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Isolation of CYP17 I, 3β-HSD and AR Genes from Spotted Sea Bass (*Lateolabrax maculatus*) Testis and Their Responses to Hormones and Salinity Stimulations

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Abstract In this study, three cDNA sequences corresponding to cytochrome P450C17 (CYP17 I), 3β-hydroxysteroid dehydrogenase (3β-HSD) and androgen receptor (AR) were isolated from spotted sea bass (*Lateolabrax maculatus*). The mRNA abundances of *CYP17 I* and *3β-HSD* increased from stage Ⅱ to stage Ⅴ with a significant increase at stage Ⅴ, and the highest abundance of *AR* mRNA was detected at stage Ⅲ in testicular development cycle. *CYP17 I*, *3β-HSD* and *AR* transcripts were obviously abundant in steroidogenesis tissues such as testis, brain, head kidney among others. Strong and positive signals were observed mainly in interstitial cell regions of *L. maculatus* testis as were measured with *in situ* hybridization method. Significant increases of *CYP17 I* and *3β-HSD* transcripts were detected after 12–48 h hCG (human chorionic gonadotropin) and GnRHa (gonadotropin-releasing hormone analogue) treatments. However, an opposite relationship was found for *AR* in testis at the same time. In addition, decreasing trends of *CYP17 I* and *3β-HSD* mRNA were observed in testis of *L. maculatus* in freshwater group (FW) from day 2 to day 6, and mRNA abundance of *AR* increased in brackish water (BW) group from day 4 to day 8. These findings revealed that these three steroid synthesis genes are import for testicular development, hormone and salinity treatment, and provided also an insight into the mechanism of reproductive endocrine of *L. maculatus*.

Key words *L. maculatus*; hCG; GnRHa; salinity; steroidogenic enzyme; spermatogenesis

1 Introduction

Steroids play critical roles throughout the reproductive cycle in teleost and androgen is essential for breeding behavior and spermatogenesis in male fish (Leet *et al*., 2011). Androgen mainly functions by activating the androgen receptor (AR) on cell membrane (Davey *et al*., 2016). The abundance of *AR* mRNA was higher in testis than that in ovary and intersex gland in protogynous wrasse (*Halichoeres trimaculatus*) and sex reversal orange-spotted grouper (*Epinephelus coioides*), indicating that the increase of mRNA abundance may relate to the sexual reversal (Kim *et al*., 2002; Shi *et al*., 2012). Although *AR* has been isolated and characterized from a number of species (Ikeuchi *et al*., 2001; Ogino *et al*., 2004), how it remained unclear if the expression of *AR* is affected by external compounds. Biosynthesis of steroids requires a series of steroidogenic enzymes, among them, 17ahydroxylase/17,20-lyase and 3β-hydroxysteroid dehydrogenase are critical for the production of androgens and estrogens in teleost (Halm *et al*., 2003). *CYP17 I* and *3β-HSD* mainly distributed in gonad (testis and ovary) and other steroidogenesis tissues like head kidney and brain (Wang and Ge, 2004; Senthilkumaran *et al*., 2009).

Spotted sea bass (*Lateolabrax maculatus*) is an important marine fish species for aquaculture in China, South Korea and Japan. Considering the asynchronous of female and male gonadal development in cage cultivation, it was difficult to obtain enough mature male fish individuals for artificial breeding when the female fish ovulated (Zhang *et al*., 2001). External hormones (human chorionic gonadotropin and gonadotropin-releasing hormone analogue) have been widely used in aquaculture to accelerate testicular development and spermatogenesis in many fish species (Choi *et al*., 2007). Juvenile *L. maculatus* inhabits the coastal water but matures in open-ocean. Some *L. maculatus* are cultured in brackish water and freshwater cages in China. However, harms on juvenile spotted sea bass have been observed in low salinity water, which include high rates of mortality and abnormality in Guangzhou Estuary (Alderdice, 1998). Gonadal maturation and reproduction were inhibited in fathead minnows (*Pimephales promelas*) and Nile tilapia (*Oreochromis niloticus*)

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(Schofield *et al*., 2011; Hoover *et al*., 2013) during salinity acclimation, therefore it is important to clarify the impact of salinity on reproductive process of spotted sea bass. The objectives of present study were to 1) isolate *L. maculatus CYP17 I*, *3β-HSD* and *AR* cDNAs; 2) investigate the expression patterns of these three genes in different tissues at different development stages; 3) illuminate the effects of hormone and salinity change on the mRNA abundance in matured *L. maculatus* testes.

2 Materials and Methods

2.1 Fish Treatment and Sampling

2.1.1 Testicular development cycle and genic tissue distribution sampling

In *L. maculatus* spawning season (September–December), six male fish individuals were obtained every month for testicular development cycle analysis. Fish individuals were acclimatized for 3 days in laboratory and anesthetized with MS-222. Testes were removed rapidly and kept at −80℃. In addition, 13 tissues (testis, liver, stomach, gills, heart, caecus, spleen, kidney, head kidney, intestine, brain, pituitary and muscle) of four fish individuals were collected as described above for tissue distribution analysis.

2.1.2 Hormone administration and sampling

Seventy-one male *L. maculatus* individuals with an average body weight of 671.21 g ± 75.25 g and an average body length of 37.78 ± 1.57 cm were collected from a commercial fish farm in Qingdao in November. Fish individuals were acclimatized in 16 tanks with natural sea water under controlled conditions (dissolved oxygen > 6 mg L^{-1}; temperature 17.5℃ ± 0.7℃; salinity 29 ± 0.8; 13 h light:11 h dark cycle) for 3 days. Fish individuals were then randomly divided into three groups, 23 each. Two groups were intraperitoneally injected with 3.5 μg kg^{-1} body weight GnRHa and 1000 units per kg body weight hCG, respectively (Zhang *et al*., 2001) while the control group was injected with physiological saline (PS, 0.7% NaCl). Four male fish individuals each group were anesthetized with 0.2% MS-222 at 0 h, 6 h, 12 h, 24 h and 48 h, respectively. Testis were sectioned into two parts, one part was fixed in Bouin's solution for identifying development stage and the other was frozen at −80℃ until use.

