Effects of Stocking Density and Water Management on Stress Responses and Reproductive Performance of Bighead Catfish (*Clarias macrocephalus*) Female Broodstock after Hormone Injection

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ABSTRACT

Stress responses and reproductive performance of female bighead catfish (*Clarias macrocephalus* Günther, 1864) broodfish in response to different stocking densities and water systems after hormone injection were investigated. The experiment followed a 4×2 factorial in a completely randomized design, wherein the female broodfish were injected with 30 µg LHRHa and 5 mg domperidone per kg of fish. They were then stocked at four different densities: 5 fish·m^{-2}, 10 fish·m^{-2}, 15 fish·m^{-2}, or 20 fish·m^{-2}. They were provided with either a water flow-through or stagnant water (without water exchange). The results revealed that neither the densities nor the water systems affected the stress response, as measured by blood plasma cortisol. Regarding reproduction-related traits, blood 17β estradiol concentration and the percentage of females spawned were not significantly influenced by these two factors ($p>0.05$). Interestingly, the water flow-through system tended to induce faster ovulation and spawning compared to the no water exchange system. Additionally, the water flow-through system also significantly ($p<0.05$) contributed to better reproductive outcomes, namely higher egg hatching rates and larval survival, compared to the no water exchange system. The loss of spawned female within 72 h after spawning was not affected by density or water system. In conclusion, this study suggests that female broodfish should be kept at a stocking density of 20 fish·m^{-2} in the water flow-through system following hormone injection for optimal reproductive performance.

Keywords: 17β estradiol, Artificial breeding, Blood cortisol, *Clarias macrocephalus*, Hormone injection

INTRODUCTION

The bighead catfish (*Clarias macrocephalus* Günther, 1864) is an economically important freshwater fish in Thailand. It is primarily used for the producing a hybrid walking catfish (female *C. macrocephalus* × male African catfish, *Clarias gariepinus* (Burchell, 1822) which contributes significantly to the annual freshwater fish production in Thailand. As a result, the demand for seed of this hybrid catfish has increased dramatically each year.

The artificial breeding protocol for producing this hybrid involves a single injection of the synthetic hormone LHRHa (Luteinizing hormone Releasing Hormonein analogue) along with domperidone. Typically, the injected female spawns about 14–16 h after the hormone injection (Na-Nakorn, 1997). Artificial insemination is then carried out using the striped eggs and milt obtained from homogenized African catfish testes (Na-Nakorn, 1995). Despite the high success rate, concerns have been raised about the management of injected females before striping, as they are often

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densely stocked in small storage tanks with shallow water depth, leading to stress-related issues such as excessive mucous secretion, severe muscle lesions, and a mortality rate of about 20–30% among female broodfish during the breeding process. This stressful environment could also negatively impact spawning success, hatching rates, and larval survival.

Although there have been no previous reports on the aforementioned issues, it is well known that *Clarias* spp. possess sharp spines on each pectoral fin, which can cause serious wounds to one another, especially under stressful conditions. Several environmental conditions have been reported to affect stress in teleost. For example, crowding and/or disturbances during harvesting have been shown to cause stress in fish, as indicated by a rapid increase in blood plasma cortisol levels (Wendelarr Bonga, 1997; Barton, 2002). Cortisol is widely recognized as the stress hormone, and density-dependence is one of the most important factors affecting stress in most teleosts (Marco *et al*., 2008). Stress has a direct effect on reproduction, by inhibiting the gametogenesis pathway, decreasing the synthesis of gonadotropin from pituitary gland, which results in low concentrations of blood plasma gonadotropin (Carragher *et al*., 1989) and steroid hormones synthesized by the ovaries-especially 17β estradiol, which is crucial for complete vitellogenesis (Clearwater and Pankhurst, 1997; Haddy and Pankhurst, 1999, Kubokawa *et al*., 1999). Stress has also associated with delayed ovulation in fish, leading to smaller fertilized eggs of poorer quality (Campbell *et al*., 1992; 1994). In female bighead catfish, the peak of the spawning season was observed in August, as indicated by the highest proportion of post-vitellogenic oocytes and peak concentration of 17β estradiol during a full spawning cycle (Ratanasatian *et al*., 2014). In *Oreochromis mossambicus*, peak concentrations of 17β estradiol were noted during the reproductive cycle, while serum cortisol levels increased at the end of the vitellogenesis phase and remained significantly high until the post-spawning phase (Ganesh, 2014).

