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ORIGINAL ARTICLE

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Blood physiological responses and steroidogenetic effects of decreasing salinity on maturing male spotted sea bass (*Lateolabrax maculatus*)

Meili Chi[1,2] | Meng Ni[2] | Yongyi Jia[2] | Zhimin Gu[2] | HaiShen Wen[1]

[1]The Key Laboratory of Mariculture, Ocean University of China), Ministry of Education, Qingdao, China

[2]Agriculture Ministry Key Laboratory of Healthy Freshwater Aquaculture, Key Laboratory of Freshwater Aquaculture Genetic and Breeding of Zhejiang Province, Zhejiang Institute of Freshwater Fisheries, Huzhou, China

Correspondence
HaiShen Wen, Ocean University of China, Qingdao, Shandong, China.
Email: wenhaishen@ouc.edu.cn
and
Zhimin Gu, Zhejiang Institute of Freshwater Fisheries, Huzhou, Zhejiang, China.
Email: guzhimin2006@163.com

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Abstract

To illuminate the physiology influence of salinity on spotted sea bass (*Lateolabrax maculatus*) during its reproductive period, blood physiological responses and steroidogenetic effects during its acclimation from seawater to brackish water or freshwater were studied. The levels of steroid hormones and mRNAs in the pituitary and testis were also evaluated during this period. Results revealed that levels of serum osmolality were significantly reduced in both brackish water and freshwater groups during 1- to 8-days acclimation. The contents of *PRLR* and *GR* gene in testis were significantly suppressed during 4–8 days in brackish water and freshwater groups, while *PRL* gene expressions in pituitary were significantly promoted during 1–2 days in these two groups under salinity acclimation. On the other hand, the maturity status of testes after acclimating in freshwater for 8 days was reduced when compared with those in seawater group. Change trends of serum T, FSH and LH concentrations were diverse in different salinity groups. In addition, decreased *AMH* and *FTZ-F1* mRNA transcripts were observed in testes of spotted sea bass in FW group after 2-day administration. It was worth noting that transcript levels of *GTHα*, *FSHβ* and *LHβ* subunits in pituitary increased largely during first 2 days of salinity acclimation in brackish water and FW groups. These results suggested that spotted sea bass were capable of a stress-induced stimulation even salinity change rapidly (8 ppt/12 hr) from 30‰ to 0‰. But decreases in salinity may affect hypothalamus–pituitary–gonad axis of spotted sea bass and had negative effects on its testicular function.

KEYWORDS
osmolality, reproduction, salinity, spotted sea bass, steroidogenesis

1 | INTRODUCTION

Spotted sea bass is a euryhaline teleost, juveniles living in coastal waters or estuaries, maturing or spawning fish moving to open-ocean or some high salinity areas (Sun, Zhu, Zhou, & Chen, 1994). Considering the culture situation of spotted sea bass in China, mostly in brackish water, some in seawater net cage and even some in freshwater lakes after desalination, physiological survival can be achieved for this seawater fish at low salinity. However, it is important to note that harms on spotted sea bass were observed in low salinity water, such as high rates of mortality and abnormality in juvenile fish in Guangzhou estuary (Alderdice, 1998). In addition, the changes in total egg production and fertilization per cent in fathead minnows (*Pimephales promelas*) (Hoover, Weisgerber, Pollock, Chivers, & Ferrari, 2013) and reduced number of vitellogenic oocytes in Nile tilapia,

Oreochromis niloticus (Schofield, Peterson, Lowe, Brown-Peterson, & Slack, 2011), during salinity acclimation suggested that sexual maturation and reproduction may be sensitive to salinity changes (Kim et al., 2013). So, it is very necessary to assess any impact of salinity on reproduction in spotted sea bass.

Euryhaline fish face osmotic challenges both in freshwater and in seawater, and they have to regulate their internal water and ion concentration in order to maintain homoeostasis (Evans, 2008). Osmoregulation has been studied in many euryhaline teleosts, such as black porgy *Acanthopagrus schlegeli* (Tomy et al., 2009), golden pompano *trachinotus ovatus* (Ma et al., 2016), black-chinned tilapia (*Sarotherodon melanotheron*) (Tine, Guinand, & Durand, 2012) and salmonids (Flores & Shrimpton, 2012; McCormick, Regish, & Christensen, 2009). Prolactin (PRL) and its receptor (PRLR) are critical for the survival of euryhaline teleost involving in ion uptake (Juan et al., 2002; Yamaguchi et al., 2017). Besides, cortisol plays a very important role in stress adaptation involving in immune, metabolism and other biological process (Szisch, Papandroulakis, Fanouraki, & Pavlidis, 2005), and its intracellular receptors glucocorticoid receptor (GR) and mineralocorticoid receptor (MR) exert their corresponding biological processes after binding with serum cortisol (Prunet, Sturm, & Milla, 2006). *PRLR* gene expression in kidney and gill tissues after 1, 2, 4, 6 and 8 days in seawater, brackish water and freshwater group had been reported in spotted sea bass (Zhang, 2015) in which higher *PRLR* mRNA level was found in kidney and gill of freshwater group than those in seawater and brackish water groups, suggesting that *PRLR* may play a key role in salinity regulation. Although *PRLR* and *GR* levels in fish tended to be high in osmoregulatory tissues such as gill, intestine during salinity change (Khong, Kuah, Jaya-Ram, & Shu-Chien, 2009; Shaw et al., 2007), transcripts of *PRLR* and *GR* were also found in other tissues including gonad, liver and brain (Bury et al., 2003; Kawauchi, Sower, & Moriyama, 2009), and the exact function during salinity change in these tissues is still unknown.

Follicle-stimulating hormone (FSH) and luteinizing hormone (LH) are the primary mediators of sex steroid synthesis (Planas & Swanson, 2007). Their specific cell surface receptors (FSHR and LHR) lead to the production of second messenger molecules and stimulate the activity of steroidogenic enzymes after binding to corresponding hormone. In testis, generation of testosterone was also regulated by many steroidogenic enzymes (Racine et al., 1998). Steroidogenic acute regulatory protein (StAR), as an important steroidogenic enzyme, regulates the timing and rate of steroid synthesis in many species (Bauer, Bridgham, Langenau, Johnson, & Goetz, 2000). Anti-Müllerian hormone (AMH) negatively modulates the differentiation and function of Leydig cells by downregulating several steroidogenic enzymes (Wang & Orban, 2007). And FTZ-F1 is homologous to mammalian steroidogenic factor 1 (SF-1) and has been proved to involve in regulating steroidogenic enzyme expression in teleost (Chi et al., 2014). Gaining insight into expression changes in these steroidogenic genes will contribute to understand the tendency of reproductive endocrine change brought by external stresses.