2.1.3 Salinity administration and sampling

Seventy-nine male *L. maculatus* (803.5 g ± 49.8 g, 40.5 cm ± 1.4 cm) individuals were obtained from the same place as hormone administration in November. Fish individuals were acclimated for 5 days at 17–19℃ and salinity 29–30 and under 13 h light:11 h dark cycle. These fish individuals were fed commercial diet pellets (TongWei, China) daily. Four male fish individuals were sampled as initial control (0d point). Then other fish individuals were randomly divided into three groups, 25 each. One group was maintained in seawater as control (SW, salinity 30). In brackish water (BW) group, salinity was reduced from 30 to 15 at a rate of 4 per 12 h. Salinity change rate for freshwater group (FW) was two times of the rate for BW group (8 per 12 h) from 30 to 0. Salinity acclimation was carried out through 4 times of water replacement at 8:00 am and 8:00 pm during the first two days. Salinity was decreased by reducing the proportion of sea water with aerated dechlorinated tap fresh water. Treating salinities in BW and FW groups (15 and 0) were achieved at 3 d. Four male fish individuals each group were sampled at 6:00 pm on 1 d, 2 d, 4 d, 6 d and 8 d after grouped. The samples were sectioned and frozen at -80℃ till use.

2.2 Total RNA Extraction and Reverse Transcription

All tissues from *L. maculatus* were used for total RNA extraction using Universal RNA Extraction Kit (Takara, Japan) following the manufacturer's instructions. The concentration of RNA each sample was quantified on the nucleic acid analyzer (OSTC, China). Agarose gel electrophoresis (1.5%) was applied to detect RNA integrity. The first-strand cDNA was synthesized by using SMART® MMLV Reverse Transcriptase (Takara, Japan) according to the manufacturer's protocol.

2.3 Isolation *of CYP17 I*, *3β-HSD* and *AR* cDNAs from *L. maculatus* Testis

Three pairs of degenerate primers were designed to amplify spotted seabass *CYP17 I*, *3β-HSD* and *AR* cDNAs (Table 1). The PCR was performed in a volume of 255 μL. PCR products were electrophoresed, purified, inserted into vectors, transformed into the competent cells of *E. coli* and sequenced. In order to obtain the full-length sequences of these three cDNAs, the RACE (Rapid Amplification of cDNA Ends) method was used. Specific primers and nesting primers for each cDNA (Table 1) were designed based on the nucleotide sequence obtained. The following steps were operated as described in previous study (Chi *et al*., 2015). Multi-sequences with deduced amino acid sequences of *CYP17 I*, *3β-HSD* and *AR* were gained from NCBI and aligned using Clustal W. MEGA 5.0 software package was applied to construct and analyze phylogenetic tree using the UPGMA method with 1000 bootstrap trials. A full-length cDNA was called a gene hereafter for the convenience of writing.

2.4 Preparation and Labelling of cRNA Probes and *in Situ* Hybridization

CYP17 I and *3β-HSD* probes were designed against nucleotide sequences got from *L. maculatus* in this study. Forward and reverse primers are listed in Table 1. PCR products were electrophoresed, purified, inserted into *p*SPT18 vector, amplified in *E. coli* and sequenced. Plasmid DNA was then purified with Plasmid Mini Kit (Qiagen) and linearized using restriction enzyme *Sac* I (*Spe* I) and *Xba* I (Fermentas). Probes were then synthesized using the cDNA as templates with DIG RNA Labeling Kit following manufacturer's instructions. After purification, probes were quantified though dot blot hybri dization. Before *in situ* hybridization, slides were pre-hybridized with hybridization buffer at 55℃ for 3 h. Probes

Table 1 Primers used for isolation of cDNA and determination of mRNA abundance of reproductive genes of spotted sea bass

Primer	Sequence (5'→ 3')	Usage
CYP17 I-deg-for	ATCTTCCCCTGGCTGCARATHTTYCC	Core fragment
CYP17 I-deg-rev	TCTTGGCCAGGGC CTCNCCNARRCA	Core fragment
CYP17 I-clon-rev	CGGCAGTAGGAGGAGTTGAAGCA	5'RACE
CYP17 I-clon-for	CTACCTGCCGTTTGGTGCCG	3'RACE
CYP17 I-expre-for	GCTTTGTCCTCTCTGTCCCA	Expression
CYP17 I-expre-rev	TGCTCTTGTTTCTCTCCCAGC	Expression
3β-HSD-deg-for	TGTGTGTGGTRACRGGAGCRTGTGG	Core fragment
3β-HSD-deg-rev	GTAGGAGAAGGTGAANGGRGTGTTNAGCAT	Core fragment
3β-HSD-clone-rev	ATGTCTCTGADAGGTGATGTGTGTGTGG	5'RACE
3β-HSD-clone-for	TTATTTCATTTCTGATGACACGCCACC	3'RACE
3β-HSD-nest-for	GGCAGCTCATCACCATGCTTAACAC	3' nesting RACE
3β-HSD-expre-for	TCCTTCATCTACACCAGCACC	Expression
3β-HSD-expre-rev	GCAAACTTCAGAGCACAGTCA	Expression
AR-deg-for	GAGAGCACATGTTCCCTATGGARTTYTT	Core fragment
AR-deg-rev	CCCAGGGCGAACACCATNACNCCCATCC	Core fragment
AR-clone-for	GGATGGGGGTGATGGTGTTTGCCCTGGG	3'RACE
AR-expre-for	CTCGGAGCACGTAAACTAAA	Expression
AR-expre-rev	CCAGGATGTTGAGGAAGACCA	Expression
18S-expre-for	GGGTCCGAAGCGTTTACT	Expression
18S-expre-rev	TCACCTCTAGCGGCACAA	Expression
Short-UPM	CTAATACGACTCACTATAGGGC	5'RACE, 3'RACE
CYP17 I-hybri-for	GGACACTGTGGCAAAGGAC	*In situ* hybridization
CYP17 I-hybri-rev	CTGGATGACGGGATGATC	*In situ* hybridization
3β-HSD-hybri-for	GTGGATTCCTGGGAAAGAG	*In situ* hybridization
3β-HSD-hybri-rev	CCCACATAGACAGGATTCACTCGG	*In situ* hybridization

