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The effect of salinity on growth performance, digestive and antioxidant enzymes, humoral immunity and stress indices in two euryhaline fish species: Yellowfin seabream (*Acanthopagrus latus*) and Asian seabass (*Lates calcarifer*)

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ABSTRACT

A 56-day research was performed to examine the effect of water salinity (WS) on performance and some physiological responses of yellowfin seabream (*Acanthopagrus latus*, 12.5 g) and Asian seabass (*Lates calcarifer*, 33.5 g) juveniles. Fishes were reared at five WS including 6, 12, 24, 35 and 48‰. Increasing WS from 6 to 12‰ improved growth performance in *A. latus* juveniles; however, elevation of WS over 24‰ suppressed growth rate in this species. Increment of WS over 12‰ gradually reduced growth and feed utilization in L. *calcarifer*. In *A. latus*, fish reared at 24 and 35‰ had the greatest total protease activity, but those in 6‰ had the lowest value ($P < 0.05$). Lipase activity in *A. latus* gradually increased with enhancing WS up to 35‰ then it was decreased. The activities of total protease and lipase in L. *calcarifer* increased with enhancing WS form 6 up to 24‰ then their activities were gradually decreased ($P < 0.05$). The activity of catalase (CAT) in the liver of *A. latus* juveniles pronouncedly decreased with increasing WS. The glutathione peroxidase (GPX) and superoxide dismutase (SOD) activities as well as lipid peroxidation (TBARs) content were gradually increased by increment of WS in the liver of *A. latus* ($P < 0.05$). Regarding L. *calcarifer*, the activity of CAT and GPX remarkably decreased from 6 to 24‰, then their activity gradually increased from 35 to 48‰ ($P < 0.05$). However, SOD activity and TBARs content significantly increased from 6 to 24‰ then remarkably decreased in the liver of L. *calcarifer*. Humoral immune responses including lysozyme, alternative complement pathway activity and total immunoglubuns provoked by increment of WS in both species. Stress indices including plasma total protein, glucose, lactate, cortisol and total osmolality in both species remarkably affected by WS. According to the results of the present study the intermediate salinities (brackish water) between 6 and 12‰ are recommended for the culture of these euryhaline species.

1. Introduction

Iran has a great potential for fish mariculture inside net-cages especially in Persian Gulf and Oman sea regions mainly due to suitable environmental, geographical and socio-economic conditions. On the other hand, due to several environmental (*e.g.* climate change, losses of ecosystems, decrease in groundwater levels, long term and frequent droughts and drastic reduction in precipitation) and socio-economic challenges (*e.g.* elevation of demands for freshwater and deterioration of water quality) during recent years, mariculture has been considered as a key production method for supplying high quality protein source. In addition, there are lots of inland water resources in the south of Iran with a wide range of salinities that have a great potential for developing aquaculture especially for euryhaline fish species.

During recent years different native and introduced marine fish species such as yellowfin seabream (*Acanthopagrus latus*) and Asian seabass (*Lates calcarifer*) were considered as candidates for aquaculture diversification in Iran. Yellowfin seabream is a widespread euryhaline

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carnivorous fish species and can tolerate a wide range of salinities from fresh water to hypersaline waters (60‰) (Movahedinia et al., 2009; Eskandari et al., 2013). Asian seabass also is a euryhaline carnivorous species and can tolerate salinities from freshwater to at least 55‰ (Shirgur and Siddiqui, 1998). Also, Asian seabass has been identified as worldwide candidate species for mariculture in tropical and subtropical regions. Both fish species have desirable characteristics for aquaculture such as great resistance to environmental changes (*e.g.* temperature and salinity), tolerance to the high stocking density, suitable growth rate and feed conversion ratio (Mathew, 2009; Vahabnezhad et al., 2016). In addition, during recent years both species have been alternatively cultured in earthen ponds dedicated for shrimp culture in the South of Iran, where shrimp aquaculture extremely suffered from white spot disease.

Euryhaline fish species have a great capacity to keep stable ionic composition of body's fluids in a broad range of WS (Marshall and Grosell, 2006) through morphological, cellular and physiological modification of their gastrointestinal epithelium, kidney and gills (Marshall and Grosell, 2006; Evans, 2008). Furthermore, previous studies demonstrated the effects of WS on a wide range of physiological responses such as growth performance, reproduction, digestive enzymes activity, antioxidant capacity and immune-competence in different euryhaline fish species such as gilthead seabream (*Sparus aurata*, Moutou et al., 2004), yellowfin seabream (Eskandari et al., 2013), large yellow croaker (*Pseudosciaena crocea*, Wang et al., 2016), sable fish (*Anoplopoma fimbria*, Kim et al., 2017) and yellow drum (*Nibae albiflora*, Tian et al., 2020). It has been estimated that from 10 to 50% of the total energy budget of the fish consumed for osmoregulation (Boeuf and Payan, 2001). In this sense, it has been suggested that the least energetic cost of osmoregulation is required in an isotonic environment which results in growth improvement.

Each fish species seems to have species-specific optimum WS for growth and other parameters such as temperature, feed intake, sex and developmental stage, may also affect growth performance (Boeuf and Payan, 2001). It has been proved that digestive capacity of fish can be change due to changes in water drinking rates in different WS that may affect digestive enzymes activities as a consequence of the salinity contents, ions composition and pH of the gut (Giffard-Mena et al., 2006; Psochiou et al., 2007). On the other hand, the alterations of WS can result in stressful condition that could induce the generation of reactive oxygen species (ROS), which may lead to oxidative stress and seriously affect humoral (*e.g.* natural antibodies, lysozyme, peroxidases and complement activity) and cellular immune (*e.g.* phagocytosis and immune cells differentiations) parameters (Dominguez et al., 2005; Yin et al., 2011; Kim et al., 2017). Moreover, the WS over tolerance limits of fish may trigger stressful condition that may compromise immune system and disease resistance (Zhang et al., 2011; Árnason et al., 2013; Choi et al., 2012).

Thus, the current research was carried out to evaluate some selected physiological responses of *A. latus* and L. *calcalifer*.

2. Materials and methods

2.1. Research setup

The current research was performed in the Marine Fish Research Station of the South Iran Aquaculture Research Center (SIARC), Khuzestan, Sarbandar, Iran. The juveniles of *A. latus* were propagated at SIARC facilities; but L. *calcarifer* juveniles were purchased from a private marine fish hatchery and transferred into the lab and stocked into a 5000 L polyethylene tank.

Five WS including 6, 12, 24, 35 and 48‰ were chosen according to the salinity ranges of brackish water bodies (*Ca.* 6–12‰), marine water (*Ca.* 35‰) and hypersaline bays (*Ca.* 50‰) exist in the local water bodies of Khuzestan province (located in the south-west of Iran). The husbandry system was supplied with sand-filtered and disinfected water with the prescribed WS. The prescribed WS were adjusted by diluting water (48‰) of Persian Gulf (Khore Musa bay, North-West of Persian Gulf, Khuzestan, Iran) with fresh water in 5-t polyethylene tanks. After preparation of the prescribed WS they were pumped into the husbandry system.

