Fish Physiol Biochem
https://doi.org/10.1007/s10695-019-00684-9

Growth, physiological, and molecular responses of golden pompano *Trachinotus ovatus* (Linnaeus, 1758) reared at different salinities

Bo Liu · Hua-Yang Guo · Ke-Cheng Zhu · Liang Guo · Bao-Suo Liu · Nan Zhang · Jing-Wen Yang · Shi-Gui Jiang · Dian-Chang Zhang

Received: 18 November 2018 / Accepted: 17 July 2019

Abstract Golden pompano (*Trachinotus ovatus*) is a commercially important marine fish and is widely cultured in the coastal area of South China. Salinity is one of the most important environmental factors influencing the growth and survival of fish. The aims of this study are to investigate the growth, physiological, and molecular responses of juvenile golden pompano reared at different salinities. Juveniles reared at 15 and 25‰ salinity grew significantly faster than those reared at the other salinities. According to the final body weights, weight gain rate, and feed conversion ratio, the suitable culture salinity range was 15–25‰ salinity. The levels of branchial NKA activity showed a typical "U-shaped" pattern with the lowest level at 15‰ salinity, which suggested a lower energy expenditure on osmoregulation at this level of salinity. The results of this study showed that the alanine aminotransferase, aspartate aminotransferase, and cortisol of juveniles at 5‰ were higher than those of other salinity groups. Our results showed that glucose-6-phosphate dehydrogenase significantly increased at 5‰ and 35‰ salinity. Our study showed that osmolality had significant differences in each salinity group. *GH*, *GHR1*, and *GHR2* had a wide range of tissue expression including the liver, intestine, kidneys, muscle, gills and brain. The expression levels of *GH*, *GHR1* and *GHR2* in the intestine, kidneys, and muscle at 15‰ salinity were significantly higher than those in other three salinity groups. Based on the growth parameters and physiological and molecular responses, the results of the present study indicated that the optimal salinity for rearing golden pompano was 21.36‰ salinity.

Keywords *Trachinotus ovatus* · Salinity · Growth · Physiological responses · Osmoregulation-related genes

B. Liu · H.-Y. Guo · K.-C. Zhu · L. Guo · B.-S. Liu · N. Zhang · J.-W. Yang · S.-G. Jiang · D.-C. Zhang (✉)
Key Laboratory of South China Sea Fishery Resources Exploitation and Utilization, Ministry of Agriculture and Rural Affairs; South China Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences, Guangzhou 510300 Guangdong, China
e-mail: zhangdch@scsfri.ac.cn

B. Liu
College of Fisheries and Life Science, Shanghai Ocean University, Shanghai 200090, China

H.-Y. Guo · K.-C. Zhu · L. Guo · B.-S. Liu · N. Zhang · J.-W. Yang · S.-G. Jiang · D.-C. Zhang
Guangdong Provincial Engineer Technology Research Center of Marine Biological Seed Industry, Guangzhou, Guangdong, China

S.-G. Jiang · D.-C. Zhang
Guangdong Provincial Key Laboratory of Fishery Ecology and Environment, Guangzhou, Guangdong, China

Introduction

Growth of teleost fishes is under direct the control of many environmental factors (Zacharia and Kakati 2004; Taylor et al. 2005; Hora et al. 2016). Salinity, one of the primary environmental factors, affects fish growth and physiological performance (Boeuf and Payan 2001; Lee et al. 2017; Zhang et al. 2017; Zhang et al. 2018). Many studies have been conducted to understand the influence

Published online: 08 August 2019

of salinity on growth in aquaculture (Laverty and Skadhauge 2012; Ran et al. 2017). The available data show that salinity does not affect the growth of some euryhaline fish species, while other species had increased growth in low or high salinities (Ma et al. 2014; Lisboa et al. 2015). Suitable salinity affects fish growth, with better growth observed in many species such as *Acanthopagrus butcheri* (Partridge and Jenkins 2002), *Bostrychus sinensis* (Zhang et al. 2017), *Hippoglossus hippoglossus* (Imsland et al. 2008), and *Trachinotus marginatus* (Abou Anni et al. 2016). Thus, determining the optimal ranger of salinities for culturing of euryhaline fish, which can survive large fluctuations in ambient salinity, may be important in developing a rearing protocol for these species (Arnason et al. 2013; Zhang et al. 2017).

Euryhaline fishes possess the ability to adapt to a wide range of environmental salinities (Arnason et al. 2013; Yamaguchi et al. 2018). Because osmoregulation is an energy-demanding process, isoosmotic salinities minimize the osmoregulatory stress and osmoregulatory costs and increase the energy available for growth or survival (Imanpoor et al. 2012). Na^+/K^+-ATPase (NKA) is an ion-transporting enzyme and is expressed at an extremely high level in salt-transporting tissues, such as the gills. The lowest activity of the gill NKA occurs when the salinity of the medium is close to or slightly above that of the blood, and the gill NKA activity is used as an indicator of osmoregulatory energetics (Zhang et al. 2017). In teleost, the number of branchial chloride cells, their shape, and the expression level of ion transport proteins involved in salt secretion have been shown to be adjusted according to salinity (McCormick et al. 2009; Amiri et al. 2018), which is particularly important for euryhaline fishes because they must maintain water and ion homeostasis in their gills (William 2014).

Salinity stress triggers a series of physiological changes, which are classified as primary, secondary, and tertiary responses (Mattioli et al. 2017). Increased plasma growth hormone, cortisol, and thyroxine are among the primary responses to salinity stress (Almeida et al. 2013; Tsui et al. 2013; Hajirezaee et al. 2018). Among the secondary responses are metabolic responses, such as changes in the glycemia and hematological responses, as well as responses that affect the hydromineral balance, such as changes in the concentrations of sodium chloride, potassium, and plasma osmolality (Shui et al. 2018; Silva Aires et al. 2018). Tertiary responses are changes that lead to a drop in productive performance and decreased disease resistance (Chang et al. 2016; Downie and Kieffer 2016). In this sense, water salinity can influence the activity of enzymes (Ahmmed et al. 2017; Tran-Ngoc et al. 2016) and alter locomotor activity and food intake, with direct consequences on animal growth (Nguyen et al. 2014; Ray and Lotz 2017; Montory et al. 2018). Salinity also has a direct influence on hematological and biochemical variables (Breves et al. 2010a), which provides important information regarding the clinical status and energy of fish. Furthermore, variables in blood chemistry can aid in the assessment of eating disorders and the action of environmental stressors (Oliveira-Ribeiro et al. 2000; Yin et al. 2018), making it possible to evaluate physiological changes during the adaptation of fish to a challenging environment (Stewart et al. 2016).

Though much is known about the molecular effectors of osmotic acclimation, comparatively little is known of the signalling and regulatory networks in fish that integrate and transduce environmental cues to initiate the physiological acclimation response (Whitehead et al. 2012). Growth hormone (GH) is secreted by the pituitary and is involved in many physiological functions in fish, most of which are associated with somatic growth and stress resistance (Ababutain 2011; Bertucci et al. 2017; Yuan et al. 2017). The osmoregulatory function of the growth hormone receptor (GHR) in the gills and kidneys has been well established (Weng et al. 1997). The *GHR1* and *GHR2* are highly expressed in the fish liver and mediate action of GH (Ozaki et al. 2006; Rhee et al. 2012). Previous studies have documented the effects of salinity on the expression of GH and GHR in several fishes, including *Acanthopagrus schlegelii* (Tomy et al. 2009) and *Sparus auratus* (Laiz-Carrión et al. 2009). Two GHRs in the olive flounder *Paralichthys olivaceus* (Nakao et al. 2004) play different roles in endocrine function. Since osmoregulation affects growth and energy expenditure, it is expected that salinity changes modulate the expression of genes involved in the regulation of somatic growth (Bertucci et al. 2017). When participating in the stress response and regulation of the body's salinity changes in the external environment, gene expression increases or decreases. However, few studies have been performed on the relationship between the osmoregulation-related genes and long-term difference under salinity conditions.

Golden pompano, *Trachinotus ovatus* belongs to the Carangidae family. Golden pompano is distributed in tropical and subtropical areas of Southeast Asia and the Mediterranean Sea. Because of its delicious taste and rapid growth, golden pompano is one of the most important marine fishes that are commercially cultured in South China (Sun et al. 2013; Guo et al. 2014; Tan et al. 2016). To date, the life cycle of *T. ovatus* has been poorly described, and several key issues related to larval rearing, such as food and feeding, the development of the larval digestive system, and weaning, have been recently successfully addressed (Ma et al. 2014; Ma et al. 2015), though further studies are still required, especially to determine the suitable environmental conditions for its production at a commercial level during the grow-out phase (Ma et al. 2016a). Currently, salinity fluctuations hinder the farming efficiency of this species since most pompano are cultured in outdoor ponds and floating sea cages in China where heavy rainfall can reduce the local salinity by 30–50% during the nursery phase (Ma et al. 2016b). The aims of this study were to examine the growth performance, survival, biochemical and physiological characteristics, and mRNA expression of osmoregulation-related genes in *T. ovatus* at different salinities. These results will not only delineate the relationship among salinity, growth performance, and expression of osmolality and metabolism-related genes but also determine the suitable rearing salinity for *T. ovatus*.

