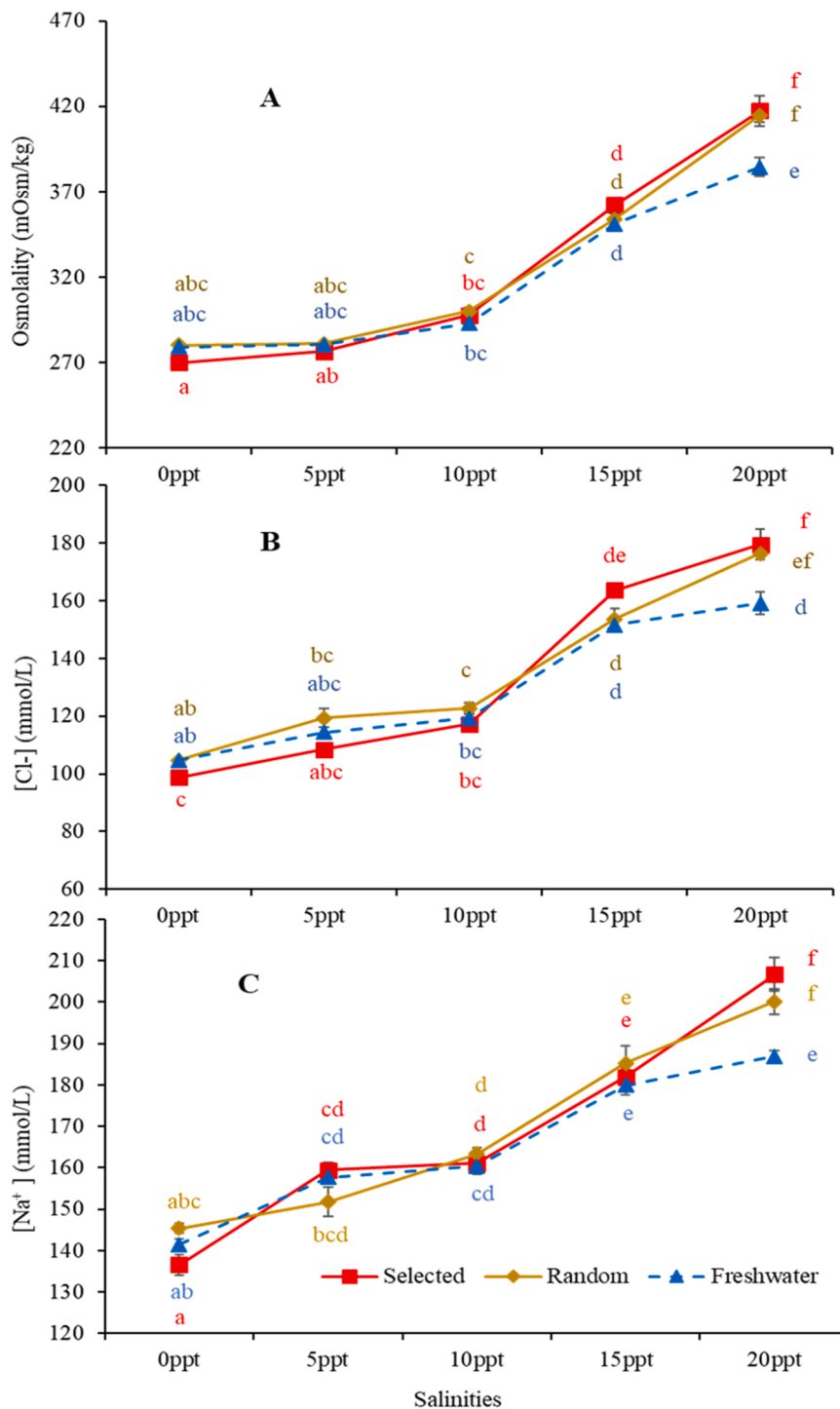


Fig. 4. Growth performances of three fish groups under saline conditions on fry to fingerling stage; Survival rate (A), WG: weight gain (B), DWG: daily weight gain (C), SGR: specific growth rate (D), FCR: Feed Conversion Ratio (F). Values are given least-square means (LSM)  $\pm$  SEM. Different letters indicate significant difference ( $P < 0.05$ ).



