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Effects of dietary salt supplementation on growth, body composition, tissue electrolytes, and gill and intestinal Na^+/K^+ ATPase activities of black sea bass reared at low salinity

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ABSTRACT

Two feeding trials were conducted to investigate the effects of dietary sea salt supplementation on growth and survival of black sea bass *Centropristis striata* reared under sub-optimal salinity conditions at 15 and 10 g L^{-1}. In experiment 1, six iso-nitrogenous (46% crude protein) and iso-lipidic (10% lipid) test diets containing a mineral premix were formulated to supplement graded levels (0, 2.5, 5, 7.5, 10, and 12.5% dry wt.) of natural sea salt (99.9% NaCl). Diets were fed twice daily to juveniles (mean wt. = 8 g) held in triplicate 75-L tanks at a salinity level of 15 g L^{-1} under controlled laboratory conditions for 56 days. In experiment 2, six natural salt-based diets as in experiment 1 and another diet with 0% salt and no mineral mix were formulated. Diets (experiment 2) were fed to triplicate groups of fish (mean wt. = 9.37 g) held at 10 g L^{-1} salinity for 70 days. In both experiments, final survival was 78–97%, with no treatment differences. At 15 g L^{-1} (experiment 1), no differences in weight gain were observed among dietary salt treatments. In contrast, a 5% salt diet significantly ($P < 0.05$) improved fish growth (vs 0% dietary salt) of fish raised at 10 g L^{-1} (experiment 2), a salinity producing lower plasma osmolality (237–285 mOsm L^{-1}) and osmoregulatory stress than at 15 g L^{-1} (330–350 mOsm L^{-1}). At the end of experiment 2, fish fed >5% salt at 10 g L^{-1} showed higher survival when challenged with a further reduction in salinity to 4 g L^{-1}. Gill Na^+/K^+ ATPase activities appeared to be higher in fish fed the diet with highest level (12.5%) of supplemental salt, whereas intestinal Na^+/K^+ ATPase activity appeared to be maximal at lower levels of dietary salt. Irrespective of dietary salt treatment, black sea bass juveniles maintained good growth and survival and normal whole body electrolyte concentrations at rearing salinities of 10 and 15 g L^{-1}, indicating that these fish are good osmoregulators and could be potentially cultured under these low salinities. The results indicate that dietary salt supplementation at 5% improved growth and averted mortality under low salinity conditions. These findings may enable black sea bass to be grown in recirculating aquaculture systems in low-salinity brackish water.

Relevance with commercial aquaculture: These results indicate that dietary salt supplementation may be used to avert mortality when a producer is faced with adversely low salinity conditions due to weather and tides as may occur in coastal black sea bass fish farms sourcing brackish water from tidal creeks.

1. Introduction

Due to high market price and demand and increasingly restrictive fishing regulations, development of culture techniques for black sea bass *Centropristis striata* from the Atlantic coast populations has been the focus of aquaculture studies from Florida to New Hampshire. The University of North Carolina Wilmington (UNCW) research results on black sea bass aquaculture have been promising. They show that black sea bass can be bred reliably in captivity, raised from fingerling to adult stages in recirculating aquaculture system (RAS) using sustainable, low fish meal-based diets, and that cultured product can garner lucrative niche markets (Berlinsky et al., 2001; Watanabe et al., 2003, Watanabe, 2011; Wilde et al., 2008; Alam et al., 2008, 2012). Interest in commercial production of black sea bass is developing among private growers in Eastern USA such as, North Carolina, Virginia, and Maine (Watanabe, 2011). Currently, startup growers in these states utilize coastal property with direct access to full-strength seawater for their production facilities. In North Carolina, however, coastal land away from open waters in headwaters of tidal creeks provides sites that are more available and affordable to aquaculturists. These coastal sites provide direct access to continuous sources of brackish water and they offer significant near-term opportunities to establish commercial recirculating aquaculture system (RAS) production facilities for marine finfish. They do not require

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access to full strength seawater, or sophisticated (and more costly) zero-discharge technology to be economically viable. Production of fish using low salinity brackish water improves siting options for fish production facilities. Reducing the salt requirements of growing marine finfish in inland RAS mitigates environmental impacts of salty effluent discharge.

When marine finfish live in seawater, they receive excess salt from the water that they drink, which must be removed by their gills and intestines. When they live in a low-salinity environment, a passive outward flux of ions (e.g., Na and Cl) to the water occurs through the gills, intestine and kidneys. To compensate, they actively take up salt through their skin, kidneys, gills and through their food (Gatlin et al., 1992; Karnaky, 1998; Perry et al., 2006). Supplementing salt through the feed can therefore potentially conserve energy required for osmoregulation and divert more energy to growth.