Materials and methods

Fish, experimental design, and experimental conditions

Experimental fish were obtained from the Tropical Fisheries Research and Development Centre, South China Sea Fisheries Research Institute, Chinese Academy of Fishery Science, Lingshui (Hainan, China). The mean body weight of the experimental fish was 12.07 ± 0.13 g, and the standard length of the fish was 8.22 ± 0.19 cm. Fish were initially stocked into 3000-L tanks.

In the experiments, pH, oxygen, and temperature were measured daily by a HQ30d (HACH30d, Loveland, CO, USA). Oxygen saturation was close to 100% at all times and the water flow was adjusted to keep ammonia well below critical levels. All tanks were supplied with filtered water (31‰). Two air stones were used in each tank to maintain dissolved oxygen close to saturation. Starting from the second day, salinity in each rearing tank was decreased or increased by 4‰ per day by adding freshwater or sea salt until reaching the target salinity of 5‰, 15‰, 25‰, and 35‰ in three replicates. After reaching the target salinity, the amount of water exchange per day was 90%. A total of 25 fish were stocked into each 600-L experimental tank. Experimental fish were fed commercial pellets (Hengxing, Guangzhou, China) two times per day at 07:00 and 17:00 h to apparent satiation. During the 56-day feeding trial, the number and weight of dead fish and feed consumption were recorded every day. Experimental tanks were cleaned daily by siphoning the bottom of the tank to remove uneaten feed and feces. During the experimental period, the water temperature was maintained at 26.9–28.4 °C. The pH ranged between 7.17 and 8.23, and dissolved oxygen was more than 6.16 mg/L.

At the end of the feeding trial, fish were fasted for 24 h before sampling and were anesthetized with 100 mg/L eugenol (Shanghai Medical Instruments Co., Ltd., Shanghai, China). Blood samples were obtained from the caudal vein of nine fish from each group, using a 2.5-mL syringe without heparin. After centrifugation (3000×*g*, 4 °C, 10 min), serum was separated from coagulated blood and stored at − 80 °C. Finally liver, intestine, kidneys, muscle, gills, and brain were sampled from each group (9 fish). All experiments in this study were approved by the Animal Care and Use Committee of South China Sea fisheries Research Institute, Chinese Academy of fishery Sciences (no. SCSFRI96-253) and performed according to the regulations and guidelines established by this committee.

Growth

At the end of the experiment, the total numbers and mean body weight of fish in each tank were determined. The survival rate (SR), weight gain rate (WG), specific growth rate (SGR), and feed conversion ratio (FCR) of each tank were calculated. Hepatosomatic index (HSI), viscerosomatic index (VSI), and condition factor (CF) were determined from nine individual fish each group by

obtaining tissues (viscera and liver) and expressing ratios as a percent of body weight.

The parameters were calculated as per the following formulae:

$$\text{Weight gain rate (WG, \%)} = 100 \times (\text{final body weight} - \text{initial body weight}) / \text{initial body weight}$$

$$\text{Feed conversion ratio (FCR, \%)} = 100 \times \text{dry diet feed (g)} / \text{wet weight gain (g)}$$

$$\text{Specific } \textit{growth rate } \left(SGR, \%day^{-1}\right) = 100 \times (Lnfinial\ individual\ weight - Lninitial\ individual\ weight) / number\ of\ days$$

$$\text{Feed conversion ratio (FCR, \%)} = 100 \times \text{dry diet feed (g)} / \text{wet weight gain (g)}$$

$$\text{Survival rate (SR, \%)} = 100 \times (\text{finial number of fish}) / (\text{initial number of fish})$$

$$\text{Condition factor } \left(\text{CF, g/cm}^3\right) = 100 \times (\text{body weight, g}) / (\text{body length, cm})^3$$

$$\text{Hepatosomatic index (HSI, \%)} = 100 \times (\text{liver weight, g}) / (\text{whole body weight, g})$$

$$\text{Viscerosomatic index (VSI, \%)} = 100 \times (\text{viscera weight, g}) / (\text{whole body weight, g})$$

Serum osmolality and serum parameters

Blood samples were collected immediately from the caudal veins of nine fish per group. Following centrifugation (3000×*g*, 4 °C, 10 min), serum was separated for analysis of the serum biochemical indices and serum osmolality. Serum osmolality was determined using a BS-100 freezing point osmotic pressure instrument (Shanghai Yida Medical Devices Co., Ltd., China). The remaining serum was stored at − 80 °C for analysis of the serum biochemical indices. Triglyceride (TG), cholesterol (CHO), total protein (TP), albumin (ALB), alanine aminotransferase (ALT), aspartate aminotransferase (AST), glucose (GLU), lactate (LD), malondialdehyde (MDA), lysozyme (LYZ), phosphofructokinase (PHK), glucose-6-phosphate dehydrogenase (G6PDH), and aldose reductase (AR) were measured with a Mindray BS-420 automatic biochemical instrument (Shenzhen Mindray Biological Medical Electronics Co., Ltd., China). The serum ionic (Na^+, K^+, Cl^-) levels were measured using the electrode method (URIT-910A, Guilin Uritest Medical Electronic Co. Ltd., China). Triiodothyronine (T3) and thyroxine (T4) were measured by using a commercial ELISA kit (URIT-910A, Guilin Uritest Medical Electronic Co. Ltd., China). The serum cortisol (COR) was measured by enzyme-linked immunosorbent assay (Abebio, Wuhan, China). First, the microtiter plate was coated with purified cortisol antibody for 2 h, and then 25 μL of serum sample, 25 μL of standard, and 100 μL of HRP were added to each well and allowed to stand for 1 h. Thoroughly wash, add 100 μL of color development solution, develop color for 15 min. Finally, stop solution was added and the absorbance of each well was measured at 450 nm in Hi Well Diatek DR-200BS enzyme micro-plate reader (Hiwell Diatek Instruments, Wuxi, China).

Expression profiles of osmoregulation-related genes

Total RNA samples were extracted using the TRIzol reagent (Invitrogen, Waltham, MA, USA) following the manufacturer's instructions. The quality and quantity of RNA were measured by 1.2% agarose gel electrophoresis and a NanoDrop 2000 (Thermo Scientific, Waltham, MA, USA), respectively. cDNA was synthesized from total RNA using a PrimeScript™ Reverse Transcriptase kit (TaKaRa, Dalian, China) according to the manufacturer's instructions and subsequently stored at − 80 °C. The working solution of the cDNA samples was diluted to 100 ng/μL and stored at − 20 °C until use.

The DNA sequences of *GH*, *GHR1*, and *GHR2* were obtained from the genome database of *T. ovatus* (Accession No. PRJEB22654 under ENA; Sequence Read Archive under BioProject PRJNA406847). To verify the accuracy of the sequences, DNA-specific primers were designed with the Primer Premier 5.0 software (Lalitha 2000). The PCR reactions were performed using a GradientMaster cycler (Eppendorf, Hamburg, Germany) system with a total volume of 25 μL of PCR mixture that contained 2.5 μL 10 × reaction buffer with 15 mM $MgCl_2$, 2 μL of 10 mM dNTP mix, 1.5 μL 25 μM each primer, 1 μL template cDNA, 16.5 μL Milli-Q water, and 0.5 μL BioReady ExTaq DNA Polymerase (5 U/μL) (TaKaRa, Dalian, China). The PCR cycles were conducted as follows: an initial denaturation at 94 °C for 3 min, followed by 30 cycles of 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 2 min, and a final extension at 72 °C for 10 min. The PCR products were analyzed by electrophoresis in a 1.2% agarose gel.

The expression patterns of *GH*, *GHR1*, and *GHR2* mRNAs were analyzed by quantitative real-time (qRT)-PCR performed on an Applied Light Cycler (Roche Diagnostics, Shanghai, China). Specific primer pairs for *GH*, *GHR1*, *GHR2* and the reference gene *EF-1α* (*elongation factor 1 alpha*) gene primers are listed in Table 1.

A 12.5-μL reaction volume contained 6.25 μL 2 × Light Cycler 480 SYBR Green I Master mix (Roche Diagnostics, Shanghai, China), 1 μL first-strand cDNA template, 0.5 μL each primer, and 4.25 μL of Milli-Q water. The thermal profile for qRT-PCR was 95 °C for 30 s followed by 40 cycles at 95 °C for 5 s, 60 °C for 20 s. The relative expression levels were calculated using the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen 2001).

Gill NKA activity

Gill tissues of the fry in the four groups were obtained at the end of the experiment. Gill tissue was homogenized and supplemented with a 0.70% NaCl solution. Thereafter, the mixture was centrifuged at 2500 r/min for 10 min at 4 °C and the supernatant was collected for determination. The NKA activity was determined by measuring the release of inorganic phosphate (Pi) from ATP according to the kit protocol, and the amount of inorganic phosphorus was measured at 636 nm (Nanjing Jiancheng Institute of Biological Engineering, China).