Therefore, this research aims to assess the effects of density and water management provided for female bighead catfish brooders after hormone injection on blood cortisol, sex steroids, and other reproduction-related traits. The information obtained will benefit the improvement of conventional practices, potentially enhancing the welfare of the broodfish and increasing production outputs while reducing the mortality rate of the broodfish.

MATERIALS AND METHODS

Experimental set-up

The experiment was conducted using a 4×2 factorial in a Completely Randomized Design, whereby the two factors examined were the stocking densities of hormonally injected female brooders (5, 10, 15, and 20 fish·m^{-2}) (Wedemeyer, 1976; Strange *et al*., 1978; Burton and Murray, 1979; El Naggar *et al*., 2006) and two water management practices, namely water flow through and stagnant water without replacement.

Broodfish preparation

Five hundred one-year-old female broodfish of *Clarias macrocephalus*, each with an average weight of 200–300 g, were reared in a 0.32-hectare earthen pond for three months, from November 2008 to January 2009. They were fed commercial pelleted feed (Charoen Pokphand Co., Ltd. Thailand), containing 30% protein at 3% of their body weight once a day at 3.00 p.m. This was reduced to 1% in mid-December 2008. In February 2009, gravid females were selected based on the appearance of swollen, soft bellies and pinkish, protruded urogenital pores. Two-years-old male African catfish, reared the same manner as the female brooders but in separate ponds, were used to obtain milt for egg fertility evaluation. The healthy fish with slender bodies and slightly long, pinkish urogenital papilla were selected.

Experimental storage tank systems

Oval-shaped fiberglass containers with a bottom area of 1 square meter were used as experimental tanks. The selected matured females were randomly stocked in each tank at assigned densities. The tanks were filled with dechlorinated water at a depth not exceeding the bodies of the

female broodfish. For tanks assigned to the flow-through water system, water inlets and outlets were installed with a water exchange rate set at 0.5–1 $L \cdot s^{-1}$. The experiment was conducted with 3 replications.

Hormone injection

Females were intra-muscularly injected above the lateral line with 30 µg LHRHa (Suprefact) and 5 mg Motilium per kg fish. They were then stocked in the experimental tanks. Spawning readiness was monitored 11 h after injection and onwards by examining the presence of a few eggs at the tank bottom. Each fish was individually checked by gently pressing at the belly, and those that easily stripped eggs were individually stripped, with the eggs used for further artificial fertilization. During this step, the time taken to strip each female and the number of spawned females per replication were recorded.

Simultaneously, 35 gravid male African catfish were kept in a 5 m^3 tank with a water depth of 10 cm, supplied with flowing water at a rate of 1–2 $L \cdot min^{-1}$. The injection was done using 10 µg of Suprefact and 5 mg of Motilium per kg of fish, concurrently with the injection of all females.

Milt preparation and evaluation of milt quality

At 11 h post-injection, the testes from 25 euthanized male fish were collected. Only those that were fully expanded and whitish were selected, pooled together, and minced in Ringer solution at a ratio of 1 ml milt to 2 ml of Ringer solution (Mongkonpunya *et.al*., 1995). The remaining tissues and debris were then removed. Subsequent milt quality evaluation was conducted using the live-dead staining method with Eosin-Nigrosin (Na-Nakorn, 1995), revealing an average survival rate of 89.2±0.79%, based on five measurements.

Artificial fertilization

Approximately 1 g of eggs from each female was fertilized with 0.1 mL of the pooled milt suspension using the semi-dry method. The fertilized eggs were then evenly spread over a fine mesh suspended in a tray measuring 25 cm in width, 40 cm in length, and 8 cm in depth, filled with 5 L of water. The total number of hatched eggs was recorded, with any unfertilized eggs being carefully removed from the tray. Later, the numbers of fry with absorbed yolk sacs was monitored to assess their survival until first-feeding.

The following parameters were calculated:

% spawning female
= (number of spawned females/ total number of injected females) × 100

% hatching
= (number of hatchlings/total number of eggs incubated) × 100

% survival
= (number of first feeding larvae/ number of hatchlings) × 100.