Hence, the objectives of this study were to (a) examine osmoregulatory-relative changes in maturing male spotted sea bass held at different salinities, (b) study effects of salinity on testicular morphology and reproductive hormones (T, FSH and LH) in spotted sea bass and (c) investigate expression patterns of mRNA encoding enzymes and/or modulators of steroidogenesis in pituitary and testis during salinity acclimation.

2 | MATERIALS AND METHODS

2.1 | Animal and experimental design

Eight-nine maturing male spotted sea bass (803.5 ± 49.8 g, 40.5 ± 1.4 cm) were obtained from a commercial farm of Qingdao, Shandong, China, in November (salinity: 29–30‰, temperature: 17–19°C). Fish were acclimated to seawater for 5 days under 13 hr light: 11 hr dark cycle at 17.5 ± 0.7°C and fed daily diet of commercial pellets.

After acclimation period, four male fish were sampled as initial control (0 day point); then, fish were randomly divided into three groups, 25 fish in five tanks for each group. One group still maintained in seawater (SW, 30‰). Salinity of fish in brackish water (BW) group was reduced from 30‰ to 15‰ with rate of 4 ppt/12 hr, while salinity change rate for freshwater (FW) group was double that of BW group (8 ppt/12 hr) from 30‰ to 0‰ according to the method of Ye (1997) with some modification. Salinity acclimation was carried out by decreasing the proportion of SW with aerated dechlorinated tap freshwater for four times at 8:00 a.m. and 8:00 p.m. during first 2 days. Treatment salinities in BW and FW groups (15‰ and 0‰) were achieved at 3 days. Four male fish for each group were anaesthetized in 100 mg/L tricaine methane sulphonate (MS-222, Sigma, St. Louis, MO) at 6:00 p.m. at 1, 2, 4, 6 and 8 days postgrouped. Pituitary and testis were quickly removed under sterile conditions, and testes were sectioned in two parts, one was fixed in Bouin's solution for haematoxylin and eosin (HE) staining to identify the development stage and the other part was frozen at −80°C until analysis.

2.2 | Gonadal histology

Testes were fixed in Bouin's solution for more than 24 hr and then dehydrated in a graded series of ethanol, embedded and cut by microtome (LEICA-RM2016), then haematoxylin and eosin (H.E.) stained and photographed by light microscopy (Nikon-E200, Japan). The testicular developmental stages were determined mainly based on the former study (Chi et al., 2014; Shi et al., 2011).

2.3 | Analysis of serum parameters

Blood from spotted sea bass acclimated to FW, BW and SW was collected from caudal vein and allowed to clot at 4°C for 6–8 hr. Serum was then separated by centrifugation at 16,000 g for 10 min and stored at −80°C until analysis. Serum osmolality was examined using Osmometer 800CLG (SLAMMED, Germany). Levels of cortisol, T, FSH and LH were measured using Iodine (^{125}I) Radioimmunoassay

Kits (Tianjin Nine Tripods Medical & Bioengineering Co., Ltd., Sino-US joint venture enterprise), according to the method described by Shi (2011).

2.4 | Total RNA extraction and reverse transcription

Total RNA was extracted from tissue samples by homogenizing in RNAiso (Takara, Japan), precipitating isopropanol and washing in 75% ethanol. After DNase treatment, the concentration and purity of each sample were quantified by the Nucleic acid analyser, Biodropsis BD–1000 (OSTC, China), and a 1.5% agarose gel was applied to detect RNA integrity. First-strand cDNA was synthesized by reverse transcribing 2 μg of the total RNA using *M*-MLV Reverse Transcription Kit (Promega, USA) according to the manufacturer's protocol.

2.5 | Real-time PCR analysis

The mRNAs of *PRL*, *GTHα*, *FSHβ* and *LHβ* subunits in pituitary and *PRLR*, *GR*, *StAR*, *AMH* and *FTZ-F1* genes in testis in SW, BW and FW acclimation were quantified with an Applied Biosystems StepOne™ real-time PCR system (Applied Biosystems, Foster City, CA, USA). The primers used for qPCR are summarized in Table 1. The primers for real-time PCR were selected after considering intron–exon boundaries to exclude genomic DNA. They were optimized for concentration and annealing temperature. qPCR reactions were performed as follows: 1 cycle at 50°C for 2 min and 95°C for 10 min, followed by 40 cycles at 95°C for 15 s and annealing temperature of each gene for 30 s. Each qRT-PCR was carried out in triplicate with the SYBR green (TAKARA, Japan). The mRNA value was normalized using the expression of *18S* rRNA which was previously shown to be a valid housekeeping gene in spotted sea bass (Chi et al., 2014). After the PCR program, $2^{-\Delta\Delta CT}$ method was used to yield the average fold change of target genes.

2.6 | Statistical analysis

Values were expressed as mean ± *SE* of the mean (*SEM*). Data were compared using one-way analysis of variance (ANOVA; Duncan's multiple range tests method). Samples from SW, BW and FW groups were assessed relative to that of initial control (0 day point). In all cases, significance was accepted at $p < 0.05$.

3 | RESULTS

3.1 | Effect of salinity on osmoregulatory parameters and relative gene expression

3.1.1 | Osmolality

In SW group, osmolality was around 429.1 mOsm/kg during the salinity acclimation. In BW and FW groups, serum osmolality changed dramatically and differed from SW group at each sampling point ($p < 0.05$) (Figure 1). Osmolality decreased to the lowest level at 4 days in BW group (277.75 ± 3.54 mOsm/kg) and 6 days in FW group (229.5 ± 11.1 mOsm/kg). At 8 days after transferring, the mean osmolality in BW and FW groups was 325.8 mOsm/kg and 309.8 mOsm/kg, which were still much lower than that in SW group (424.5 mOsm/kg).

3.1.2 | Serum cortisol

Levels of cortisol in BW and FW groups changed significantly during salinity administration period (1 and 2 days). There were around 1.9- and 1.7-fold increase than that of SW group at 1 days (Figure 2). Then, cortisol levels in BW and FW groups declined slightly, but significant increasing still could be detected at 2 days ($p < 0.05$). Cortisol in two treated groups recovered to a similar level of SW group at the end of treatment period (during 4–8 days).