Statistical analysis

Data are expressed as the means ± standard error of mean (SEM). The mean values among salinity treatments were compared using one-way analysis of variance (ANOVA), followed by Tukey's test. The significance level adopted was 95% ($P < 0.05$). All statistical analyses were performed using SPSS 22.0 (SPSS Inc., Chicago, USA). The final body weight and NKA activity were subjected to a quadratic regression analysis to analyze the correlation among the weight, NKA activity, and salinity levels of juvenile golden pompano.

Results

Effects of salinity on growth of *T. ovatus*

Salinity affected the final body weight (FBW), survival rate (SR), weight gain rate (WGR), specific growth rate (SGR), feed conversion ratio (FCR), condition factor (CF), hepatosomatic index (HSI), and viscerosomatic index (VSI). Juvenile *T. ovatus* reared at 15‰ salinity for 56 days showed higher FBW and WGR than those reared at the other salinity levels; FBW and WGR were significantly higher at 25‰ salinity than in those reared at 5 and 35‰ salinity. FCR and HSI were significantly higher in juvenile pompanos reared at 5‰ salinity than in those reared at 15‰ salinity. No significant differences were observed in SR, CF, and VSI among juvenile pompano reared at different salinities. There were no significant differences in the SR, SGR, FCR, CF, HSI, and VSI between 25 and 35‰ salinity (Table 2). Based on final body weight, the optimal salinity of golden pompano was estimated to be 21.36‰ salinity (Fig. 1).

Table 1 Primers for real-time fluorescence quantification PCR

Primers	Sequence (5′ → 3′)	Size (bp)	Amplification target
GH-F	CAGCCAATCACAGACAGCC	262	Expression of reference genes
GH-R	GGAACTCCCAAGACTCCACTAA		
GHR1-F	GGTGGAGTTCATTGAGGTGGAT	111	Expression of reference genes
GHR1-R	TGGTGGCTGACAGGTTGG		
GHR2-F	CACCACCTCTACCTCCTCTG	93	Expression of reference genes
GHR2-R	CCCTCTTCGGCGTTCATA		
EF-1α-F	CCCCTTGGTCGTTTTGCC	170	Expression of reference genes
EF-1α-R	GCCTTGGTTGTCTTTCCGCTA		

Effects of salinity on the serum osmolality and ion concentration of *T. ovatus*

The serum osmolality and electrolyte contents significantly differed among the salinity groups. The serum osmolality increased with increasing salinity. Juveniles reared at 25 and 35‰ salinity showed significantly higher serum Na^+ concentrations than those maintained at 5 and 15‰ salinity. However, there were no significant differences in serum K^+ and Cl^- among all treatments ($P \geq 0.05$, Table 3). There was a significant difference in NKA activity between salinity groups at the same treatment time. The lowest NKA activity was observed when fish were cultured at 18.44‰ salinity (Fig. 2).

Effects of salinity on the serum biochemical indices in *T. ovatus*

The contents of triglyceride (TG), cholesterol (CHO), total protein (TP), albumin (ALB), alanine aminotransferase (ALT), aspartate aminotransferase (AST), glucose (GLU), lactate (LD), malondialdehyde (MDA), and lysozyme (LYZ) are provided in Table 4. The results showed that the salinity level had no significant effects on TG, TP, ALB, GLU, MDA, and LYM. However, TP, ALT, and AST were significantly highly increased in fish in the high salinity groups compared with fish at 5‰ salinity.

Table 2 Effects of salinity on growth of *Trachinotus ovatus*

	Salinity (‰)			
Parameter	5	15	25	35
IBW	12.07 ± 0.20^a	12.20 ± 0.22^a	12.00 ± 0.15^a	11.93 ± 0.20^a
FBW	54.33 ± 0.14^d	88.07 ± 0.19^a	78.53 ± 0.17^b	67.69 ± 0.64^c
SR	100.00 ± 0.00^a	100.00 ± 0.00^a	100.00 ± 0.00^a	100.00 ± 0.00^a
WGR	350.54 ± 8.80^d	620.15 ± 11.77^a	554.60 ± 7.07^b	467.64 ± 13.00^c
SGR	2.69 ± 0.065^b	3.52 ± 0.058^a	3.33 ± 0.22^{ab}	3.06 ± 0.27^{ab}
FCR	2.21 ± 0.05^a	1.54 ± 0.05^b	1.63 ± 0.076^b	1.82 ± 0.15^b
CF	3.73 ± 0.045^a	3.55 ± 0.068^a	3.66 ± 0.18^a	3.79 ± 0.09^a
HSI	1.26 ± 0.15^a	0.80 ± 0.049^b	1.07 ± 0.056^a	1.06 ± 0.056^a
VSI	5.36 ± 0.28^a	5.18 ± 0.10^a	4.89 ± 0.047^a	5.11 ± 0.23^a

Initial body weights (IBW, g), final body weights (FBW, g), survival rate (SR, %), weight gain rate (WGR, %), specific growth rate (SGR, %/day), feed conversion ratio (FCR, %), condition factor (CF, g/cm^3), hepatosomatic index (HSI, %), and viscerosomatic index (VSI, %) of *T. ovatus* reared at different salinities for 56 days. Data are expressed as mean ± SE ($N = 9$). Different letters in the same line indicate significant different mean values among salinity treatments ($P < 0.05$)

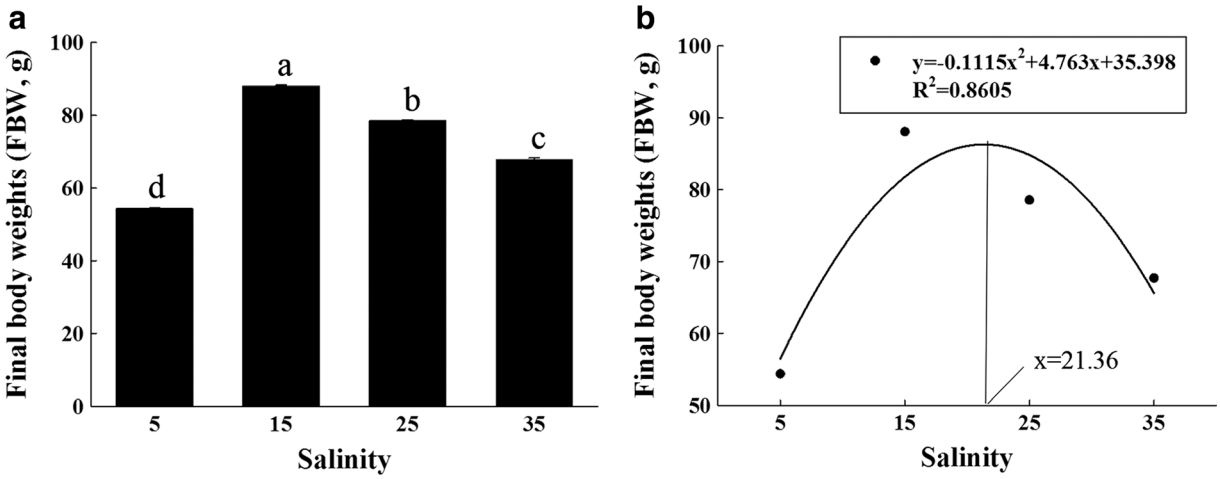

Fig. 1 Effects of the salinity level on the final body weights (FBW) of *Trachinotus ovatus*. Relation between final body weights and salinity. The columnar analysis (**a**) and the quadratic regression analysis (**b**) were analyzed with Tukey's test following one-way ANOVA ($P < 0.05$, $N = 9$)

Effects of salinity on carbohydrate metabolism in *T. ovatus*

The carbohydrate metabolism parameters of *T. ovatus* reared at the different salinity levels are displayed in Table 5. In general, the G6PDH concentrations were significantly lower in fish reared at 5‰ salinity compared with fish at 25‰ salinity. The PHK and AR concentrations were similar among all of the experimental groups.

Effects of salinity on the hormone levels in *T. ovatus*

The hormone levels of *T. ovatus* reared at the different salinities employed are shown in Table 6. The T3 in the four groups did not exhibit significant differences. However, the T4 level was significantly higher in fish in the high salinity groups. Nevertheless, a significantly higher COR was observed at 5‰ salinity compared to the other salinity groups.

Expressions of GH, GHR1, and GHR2 mRNA in the muscle, liver, intestine, brain, gills, and kidneys at different salinities

The expression levels of *GH* in the liver, intestine, kidneys, muscle, gills, and brain at 15‰ salinity were significantly higher than those of the other three groups, and the *GH* level at 25‰ salinity was higher than those at 5 and 35‰ salinity, except for brain tissue (Fig. 3a).

