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Reproductive Parameters and Larval Growth of Bighead Catfish (*Clarias microcephalus* Günther, 1864) from Wild and Cultured Broodstock Strains

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Abstract

Bighead catfish, *Clarias macrocephalus*, is an important species for aquaculture. Nevertheless, information on population variation in their reproductive characteristics and larval growth is limited. This research aimed to evaluate the reproductive performance and larval growth of three bighead catfish broodstock strains collected in Ca Mau (CM) and Hau Giang (HG) provinces (wild strains), and Can Tho (CT, domesticated strain) in the Mekong Delta, Vietnam. The three groups of fish were cultured under optimal maturation conditions in a recirculating system for three months. Then, 16-18 pairs from each broodstock source were artificially propagated. Relative fecundity differed among the sources, from 48,600 (CM) to 69,300 (CT) eggs/kg female ($P< 0.05$). The three broodstock sources also differed in fertilization and hatching rates. In addition, wild breeders (CM and HG) had slightly larger eggs, and their offspring had larger sizes at hatching and larger yolk sac volumes than those of cultured (CT) breeders ($P< 0.05$ for all tests, except for egg sizes). In the larval rearing experiment, 2 day-old larvae were stocked in 40 L-rectangle tanks (1,000 individuals/tank) in a recirculating water system. Larvae were fed with Moina combined with commercial feed. After 40 days, survival rates of the three fish groups ranged from 46.7% (CT) to 54.7% (HG). The final weights varied from 177 mg (CM) to 201 mg (HG) and 202 mg (CT). However, the effects of broodstock sources on the growth and survival rates of the offspring were not statistically significant ($P> 0.05$).

Keywords

Clarias macrocephalus, domesticated broodstock, larval rearing, reproduction, wild population.

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Introduction

Clarias catfish are highly economic and important species for aquaculture in Southeast Asia (Na-Nakorn & Brummett, 2009). In the Mekong Delta, Viet Nam, there are two indigenous species of the

Clarias genus, bighead catfish, *Clarias macrocephalus*, and walking catfish, *Clarias batrachus* (Tran *et al.*, 2013). Meanwhile, the African catfish, *Clarias gariepinus*, was introduced into Viet Nam in 1975 for producing hybrid catfish (female *C. macrocephalus* x male *C. gariepinus*) for farming purposes (Duong *et al.*, 2017). This hybrid catfish has been cultured in Viet Nam and other Southeast Asian countries for many years (Yi *et al.*, 2003) due to its fast growth, strong tolerance to high fluctuations of environmental conditions, high resistance to diseases, and ability to grow under high stocking densities (Duong Thuy Yen *et al*., 2014a). Nevertheless, bighead catfish, *C. macrocephalus*, is still more preferred in markets, even at higher prices, because its meat quality is better than the hybrid catfish and other *Clarias* species (Na-Nakorn & Brummett, 2009). However, the poor performance in the growth and survival of bighead catfish during the early life stages is one of the major constraints to the sustainable farming of this species (Duong Thuy Yen *et al*., 2014a).

The high genetic quality of seeds for fish farming is essential for the success of aquaculture production. A long history of the domestication of a fish species can result in inbreeding depression such as decreases in growth rates, sperm quality, and disease resistance, and high levels of deformities (Tave, 1999). Empirical studies on inbreeding depression have been reported for turbot (*Scophthalmus maximus* L.) (Bouza *et al.*, 1997) and three-spined sticklebacks (*Gasterosteus aculeatus*) (Nielsen *et al*., 2010; Mehlis *et al.*, 2012). Inbreeding has not been reported for bighead catfish. However, bighead catfish has been domesticated for over 30 years in the Mekong Delta, where many hatcheries have used males and females that originated from the same broodstock sources (Duong & Scribner, 2018). Thus, it is hypothesized that domesticated broodstock can have some levels of inbreeding. Genetic evaluation based on microsatellites indicated that cultured bighead catfish populations showed slightly lower genetic diversity compared to wild ones (Duong & Scribner, 2018). However, quantitative information on the reproductive parameters of broodstock and early life growth performances of offspring produced from different broodstock sources of *C. macrocephalus* are unknown in the Mekong Delta. For the sustainable development of aquaculture, fish production can be enhanced through genetic improvement programs (Tave, 1993; Dunham, 2011). The first basic step of genetic enhancement is to choose good broodstock sources from different wild and domesticated fish strains, which requires research on strain evaluations (Dunham, 2011). A previous study in Thailand found that a domesticated strain, namely KU, had better growth than the wild strain NE (Muiocha *et al.*, 2017).

