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Article

Gonadal development of striped catfish (*Pangasianodon hypophthalmus*) in climate-caused salinity intrusion

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Abstract: Salinity intrusion is one of the major climate-caused problems globally. The present work evaluated the impacts of salinity on the gonadal improvement in striped catfish (*Pangasianodon hypophthalmus*). The fingerlings of striped catfish (8.63 g) were cultured at three different salinity levels (0, 4, and 8 ppt) for two years. Gonadal development was assessed by gonadosomatic index (GSI) and histological analysis over six months at the end of two years of rearing from April to September. The gonad histological observations and the associated changes in GSI showed that the male and female striped catfish cultured in different salinity conditions could complete testicular and ovarian maturation at 4 and 8 ppt salinity by June and July, as they have grown at 0 ppt salinity. The current findings indicate that despite a slow rate of development, salinity up to 8 ppt showed a positive impact on the gonadal development of striped catfish.

Keywords: gonadosomatic index; salinity; reproduction; Thai pangas; aquaculture

1. Introduction

Aquaculture is the most notable and distinctive industry that has been recognized as a substantial source of protein all over the world and is always striving to guarantee safe food for people (Hossain *et al*., 2016; Gui *et al*., 2018). Climate change is a major impediment to global food production, poses a substantial danger to food security (Hamdan *et al*., 2015; Myers *et al*., 2017). Several studies have reported that climate changes negatively impacted sustainable aquaculture production (Blanchard *et al*., 2017; Troell *et al*., 2017), putting the entire aquaculture production cycle at risk.

Salinity intrusion is one of the prime climate-caused problems globally. Along with other natural phenomena (cyclones and storm surges), salinity intrusion due to rising sea levels are induced by climate change (Rabbani *et al*., 2013; Pörtner *et al*., 2019). Because of salinity intrusion, a huge threat has been imposed on the existing biodiversity in coastal areas. Environmental salinity is a crucial factor affecting aquatic organism persistence and physiological processes (Alam *et al*., 2020: Su *et al*., 2023). Every aquatic organism has several limiting factors that influence its abundance in aquatic media, and salinity is one of them. Salinity intrusion in coastal

areas alters not just the features of the water and soil but also the fish reproductive cycle, including commercially important aquaculture species. Salinity potentially acts as a stressor in aquaculture conditions (Varsamos *et al*., 2004). It is one of the most important environmental factors affecting fish hatchery production (Aktas *et al*., 2004). Salinity affects the success of egg incubation and larval rearing of fish (Kujawa *et al*., 2017). Typically, egg fertilization, embryonic development, and larval growth of aquatic organisms are mostly dependent on the water salinity range for specific aquatic environments (Bœuf and Payan, 2001). Acclimation from freshwater to seawater environments is a species-specific feature among fish and varies among developmental stages (Villegas, 1990; Suresh and Lin, 1992). Hence, salt-tolerant species are needed to bring variety to aquaculture practice and the physiological factors of these species should also be studied. However, studies on the influences of salinity on the gonadal development of fish are limited.

Reproduction is a continual process of development that calls for adaptations in the areas of energy use, ecology, anatomy, biochemistry, and endocrinology (Caputo *et al*., 2001). The GSI is a reliable indication of reproductive activity that can be used to pinpoint the stages of gonadal development (Hismayasari *et al*., 2015). It shows the timing of fish reproduction (Mohan and Jhajhria, 2001; Shankar and Kulkarni, 2005). Fish spawning activities may be predicted based on monthly fluctuations in GSI and are commensurate to each stage of maturation (Zhang *et al*., 2009). The GSI is a useful tool for estimating the fish spawning phase (Bladon *et al*., 2019) found that a considerable reduction in GSI from high to low is a signal of egg-depositing activity in fish. Once again, histological research on fish's gonadal alterations is a vital tool for determining the level of reproduction, which encompasses several developmental stages. The sexual maturation of Hilsa was discovered to be strongly correlated with the histological alteration in the gonad (Ünver and Saraydin, 2004).

