Influence of Salinity on the Survival and Growth of Juvenile Coregonus Ussuriensis Berg

Tianqing Huang
Heilongjiang River Fishery Research Institute of Chinese Academy of Fishery Sciences
Enhui Liu
Heilongjiang River Fishery Research Institute of Chinese Academy of Fishery Sciences
Wei Gu
Heilongjiang River Fishery Research Institute of Chinese Academy of Fishery Sciences
Bingqian Wang
Heilongjiang River Fishery Research Institute of Chinese Academy of Fishery Sciences
Fulin Dong
Heilongjiang River Fishery Research Institute of Chinese Academy of Fishery Sciences
Zuoyu Zou
Haerbin Academy of Agriculture Sciences
Gefeng Xu (xugefeng@hrfri.ac.cn)
Heilongjiang River Fishery Research Institute of Chinese Academy of Fishery Sciences

Research Article

Keywords: Coregonus ussuriensis Berg, Salinity, Osmoregulation, Gill, Liver, Gene expression

Posted Date: May 26th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-524713/v1

Abstract

To explore the suitable salinity range of *Coregonus ussuriensis* Berg, we investigated the effect of induced salinity change in captivity on *C. ussuriensis* with an initial body weight of 35 ± 1.5 g. After 30 days of salinity acclimation, the survival, growth performance, blood biochemical profiles, antioxidative capacity, and tissue structure of juveniles under four salinity conditions (8‰, 16‰, 24‰, and 32‰) were investigated. Our results revealed that serum penetration, blood glucose, and serum Na^+, Cl^-, and Mg^{2+} gradually increased with increasing salinity until 32‰ salinity, when a significant difference was observed, whereas the K^+ concentration showed a downward trend. The tissue sections showed that under high salinity (32‰), the liver and gill tissues of the fish were severely damaged and the vacuolation was serious. The levels of superoxide dismutase, glutathione peroxidase, and serum cortisol gradually increased with increasing salinity. A gene expression analysis showed that the increase in salinity induced higher expression of stress-, growth-, and inflammation-related genes (*HSP70*, *Gh* and *Igf-1*, and *IL-1β*, respectively). The downregulation of stress-related gene expression at 32‰ salinity may indicate that this level of salinity exceeded the regulatory capacity of *C. ussuriensis*. We concluded that *C. ussuriensis* may survive in an estuary under 0–24‰ salinity. Our findings provide insights into the physiological adaptation of *C. ussuriensis* to salinity change. These results could improve our knowledge of the stress response and resilience of estuarine fish to hyposalinity and hypersalinity stress.

Introduction

Salinity is an important environmental factor that affects the distribution and community structure of aquatic species and is closely related to physiological functions, growth performance, and immune regulation in fish farming (Lein et al. 1997; Fang et al. 2019). Owing to the variation in the salinity of aquatic environments, teleost fish have evolved various physiological strategies for adapting to salinity (El-Leithy et al. 2019; Geven et al. 2017). When fish live in an environment with fluctuating salinity, various enzymes and transporters participate in the process of salinity adaptation and osmotic adjustment to maintain the body's permeability and ion homeostasis (Pungpung et al. 2007). Tolerance depends on the osmotic adjustment ability of the fish (Rubio et al. 2005). The liver can promote the decomposition of glycogen into glucose to maintain normal blood sugar levels and provide energy for gills and other osmotic adjustment organs under salinity stress (Zhang et al. 2017). However, compared with other osmotic mediators (gills and intestines), there have been fewer studies focusing on the liver. In the present study, the indicators of enzyme activity in the liver were investigated and the physiological responses of juvenile fish were explored under different salinity stress conditions. Salinity usually affects the growth rate of teleost fish because part of the energy used for growth is consumed during osmotic adjustment (Naglaa et al. 2017; Gonzalez et al. 2011). In addition, changes in salinity can cause an increase or decrease in certain blood indicators, such as an increase in cortisol, and these changes can affect oxygen transport in the gills (Yada et al. 2002).

Although the molecular effectors of teleosts under salinity stress have been reported (Mattioli et al. 2017; Whitehead et al. 2012), the influence of salinity variation on *Coregonus ussuriensis* Berg (Salmoniformes,

Coregoninae) is unclear. Thus, in the present study, we investigated the expression levels of a stress-related gene (heat shock protein 70, *HSP70*), growth-related genes (growth hormone, *Gh* and insulin-like growth factor 1, *Igf-1*), and an inflammation-related gene (interleukin-1β, *IL-1β*).

C. ussuriensis is mostly distributed in the Heilongjiang Valley in northeast China and the waters of Siberia and Sakhalin in Russia (Bochkarev et al. 2017). In 1998, *C. ussuriensis* was listed in the “Red Data Book of China’s Endangered Animals” (Wang et al. 1998). Owing to its high economic value, *C. ussuriensis* is considered a promising new fish species for culturing in China. An analysis of otolith growth ring characteristics showed that *C. ussuriensis* has annual (seasonal) migration characteristics and migration history between rivers and seas or between freshwaters and estuaries (Wang et al. 2019). However, the range of adaptation for salinity in *C. ussuriensis* has not yet been reported. In the present study, we aimed to explore the adaptation of *C. ussuriensis* to different salinities through the analysis of physiological and genes expression. Our results provide a reference for supplementing the breeding and release of *C. ussuriensis*.

