Aquaculture 476 (2017) 37–43

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aquaculture

Optimal salinity for rearing Chinese black sleeper (*Bostrychus sinensis*) fry

Yu Ting Zhang, Sheng Huang, Heng Tong Qiu, Zhe Li, Yong Mao, Wan Shu Hong*, Shi Xi Chen*

State Key Laboratory of Marine Environmental Science, Xiamen University, Fujian 361005, P. R. China
College of Ocean and Earth Sciences, Xiamen University, Fujian 361005, P. R. China
Fujian Collaborative Innovation Center for Exploitation and Utilization of Marine Biological Resources, Fujian 361005, P. R. China

ARTICLE INFO

Keywords:
Bostrychus sinensis
Salinity
Growth
Insulin-like growth factor-1 receptor
Na^+-K^+-ATPase activity

ABSTRACT

The Chinese black sleeper (*Bostrychus sinensis*) is a commercially important fish in southeastern China, but the effects of salinity on the growth of *B. sinensis* fry remain unclear. In the present study, the three month-old *B. sinensis* fry were reared for 5 weeks at 5, 15, 25 and 35 parts per thousand (ppt) salinities to evaluate the effects of salinity on the survival rate, specific growth rate, condition factor, feed intake (FI), food conversion efficiency (FCE), plasma osmolality, plasma Cl^- concentration and the gill Na^+-K^+-ATPase (NAK) activities. In addition, since the insulin-like growth factor (IGF) system plays important roles in osmoregulation and promoting growth in teleosts, the complete *B. sinensis* insulin-like growth factor-1 receptor (*igf-1r*) gene was cloned and *igf-1r* mRNA levels in the muscle and gill were measured at the end of this experiment. Our results showed that the fry reared at lower salinities (5 and 15 ppt) grew significantly faster than those at higher salinities (25 and 35 ppt), and the fry reared at 15 ppt exhibited lower FI and higher FCE than those reared at 5 ppt. A typical 'U-shaped' pattern of gill NAK activity levels with the lowest one at 15 ppt was observed, suggesting that a lower energy expenditure on osmoregulation at this level of salinity. The length of the complete *igf-1r* cDNA sequence was 6864 bp, and it had a wide range of tissue expression including muscle and gill. After 5 weeks of rearing, muscle *igf-1r* mRNA levels were similar among the four salinity groups, while the gill *igf-1r* mRNA level at 5 ppt was significantly higher than that at 15 ppt. Taken together, the results from the present study indicated that the optimal salinity for rearing *B. sinensis* fry was 15 ppt, and that IGF-1 might serve as a hyperosmoregulatory hormone in long-term low salinity acclimation in *B. sinensis*.

1. Introduction

Growth in teleost fishes is directly under the control of many environmental factors, such as salinity, temperature and photoperiod (Boeuf and Payan, 2001; Zacharia and Kakati, 2004; Taylor et al., 2005). Salinity is one of the most important abiotic factors affecting the growth and survival of aquatic organisms, and many authors have demonstrated the influence of external salinity on growth capacities in fish (see the review by Boeuf and Payan, 2001). Thus, defining the optimal salinities for the culture of euryhaline fish that can survive large fluctuations in ambient salinity may be fundamental in developing a rearing protocol for these species.

As osmoregulation is an energy demanding process, isoosmotic salinities minimize osmoregulatory stress and osmoregulatory costs and increase the energy available for growth and/or survival (Imanpoor et al., 2012). Na^+-K^+-ATPase (NKA) is an ion translocating enzyme and is expressed at an extremely high level in salt transporting tissues such as gills. The lowest activity of the gill NAK occurs when the salinity of the medium is close to or slightly above that of the blood, and gill NKA activity is used as an indicator of osmoregulatory energetics (Gaumet et al., 1995; Morgan and Iwama, 1998; Imsland et al., 2003).

Many hormones, including insulin-like growth factor-1 (IGF-1), growth hormone (GH), thyroid hormones and prolactin are known to be involved in both osmoregulation, and growth control and regulation. IGF-I is produced mainly in the fish liver under the influence of GH released from the adenohypophysis and binding to GH receptors (Link et al., 2010). IGF-I promotes body growth among vertebrates, and many studies suggest that IGF-1 may be a useful growth index (Beckman, 2011). Moreover, in many teleost species the GH/IGF-I axis is also involved in osmoregulation (Wood et al., 2005; Yada et al., 2012). For example, transfer of some euryhaline fishes from freshwater to seawater increases circulating IGF-I (Link et al., 2010). The biological effects of the IGF system are mediated mainly by the interaction of the IGF-1 ligand with the IGF-1 receptor (*igf-1r*) modulated through IGF binding proteins (Escobar et al., 2011). Once the *igf-1r* is activated by the binding of IGF-1, it triggers a signaling transduction cascade to the cell

* Corresponding authors at.
E-mail addresses: wshong@xmu.edu.cn (W.S. Hong), chenshixi@xmu.edu.cn (S.X. Chen).

http://dx.doi.org/10.1016/j.aquaculture.2017.04.004
Received 19 December 2016; Received in revised form 17 March 2017; Accepted 1 April 2017
Available online 04 April 2017

nucleus thus modulating some cellular functions through the regulation of transcription factors (Valenciano et al., 2012).

The Chinese black sleeper (*Bostrychus sinensis*) is a euryhaline fish, which can be found occasionally in freshwater (Kottelat et al., 1993; Peh et al., 2009), but it usually inhabits marine ecosystems such as coral reefs (Nguyen and Nguyen, 2006). Previous research establishes that adult *B. sinensis* has high tolerance of exposure to air (Ip et al., 2001) or environmental ammonia (Anderson et al., 2002), and can tolerate a progressive increase in salinity from 5 parts per thousand (ppt) water to 30 ppt (Peh et al., 2009). However, there is a dearth of knowledge on the osmoregulatory capacity of *B. sinensis* fry. As a commercially important fish in southeastern China, the rearing of *B. sinensis* fry is a critical step for its farming. The optimum stocking density, shelter and diet for rearing *B. sinensis* fry is established (Zhang et al., 2015), but the effects of salinity on the growth of *B. sinensis* fry remain unclear. Hence, the present study aimed to understand the optimal salinity for rearing *B. sinensis* fry.