The striped catfish (*P. hypophthalmus*) is a well-known aquaculture species globally. Its high growth rate, easy rearing at great densities, and easy seed production in hatchery made the fish a good candidate for aquaculture. This fish also has high culture potential under coastal aquaculture practice. This fish might be desirable for farming in brackish water with a salinity of up to 10 ppt (Jahan *et al*., 2019; Hossain *et al*., 2021; Hossain *et al*., 2022). Investigating the influences of salinity on the striped catfish gonadal development could provide an understanding of the reproductive status of this fish due to climate change-induced salinity intrusion on freshwater aquaculture. Hence, the aim of the current study was to assess the influences of environmental salinity on the gonadal development of *P. hypophthalmus* using GSI and histological techniques.

2. Materials and Methods

2.1. Ethical approval

The present study was approved by the Animal and Ethical Committee, Bangladesh Agricultural University (No.: BAU-FoF/2020/004). The legitimate authority of the university approved the study, including all the methodologies and fish for their use for scientific purposes.

2.2. Experimental fish

In October 2019, a total of 200 healthy 60-day-old active fingerlings of striped catfish were obtained from Bangladesh Fisheries Research Institute, Mymensingh. The average weight and total length of the fingerlings were 8.63 g and 10.68 cm, respectively. Acclimatization of the fingerlings was done in the laboratory in three concrete tanks (2 m × 2 m × 0.30 m = length × width × height) containing 1000 L of freshwater for 15 days. After acclimatization in freshwater, the fish (n = 60) were subjected to varying salinity conditions, i.e., 0, 4, and 8 ppt, for two years. To prepare the desired salinity concentrations, freshwater and brine water (260 ppt) were mixed. Varying salinity concentrations were measured using a refractometer. Fish were fed with commercial floating starter feed at 5% of their body weight twice a day. Every month, the tanks were cleaned, and about half of the water in the system was drained out in order to lessen the nitrogen waste and sediment accumulation.

2.3. Sampling and analytical protocol

The male (n = 3) and female (n = 3) striped catfish were sampled every month over the six months from April to September 2021. Fish were randomly selected and anesthetized with Aquadine (Fish Stabilizer, International Fish S.O.S. Assoc. USA). The body weight and total length of the fish were respectively measured to the nearest 0.01 g and 0.01 cm and then euthanized. Dissection of the fish was done to obtain the gonads, which were then separately weighed and utilized for the gonadosomatic index (GSI) determination using the following formula: Gonadosomatic index (GSI) = (Gonad weight/body weight) ×100

2.4. Histo-pathological studies

On the sampling day, after the fish were dissected, testes and ovary tissues were carefully collected. For histology investigations, transverse sections of gonad samples were extracted from the posterior, middle, and

anterior parts of the gonads of each fish. After eliminating fat, blood, and other adhering, the tissue samples were fixed in labeled bottles containing aqueous Bouin's fluid for 24 hours. Finally, the tissue samples were then gathered and preserved in 70% alcohol and kept at 4°C until further analysis (Hossain *et al*., 2016).

2.5. Tissue processing, sectioning, staining, and analysis

Using ethanol series, the tissue samples were subjected to dehydration and cleared through pure chloroform. Then, the tissue samples were embedded in paraffin wax (60-70°C melting points) and a prepared block for sectioning. Using a rotary microtome (MICROM HM 325, Thermo Scientific), the tissue samples were cut at approximately 5-10 µm thickness and fixed on slides. The slides were then kept overnight for drying in the open air. Finally, with the use of Eosin and Hematoxylin, the slides were stained and mounted in DPX to preserve them in the long term. Observation of the stained sections was done using a digital light microscope (Micros, MCX100, Austria) at 100× and 400× magnifications, and pictures were taken using a digital microscopic camera (AmScope MA1000). The developmental stages of gonads and histological changes were carefully observed and compared with the control samples.