were degenerated at 95℃ for 5 min and cooled down on ice immediately. The probes were then diluted to a concentration of 1 μg mL^{-1}. Probes were placed onto tissue sections and slides were hybridized at 55℃ for more than 16 h. Slices were gently oscillated and cleaned with 0.2×SSC (55℃, 2×60 min), NTE (37℃, 2×5 min), RNase (20 mg mL^{-1}, 37℃, 30 min), NTE (37℃, 2×5 min), 0.2× SSC (55℃, 60 min) and 1×PBS (5 min). The DIG chemiluminescent detection kit for nucleic acids was applied. Slices were then stained with eosin and dehydrated in a series of graded ethanol, and photographed under light microscopy (Nikon-E200, Japan) (Tompsett *et al*., 2009).

2.5 Gene Expression Analysis

Real-time PCR was performed with the SYBR green (TAKARA, Japan) on Roche 480 Light Cycler System to measure the relative mRNA abundance of *CYP17 I*, *3β-HSD* and *AR* in reproductive cycle, tissue distribution, hormone and salinity administration. The *18S* ribosomal RNA gene was chosen as the internal control. Expression analysis primers are listed in Table 1. qPCR amplification was carried out in triplicate along with a no-template control in a total volume of 25 μL. The thermal cycling included an initial cycle at 95℃ for 2 min, followed by 40 cycles of denaturing at 95℃ for 15 s, annealing at 56℃ for 15 s and extending at 72℃ for 15 s. After PCR, the $2^{-\Delta\Delta CT}$ method was used to analyze the mRNA abundances of these genes.

2.6 Statistical Analysis

All data were expressed as mean±standard error (SEM). One-way ANOVA was employed followed by Duncan's multiple range tests using the SPSS 13.0. Significance was accepted if $P < 0.05$. Samples in development cycle were relatively expressed to that of stage II. In hormone administration experiment, mRNA abundances in hormone administration group were relative to that of PS-injected group at the same time. In salinity administration experiment, samples from salinity detection groups were expressed as the relative to that of the initial control (0 d point).

3 Results

3.1 Isolation and Characterization of *CYP17 I*, *3β-HSD* and *AR* cDNAs from *L. maculatus* Testis

The complete coding sequences of *CYP17 I* were determined from testis of *L. maculatus*. *CYP17 I* cDNA, 1650 bp in length, contained a 1551 bp open reading frame (ORF) encoding a predicted protein of 516 amino acids. The conserved regions of CYP17 I included a transmembrane region, a conservative P450c17 specific region, an Ozols tridecapeptide region and a heme-iron ligand signature region (Fig.1A). The cDNA of *3β-HSD* was 1464 bp in length, containing a 1122 bp ORF which encoded a predicted protein of 373 amino acids. In addition, the conservative Rossmann's fold and active site/substrate pocket region was boxed in Fig.1B. The *AR* cDNA was a 1489bp sequence that encoded a predicted protein with 372 amino acids. After comparing *L. maculatus AR* deduced amino acid sequences with others available in NCBI, transcriptional activation domain, hormone ligand-binding domain and DNA-binding domain were indicated in Fig.1C. The sequences obtained were submitted to Gen-