2. Materials and methods

2.1. Fish, experimental design and rearing conditions

The experiment was first conducted between July and August, 2013 in Dongshan, Fujian Province, China. Three month-old *B. sinensis* fry used in this study were obtained from hatchery. The average initial weight and length of fry were 0.377 ± 0.051 g and 3.263 ± 0.15 cm. Before the experiment, the fry were reared in an earth pond under dissolved oxygen conditions of 5.84 mg L^{-1} ± 0.184, a temperature of 29.4 °C ± 0.703 and a salinity of 12.5 ppt ± 0.750. 120 healthy fry were divided into four treatment groups of different salinities (5, 15, 25 and 35 ppt, with no replicates). Prior to the experiments, the fry of each group were acclimatized to their own rearing salinities for five days. Different salinities were attained by exposing individuals to progressively increasing or decreasing salinity (5 ppt per day) until all treatments reached the target salinity. Salinities were realized by dilution of full-strength seawater with dechlorinated fresh water. Each group (30 individuals) was reared in a 0.65 ∗ 0.45 ∗ 0.40 m^3 (140 L) plastic tank with 120 L of filtrated seawater and PVC pipes (56.5 cm in length, 10.7 cm in diameter) were used as the shelters. The values of dissolved oxygen, temperature, total dissolved solids and electrical conductivity were 5.37 mg L^{-1} ± 0.340, 28.29 °C ± 0.518, 9.6 mg L^{-1} ± 0.5 and 4.84 S m^{-1} ± 0.5 for the 5 ppt group; 5.27 mg L^{-1} ± 0.256, 28.29 °C ± 0.457, 24.7 mg L^{-1} ± 0.5 and 12.84 S m^{-1} ± 0.5 for the 15 ppt group; 5.22 mg L^{-1} ± 0.279, 28.34 °C ± 0.472, 38.71 mg L^{-1} ± 0.5 and 19.84 S m^{-1} ± 0.5 for the 25 ppt group; and 5.33 mg L^{-1} ± 0.281, 28.41 °C ± 0.495, 51.9 mg L^{-1} ± 0.5 and 26.84 S m^{-1} ± 0.5 for the 35 ppt group. The fry were fed with dry commercially formulated pellet diets (1.0 mm in diameter each) twice a day at 0830 and 1800 h as previously described (Zhang et al., 2015). The daily feed ration was 12% of the total fish weight. Two hours after feeding the number of residual pellet diets was counted to calculate the feed intake. Then the feces and residual feed were removed using a syphon. The water refreshment rate in each tank was 60 L per day using a syphon to keep nitrogenous waste products at acceptable low levels.

The same experiment was repeated (with two replicates) between July and August, 2016 in the same location as the first experiment. The average initial weight and length of fry were 0.374 ± 0.074 g and 3.279 ± 0.32 cm. The rearing conditions and procedures were similar to those of the first experiment.

2.2. Measurements of growth

The weights and lengths of the *B. sinensis* fry (n = 15) in each group were measured every week. The fry were not fed the day before sampling. At the end of the experiment, the survival rate (SR), condition factor (CF), specific growth rate (SGR), feed intake (FI) and food conversion efficiency (FCE) of each group were calculated.

The CF was calculated as: $CF = (W/L^3) \times 100$ where W is weight and L is length.

The SGR was calculated following the formula (Gunnarsson et al., 2012; Houde and Schekter, 1981): $SGR = (e^g - 1) \times 100$ where $g = (\ln(W2) - \ln(W1)) \times (t2 - t1)^{-1}$ and W2 and W1 are the weights on days t2 and t1.

FI was calculated for the fish in each tank on a weekly basis, and then expressed as consumption per day on a weight per weight basis (% body weight/day).

Food conversion efficiency (FCE) was calculated as the increase in biomass in relation to total food consumption.

2.3. Cloning of igf-1r

The total RNA of gill tissue was extracted using the RNAzol reagent (Molecular Research Center Inc. (MRC), Cincinnati, OH, USA) and reverse transcribed into first strand cDNA using the SMART RACE cDNA Amplification Kit (Clontech) following the manufacturer's instructions. The partial sequence of *igf-1r* was obtained from brain transcriptomic data. In order to confirm the sequence, two primers were designed (Supplemental Table 1). Then the PCR amplification was carried out in 20 μL volume under the following cycling conditions: 94 °C for 3 min (1 cycle); 94 °C for 30 s, 56 °C for 30 s and 72 °C for 1 min (35 cycles) followed by a final DNA extension at 72 °C for 10 min using recombinant Taq™ DNA polymerase (TaKaRa). Then all PCR products were purified from agarose gel and sub-cloned into vector pmd19-t (TAKARA, Japan), and then transformed into Escherichia coli DH5α (Promega, Madison, WI, USA). The plasmid DNA of several positive clones was prepared for DNA sequencing (Invitrogen Ltd, Guangzhou, China). Based on the confirmed partial cDNA sequence obtained, gene-specific primers were designed for further extension using 5′- and 3′-RACE (Supplemental Table 1). The first PCR amplification for 5′ or 3′ RACE was performed using a universal primer in the kit and a gene specific primer. If no specific band was obtained, these initial 5′ or 3′ RACE products were diluted and used for nested PCR amplification with gene-specific nested primers, in combination with a nested universal primer. All RACE reactions were carried out following the manufacturer's instructions. RACE products were sub-cloned and sequenced as described above.

2.4. Phylogenetic analysis

After obtaining the cDNA for the *B. sinensis igf-1r*, a BLAST homology search was performed using deduced amino acid sequences. The alignment of known *igf-1r* was performed using the MEGA 6.0 program and the Clustal W method. Then a phylogenetic tree was constructed using the neighbor-joining method with a bootstrap value of 1000 trials for each position.

2.5. Tissue distribution of igf-1r

Adult fish were anesthetized in 0.01% MS222 (Sigma–Aldrich) buffered solution with an equal amount of sodium bicarbonate, and then decapitated humanely. The olfactory bulb, telencephalon, mesencephalon, hypothalamus, cerebellum, medulla oblongata, pituitary, olfactory sac, spleen, gill, liver, intestine, heart, muscle, skin, kidney, testis and ovary were collected and immediately dipped into liquid nitrogen and stored at -80°C. Total RNA extraction and cDNA synthesis were conducted as described in Section 2.3.

The PCR amplification was performed in 20 μL volumes using recombinant Taq™ DNA polymerase (TaKaRa). The gene-specific primers that were used to amplify a fragment of *igf-1r* and *β-actin* are listed in Supplemental Table 1. Thermal cycling conditions for *igf-1r* and *β-actin* were as follows: initial activation of 10 min at 95 °C, followed by

Table 1
Effects of the four different salinities (5, 15, 25 and 35 ppt) on the growth performance of *B. sinensis* fry.

Date	Salinity(ppt)	SR(%)	CF(%)	SGR(%)	FI(%)	FCE
2013/7/5–2013/8/8(5 weeks)	5	100.0	1.33 ± 0.07^{a}	6.94 ± 0.35^{b}	8.35 ± 0.89^{b}	0.54 ± 0.06^{a}
	15	96.7	1.34 ± 0.13^{a}	6.98 ± 0.51^{b}	6.81 ± 1.09^{a}	0.68 ± 0.05^{b}
	25	96.7	1.33 ± 0.16^{a}	6.06 ± 0.54^{a}	7.58 ± 1.27^{a}	0.57 ± 0.18^{ab}
	35	93.3	1.27 ± 0.07^{a}	6.04 ± 0.60^{a}	7.04 ± 1.38^{a}	0.61 ± 0.16^{ab}
2016/7/1–2016/8/4(5 weeks)	5	86.7	1.36 ± 0.14^{a}	6.94 ± 0.64^{b}	8.00 ± 0.34^{c}	0.59 ± 0.02^{a}
	15	93.3	1.43 ± 0.15^{a}	6.94 ± 0.65^{b}	7.10 ± 0.11^{a}	0.66 ± 0.01^{b}
	25	93.3	1.48 ± 0.18^{a}	6.62 ± 0.46^{ab}	7.68 ± 0.85^{bc}	0.59 ± 0.06^{a}
	35	93.3	1.37 ± 0.22^{a}	6.04 ± 1.14^{a}	7.32 ± 0.40^{ab}	0.58 ± 0.03^{a}

SR, survival rate; CF, condition factor; SGR, specific growth rate; FI, feed intake; FCE, food conservation efficiency. Data are expressed as the mean ± SD. Values sharing a common superscript letter are not significantly different ($p < 0.05$).