The objective of this study was to compare the reproductive parameters and larval growth and survival of wild and cultured broodstock strains of bighead catfish in the Mekong Delta. The information obtained would be helpful for further genetic improvement programs of bighead catfish.

Materials and Methods

Broodstock sources

Adult fish (100-200g/individual) were collected from cultured and wild sources. Cultured adults were bought from a hatchery in Can Tho (CT) city. Wild fish were collected from conservation areas of Lung Ngoc Hoang in Hau Giang (HG) and U Minh Ha in Ca Mau (CM). These fish were immediately transported alive to the hatchery in Can Tho University for broodstock conditioning.

Broodstock conditioning culture

Males and females of each broodstock source were cultured separately in 1 m^3-tanks with a recirculating water system. Stocking densities were 50 individuals/tank. Fish were fed with pelleted feed containing 41% crude protein at a feeding rate of 1% of their body weight. The breeders were checked for maturation after two months and mature individuals were chosen (16-18 pairs for each broodstock source) for induced spawning. The maturation of breeders was evaluated based on their external appearance of a big and soft belly, and reddish urogenital papilla

for females; and a long and pink genital spine for males.

Experiment 1: Effects of broodstock sources on the reproductive parameters

Sixteen to 18 pairs of breeders from each fish source were injected with hormones (**Table 1**). Each individual was weighed and labelled.

After the final injection, fish were kept separate in small tanks. Females ovulated after 11 to 12 hours at water temperatures of 26-27°C. Then, females were stripped to collect eggs and males were dissected to collect testis. Before fertilization, two samples of eggs were randomly collected, weighed, and preserved in 2.5ppm formalin for measuring egg sizes and counting egg numbers (to estimate absolute and relative fecundity). All remaining eggs were fertilized with sperm (separated for each pair of female and male) and softly mixed by using a dry feather to prevent the fertilized eggs from sticking together. The adhesiveness of the fertilized eggs was immediately removed by soaking them in urea solution (3 g of salt and 4 g of urea diluted in 1 liter of water) for 15 minutes and then tannin solution (1 g/liter) for 10 seconds. The eggs were rewashed three times with tap water before incubation in a Joung Jar system (for the larval rearing experiment later)**.** In addition, two samples (replicates) of 100 to 200 fertilized eggs were taken randomly from each treatment and placed in 30 × 20cm plastic trays to determine the fertilization rates after 8-10h. The hatching and deformity rates were also calculated based on these fertilized eggs.

Data calculation

The reproductive parameters were evaluated as follows:

Relative fecundity

$$Relative\ fecundity\ (\text{eggs/kg}) = \frac{\text{Number of stripped eggs}}{\text{Body weight of female}}$$

$$Ovulation\ rate\ (\%) = \frac{\text{Number of ovulated females}}{\text{Number of injected females}} \text{ x } 100$$

Fertilization rate

$$Fertilization\ rate\ (\%) = \frac{\text{Number of fertilized eggs}}{\text{Number of eggs incubated}} \text{X} 100$$

Hatching rate

$$Hatching\ rate\ (\%) = \frac{\text{Number of larvae}}{\text{Number of fertilized eggs}} \text{X} 100$$

Deformity rate

$$Deformity\ rate\ (\%) = \frac{\text{Number of deformed larvae}}{\text{Number of larvae}} \text{X} 100$$

Yolk sac volume

The length and width of the yolk sac of 30 larvae of each treatment were measured by using a stereoscopic microscope to determine the yolk sac volume as follows (Alderdice *et al*., 1979):

$$Yolk\ sac\ volume\ (mm^3) = \frac{4}{3} \times (\frac{W}{2})^2 \times (\frac{L}{2})$$

where W is the width of the yolk sac (mm) and L is the length of the yolk sac (mm).