Materials And Methods

Fish collection and management

Test *C. ussuriensis*, with a body length of 15 ± 1.5 cm and body weight of 30 ± 3.5 g were obtained from the Bohai Coldwater Fisheries Research Station (Heilongjiang Province, P.R. China). Before the start of the experiment, 250 fish were randomly distributed in a semi-recirculation system consisting of five circular polythene tanks (70 cm diameter, 20 cm depth, 75 L water volume) and acclimated to the new rearing environment for 2 weeks. During the experiment and acclimation period, the photoperiod was set at 12 h light: 12 h dark (12 L : 12 D) and the light intensity at 70 lx. Filtered freshwater was used in the trial: dissolved oxygen was 7.8–10.0 mg/L, pH 7.4, water temperature was maintained at 10 ± 0.2 °C, and ammonia-N was < 0.1 mg/L throughout the study. Fish were fed to apparent satiation twice daily (08:00 and 14:00 h), and the amount of bait was 2 % of the mass of the fish. During the acclimation and test period, the water was changed weekly and the amount of water was changed from 30 % to 40 %. The seawater used to change the experimental water was pre-prepared, the salinity difference was < 0.5, and the temperature difference was < 0.5 °C.

Salinity experimental design

To simulate the environmental salinity of estuaries and offshore watersheds, five test groups were designed with salinities of 0‰ (S0; freshwater control), 8‰ (S8), 16‰ (S16), 24‰ (S24), and 32‰ (S32). In the test groups, the salinity was increased by 4‰ every day until the set salinity was reached and the test period was 30 days. Groundwater was used as the test water source, and the salinity was measured with a salinometer (error ≤ 0.5). Each test group had three replicates and a parallel stocking of 15 juvenile fish, with a total of 45 fish. Feeding was stopped 24 h before sampling. For anaesthesia, 100 mg/mL tricaine methanesulfonate (MS-222) was used.

Serum physiological determination

After the fish were anaesthetised, blood was collected from their tail vertebrae on an ice tray; 1 ml of blood was taken from each tail, and nine fish were taken from each group. The blood was centrifuged at 3500 × *g* at 4°C for 15 min. A fully automatic biochemical analyser (Olympus AU 600, Japan) was used to determine the osmolality, Na^+, K^+, Mg^{2+}, Ca^{2+}, Cl^-, and P^+, as well as the activities of glucose, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and urea nitrogen (UREA).

Histological analysis

Liver and gill samples from the five experimental groups were analysed. Samples were fixed with Bouin's solution, dehydrated with ethanol, made transparent with xylene, and embedded in paraffin. The samples were cut with a microtome (KD1508), stained with haematoxylin-eosin, and mounted in neutral resin. The prepared sections were observed and photographed using an Olympus CX41 microscope.

Physiological index determination

The livers of six fish from each group were collected and stored at −80°C for enzyme activity analysis, as previously described by Sandstrom et al. (1994). Superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-PX) activity were determined out using kits (Nanjing Jiancheng Biological Engineering Research Institute, Nanjing, China). Protein levels in the homogenate were determined using the Coomassie blue method. Serum cortisol levels were measured using commercially available radioimmunoassay equipment (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). The growth index was calculated as follows:

$$SGR = 100\% \times (\ln FW - \ln IW)/T$$

$$\text{Survival rate} = 100\% \times (N_T/N_0)$$

where SGR is the specific growth rate, IW and FW are the initial and final body weights of the juvenile fish (g), respectively, T is the experiment time (d), N_0 is the number of tails of *C. ussuriensis* at the beginning of the experiment, and N_T is the number of surviving juvenile fish at the end of the experiment.

RNA isolation and cDNA synthesis

RNA was extracted from the collected liver samples of *C. ussuriensis* using TRIzol® (Invitrogen) according to the manufacturer's instructions (1 mL/100 mg tissue). Its concentration was measured using ScanDrop at 260/230 nm (Analytikjena, Germany) and purity was assessed at a 260/280 ratio. Reverse transcription was used to generate cDNA using the PrimeScript RT Reagent Kit (TaKaRa, Japan) according to the manufacturer's instructions. The obtained cDNA was stored at −20°C until further use as the template in the amplification reaction.

Real-time PCR

Quantitative real-time PCR was performed using TB Green® Premix Ex TaqTM (TaKaRa, Japan). The conditions were as follows: 95°C for 30 s, 39 two-step cycles at 95°C for 5 s and 60°C for 30 s, followed by

95°C for 10 s, 65°C for 5 s, and 95°C for 5 s. Beta-actin was used as the reference gene. Primers were designed using Primer Premier software 5.0. Primers used for real-time PCR are listed in Table 1. For each cDNA sample, all target and reference genes were independently amplified in triplicates on the same plate and the same experimental run. The melting curve analysis showed that there were no dimers or other non-specific PCR products in any of the reactions performed. Ct values were measured using the CFX96 C1000 touch Thermal Cycler (Bio-Rad, USA), and the value of the target sequence normalised to the reference sequence was calculated as $2^{-\Delta\Delta Ct}$.

Statistical Analysis

Graphs were plotted using GraphPad Prism 7.0. The least significant difference test and one-way ANOVA were used to analyse the differences between groups. Results are expressed as the mean ± SE. Statistical analysis was performed using SPSS version 13.0. Statistical significance was set at $P < 0.05$.