30 (*igf-1r*) or 28 (*β-actin*) cycles of 30 s at 95 °C; 30 s at 58 (*igf-1r*) or 60 °C (*β-actin*); 30 s at 72 °C, and a final extension of 10 min at 72 °C. The amplified products (10 μL) were resolved in a 1.5% agarose gel, and the DNA was visualized using ethidium bromide, with a u.v. transilluminator, and then photographed.

2.6. Expression of igf-1r transcripts in the muscle and gill using real-time qPCR

At the end of the experiment, five fry from each group were anesthetized and humanely decapitated. The muscle and gill were collected and immediately dipped into liquid nitrogen and stored at − 80 °C. Total RNA extraction and cDNA synthesis were conducted as described in Section 2.3. Then the real-time qPCR was performed. Specific primers for detecting *igf-1r* (Supplemental Table 1) were designed and examined for their specificity and amplification efficiency on serial dilutions of respective target gene plasmid DNA ($10^3 – 10^8$ copies/μL). All qPCR was performed using premix (Fermentace). Ct values were determined in a 7500 fast Real-Time PCR System (Applied Biosystems, USA) using default settings and baseline, and thresholds were adjusted manually where necessary. The relative mRNA levels of *igf-1r* were determined using the comparative Ct method (Schmittgen and Livak, 2008) with the *β-actin* gene as the internal control. The specificity and efficiency of *β-actin* specific primers are described in a previous study (Lai et al., 2014) (Supplemental Table 1).

2.7. Measurements of gill NAK activity

Gill tissues of the fry in the four groups were obtained at the end of the experiment. Gill tissue was homogenized using a Polytron-type homogenizer and supplemented with 0.70% NaCl solution. Thereafter, the mixture was centrifuged at 2,500 rpm for 10 mins and the supernatant was collected for determination. NAK activity was determined by measuring the release of inorganic phosphate (Pi) from ATP with malachite green dye method according to the kit protocol (Nanjing Jiancheng Institute of Biological Engineering, China) (Zhao et al., 2013). The kit was confirmed to be available to measure gill NKA activity in *B. sinensis* fry (Supplemental Fig. 1).

2.8. Measurements of plasma osmolality and Cl^- concentration

Blood samples were taken from the caudal vein with a heparinized syringe and transferred into test tubes on ice. Plasma was separated by centrifugation, collected in microfuge tubes and stored at − 20 °C until analysis. Plasma osmolality was determined using a freezing point osmotic pressure instrument (LOSER-OM806 M, Löser Messtechnik, Berlin, Germany). The Cl^- concentration of the plasma was measured as described previously (Zhang et al., 2014).

2.9. Statistical analysis

All data were analyzed using one-way ANOVA followed by Fisher's PLSD post hoc test to assess statistical differences among the individual groups. The statistical analyses were run using the SPSS (version 21.0) statistical software package.

3. Results

3.1. Growth of B. sinensis fry

The SRs of the fry in the four groups were all above 86.7%, while 100% mortality occurred in freshwater after only 3 days of rearing. The CFs in the four groups did not exhibit significant differences. The fry at 5 and 15 ppt showed higher SGRs than those at 25 and 35 ppt, indicating that both 5 and 15 ppt were suitable salinities for the growth of *B. sinensis* fry. Nevertheless, a significantly higher FI and lower FCE were observed at 5 ppt, compared to 15 ppt (Table 1). Therefore 15 ppt was taken as the optimal salinity for rearing *B. sinensis* fry, when the feeding cost is taken into consideration.

3.2. Gill NAK activity, plasma osmolality and plasma Cl^- concentration

A typical 'U-shaped' pattern of gill NAK activities is shown in Fig. 1A. The lowest NAK activity (0.11 μmol Pi h^{-1} mg^{-1} protein) was found in the 15 ppt group, which was significantly lower than in 5 and 35 ppt (0.29 and 0.34 μmol pi h^{-1} mg^{-1} protein). There were no significant differences between the 25 ppt (0.23 μmol pi h^{-1} mg^{-1} protein) and the other groups. Plasma osmolality in four groups remained in the range 326–339 mosmol kg^{-1} with no significant differences (Fig. 1B). Plasma Cl^- concentrations significantly decreased as the ambient salinity increased from 5 to 35 ppt (Fig. 1C).

3.3. Cloning and phylogenetic analyses of B. sinensis igf-1r

The complete *B. sinensis igf-1r* sequence was obtained (GenBank accession no. KT961623). The length of the complete *igf-1r* cDNA sequence was 6864 bp, which included a 794 bp 5′-untranslated region (UTR), an open reading frame (ORF) of 4275 bp and a 1795 bp 3′-UTR. The ORF encoded a putative protein of 1424 amino acid residues (Supplemental Fig. 2). Sequence analysis revealed that the *B. sinensis igf-1r* cDNA was organized into several major domains including a signal peptide sequence of 30 amino acids, an extracellular alpha subunit of 708 amino acids and an intracellular beta subunit of 682 amino acids (Fig. 2). Moreover, a tyrosine kinase domain of 266 amino acids, a cysteine domain of 154 amino acids and a transmembrane domain of 23 amino acids were found using a Conserved Domain Search (CD-search) and the TMHMM program (Supplemental Fig. 2). The phylogenetic tree showed that *B. sinensis igf-1r* was classified into the fish clade and belonged to the Igf-1rb (Fig. 3).