2.6. Statistical analysis

All data were presented as mean ± SD. To assess for statistically significant variations across salinity groups and months, data was analysed using two-way analysis of variance (ANOVA), followed by Tukey's test. All statistical analyses were performed using SPSS version 20.0 for Windows (SPSS Inc., Chicago, IL, USA) with a significance level of $p < 0.05$.

3. Results

3.1. Gonadosomatic index (GSI) of males

Body weight (BW) and gonado-somatic index (GSI) of male striped catfish reared in different salinity levels (0, 4, and 8 ppt) are shown in Table 1. Figure 1 provides the GSI over six months study period for males. The GSI increased gradually from April and peaked in June, and the lowest GSI was seen in September. The gonad histological observations and the associated changes in GSI showed that the male striped catfish cultured in different salinity conditions could complete testicular maturation at 4 and 8 ppt salinity by June and July, as they have grown at 0 ppt salinity.

3.2. Gonadal development in males

The spermatogonia, spermatocytes, spermatozoa, and spermatids were considered for the determination of testicular development (Figure 2). Histological observations indicated four main stages of testicular maturity: Stage I [developing stage in April and May, where primary spermatocyte (Sc1), secondary spermatocyte (Sc2), primary spermatogonia (Sg1), secondary spermatogonia (Sg2), immature stage, spermatids (St), and developing germinal epithelium were observed at different salinity levels], Stage II [spawning capable stage in June predominance of spermatozoa (Sz) in lumen seminiferous tubules, spermatozoa (Sz), testicular lumen (lu), presence of cysts (Cy), germinal epithelium (GE) continuous throughout, and residual lumen were observed]**,** Stage III [regression stage in July and August, Leydig cells, residual spermatozoa (Sz), presence of cysts (Cy), spermatids, immature stage, residual germinal epithelium (GE), residual cyst (Cy), and germinal epithelium (GE) were observed in all the tissues examined], and Stage IV [undeveloped stage in August and September, where an immature stage and residual spermatozoa (Sz) were found].

Table 1. Body weight (BW) and gonado-somatic index (GSI) of male striped catfish (*Pangasianodon hypophthalmus*) reared in different salinity levels (0, 4, and 8 ppt).

Parameters	Salinity (ppt)	Sampling months					
		April	May	June	July	August	September
BW (g)	0	975 ± 28^{a}	950 ± 55^{a}	1090 ± 65^{a}	1350 ± 78^{a}	1300 ± 72^{a}	1280 ± 67^{a}
	4	670 ± 32^{ab}	760 ± 47^{ab}	870 ± 45^{b}	810 ± 68^{b}	805 ± 52^{ab}	810 ± 53^{b}
	8	480 ± 35^{b}	560 ± 28^{b}	615 ± 56^{c}	630 ± 56^{c}	625 ± 47^{b}	590 ± 36^{c}
GSI	0	0.61 ± 0.07^{a}	1.30 ± 0.05^{a}	1.38 ± 0.04^{a}	1.13 ± 0.13^{a}	0.81 ± 0.09^{a}	0.37 ± 0.04^{a}
	4	0.38 ± 0.04^{b}	0.73 ± 0.07^{b}	0.83 ± 0.13^{b}	0.80 ± 0.08^{b}	0.43 ± 0.04^{b}	0.35 ± 0.04^{a}
	8	0.32 ± 0.03^{b}	0.76 ± 0.08^{b}	$0.80\pm0.0.08^{b}$	0.50 ± 0.05^{c}	0.39 ± 0.13^{b}	0.32 ± 0.03^{a}

BW; body weight, GSI; gonado-somatic index. Values of a single parameter in a column with different alphabetical superscripts are significantly ($p < 0.05$) different. All values expressed as mean ± SD (n = 10).