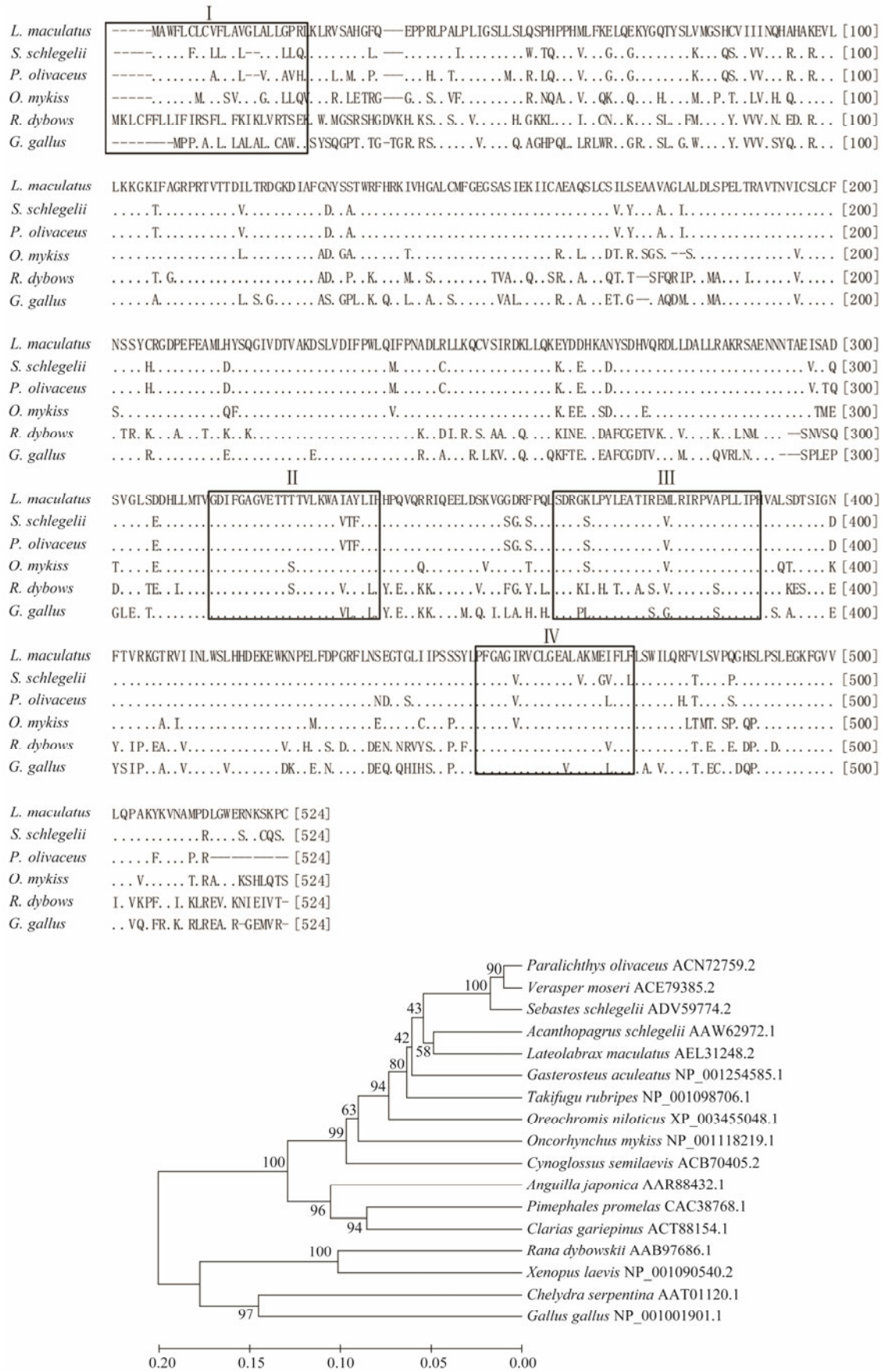

Fig.1A Alignment analysis and phylogenetic tree construction of spotted sea bass *CYP17 I* genes. Phylogenetic analyses were conducted in MEGA version 5.0 with bootstrap values calculated from 1000 resamplings. Protein sequences used for comparison and their GenBank accession numbers were listed at the right of the branches. The putative conserved domains are boxed. I, transmembrane region; II, conserved P450c17 specific region; III, Ozols tridecapeptide region; IV, heme-iron ligand signature region.

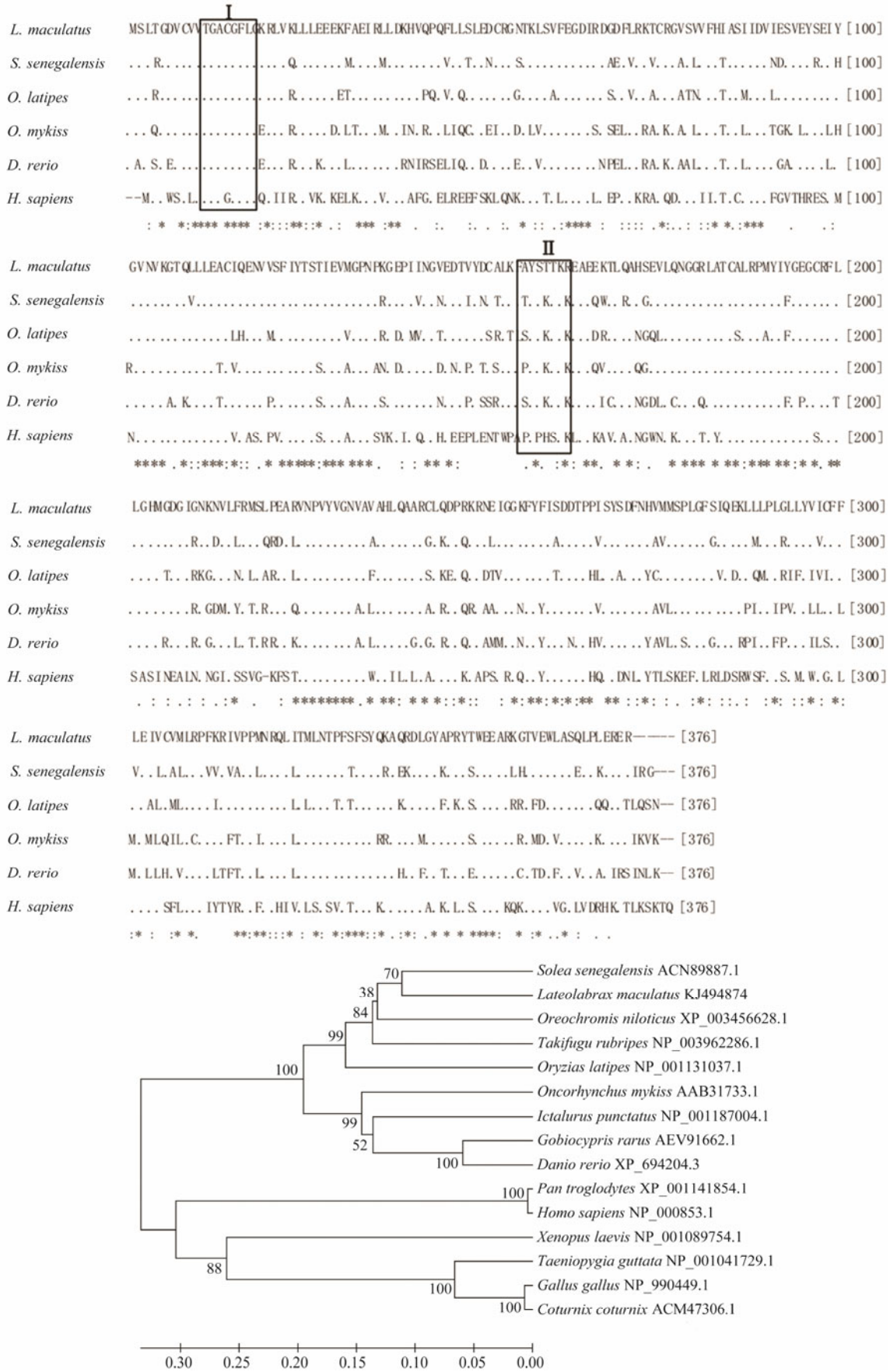

Fig.1B Alignment analysis and phylogenetic tree construction of spotted sea bass *3β-HSD* genes. Phylogenetic analyses were conducted in MEGA version 5.0 with bootstrap values calculated from 1000 resamplings. Protein sequences used for comparison and their GenBank accession numbers were listed at the right of the branches. The putative conserved domains are boxed. I, Rossmann's fold; II, active site/substrate pocket.