After stripping the females were returned to their respective tanks. Dead fish were routinely removed, and number of surviving females was recorded 72 h post-stripping.

Measurement of hormones

Blood samples were collected for hormone analysis from a randomly selected female in each replicate at three distinct time points: before starting hormone injection, 7 h after hormone injection, and at ovulation. Among the 144 total samples, only cortisol and 17β estradiol were measured. The analysis was conducted using radioimmunoassay (RIA) following the method described by Sufi *et al*. (1986), and employing the test kit from Adaltis Italia S.p.A. Gamma rays were measured using a gamma counter, and data interpretation was performed using the gamma counter GMS version 3.05 program: GAMMA-C12.

Water quality measurement

The water quality in female storage tanks was assessed simultaneously with blood sample

collection. Dissolved oxygen (DO), pH, and temperature were measured using YSI Model 63 DO meter (Cole-Parmer, IL, USA). A test kit was used to measure total ammonia nitrogen (TAN).

Statistical analysis

All data were analyzed using 2-way analysis (ANOVA). Subsequent mean comparisons were performed using Duncan's New Multiple Range Test. All tests were considered significant at $p<0.05$.

RESULTS AND DISCUSSION

Time at ovulation, percent female spawned and survival of female broodfish at 72 h post-injection

The female brooders could be easily stripped (referred to as 'spawned') between 11 and 13 h after injection in the groups receiving water flow-through, and between 14 and 16 h for those in stagnant water. No variation was observed among females stocked at different densities (Table 1). Since the exact spawning time was not recorded, statistical analysis was not performed. However, it is worth mentioning that there was a tendency toward shorter latency times in the groups that received water flow-through. Similarly, the percentage of spawning females was not affected by either the water systems or density, with percentages ranging between 80% and 90%. The survival of females 72 h post-spawning showed a near-significant difference ($p = 0.063$) between water systems, with 84%–95% survival for no water exchange; 60%–95% for water flow-through) (Table 1). No water exchange seemed to favor survival, and within each water system, higher density tended to result in better survival. However, since the results were not strongly supported by statistical analysis, further implications of these findings should be approached with caution.

Egg hatching and larval survival rates

Apparently, water flow-through system had significant effects on enhancing hatching rates and survivability of larvae (Table 2). Notably, female density significantly affected only hatching rates, but not larval survivability. A density of 20 females·m^{-2} yielded the highest hatching rate of fry, although the difference was not significant compared to the 15 females·m^{-2} density.

Blood plasma cortisol and 17β estradiol

Before hormone injection, blood plasma cortisol levels ranged from 41.19±43.68 ng·mL^{-1} to 88.44±31.03 ng·mL^{-1}. Following hormone injection, an increasing trend was observed, with no statistically significant ($p>0.05$) effects attributable to female densities, water systems, or the interaction between

Table 1. Effects of female densities and water systems, and their interaction on time at ovulation, spawning percentage, and female survival rate 72 h post-spawning in female *Clarias macrocephalus* broodfish.

Water system	Density (fish·m^{-2})	Time at ovulation (h)	% Spawning females	% Survival of females at 72 h after spawning
Water flow-through	5	11–13	80.0±0.0	60.0±0.0
	10	11–13	83.3±11.5	86.7±11.5
	15	11–13	88.9±7.7	82.2±3.9
	20	11–13	90.0±0.0	95.0±5.0
No water exchange	5	14–16	80.0±20.0	93.3±11.5
	10	14–16	80.0±10.0	90.0±10.0
	15	14–16	86.7±6.6	84.4±15.4
	20	14–16	88.3±10.4	95.0±5.0
p-value (density*water system)	-	-	0.908	0.099
p-value (density)	-	-	0.552	0.154
p-value (water systems)	-	-	0.916	0.063

Table 2. Effects of female densities, water systems, and their interaction on % hatching and % survival of the larvae from *Clarias macrocephalus* females fertilized with *C. gariepinus* sperm.