TABLE 1 Primers used for mRNA qPCR analysis of genes from spotted sea bass

Gene	Forward primer	Reverse primer	Tm	Accession Num.
GTHα	AAACATGGGCTGTGAGGAGT	CGGGATCGTCATTGTCTTCA	60	JQ995530
FSH β	CCAACCAACATCAGCATCCC	CCCACTGGACATCCTTGAATG	61	JX185720
LH β	GAGTTTGTTTCTGGGAGCCTC	TGGTTGTTTCCACTGGGTGA	62	JX185719
StAR	AATGGGGGAGTGGAACCCTAAA	AGCGGACGCTGACAAAGTC	62	JQ995529
AMH	CCGTGCGTATGAGGTGC	GTTGGCGGTGTTTGGAC	61	JQ290346
FTZ-F1	TGCCTCAAGTTCCTGGTCCT	CGTTTGCTGCGGGTAGTTAC	62	KC534882
PRL	GCAGGAGCACTACAAGAGTCT	GGAAGTTGGTCAGTTGGGAGA	61	KC534883
PRLR	TGACTGTAATGGAGGACAAGGG	GACCGCAGGCTGAAAATGT	62	KC534884
GR	ATGAAGGTCCTGTTACTGCTCA	CTCGCTTGACGATGGCTTT	62	KC534885
18S	GGGTCCGAAGCGTTTACT	TCACCTCTAGCGGCACAA	59	AB089346

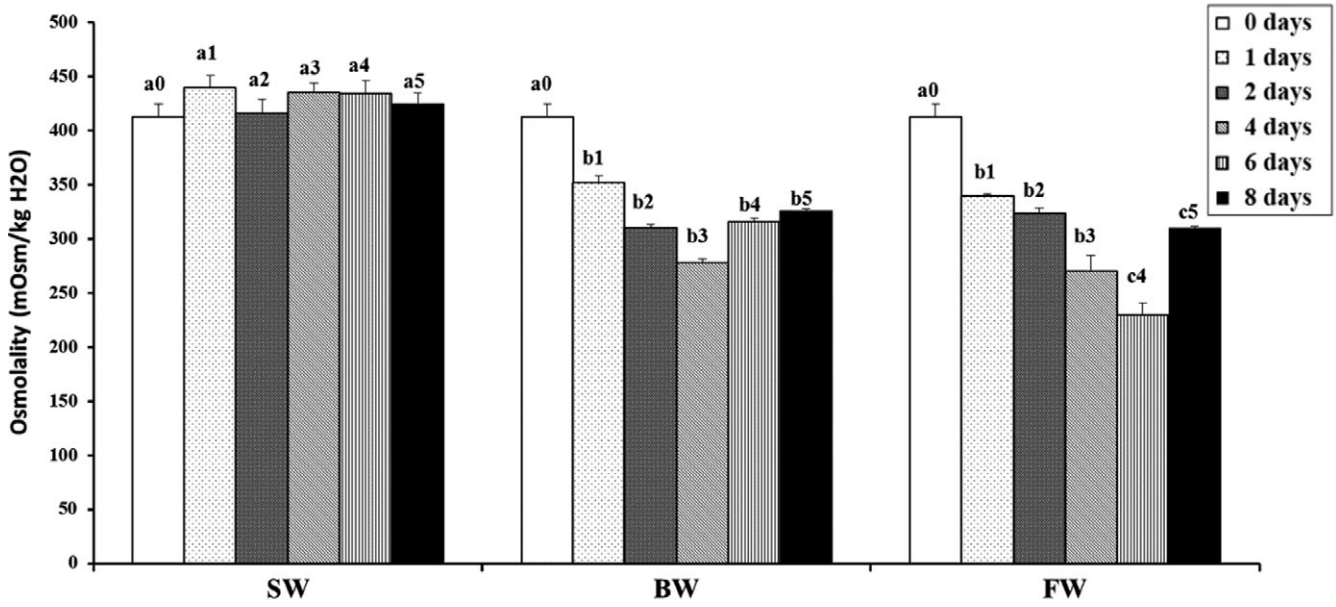

FIGURE 1 Osmolality of spotted sea bass acclimated to SW, BW and FW groups (N = 4 for all groups). Different number was used to distinguish experiment point. Different letters indicate significant differences ($p < 0.05$) using Duncan's multiple range tests following one-way ANOVA. Values are means ± *SEM*. SW, seawater BW, brackish water; FW, freshwater.