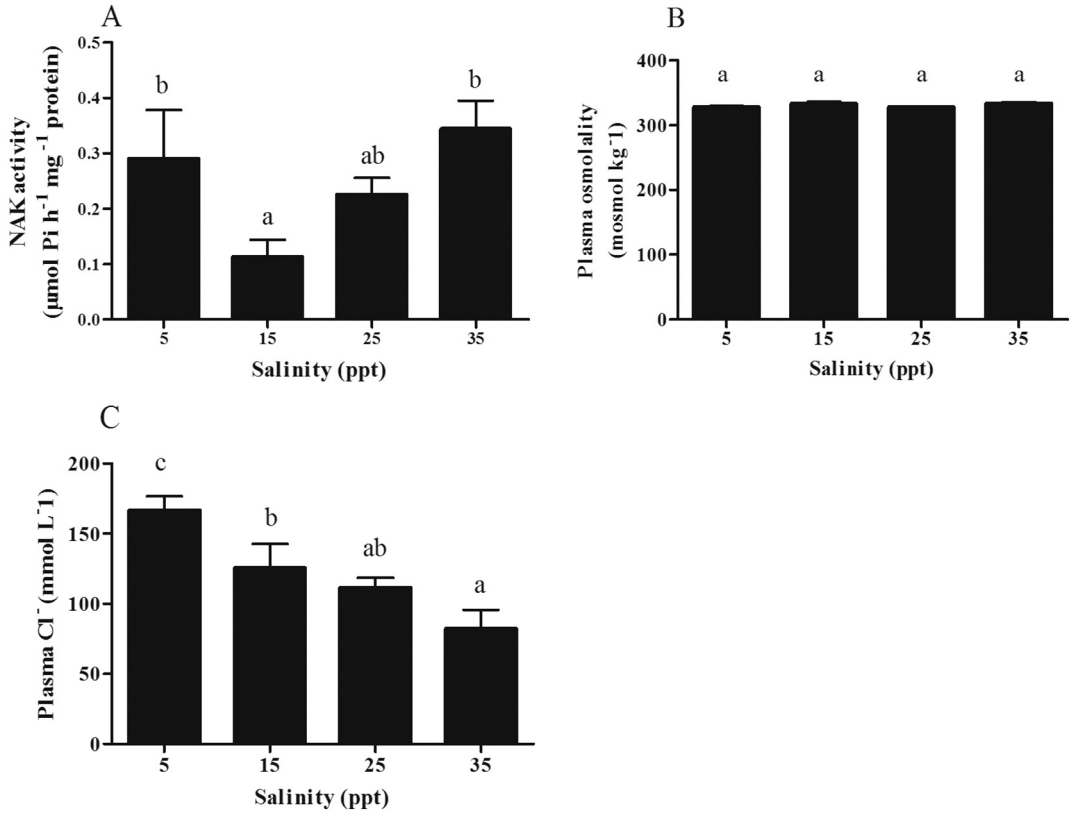

Figure 1. Gill Na^+-K^+-ATPase (NAK) activities (A), plasma osmolality (B) and plasma Cl^- concentration (C) of *B. sinensis* fry in the four different salinity groups (5, 15, 25 and 35 ppt). Data are expressed as the mean ± SEM (n ≥ 3). Bars marked with different letters are significantly different from each other ($p < 0.05$).

3.4. Tissue distribution of igf-1r mRNA

Expressions of *igf-1r* were found in all the tissues examined except mesencephalon, and some tissues including hypothalamus, cerebellum, testis and ovary expressed high levels of *igf-1r* mRNA. Importantly, both the muscle and gill expressed *igf-1r* mRNA, even though the expression was relatively low (Fig. 4).

3.5. Expressions of igf-1r mRNA in the muscle and gill at different salinities

No significant differences of *igf-1r* levels in the muscle were found in the four groups (Fig. 5A). However, the expression level of *igf-1r* in the gill at 5 ppt was the highest among the four groups and significantly higher than that at 15 ppt (Fig. 5B). Even though the gill *igf-1r* levels at higher salinities (25 and 35 ppt) were lower than at 5 ppt, the differences between them were not significant (Fig. 5B).

4. Discussion

Many authors have reported on the influence of water salinity on fish growth and it appears that most marine fish present higher developmental or growth rates at lower salinity (see the review by Boeuf and Payan, 2001). For instance, the tolerance and the optimal salinity for growth are 0–35 and 15 ppt for *Sparus sarba* (Woo and Kelly, 1995), 0–35 and 12 ppt for *Salaria fluviatilis* (Plaut, 1999) and 0–20 and 5 ppt for *Micropogonias undulates* (Peterson et al., 1999). Previous research establishes that adult *B. sinensis* can tolerate a progressive increase in salinity from 5 to 30 ppt, and it is a hyperosmotic regulator at 5 ppt and hypoosmotic hypoionic regulator in seawater (Peh et al., 2009). Similarly, the results from the present study indicated that *B. sinensis* fry could tolerate a wide range of salinity from 5 to 35 ppt, but did not survive in freshwater. The highest SGRs were observed at 5 and 15 ppt, while significantly lower SGRs were obtained in the higher salinity groups. In addition, the fry at 15 ppt showed significantly less FI and higher FCE than those at 5 ppt, although the SGRs in both groups were similar. Hence, to obtain higher growth rates with low feeding cost, it is suggested to rear *B. sinensis* fry at a salinity of 15 ppt.

In teleosts, two different patterns of NAK activities are reported in response to changes in salinity: a linear and a 'U-shaped' relationship (Jensen et al., 1998; McCormick, 2001; Laiz-Carrion et al., 2005; Vargas-Chacoff et al., 2011). In terms of the 'U-shaped' relationship, lowest activity of the gill enzyme occurs when the salinity of the medium is close to or slightly above that of the blood (Imsland et al., 2003). For instance, gill NAK activities of the euryhaline killifish *Fundulus heteroclitus* and the pupfish *Cyprinodon salinus* are lower at near isosmotic salinity conditions (15–16 ppt) compared to either

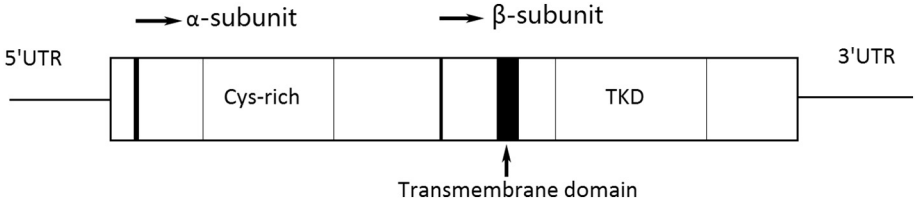

Figure 2. Schematic diagram of *B. sinensis igf-1r*. The α-subunit contains a conserved cysteine-rich (Cys-rich) region; the β-subunit contains a tyrosine-kinase domain (TKD) and a transmembrane domain.