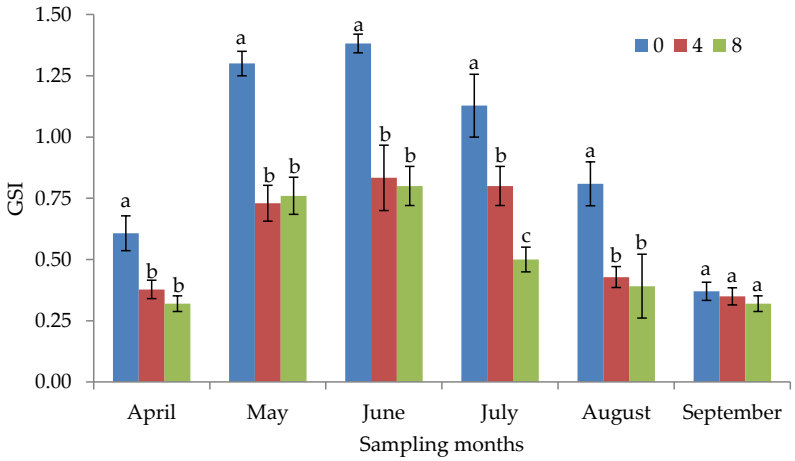

Figure 1. Gonadosomatic index (GSI) of male striped catfish (*Pangasianodon hypophthalmus*) reared in different salinity levels (0, 4, and 8 ppt) for two years. All values are expressed as mean ± SD (n = 10). Different salinity groups with different alphabetical superscripts are significantly ($p < 0.05$) different.

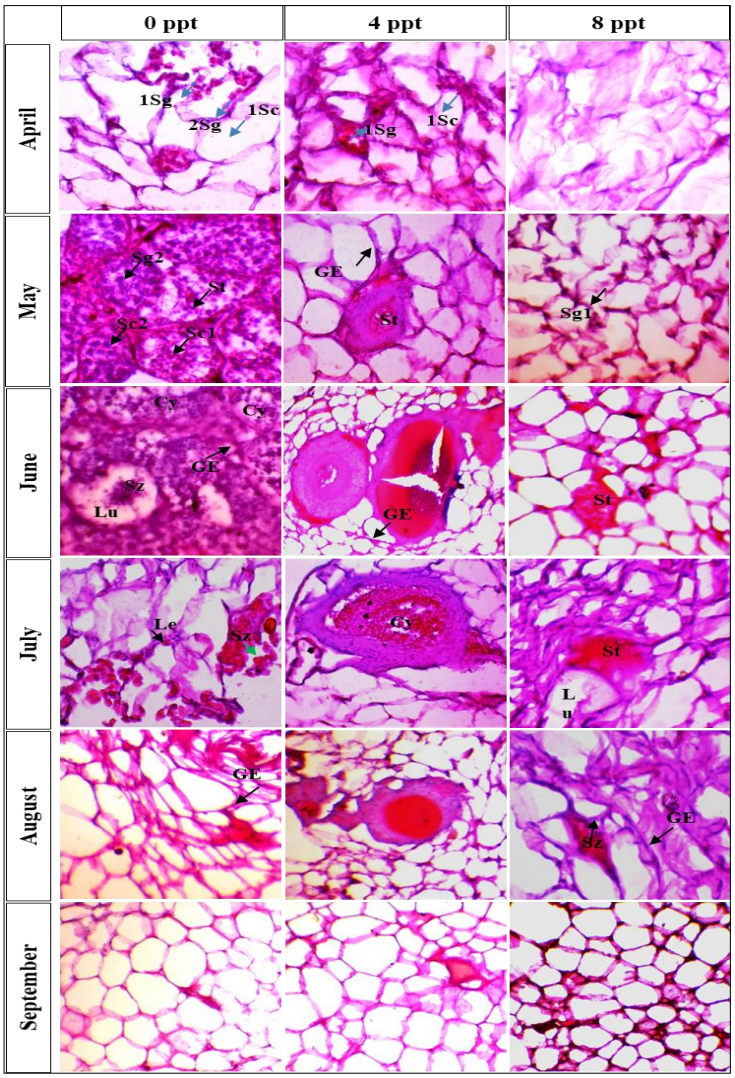

Figure 2. Gonadal development in male striped catfish (*Pangasianodon hypophthalmus*) reared in different salinity levels (0, 4, and 8 ppt) for two years. Sg1; primary spermatogonia, Sg2; secondary spermatogonia, Sc1; primary spermatocyte, Sc2; secondary spermatocyte, St; spermatids, GE; germinal epithelium, Cy; presence of cysts, Sz; spermatozoa, and lu; testicular lumen.