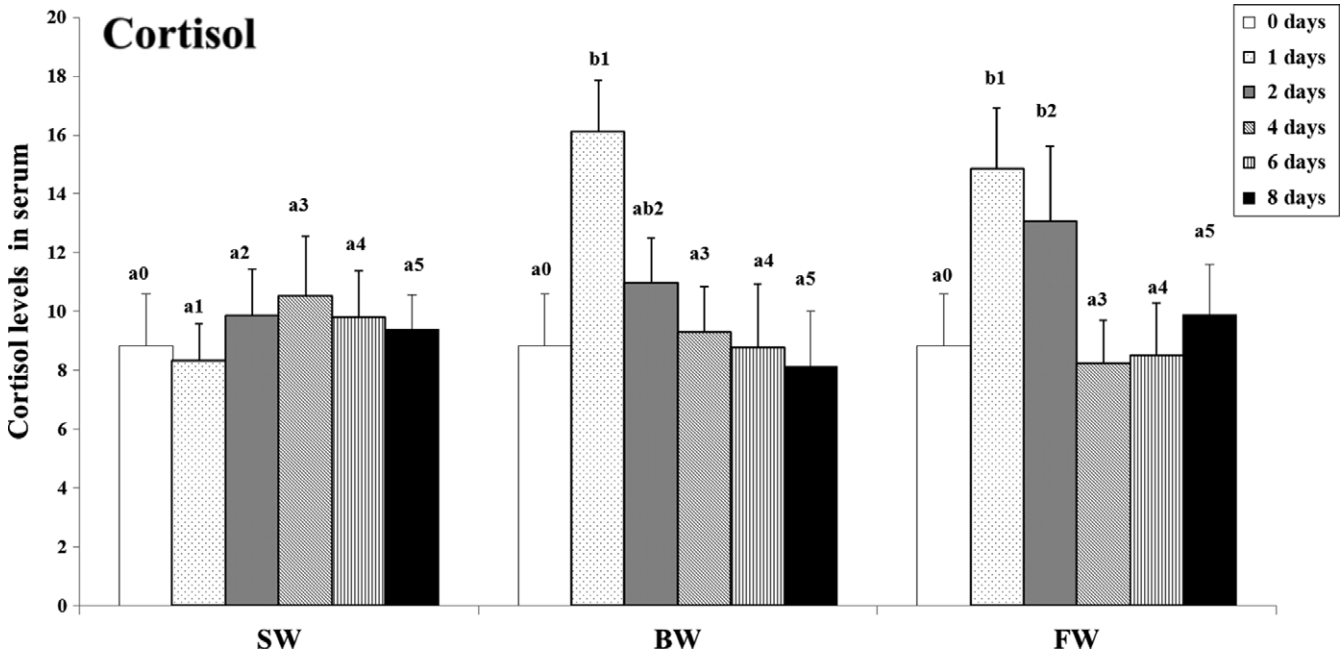

FIGURE 2 Effects of salinity on cortisol level of spotted sea bass in SW, FW and BW groups (N = 4). Dissimilar letters indicate significant differences among the test time-points (one-way ANOVA with Duncan's multiple range tests, $p < 0.05$). Values are mean ± *SEM*. FW, freshwater; BW, brackish water; SW, seawater

3.1.3 | Expression of *PRL* in pituitary and *PRLR* and *GR* genes in testes

In BW and FW groups, *PRL* mRNA levels were strongly increased at 1 and 2 days, peaking at 1 d with levels of 11.8- and 17.6-fold higher than those in SW group ($p < 0.01$); at 2 days, the levels were decreased to 10.9- and 11.5-fold higher. Then, during 4–8 days, transcript levels returned to level of the SW group, and significance could only be detected in BW group at 4 days ($p < 0.05$) (Figure 3a). Inversely, *GR* and *PRLR* genes in testes were strongly inhibited in response to salinity change during 4–8 days in BW group and 2–8 days in FW group ($p < 0.05$) (Figure 3b and c).

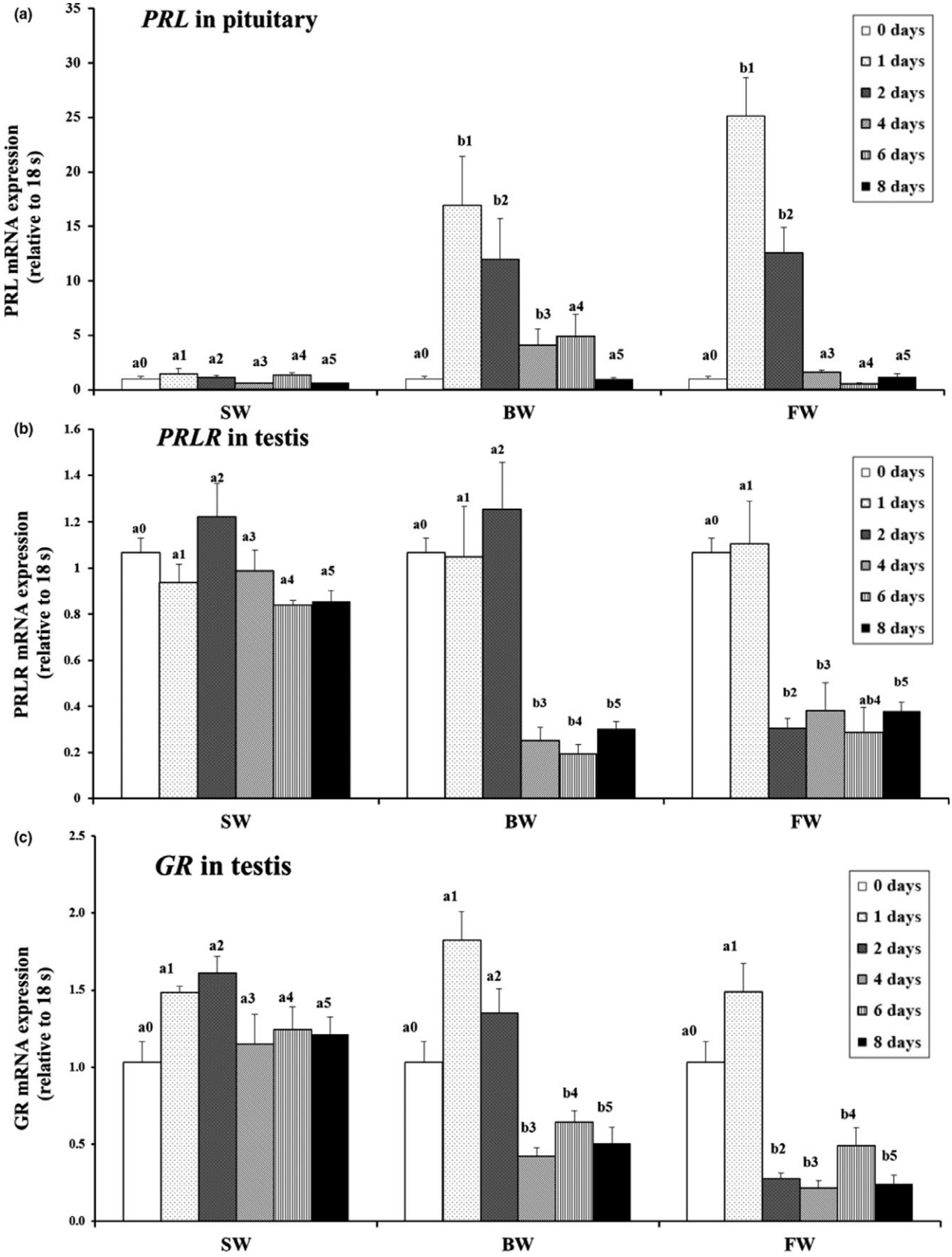

FIGURE 3 mRNA expression levels of *PRL* gene in pituitary, *PRLR* and *GR* genes in the testis of spotted sea bass acclimated to seawater (SW), brackish water (BW) and freshwater (FW). Data are expressed as the means ± *SEM*. Different number was used to distinguish experiment point. Different letters indicate significant differences at $p < 0.05$ by Duncan's multiple range tests

3.2 | Effect of salinity on histology, steroid hormones and steroidogenetic gene expression

3.2.1 | Histological analysis

The histological photomicrographs of spotted sea bass testes are shown in Figure 4. Typical late spermatogenesis stage testis was detected in seawater group at 0 day (Figures 4a a nd c). Seminiferous tubules were filled with mature spermatozoa and spermatids, which could be evidently distinguished. While the maturity status of testis was reduced after acclimating in freshwater for 8 days when compared to SW group, photomicrographs showed spermatocytes, spermatids and spermatozoa in testes, and spermatocytes and spermatids were the dominant germ cells (Figures 4b and d).