**Fig. 5.** The change of osmolality level (A), ion Cl<sup>-</sup> (B) and ion Na<sup>+</sup> (C) of three fish groups under saline stress. Values are given least-square means (LSM) ± SEM. Different letters indicate significant difference (P < 0.05).

harvest weight (18 %) and survival rate (11.4 %). For harvest weight, we found lower values of the coefficient of variation in the selected group in both generations, with 12.7 % in G1 generation and 21.8 % in G2 generation, while the values for the random (with 28.8 % in G1 and 25.5 % in G2) and freshwater (25.1 % in G2) groups were similar.

#### 4. Discussion

##### 4.1. Effect of the salinity on the survival and growth performance of striped catfish

Salinity is one of the critical factors affecting the growth rate, survival rate, geographical distribution and metabolism of fish (Amornsakun et al., 2017). The analyses in our experiment confirm that salinity can affect the survival and the growth performances of striped catfish in various developmental stages. These impacts have already been shown

**Table 1**

Heritability and response to selection for body weight (given same age –11 months at harvest time) after one generation of selection under salinity of 10ppt.

Parameters	G1 generation		G2 generation		
	Selected	Random	Selected	Random	Freshwater
BW (g)	1380	793	1106	935	827
	± 7.3 <sup>a</sup>	± 10.6 <sup>b</sup>	± 12.8 <sup>c</sup>	± 13.6 <sup>d</sup>	± 12.3 <sup>e</sup>
SR (%)	–	–	76.9	69.0	57.1
	–	–	± 4.8 <sup>a</sup>	± 1.5 <sup>a</sup>	± 1.9 <sup>b</sup>
S for BW (g)	587	–	–	–	–
R for BW (g, %)	–	–	171 (18.0)	–	–
R for SR (%)	–	–	7.9 (11.4)	–	–
h <sup>2</sup> (BW)	0.29	–	–	–	–

Values are given as least square means (LSM) ± SEM, BW = body weight, SR = survival rate, S = selection differential, R = selection response, h<sup>2</sup> = realized heritability. Different letters indicate significant differences within the same row (P < 0.05).

in previous works: increasing the salinity reduced the probability of eggs reaching an observable developmental stage and hatching rates, delayed the hatching time and development of fertilized eggs, and increased the deformity rate of the larvae (Borode et al., 2002; Huong and Quyen, 2012; Hossain et al., 2021). Nevertheless, for some freshwater fish, slightly raised salinities could lead to faster growth compared to freshwater condition (Gilles and Patrick, 2001). An hypothesis potentially explaining this observation is that the energetic cost of osmoregulation is lower in an isosmotic medium (for most freshwater fish, the iso-osmotic salinity normally ranges from around 10–12 ppt (Varsamos et al., 2005)), where the gradients between blood and water are minimal, and that energy savings are substantial enough to increase the growth (Gilles and Patrick, 2001). In the current study, we obtained the best growth performances from larva to fingerling stages for salinities of 5 and 10 ppt, and the performances started to decline for higher salinities. This decline of the performances for higher salinities have been observed in other studies. Nguyen et al. (2014) reported a poor survival rate (38.2 %) for striped catfish in 18 ppt and Amornsakun et al. (2017) observed a poor survival rate (16.7 %) for snake head fish (*Channa striatus*) at 14 ppt. Consequently, our results support the statement that striped catfish can tolerate low to moderate saline conditions, with minor and sometimes positive effects on their survival and growth performances.

#### 4.2. Improvement of the salinity tolerance of striped catfish using selection

The results presented in the preceding section show the effect of the salinity on the measured parameters (Figs. 2–4). In addition, we also observed differences in salinity tolerance of striped catfish in our selection program. In general, the group with the offspring of the fish selected on size (“selected” group) tended to perform better in terms of growth and survival than the offspring of the fish from the randomly chosen fish (“random” group). We interpret this difference as a response to the selection in the previous generation (see also next paragraph). Furthermore, the fish originating from parents reared in saline conditions performed better than those coming from parents kept in freshwater did. Several hypotheses might support these observations. A first hypothesis is that fish raised in a saline environment might have a better tolerance to stressful osmotic conditions in comparison to those raised in freshwater. Indeed, stress due to the environmental salinity is known to induce a costly adjustment of the osmoregulation, which consumes up to 50 % of total energy (Gilles and Patrick, 2001; Hasan et al., 2017). This energetic expense has a negative impact on the growth rate. As a first step to confirm this hypothesis, we have observed in our study that striped catfish are able to regulate internal osmolality to adapt to the salinity fluctuations. More importantly, we have observed significantly higher osmolalities in the selected and the random groups than in the