Two hundred and twenty five *A. latus* (initial weight (BW_i) = 12.5 ± 0.02 g) were randomly stocked into fifteen 250-L cylindrical polyethylene tanks (15 fish in each tank). In addition, 225 of L. *calcarifer* (BW_i = 33.5 ± 0.06 g) juveniles were also distributed into 15 tanks (15 fish in each tank). For each fish species, each WS treatment was replicated in three tanks. Fish were acclimatized with the WS for two weeks. Fish were fed with a commercial feed (size 3.0 mm, 480 g kg^{-1} protein, 150 g kg^{-1} lipid, 100 g kg^{-1} ash, 100 g kg^{-1} moisture, Beyza Feed Mill 21, Shiraz, Iran). After two weeks of acclimation, fish were reared in the prescribed WS for 8 weeks. Fish were fed twice daily (0900 and 1400) with the commercial diet to visual satiation making sure that no feed was left uneaten. The mean values (mean ± standard deviation) for temperature, pH and dissolved oxygen were 25.2 ± 1.5 °C, 8.2 ± 0.2 and 7.1 ± 1.0 mg L^{-1}, respectively. The photoperiod was 12 L: 12D (Light: Darkness) during the trial. The light intensity was supplied by some white light florescent lamps (40 W) that was fixed at 1.5 m above the tanks' surface to provide 700 lx light on the tanks' surface. About 50% of water of system was exchanged with new water daily.

2.2. Sample collection

At the end of the husbandry trial, fish were unfed for 24 h then their weight (g) and length (mm) were individually measured. Six specimens of each tank (18 fish for each treatment) were anesthetized with 2-phenoxyethanol and their blood was collected from their caudal vein with heparinized syringes. The collected blood was centrifuged (4000 *g*, 10 min, at room temperature), and plasma separated. After blood collection, the same fish were sacrificed with an overdose (1000 mg L^{-1}) of the anesthetic to measure their liver and visceral weights for evaluating hepatosomatic index and viscerosomatic index. In addition, the liver and whole gut (pyloric caeca, foregut, midgut and hindgut) of the sacrificed fish were dissected on an ice, transferred into cryotubes and snap-frozen with liquid nitrogen. Finally, cryotubes contained plasma or tissues were kept at −80 °C until their analyses.

2.3. Growth parameters

The following formula were applied for determination of the growth, somatic indices and feed utilization including: weight gain (WG, %) = $((BW_f - BW_i)/BW_i) \times 100$; specific growth rate (SGR, %) = $((\ln BW_f - \ln BW_i) / t) \times 100$, where t is experimental period = 56 days; survival (%) = number of fish in each group remaining on day 56/initial number of fish) × 100; feed intake (FI, g) = total feed intake per tank (g) / number of fish; feed conversion ratio (FCR) = feed intake (g) / weight gain (g); Fulton's condition factor (K, %) = (BW_f / standard lenrth^3) × 100; hepatosomatic index (HSI, %) = (liver weight/ BW_f) × 100 and viscerosomatic index (VSI, %) = (visceral weight/ BW_f) × 100, where BW_i and BW_f were initial and final body weight, respectively.

2.4. Digestive and antioxidant enzymes measurements

The gut samples were homogenized according to Gisbert et al. (2016). The samples were homogenized in ice-cold Mannitol-Tris buffer (1:30, *w/v*) contained 50 mM Mannitol and 2 mM Tris-HCl (pH 7.0) for 30 s. After that 100 μl of $CaCl_2$ (0.1 M) was added to the homogenate and centrifuged (10,000 *g*, 10 min, 4 °C). Finally the supernatants were separated, aliquoted and kept at −80 °C. Total alkaline proteases were assayed using the azo-casein (0.5%) as substrate in Tris-HCL buffer (50 mM, pH 8.0, room temperature) according to Walter (1984). The activity of α-amylase was measured by starch (1% in a buffer contain 0.02 M Na2HPO4 and 0.006 M NaCl, pH 6.9) as substrate using the 3,5-

dinitrosalicylic acid according to Worthington (1991). Bile-salt dependent lipase activity was measured using 0.4 mM 4-nitrophenylmyristate as substrate (at room temperature) according to Gawlicka et al. (2000).

The frozen livers were homogenized in ice-cold physiological buffer saline (1:10, *w/v*; NaCl 0.9%, pH 7.0) then centrifuged (2900 *g*, 15 min, 4 °C) and the supernatants were separated, aliquoted and kept in – 80 °C (Jaroli and Sharma, 2005). The activity of the antioxidant enzymes including glutathione peroxidase (Noguchi et al., 1973), superoxide dismutase (McCord and Fridovich, 1969) and catalase (Abei, 1984) as well as thiobarbituric acid reactive substances (TBARs) concentration (Buege and Aust, 1978) in the liver were measured according to standard methods. Liver enzymes including alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) were measured by means of an autoanalyser (Technicon RA-1000, Technicon Instruments) by applying commercial kits (Pars Azmoon Kit, Iran). Bradford method (Bradford, 1976) used for determining the soluble protein content of the homogenates. All digestive, antioxidant enzymes activities and protein content were measured in triplicate by a microplate scanning spectrophotometer (PowerWave HT, BioTek®, USA). The specific activities of digestive enzymes, antioxidant enzymes and enzymes in the liver (ALT, AST, ALP and LDH) were presented as U mg protein^{-1} of the tissue.

2.5. Plasma immune and biochemical factors

Lysozyme activity in plasma was evaluated by turbidimetric method using lyophilized *Micrococcus luteus* in sodium citrate buffer (0.02 M, pH 5.8) as described by Ellis (1990). Plasma total immunoglobulin (Ig) was measured in plasma by precipitation method (Siwicki et al., 1994). First of all, total protein content in serum and mucus was determined by a commercial kit (Zist Shimi kits, Iran) according to manufacturer's protocol. Then, 100 µl of samples were mixed with 100 µl of 12% polyethylene glycol and agitated for 2 h at room temperature. After that, the mixture was centrifuged and total protein content was determined in the supernatant by the commercial kit. The total Ig content in the samples was calculated by subtracting quantity of protein in the supernatant from the total protein value in the samples.

The alternative complement pathway hemolytic activity (ACH50) was estimated suing rabbit red blood cell (RARBC) as target cells in the presence of EGTA and Mg^{+2} according to Tort et al. (1996). The 50% lysis of RARBC was determined by drawning hemolysis curve graph through plotting the hemolysis degree against the volume of plasma.

Plasma cortisol content was determined by radioimmunoassay (RIA) using a commercial kit (IM1841, Beckman Coulter, Immunotech) (Ellis et al., 2004). Plasma glucose, total protein, lactate and glucose were measured by means of an autoanalyser (Technicon RA-1000, Technicon Instruments) using clinical kits (Pars Azmoon Kit). Plasma osmolality (mOsmol kg^{-1}) was measured using an osmometer (Knauer, K-7400, Germany).