3.3. Gonadosomatic index (GSI) of females

Body weight (BW) and gonado-somatic index (GSI) of female striped catfish reared in different salinity levels (0, 4, and 8 ppt) are shown in Table 2. Figure 3 provides the GSI over a 6-month study period for females. The GSI increased gradually from April and peaked in June and July. The lowest GSI was seen from August to September. The gonad histological observations and the associated changes in GSI showed that the female striped catfish cultured in different salinity conditions could complete ovarian maturation at 4 and 8 ppt salinity by June and July, as they have grown at 0 ppt salinity.

Table 2. Body weight (BW) and gonado-somatic index (GSI) of female striped catfish (*Pangasianodon hypophthalmus*) reared in different salinity levels (0, 4, and 8 ppt).

Parameters	Salinity (ppt)	Sampling months					
		April	May	June	July	August	September
BW (g)	0	1050±79^{a}	1250±86^{a}	1320±98^{a}	1310±82^{a}	1230±74^{a}	1200±63^{a}
	4	700±62^{ab}	860±57^{ab}	970±63^{b}	910±59^{b}	905±62^{b}	820±71^{ab}
	8	520±58^{b}	560±46^{b}	620±26^{c}	610±23^{c}	605±61^{c}	610±51^{b}
GSI	0	2.72±0.17^{a}	5.34±0.23^{a}	6.75±0.45^{a}	6.69±0.37^{a}	1.52±0.52^{a}	1.66±0.40^{a}
	4	1.48±0.48^{b}	1.78±0.78^{b}	2.58±0.78^{b}	2.61±0.61^{b}	0.77±0.14^{b}	0.99±0.36^{ab}
	8	1.20±0.20^{b}	1.32±0.32^{b}	1.78±0.58^{b}	2.24±0.24^{b}	0.53±0.16^{b}	0.50±0.15^{b}

BW; body weight, GSI; gonado-somatic index. Values of a single parameter in a column with different alphabetical superscripts are significantly ($p < 0.05$) different. All values expressed as mean ± SD (n = 10).