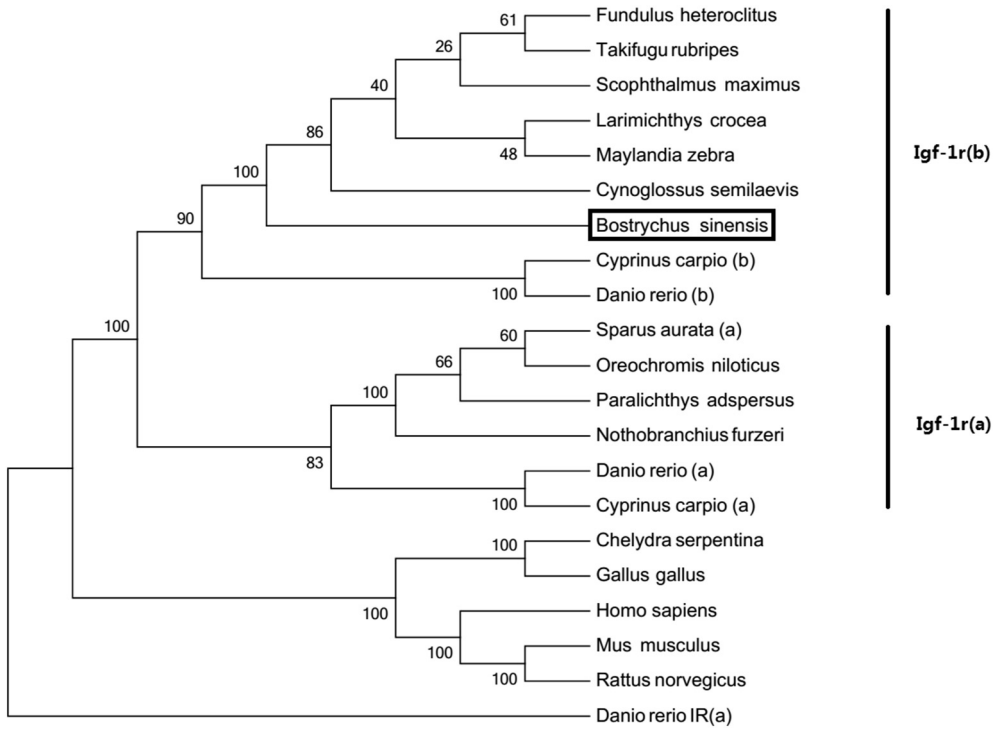

Figure 3. Phylogenetic analysis of *B. sinensis igf-1r*. Multiple species' amino acid sequences of *igf-1r* were aligned using Clustal W. *Danio rerio* insulin receptor a (IRa) is used as an outgroup for the tree. The GenBank accession numbers for sequence data analyzed are: *Chelydra serpentine*, AGJ91144.1; *Cynoglossus semilaevis*, XP_008309033.1; *Cyprinus carpio* (a), AAN52151.1; *Cyprinus carpio* (b), AAN52152.1; *Danio rerio* (a), AAI63723.1; *Danio rerio* (b), NP_694501.1; *Fundulus heteroclitus*, XP_012724167.1; *Gallus gallus*, AGG38008.1; Homo sapiens, AAI13611.1; *Larimichthys crocea*, KKF30174.1; *Maylandia zebra*, XP_004539804.1; *Mus musculus*, NP_034643.2; *Nothobranchius furzeri*, ADV72543.1; *Oreochromis niloticus*, XP_003440646.2; *Paralichthys adspersus*, ACJ66864.1; *Rattus norvegicus*, NP_434694.1; *Scophthalmus maximus*, CAA12278.1; *Sparus aurata* (a), AJE25612.1; *Takifugu rubripes*, XP_003969481.1; *Danio rerio* IRa, NP_001136144.1.

freshwater or seawater (Stuenkel and Hillyard, 1981; Morgan and Iwama, 1998). In the present study, the pattern of gill NAK showed a typically 'U-shaped' relationship, and the lowest activity was observed at 15 ppt, probably because the osmolality of seawater at 15 ppt (approximately 440 mosmol kg^{-1}) is close to fry plasma osmolality (311–342 mosmol kg^{-1}). Many studies have shown that gill NAK activity relates directly to Cl^- fluxes across gills and how a decrease in NAK activity at reduced salinities is accompanied by a decrease in plasma Cl^- (Woo and Kelly, 1995; Foss et al., 2001; Fielder et al., 2007; Partridge and Lymbery, 2008; Blanco Garcia et al., 2015). However, in juvenile yellowtail kingfish (*Seriola lalandi*), higher Cl^- concentration and lower gill NAK activity were observed at low salinities (Blanco Garcia et al., 2015). Similarly, no positive correlation between gill NAK activity and plasma Cl^- concentration was found in the present study, where plasma Cl^- concentration decreased at higher salinities. Interestingly, in adult *B. sinensis*, plasma Cl^- concentration remains unchanged after long-term acclimation to seawater (Peh et al., 2009). Thus, future studies on the branchial permeabilities and chloride transporter proteins in both adult and juvenile *B. sinensis* should be undertaken.

It is widely accepted that rearing fish in salinities near their isoosmotic point has an energy-saving effect, since salinities that differ from the internal osmotic concentration of the fish must impose energy regulatory costs for active ion transport (Kuhlmann and Quantz, 1980; Febry and Lutz, 1987; Gaumet et al., 1995; Imsland et al., 2003). In the present study, the SGR of *B. sinensis* fry at 15 ppt was higher than those at 25 and 35 ppt, suggesting a lower energy expenditure on osmoregulation at isosmotic salinity. However, the SGR of the fry at 5 ppt was close to that at 15 ppt, although a significantly higher NAK activity was observed in 5 ppt. We supposed that this may be due to higher FI of the fry at 5 ppt, which resulted in more overall energy input. Similarly, the growth rate of the turbot (*Scophthalmus maximus*) is better at 10–19 ppt, compared to fish in full strength sea water, and the turbot eat more at lower salinity (Gaumet et al., 1995). In contrast, in the cod (*Gadus*

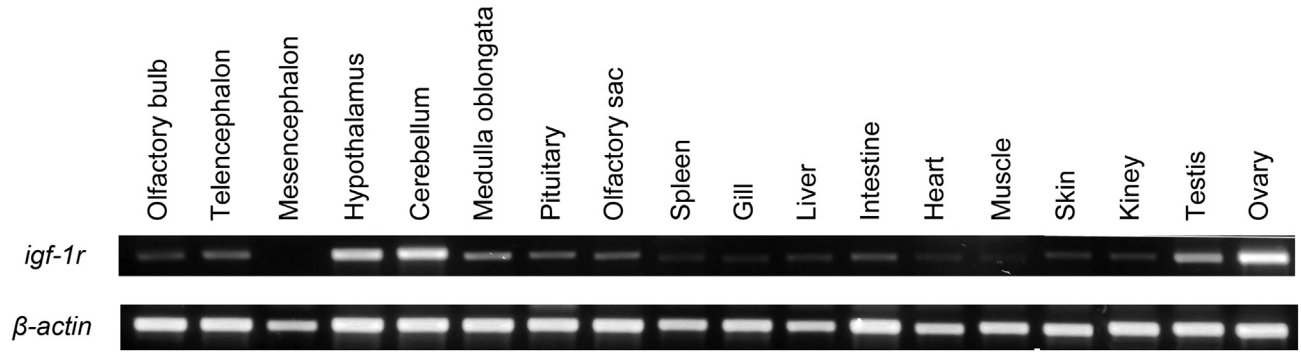

Figure 4. Expression of *B. sinensis igf-1r* transcripts in various tissues analyzed using RT-PCR with gene specific primers for *B. sinensis igf-1r* and *β-actin* listed in Supplemental Table 1.Expression of *B. sinensis igf-1r* transcripts in various tissues analyzed using RT-PCR with gene specific primers for *B. sinensis igf-1r* and *β-actin* listed in Supplemental Table 1.