2.6. Statistical assessments

Data were analyzed using the SPSS ver. 16.0 (Chicago, IL, USA). After confirmation of normality and homogeneity of data by kolmogorov-smirnov and Leven tests, respectively, one-way ANOVA and Duncan's multiple-range as post-hoc test were performed. The Pearson product moment correlation test was applied to evaluate any correlation among parameters. The $P < 0.05$ was considered as significant for all statistical tests.

3. Results

3.1. Growth performance

The survival rate in both species was not affected by WS (Tables 1 and 2). Increasing WS from 6 to 12‰ remarkably improved growth

Table 1
Growth, feed utilization and biometric parameters of *A. latus* juveniles reared in water salinities at the end of growth trial (mean ± SEM, $n = 3$ tank).

Treatments	Water salinities (‰)					
	6	12	24	35	48	*P* value
BW_i (g)	12.7 ± 0.03	12.6 ± 0.06	12.7 ± 0.0	12.6 ± 0.02	12.7 ± 0.04	0.184
BW_f (g)	23.3 ± 0.6^{b}	25.3 ± 0.7^{a}	24.8 ± 0.08^{ab}	22.9 ± 0.15^{b}	23.4 ± 0.13^{b}	0.009
TL_f (cm)	9.6 ± 0.05	9.8 ± 0.07	9.5 ± 0.02	9.8 ± 0.05	9.5 ± 0.03	0.315
WG (%)	83.7 ± 5.11^{b}	100.2 ± 4.72^{a}	95.1 ± 0.7^{ab}	81.5 ± 0.94^{b}	84.0 ± 1.61^{b}	0.008
SGR (% BW_i day^{-1})	1.01 ± 0.05^{b}	1.16 ± 0.04^{a}	1.11 ± 0.01^{ab}	0.99 ± 0.01^{b}	1.01 ± 0.01^{b}	0.008
SUR (%)	100 ± 0.0	93.3 ± 0.8	100 ± 0.0	100 ± 0.0	100 ± 0.0	0.072
FI (g $fish^{-1}$)	16.7 ± 0.1	18.5 ± 1.25	17.7 ± 00.16	15.2 ± 1.6	18.2 ± 0.0	0.162
FCR	1.56 ± 0.08	1.58 ± 0.09	1.66 ± 0.03	1.5 ± 0.2	1.7 ± 0.03	0.613
K (%)	3.4 ± 0.06	3.5 ± 0.07	3.4 ± 0.1	3.6 ± 0.17	3.5 ± 0.2	0.696
HSI (%)	1.7 ± 0.15	1.6 ± 0.27	1.8 ± 0.1	2.1 ± 0.21	2.0 ± 0.1	0.323
VSI (%)	8.2 ± 0.53	7.7 ± 0.43	8.0 ± 0.74	9.4 ± 0.45	8.4 ± 0.33	0.217

Abbreviation: BW_i: initial body weight; BW_f: final body weight; SL_f: final standard length; WG: weight gain; SGR: specific growth rate; SUR: survival FI: feed intake; FCR: feed conversion ratio; K: Fulton's condition factor; HSI: hepatosomatic index; VSI: viscerosomatic index.
A different superscript in the same row denotes statistically significant differences ($P < 0.05$).

Table 2
Growth, feed utilization and biometric parameters of *Lates calcarifer* juveniles reared in water salinities at the end of growth trial (mean ± SEM, n = 3 tank).

Treatments	Water salinities (‰)					
	6	12	24	35	48	P value
BW_i (g)	34.2 ± 0.11	33.8 ± 0.02	34.0 ± 0.08	33.9 ± 0.02	33.8 ± 0.06	0.09
BW_f (g)	69.1 ± 0.34^{a}	69.1 ± 0.4^{a}	66.1 ± 0.27^{b}	66.2 ± 0.11^{b}	60.4 ± 0.53^{c}	0.001
TL_f (cm)	16.2 ± 0.21	16.0 ± 0.17	15.9 ± 0.48	15.6 ± 0.46	15.5 ± 0.31	0.627
WG (%)	102.2 ± 1.69^{a}	104.6 ± 1.31^{a}	94.5 ± 1.23^{b}	95.1 ± 1.44^{b}	78.9 ± 1.9^{c}	0.001
SGR (% BW_i day^{-1})	1.17 ± 0.01^{a}	1.19 ± 0.01^{a}	1.10 ± 0.01^{b}	1.11 ± 0.02^{b}	0.97 ± 0.01^{c}	0.001
SUR (%)	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	1.000
FI (g $fish^{-1}$)	37.6 ± 0.3	35.8 ± 1.6	33.2 ± 2.66	36.3 ± 0.53	34.0 ± 0.4	0.118
FCR	1.07 ± 0.01^{a}	1.01 ± 0.03^{a}	1.04 ± 0.09^{a}	1.12 ± 0.01^{a}	1.28 ± 0.01^{b}	0.006
K (%)	2.05 ± 0.04	2.1 ± 0.08	2.1 ± 0.02	2.13 ± 0.06	2.24 ± 0.1	0.369
HSI (%)	1.2 ± 0.12	1.5 ± 0.17	1.5 ± 0.1	1.6 ± 0.23	1.5 ± 0.15	0.147
VSI (%)	7.5 ± 0.17	6.3 ± 0.39	6.9 ± 0.62	8.1 ± 0.84	8.6 ± 0.48	0.072

Abbreviation: BW_i: initial body weight; BW_f: final body weight; SL_f: final standard length; WG: weight gain; SGR: specific growth rate; SUR: survival FI: feed intake; FCR: feed conversion ratio; K: Fulton's condition factor; HSI: hepatosomatic index; VSI: viscerosomatic index.
A different superscript in the same row denotes statistically significant differences ($P < 0.05$).

performance in *A. latus* juveniles; however, augmentation of WS over 24‰ reduced growth parameters in this species. Feed intake, FCR and somatic indices including K, HSI and VSI did not change by WS in *A. latus* ($P > 0.05$). Regarding L. *calcarifer,* increasing WS over 12‰ gradually decreased growth parameters and fish reared at 48‰ had the lowest BW_f (60.4 g) and SGR (0.97% BW_i day^{-1}) ($P < 0.05$, Table 2). Feed consumption did not affect by WS, but FCR (1.28) remarkably increased in L. *calcarifer* reared at 48‰. Somatic indices were not changed by WS. There were negative correlations between WS and BW_f ($r = -0.924$; $P = 0.0001$), SGR ($r = -0.829$; $P = 0.0001$) and WG ($r = -0.895$; $P = 0.0001$), but the correlation between WS and FCR was positive ($r = 0.691$; $P = 0.004$). There were also remarkable negative correlations between FCR and BW_f ($r = -0.757$; $P = 0.001$), SGR ($r = -0.773$; $P = 0.001$) and WG ($r = -0.769$; $P = 0.001$).