The expression levels of *GHR1* in the intestine, kidneys, muscle, and gills at 15 and 25‰ salinity were significantly higher than those at 5‰ salinity. However,

Table 3 Effects of salinity on the serum osmolality and ion concentration in *Trachinotus ovatus*

	Salinity (‰)			
Parameter	5	15	25	35
Serum osmolality	341.00 ± 6.50^{c}	371.00 ± 2.65^{b}	385.33 ± 4.91^{b}	420.67 ± 5.33^{a}
Serum K^+	3.35 ± 0.68^{a}	2.79 ± 0.29^{a}	4.69 ± 0.77^{a}	3.59 ± 0.42^{a}
Serum Na^+	140.90 ± 7.20^{b}	159.07 ± 1.20^{b}	184.77 ± 3.24^{a}	194.60 ± 11.38^{a}
Serum Cl^-	106.50 ± 4.50^{a}	120.70 ± 1.79^{a}	162.03 ± 10.13^{a}	173.47 ± 18.42^{a}

Serum osmolality (mOsm/kg H_2O) and serum ionic (Na^+, K^+, Cl^-) composition (in mmol/L) in *T. ovatus* acclimated to different salinities for 56 days. Data are expressed as mean ± SE ($N = 9$). Different letters in the same line indicate significant different mean values among salinity treatments ($P < 0.05$)

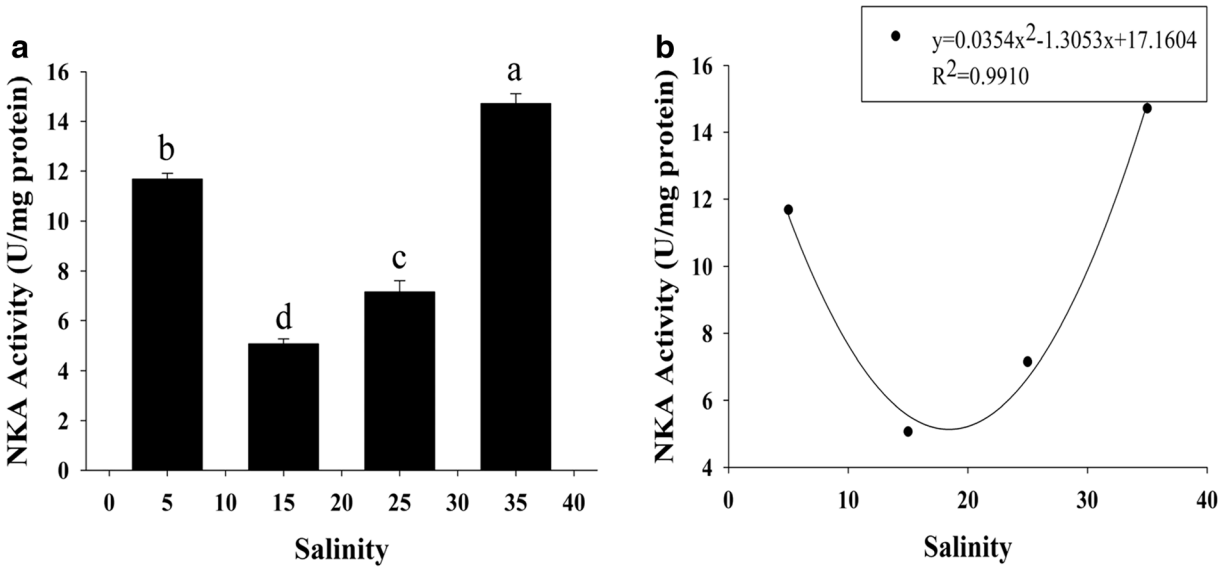

Fig. 2 Effects of the salinity level on the gill Na^+/K^+-ATPase (NKA) activities of *Trachinotus ovatus*. Relation between NKA and salinity. The columnar analysis (**a**) and the quadratic regression analysis (**b**) were analyzed with Tukey's test following one-way ANOVA ($P < 0.05$, $N = 9$)

the *GHR1* levels in the liver at 35 ‰ salinity were higher than those of the other three groups (Fig. 3b).

The expression levels of *GHR2* in the liver, intestine, kidneys, muscle, and brain at 5‰ were significantly lower than those in the other three salinity groups. However, the *GHR2* level in the gills was significant higher than those in the three groups (Fig. 3c).

Discussion

Salinity affects fish growth, and euryhaline fish have better survival or growth rates at suitable salinity (Boeuf and Payan 2001; Wu et al. 2017). For instance, the tolerance and the optimal salinity for growth are 5–35‰ salinity and 15‰ salinity for *Bostrychus sinensis*

Table 4 Effects of salinity on the serum biochemical indices in *Trachinotus ovatus*

	Salinity (‰)			
Parameter	5	15	25	35
TG (mmol/L)	1.91 ± 0.018^{a}	2.16 ± 0.14^{a}	1.88 ± 0.20^{a}	1.68 ± 0.091^{a}
CHO (mmol/L)	4.30 ± 0.40^{a}	3.77 ± 0.18^{ab}	2.71 ± 0.40^{c}	3.19 ± 0.18^{bc}
TP (g/L)	31.50 ± 3.42^{a}	34.95 ± 0.51^{a}	28.92 ± 3.78^{a}	30.54 ± 2.36^{a}
ALB (g/L)	8.98 ± 1.04^{a}	8.55 ± 0.17^{a}	6.63 ± 0.96^{a}	7.21 ± 0.46^{a}
ALT (U/L)	18.75 ± 5.05^{a}	13.90 ± 2.20^{ab}	10.30 ± 3.40^{ab}	7.03 ± 1.77^{b}
AST (U/L)	240.50 ± 1.17^{a}	172.19 ± 1.92^{b}	165.50 ± 0.37^{c}	85.09 ± 0.28^{d}
GLU (mmol/L)	9.86 ± 1.33^{a}	7.83 ± 0.23^{a}	11.59 ± 1.70^{a}	9.49 ± 0.82^{a}
LD (mmol/L)	3.03 ± 0.80^{a}	2.68 ± 0.43^{a}	1.98 ± 0.52^{a}	1.67 ± 0.21^{a}
MDA (nmol/ml)	5.40 ± 1.01^{a}	4.06 ± 0.89^{a}	4.78 ± 0.93^{a}	3.57 ± 0.21^{a}
LYZ (mg/L)	4.19 ± 1.75^{a}	2.55 ± 0.47^{a}	2.27 ± 0.41^{a}	2.00 ± 0.17^{a}

Triglyceride (TG, mmol/L), cholesterol (CHO, mmol/L), total serum protein (TP, g/L), albumin (ALB, g/L), alanine aminotransferase (ALT, U/L), aspartate aminotransferase (AST, U/L), glucose (GLU, mmol/L), lactate (LD, mmol/L), malondialdehyde (MDA, mmol/L), and lysozyme (LYZ, mg/L) in the *T. ovatus* acclimated to different salinities for 56 days. Data are expressed as mean ± SE ($N = 9$). Different letters in the same line indicate significant different mean values among salinity treatments ($P < 0.05$)

Table 5 Effects of salinity on carbohydrate metabolism in *Trachinotus ovatus*

	Salinity (‰)			
Parameter	5	15	25	35
PFK (U/L)	0.62 ± 0.10^{a}	0.93 ± 0.13^{a}	0.74 ± 0.23^{a}	0.62 ± 0.27^{a}
G6PDH (U/L)	1.16 ± 0.040^{a}	1.08 ± 0.029^{ab}	1.03 ± 0.027^{b}	1.06 ± 0.022^{ab}
AR (mU/L)	3.60 ± 0.92^{a}	1.98 ± 0.21^{a}	4.20 ± 0.94^{a}	2.83 ± 0.42^{a}

Phosphofructokinase (PHK, U/L), glucose-6-phosphate dehydrogenase (G6PDH, U/L), and aldose reductase (AR, mU/L) in the *T. ovatus* acclimated to different salinities for 56 days. Data are expressed as mean ± SE ($N = 9$). Different letters in the same line indicate significant different mean values among salinity treatments ($P < 0.05$)

(Zhang et al. 2017), the tolerance and the optimal salinity for growth are 5–35‰ salinity and 15‰ salinity for *Centropomus parallelus* (Tsuzuki et al. 2007), and the tolerance and optimum salinity for growth are 15–32‰ salinity and lower than 32‰ salinity for *H. hippoglossus* L. (Imsland et al. 2008). In the present study, we showed that juvenile *T. ovatus* have a survival rate of 100% in all of salinity treatments and that growth occurred in a wide range of salinities from 5 to 35‰ salinity. Previous studies showed that juveniles displayed a good survival rate from 10 to 34‰ salinity, but the highest survival rate was 94.28% (Ma et al. 2016a). This difference may be related to the size of the experimental fish and the rearing cycle. A "U-shaped" relationship between salinity and final weight was observed through the regressions performed, such that the optimal salinity for rearing golden pompano was 21.36‰ salinity. However, the growth of *T. ovatus* was inhibited, and no death occurred under low-salt conditions, indicating that *T. ovatus* can adapt to a low-salt environment. Studies have found survival at low salinity, but restrained growth in an euryhaline flounder (*Paralichthys orbignyanus* L.) in southern Brazil and in *Hippocampus reidi* (Sampaio and Bianchini 2002; Hora et al. 2016).

This study found hepatosomatic index (HSI) was affected by salinity. AST and ALT levels may increase in the serum when tissue damage and dysfunction occur (Canli and Canli 2015). The release of AST and ALT from cells into the blood could be used to infer the extent to which the body's cells and tissues were damaged (Guo et al. 2018). In humans, the increase in ALT and AST is often associated with the extent and severity of cellular damage, and ALT and AST levels provide further information about severity of liver disease (Lin et al. 2010). The present results showed that the AST and ALT activities of juveniles at 5‰ salinity were higher than those in the other salinity groups, which indicated that low salinity might cause hepatic injury. There was no death in juveniles during the experiment, indicating that salinity did not exceed the regulatory range of its physiological mechanism.