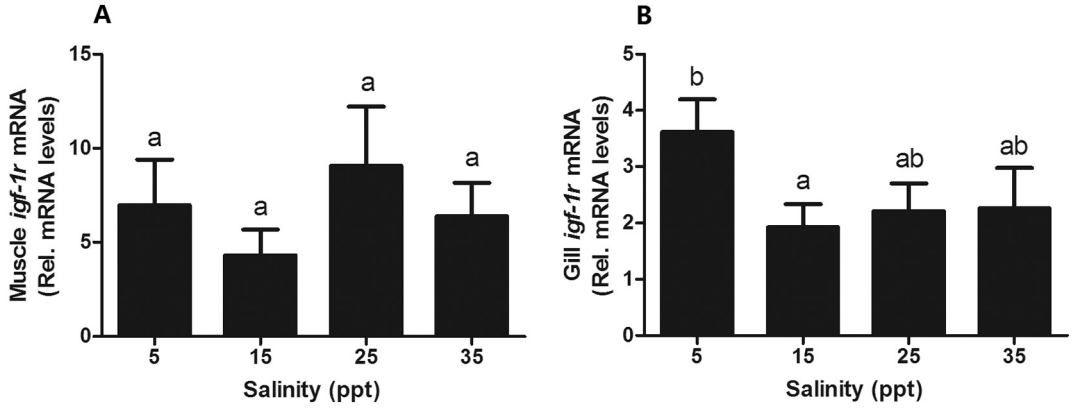

Figure 5. Muscle *igf-1r* mRNA levels (A) and gill *igf-1r* mRNA levels (B) of *B. sinensis* fry in the four different salinity groups after 5 weeks of rearing. Data are expressed as the mean ± SEM (n = 5). Bars marked with different letters are significantly different from each other ($p < 0.05$).

morhua) better growth rates at lower salinity conditions (7 ppt) result from an increase in FCE, but not an increase in FI (Lambert et al., 1994).

Besides the energy cost, hormonal stimulation such as IGF-1 is also involved in the variation in growth under different ambient salinities (Boeuf and Payan, 2001). The biological responses of IGF-1 are mainly mediated by the binding and activation of the *igf-1r* (Escobar et al., 2011). Two isoforms of *igf-1r* genes are reported in some teleosts, such as *Danio rerio* (Ayaso et al., 2002; Maures et al., 2002), *Paralichthys olivaceus* (Nakao et al., 2002), *Oncorhynchus kisutch* (Chan et al., 1997) and *Oncorhynchus mykiss* (Greene and Chen, 1999), while only one *igf-1r* gene that belongs to the Igf-1ra is found in Chilean flounder (*Paralichthys adspersus*) (Escobar et al., 2011). In the present study, we cloned one *igf-1r* gene that was classified into the Igf-1rb group based on phylogenetic analysis. Experimental trials to isolate additional *igf-1r* cDNAs or transcriptomic data obtained from the brain and olfactory sac did not provide any evidence for the existence of any additional *igf-1r* genes in *B. sinensis*.

In this study, we found that *B. sinensis igf-1r* had a wide range of tissue expression and this was consistent with the previous data from other teleosts, which indicates the pleiotropic role of IGF-1(Duan et al., 1993; Reinecke et al., 1997; Duval et al., 2002; Tse et al., 2002; Vong et al., 2003; Clay et al., 2005; Patruno et al., 2006). For example, the abundant expressions of *igf-1r* in the testes and ovary agree with their important roles such as regulators in hormone synthesis and secretion, germ cell proliferation and differentiation (Weber and Sullivan, 2000; Escobar et al., 2011). Moreover, the participation of IGF-1 in osmoregulation, seawater adaptability, skeletal muscle satellite cell proliferation and differentiation is reported in teleosts (Datuin et al., 2001; Ng et al., 2001; Wood et al., 2005; Castillo et al., 2006; Yada et al., 2012). In the present study, *igf-1r* was detected in the muscle and gill, which suggested that IGF-1 might also play a role in osmoregulation and promoting growth through *igf-1r* in *B. sinensis*.

As well as showing the function of promoting body growth, the conclusive evidence for the role of IGF-I as a seawater-adaptive hormone in salmonids is offered as a result of injection experiments in which IGF-I improves hypoosmoregulatory ability (McCormick, 1996; Seidelin et al., 1999; Tipsmark et al., 2007). However, it is recently reported that, in the striped bass (*Morone saxatilis*), circulating IGF-1 is upregulated during freshwater acclimation and downregulated during short-term (24 h) seawater acclimation, and short-term (24 h) exogenous treatment of freshwater-fish with IGF-I induces physiological freshwater levels of the hormone after seawater transfer and produces an anti-seawater adaptive effect, thus suggesting that it acts as a hyperosmoregulatory hormone (Tipsmark et al., 2007). Since the upregulation of circulating IGF-I observed in the striped bass in response to salinity changes is transitory (24 h) and not prolonged (96 h), it is suggested that IGF-I may serve dual roles, both as a hyperosmoregulatory hormone during rapid salinity acclimation and as a growth-promoting and developmental hormone in both freshwater- and seawater-acclimated fish (Tipsmark et al., 2007). Interestingly, a higher gill *igf-1r* level in *B. sinensis* fry was observed at 5 ppt, compared to 15, 25 and 35 ppt. Since the upregulation of gill *igf-1r* level at 5 ppt would result in enhancing IGF-1's biological effects in the gill, we supposed that in addition to promoting growth, *B. sinensis* IGF-1 may also serve as a hyperosmoregulatory hormone through *igf-1r* in the gill in long-term low salinity acclimation.

Taken together, it is likely that *B. sinensis* fry are more adaptive to low salinity than high ones and this may result from their ecological adaption. *B. sinensis* is a seasonal breeding fish, inhabiting intertidal zones: the spawning season of this species extends from April to October in the coastal waters of Southeastern China, with a peak spawning period between May and July (Hong et al., 2006). Accordingly, the development and growth of the *B. sinensis* fry occur from pre-summer to autumn. The major rainy season can last for more than 60 days (from April to June) in the southeastern coastal areas of China, and during this period, rainfall is highly abundant and accounts for 30 to 60% of the total annual rainfall (Yihui and Zunya, 2008). Moreover, in autumn, with the rapid southward retreat of the East Asian summer monsoon, abundant rainfall can still appear in the southeastern coastal areas of China with the impacts of tropical cyclones and other tropical weather systems (Yihui and Zunya, 2008). Therefore, from pre-summer to autumn, high precipitation often decreases the salinity in the coastal waters of southeastern China. We supposed that in order to adapt to this sudden decrease of ambient salinity *B. sinensis* fry has evolved a better low salinity acclimation system.

In summary, in this study we found that salinity affects the growth performance of *B. sinensis* fry during long-term rearing, and a salinity of 15 ppt was optimal for rearing *B. sinensis* fry based on the SGR and FCE. Moreover, a typical 'U-shaped' pattern of gill NAK activity with the lowest level at 15 ppt was observed, suggesting that 15 ppt salinity is close to the isoosmotic point and costs the lowest energy for osmoregulation in *B. sinensis* fry. The fry at 5 ppt salinity showed a significantly higher gill *igf-1r* mRNA level, suggesting that the *B. sinensis* IGF-1 might serve as a hyperosmoregulatory hormone in long-term low salinity acclimation.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.aquaculture.2017.04.004.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (No. 41276129) and the Xiamen Southern Oceanographic Center (No. 13GST001NF13). Professor John Hodgkiss of The University of Hong Kong is thanked for his help with the English in this manuscript.

References

Anderson, P.M., Broderius, M.A., Fong, K.C., Tsui, K.N., Chew, S.F., Ip, Y.K., 2002. Glutamine synthetase expression in liver, muscle, stomach and intestine of *Bostrichthys sinensis* in response to exposure to a high exogenous ammonia concentration. J. Eep. Biol. 205, 2053–2065.

Ayaso, E., Nolan, C.M., Byrnes, L., 2002. Zebrafish insulin-like growth factor-I receptor: molecular cloning and developmental expression. Mol. Cell. Endocrinol. 191, 137–148.