3.2.2 | Changes of serum reproductive hormones

Spotted sea bass testosterone hormone levels presented "U" shape pattern in response to differential salinity. It was at low level around 2–4 days and then started recovering at 4 days after acclimation, especially in FW group (Figure 5a). FSH levels were significantly decreased during 2–8 days in BW and FW groups when compared to that of SW group (Figure 5b) ($p < 0.05$). However, the change in LH hormone was different from that of FSH, increasing transitorily at 2 days then decreasing significantly (Figure 5c). Serum T, FSH and LH levels remained relatively constant during the whole salinity acclimation in SW group.

3.2.3 | Expression of *GTHs* genes in pituitary

Transcript levels of three GTH subunits (*GTHα*, *FSHβ* and *LHβ*) showed large increase relative to SW group during 1–2 days in BW and FW groups (Figure 6). mRNA expression at 1 day was much higher than that of 2 days in these three groups except *FSHβ* and *LHβ* subunits in FW group. It was worth noting that expression levels of *GTHα* and *LHβ* subunits were significantly increased at 6 days in BW group ($p < 0.05$).

3.2.4 | Expression of *StAR*, *AMH* and *FTZ-F1* genes in testes

Expressions of *StAR* mRNA in testes were rarely changed during the whole treatment, except the significant increasing detected at 4 days in FW group (Figure 7a). Significant decreases of *AMH* mRNA were detected at 4 days in BW group and at 2–4 days in FW group when compared to SW group ($p < 0.05$). Further, 1.5- and 1.7-fold increases were measured at 8 days in FW group than those of SW and BW groups, respectively ($p < 0.05$, Figure 7b). Different trends of *FTZ-F1* expression were detected in BW and FW groups. It increased first and then decreased in BW group, and significant increasing at 4 days was detected when compared with other two groups. While in FW group, *FTZ-F1* RNA expression was decreased to some extent, two significant decreasing time-points were discovered at 2 and 6 days (Figure 7c). In addition, three gene (*StAR*, *AMH* and *FTZ-F1*) transcript levels remained relatively constant during the whole administration in SW groups.

4 | DISCUSSION

Spotted sea bass is a euryhaline teleost that lives in a wide range of salinity. In this study, decreasing salinity significantly inhibited osmolality levels of spotted sea bass in BW and FW groups which corresponded to previous studies in milkfish (*Chanos chanos*) (Lin, Chen, & Lee, 2003), black porgy (Tomy et al., 2009), tilapia (*Oreochromis*

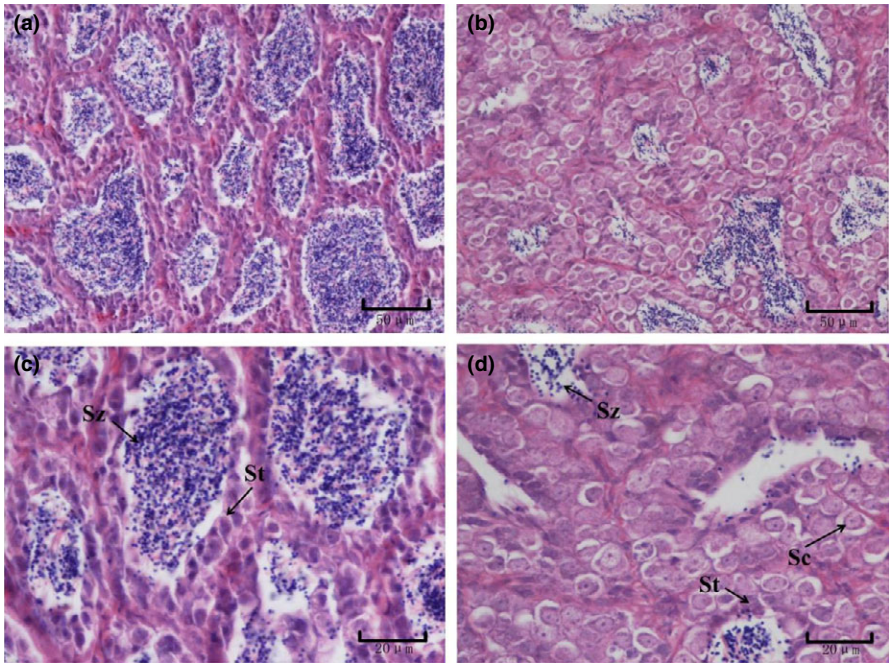

FIGURE 4 Histological photomicrographs of spotted sea bass testes applied in this study. (a) and (c): The testis of spotted sea bass in seawater group at 0 days, (a): bar = 50 μm, (c): bar = 20 μm. (b) and (d): The testis of spotted sea bass in freshwater group at 8 days, (b): bar = 50 μm, (d): bar = 20 μm. Sc: spermatocytes St: spermatid; Sz: spermatozoa