Glycogen metabolism is the principal energy source in both vertebrates and invertebrates, especially during environmental fluctuations (Chang et al. 2007). Studies have found that glycolytic metabolism leads to some adaptability after exposure to different salinities. Our results showed that related enzyme activities were significantly increased at lower and higher salinities,

Table 6 Effects of salinity on the hormone levels in *Trachinotus ovatus*

	Salinity (‰)			
Parameter	5	15	25	35
T3 (ng/mL)	2.43 ± 0.13^{a}	2.41 ± 0.29^{a}	1.82 ± 0.073^{a}	1.73 ± 0.22^{a}
T4 (ng/mL)	5.01 ± 0.14^{d}	6.95 ± 0.18^{c}	8.71 ± 0.25^{b}	10.06 ± 0.069^{a}
COR (ng/mL)	141.36 ± 2.50^{a}	37.46 ± 0.51^{d}	41.90 ± 0.30^{c}	79.52 ± 0.55^{b}

Triiodothyronine (T3, ng/mL), thyroxine (T4, ng/mL), and cortisol (COR, ng/mL) in the *T. ovatus* acclimated to different salinities for 56 days. Data are expressed as mean ± SE ($N = 9$). Different letters in the same line indicate significant different mean values among salinity treatments ($P < 0.05$)

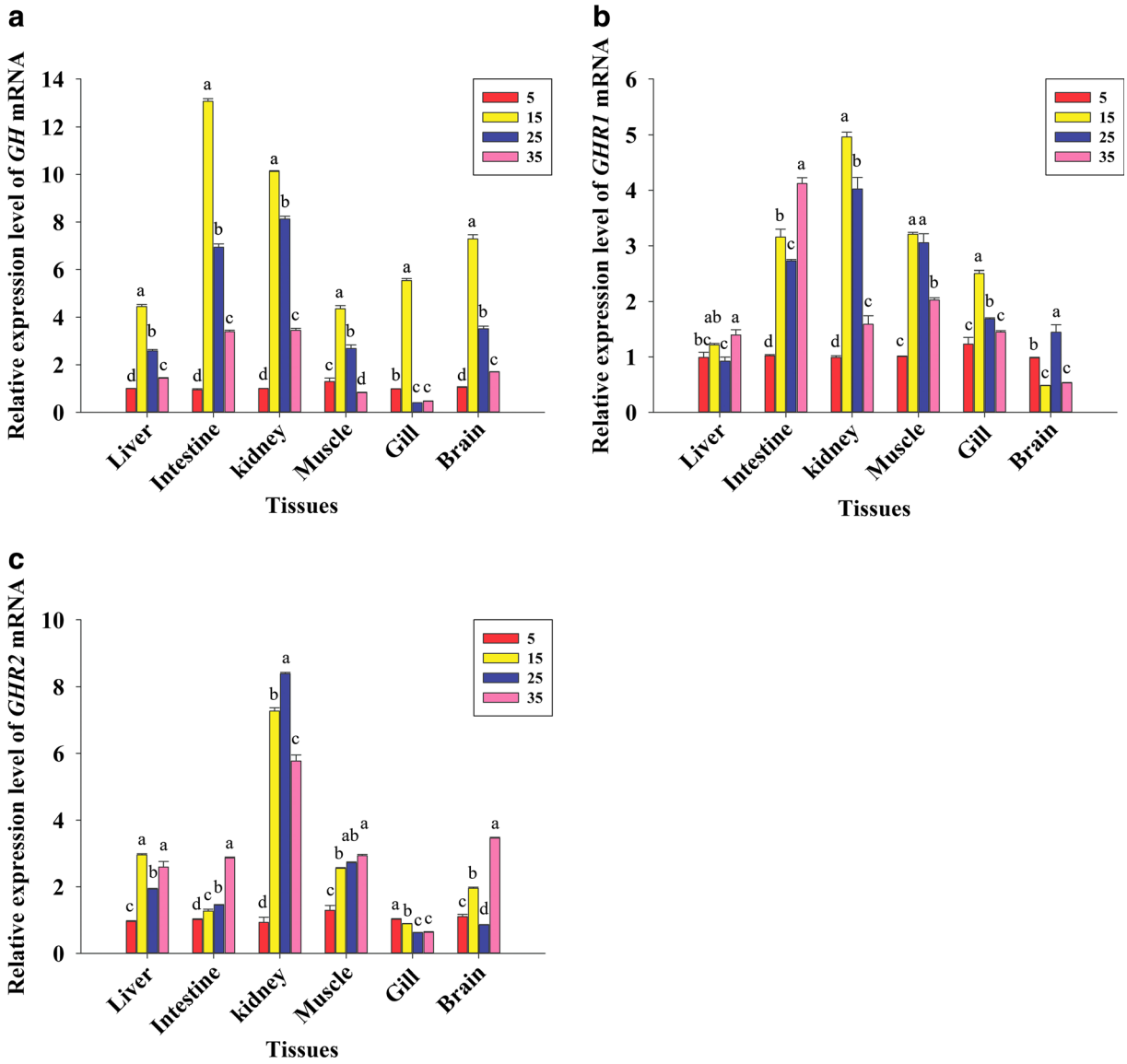

Fig. 3 Temporal expression of *GH* (**a**), *GHR1* (**b**), and *GHR2* (**c**) in the liver, intestine, kidneys, muscle, gills, and brain at the four different salinity levels after 56 days of rearing. *EF-1α* expression was used as an internal control for real-time PCR. Data are expressed as the mean ± SE ($N = 9$). Bars marked with different letters are significantly different from each other ($P < 0.05$)

indicating that the activity of glucose metabolism was enhanced by the increase of the osmotic regulation pressure. These results were in contrast with those in *Oreochromis mossambicus* (Nakano et al. 1998) and *O. niloticus* (Nakano et al. 1997). The glucose metabolism enzyme activities were improved during the poor growth of the salinity treatment group, indicating that the energy consumption increased in the salinity environment and the food intake did not significantly increase, which would inevitably lead to a decrease of growth. Therefore, we believe that salinity affects the growth of juveniles, in part, because the energy consumed by osmotic regulation increases, affecting the distribution of the energy intake, thereby affecting growth.

In the process of osmotic pressure regulation, the morphology and function of chlorine cells in the gills and changes of the NKA activity are regulated by the endocrine system, and hormones, such as COR and thyroxine, directly or indirectly participate in the morphology and function of chlorine cells (McCormick et al. 2009). COR not only participates in osmotic

regulation, but also acts as a stress indicator that participates in other important physiological functions, such as metabolism, growth, reproduction, and food intake. COR enhances the NKA activity, promotes chloride cell maturation and proliferation, and increases fish tolerance to salinity (McCormick et al. 2009). Our results showed that serum COR in juvenile fish of lower salinity was higher than in other salinity groups, but there was no significant correlation with NKA activity. A similar result was reported in killifish (Scott et al. 2006). COR reduced the concentration in gill chloride cells and eliminated the effect of ions in the body. They suggested that the osmotic pressure adjustment of COR could be regulated at low salinity. Therefore, the correlation between COR and NKA was uncertain, and its specific mechanism needs further study.

In teleosts, two different patterns of NKA activities are reported in response to changes in salinity: linear and "U-shaped" relationships. For the "U-shaped" relationship, the lowest activity of the gill enzyme occurs when the salinity of the medium is close to or slightly above that of the blood. The change of osmolality increases with the increase of salinity during the adaptation to salinity (Schmitz et al. 2017). Euryhaline teleost fishes are generally famous for their good ability to regulate and maintain their plasma ionic composition and osmotic concentration after changes in the salinity of ambient water (Divino et al. 2016; Yamaguchi et al. 2018). Our study showed that there were no significant differences between the K^+ and Cl^- concentrations in each salinity group, indicating that *T. ovatus* had strong ability of regulating osmotic pressure. When the water environment was similar or equal to the osmotic pressure, the lowest energy was required for fish osmotic pressure regulation, and the energy saved can be used for growth. At the same time, NKA played a very important role in the regulation of the ion concentration in the fish body (Hiroi and McCormick 2012). Although salinity could significantly affect NKA activity, NKA energy was not negatively correlated with the specific growth rate and feed conversion ratio, which was similar to the results in *O. mossambicus* (Chourasia et al. 2018). Therefore, more accurately reflect physiological changes under the attendant conditions of changing salinity.