Water system	Density (fish·m^{-2})	% Hatching	% Survival
Water flow-through	5	80.6±2.4	82.9±2.6
	10	82.9±1.3	84.3±3.3
	15	84.3±1.9	83.6±2.7
	20	84.4±1.1	84.2±3.3
No water exchange	5	63.9±3.3	74.8±2.7
	10	67.2±1.4	74.5±0.8
	15	67.8±1.5	74.0±0.8
	20	71.1±2.2	74.5±1.1
p-value (density*water system)	-	0.481	0.882
Density (averaged across water systems)			
	5	72.3±9.5^{c}	79.2±5.4
	10	75.1±8.6^{b}	78.9±6.5
	15	76.1±9.2^{ab}	78.5±5.2
	20	77.6±7.5^{a}	79.3±5.7
p-value (density)	-	0.002	0.937
Water system (averaged across female density)			
Water flow-through	-	83.1±2.2^{a}	83.8±2.6^{a}
No water exchange	-	67.5±3.3^{b}	74.2±1.8^{b}
p-value (water system)	-	<0.001	<0.001

Note: Mean±SD in each column within each category, superscripted with different lowercase letters, indicate highly significant ($p<0.05$) differences between treatments.

these two factors. Notably, there was substantial variation within each treatment combination, as evidenced by large SD with mean values ranging from 83.5±36.1 ng·mL^{-1} to 141.8±26.1 ng·mL^{-1}. Similar patterns were observed in blood cortisol levels at ovulation, with a range from 88.0±43.2 ng·mL^{-1} to 198.5±83.6 ng·mL^{-1}. It could be stated that there is a high sensitivity to stress that varied specifically among individual females.

The blood concentration of 17β estradiol was unaffected by female densities, water systems, or the interaction between these factors. Prior to hormone injection, it ranged from 3.35±0.55 ng·mL^{-1} to 6.94±2.47 ng·mL^{-1}, while after hormone injection, it varied between 2.54±0.65 ng·mL^{-1} and 5.41± 1.70 ng·mL^{-1}. During ovulation, the concentration ranged from 3.88±2.87 ng·mL^{-1} to 7.29±2.42 ng·mL^{-1}. Large variations occurred within each treatment combination. Notably, the effect of female density was almost significant ($p = 0.05$) at ovation, whereby the hormone concentration tended to increase with densities (Table 3).

Water quality

All water quality parameters in the female storage tanks during the experimental period are presented in Tables 4, 5, 6, and 7, respectively. At the beginning of the experiment, all water quality parameters, such as dissolved oxygen (DO), pH, and temperature, were not significantly ($p>0.05$) different between treatments, except for total ammonia nitrogen (TAN), which was non-detectable.

However, a highly significant ($p<0.001$) difference in DO between treatments was observed at 7 h after hormone injection and at ovulation

Table 3. Effects of female densities, water systems, and their interaction on levels of blood plasma cortisol and 17β estradiol of female broodfish *Clarias macrocephalus* during the experimental periods.

Water system	Density (fish·m^{-2})	Cortisol (ng·mL^{-1})			17β Estradiol (ng·mL^{-1})		
		Before hormone injection	After hormone injection at 7 h	At ovulation	Before hormone injection	After hormone injection at 7 h	At ovulation
Water flow-through	5	6.12±2.22	4.46±0.41	4.85±0.69	6.12±2.22	4.46±0.41	4.85±0.69
	10	3.35±0.55	2.54±0.65	4.82±2.58	3.35±0.55	2.54±0.65	4.82±2.58
	15	6.94±2.47	5.41±1.70	5.41±0.28	6.94±2.47	5.41±1.70	5.41±0.28
	20	5.08±3.92	4.01±2.67	6.91±1.33	5.08±3.92	4.01±2.67	6.91±1.33
No water exchange	5	3.74±1.49	3.66±0.49	3.88±2.87	3.74±1.49	3.66±0.49	3.88±2.87
	10	5.72±4.75	4.00±1.69	4.13±0.99	5.72±4.75	4.00±1.69	4.13±0.99
	15	6.59±4.20	4.23±0.88	6.94±1.48	6.59±4.20	4.23±0.88	6.94±1.48
	20	4.78±0.78	5.14±3.92	7.29±2.42	4.78±0.78	5.14±3.92	7.29±2.42
p-value (density*water system)	-	-	0.557	0.629	-	0.557	0.629
p-value (density)	-	-	0.538	0.050	-	0.538	0.050
p-value (water systems)	-	-	0.849	0.933	-	0.849	0.933

(Table 4). The water flow-through system maintained a very highly significant ($p<0.001$) higher level of DO compared to the no water exchange system throughout the experimental periods. In the water flow-through system, DO level remained relatively stable from 7 h after hormone injection until ovulation, maintaining an optimal level (>5 mg·L^{-1}). Conversely, in the no-water exchange system, DO levels continuously declined, reaching below 3 mg·L^{-1} at the time of ovulation (Table 4). Notably, there was a significant interaction between female densities and water systems ($p<0.001$), with the lowest DO observed at a density of 20 fish·m^{-2} in the no water exchange system.