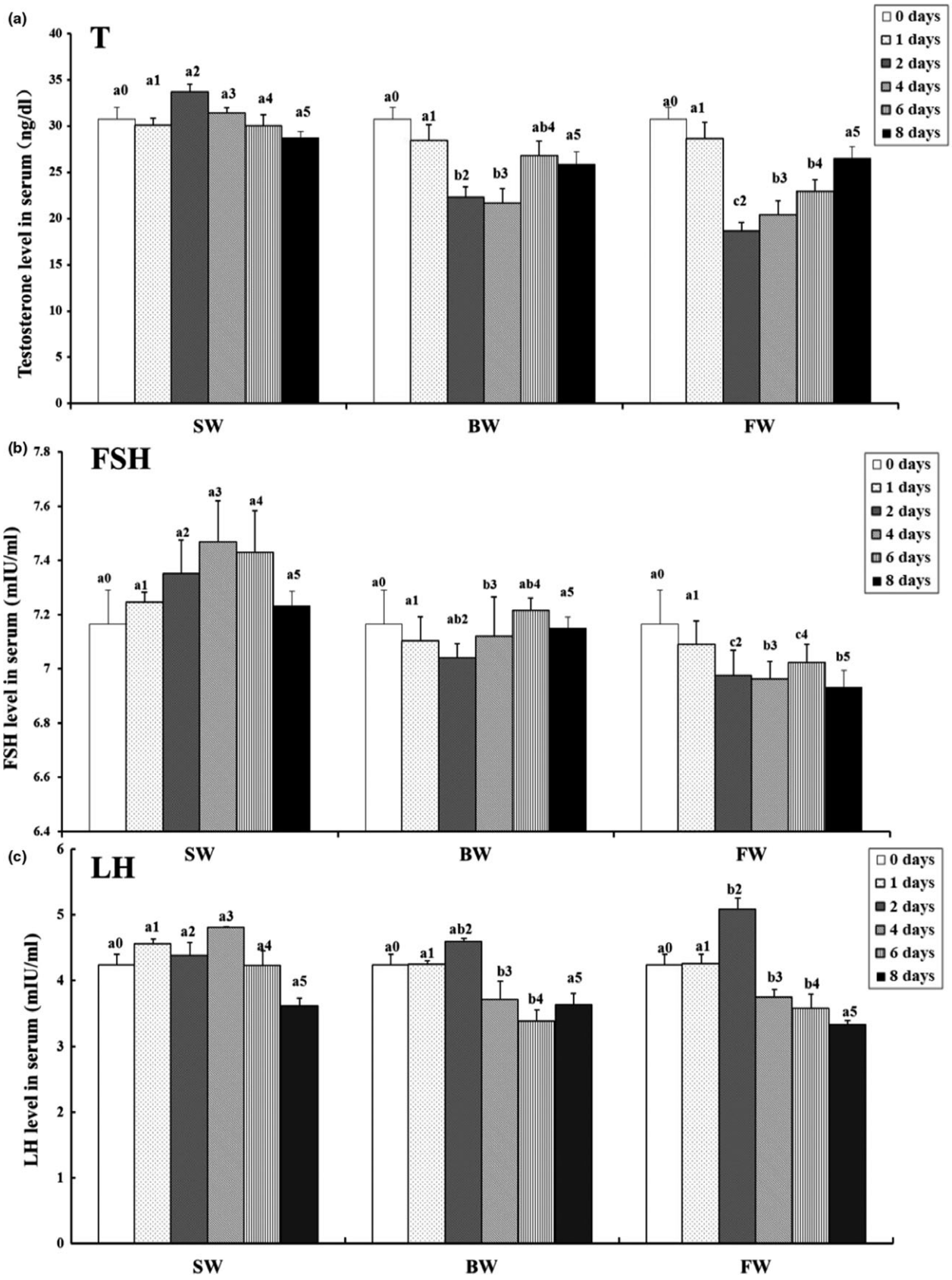

FIGURE 5 Effects of salinity on testosterone (T, a), follicle-stimulating hormone (FSH, b) and luteinizing hormone (LH, c) levels of spotted sea bass serum in SW, FW and BW groups. Dissimilar letters indicate significant differences among the test time-points (one-way ANOVA with Duncan's multiple range tests, $p < 0.05$). Values are mean ± *SEM*. FW, freshwater; BW, brackish water; SW, seawater.