In teleosts, GH has several functions including hydromineral balance. The roles of *GH* in osmoregulation have been studied in several teleosts (Sakamoto and McCormick 2006; Laiz-Carrión et al. 2009). In our study, we found that *GH* was expressed in a wide range of tissues, which was consistent with previous data from other teleosts, indicating the pleiotropic role of *GH* (Laiz-Carrión et al. 2009; Yada et al. 2012; Yuan et al. 2017). In our study, *T. ovatus* were better adapted to higher salinities than those reared at 5‰ salinity, showing higher *GH* mRNA levels at higher salinity conditions. Similarly, studies showed that the tilapia *Oreochromis mossambicus* had the highest levels of *GH* mRNA in seawater (Riley et al. 2002; Riley et al. 2003). By contrast, studies on *Sparus sarba* (Deane and Woo 2004) and *Mylio macrocephalus* (Deane and Woo 2005) adapted to higher salinity conditions showed higher *GH* mRNA levels under lower salinity conditions. This difference may be related to the fish species and experimental period. In our study, the low levels of *GH* mRNA observed may explain the better growth was observed. In addition to its somatotropic role, *GH* is also involved in salinity adaptation in fish (Borski et al. 1994; Sakamoto et al. 1997; Seale et al. 2002). Several studies have shown that the increase in *GH* expression in response to an increase in salinity is transitory (Seale et al. 2002; Ágústsson et al. 2003). In fact, *GH* mRNA seems to be only expressed in the early stages of acclimation to salinity. However, our analyses were performed on natural populations that have adapted to relatively stable salinity conditions.

GH synthesis is stimulated by growth hormone-releasing hormone (GHRH), which acts by interacting with *GHR*. Our expression results showed the highest levels of *GHR* expression in the liver, intestine, and kidneys; therefore, they were extremely suggestive. Because *GH* is involved in the regulation of energy metabolism, the upregulation found in *T. ovatus* could be due to a mobilization of energy stores that are required for osmoregulation. High mRNA expression levels of *GH*, *GHR1*, and *GHR2* were produced, which could be explained by the key role that *GH* plays in the hyperosmolar tolerance in fish (Reinecke 2010; Ababutain 2011; Reindl and Sheridan 2012). The activity of *GH* seems to be related to a branchial osmoregulatory function, including the activity and distribution of chloride cells (Olson 2002), and several ion transport mechanisms are associated with these cells, including the sodium pump enzyme (Deane and Woo 2009). The osmoregulatory function of *GHR* under a hyperosmotic environment had been confirmed in salmon (Kiilerich et al. 2007), flounder (Meier et al. 2009), and tilapia (Breves et al. 2010a). A study found that the increase of branchial *GHR* mRNA expression was associated with

the seawater adaptation mechanism in Nile tilapia (Breves et al. 2010b). We found that the *GHR1* and *GHR2* mRNA levels were decreased in these tissues in fish adapted to salinity compared to the *GH* mRNA level, and a significant negative correlation was found between the circulating GH level and *GHR* mRNA levels. Negative regulation of *GHR* by GH had been reported in the rat kidneys (Butler et al. 1996) and chicken liver (Mao et al. 1997). Taken together, these results indicate that *GH* and *GHRs* might play an essential role in osmoregulation in these fish species. However, the exact mechanism for the expression of *GH* and *GHRs* under salinity requires further study.

Conclusions

The present study reinforces the understanding that *T. ovatus* is tolerant to a wider range of salinities than previously reported (5–35‰ salinity) and grows better when reared at 15‰ and 25‰ salinity at low and high salinities for 56 days. Salinity had significant effects on the growth performance, NKA activity, serum osmolality, serum ion concentration, serum biochemical indices, carbohydrate metabolism, and hormones levels of *T. ovatus*. *GH*, *GHR1*, and *GHR2* were ubiquitously expressed in the different tissues of *T. ovatus*. The mRNA expression levels of these genes showed a sharp increase in liver, kidneys, and intestine after rearing at different salinities, indicating that they play a complementary role in promoting growth and regulating osmotic pressure in *T. ovatus* under different salinities. In summary, the upregulation of *GH*, *GHR1*, and *GHR2* transcripts of *T. ovatus* suggest that these genes are involved in osmotic regulation mechanisms, and further studies are needed to uncover the role of those genes in the osmoregulation and growth of *T. ovatus*.

Acknowledgements This work was supported by the Guangdong Provincial Science and Technology Project (2019B030316030), China Agriculture Research System (CARS-47), China-ASEAN Maritime Cooperation Fund, National Infrastructure of Fishery Germplasm Resources Project (2019DKA30407) and Guangdong Provincial Special Fund For Modern Agriculture Industry Technology Innovation Teams.

Funding information This study was supported by the China Agriculture Research System (CARS-47-G07), China-ASEAN maritime cooperation fund, Natural Science Foundation of Guangdong Province (2015A030313818), Sanya city scientific and technology cooperation of academic and regional (2015YD06), National Science & Technology Infrastructure platform (2018DKA30470), and Guangdong Provincial Special Fund For Modern Agriculture Industry Technology Innovation Teams.

Compliance with ethical standards All experiments in this study were approved by the Animal Care and Use Committee of South China Sea fisheries Research Institute, Chinese Academy of fishery Sciences (no. SCSFRI96-253) and performed according to the regulations and guidelines established by this committee.

References

Ababutain IM (2011) Antimicrobial activity of ethanolic extracts from some medicinal plant. Aust J Basic Appl Sci 5:678–683

Abou Anni IS, Bianchini A, Barcarolli IF, Varela Junior AS, Robaldo RB, Tesser MB, Sampaio LA (2016) Salinity influence on growth, osmoregulation and energy turnover in juvenile pompano *Trachinotus marginatus* Cuvier 1832. Aquaculture 455:63–72

Ágústsson T, Sundell K, Sakamoto T, Ando M, Björnsson BT (2003) Pituitary gene expression of somatolactin, prolactin, and growth hormone during Atlantic salmon parr-smolt transformation. Aquaculture 222:229–238

Ahmmed MK, Ahmmed F, Kabir KA, Faisal M, Ahmed SI, Ahsan MN (2017) Biochemical impacts of salinity on the catfish, *Heteropneustes fossilis* (Bloch, 1794), and possibility of their farming at low saline water. Aquac Res 48:4251–4261

Almeida DV, Martins CMG, Figueiredo MA, Lanes CFC, Bianchin A, Marins LF (2013) Growth hormone transgenesis affects osmoregulation and energy metabolism in zebrafish (*Danio rerio*). Transgenic Res 22:75–88

Amiri BM, Xu EG, Kupsco A, Giroux M, Hoseinzadeh M, Schlenk D (2018) The effect of chlorpyrifos on salinity acclimation of juvenile rainbow trout (*Oncorhynchus mykiss*). Aquat Toxicol 195:97–102

Arnason T, Magnadottr B, Bjornsson B, Steinarsson A, Bjornsson BT (2013) Effects of salinity and temperature on growth, plasma ions, cortisol and immune parameters of juvenile Atlantic cod (*Gadus morhua*). Aquaculture 380-383:70–79

Bertucci JI, Tovar MO, Blanc AM, Gómez-Requeni P, Unniappan S, Canosa LF (2017) Influence of water salinity on genes implicated in somatic growth, lipid metabolism and food intake in Pejerrey (*Odontesthes bonariensis*). Comp Biochem Physiol B Biochem Mol Biol 210:29–38

Boeuf G, Payan P (2001) Review: how should salinity influence fish growth? Comp Biochem Physiol C Toxicol Pharmacol 130:411–423

Borski RJ, Yoshikawa JSM, Madsen SS, Nishioka RS, Zabetian C, Bern HA, Grau EG (1994) Effects of environmental salinity on pituitary growth hormone content and cell activity in the euryhaline tilapia, *Oreochromis mossambicus*. Gen Comp Endocrinol 95:483–494

Breves JP, Fox BK, Pierce AL, Hirano T (2010a) Gene expression of growth hormone family and glucocorticoid receptors, osmosensors, and ion transporters in the gill during seawater acclimation of Mozambique tilapia, *Oreochromis mossambicus*. J Exp Zoology A 313,432–441

Breves JP, Hasegawa S, Yoshioka M, Fox BK, Davis LK, Lerner DT, Takei Y, Hirano T, Grau EG (2010b) Acute salinity challenges in Mozambique and Nile tilapia: differential responses of plasma prolactin, growth hormone and branchial expression of ion transporters. Gen Comp Endocrinol 167: 135–142

Butler AA, Funk B, Breier BH, LeRoith D, Roberts CT Jr, Gluckman PD (1996) Growth hormone (GH) status regulates GH receptor and GH binding protein mRNA in a tissue- and transcript-specific manner but has no effect on insulin-like growth factor-I receptor mRNA in the rat. Mol Cell Endocrinol 116:181–189

Canli EG, Canli M (2015) Low water conductivity increases the effects of copper on the serum parameters in fish (*Oreochromis niloticus*). Environ Toxicol Pharmacol 39: 606–613

Chang JCH, Wu SM, Tseng YC, Lee YC, Baba O, Hwang PP (2007) Regulation of glycogen metabolism in gills and liver of the euryhaline tilapia (*Oreochromis mossambicus*) during acclimation to seawater. J Exp Biol 210:3494–3504

Chang CH, Lo WY, Lee TH 2016. The antioxidant peroxiredoxin 6 (Prdx6) exhibits different profiles in the livers of seawater- and fresh water-acclimated milkfish, *Chanos chanos*, upon hypothermal challenge. Frontiers in Physiology, 29