Results

Survival rate and growth performance

In the salinity stress experiment, the survival rate and SGR of the high-salinity group were significantly different from those of S0. As shown in Table 2, *C. ussuriensis* was able to survive normally during the experimental period up to S24 salinity. In the S32 group, the survival rate of *C. ussuriensis* significantly decreased ($P < 0.05$), with 36 deaths in total and a survival rate of 21.04%. As shown in Table 2, the final weight of *C. ussuriensis* was 53.33 ± 5.53 g, and the specific growth rate was 1.24%. However, in the S32 group, a negative growth phenomenon was observed. The SGR presented a similar pattern of change.

Blood biochemical parameters

The serum physiological indexes of *C. ussuriensis* under salinity treatment are shown in Table 3. With the increase in salinity, the serum osmolality showed a gradual increasing trend, which was significantly different from that of S0 at S32. The change trend in the concentration of Na^+ and Mg^{2+} in serum was basically the same as that of osmolality, whereas the concentration of K^+ gradually decreased as the salinity gradient increased. The change in Cl^- was the most pronounced and was significantly different compared with that of S0 at S24. However, the Ca^{2+} and P^+ levels did not significantly change. As shown in Table 4, there was no significant difference in the glucose levels of *C. ussuriensis* serum at S24, whereas the glucose concentration significantly increased at S32. The concentration of AST gradually decreased with increasing salinity, but the salinity gradient did not significantly affect ALT and UREA concentrations.

Observation of tissue sections

Compared with S0, with the increase in salinity, the liver tissues of *C. ussuriensis* changed to varying degrees (Fig. 1). When the salinity was S0, the hepatocytes were arranged compactly and neatly with clear structures and evident boundaries. With the gradual increase in salinity, the hepatocyte structure had a tendency to shrink, but under S8 and S16 conditions, there was no evident damage. Under S24 and S32

conditions, the liver was characterised by empty vacuoles and fuzzy hepatocyte boundaries. The liver tissue was clearly injured.

At S0, the gill filament epithelium of juvenile *C. ussuriensis* comprised multiple layers of epithelial cells, including chloride cells and pavement cells. Pavement cells, pillar cells, and blood channels were distributed on the epithelial cells of the gill fragments. As shown in Fig. 2, with the gradual increase in salinity, chloride cells and epithelial cells showed varying degrees of vacuolisation, and gill filament cells had the most severe vacuolisation at S32.

Research on enzyme activity

There was a significant difference ($P < 0.05$) in SOD activity in the increasing salinity gradient. Compared with S0, with the increase in salinity, SOD activity first increased and then decreased peaking at S24 (Fig. 3A). There was no significant difference in CAT enzyme activity during the entire test period (Fig. 3B). The activity of GSP-PX peaked at S32 (Fig. 3C). The serum cortisol content gradually increased with increasing salinity (Fig. 3D).

Gene expression

Different gene expression patterns were observed in the liver at different salinities. As shown in Fig. 4, *HSP70* expression in the liver increased 11.46-fold at S8. The relative expression at S32 decreased by 0.75-fold compared with that at S0 (Fig. 4A). The expression of the growth-related gene *Gh* increased with increasing salinity peaking at S32, which was 16.26-fold higher than that at S0 (Fig. 4B). The expression of *Igf-1* was the highest at S16, which was 2.97-fold higher than that at S0, but then showed a downward trend (Fig. 4C). The expression of the inflammation-related gene *IL-1β* in the liver is shown in Fig. 4. Generally, the expression of *IL-1β* was 22.10- and 17.79-fold higher at S16 and S24 than that at S0, respectively (Fig. 4D).

Discussion

Artificial propagation technology for *C. ussuriensis* is still in its preliminary exploration stage. To supplement the basic biological data of this species and provide theoretical guidance for artificial breeding, a salinity domestication experiment was carried out on *C. ussuriensis*. Our results confirmed the previous speculation that the fish has a migration history between rivers and the sea or between freshwaters and estuaries (Wang et al. 2019). Similar to other salmonids, *C. ussuriensis* has some hypotonic regulatory mechanisms when exposed to salt water (Mccormick et al. 1989; Zhi et al. 2009).

Haematological parameters are important for determining the physiological status and health condition of fish (Harikrishnan et al. 2003; Garcia F et al. 2007). A previous study found that when the environmental salinity changed, the serum osmolality, Na^+, and Cl^- concentrations of euryhaline fish showed an upward trend with the increase in salinity (Sakamoto et al. 2001), which was consistent with the results of the present study (Table 3). The trend in the osmotic pressure, Na^+, Cl^-, and Mg^{2+} in the *C. ussuriensis* serum was basically the same as the osmolality trend, whereas the K^+ concentration gradually decreased as the

salinity gradient increased. We considered that *C. ussuriensis* may maintain proton balance by absorbing K^+ during the process of osmotic adjustment and avoid the damage caused by high salt levels in the body. The activity of ALT and AST in plasma can reflect hepatopancreatic damage (Jiang et al. 2014; Yan et al. 2016). The present study showed that AST activity increased to its highest level at S32, indicating that the fish were damaged at this salinity. However, the ALT activity showed no evident change during salinity adaptation.