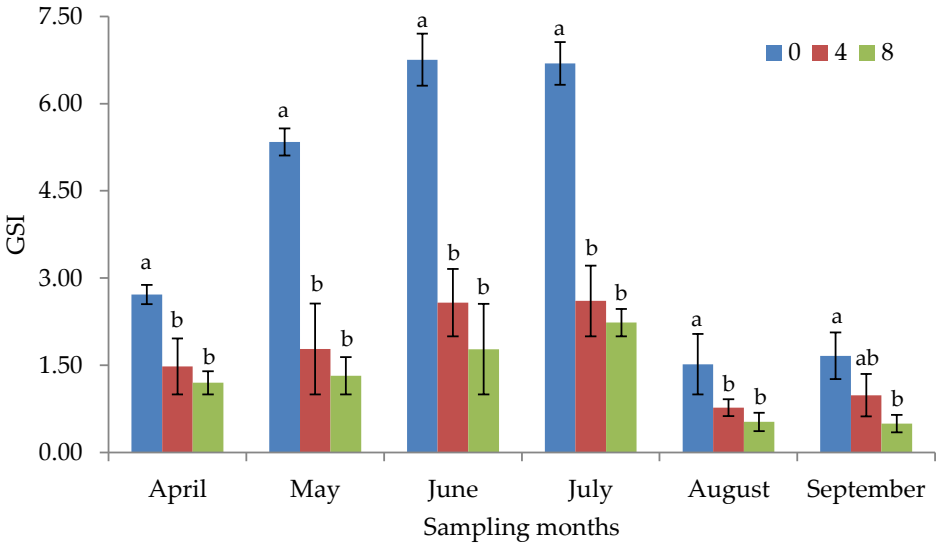

Figure 3. Gonadosomatic index (GSI) of female striped catfish (*Pangasianodon hypophthalmus*) reared in different salinity levels (0, 4, and 8 ppt) for two years. All values are expressed as mean ± SD (n = 10). Different salinity groups with different alphabetical superscripts are significantly (p < 0.05) different.

3.4. Gonadal development in females

Histological observations indicated the finding of four main stages of ovarian maturity. In Stage I, oogonia, chromatin nucleolar oocyte with a spherical nucleus occupying a greater part, perinucleolar oocyte, and primary growth oocyte were observed. Stage II showed the presence of secondary and primary vitellogenic oocytes and cortical alveolar oocytes. Tertiary vitellogenic oocytes and mature oocytes of Stage III were observed in different months at different salinity levels. Old post-ovulatory follicles, degenerating atretic oocytes, and primary growth oocytes in Stage IV were found (Figure 4).