The density of females did not affect pH, while water systems and the interaction of the two factors showed significant effects at 7 h after injection. It was apparent that pH was quite constant across densities where water flow-through system was applied. On the contrary, without water exchange, pH significantly dropped when densities increased. However, average pH in all treatment combinations was still in an optimum range (7.41–7.68). At ovulation, only the effect of water systems was significant whereby no water exchange resulted in decrease of pH, although it was still within an acceptable range (Table 5).

Similarly, water temperature at 7 h after injection was affected by female densities, water systems, and their interaction. Overall, the water flow-through system maintained water temperature at about 30 °C while the temperature of the treatments without water exchange significantly varied among densities, with relatively low temperatures (28.1±0.15 °C–28.6±0.29 °C). At ovulation, only water systems significantly affected temperature, with higher temperatures (31.8±0.06 °C –32.0±0.10 °C) observed in the water flow-through system (Table 6).

The initial TAN levels in all treatments were undetectable (Table 7). In the no-water exchange system, TAN rapidly increased from the start of the experiment until ovulation, reaching 2.50±0.00 mg·L^{-1} at 7 h and 5.00±0.00 mg·L^{-1} at ovulation, exceeding the optimal value (>0.5 mg·L^{-1}). In the water flow-through system, TAN was only detected at 7 h at a density of 20 fish·m^{-2} and was present at all densities at ovulation, but remained below 0.50 mg·L^{-1}. The water system was identified as a highly significant ($p<0.001$) factor influencing TAN differences at ovulation. The water flow-through treatments exhibited a maximum of 0.25 mg·L^{-1} TAN, significantly lower than the no-water exchange treatments (5.00 mg·L^{-1}) (Table 7).

Table 4. Effects of female densities and water systems, and their interaction on dissolved oxygen (mg·L^{-1}) in female storage tanks during the period of experiment.

Water system	Density (fish·m^{-2})	DO (mg·L^{-1})		
		Before hormone injection	After hormone injection at 7 h	At ovulation
Water flow-through	5	6.56	5.56±0.08^{a}	5.48±0.03^{ab}
	10	6.56	5.43±0.04^{b}	5.61±0.16^{a}
	15	6.56	5.32±0.07^{b}	5.34±0.06^{bc}
	20	6.56	5.36±0.06^{b}	5.42±0.05^{c}
No water exchange	5	6.56	5.04±0.06^{c}	2.90±0.07^{d}
	10	6.56	4.45±0.11^{d}	2.38±0.06^{e}
	15	6.56	4.29±0.06^{e}	2.12±0.03^{f}
	20	6.56	4.07±0.06^{f}	1.10±0.06^{g}
p-value (density*water system)	-	-	<0.001	<0.001
p-value (density)	-	-	<0.001	<0.001
p-value (water systems)	-	-	<0.001	<0.001

Note: Wherever interaction is significant, mean comparison within each main effect (water system or density) is not shown; Mean±SD in each column within each category, superscripted with different lowercase letters indicate highly significant ($p<0.05$) differences between treatments.

Table 5. Effects of female densities and water systems, and their interaction on water pH in female storage tanks during the period of experiment.