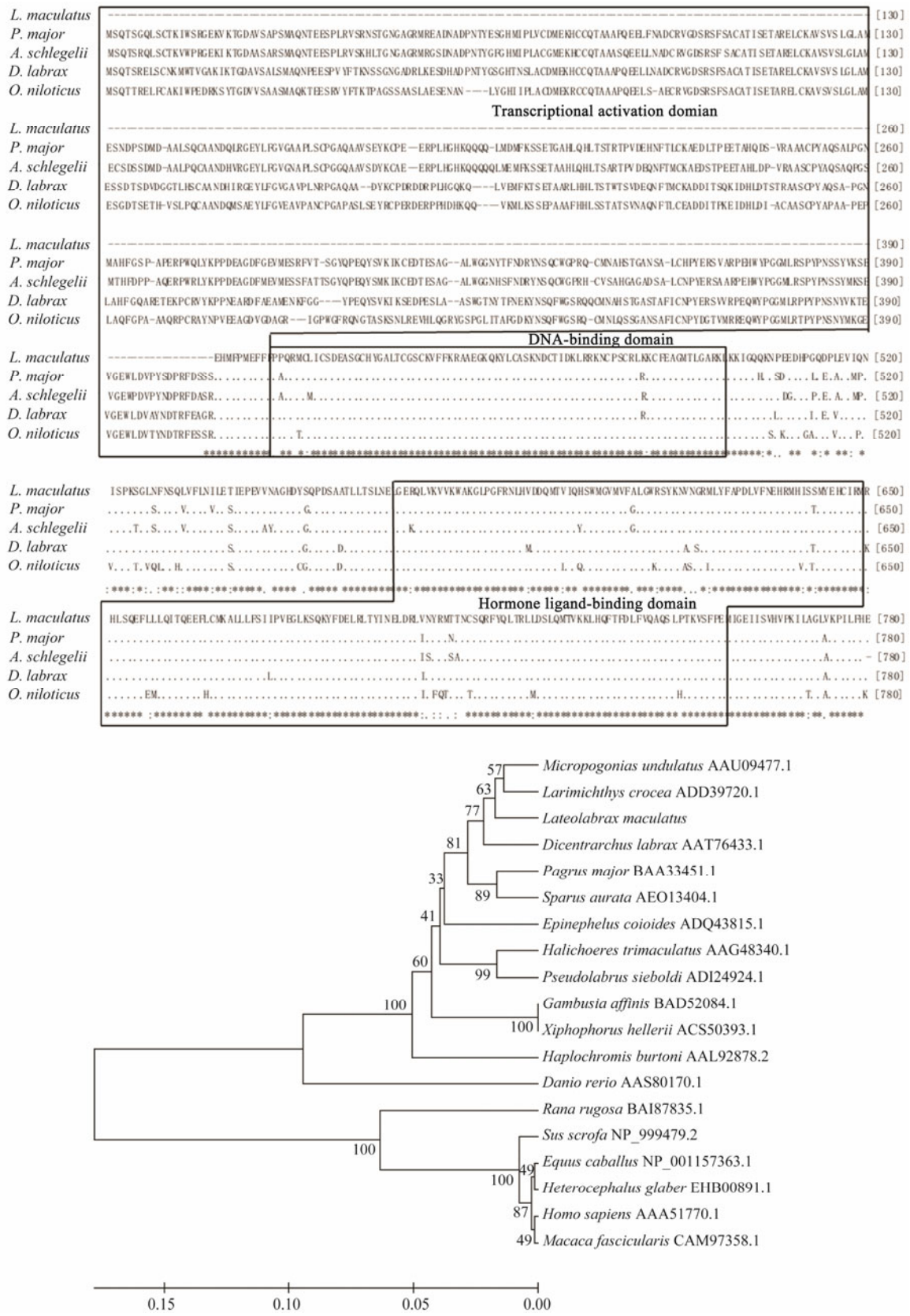

Fig.1C Alignment analysis and phylogenetic tree construction of spotted sea bass *AR* genes. Phylogenetic analyses were conducted in MEGA version 5.0 with bootstrap values calculated from 1000 resamplings. Protein sequences used for comparison and their GenBank accession numbers were listed at the right of the branches. The putative conserved domains are boxed. Transcriptional activation, hormone ligand-binding and DNA-binding domains are boxed.

Bank with accession numbers JN089771.2 for *CYP17 I*, KJ494874.1 for *3β-HSD* and KC534880 for *AR*. Phylogenetic analyses of vertebrate CYP17 I, 3β-HSD and AR proteins clearly showed that the *L. maculatus* sequences

grouped with those of other teleost, suggesting that they are homologous to those of other perciformes species.

3.2 Localization of *CYP17 I* and *3β-HSD* Transcripts in *L. maculatus* Testis by Using *in Situ* Hybridization

There was no positive signal detected after treating with sense probes of *CYP17 I* and *3β-HSD* in *L. maculatus* testis (Figs.2A and C). The positive signals of these two genes appeared in testis at stage II when the antisense probes were used. Strong hybridization signals were observed mainly in interstitial cell regions (Figs.2B and D) which were based on the experimental results got from histologic sectioning (Fig.2E).