The gill is a multifunctional organ in fish. In addition to gas exchange and nitrogenous waste excretion, gills also participate in regulating the maintenance of inorganic salt ions and acid–base balance (Evans et al. 2005; Evans et al. 2005). The gill tissue structure of fishes living in water bodies differs according to salinity. For example, euryhaline teleost fishes adapt to the salinity by adjusting the distribution, structure, and number of chloride cells in the gill filament epithelium (Hirai et al. 1999; Toyoji et al. 2008). Chloride cells are mainly located in the epithelium of the gill filaments, and some are distributed on the gill slices (Evans et al. 2005; Perry et al., 1998). When the ion balance in the environment changes, the morphology, distribution, number, and internal structure of the gill epithelial cells undergo evident adaptive changes to maintain osmotic pressure balance in the body (Huang et al. 2007). The results of the present study showed that with gradual increase in salinity, chloride and epithelial cells showed varying degrees of vacuolisation. Among them, the gill filament cells had the most severe vacuolisation at S32 (Fig. 1). Similar results were reported by Shirangi et al. (2016). Our results indicated that S32 was the salinity threshold for physiological tolerance in *C. ussuriensis*.

The liver is the main immune organ in teleosts. Although it is not directly involved in osmotic adjustment, it is the main component of glycogen/glucose metabolism, and its metabolism is enhanced during the osmotic adaptation process, which is useful for the osmotic adjustment of biofuels (Vijayan et al. 1996; Peragón et al. 1998). In the present study, salinity had a certain degree of influence on the liver of juvenile *C. ussuriensis*. When observed under normal conditions (S0), the liver tissue structure was normal. As salinity increased, liver cells gradually shrunk. When the salinity was S32, the liver was characterised as empty vacuoles (Fig. 2), which could be related to the physiological changes in *C. ussuriensis*. We consider that salinity had exceeded the liver's physiological regulation ability.

A sudden increase or decrease in environmental salinity can cause the formation of reactive oxygen species (ROS in cells, causing oxidative damage to the body (Halliwell and Aruoma. 1991), but antioxidant enzymes have evolved to reduce or eradicate radicals from the cell (Tomanek et al. 2011). For example, SOD is a key enzyme in the antioxidant defence system of fish and can reduce body damage by removing excessive O^{2-} (Sinha et al. 2015; Sui et al. 2016; El-Leithy et al. 2019). In the present study, changes in salinity caused the SOD activity to initially increase and then decrease (Fig. 3), indicating that changes in salinity activated the antioxidant capacity of juveniles. These results are similar to those reported by another study on fish (Conides et al. 2010). CAT and GSH-PX also minimise the damage caused by stress through regulation (Barber et al. 2006; Md. Afzal et al. 2016). In the present study, the content of CAT was not significantly different under different salinity treatments and we consider that CAT may not work under salinity stress. Serum cortisol is an important physiological indicator of stress in fish. It is a glucocorticoid

hormone synthesised and released by the head kidney cells under the action of the adrenocorticotropic hormone secreted by the hypothalamus–pituitary–adrenal axis (Zong et al. 2019). The results of the present study demonstrated that as salinity increased, the cortisol concentration gradually increased peaking at S32 compared with that at S0, indicating that salinity induced a stress response in the fish body.

HSP70 plays a key role in a variety of stressful environmental conditions, such as those related to temperature, hypoxia, and salt. Exposure to stressful environments causes the upregulation of gene expression as a protective mechanism. In a salinity stress experiment on blackchin tilapia (*Sarotherodon melanotheron*), it was found that *HSP70* was positively correlated with Na^+ and K^+/ATPase gene expression (Tine et al. 2010). Deane and Woo (2004) also showed that an increase in salinity caused the upregulation of *HSP70*. In the present study, *HSP70* was significantly upregulated at S8, which was similar to the results of El-Leithy et al. (2019) in Nile tilapia (*Oreochromis niloticus*). Hence, we suggest that *HSP70* plays a key role in osmoregulation in *C. ussuriensis*.

Gh is secreted by the pituitary gland and is involved in many physiological functions in fish, most of which are related to somatic growth and stress (Ababatin 2011; Bertucci et al. 2017; Yuan et al. 2017). A previous study reported that the *Gh* gene of teleosts was highly expressed with increased salinity in *Trachinotus ovatus* (Liu et al. 2019), suggesting that *Gh* may provide energy for osmotic regulation by regulating energy metabolism. Similar results were obtained in the present study. The significantly high expression of *Gh* at S32 may be a stress response of fish. *Igf-1* plays an important role in hypo-osmoregulatory functions during seawater adaptation (Tipsmark et al. 2007). *Igf-1* may activate salt secretion by affecting the function of the transporter, which is part of the response of teleosts to acute salt stress. We consider that this gene has an osmoregulatory effect in *C. ussuriensis* during salinity adaptation.

Salinity stress has an indirect relationship with immune response, and previous studies have shown that moderate salinity stress may enhance the immune defences of fish (Tort 2011; Schmitz et al. 2017). IL-1β is a key early proinflammatory cytokine that plays a key role in the fish immune response. In the current study, *IL-1β* was significantly upregulated at all salt concentrations. Furthermore, El-Leithy et al. (2019) observed a significant increase in *IL-1β* expression during salinity adaptation in Nile tilapia. The role of inflammation-related genes during osmoregulation in *C. ussuriensis* requires further in-depth research.

In summary, combined with the analysis of physiological indicators and the observation of tissue sections, we found that juvenile *C. ussuriensis* have strong adaptability to moderate salinity (S8–S24), under which they can adapt and grow normally. However, at S32, the fish body produced a stronger stress response to the external environment. Thus, our findings will provide a theoretical reference for the breeding and release of *C. ussuriensis* in the future.