Experiment 2: Effects of broodstock sources on larval development

Experimental system and stocking

Three treatments of larvae produced from the three broodstock sources mentioned above were used for this experiment. The experiment was designed in 12 rectangle tanks (containing 40 L water) with a recirculating water system. Larvae (2 days old) were stocked with 1,000 individuals per tank. All treatments were completely randomized in design with four replications. Aeration was applied during the rearing period of 40 days.

Food and feeding

Larvae were fed from the third-day post-hatch. At first, they were fed twice a day (in the early morning and late afternoon) with Moina for 10 days. Afterwards, the combination of Moina and commercial feed (40% of crude protein) was used until the fish could utilize 100% commercial feed. At the stage of using the artificial feed, they were fed four times a day. The amount of feed was adjusted depending on their utilization.

Water quality monitoring

Water temperatures were recorded twice a day using a thermometer, and pH was measured

Table 1. Hormone injections for bighead catfish males and females

	Preliminary dose	Interval time	Decisive dose
Females	500 IU of HCG/kg female	7-8 hours	3,500 IU of HCG and 2 mg of PG/kg female
Males	None		1,500 IU of HCG/kg male

Note: HCG = Human chorionic gonadotropin; PG = Pituitary gland

every three days using a pH meter (HANNA HI98107).

Data collection

For the fish growth parameters, before stocking, at least 30 larvae from each treatment were randomly sampled to record the initial length and weight. The total length and the bodyweight were measured by using a ruler (the nearest 0.1mm) and a digital balance with 0.01g accuracy, respectively.

At the end of the culture period, at least 40 individuals from each tank were randomly sampled and measured for their size. Weight and length data collected during the experiment were used for the determination of the growth rate. The growth and survival performances of the larvae were evaluated using the terms of daily weight gain (DWG), daily length gain (DLG), and survival rates. Variation in the growth of the fish was evaluated by comparing the coefficient of variation in the weight of the fish among treatments, in which:

$$Daily\ weight\ gain\ (g/day) = \frac{Final\ body\ weight(g) - Initial\ body\ weight(g)}{Rearing\ period\ in\ days}$$

$$Daily\ length\ gain\ (mm/day) = \frac{Final\ body\ length(mm) - Initial\ body\ length(mm)}{Rearing\ period\ in\ days}$$

$$Survival\ rate\ (\%) = \frac{Number\ of\ survival\ fish}{Number\ of\ fish\ stocked} \times 100$$

$$Coefficients\ of\ variation\ (\%) = \frac{Standard\ deviation\ (in\ weight\ or\ length)}{Mean\ (weight\ or\ length)} \times 100$$

Statistical analyses

The data on the reproductive parameters, growth, and survival rates were analyzed using a one-way analysis of variance (ANOVA). Then, mean values were compared among the three fish groups by DUNCAN multiple range tests at a significance level of $P< 0.05$. These statistical tests were performed using SPSS 20. In addition, regression of female body weight (g) on relative fecundity (eggs/kg female) was analyzed for each fish group and the pooled three groups using the regression function in Microsoft Excel.

Results and Discussion

Effects of broodstock sources on reproductive parameters

Breeders had a high variation in weight, ranging from 82 to 324g for females and 90 to 255g for males. Fish from CM had insignificantly larger sizes than those from CT and HG ($P> 0.05$) (**Table 2**). However, the relative fecundity of CM fish (48,600 ± 11,870 eggs/kg female) was significantly lower than that of CT and HG (from 68,200 to 69,300 eggs/kg female) ($P< 0.05$). The relative fecundity of these breeders was in the upper range of the species reported in previous literature, from 40,000 to 50,000 eggs/kg female (Ali, 1993; Nguyen Van Kiem & Pham Minh Thanh, 2013). The linear regression of relative fecundity on the body weights of all three groups of females indicated a negative relationship between them (**Figure 1**), which explains why larger CM females had lower relative fecundity compared to the other groups. This negative relationship was also reported in climbing perch *Anabas testudineus* (Duong Thuy Yen & Pham Thanh Liem, 2014).