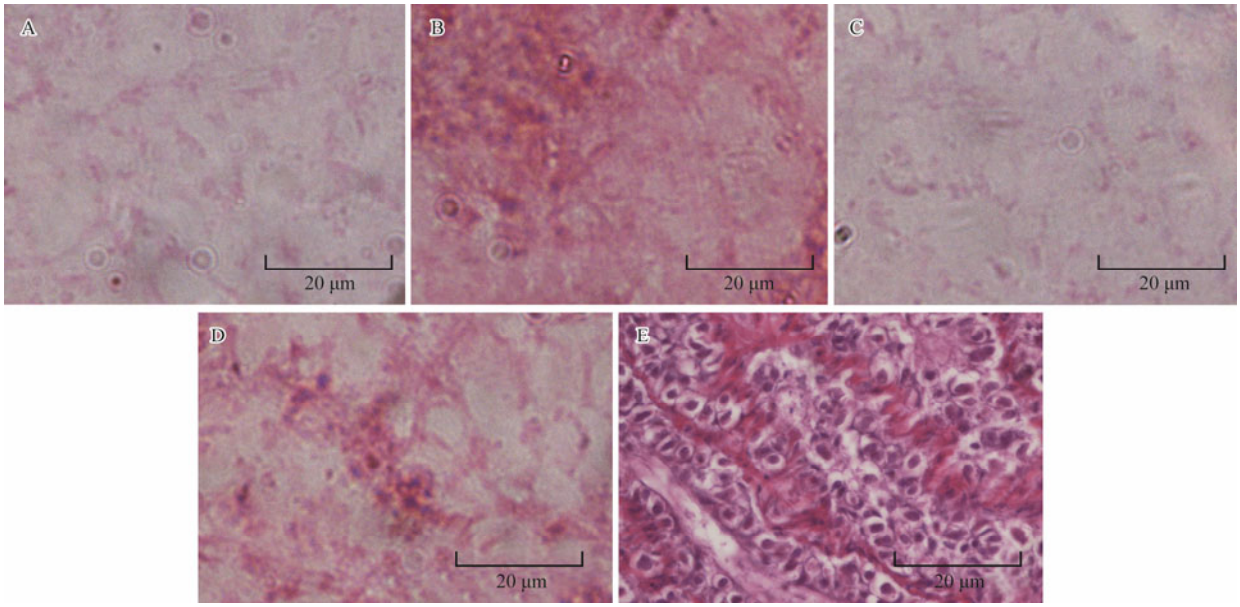

Fig.2 Localization of *CYP17 I* (A and B) and *3β-HSD* (C and D) transcripts in spotted sea bass testis at stage II using *in situ* hybridization. *In situ* hybridization with sense probe was the negative control (Figs.2A, C). The corresponding DIG-labeled antisense RNA probe was used for *CYP17 I* and *3β-HSD* transcripts (Figs.2B, D). HE staining section photograph was showed in Fig.2E. Scale bars are shown each panel.

3.3 The mRNA Abundance of *CYP17 I*, *3β-HSD* and *AR* in Testicular Development Cycle of Male *L. maculatus*

Twenty-four male Japanese sea bass were selected to test the expression of these three genes at different stages (N=4 for stage II, N=4 for stage III, N=5 for stage VI, and N=11 for stage V) according to the research conducted by Chi (2014). In testis, the expression level of *CYP17 I* increased from stage II to stage V and peaked at stage V. The significant change can be detected at stage V in comparison with stage II ($P<0.05$). The tendency of *L. maculatus 3β-HSD* expression was consistent with that of *CYP17 I*, and the transcript of *3β-HSD* significantly increased at stage V ($P<0.05$). Meanwhile, the expression of *AR* increased at stage III and VI, then declined slightly at stage V (Fig.3).

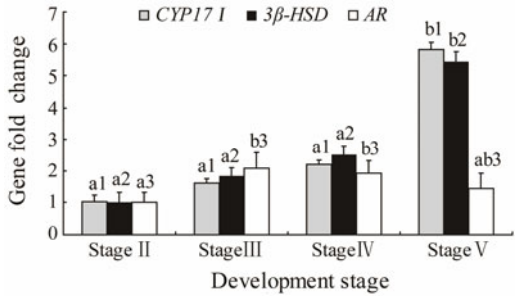

Fig.3 mRNA abundances of *CYP17 I*, *3β-HSD* and *AR* in spotted sea bass in testicular development cycle. Data are the relative value to that of stage II. Values are expressed as mean±SEM ($n=6$). Values with different letters indicate statistical significances in difference determined by one-way ANOVA and Duncan's multiple tests.

3.4 The mRNA Abundance of *CYP17 I*, *3β-HSD* and *AR* in Different Tissues of Male *L. maculatus*

CYP17 I, *3β-HSD* and *AR* expressed in all tissues tested. *CYP17 I* transcripts presented highly in testis, pituitary, brain and head kidney. *L. maculatus 3β-HSD* transcripts

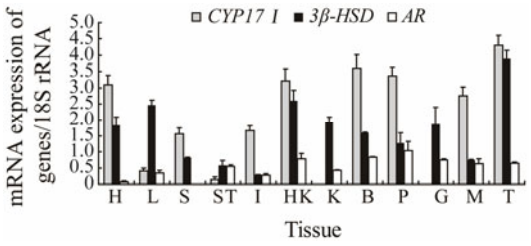

Fig.4 Tissue expression patterns of *CYP17 I*, *3β-HSD* and *AR* in male spotted sea bass. Gene expression was profiled thorough qPCR using specific primers, and 18S ribosomal RNA gene was used as internal control. The tissues include heart (H), liver (L), spleen (S), stomach (ST), kidney (K), brain (B), intestine (I), pituitary (P), muscle (M), head kidney (HK), gills (G) and testis (T).

were highly abundant in testis, head kidney, liver and heart but weak in brain and gills. Strong *AR* expression was observed in brain, pituitary, gills, testis, muscle and head kidney (Fig.4).

3.5 Changes of *CYP17 I*, *3β-HSD* and *AR* Expressions in Response to hCG and GnRHa Treatments

Changes of *CYP17 I*, *3β-HSD* and *AR* gene expressions in testis were investigated by qPCR. It was showed that mRNA abundance of *CYP17 I* was significantly increased by hCG and GnRHa treatments at 12 h, 24 h and 48 h in comparison with PS treatment (Fig.5A, $P<0.05$). For *3β-HSD*, its mRNA rose first and then fell in 48 h after hCG administration, and the highest expression was detected at 12 h. The significant increases can be detected in all GnRHa treatment groups from 6 h to 48 h ($P<0.05$, Fig.5B). However, for *AR*, the significant decrease can only be detected in hCG treatment group at 24 h ($P<0.05$, Fig.5C).