Declarations

Author Declarations

Funding

This work was supported by the Central Public-interest Scientific Institution Basal Research Fund, HRFRI (NO.HSY202103M), China Agriculture Research System (CARS-46) and Central Public-interest Scientific Institution Basal Research Fund, CAFS (NO. 2020TD32)Special project on agricultural financial fund from the Ministry of Agriculture and Rural Affairs of China entiled “Survey of fishery resources and environment in key waters of Northeast China”.

Conflicts of interest

There are no conflicts of interest to declare.

Ethics approval

All experiments were performed in accordance with the European Communities Council Directive (86/609/EEC). Fish were bred following the guidelines of the Animal Husbandry Department of Heilongjiang Province, P.R. China. All efforts were made to minimise suffering.

Consent to participate

Not applicable.

Consent for publication

Not applicable.

Data availability

The data used to support the findings of this study are available from the corresponding author upon request.

Code availability

Not applicable.

Authors' contributions

Tianqing Huang performed the experiments; Enhui Liu analysed the data and checked the manuscript; Wei Gu and Fulin Dong cultured and sampled the fish; Zuoyu Zou drafted the article; Bingqian Wang reviewed the manuscript; Gefeng Xu designed the experiments. All authors contributed to the manuscript at various stages. All authors have read and approved the manuscript.

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Tables

Table1. Primers sequences used in qRT-PCR

Gene name	Forward primer(5'-3')	Reverse primer (5'-3')	Targe size (BP)	Accession no.
HSP70	GACATCAGCCAGAACAAGCG	AGAGGGAGTCAATCTCAATGCTG	109	AJ632154.1
Gh	CGTACTGAGCCTGGATGACAAT	CGACCTTGTGCATGTCCTTCT	137	X14305.1
Igf-1	AGTTCACGGCGGTCACATAA	CACAGTACATTTCGAGCCGC	85	M81904.1
IL-1β	GTTGTGTTCCACAGCGACAC	TAGTCTGGGTCGAGGGTGAG	86	BT059619.1

Table2. Survival and growth of juvenile *Coregonus ussuriensis* Berg in different salinity group *n*=45; ±SE

Salinity	Survival rate/%	IW/g	FW/g	SGR/%
S0	100^{a}	35.08 ± 3.73^{ab}	53.33 ± 5.53^{a}	1.24^{a}
S8	100^{a}	37.47 ± 2.22^{c}	49.44 ± 3.80^{a}	1.17^{ab}
S16	100^{a}	34.15 ± 2.23^{a}	50.00 ± 5.59^{a}	1.22^{a}
S24	100^{a}	36.29 ± 1.51^{bc}	47.00 ± 3.94^{a}	1.02^{b}
S32	21.4^{b}	34.66 ± 3.65^{ab}	30.77 ± 5.28^{b}	-0.47^{c}

Values in each column with different superscripts are significantly different (P<0.05).

Table3. Effects of salinity on the serum biochemical indices in juvenile *C. ussuriensis* Berg *n*=9; ±SE

Salinity	osmolality （m/L）	Na^+（mmol/L）	K^+ (mmol/L)	Mg^{2+} (mmol/L)	Ca^{2+} (mmol/L)	Cl^- (mmo/L)	P^+ (mmol/L)
S0	296.98± 5.37 [a]	151.33± 3.09 [a]	2.39± 0.24 [a]	1.24± 0.23 [a]	2.97± 0.14[a]	121.33± 2.49 [a]	5.60± 1.08[a]
S8	293.14± 8.75 [a]	154.33± 1.24 [a]	1.74± 0.30 [ab]	1.23± 0.19 [a]	2.89± 0.44[a]	123.67± 0.94 [ab]	5.03± 1.31[a]
S16	301.0 ±13.29 [a]	156.00± 5.71 [a]	1.51± 0.37 [b]	1.37± 0.54 [a]	2.85± 0.14[a]	130.67± 3.85 [bc]	3.62± 0.23[a]
S24	307.05± 7.32 [a]	153.67± 1.25 [a]	1.46± 0.39 [b]	1.61± 0.26 [a]	3.37± 0.12[a]	133.00± 3.74 [c]	4.15± 0.46[a]
S32	407.78± 18.89 [b]	204.67± 7.85[b]	1.24± 0.68 [b]	2.79± 0.04 [b]	3.21± 0.16[a]	168.67± 4.10 [d]	4.57± 1.10[a]

Values in each column with different superscripts are significantly different (P<0.05).

Table 4. Serum glucose and enzyme activity of juvenile *Coregonus ussuriensis* Berg in different salinity group n=9; ±SE

Salinity	GLU （mmol/L）	ALT(IU/L)	AST(IU/L)	AST/ALT	UREA （mmol/L）
S0	5.67±0.16 [a]	32.33±0.94 [a]	651.00±61.25[a]	20.20±2.45[a]	0.50±0.08[a]
S8	4.80±0.35 [a]	32.33±1.24 [a]	577.67±37.18[a]	25.56±7.72[a]	0.70±0.12[a]
S16	4.60±0.49 [a]	24.00±1.41 [b]	777.67±61.22[a]	26.72±3.66[a]	0.63±0.12[a]
S24	5.10±0.28 [a]	23.67±0.94 [b]	724.00±31.90[a]	20.49±2.79[a]	0.80±0.21[a]
S32	10.73±1.59 [b]	18.67±0.47 [c]	705.67±28.66[a]	30.11±7.15[a]	0.70±0.28[a]

Values in each column with different superscripts are significantly different (P<0.05).