Beckman, B.R., 2011. Perspectives on concordant and discordant relations between insulin-like growth factor 1 (IGF1) and growth in fishes. Gen. Comp. Endocrinol. 170, 233–252.

Blanco Garcia, A., Partridge, G.J., Flik, G., Roques, J.A., Abbink, W., 2015. Ambient salinity and osmoregulation, energy metabolism and growth in juvenile yellowtail kingfish (*Seriola lalandi* Valenciennes 1833) in a recirculating aquaculture system. Aquac. Res. 46, 2789–2797.

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

Castillo, J., Ammendrup-Johnsen, I., Codina, M., Navarro, I., Gutierrez, J., 2006. IGF-I and insulin receptor signal transduction in trout muscle cells. Am. J. Physiol. Regul. Integr. Comp. Physiol. 290, 1683–1690.

Chan, S.J., Plisetskaya, E.M., Urbinati, E., Jin, Y., Steiner, D.F., 1997. Expression of multiple insulin and insulin-like growth factor receptor genes in salmon gill cartilage. P. Natl. Acad. Sci. USA. 94, 12446–12451.

Clay, L.A., Wang, S.Y., Wolters, W.R., Peterson, B.C., Waldbieser, G.C., 2005. Molecular characterization of the insulin-like growth factor-I (IGF-I) gene in channel catfish (*Ictalurus punctatus*). Biochim. Biophys. Acta 1731, 139–148.

Datuin, J.P., Ng, K.P., Hayes, T.B., Bern, H.A., 2001. Effects of glucocorticoids on cartilage growth and response to IGF-I in the tilapia (*Oreochromis mossambicus*). Gen. Comp. Endocrinol. 121, 289–294.

Duan, C., Duguay, S.J., Plisetskaya, E.M., 1993. Insulin-like growth factor I (IGF-I) mRNA expression in coho salmon, Oncorhynchus kisutch: tissue distribution and effects of growth hormone/prolactin family proteins. Fish Physiol. Biochem. 11, 371–379.

Duval, H., Rousseau, K., Elies, G., Le Bail, P.Y., Dufour, S., Boeuf, G., Boujard, D., 2002. Cloning, characterization, and comparative activity of turbot IGF-I and IGF-II. Gen. Comp. Endocrinol. 126, 269–278.

Escobar, S., Fuentes, E.N., Poblete, E., Valdés, J.A., Safian, D., Reyes, A.E., Álvarez, M., Molina, A., 2011. Molecular cloning of IGF-1 and IGF-1 receptor and their expression pattern in the Chilean flounder (*Paralichthys adspersus*). Comp. Biochem. Physiol. B. 159, 140–147.

Febry, R., Lutz, P., 1987. Energy partitioning in fish: the activity related cost of osmoregulation in a euryhaline cichlid. J. Exp. Biol. 128, 63–85.

Fielder, D.S., Allan, G.L., Pepperall, D., Pankhurst, P.M., 2007. The effects of changes in salinity on osmoregulation and chloride cell morphology of juvenile Australian snapper, *Pagrus auratus*. Aquaculture 272, 656–666.

Foss, A., Evensen, T.H., Imsland, A.K., Øiestad, V., 2001. Effects of reduced salinities on growth, food conversion efficiency and osmoregulatory status in the spotted wolffish. J. Fish Biol. 59, 416–426.

Gaumet, F., Boeuf, G., Severe, A., Leroux, A., Mayergonstan, N., 1995. Effects of salinity on the ionic balance and growth of juvenile turbot. J. Fish Biol. 47, 865–876.

Greene, M.W., Chen, T.T., 1999. Characterization of teleost insulin receptor family members. II. Developmental expression of insulin-like growth factor type I receptor messenger RNAs in rainbow trout. Gen. Comp. Endocrinol. 115, 270–281.

Gunnarsson, S., Imsland, A.K., Siikavuopio, S.I., Arnason, J., Gustavsson, A., Thorarensen, H., 2012. Enhanced growth of farmed Arctic charr (*Salvelinus alpinus*) following a short-day photoperiod. Aquaculture 350, 75–81.

Hong, W.S., Chen, S.X., Zheng, W.Y., Xiao, Y., Zhang, Q.Y., 2006. Hermaphroditism in cultured Chinese black sleeper (*Bostrichthys sinensis L.*). J. World Aquacult. Soc. 37, 363–369.

Houde, E.D., Schekter, R.C., 1981. Growth rates, rations and cohort consumption of marine fish larvae in relation to prey concentrations. Rapp. P.-V. Réun. - Cons. Perm. Int. Explor. Mer. 178, 441–453.

Imanpoor, M.R., 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, A.K., Gunnarsson, S., Foss, A., Stefansson, S.O., 2003. Gill Na^+, K^+-ATPase activity, plasma chloride and osmolality in juvenile turbot (*Scophthalmus maximus*) reared at different temperatures and salinities. Aquaculture 218, 671–683.

Ip, Y.K., Chew, S.F., Leong, I.A., Jin, Y., Lim, C.B., Wu, R.S., 2001. The sleeper *Bostrichthys sinensis* (family Eleotridae) stores glutamine and reduces ammonia production during aerial exposure. J. Comp. Physiol. Part B 171, 357–367.

Jensen, M.K., Madsen, S.S., Kristiansen, K., 1998. Osmoregulation and salinity effects on the expression and activity of Na^+, K^+-ATPase in the gills of European sea bass, *Dicentrarchus labrax* (L.). J. Exp. Zool. 282, 290–300.

Kottelat, M., Whitten, A.J., Kartikasari, S.N., Wirjoatmodjo, S., 1993. Freshwater Fishes of Western Indonesia and Sulawesi. Periplus Editions, Hong Kong, pp. 221.

Kuhlmann, D., Quantz, G., 1980. Some effects of temperature and salinity on the embryonic development and incubation time of the turbot, *Scophthalmus maximus L.*, from the Baltic Sea. Meeresforschung 28, 172–178.

Laiz-Carrion, R., Guerreiro, P.M., Fuentes, J., Canario, A.V., Martin Del Rio, M.P., Mancera, J.M., 2005. Branchial osmoregulatory response to salinity in the gilthead sea bream, Sparus auratus. J. Exp. Zool. Part A 303, 563–576.

Lambert, Y., Dutil, J.D., Munro, J., 1994. Effects of intermediate and low-salinity conditions on growth-rate and food conversion of Atlantic cod (*Gadus Morhua*). Can. J. Fish Aquat. Sci. 51, 1569–1576.

Lai, X.J., Hong, W.S., Liu, F., Zhang, Y.T., Chen, S.X., 2014. Cloning and expression of prostaglandin E receptor subtype 1 (ep1) in Bostrichthys sinensis. Fish Physiol. Biochem. 40, 1281–1288.

Link, K., Berishvili, G., Shved, N., D'Cotta, H., Baroiller, J.F., Reinecke, M., Eppler, E., 2010. Seawater and freshwater challenges affect the insulin-like growth factors IGF-I and IGF-II in liver and osmoregulatory organs of the tilapia. Mol. Cell. Endocrinol. 327, 40–46.