3.2. Digestive enzymes

Digestive enzymes activities strikingly affected by WS in both species (Table 3). In *A. latus*, fish reared at 24 and 35‰ had the greatest total protease activity, but those in 6‰ had the lowest value ($P < 0.05$). Lipase activity in *A. latus* gradually increased with enhancing WS up to 35‰ then it was decreased. The activities of total protease and lipase in L. *calcarifer* increased with enhancing WS form 6 up to 24‰ then they were gradually decreased ($P < 0.05$). The Pearson product-moment correlation method also revealed that WS was positively correlated to lipase ($r = 0.800$; $P = 0.0001$) and protease ($r = 0.693$; $P = 0.004$) activities, respectively in *A. latus* and L. *calcarifer*. The activity of α-amylase was not affected by WS in both species.

3.3. Antioxidant enzymes

The activity of CAT in the liver of *A. latus* juveniles pronouncedly decreased with increasing WS (Table 4). The GPX activity and TBARs content were gradually increased by increment of WS in the liver of *A. latus* ($P < 0.05$). The SOD activity in the liver of *A. latus* increased with the increment of WS up to 35‰ then it was decreased at 48‰. Moreover, WS positively correlated with SOD ($r = 0.799$; $P = 0.0001$), GPX ($r = 0.918$; $P = 0.0001$) and TBARs ($r = 0.950$; $P = 0.0001$), but its correlation with CAT was negative ($r = -0.927$; $P = 0.0001$). Regarding L. *calcarifer*, the activity of CAT and GPX remarkably decreased from 6 to 24‰, then their activity gradually increased from 35 to 48‰ ($P < 0.05$). There were also negative correlations between WS and CAT ($r = -0.668$; $P = 0.006$) and GPX ($r = -0.606$; $P = 0.018$). However, SOD activity and TBARs content significantly increased from 6 to 24 then remarkably decreased in the liver of L. *calcarifer*. Positive correlation among WS and SOD ($r = 0.648$; $P = 0.009$) as well as TBARs ($r = 0.654$; $P = 0.005$) content in the liver was found in L. *calcarifer*.

Table 3
Digestive enzymes activities (U mg $protein^{-1}$) (mean ± SE, n = 3 tank) of *A. latus* and *Lates calcarifer* juveniles reared in water salinities at the end of growth trial.

Treatments	Water salinities (‰)					
	6	12	24	35	48	P value
A. latus						
Total protease	0.86 ± 0.01^{d}	1.05 ± 0.01^{c}	1.89 ± 0.0^{a}	1.97 ± 0.0^{a}	1.14 ± 0.0^{b}	0.001
α-amylase	0.18 ± 0.0	0.17 ± 0.0	0.19 ± 0.0	0.19 ± 0.0	0.18 ± 0.0	0.452
Lipase	2.14 ± 0.02^{d}	2.25 ± 0.01^{c}	2.5 ± 0.04^{b}	2.95 ± 0.02^{a}	2.63 ± 0.0^{ab}	0.001
L. calcarifer						
Total protease	0.98 ± 0.01^{d}	1.03 ± 0.18^{d}	1.69 ± 0.05^{a}	1.53 ± 0.02^{b}	1.46 ± 0.08^{c}	0.001
α-amylase	0.13 ± 0.0	0.14 ±0.0	0.13 ± 0.0	0.13 ± 0.0	0.13 ± 0.0	0.772
Lipase	2.1 ± 0.06^{d}	2.36 ± 0.02^{b}	2.92 ± 0.17^{a}	2.23 ± 0.04^{c}	2.18 ± 0.08^{cd}	0.001

A different superscript in the same row denotes statistically significant differences ($P < 0.05$).

Table 4
Liver antioxidant (U mg $protein^{-1}$) (mean ± SE, n = 3 tank) and glutathione levels (nmol g^{-1}tissue) of *A. latus* and *Lates calcarifer* juveniles reared in water salinities at the end of growth trial.

Treatments	Water salinities (‰)					
	6	12	24	35	48	P value
A. latus						
CAT	1.27 ± 0.02^{a}	1.15 ± 0.03^{b}	0.95 ± 0.0^{c}	0.67 ± 0.07^{d}	0.74 ± 0.03^{d}	0.001
GPX	2.22 ± 0.03^{d}	2.45 ± 0.04^{c}	2.85 ± 0.01^{b}	3.51 ± 0.1^{a}	3.32 ± 0.07^{a}	0.001
SOD	2.74 ± 0.01^{d}	2.92 ± 0.01^{c}	3.02 ± 0.0^{c}	4.21 ± 0.07^{a}	3.68 ± 0.03^{b}	0.001
TBARs	0.45 ± 0.03^{d}	0.59 ± 0.02^{c}	0.98 ± 0.02^{b}	2.01 ± 0.02^{a}	2.0 ± 0.04^{a}	0.001
L. calcarifer						
CAT	2.04 ± 0.01^{a}	1.69 ± 0.0^{b}	0.81 ± 0.02^{e}	1.04 ± 0.01^{d}	1.24 ± 0.01^{c}	0.001
GPX	2.92 ± 0.0^{a}	2.53 ± 0.0^{b}	2.03 ± 0.01^{e}	2.17 ± 0.0^{d}	2.37 ± 0.0^{c}	0.001
SOD	1.64 ± 0.01^{e}	2.31 ± 0.01^{d}	3.55 ± 0.03^{a}	3.22 ± 0.01^{b}	2.87 ± 0.02 ^{c}	0.001
TBARs	0.69 ± 0.0^{e}	0.94 ± 0.0^{d}	1.75 ± 0.01^{a}	1.56 ± 0.0^{b}	1.4 ± 0.0^{c}	0.001

Abbreviations: CAT: catalase; GPX: glutathione peroxidase; SOD: superoxide dismutase; GSH: glutathione; $TBAR_S$: thiobarbituric acid reactive substances.
A different superscript in the same row denotes statistically significant differences ($P < 0.05$).

3.4. Liver enzymes

The activities of ALP, ALT and AST in the liver of *A. latus* gradually increased with augmentation of WS (Table 5). The values of LDH activity in the liver of *A. latus* reared at 12 to 35‰ WS were higher than other groups ($P < 0.05$). There were positive correlations between WS and ALP ($r = 0.931$; $P = 0.0001$), ALT ($r = 0.952$; $P = 0.0001$) and AST ($r =$

Table 5
Liver enzymes (U mg $protein^{-1}$) (mean ± SE, n = 3 tank) of *A. latus* and *Lates calcarifer* juveniles reared in water salinities at the end of growth trial.