freshwater group at high level of salinity (20 ppt) (Fig. 5), indicating a better tolerance to stressful osmotic conditions for the fish raised in a saline environment and a lower energetic expense for osmoregulation. We can propose another hypothesis to explain the differences in growth between the offspring of the fish reared in freshwater and those coming from the randomly selected sample in saline conditions: although not selected on growth, fish from the random selection sample are selected on survival. This selection might contribute to select favorable alleles for growth at the same time if growth and survival are correlated traits. Further studies are needed to potentially support this hypothesis, such as a genome-wide association study (GWAS) designed to identify quantitative trait loci (QTL) underlying the difference between the two sets of fish. Alternatively, we might consider using candidate genes approach (maybe targeting genes involved in osmoregulation) to explain part of the genetic difference between rearing groups.

Coming back to the comparison between the selected and the random groups in saline conditions, our hypothesis is that mass selection in the previous generation has increased the frequency of favorable alleles for salinity tolerance in the selected group. The transmission of these variants to the next generation should lead to the observed better performances for the offspring of the selected fish than for those of the randomly selected fish. These results are therefore a confirmation that selection in saline conditions could be a first step towards genetic adaptation to the new environmental conditions (Donelson et al., 2019). Several previous studies provide additional support to this hypothesis. A genetic component of the salinity tolerance has been observed in several studies and species, such as Arctic charr (*Salvelinus alpinus*) (Norman et al., 2011) and Nile tilapia (*Oreochromis niloticus*) (Gu et al., 2018; Rengmark et al., 2007). In striped catfish, a number of putative genes potentially related to salinity tolerance have been identified using RNA sequencing data (Thanh et al., 2014; Viet et al., 2016). The increased salinity tolerance obtained in selection programs has been reported for tilapia in several previous studies (Jaspe and Caipang, 2011; Tran et al., 2008). Similarly, Afonso et al. (1998) have provided an extensive review of successful selection programs for stress tolerance on rainbow trout and common carp. Progressive genetic adaptation to salinity has been documented in laboratory strains of guppy (*Poecilia reticulata*) (Shikano et al., 2001; Shikano and Fujio, 1998a, 1998b). Purcell et al. (2008) also showed that mosquitofish (*Gambusia affinis*) living in saline environment required genetic adaptation through natural selection for higher individual salinity tolerance than fish from freshwater conditions where no such selection for salinity tolerance exists. This process can sometimes occur remarkably rapidly in a few generations if such favorable variants exist in the challenged population, (Barrick and Lenski, 2013), as seems to be the case in our experiment. Furthermore, gametes, embryos and larvae might undergo selection for alleles that provide advantage in the parental environment, particularly in highly fecund species as fish (Torda et al., 2017). High phenotypic variance coupled with a reasonable heritability indicate that the high genetic diversity underlying a trait could be efficiently used in a selection program (Tahapari et al., 2018). In our study, the large phenotypic variation observed for growth (Table 1) and the corresponding reasonable heritability (0.29) suggest opportunities for a successful selection program targeting an improved tolerance and a progressive adaptation to the emerging saline conditions.

#### 4.3. Heritability and response to selection

Our study demonstrated that genetic selection effectively improved the performance of striped catfish in brackish water of moderate salinity (10 ppt). The gain achieved for body weight in this population of striped catfish averaged 18.0 % after one generation of selection. This achievement was higher than those reported in striped catfish from a selection program conducted under favorable freshwater pond environment with only 9.3 % per generation (Vu et al., 2019). However, this is in line with genetic gain found in other fish species, such as tilapias

(Bentsen et al., 2017), common carp (Ninh et al., 2013), channel catfish (Rezk et al., 2003) and Atlantic salmon (Thodesen et al., 1999). In general, the genetic gain achieved after one generation in our breeding program under brackish water was comparable to those reported in aquatic animals, which are about 12.7 % on average (Gjedrem and Rye, 2018).