Ovulation rates of the three broodstock groups were high, varying from 75.0% (HG) to 88.9% (CT). The success rate of fish ovulation under the same hormone injection depends largely on the stage of the oocytes (Nguyen Van Kiem & Pham Minh Thanh, 2013). Oocytes should be at the germinal vesicle migration stage as in other fish species (Shiraishi *et al.*, 2005). In this study, the maturation of females was evaluated by the

Table 2. Reproductive traits (Mean ± SD) of the three broodstock sources

Parameters	Ca Mau	Can Tho	Hau Giang
Number of injected pairs	17	18	16
Weight of females (g)	208.6^{a} ± 50.0	185.2^{a} ± 54.9	165.4^{a} ± 57.8
Weight of males (g)	194.3^{b} ± 54.0	117.8^{a} ± 54.0	127.0^{a} ± 24.6
Ovulation rate (%)	88.2	88.9	75.0
Relative fecundity (eggs/kg female)	48,600^{a} ± 11,870	69,300^{b} ± 16,386	68,200^{b} ± 18,418
Fertilization rate (%)	84.7^{b} ± 13.1	82.8^{b} ± 8.4	73.8^{a} ± 11.8
Hatching rate (%)	85.7^{b} ± 8.7	80.1^{b} ± 13.9	61.8^{a} ± 10.8
Egg diameter (mm)	1.68^{a} ± 0.05	1.66^{a} ± 0.04	1.71^{a} ± 0.03
Body length of hatchlings (mm)	5.45^{c} ± 0.18	5.00^{a} ± 0.22	5.24^{b} ± 0.14
Yolk sac volume (mm^{3})	0.52^{b} ± 0.05	0.50^{a} ± 0.08	0.56^{b} ± 0.05

Note: Means in the same rows with the same superscript letters were not significantly different (P> 0.05).