Treatments	Water salinities (‰)					
	6	12	24	35	48	P value
A. latus						
ALP	0.93 ± 0.0^{d}	1.08 ± 0.02^{c}	1.49 ± 0.0^{b}	1.79 ± 0.02^{a}	1.72 ± 0.03^{a}	0.001
ALT	1.34 ± 0.01^{c}	1.41 ± 0.02^{c}	1.87 ± 0.01^{b}	2.83 ± 0.06^{a}	2.7 ± 0.02^{a}	0.001
AST	1.1 ± 0.01^{c}	1.16 ± 0.02^{c}	1.56 ± 0.03^{b}	1.9 ± 0.04^{a}	1.93 ± 0.2^{a}	0.001
LDH	0.33 ± 0.0^{c}	0.48 ± 0.0^{a}	0.52 ± 0.02^{a}	0.51 ± 0.02^{a}	0.4 ± 0.01^{b}	0.001
L. calcarifer						
ALP	0.66 ± 0.01^{e}	0.84 ± 0.02^{d}	1.9 ± 0.06^{a}	1.53 ± 0.02^{b}	1.35 ± 0.01^{c}	0.001
ALT	1.53 ± 0.02^{e}	2.15 ± 0.04^{d}	3.8 ± 0.11^{a}	3.15 ± 0.03^{b}	2.74 ± 0.01^{c}	0.001
AST	1.01 ± 0.0^{d}	1.43 ± 0.02^{c}	2.02 ± 0.07^{a}	1.74 ± 0.02^{b}	1.51 ± 0.02 ^{c}	0.001
LDH	0.49 ± 0.0^{c}	0.51 ± 0.01^{c}	1.3 ± 0.05^{a}	1.03 ± 0.01^{b}	0.95 ± 0.0^{b}	0.001

Abbreviations: ALP: alkaline phosphatase; ALT: alanine aminotransferase; AST: aspartate aminotransferase; LDH: lactate dehydrogenase.
A different superscript in the same row denotes statistically significant differences ($P < 0.05$).

0.959; $P = 0.0001$). Regarding L. *calcarifer* juveniles, the activities of ALP, ALT, AST and LDH increased in the liver form 6 to 24‰, then their activities gradually decreased with increment of WS ($P < 0.05$). Furthermore, the activities of liver enzymes in fish reared at lower WS including 6 and 12‰ were lower than those reared at higher WS (35 and 48‰). According to the Pearson test WS had positive correlations with ALP ($r = 0.607$; $P = 0.016$), ALT ($r = 0.560$; $P = 0.03$) and LDH ($r = 0.623$; $P = 0.013$).

3.5. Humoral immune parameters

The humoral immune parameters pronouncedly affected by WS in both species (Figs. 1 and 2). Plasma lysozyme activity in *A. latus* linearly increased with the increment of WS from 6 to 35‰ then it was decreased at 48‰ (Fig. 1a). The plasma ACH50 (Fig. 1b) and total Ig content (Fig. 1c) in *A. latus* noticeably increased with enhancement of WS ($P < 0.05$). Moreover, WS positively correlated with plasma lysozyme ($r = 0.777$; $P = 0.0001$), ACH50 ($r = 0.925$; $P = 0.0001$) and total Ig ($r = 0.823$; $P = 0.0001$). There were also negative correlations between plasma cortisol level and plasma ACH50 ($r = -0.552$; $P = 0.033$) and total Ig ($r = -0.512$; $P = 0.051$). Regarding L. *calcarifer*, plasma lysozyme activity (Fig. 2a) increased from 6 to 24‰ then its activity reduced in fish reared at 35 and 48‰. Plasma ACH50 (Fig. 2b) and total Ig content (Fig. 2c) were increased with enhancing WS from 6 to 35‰ then they were remarkably reduced in fish reared at 48‰. Positive correlations also found among WS and plasma lysozyme ($r = 0.525$; $P = 0.028$), ACH50 ($r = 0.822$; $P = 0.0001$) and total Ig ($r = 0.712$; $P = 0.003$). In addition, the correlations among plasma cortisol and lysozyme ($r = 0.956$; $P = 0.0001$), ACH50 ($r = 0.793$; $P = 0.0001$) and total Ig ($r = 0.872$; $P = 0.0001$) were positive.

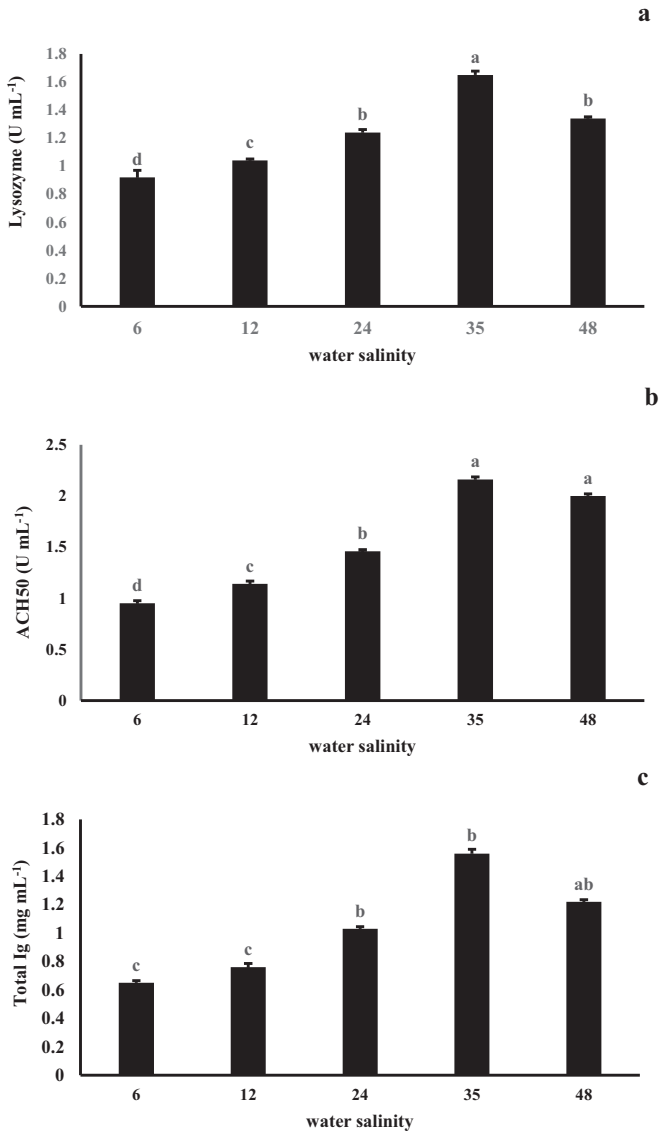

Fig. 1. Effect of water salinity (6, 12, 24, 35 and 48‰) on plasma immune parameters of *A. latus* including: (a) lysozyme (b) ACH50 and (c) total Ig. (Values are presented as the mean ± S.E., $n = 9$). Different superscripts on the bars denotes statistically significant differences ($P < 0.05$).