Furthermore, our results also showed that selection for increased growth performance in saline conditions seemed to enhance the survival rate of striped catfish during the grow-out phase (11.4 %), although this increase was not significant. The survival gain obtained in the present study was also remarkably higher than that reported for striped catfish selection program carried out under freshwater condition of only 7.4 % (Vu et al., 2019). In the present study, selection in G1 generation was obviously made among the surviving fish under saline stress, making survival part of the tolerance to salinity and, as such, a selected trait. Survival is also an economically important trait for striped catfish in brackish water system because it affects the number of fish harvested and marketed, and hence the production yield per unit of culture and the farmers' income. In other species, responses to selection for both body weight and grow-out survival were positive in giant freshwater prawn (Vu et al., 2017), negative in Pacific white shrimp (Zhang et al., 2017) and non-significant in common carp (Dong et al., 2015; Ninh et al., 2014). In Nile tilapia, the long term-selection program for high growth in the Genetically Improved Farmed Tilapia (GIFT) strain over 10 generations did not cause any changes in grow-out survival in pond (Hamzah et al., 2017).

The genetic gains of body weight and survival obtained in our selection program under saline condition suggest that selection to improve ability of striped catfish to adapt to saline stress can be achieved. These results also demonstrate for the first time that at least two major production traits (survival and growth) associated with the ability to adapt to salinity were positively modified after only a single generation of selection, indicating again that this population of striped catfish have the genetic potential to adapt to salinity changes. Similarly, improved tolerance of striped catfish observed in this study has been found for disease resistance to specific pathogens for salmonids (Leeds et al., 2010; Storset et al., 2007) and tilapia (Shoemaker et al., 2017; Sukhavachana et al., 2019). Among these studies, several have demonstrated moderate to high genetic gains per generation, for example 18.7 % for infectious pancreatic necrosis resistance in Atlantic salmon (Storset et al., 2007), and 9 % for streptococcosis resistance in red tilapia (Sukhavachana et al., 2019).

Although culture of this improved strain of striped catfish in current hatcheries conditions (*i.e.* in freshwater) could be an objective, it is likely that saline-tolerant strains of striped catfish such as the one developed in our program could also perform well in either low salinity or freshwater environment. Specifically, growth performance of fish from the selected group was slightly higher than those from the random and freshwater groups in 5 ppt and 0 ppt treatments although selection program was implemented in 10 ppt. These findings seem to be in agreement with the hypothesis proposed by Falconer (1990) based on a review of 24 studies in both farmed animals and model species that antagonistic selection (*i.e.* selection under unfavorable environments as saline stress in our study) may produce genotypes that can perform well across a range of production system environments. In contrast, selection under favorable environmental conditions (*i.e.* synergistic selection) may result in sensitive genotypes that do not thrive under stressed conditions. This hypothesis was supported in the study of Thoa et al. (2016): a genetic line of Nile tilapia having undergone five generations of selection under a moderate saline water environment could be cultured successfully in freshwater systems. It suggests that our breeding program to improve growth performance of striped catfish under saline conditions can provide catfish seeds for both freshwater and saline water production systems.

The moderate heritability estimates obtained for body weight under saline condition in our study is generally in line with those reported in

other selection programs for striped catfish in favorable freshwater environment with a range from 0.21 to 0.34 after three generations of selection (Sang et al., 2012; Vu et al., 2019). In a selection program on channel catfish (*Ictalurus punctatus*) grown in earthen ponds, realized heritability for three generations of selection were 0.17 and 0.19 for Kansas and Marion strains, respectively (Rezk et al., 2003). In a breeding program on Nile Tilapia, Tran et al. (2008) reported heritability estimates of 0.24 and 0.19 for harvest weight after three generations with selection in freshwater and in brackish water respectively. The heritability achieved for body weight after one generation of selection in the current study suggests that the population will continue responding to future selection rounds under saline conditions.

## 5. Conclusion

The results from this study demonstrate that a selective breeding for increased saline tolerance of striped catfish can already be effective after one generation of selection under saline environment. The improved salinity tolerance of striped catfish developed in the present study is of practical significance in the context of Mekong Delta aquaculture, where the striped catfish breeding activities are projected to be greatly affected by salinity intrusion due to climate change. The selected animals originating from this study also constitute a valuable genetic resource for subsequent studies targeting a better understanding of the physiology and genomic characteristics of this improved strain as well as the mechanisms of osmoregulatory adaptation to salinity in striped catfish.

## Author statement

All authors have seen and approved the final version of the manuscript being submitted. They warrant that the article is the authors' original work, hasn't received prior publication and isn't under consideration for publication elsewhere.

## Declaration of Competing Interest

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

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