Water system	Density (fish·m^{-2})	pH		
		Before hormone injection	After hormone injection at 7 h	At ovulation
Water flow-through	5	7.50	7.58±0.01^{a}	7.67±0.02
	10	7.50	7.63±0.02^{a}	7.65±0.04
	15	7.50	7.61±0.02^{a}	7.68±0.02
	20	7.50	7.61±0.01^{a}	7.67±0.03
No water exchange	5	7.50	7.46±0.03^{b}	7.35±0.03
	10	7.50	7.41±0.03^{c}	7.35±0.02
	15	7.50	7.42±0.03^{bc}	7.33±0.02
	20	7.50	7.41±0.03^{c}	7.31±0.02
p-value (density*water system)	-	-	0.027	0.100
p-value (density)	-	-	0.672	0.572
Water systems (averaged across densities)				
Water flow-through				7.67±0.02^{a}
No water exchange				7.33±0.0^{b}
p-value (water system)	-	-	<0.001	<0.001

Note: Wherever interaction is significant, mean comparison within each main effect (water system or density) is not shown; Mean±SD in each column within each category, superscripted with different lowercase letters indicate highly significant ($p<0.05$) differences between treatments.

Table 6. Effects of female densities and water systems, and their interaction on water temperature (°C) in female storage tanks during the period of experiment.

Water system	Density (fish·m^{-2})	Water temperature (°C)		
		Before hormone injection	After hormone injection at 7 h	At ovulation
Water flow-through	5	29.8	29.9±0.26^{b}	32.0±0.10
	10	29.8	30.9±0.21^{a}	31.9±0.15
	15	29.8	30.6±0.26^{a}	32.0±0.11
	20	29.8	30.5±0.26^{a}	31.8±0.06
No water exchange	5	29.8	28.3±0.17^{cd}	29.7±0.15
	10	29.8	28.1±0.15^{d}	29.7±0.06
	15	29.8	28.3±0.10^{cd}	29.7±0.20
	20	29.8	28.6±0.29^{c}	29.7±0.12
p-value (density*water system)	-	-	0.002	0.456
Water system (averaged across densities)				
Water flow-through				31.9±0.12^{a}
No water exchange				29.7±0.12^{b}
p-value (water system)	-	-	<0.001	<0.001
p-value (density)	-	-	0.013	0.730

Note: Wherever interaction is significant, mean comparison within each main effect (water system or density) is not shown; Mean±SD in each column within each category, superscripted with different lowercase letters indicate highly significant ($p<0.05$) differences between treatments.

Table 7. Effects of female densities and water systems, and their interaction on total ammonia nitrogen (TAN) (mg·L^{-1}) in female storage tanks during the period of experiment.

Water system	Density (fish·m^{-2})	TAN (mg·L^{-1})		
		Before hormone injection	After hormone injection at 7 h	At ovulation
Water flow-through	5	ND	ND	0.17±0.14
	10	ND	ND	0.17±0.14
	15	ND	ND	0.25±0.00
	20	ND	0.08±0.14	0.25±0.00
No water exchange	5	ND	2.50±0.00	5.00±0.00
	10	ND	2.50±0.00	5.00±0.00
	15	ND	2.50±0.00	5.00±0.00
	20	ND	2.50±0.00	5.00±0.00
p-value (density*water system)	-	-	-	0.585
p-value (density)	-	-	-	0.585
Water systems (averaged across densities)				
Water flow-through				0.21±0.10^{b}
No water exchange				5.00±0.00^{a}
p-value (water system)	-	-	-	<0.001

Note: Mean±SD in a column superscripted with different lowercase letters indicate highly significant ($p<0.05$) differences between treatments; ND = not detectable

To the best of our knowledge, no prior studies have investigated the impact of the environment following hormone injections, despite its significance for breeding success and the welfare concerns of brooders. Poor water quality and crowding are among the stress-inducing factors. The fish reproductive response to stress varied by species; for example, a decrease in sex hormone levels was observed in rainbow trout (*Oncorhynchus mykiss*) and black bream (*Acanthopagrus butcheri*) in response to stress (Clearwater and Pankhurst, 1997; Haddy and Pankhurst, 1999). Conversely, increased levels of cortisol, which are associated with stress, enhanced estradiol secretion and eventually reproduction in *Anguilla anguilla* (Huang *et al*., 1999) and catfish (reviewed by Murugananthkumar and Cheni-Chery, 2022).