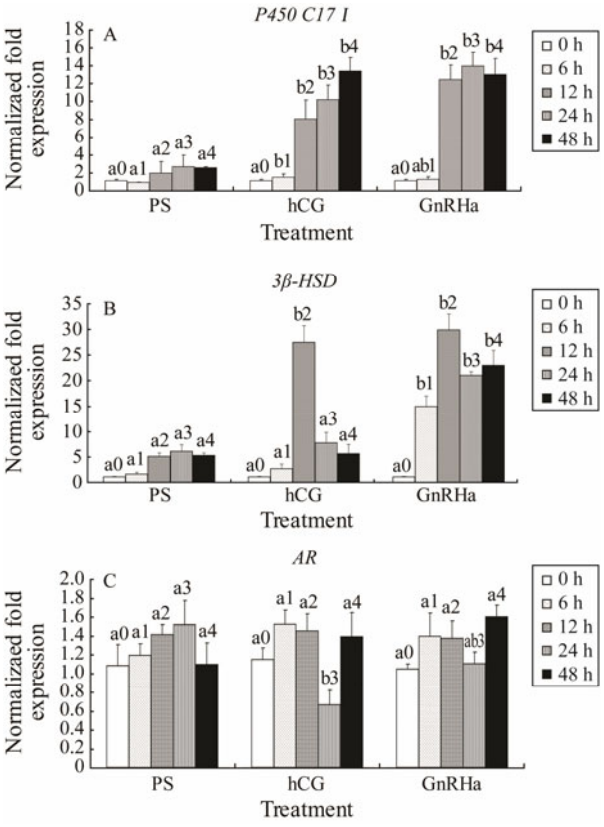

Fig.5 Regulation of the *CYP17 I*, *3β-HSD* and *AR* expressions in spotted sea bass testis by hormone treatment. Data are the relative values to that of PS-injected fish individuals (Mean ± SEM, $n=4$). Different letters indicate significant differences between groups at the same time after PS/hormones injection ($P<0.05$, one-way ANOVA, followed by Duncan's test).

3.6 Expression of *CYP17 I*, *3β-HSD* and *AR* in Testis During Salinity Administration

Expression of *CYP17 I* in testes was significantly inhabited in BW and FW groups from 1 d to 8 d when compared with that in SW group ($P<0.05$, Fig.6A). Significant decreases of *3β-HSD* mRNA were detected at 2 d and 4 d in BW group and at 2 d, 4 d and 6 d in FW group when compared to SW group ($P<0.05$, Fig.6B). Different trends of *AR* expression were detected in BW and FW groups. Its expression increased first and then decreased in BW group. The significant increases at 4 d, 6 d and 8 d were detected when compared with the other two groups ($P<0.05$). While in FW group, *AR* mRNA levels remained relatively constant throughout the administration (Fig.6C).

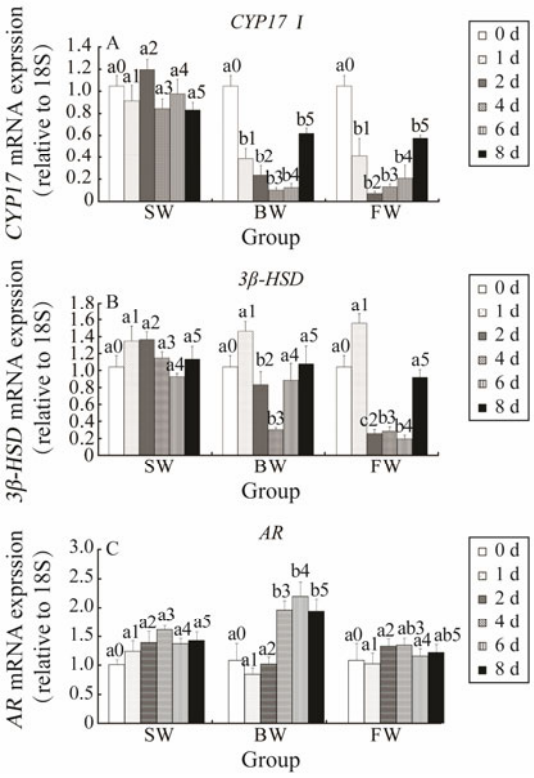

Fig.6 mRNA abundances of *CYP17 I* (A), *3β-HSD* (B) and *AR* (C) in the testis of spotted sea bass acclimated to seawater (SW), brackish water (BW) and fresh water (FW). Data are expressed as means ± SEM ($n=4$). Different numbers are used to distinguish experiment points. Different letters indicate significant differences at $P<0.05$ by Duncan's multiple range tests.

4 Discussion

In present study, *CYP17 I*, *3β-HSD* and *AR* cDNAs were isolated from the testis of *L. maculatus*. Multi-alignment and phylogenetic studies of these three genes revealed that *L. maculatus* was highly homologous with other fish species, especially those in Perciformes. *L. maculatus CYP17 I* included only one potential polyadenylation signal (AATAAA) in 3'-untranslational region, while two or more signals were reported in zebrafish (*Danio rerio*) (Wang and Ge, 2004), rice field eel (*Monopterus albus*) (Yu *et al*., 2003) and half-smooth tongue sole (*Cynoglossus semilaevis*) (Chen *et al*., 2010). More than one splice variants of *CYP17* were found in these studies. It has been suggested that longer *CYP17* may be polyadenylated to be a shorter one at 3'-UTR. In contrast to many other vertebrates (Thomas *et al*., 2015; Chen *et al*., 2015), only one *3β-HSD* copy was isolated from *L. maculatus* testis in this

study. Although the complete coding sequence of *AR* was not acquired in this study, the hormone ligand-binding and DNA-binding domains were completely isolated. It is important to notice that the 5' sequence was also difficult to obtain in sea bass (*Dicentrarchus labrax*), which may be due to the high GC-content or other reasons (Blázquez and Piferrer, 2005).