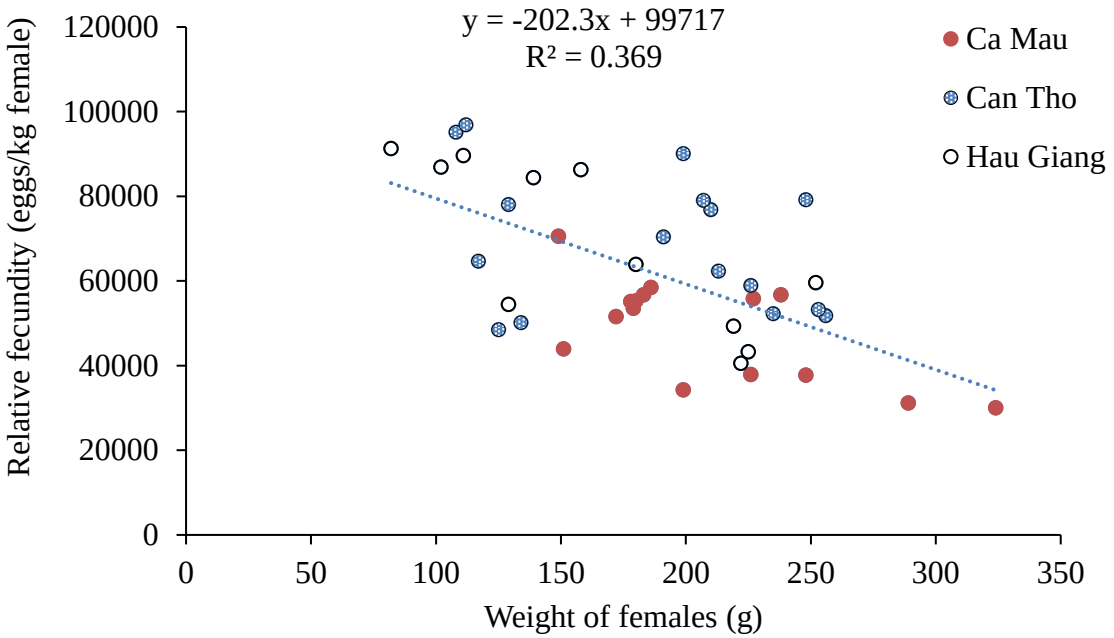

Figure 1. Regression of female body weight on relative fecundity of the three bighead catfish populations

external appearance of a soft belly and reddish urogenital papilla. Some females (2 in the CM and CT groups, and 4 in the HG group) did not ovulate, maybe because their oocytes did not reach the germinal vesicle migration stage. In addition, handling during hormone injection can cause stress to the breeders, negatively affecting their ovulation (Pham Minh Thanh & Nguyen Van Kiem, 2009).

Fertilization rates (%) of CM (84.7%) and CT (82.8%) were significantly higher than that of HG fish (73.8%). A similar trend was observed for the hatching rate in which the HG source was significantly lowest (61.8%). Lower values of fertilization and hatching rates of bighead catfish in HG indicated that those breeders were not in good mature conditions compared to the other

fish sources. Particularly, the maturity of the male bighead catfish and the other clariid species is difficult to determine based on external morphology because of their testis's special structure (Nguenga *et al.*, 1996; Pham Thanh Liem *et al.*, 2015). An immature male (transparent testis) could decrease fertilization and hatching rates. In addition, the quality of eggs indicated by their color also affects fertilization and hatching rates (Pham Minh Thanh & Nguyen Van Kiem, 2009). Good mature females produced mostly brown or dark yellow eggs, while in some females, a portion of immature eggs with light yellow color was also stripped, resulting in low fertilization and hatching rates.

Egg diameter, larval size at hatching, and yolk sac volume are indicators of maternal effects, which are important determinants of larval growth and survival (Heath *et al*., 1999; Johnston & Leggett, 2002). In this study, the egg diameters of CT cultured fish (1.66 mm) were smaller than those of wild fish in CM (1.68mm) and HG (1.71mm), but this difference was not significant ($P > 0.05$). However, the sizes of newly hatched larvae and yolk sac volumes statistically differed among fish groups ($P < 0.05$). The smallest size of larvae (5.0mm) and yolk sac volume (0.5mm^3) were observed in the cultured CT strain, compared to wild fish in CM and HG (larval sizes 5.45 and 5.24mm; yolk sac volumes 0.52 and 0.56mm^3, respectively). These differences were consistent with variations in egg sizes among bighead catfish strains (**Table 1**).

Effect of broodstock sources on the growth and survival of fry

Environmental parameters

During the rearing period, water temperatures fluctuated from 27.3 to 28.2^{o}C in the morning and 29.1 to 30.8^{o}C in the afternoon. This range is suitable for the normal growth and development of fish. Generally, temperatures varied more within a day and across experimental days (rainy versus sunny days) than among the three treatments.

Values of pH were stable during the experiment and similar among treatments, ranging from 7.1 to 7.4. In addition, with aeration and water flow, water conditions were good for the growth of bighead catfish larvae.

Growth of fry

After 40 days, fish in CM reached 177 ± 37mg in weight and 26.50 ± 3.15mm in length, while the CT and HG groups had similar final weights (202 and 201mg) and lengths (26.96 and 28.15mm, respectively) (**Table 3**). However, differences in growth parameters of the three fish groups were not significant ($P > 0.05$). Growth rates (daily weight gain, DWG, and length gain, DLG) of bighead catfish in this study were smaller than fingerlings of the same species in a previous study where DWG obtained ~ 8 mg/day (Duong Thuy Yen *et al.*, 2020). A high stocking density (1000 individuals/40L) could have negatively affected the growth of fish in the present study.