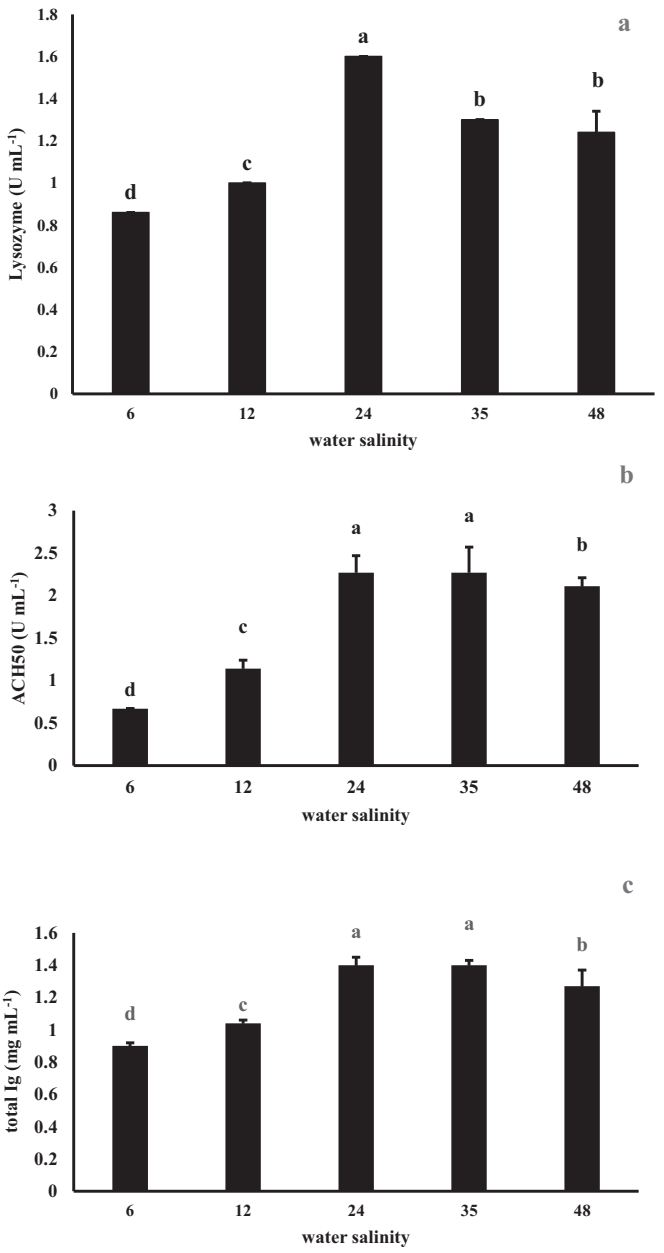

Fig. 2. Effect of water salinity (6, 12, 24, 35 and 48‰) on plasma immune parameters of L. *calcarifer* including: (a) lysozyme (b) ACH50 and (c) total Ig. (Values are presented as the mean ± S.E., n = 9). Different superscripts on the bars denotes statistically significant differences ($P < 0.05$).

3.6. Plasma biochemical parameters

Plasma total protein in *A. latus* linearly increased with the

Table 6
Plasma biochemical parameters (mean ± SE, n = 3 tank) of *A. latus* and *Lates calcarifer* juveniles juvenile reared in water salinities at the end of growth trial.

Treatments	Water salinities (‰)					
	6	12	24	35	48	P value
A. latus						
Total protein (g L^{-1})	7.6 ± 0.11^{c}	8.3 ± 0.0^{b}	8.4 ± 0.08^{b}	9.2 ± 0.02 ^{a}	8.9 ± 0.01^{a}	0.001
Lactate (mmol L^{-1})	1.36 ± 0.0^{e}	1.46 ± 0.0^{d}	1.88 ± 0.0^{c}	2.85 ± 0.01^{a}	2.14 ± 0.0^{b}	0.001
Glucose (mmol L^{-1})	4.24 ± 0.0^{d}	4.36 ± 0.0^{c}	4.69 ± 0.0^{b}	4.77 ± 0.0^{a}	4.66 ± 0.02^{b}	0.001
Cortisol (nmol L^{-1})	2.35 ± 0.0^{b}	2.44 ± 0.02^{a}	2.46 ± 0.0^{a}	2.30 ± 0.0^{c}	2.32 ± 0.0^{bc}	0.001
Total osmolality (mOsm Kg^{-1})	519.3 ± 8.8^{d}	552.7 ± 5.84^{c}	575.7 3.48± ^{b}	598.6 ± 4.1^{a}	609.3 ± 0.88^{a}	0.001
L. calcarifer						
Total protein (g L^{-1})	7.7 ± 1.0^{b}	7.7 ± 0.14^{b}	8.1 ± 0.15^{a}	7.8 ± 0.13^{b}	6.5 ± 0.07^{c}	0.001
Lactate (mmol L^{-1})	1.97 ± 0.0^{d}	2.07 ± 0.01^{c}	2.36 ± 0.0^{a}	2.22 ± 0.0^{b}	2.12 ± 0.0^{c}	0.001
Glucose (mmol L^{-1})	4.18 ± 0.02^{d}	4.34 ± 0.02^{c}	4.9 ± 0.02^{a}	4.58 ± 0.01^{b}	4.5 ± 0.01^{b}	0.001
Cortisol (nmol L^{-1})	2.5 ± 0.0^{c}	2.58 ± 0.02^{c}	2.88 ± 0.02^{a}	2.72 ± 0.01 ^{b}	2.61 ± 0.01^{c}	0.001
Total osmolality (mOsm Kg^{-1})	447.3 ± 0.88^{e}	467.0 ± 0.57^{d}	491.6 ± 0.33^{c}	500.6 ± 0.88^{b}	513.0 ± 1.0^{a}	0.001

A different superscript in the same row denotes statistically significant differences ($P < 0.05$).

augmentation of WS (Table 6). The contents of plasma lactate and glucose were gradually increased from 6 to 35‰ then their contents significantly decreased in fish reared at 48‰ ($P < 0.05$). The plasma cortisol level in fish reared at 12 and 24‰ was higher than the other groups. Moreover, WS positively correlated with plasma total protein ($r = 0.751$; $P = 0.001$), glucose ($r = 0.759$; $P = 0.001$), lactate ($r = 0.820$; $P = 0.001$) and osmolality ($r = 0.949$; $P = 0.0001$). There were also negative correlations among plasma cortisol and plasma total protein ($r = -0.712$; $P = 0.003$). Regarding *L. calcarifer*, the contents of plasma total protein, lactate, glucose and cortisol gradually increased from 6 to 24‰, then their concentrations gradually decreased with increasing WS ($P < 0.05$). There was also a negative correlation between WS and plasma total protein ($r = -0.617$; $P = 0.014$). Plasma total osmolality linearly increased with the enhancement of WS and there was a positive correlation between them ($r = 0.965$; $P = 0.0001$).