Figures

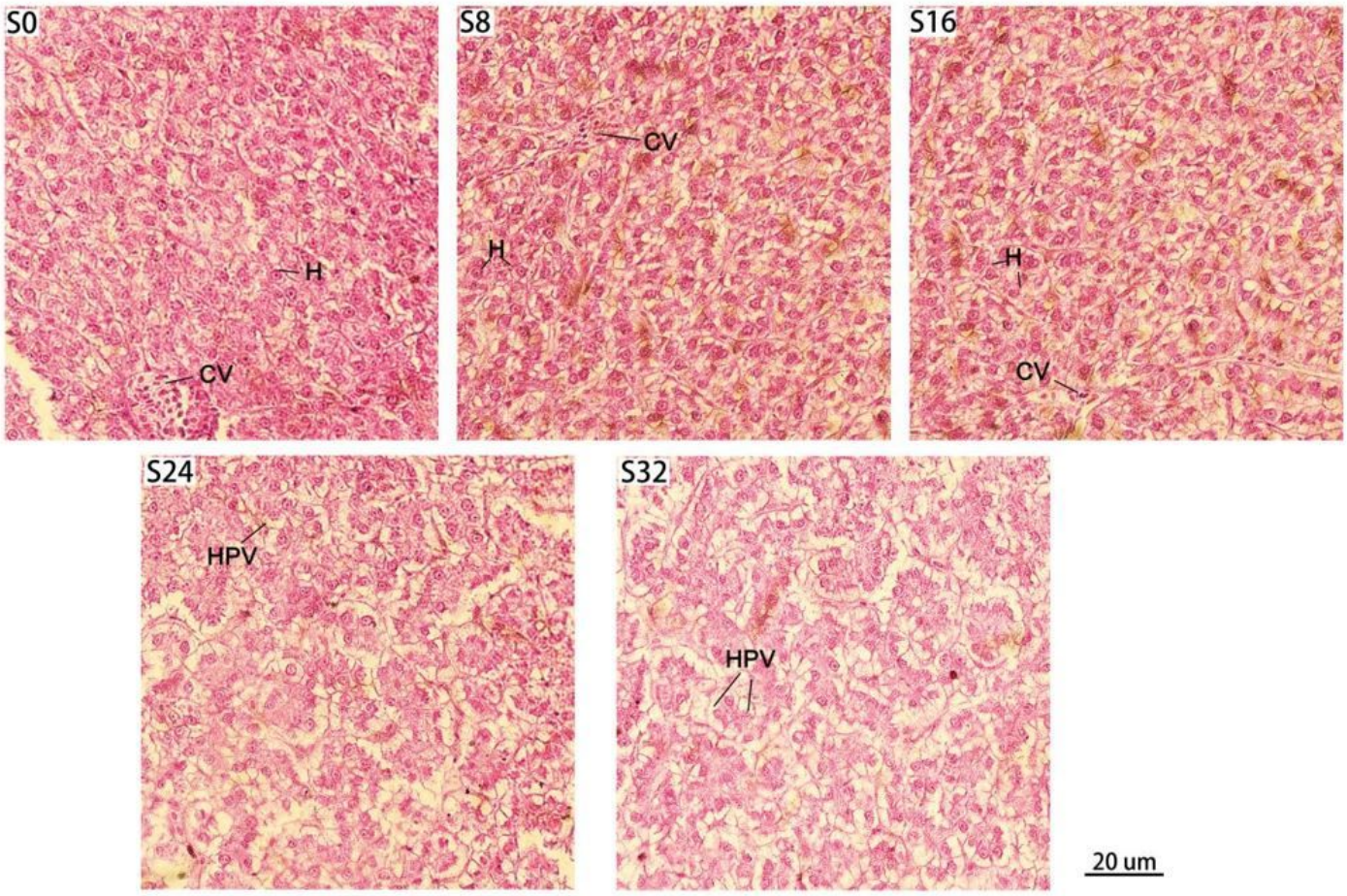

Figure 1

Microstructure of liver tissue in C. ussuriensis exposed to different salinity. CV, central veins; H, hepatocyte; HPV, hepato cellular vacuolation