In the present investigation, both crowding and poor water quality in the no-water exchange groups did not significantly impact cortisol levels. This contrasts with a previous study (Marco *et al*., 2008) that reported an increase in stress due to crowding, leading to elevated plasma cortisol levels at higher density. In teleosts, basal or resting cortisol levels have been reported to range from 5 ng·mL^{-1} to 30 ng·mL^{-1} (Tintos *et al*., 2006). In the current study, serum cortisol levels were comparatively high even at the beginning of the study before hormone injection. This may indicate an association between high cortisol levels and gonad maturation in bighead catfish, and may imply that further exposition to stress did not further increase cortisol levels or 17β estradiol. It is also possible that crowding might not affect cortisol secretion of the bighead catfish used in this study. This hypothesis is supported by a report on North African catfish (*C. gariepinus*), which suggested that fish density did not affect plasma cortisol levels during the reproduction period (Nieuwegiessen *et al*., 2008).

The serum 17β estradiol levels in the bighead catfish observed in this study are significantly elevated compared to the peak concentration of 0.7±0.4 ng·mL^{-1} recorded in August, as reported by Ratanasatian *et al*. (2014). This peak was identified as the peak of the spawning season based on the highest proportion of post-vitellogenic oocytes in their ovaries (Ratanasatian *et al*., 2014). Ganesh (2014) reported a peak concentration of approximately 0.4 ng·mL^{-1} during the reproductive cycle of *Oreochromis mossambicus*, while the highest concentration reported for striped catfish (*Pangasianodon hypophthalmus*) was 0.8 ng·mL^{-1}. The remarkably elevated level of 17β estradiol in the present study may be attributed to hormone injection.

This study demonstrates the significant impact of a water flow-through system on accelerating faster spawning (11–13 h) and enhancing egg quality. Improved egg-hatching rates and survival of first-feeding larvae serve as indicators of these advancements. The superior water quality in a flow-through system is a crucial factor influencing better reproductive output. Conversely, a no-water exchange system with poor water quality can lead to delays in ovulation and spawning completion. Iwama *et al*. (1977) reported that conditioning broodfish in a suboptimal environment directly influences egg quality, resulting in smaller eggs with lower hatching rates, decreased survival, and delayed spawning due to increased atresia in the ovaries. Keeping matured broodfish in captivity for an extended period before breeding has been shown to produce smaller eggs in rainbow trout and lower survival rates in larvae of both rainbow trout and brown trout (*Salmo trutta*) compared to those in the control group (Campbell *et al*., 1992; 1994).

The water flowing-through system maintained optimal water quality, particularly regarding dissolved oxygen (DO≥3 mg·L^{-1}) and total ammonia nitrogen (TAN<0.05 mg·L^{-1}), as per Boyd's recommendations for aquaculture (Boyd, 1990). Notably, tanks with water flow-through exhibited a significantly higher temperature compared to those without water exchange. This temperature difference may be due to the supplied water originating from an outdoor tank, which could enhance the metabolism of the brooders and potentially lead to faster ovulation when compared to females in the cooler temperatures of the no-water exchange system.

The evaluation of female fish loss within 72 h post-spawning is crucial in assessing the significant costs associated with fish breeding. Although no notable impacts of density and water system on the loss of female broodfish were observed, a density of 5 fish·m^{-2} in a water flow-through system resulted in a low survival rate (60±0%). This decline in survival can be attributed to aggressive behaviors such as biting and the use of sharp spines on the pectoral fins to injure each other. Our observations suggest that these aggressive interactions are the primary cause of the highest losses. Factors influencing these behaviors include the availability of space and the introduction of new running water. The current results emphasizes that increased space and fresh flowing water are important environmental factors that directly influence the intensity of aggressive behaviors, thereby contributing to the likelihood of female fish loss.

In contrast, both the highest and lowest variations in survival (95±5%) occurred at a density of 20 fish·m^{-2} in both water systems. The crowding effect limits the space available for active movement, which in turn minimizes opportunities for aggressive behavior.

CONCLUSIONS

In conclusions, this research demonstrates that a water flow-through system can have a significant impact on accelerating ovulation and spawning, leading to improved egg quality and higher hatching and larval survival rates. The study recommends stocking post-hormone-injected female broodfish at a density of 20 fish·m^{-2} in a storage tank with a water flow-through system. It is important to note that the experimental conditions did not result in elevated cortisol levels, suggesting that the conditions did not cause stress to the brooders. Overall, this approach appears to be a promising best practice for addressing animal welfare concerns in breeding and aquaculture.

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