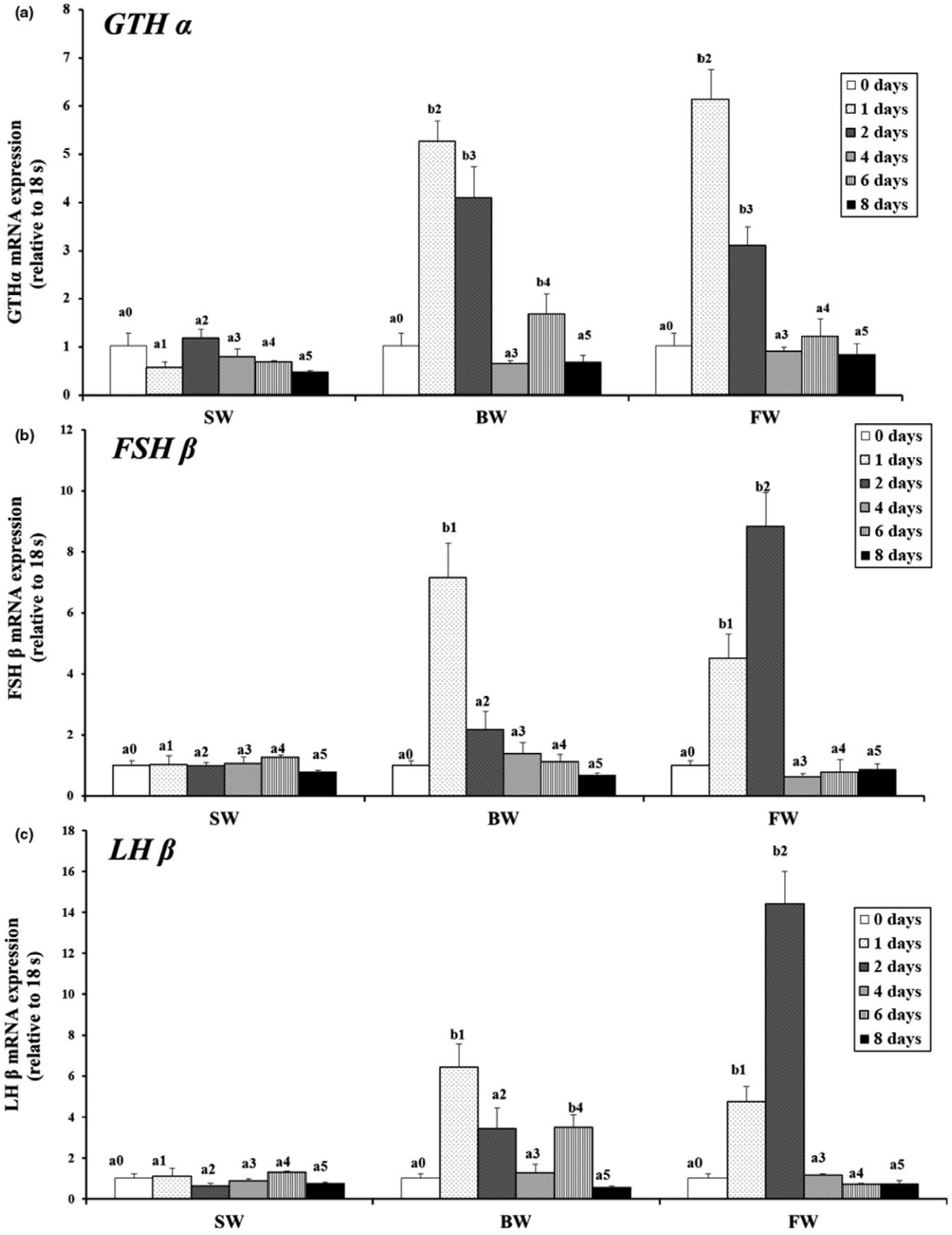

FIGURE 6 mRNA expression levels of *GTHα* subunit (a), *FSHβ* subunit (b) and *LHβ* subunit (c) in the pituitary of spotted sea bass acclimated to seawater (SW), brackish water (BW) and freshwater (FW). Data are expressed as the means ± *SEM* (n = 4). Different number was used to distinguish experiment point. Different letters indicate significant differences at $p < 0.05$ by Duncan's multiple range tests.

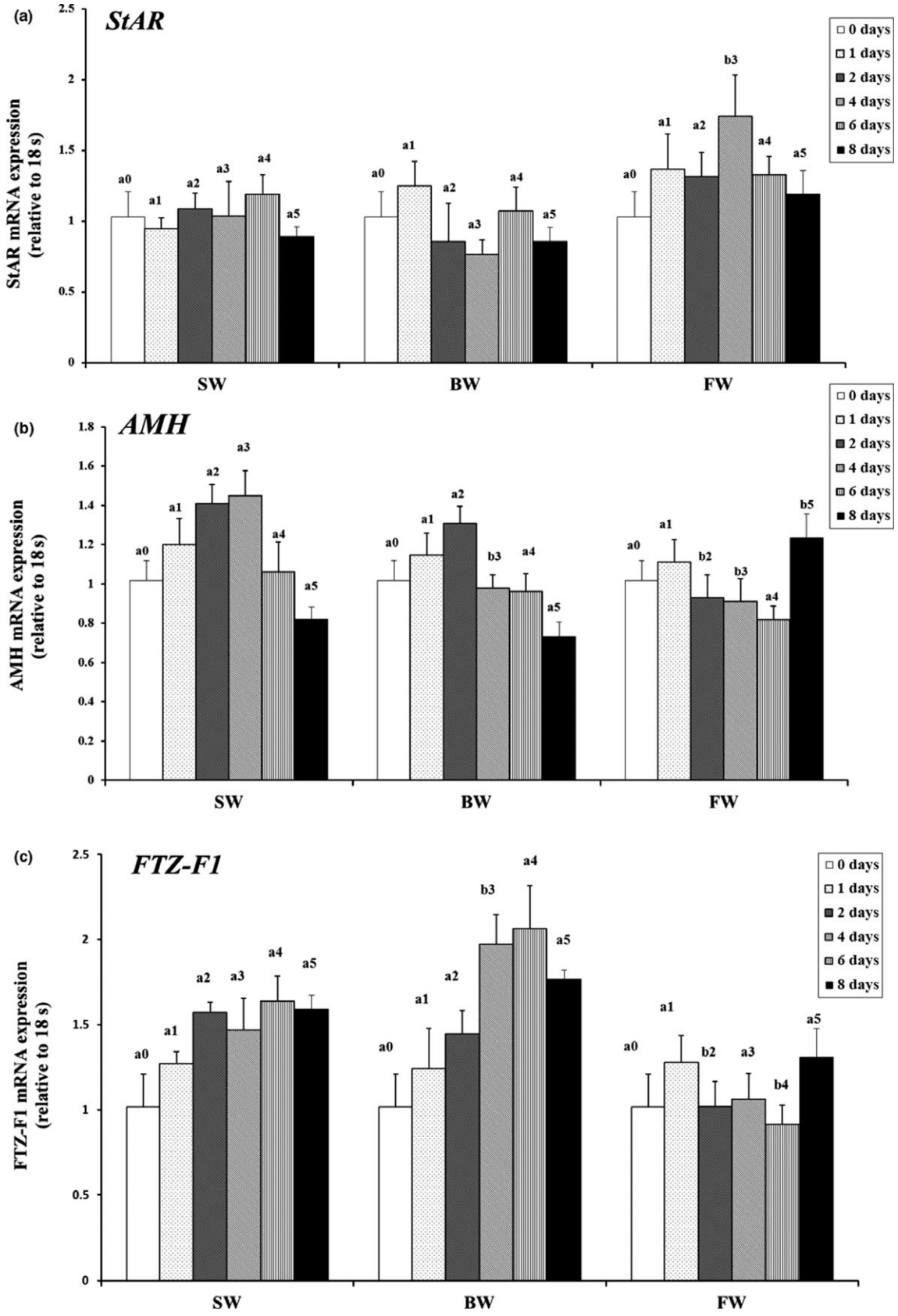

FIGURE 7 mRNA expression levels of *StAR* (a), *AMH* (b) and *FTZ-F1* (c) genes in the testis of spotted sea bass acclimated to seawater (SW), brackish water (BW) and freshwater (FW). Data are expressed as the means ± *SEM*. Different number was used to distinguish experiment point. Different letters indicate significant differences at $p < 0.05$ by Duncan's multiple range tests.

mossambicus) (Lin, Huang, Yang, Lee, & Hwang, 2004) and other euryhaline teleosts. Furthermore, osmolality levels of spotted sea bass in BW and FW groups remained low after 8-d acclimation when compared to seawater group. Similar result was also described in Senegalese sole (*Solea senegalensis*) which was transferred from water of 38‰ to 15‰ and 5‰ of low salinity water (Arjona, Vargas-Chacoff, Ruiz-Jarabo, Martín del Río, & Mancera, 2007). Those low osmolality levels in BW and FW groups were maintained within normal physiological range, indicating that spotted sea bass could successfully adapt to these salinities (Seo, Lee, & Kaneko, 2009).