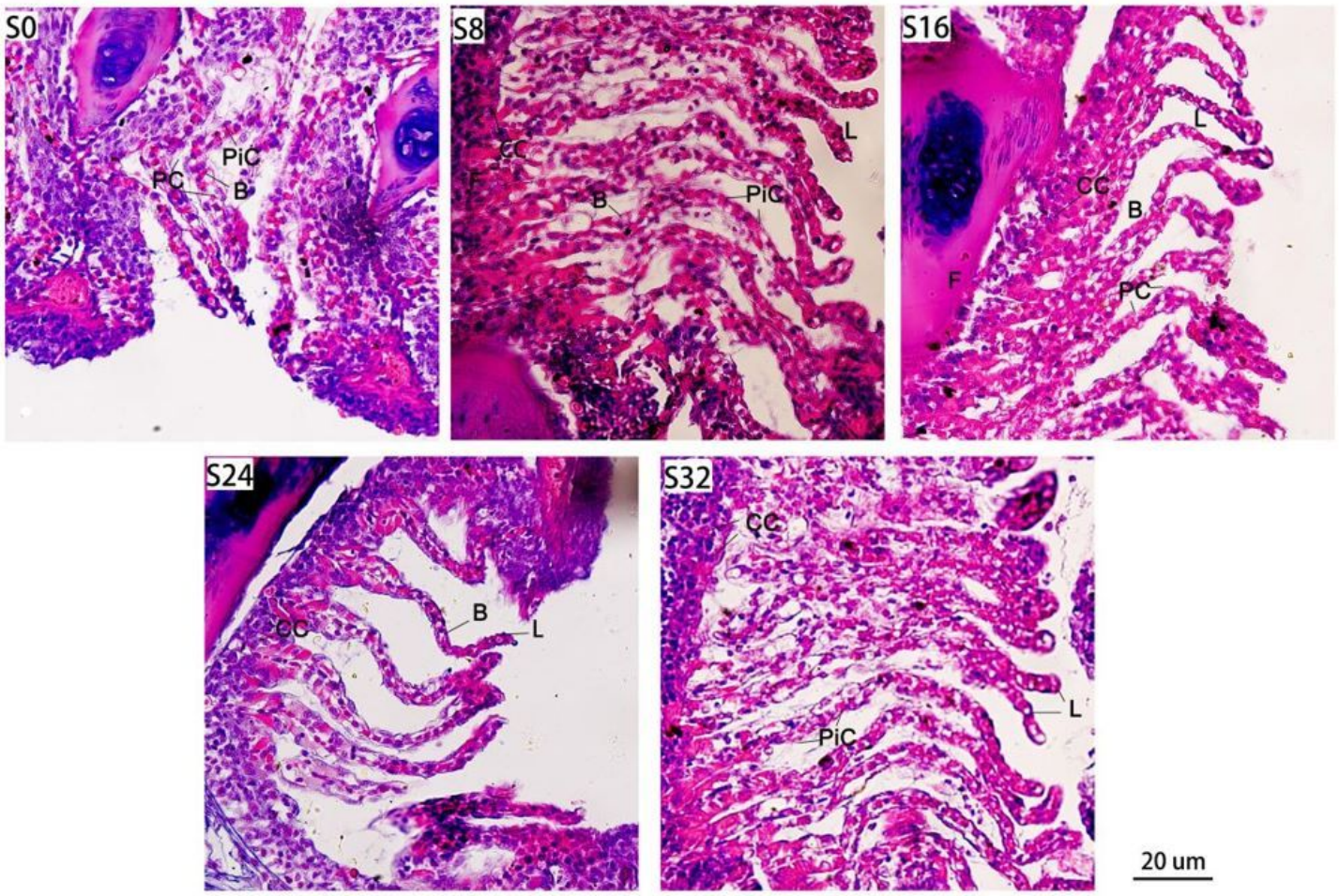

Figure 2

Microscopic section of gill tissue of C. ussuriensis exposed to different salinity. F, filament; L, lamellae; CC, chloride cells; PC, pavemen cells; Pi C, pillar cells; B, blood channel and blood cell