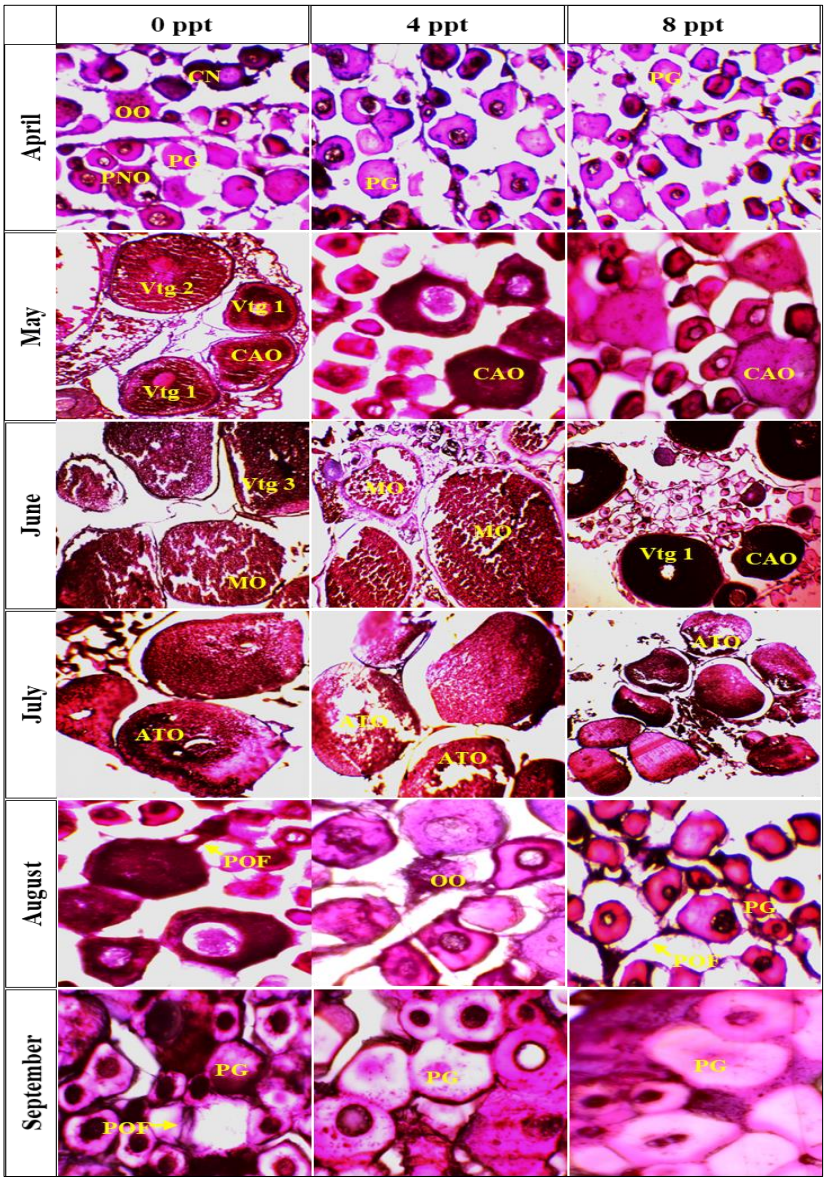

Figure 4. Gonadal development in female striped catfish (*Pangasianodon hypophthalmus*) reared in different salinity levels (0, 4, and 8 ppt) for two years. OO; oogonia, CNO; chromatin nucleolar oocytes, PNO; perinucleolar oocyte, PG; primary growth oocyte, CAO; cortical alveolar oocytes, Vtg1; primary vitellogenic oocyte, Vtg2; secondary vitellogenic oocyte, Vtg3; tertiary vitellogenic oocyte, MO, mature oocyte, POF; post-ovulatory follicles, ATO; degenerating atretic oocyte.

4. Discussion

Salinity has different forms of effects on fish physiology. To minimize the adverse effects of salinity, growth, survivability, gonadal development, and reproductive biology of fish should be studied. Histological studies provide the most objective and reliable data on gonad staging and are likewise crucial in evaluating the maturation cycle (Tingaud-Sequeira *et al*., 2008; Shahjahan *et al*., 2020). In the aquatic environment, variation of salinity is a significant abiotic stress that considerably affects fish growth, development, reproduction, and metabolic activities (Zhang *et al*., 2017; Yang *et al*., 2019). In addition, salinity affects the success of fertilization, egg incubation, and larval rearing of fish (Kujawa *et al*., 2017). The fish gonadal development and maturation timing are paramount issues in aquaculture and may limit reproductive success. The present study gives information on the striped catfish gonadal development in captivity in varying salinity conditions.

In this study, four development stages in two years old males were noted in histological analyses. Histological study on fish gonadal changes is an important tool for measuring the status of reproduction, which includes numerous developmental phases. The examination of the vitellogenic stage of the egg, increasing gonad weight, and gonad histology are critical components of reproductive biology that reflect the degree of maturity and offer

an estimate of the spawning period (Brown-Peterson *et al*., 2011). In April, the developing stage, primary spermatogonia, secondary spermatogonia, and primary spermatocytes were found at 0 ppt. At 4 and 8 ppt salinity, primary spermatogonia and immature stage were respectively observed. In May, at 0 ppt salinity, secondary spermatogonia, primary spermatocyte, secondary spermatocyte, and spermatids were noticed. At 4 ppt salinity, spermatids and developing germinal epithelium were noted. In June, at 0 ppt salinity, a high GSI value – peak spawning capable stage, was observed, which was associated with the presence of predominance of spermatozoa, spermatozoa in lumen seminiferous tubules, cysts, and testicular lumen. At 4 ppt salinity, continuous throughout of germinal epithelium was found, whereas at 8 ppt, the presence of spermatids was noticed. In July, Leydig cells and residual spermatozoa were found at 0 ppt. At 4 and 8 ppt salinity, the presence of cysts and spermatids were observed, respectively. In August, at 0 ppt salinity, immature stage and residual germinal epithelium were noted. At 4 ppt, residual cyst and germinal epithelium were observed; at 8 ppt, residual spermatozoa and germinal epithelium were found. The undeveloped stage was observed in September by the presence of the immature stage at 0, 4, and 8 ppt, where GSI values were lowest. The spermatogenesis stage was detected from April to June, following the spawning stage that took place in June as well as at the beginning of July. The spawning stage that had commenced in June continued until July. Hilsa's sexual maturation was observed to be highly associated with gonad histological changes (Ünver and Saraydin, 2004).