4. Discussion

4.1. Growth and feed utilization

It has been reported that the blood osmolality of euryhaline fish species is about 12‰ (Mylonas et al., 2009). Furthermore, a plethora of research demonstrated that the growth of euryhaline fish species enhance in intermediate salinities because these WS closer to the isosmotic point that consequently reduce metabolic demands for osmoregulation and may channel more energy for growth (Woo and Kelly, 1995; Imsland et al., 2001; Mylonas et al., 2009; Boeuf and Payan, 2001). In addition, it has been speculated that WS can affect stimulation of osmoregulatory hormones related to growth (*e.g.* growth hormone and insulin-like growth factor) (McCormick, 2001; Yada et al., 2012). Our findings indicated the intermediate WS (*i.e.* brackish water) elevate growth performance in both species as also reported in different euryhaline fish species such as golden-line sea bream (*Sparus sarba*, Woo and Kelly, 1995), black sea bream (*Mylio macrocephalus*, Kelly et al., 1999), turbot (*Scopthalmus maximus*, Imsland et al., 2001), black bream (*Acanthopagrus butcheri*, Partridge and Jenkins, 2002), gilthead seabream (Laiz-Carrión et al., 2005), red seabream (*Pagrus pagrus*, Vargas-Chacoff et al., 2011), opossum pipefish, (*Microphis brachyurus*, Martinez-Cardenas et al., 2014) and European seabass (*Dicentrarchus labrax*, Goda et al., 2019).

In the present study WS did not affect FI in both species as previously reported in Atlantic cod *Gadus morhua* (Lambert et al., 1994), cobia (*Rachycentron canadum*, Chen et al., 2009) and black bream, *Acanthopagrus butcheri*, reared from 12 to 48‰ (Partridge and Jenkins, 2002). However, some studies reported that increasing WS remarkably reduced FI in gilthead seabream (Conides et al., 1997) and Atlantic halibut (*Hippoglossus hippoglossus* L.; Imsland et al., 2008). The results of the present study showed that FCR remarkably increased in L. *calcalifer* reared at 48‰ that may be resulted from alternations of the gut evacuation rate due to extra drinking water rate, low retention of nutrients and high excretion of metabolites at hyperosmotic environment (Lambert et al., 1994; Barman et al., 2005). Similar results also demonstrated in fish reared at high WS such as black bream (60‰, Partridge and Jenkins, 2002), Atlantic halibut (32‰, Imsland et al., 2008) and yellow drum (*Nibea albiflora*; 42‰; Tian et al., 2020). Furthermore, Barman et al. (2005) reported that FCR values linearly enhanced with increasing WS from 10 to 30‰ that resulted in linear growth reduction in gray mullet (*Mugil cephalus*) fry.

4.2. Digestive enzymes activities

It has been speculated that WS can affect the activation of each enzyme's zymogen separately as it takes place in the gut lumen and it can alter the gut's physicochemical condition by changing the pH, ion concentrations or ions composition of the gut contents (Usher et al., 1988; Boeuf and Payan, 2001; Moutou et al., 2004). Moreover, rearing fish at different WS may alter drinking rates that consequently affect the salinity of the gut contents, gut evacuation rate and it presumably could affect digestive enzyme activity (Usher et al., 1988). In the present study, the activities of total proteases and lipase enhanced with increasing WS in *A. latus* and L. *calcarifer* up to 35 and 24‰, respectively then their activities were reduced at higher WS. Similarly, Moutou et al. (2004) reported that the activities of pancreatic proteases (*e.g.* total alkaline proteinase and chymotrypsin) were lower in gilthead sea bream reared at 20‰ compared to 33‰, but growth performance was better in fish reared at 20‰. In addition, Woo and Kelly (1995) reported golden-line seabream reared at 7‰ had higher trypsin activity compared to those exposed to 15 and 35‰, but fish reared at 15‰ had higher growth rate than other groups. Thus, these results suggested that better growth rate of fish could not be explained by changes in digestive capacity and could be related to reduction of metabolic cost of osmoregulation (Woo and Kelly, 1995; Moutou et al., 2004).

4.3. Oxidative stress responses

It has been reported that the alterations in WS can induce ROS generation and result in oxidative stress in aquatic animals (Lushchak, 2011). Our knowledge is restricted about the effects of WS on fish oxidative stress responses and previous studies demonstrated contradictory findings regarding effects of WS on antioxidant enzymes activities in different fish species (Ma et al., 2016; Kim et al., 2017; Tian et al., 2020). In the present study, the levels of TBARs linearly increased in the liver of both species with enhancing WS that was attributed to increment of the liver enzymes (*e.g.* ALP, ALT, AST and LDH), plasma glucose and lactate levels suggesting an oxidative stress in both species with increasing WS. In agreement with these results, Sui et al. (2016) reported that SOD level in the blood parrotfish (*Cichlasoma synspilum* ♀ × *C. citrinellum* ♂) increased with enhancing WS, but its activity reduced after 168 h of adaptation period. Furthermore, it has been reported that reduction of WS remarkably reduced SOD activity in sablefish

(*Anoplopoma fimbria*, Kim et al., 2017) and CAT activity in golden pompano (*Trachinotus ovatus,* Ma et al., 2016). In contrast, Martinez-Alvarez et al. (2002) reported that SOD and CAT activities pronouncedly decreased in the liver of Adriatic sturgeon (*Acipenser naccarii*) as WS rose. In addition, our results demonstrated that CAT activity in the liver decreased with increasing WS in both species. In accordance with these results, Tian et al. (2020) reported that CAT activity in the liver reduced with increasing WS over 6‰, but SOD activity and lipid peroxidation in the liver was not affected by WS in yellow drum. These findings suggesting that the oxidative stress response of fish to alternations of WS are species-specific and may affected by WS, developmental stage, experimental condition and the adaptation period of fish.

4.4. Liver health condition

Liver enzymes activities are considered as sensitive biomarkers in response to the presence and/or magnitude of stress, water pollutants and diseases and can reflect the health status of fish (Wagner and Congleton, 2004). Alkaline phosphatase is a cytoplasmic membrane-mediated glycoprotein enzyme and considered as a valuable biomarker to assess the integrity of plasma membrane and endoplasmic reticulum (Wagner and Congleton, 2004). In the present study, ALP activity in liver of *A. latus* increased with enhancing WS suggesting high salinity can trigger stress condition in this species, but regarding L. *calcarifer,* intermediate (24‰) WS may be compromised fish welfare. In this sense, Tian et al. (2020) reported that the activity of ALP in the liver of yellow drum elevated with increasing WS from 6‰ to 30‰ then it was remarkably decreased in fish reared at 42‰.