Growth differentiation and survival of fish

Growth differentiation was shown by the coefficients of variation (CV) in weight and length (**Table 4**) and the weight range distribution of individuals within each fish group (**Figure 2**). Fish in the three groups had more variation in weight with CV ranging from 56.7% (CM) to 65.5% (HG), while less variation (from 19.5 to 21.4%) in length was observed. Differences in CVs among the three groups were not significant ($P > 0.05$). After 40 days of rearing, the highest frequency of fish weight in the three groups ranged from 0.1 to 0.2 g (accounting for 43.9 and 33.6% in CM and HG, respectively).

The ratios of fish with the final weight > 0.2g in the CT (36.3%) and HG (37.2%) groups were higher than that of the CM group (28.6%). The above results indicate that bighead catfish fingerlings show a high differentiation in growth among individuals. This phenomenon is common among carnivorous fish species such as snakehead (War *et al.*, 2011) and climbing perch (Duong Thuy Yen & Duong Nhut Long, 2013).

Survival rates of fish in the three groups were not statistically different ($P > 0.05$), ranging from 46.7 (CT) to 54.7 % (HG). However, survival rates varied widely among replications of each fish group as indicated by the standard deviations of their survival rates.

Table 3. Growth parameters in weight and length of the three bighead catfish fingerling groups

Group	Ca Mau	Can Tho	Hau Giang
Initial weight (mg)	$3.59^a \pm 0.50$	$3.61^a \pm 0.99$	$3.94^a \pm 1.21$
Final weight (mg)	$177^a \pm 37$	$202^a \pm 55$	$201^a \pm 36$
DWG (mg/day)	$4.34^a \pm 0.925$	$4.96^a \pm 1.37$	$4.94^a \pm 0.91$
Initial length (mm)	$7.66^a \pm 0.48$	$7.51^a \pm 0.63$	$7.74^a \pm 0.63$
Final length (mm)	$26.50^a \pm 3.15$	$26.96^a \pm 2.69$	$28.15^a \pm 1.54$
DLG (mm/day)	$0.471^a \pm 0.079$	$0.468^a \pm 0.067$	$0.510^a \pm 0.038$

Note: Means in the same rows with the same superscript letters were not significantly different (P> 0.05). DWG and DLG are daily weight gain and daily length gain, respectively.

Table 4. Coefficients of variation (CV) in weight and survival rate

Group	CV in weight (%)	CV in length (%)	Survival rate (%)
Ca Mau	$56.7^a \pm 17.2$	$19.5^a \pm 4.6$	$49.1^a \pm 12.5$
Can Tho	$61.5^a \pm 9.6$	$21.1^a \pm 3.4$	$46.7^a \pm 6.2$
Hau Giang	$65.5^a \pm 12.7$	$21.4^a \pm 2.5$	$54.7^a \pm 2.4$

Note: Means in the same rows with the same superscript letters were not significantly different (P> 0.05).