In this study, levels of cortisol, mRNA expression of *PRL* in pituitary, *GR* and *PRLR* genes in testes changed significantly after decreasing salinity in BW and FW groups. Considering their recognized roles in osmoregulatory processes in other fish (Szisch et al., 2005; Whittington & Wilson, 2013), it indicated that salinity could influence osmotic homoeostasis of spotted sea bass in both BW and FW administration. There was one day ahead difference in time of beginning function between BW and FW groups (except the expression of *PRL* gene in FW group), and this phenomenon was similar to that in rainbow trout (*Oncorhynchus mykiss*) which appeared a salinity-depend increasing of serum cortisol after salinity changed rapidly (Richards, Semple, Bystriansky, & Schulte, 2003). So, it was presumed that spotted sea bass in freshwater faced more stress and reacted more intense than that in brackish water. Although little information is available on the relationships among environmental osmolality, cortisol, PRL and their receptors in the testis, researches in tilapia had showed that levels of *PRLR* mRNA in gill and pituitary, and *GR* mRNA in the kidney and intestine raised with the increasing of extracellular osmolality (Aruna, Nagarajan, & Chang, 2012; Fiol, Sanmarti, Sacchi, & Kultz, 2009; Seale et al., 2012, 2014). In addition, rapid downregulation of testicular *GR* and *PRLR* transcripts during 4–8 days in BW group and 2–8 days in FW group were following with the increasing of cortisol and *PRL* during 1–2 days. Therefore, it is plausible that salinity acclimation may exert a strong influence on spotted sea bass testicular ionoregulatory function via modulating the expression of *GR* and *PRLR* genes (Santos et al., 2001).

Many studies investigated the effects of salinity on early life stages of teleosts (Arjona et al., 2007). However, few studies focused on the effects of different salinities during gonadal maturing phase of marine euryhaline fish (Pham, Kjørsvik, Nguyen, Nguyen, & Arukwe, 2010). Morphological and microscopic observations in this study showed that spotted sea bass exposed to freshwater for 8 days were characterized by atrophying testis to a certain extent. In agreement with the finding in this study, *Diplodus vulgaris* which acclimated to 25‰ water for one month also appeared retarding effect on the spermatogenesis activities (Moharram, 2000). Furthermore, Moharram suggested that major processes affected might include degeneration of spermatogonia and inhibition of cell division. The decreasing number of spermatozoa and an increasing number of spermatocytes and spermatids observed in this study also supported this suggestion. In addition, female marine striped mullet (*Mugil cephalus*) maturing in freshwater showed a slow rate of oocyte growth, and only few females completed vitellogenesis (Tamaru, Lee, Kelley, Miyamoto, & Moriwake, 1994). These results implied that decreasing salinity might inhibit gonadal reproductive functions of spotted sea bass.

To further illuminate the influence of salinity on reproductive endocrine of spotted sea bass, the changes in T, FSH and LH in three different salinity groups were compared in this study. As the inhibition effect on testicular development in FW group, modest decreases in serum T, FSH and LH levels were corresponding to the decrease in osmolality indicating that osmotic water gain in hypoosmotic medium may result in the whole hemodilution of hormones in spotted sea bass (Lee, Kaneko, Katoh, & Aida, 2006). Inhabiting of serum T synthesis had also been reported in mature Waigieu sea perch (*Psammoperca waigiensis*) and black bream (*Acanthopagrus butcheri*) after decreasing salinity manipulation (Haddy & Pankhurst, 2000; Pham et al., 2010), while the transient increase in testosterone level after one-day SW-to-FW transition was found in chum salmon (*Oncorhynchus keta*) (Onuma et al., 2003). It was converse with our findings that this discrepancy may be due to different salinity suitability for reproductive strategy, and chum salmon spawns in freshwater, while spotted sea bass spawns in seawater. In contrast to decreasing FSH and LH hormone levels, three GTH subunit expression levels in pituitary were significantly higher in BW and FW groups than those in seawater group during 1–2 days (Figure 6). Furthermore, the levels of chum salmon GTHα2, Iβ and IIβ mRNAs were also found significantly increased during 4-day SW-to-FW transition experiment (Onuma et al., 2003). These findings suggested that mRNA levels of gonadotropin subunits may not necessarily reflect the change in circulating hormone levels in spotted sea bass. The increasing pituitary mRNA levels may simply reflect a transient increase in utilization of mRNA for hormone synthesis, whereas serum gonadotropin levels reflected a balance between transcript production and utilization for synthesis of LH and FSH (Levavi-Sivan, Bogerd, Mañanós, Gómez, & Lareyre, 2010). It is interesting to find that LH hormone was significantly increased at 2 days in BW and FW groups which was happened at a time of three GTH subunits highly expression in pituitary. Furthermore, considering the more important role of LH than FSH in facilitating gamete maturation and spawning (Sarkar & Nath, 2012), we speculate that the increase in GTH subunits in pituitary might directly improve serum LH level transiently. The potential dynamics of synthesis/secretion/clearance during salinity acclimation are still needed to be considered.

Salinity adaptation in euryhaline teleost, such as spotted sea bass, is a complex process involving a suite of physiological and behavioural responses (Pham et al., 2010). StAR, AMH and FTZ-F1 are all important enzymes involved in steroidogenic pathway in teleost (Bauer et al., 2000; Chi et al., 2014; Wang et al., 2007). In this study, significant changes in these gene expressions were mainly concentrated on 2–6 days in FW and BW groups, while during this period, levels of serum cortisol, FSH and LH in addition with three GTH subunits, *PRL*, *PRLR* and *GR* gene expression, were all changed significantly. And almost all these factors detected in this study recovered to the control level at the end of salinity acclimation. These results indicated that these three steroidogenetic factors

could response to salinity change in testis. Expression of *StAR* and *AMH* mRNA reacted more intense in freshwater than that in brackish water. In addition, contrasting trend was existed in *FTZ-F1* gene expression in BW and FW groups. Differences in their expression pattern might reflect different physiological functions in different salinity.

5 | CONCLUSIONS

In general, changes in serum osmolality, cortisol, steroid hormone levels and histology during acute salinity stress were analysed. Meanwhile, the mRNA expression patterns of *PRL*, *GTHα*, *FSHβ* and *LHβ* subunits in pituitary and *PRLR*, *GR*, *StAR*, *AMH* and *FTZ-F1* genes in testis of spotted sea bass were also detected by qPCR analysis. As far as we know, this is the first report about the relationship between testicular steroidogenic enzymes and osmoregulation. These findings revealed spotted sea bass have excellent osmoregulatory abilities. Furthermore, decreasing salinity might directly affect on spotted sea bass hypothalamus–pituitary–gonad axis by changing gonadotropin level and testicular steroidogenesis.

ORCID

Meili Chi http://orcid.org/0000-0003-3736-7108

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