Alanine aminotransferase and AST are vital enzymes in transferring L-amino acids for gluconeogenesis and they are mediators between carbohydrate and protein metabolism (Rodwell, 1988). In the present study the activity of ALT and AST increased with enhancing WS in *A. latus*; but in L. *calcarifer*, the activity of these enzymes enhanced up to 24‰ then gradually decreased. These data indicate that both species metabolized amino acids derived from proteolysis or uses exogenous amino acids as a fuel source for gluconeogenic activity to cope with stressful condition. In accordance with these findings, Fazio et al. (2013) reported that the values of AST and ALT in the liver of gray mullet enhanced with increasing levels WS and they suggested such increment in amount of liver enzymes indicated the high permeability of the hepatocytes and cellular leakage. Furthermore, Vargas-Chacoff et al. (2009) reported that elevation of WS from 5‰ to 55‰ at 19 and 26 °C pronouncedly increased ALT in the liver of gilthead seabream; however, the activity of AST in the liver increased from 5‰ to 35‰ then decreased at 19 °C suggesting that the activity of these enzymes not only depends on WS, but also other environmental factors and health status of fish may affect them.

Lactate dehydrogenase has a key role in converting lactate, the major by-product of anaerobic glycolysis, to pyruvate *via* oxidation in the presence of NADH (Powers et al., 1991). In the present study LDH activity in *A. latus* and L. *calcarifer* increased to the maximum level at 35 and 24‰, respectively and then decreased smoothly indicating that the anaerobic metabolism was highest in these WS. Furthermore, increase in the activity of LDH in the liver associated with the amount of lactate in plasma in both species and their correlation was positive indicating anaerobic metabolism of fish in these WS for providing extra energy for the body to acclimatize to WS.

4.5. Humoral immune responses

There is a tight relationships between immunity, osmoregulation and endocrine system in fish (Yada et al., 2012). In the present study plasma lysozyme activity increased in *A. latus* with increasing WS but then it was decreased in fish reared at 48‰, but in *L. calcarifer* plasma lysozyme increased from 6 to 24‰ then it was decreased suggesting non-specific humoral immunity can be modified by alternation of WS and this response is species-specific. In this sense it has been reported that increasing WS enhanced serum/plasma lysozyme activity in brown trout (Marc et al., 1995), rainbow trout (Yada et al., 2001; Fast et al., 2002), Nile tilapia (Dominguez et al., 2005) and sablefish (Kim et al., 2017). Furthermore, Jiang et al. (2008) reported that abrupt transferring of Mozambique tilapia (*Oreochromis mossambicus*) from fresh water into hyperosmotic water (25‰) has immunostimulatory effects on its cellular (phagocytosis and respiratory burst activity) and humoral reactions (lysozyme and complement activities). In agreement with results observed in *A. latus* it has been reported that increasing WS remarkably increased plasma IgM in Nile tilapia (Elarabany et al., 2017) and Mozambique tilapia (Yada et al., 2002). In addition, in accordance with the findings observed in L. *calcarifer*, Yanjiao et al. O'Neill et al. (2011) reported that rearing of turbot (*Scophthalmus maximus* L.) at the intermediate salinity (20‰) enhanced the serum lysozyme activity, ACH50 and the head-kidney macrophages phagocytosis and disease resistance against *V. anguillarum,* but high WS (40‰) suppressed these immune parameters. Also, Cuesta et al. (2005) reported that hypo-osmotic acclimation (6‰) has a negative effect on plasma total IgM and peroxidases, while hyper-osmotic acclimation (55‰) provoked plasma total IgM level, but reduced ACH50 in gilthead seabream. In contrast, Choi et al. (2012) reported that abrupt reduction in WS from 20 to 5‰ pronouncedly trigged cellular immune responses including phagocytosis and number of circulating neutrophils and monocytes but reduced the number of circulating lymphocytes in Nile tilapia. These results might be explained by the effects of osmoregulatory hormones and mediatory role of osmoregulatory organs in the immune responses.

4.6. Plasmatic health indices

Serum/plasma total protein level is considered as a valuable indicator for general health and stress status of fish as it contains a wide range of proteic components including albumin, immunoglobulins, complements, enzymes, cytokines, transferrin and lectins (Magnadottir, 2006). In the present study, plasma total protein increased with increment of WS in *A. latus* and it was associated with increasing humoral immune parameters in this species suggesting WS provoked humoral immune responses in this species. However, plasma protein in L. *calcarifer* increased with augmentation of WS up to 24‰ and then it was decreased suggesting proteins oxidized for gluconeogenesis and providing energy for osmoregulation at high WS. This result is in agreement with other studies where serum total protein content remarkably declined with increasing WS (Kelly et al., 1999; Martinez-Alvarez et al., 2002).

The plasma glucose in *A. latus* and L. *calcarifer* specimens reared at 35 and 24‰, respectively was higher than other groups that may be related to the increasing transfer of metabolites as a stored fuel source. In addition, the reason why the amount of plasma glucose reduced in fish reared at 6–12‰ especially in L. *calcarifer* may be related to an increased need for energy substrate to fuel the higher growth seen in these treatments that resulted in plasma glucose depletion (Iwama, 1998).

In the present study, plasma cortisol was slightly (*Ca.* 0.1 nmol L^{-1}) increased in *A. latus* reared at 12 and 24‰ than other groups. In addition, plasma cortisol in L. *calcarifer* reared at 24‰ was higher (*Ca.* 0.3 nmol L^{-1}) than the other groups that was coincided with the increment of plasma lactate and intercede with hyperglycemia. These results, suggesting chronic stress situation occurred in both fishes reared at 24‰, but increasing plasma cortisol in *A. calcarifer* was associated with hyperglycemia indicating species-specific stress response to WS.

The measurement of blood osmolality and ion levels following alternation of WS can provide information regarding the osmoregulatory capacity of fish and as a predictor of successful survival and growth in a saline environment (Stewart et al., 2016). In the present study, the change in plasma osmolality in both species in different WS is a response to the external WS and it was remarkably increased at 48‰, indicative of

the strong osmoregulatory capacity of the fish. Similar findings have been observed in other euryhaline fish such as black bream (Partridge and Jenkins, 2002), gilthead seabream (Laiz-Carrión et al., 2005), rabbitfish (*Siganus rivulatus,* Saoud et al., 2007) and red seabream (Vargas-Chacoff et al., 2011).

5. Conclusion

In conclusion, the findings of the present study clearly demonstrated that both species have high osmoregulation capacities in tolerating a wide range of WS. In addition, growth performance of both species were higher at intermediate WS. The activity of total proteases and lipase in both species elevated with increasing by elevation of WS, but there were not a positive correlation between increasing digestive enzymes and growth rate that suggesting better growth rate of fish at intermediate WS mainly related to the reduction of metabolic cost of the osmoregulation. In addition, elevation of WS induced oxidative stress and enhanced liver antioxidant enzymes in both species indicating high WS compromised fish welfare and induce more energy consumption. Moreover, our findings indicated increment of WS induced humoral immune responses in both species that may suggest inflammatory response of fish under chronic stress that may retard growth rate. By considering the results of the growth parameters, lipid peroxidation index (TBARs), liver enzymes and stress indices culture of these species in brackish would be resulted in better performance and would not compromise fish welfare.

Authors statements

Mansour Torfi Mozanzadeh: Conceptualization; Data curation; Software; Formal analysis; Investigation; Visualization and Writing - original draft.

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Declaration of Competing Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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