Maures, T., Chan, S.J., Xu, B., Sun, H., Ding, J., Duan, C.M., 2002. Structural, biochemical, and expression analysis of two distinct insulin-like growth factor I receptors and their ligands in zebrafish. Endocrinology 143, 1858–1871.

McCormick, S.D., 1996. Effects of growth hormone and insulin-like growth factor I on salinity tolerance and gill Na^+, K^+-ATPase in Atlantic salmon (*Salmo salar*): interaction with cortisol. Gen. Comp. Endocrinol. 101, 3–11.

McCormick, S.D., 2001. Endocrine control of osmoregulation in teleost fish. Am. Zool. 41, 781–794.

Morgan, J.D., Iwama, G.K., 1998. Salinity effects on oxygen consumption, gill Na^+,K^+-ATPase and ion regulation in juvenile coho salmon. J. Fish Biol. 53, 1110–1119.

Nakao, N., Tanaka, M., Higashimoto, Y., Nakashima, K., 2002. Molecular cloning, identification and characterization of four distinct receptor subtypes for insulin and IGF-I in Japanese flounder, *Paralichthys olivaceus*. J. Endocrinol. 173, 365–375.

Ng, K.P., Datuin, J.P., Bern, H.A., 2001. Effects of estrogens *in vitro* and *in vivo* on cartilage growth in the tilapia (*Oreochromis mossambicus*). Gen. Comp. Endocrinol. 121, 295–304.

Nguyen, N.T., Nguyen, V.Q., 2006. Biodiversity and Living Resources of the Coral Reef Fishes in Vietnam Marine Waters. Science and Technology Publishing House, Hanoi.

Partridge, G.J., Lymbery, A.J., 2008. The effect of salinity on the requirement for potassium by barramundi (*Lates calcarifer*) in saline groundwater. Aquaculture 278, 164–170.

Patruno, M., Maccatrozzo, L., Funkenstein, B., Radaelli, G., 2006. Cloning and expression of insulin-like growth factors I and II in the shi drum (*Umbrina cirrosa*). Comp. Biochem. Physiol. B. 144, 137–151.

Peh, W.Y.X., Chew, S.F., Wilson, J.M., Ip, Y.K., 2009. Branchial and intestinal osmoregulatory acclimation in the four-eyed sleeper, *Bostrychus sinensis* (Lacepède), exposed to seawater. Mar. Biol. 156, 1751–1764.

Peterson, M.S., Comyns, B.H., Rakocinski, C.F., Fulling, G.L., 1999. Does salinity affect somatic growth in early juvenile Atlantic croaker, *Micropogonias undulatus* (L.)? J. Exp. Mar. Biol. Ecol. 238, 199–207.

Plaut, I., 1999. Effects of salinity acclimation on oxygen consumption in the freshwater blenny, Salaria fluviatilis, and the marine peacock blenny, S. pavo. Mar. Freshwater Res. 50, 655–659.

Reinecke, M., Schmid, A., Ermatinger, R., Loffing-Cueni, D., 1997. Insulin-like growth factor I in the teleost Oreochromis mossambicus, the tilapia: gene sequence, tissue expression, and cellular localization. Endocrinology 138, 3613–3619.

Schmittgen, T.D., Livak, K.J., 2008. Analyzing real-time PCR data by the comparative CT method. Nat. Protoc. 3, 1101–1108.

Seidelin, M., Madsen, S.S., Byrialsen, A., Kristiansen, K., 1999. Effects of insulin-like growth factor-I and cortisol on Na^+, K^+-ATPase expression in osmoregulatory tissues of brown trout (*Salmo trutta*). Gen. Comp. Endocrinol. 113, 331–342.

Stuenkel, E.L., Hillyard, S.D., 1981. The effects of temperature and salinity acclimation on metabolic-rate and osmoregulation in the pupfish *Cyprinodon Salinus*. Copeia 411–417.

Taylor, J.F., Migaud, H., Porter, M.J., Bromage, N.R., 2005. Photoperiod influences growth rate and plasma insulin-like growth factor-I levels in juvenile rainbow trout, *Oncorhynchus mykiss*. Gen. Comp. Endocrinol. 142, 169–185.

Tipsmark, C.K., Luckenbach, J.A., Madsen, S.S., Borski, R.J., 2007. IGF-I and branchial IGF receptor expression and localization during salinity acclimation in striped bass. Am. J. Physiol. Regul. Integr. Comp. Physiol. 292, R535–R543.

Tse, M.C., Vong, Q.P., Cheng, C.H., Chan, K.M., 2002. PCR-cloning and gene expression studies in common carp (*Cyprinus carpio*) insulin-like growth factor-II. Biochim. Biophys. Acta 1575, 63–74.

Valenciano, A., Henríquez-Hernández, L.A., Moreno, M., Lloret, M., Lara, P.C., 2012. Role of IGF-1 receptor in radiation response. Transl. Oncol. 5, 1–9.

Vargas-Chacoff, L., Calvo, Á., Ruiz-Jarabo, I., Villarroel, F., Muñoz, J.L., Tinoco, A.B., Cárdenas, S., Mancera, J.M., 2011. Growth performance, osmoregulatory and metabolic modifications in red porgy fry, *Pagrus pagrus*, under different environmental salinities and stocking densities. Aquac. Res. 42, 1269–1278.

Vong, Q.P., Chan, K.M., Leung, K., Cheng, C.H.K., 2003. Common carp insulin-like growth factor-I gene: complete nucleotide sequence and functional characterization of the 5′-flanking region. Gene 322, 145–156.

Weber, G.M., Sullivan, C.V., 2000. Effects of insulin-like growth factor-I on *in vitro* final oocyte maturation and ovarian steroidogenesis in striped bass, *Morone saxatilis*. Biol. Reprod. 63, 1049–1057.

Woo, N.Y.S., Kelly, S.P., 1995. Effects of salinity and nutritional status on growth and metabolism of *Sparus sarba* in a closed seawater system. Aquaculture 135, 229–238.

Wood, A.W., Duan, C., Bern, H.A., 2005. Insulin-like growth factor signaling in fish. Int. Rev. Cytol. 243, 215–285.

Yada, T., McCormick, S.D., 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.

Yihui, D., Zunya, W., 2008. A study of rainy seasons in China. Meteorol. Atmos. Phys. 100, 121–138.

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

Zhang, Y., Guo, X., Guo, J., He, Q., Li, H., Song, Y., Zhang, H., 2014. Lactobacillus casei reduces susceptibility to type 2 diabetes via microbiota-mediated body chloride ion influx. Sci. Rep. 4, 5654.

Zhang, Y.T., Li, Z., Chen, S.X., Mao, Y., Hong, W.S., 2015. Optimization of the culture methods for nursing Chinese black sleeper (*Bostrychus sinensis* Lacépède, 1801) fry using an orthogonal array design. J. Appl. Ichthyol. 31, 1096–1101.

Zhao, Q., Shao, L., Hu, X., Wu, G., Du, J., Xia, J., Qiu, H., 2013. Lipoxin A_4 preconditioning and postconditioning protect myocardial ischemia/reperfusion injury in rats. Mediat. Inflamm. 2013, 231351.