Chourasia TK, D'Cotta H, Baroiller JF, Slosman T, Cnaani A (2018) Effects of the acclimation to high salinity on intestinal ion and peptide transporters in two tilapia species that differ in their salinity tolerance. Comp Biochem Physiol A Mol Integr Physiol 218:16–23

da Silva Aires M, Paganini CL, Bianchini A (2018) Biochemical and physiological effects of nickel in the euryhaline crab *Neohelice granulata* (Dana, 1851) acclimated to different salinities. Comparative Biochemistry and Physiology Part C, toxicology & Pharmacology 204:51–62

Deane EE, Woo NYS (2004) Differential gene expression associated with euryhalinity in sea bream (*Sparus sarba*). Am J Phys 287:1054–1063

Deane EE, Woo NYS (2005) Upregulation of the somatotropic axis is correlated with increased G6PDH expression in black sea bream adapted to isoosmotic salinity. Ann N Y Acad Sci 1040:293–296

Deane EE, Woo NYS (2009) Modulation of fish growth hormone levels by salinity, temperature, pollutants and aquaculture related stress: a review. Rev Fish Biol Fish 19:97–120

Divino JN, Monette MY, McCormick SD, Yancey PH, Flannery KG, Bell MA, Rollins JL, Hippel FA, Schultz ET (2016) Osmoregulatory physiology and rapid evolution of salinity tolerance in threespine stickleback recently introduced to fresh water. Evol Ecol Res 17:179–201

Downie AT, Kieffer JD (2016) The physiology of juvenile shortnose sturgeon (*Acipenser brevirostrum*) during an acute saltwater challenge. Can J Zool 94:677–683

Guo H, Ma Z, Jiang S, Zhang D, Zhang N, Li Y (2014) Length-weight relationship of oval pompano, *Trachinotus ovatus* (Linnaeus 1758) (Pisces; Carangidae) cultured in open sea floating sea cages in South China Sea. Indian Jouranl of Fisheries 61:93–95

Guo HZ, Gui JY, Li M, Liu HH, Zhang MZ, Meng FX, Shi G, Wang RX, He XY, Zhao YM (2018) Effect of feeding frequency on growth performance, antioxidant status, immune response and resistance to hypoxia stress challenge on juvenile dolly varden char *Salvelinus malma*. Aquaculture 486:197–201

Hajirezaee S, Mirvaghefi AR, Farahmand H, Agh N (2018) A metabolic approach to understanding adaptation to sea water by endangered Persian sturgeon, *Acipenser persicus* fingerlings. Aquac Res 49:341–351

Hiroi J, McCormick SD (2012) New insights into gill ionocyte and ion transporter function in euryhaline and diadromous fish. Respir Physiol Neurobiol 184:257–268

Hora MSC, Joyeux JC, Rodrigues RV, Sousa-Santos LP, Gomes LC, Tsuzuki MY (2016) Tolerance and growth of the longsnout seahorse *Hippocampus reidi* at different salinities. Aquaculture 463:1–6

Imanpoor MR, Najafi E, Kabir M (2012) Effects of different salinity and temperatures on the growth, survival, haematocrit and blood biochemistry of goldfish (*Carassius auratus*). Aquac Res 43:332–338

Imsland AK, Gustavsson A, Gunnarsson S, Foss A, Arnason J, Arnarson I, Jonsson AF, Smaradottir H, Thorarensen H (2008) Effects of reduced salinities on growth, feed conversion efficiency and blood physiology of juvenile Atlantic halibut (*Hippoglossus hippoglossus* L.). Aquaculture 274: 254–259

Kiilerich P, Kristiansen K, Madsen SS, (2007) Hormone receptors in gills of smolting Atlantic salmon, Salmo salar: Expression of growth hormone, prolactin, mineralocorticoid and glucocorticoid receptors and 11β-hydroxysteroid dehydrogenase type 2. Gen Comp Endocr, 152: 295–303

Laiz-Carrión R, Fuentes J, Redruello B, Guzmán JM, Martín del Río MP, Power D, Mancera JM (2009) Expression of pituitary prolactin, growth hormone and somatolactin is modified in response to different stressors (salinity, crowding and food-deprivation) in gilthead sea bream *Sparus auratus*. Gen Comp Endocrinol 162:293–300

Lalitha S (2000) Primer Premier 5. Biotech Software & Internet Report 1:270–272

Laverty G, Skadhauge E (2012) Adaptation of teleosts to very high salinity. Comp Biochem Physiol A Mol Integr Physiol 163: 1–6

Lee, SH., Lee, MC., Puthumana, J, Park, JC., Kang, S, Hwang, DS, Shin, KH, Park, HG, Souissi, S, Om, AS, Lee, JS, Han, J, (2017). Effects of salinity on growth, fatty acid synthesis, and expression of stress response genes in the cyclopoid copepod *Paracyclopina nana*. Aquaculture, 470: 182–189

Lin JD, Lin PY, Chen LM, Fang WH, Lin LP, Loh CH (2010) Serum glutamic- oxaloacetic transaminase (GOT) and glutamic-pyruvic transaminase (GPT) levels in children and adolescents with intellectual disabilities. Res Dev Disabil 31: 172–177

Lisboa V, Barcarolli IF, Sampaio LA, Bianchini A (2015) Effect of salinity on survival, growth and biochemical parameters in juvenile Lebranch mullet *Mugil liza* (Perciformes: Mugilidae). Neotropical Ichthyology 13:447–452

Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2 (−Delta Delta C(T)) method. Methods 25:402–408

Ma Z, Guo H, Zheng P, Wang L, Jiang S, Qin JG, Zhang D (2014) Ontogenetic development of digestive functionality in T. ovatus *Trachinotus ovatus* (Linnaeus 1758). Fish Physiol Biochem 40:1157–1167

Ma Z, Guo H, Zhang D, Hu C, Jiang S (2015) Food ingestion, consumption and selectivity of pompano, *Trachinotus ovatus* (Linnaeus 1758) under different rotifer densities. Aquac Res 46:2593–2603

Ma Z, Guo H, Zheng P, Wang L, Jiang S, Zhang D, Qin JG (2016a) Effect of salinity on the rearing performance of juvenile *Trachinotus ovatus* (Linnaeus 1758). Aquac Res 47:1761–1769

Ma Z, Zheng P, Guo H, Jiang S, Qin J, Zhang D, Liu X (2016b) Salinity regulates antioxidant enzyme and Na^+-K^+-ATPase activities of juvenile T. ovatus *Trachinotus ovatus* (Linnaeus 1758). Aquac Res 47:1481–1487

Mao JN, Cogburn LA, Burnside J (1997) Growth hormone down-regulates growth hormone receptor mRNA in chickens but developmental increases in growth hormone receptor mRNA occur independently of growth hormone action. Mol Cell Endocrinol 129:135–143

Mattioli CC, Takata R, Leme FOP, Costa DC, Filho RM, Silva WS, Luz RK, (2017) The effects of acute and chronic exposure to water salinity on juveniles of the carnivorous freshwater catfish *Lophiosilurus alexandri*. Aquaculture, 481: 255–266

McCormick SD, Regish M, Christensen K (2009) Distinct freshwater and seawater isoforms of Na^+/K^+-ATPase in gill chloride cells of Atlantic salmon. J Exp Biol 212:3994–4001

Meier KM, Figueiredo MA, Kamimura MT, Laurino J, Maggion R, Pinto LS, Dellagostin OA, Tesser MB, Sampaio LA, Marins LF, (2009) Increased growth hormone (GH), growth hormone receptor (GHR), and insulin-like growth factor I (IGF-I) gene transcription after hyperosmotic stress in the Brazilian flounder *Paralichthys orbignyanus*. Fish Physiol. Biochem. 35: 501–509

Montory JA, Cumillaf JP, Cubillos VM, Paschke K, Urbina MA, Gebauer P (2018) Early development of the ectoparasite *Caligus rogercresseyi* under combined salinity and temperature gradients. Aquaculture 486:68–74

Nakano K, Tagawa M, Takemura A, Hirano T (1997) Effects of ambient salinities on carbohydrate metabolism in two species of tilapia, *Oreochromis mossambicus* and *O. niloticus*. Fish Sci 63:338–343

Nakano K, Tagawa M, Takemura A, Hirano T (1998) Temporal changes in liver carbohydrate metabolism associated with seawater transfer in *Oreochromis mossambicus*. Comparative Biochemistry and Physiology. Part B, biochemistry & molecular biology. 119:721–728

Nakao N, Higashimoto Y, Ohkubo T, Yoshizato H, Nakai N, Nakashima K, Tanaka M (2004) Characterization of structure and expression of the growth hormone receptor gene of the Japanese flounder (*Paralichtys olivaceus*). J Endocrinol 182: 157–164

Nguyen PTH, Do HTT, Mather PB, Hurwood DA (2014) Experimental assessment of the effects of sublethal salinities on growth performance and stress in cultured tra catfish (*Pangasianodon hypophthalmus*). Fish Physiol Biochem 40:1839–1848

Oliveira-Ribeiro CA, Pelletier E, Pfeiffer WC, Rouleau C (2000) Comparative up-take, bioaccumulation, and gill damages of inorganic mercury in tropical and nordic freshwater fish. Environ Res 83:286–292