The expression of *CYP17 I* and *3β-HSD* was relatively stable from stage II to stage IV, and reached the maximum at stage V in testicular development cycle, which was in accordance with the level of serum testosterone (Chi *et al*., 2014). Identical or similar results were obtained in other teleost such as Nile tilapia and rainbow trout (*Oncorhyncus mykiss*) (Nakamura *et al*., 2005; Senthilkumaran *et al*., 2009). However, in channel catfish (*Ictalurus punctatus*), the expression of *3β-HSD* was relatively stable throughout ovary development cycle (Kumar *et al*., 2000). Meanwhile, the expesssion of *AR* increased first and then declined slightly at stage V, showing a different trend from that of *CYP17 I* and *3β-HSD*. Recombining androgen receptor has a high affinity with the androgen at low concentrations for promoting both nuclear transport and transcriptional activities, however, the high androgen concentration inhibits these activities (Kemppainen *et al*., 1992). Therefore, the decrease of *AR* expressions at stage IV and stage V may be due to the increase of testosterone in *L. maculatus* testis.

L. maculatus CYP17 I predominantly expressed in the testis, brain, pituitary and head kidney, which was in accordance with that of other fish species (Kazeto *et al*., 2000; Halm *et al*., 2003; Wang and Ge, 2004). In addition, the positive signal of *CYP17 I* RNA probe was showed in testis by using *in situ* hybridization in this study. The *CYP17* signal was also detected in interstitial cells of medaka (*Oryzias latipes*) (Zhou *et al*., 2007) and germ cells of male gonad and testis of frog (*Rana rugosa*) (Iwade *et al*., 2008) with identified steroid generation function. Meanwhile, *3β-HSD* signal was also located in the testis of *L. maculatus* and Nile tilapia (Yu, 2013) by using *in situ* hybridization. It is the first time to detect the co-expression of *CYP17 I* and *3β-HSD* in *L. maculatus* testis. Results in immature and mature testis of rainbow trout using immunolocalization were also consistent with the co-expression of these two genes in this study (Kobayashi *et al*., 1998). The expression of *3β-HSD* in testis, head kidney, liver and heart was very high in *L. maculatus*, while that of *3β-HSD* mainly in gonad and brain in rare minnow (*Gobiocypris rarus*) (Liu *et al*., 2012). Although mRNA can be detected in the brain of *L. maculatus*, the content was not as high as that in rare minnow. The high expression of *AR* was mainly focused on brain, pituitary, gills, testis and head kidney. These results indicated that *CYP17 I*, *3β-HSD* and *AR* may mainly play their roles in steroidogenesis and stress-responsive tissues in male *L. maculatus*.

In hormone administration experiment, the gonadal development statue and corresponding testosterone level in serum had been improved greatly after injecting exogenous hormone into *L. maculatus* testis (Chi *et al*., 2014). *L. maculatus* testicular *CYP17 I* mRNA abundance was sharply risen after hCG and GnRHa treatments from 6 to 48 h. This increase was also found in ovary of zebrafish exposed to progesterone for 40 d and *in vitro* oocyte of snake head murrel (*Channa striatus*) exposed to hCG (Liang *et al*., 2015). However, western blotting analysis demonstrated that no significant change in the CYP17 protein content during hCG induction in snake head murrel (Sreenivasulu *et al*., 2005). In addition, the inconsistency was also observed between *3β-HSD* mRNA abundance and its corresponding protein content in Japanese eel and catfish (*Clarias gariepinus*) after hormone administration (Kazeto *et al*., 2003; Raghuveer *et al*., 2005). This may be caused by the post-transcriptional regulation in this process (Chen *et al*., 2015). Therefore, the changes of *CYP17 I* and *3β-HSD* encoding protein contents in this experiment will be the priority of further research. For *L. maculatus AR*, significant decrease can only be detected in hCG treatment group at 24 h. There was no research on the change of *AR* expression after hCG and GnRHa administrations. Some studies have been conducted by using other exogenous hormones. The expression patterns of *ARα* and *ARβ* subunit genes in western mosquito (*Gambusia affinis*) varied among tissues. There was no significant change in the expression of *ARα*, but significant decrease was detected for that of *ARβ* in ovaries after testosterone treatment. The 17β-estradiol treatment suppressed the expression of androgen receptor α but not *β* genes in liver (Huang *et al*., 2012). The increase of *AR* expression in immature Japanese eel (*Anguilla japonica*) testis after hCG treatment (Todo *et al*., 1999) was inconsistent with the findings of this study. In general, the *AR* expression after hormone treatment may undergo very complex transcriptional regulation, and may be related to different types of hormones, AR subunit genes, tissues and development status.

From 1 to 8 d of salinity acclimation, testosterone hormone level was very low from 2 to 4 d, and then started to recover to control level after 4 day's administration, especially in FW group. The maturity status of testis was reduced after acclimating in fresh water for 8 d when compared to seawater group (data not shown). The depression testicular development was entirely different from that of hormone administration as well as *CYP17 I*, *3β-HSD* and *AR* mRNA abundances. The changes of *3β-HSD* and *CYP17 I* expression in the testis were consistent with the changes of testosterone and histology, decreasing with the decrease of salinity from 1 to 6 d, but recovered on 8 d. Considering the important role of *3β-HSD* and *CYP17 I* in testosterone production, the endogenous hormone balance may be achieved after 8 d adaptation. *AR* expression was significantly increased on 4 d, 6 d and 8 d in BW group when compared with SW and FW groups, suggesting that other factors except for testosterone may be involved in the regulation of *AR* expression.

5 Conclusions

The CDS of *CYP17 I*, *3β-HSD* and *AR* were isolated

and characterized from *L. maculatus* testis. These genes also expressed in other tissues such as pituitary, brain and head kidney. Significant changes of the expressions of these three genes in testis in reproductive cycle, hormone and salinity treatments suggested that they may play important roles in testicular development and spermatogenesis of *L. maculatus*. These findings have improved our understanding of reproductive endocrine of *L. maculatus*. Further *in vitro* and *in vivo* studies will be conducted to clarify whether the changes occur at the protein level.

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