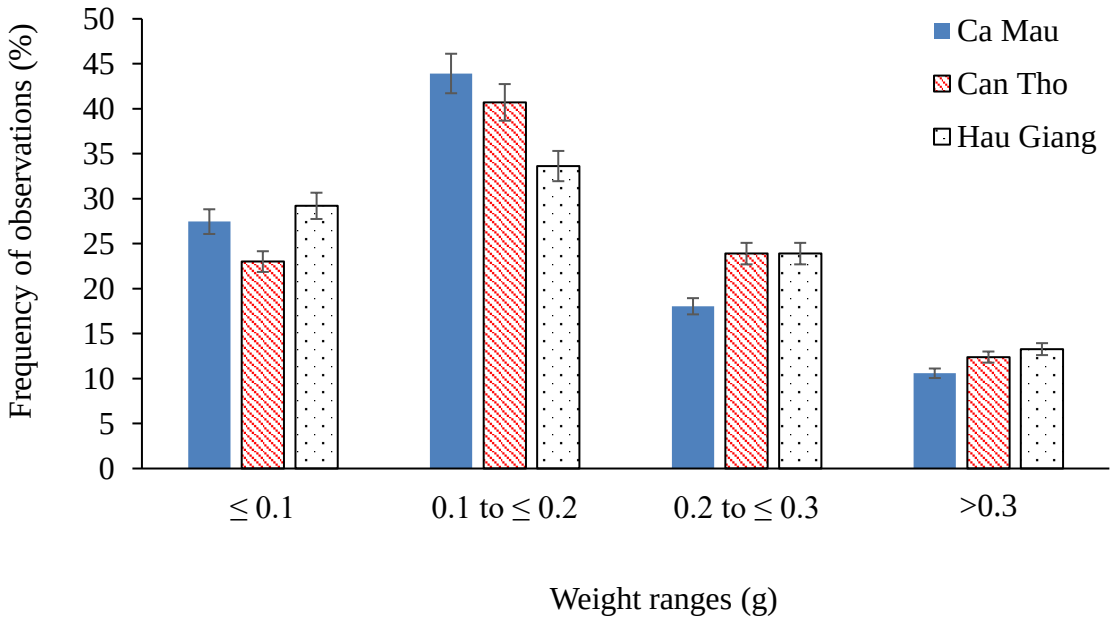

Figure 2. The weight distribution of bighead catfish fingerlings after 40 days of larval rearing (Error bars present one stardard deviation)

Survival rates of bighead catfish in the present experiment were higher than those in a previous study in which survival rates of different larval groups (reared in 500-L tanks) at 30 days ranged from 10.3 to 56.4% (Duong Thuy Yen *et al*., 2020). Better feeding management and less cannibalism could lead to higher survival in smaller rearing tanks compared to larger ones.

The growth of bighead catfish from larvae to the fingerling stage was not statistically significant among the three fish groups (P> 0.05). This result did not match our prediction.

Wild (CM and HG) groups with larger sizes of larvae at hatching and larger yolk sac volume were expected to result in faster growth of fry due to maternal effects. The period of maternal effects on offspring's growth varies among species (Sogard *et al*., 2008), often in the first two or three weeks post-hatch. In tropical clownfish, *Amphiprion melanopus*, maternal and paternal effects of bigger sizes on the faster growth of larvae were observed up to 11 days post-hatch (Green & McCormick, 2005). Similarly, larger females with larger egg sizes and larval sizes at hatching also resulted in better growth of square-head climbing perch at 21 days old (Duong Thuy Yen *et al.*, 2014b). In the present study, the period of maternal effects on offspring could not be determined due to a lack of sampling at middle points during the experiment. For bighead catfish, sample handling can cause stress and injury which can lead to disease infection of the young fish. Therefore, no middle sampling was intentionally conducted in this study.

No significant difference in offspring growth between the cultured and wild bighead catfish strains rejected the alternative hypothesis that cultured broodstock could result in lower offspring growth due to possible inbreeding depression (Tave, 1999). When no inbreeding depression occurs, cultured strains usually grow faster than wild strains (Dunham, 2011). Further evaluation of these bighead catfish sources on larger stages (juvenile and grow-out) should be conducted to provide basic information of strain comparisons for genetic improvement programs of the species. In a similar study on bighead catfish in Thailand, Muiocha *et al.* (2017) reported that a cultured strain had larger sizes than those of wild strain in a grow-out experiment from 60 days to 210 days old.

Conclusions

The three bighead catfish strains differed in relative fecundity in which the Can Tho cultured strain was the highest and wild fish from Ca Mau was the lowest. Fertilization and hatching rates were high and different among the three broodstock sources collected in Ca Mau, Hau Giang, and Can Tho. In addition, the size of newly hatched larvae and yolk sac volume were significantly higher in the wild groups (Ca Mau and Hau Giang) compared to the cultured Can Tho fish. However, the broodstock sources did not significantly affect the growth and survival rates of bighead catfish from larvae to the fingerling stage.

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