Olson KR (2002) Cell signaling and ion transport across the fish gill epithelium. J Exp Zool 293:336–347

Ozaki Y, Fukada H, Tanaka H, Kagawa H, Ohta H, Adachi S, Hara A, Yamauchi K (2006) Expression of growth hormone family and growth hormone receptor during early development in the Japanese eel (*Anguilla japonica*). Comparative Biochemistry and Physiology Part B, biochemistry & molecular biology 145:27–34

Partridge GJ, Jenkins GI (2002) The effect of salinity on growth and survival of juvenile black bream (*Acanthopagrus butcheri*). Aquaculture 210:219–230

Ran Z, Chen H, Ran Y, Yu S, Li S, Xu J, Liao K, Yu X, Zhong Y, Ye M, Yan X (2017) Fatty acid and sterol changes in razor clam *Sinonovacula constricta* (Lamarck 1818) reared at different salinities. Aquaculture 473:493–500

Ray AJ, Lotz JM (2017) Comparing salinities of 10, 20, and 30‰ in intensive, commercial-scale biofloc shrimp (*Litopenaeus vannamei*) production systems. Aquaculture 476:29–36

Reindl KM, Sheridan MA (2012) Peripheral regulation of the growth hormone-insulin-like growth factor system in fish and other vertebrates. Comparative Biochemistry and Physiology. Part A, molecular & integrative physiology. 163:231–245

Reinecke M (2010) Influences of the environment on the endocrine and paracrine fish growth hormone-insulin-like growth factor-I system. J Fish Biol 76:1233–1254

Rhee JS, Kim BM, Seo JS, Kim IC, Lee YM, Lee JS, (2012) Cloning of growth hormone, somatolactin, and their receptor mRNAs, their expression in organs, during development, and on salinity stress in the hermaphroditic fish, *Kryptolebias marmoratus*. Comp. Biochem. Physiol. A 161: 436–442

Riley LG, Richman NH III, Hirano T, Grau EG (2002) Activation of the growth hormone/insulin-like growth factor axis by treatment with 17a-methyltestosterone and seawater rearing in the tilapia, *Oreochromis mossambicus*. Gen Comp Endocrinol 127:285–292

Riley LG, Hirano T, Grau EG (2003) Effects of transfer from seawater to fresh water on the growth hormone/insulin-like growth factor-I axis and prolactin in the Tilapia, *Oreochromis mossambicus*. Comparative Biochemistry and Physiology Part B, biochemistry & Molecular Biology 136:647–655

Sakamoto T, McCormick SD (2006) Prolactin and growth hormone in fish osmoregulation. Gen Comp Endocrinol 147:24–30

Sakamoto T, Shepherd BS, Madsen SS, Nishioka RS, Siharath K, Richman NH, Bern HA, Grau EG (1997) Osmoregulatory actions of growth hormone and prolactin in an advanced teleost. Gen Comp Endocrinol 106:95–101

Sampaio LA, Bianchini A (2002) Salinity effects on osmoregulation and growth of the euryhaline flounder *Paralichtys orbignyanus*. J Exp Mar Biol Ecol 269:187–196

Schmitz M, Ziv T, Admon A, Baekelandt S, Mandiki SNM, L'Hoir M, Kestemon P (2017) Salinity stress, enhancing basal and induced immune responses in striped catfish *Pangasianodon hypophthalmus* (Sauvage). J Proteome 167: 12–24

Scott GR, Schultel PM, Wood CM (2006) Plasticity of osmoregulatory function in the killifish intestine: drinking rates, salt and water transport, and gene expression after freshwater transfer. J Exp Biol 209:4040–4050

Seale AP, Riley LG, Leedom TA, Kajimura S, Dores RM, Hirano T, Grau G (2002) Effects of environmental osmolality on

release of prolactin, growth hormone and ACTH from the tilapia pituitary. Gen Comp Endocrinol 128:91–101

Shui C, Shi Y, Hua X, Zhang Z, Zhang H, Lu G, Xie Y (2018) Serum osmolality and ions, and gill Na^+/K^+-ATPase of spottedtail goby *Synechogobius ommaturus* (R.) in response to acute salinity changes. Aquaculture and Fisheries 3:79–83

Silva Aires, M da, Paganini, CL, Bianchini, A, (2018). Biochemical and physiological effects of nickel in the euryhaline crab *Neohelice granulata* (Dana, 1851) acclimated to different salinities. Comp Biochem P hysiol C T oxicol P harmacol, 204: 51–62

Stewart HA, Noakes DLG, Cogliati KM, Peterson JT, Iversen MH, Schreck CB (2016) Salinity effects on plasma ion levels, cortisol, and osmolality in Chinook salmon following lethal sampling. Comp Biochem Physiol A Mol Integr Physiol 192:38–43

Sun L, Zhang D, Jiang S, Guo H, Zhu C (2013) Isolation and characterization of 21 polymorphic microstatellites in golden pompano *Trachinotus ovatus*. Conserv Genet Resour 5: 1107–1109

Tan X, Lin H, Huang Z, Zhou C, Wang A, Qi C, Zhao S (2016) Effects of dietary leucine on growth performance, feed utilization, non-specific immune responses and gut morphology of juvenile *Trachinotus ovatus*. Aquaculture 465:100–107

Taylor JF, Migaud H, Porter MJ, Bromage NR (2005) Photoperiod influences growth rate and plasma insulin-like growth factor-I levels in juvenile rainbow trout, O*ncorhynchus mykiss*. Gen Comp Endocrinol 142:169–185

Tomy S, Chang YM, Chen YH, Cao JC, Wang TP, Chang CF (2009) Salinity effects on the expression of osmoregulatory genes in the euryhaline black porgy *Acanthopagrus schlegeli*. Gen Comp Endocrinol 161:123–132

Tran-Ngoc KT, Schrama JW, Le MTT, Nguyen TH, Roem AJ, Verreth JAJ (2016) Salinity and diet composition affect digestibility and intestinal morphology in Nile tilapia (*Oreochromis niloticus*). Aquaculture 469:36–43

Tsui, WC, Chen, JC, Cheng, SY, (2012). The effects of a sudden salinity change on cortisol, glucose, lactate, and osmolality levels in grouper *Epinephelus malabaricus*. Fish P hysiology and B iochemistry, 38: 1323–1329

Tsuzuki MY, Sugai JK, Maciel JC, Francisco CJ, Cerqueira VR (2007) Survival, growth and digestive enzyme activity of juveniles of the fat Snook (*Centropomus parallelus*) reared at different salinities. Aquaculture 271:319–325

Weng CF, Lee TH, Hwang PP (1997) Immune localization of prolactin receptor in the mitochondria-rich cells of the euryhaline teleost (*Oreochromis mossambicus*) gill. FEBS Lett 405:91–94

Whitehead A, Roach JL, Zhang SY, Galvez F (2012) Salinity- and population-dependent genome regulatory response during osmotic acclimation in the killifish (*Fundulus heteroclitus*) gill. J Exp Biol 215:1293–1305

William KFT 2014. The role of osmotic stress transcription factor 1 in fishes. Front Zool, 11

Wu H, Liu J, Lu Z, Xu L, Ji C, Wang Q, Zhao J (2017) Metabolite and gene expression responses in juvenile flounder *Paralichthys olivaceus* exposed to reduced salinities. Fish & Shellfish Immunology 63:417–423

Yada T, McCormick SD, Hyodo S (2012) Effects of environmental salinity, biopsy, and GH and IGF-I administration on the expression of immune and osmoregulatory genes in the gills of Atlantic salmon (*Salmo salar*). Aquaculture 362-363:177–183

Yamaguchi Y, Breves JP, Haws MC, Lerner DT, Grau EG, Seal AP (2018) Acute salinity tolerance and the control of two prolactins and their receptors in the Nile tilapia (*Oreochromis niloticus*) and Mozambique tilapia (*O. mossambicus*): a comparative study. Gen Comp Endocrinol 257:168–176

Yin SJ, Zhang L, Zhang L, Wan J, Song W, Jiang X, Park YD, Si YX (2018) Metabolic responses and arginine kinase expression of juvenile cuttlefish (S*epia pharaonis*) under salinity stress. Int J Biol Macromol 113:881–888

Yuan M, Jia Q, Wang T, Lu Q, Tang L, Wang Y, Lu W (2017) Dynamic responses of prolactin, growth hormone and their receptors to hyposmotic acclimation in the olive flounder *Paralichthys olivaceus*. Gen Comp Endocrinol 254:8–13

Zacharia S, Kakati VS (2004) Optimal salinity and temperature for early developmental stages of *Penaeus merguiensis* De man. Aquaculture 232:373–382

Zhang YT, Huang S, Qiu HT, Li Z, Mao Y, Hong WS, Chen SX (2017) Optimal salinity for rearing Chinese black sleeper (*Bostrychus sinensis*) fry. Aquaculture 476:37–43

Zhang L, Feng Q, Sun L, Ding K, Huo D, Fang Y, Zhang T, Yan H (2018) Differential gene expression in the intestine of sea cucumber (*Apostichopus japonicus*) under low and high salinity conditions. Comparative Biochemistry and Physiology Part D, Genomics & Proteomics 25:34–41