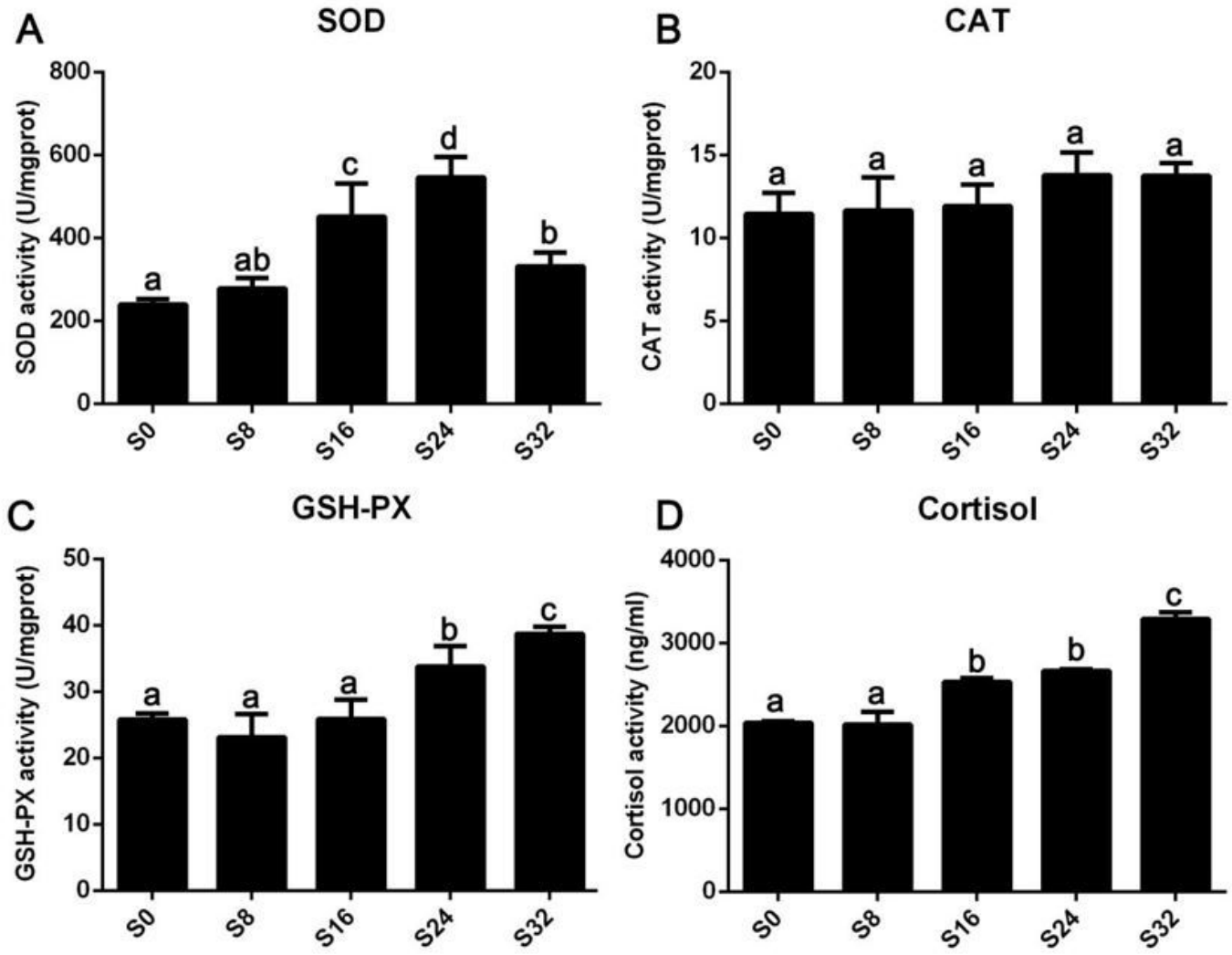

Figure 3

Investigation of physiological indexes of C. ussuriensis exposed to different salinity. n=6 A. Superoxide dismutase (SOD) activity (U/mgprot) in the liver. B. Catalase (CAT) activity (U/mgprot) in liver. C.Glutathione (GSH-PX) activity (U/mgprot) in the liver. D. Cortisol activity (U/mgprot) in the serum.

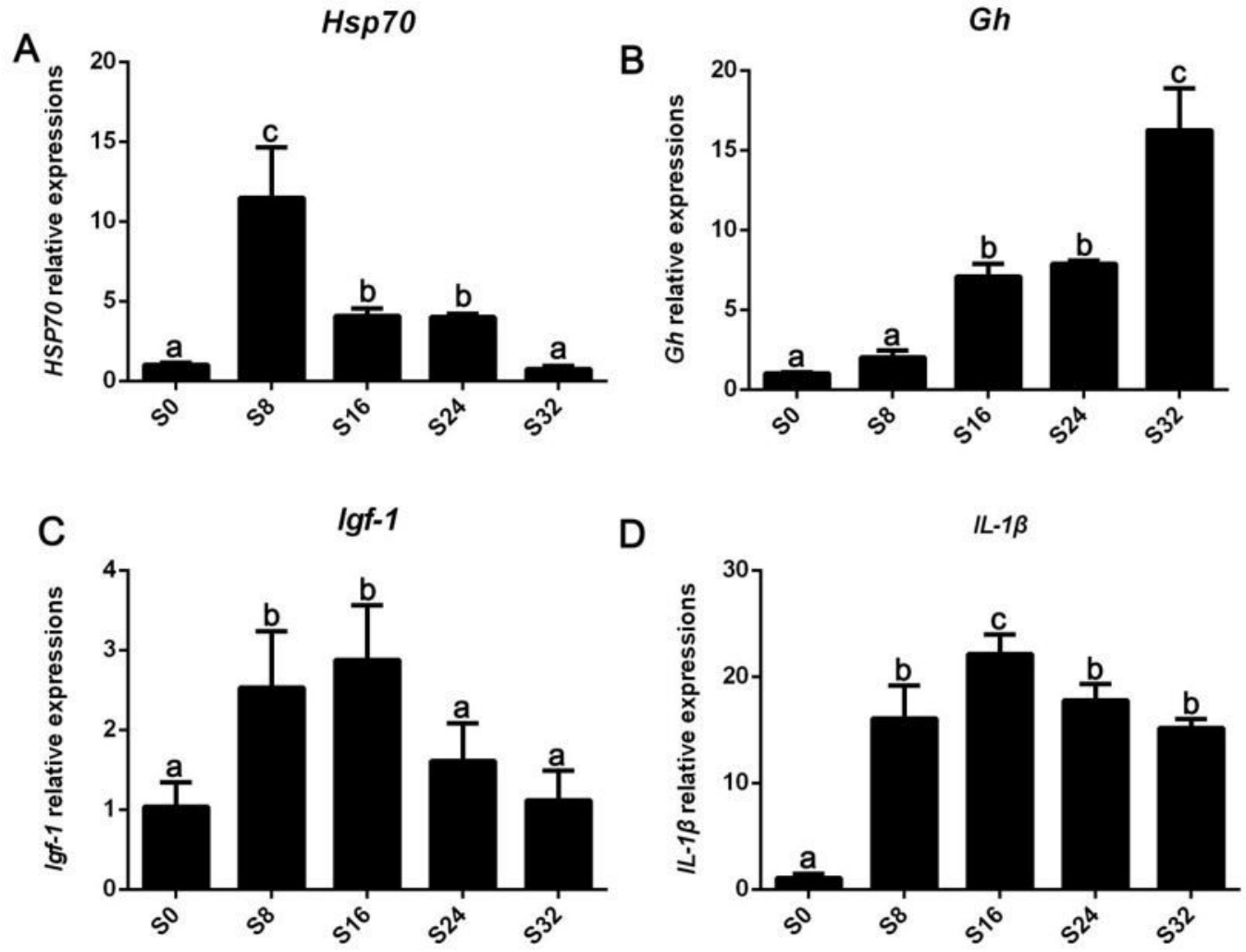

Figure 4

HSP70, Gh and IGF-1, IL-1 in liver of C. ussuriensis exposed to different salinity, n=6. $P < 0.05$ showed significant difference.