In the present study, four developmental stages of female gamete under varying salinity levels from April to September were observed. In April, at 0 ppt salinity, oogonia, chromatin nucleolar oocytes, primary growth oocytes, and perinucleolar oocytes were observed, while at 4 and 8 ppt salinity, primary growth oocyte was found. In May, at 0 ppt salinity, secondary and primary vitellogenic oocytes and cortical alveolar oocytes were noticed, whereas at 4 and 8 ppt salinity, only cortical alveolar oocytes were found, and vitellogenic stages did not start. In June, tertiary vitellogenic oocyte and mature oocyte occurred at 0 ppt. Mature oocytes were also found in 4 ppt salinity but were absent at 8 ppt. Fertility rates are impacted by ovarian degenerative processes (Fenerich-Verani *et al*., 1984; Rizzo and Bazzoli, 1995). In July, degenerating atretic oocytes were detected at 0 and 4 ppt salinity. Reproductive potential can be reduced by follicular atresia since it was observed when the GSI was still high and the atretic oocyte presence seemed adjacent to normal oocytes during ovulation (Kjesbu *et al*., 1991; Palmer *et al*., 1995). POFs are ovarian follicle remains that take place in fish ovaries after spawning (Drummond *et al*., 2000). Their primary function is said to be the reabsorption of oocytes or other cellular remnants left behind after ovulation, followed by their auto-disintegration (Lang, 1981). In August, at 0 ppt, old POFs were noted, and at 4 and 8 ppt salinity, oogonia and primary growth oocyte were observed, respectively. In September, at 0 ppt, the presence of POFs was noticed again, and primary growth oocytes were recorded at 0, 4, and 8 ppt.

In this study, at 0 ppt salinity, the GSI value in males from April to June increased, and the peak value was found in June, but the value gradually decreased, reaching a minimum in September. In females, the high values were observed in June and July, and the value decreased slowly, reaching a minimum in September. In 4 and 8 ppt salinity, the highest GSI value in males was found in June, whereas the lowest GSI value was recorded during September. In females, GSI values increased gradually, reaching a maximum from June to July, but dropped abruptly in August. The GSI parameter is used to research fish spawning biology. It also evaluates the level of ovary ripeness (Nandikeswari *et al*., 2014; Al Mahmud *et al*., 2016), and therefore, it renders an important indicator of the fish reproductive seasonality (Siddiquee *et al*., 2015; Jega *et al*., 2018). Salinity was found to increase gonadal growth in *Eriocheir sinensis* (Long *et al*., 2017) and *Crassostrea gasar* (Paixão *et al*., 2013). There are several possible explanations for this relationship: (1) the similarity of osmolality of brackish water saves less energy consumption on osmoregulation, allowing more nutrients/energy to be used for gonadal development; and (2) gonadal development is significantly fueled by calcium ion enriched brackish water. Low salinity has been linked to impaired gonadal development in *Crangon crangon* (Agnès *et al*., 2001) and *Grapsid crabs* (Bas and Spivak, 2000).

5. Conclusions

Salinity fluctuations, alongside other stressors, can influence fish growth, gonadal development, and, ultimately, survival. However, documentation on the influences of varying water salinity levels on the reproductive physiology of this commercially important species is insufficient. Overall, the gonad histological observations and the associated changes in GSI showed that the male and female striped catfish cultured in different salinity conditions could complete testicular and ovarian maturation at 4 and 8 ppt salinity by June and July, as they have grown at 0 ppt. However, further studies warrant for artificial induction of spawning by hormonal treatments, fertilization of eggs, embryonic development, and larval development in saline conditions.

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Data availability
The data that support the findings of this study are available on request from the corresponding author.

Conflict of interest
None to declare.

Authors' contribution
Farzana Hossain, Devojani Bhowmik, Wahidul Abrar: writing – original draft. SM Majharul Islam, Md Sadiqul Islam: writing – review and editing, and data analysis. Md Shahjahan: conceptualization, writing – review and editing. All authors have read and agreed to the published version of the manuscript.

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