Information on the effects of dietary mineral supplementation for euryhaline marine fish species reared in low-salinity water is limited, but promising. Dietary salt has improved growth of euryhaline fish species raised at low salinity, including red drum *Sciaenops ocellatus* (Gatlin et al., 1992), barramundi *Lates calcarifer* (Harpaz et al., 2005), European sea bass *Dicentrarchus labrax* (Eroldogan et al., 2005), tilapia hybrids (Cnaani et al., 2009) and gilthead sea bream *Sparus aurata* (Appelbaum and Arockiaraj, 2009). In barramundi, adding 4% salt to the diet stimulated activity of intestinal brush border digestive enzymes (Harpaz et al., 2005). Juvenile gilthead sea bream raised in low salinity brackish water (2.9–3.6 g L^{-1}) showed maximum growth and survival when diets were supplemented with 12% salt (dehydrated brackish geothermal well water) within a range of 0 to 16% salt (Appelbaum and Arockiaraj, 2009). Available data indicated that the black sea bass is moderately euryhaline species and that juveniles raised at salinities of 20 and 30 g L^{-1} grew at a similar rate and much faster than at a salinity of 10 g L^{-1} (Atwood et al., 2001, 2003; Cotton et al., 2003).

There is no information on the dietary salt requirements of black sea bass reared in low-salinity water and whether dietary salt supplements could be used to improve growth and survival of black sea bass reared under sub-optimal salinity conditions (i.e., <20 g L^{-1}). The main goal of the current experiment was to develop practical diets for maximum growth of black sea bass raised in low salinity brackish water. The specific objectives were to determine the effect of dietary salt supplementation at different levels on growth, survival, feed utilization and osmoregulatory ability of black sea bass reared at salinity levels of 15 g L^{-1} and 10 g L^{-1}.

2. Materials and methods

2.1. Diet formulation and preparation

2.1.1. Experiment 1 (rearing water salinity level of 15 g L^{-1})

In experiment 1, a basal diet was formulated to contain 46% crude protein and 10% crude lipid on the basis of nutrient requirement information of juvenile black sea bass (Alam et al., 2008, 2009). The basal diet also contained 2.5% of a mineral mix (Kadai, Kagoshima University, Japan) formulated for marine finfish. To determine the effects of additional dietary salt enhancement on growth and feed utilization in black sea bass reared at sub-optimal salinity (15 g L^{-1}), six test diets were formulated to supplement graded (2.5% increments) levels (0, 2.5, 5, 7.5, 10, and 12.5% dry wt.) of a premium food grade natural sea salt (Cargill Hi-Grade Evaporated Salt, 99.86% NaCl) (Cargill Salt, Minneapolis, MN) (Table 1). Alpha-cellulose and wheat starch were decreased proportionally as salt levels were increased in the diets. Levels of other ingredients were constant for all diets. In all diets, soybean meal, menhaden fish meal and poultry meal were used as protein sources on the basis of our previous findings on the utilization of alternative protein feedstuffs by black sea bass (Alam et al., 2009, 2012). All diets were formulated to have the same protein (46% crude protein) and lipid (10% lipid) levels. Diets (1.5 mm pellets) were prepared at the UNCW Aquaculture Facility (Wrightsville Beach, NC) using a Hobart mixer, meat grinder and then dried in an oven and stored in a freezer at −20 °C.

Table 1
Composition of diets (%) (experiment 1 at 15 g L^{-1} salinity).

Diets	1	2	3	4	5	6
(% Sea salt in diets)	0	2.5	5.0	7.5	10.0	12.5
Soybean meal[a]	13	13	13	13	13	13
Menhaden meal[b]	38	38	38	38	38	38
Poultry meal[c]	23	23	23	23	23	23
Wheat starch[d]	6.5	5	3.5	2	0.5	0
Wheat gluten[d]	4	4	4	4	4	4
Menhaden fish oil[e]	3	3	3	3	3	3
Soybean lecithin[f]	1	1	1	1	1	1
Vitamin premix[g]	2	2	2	2	2	2
Mineral premix[h]	2.5	2.5	2.5	2.5	2.5	2.5
Cellulose	6	5	4	3	2	0
Sea salt[i]	0	2.5	5	7.5	10	12.5
L-Methionine	0.5	0.5	0.5	0.5	0.5	0.5
L-Lysine	0.5	0.5	0.5	0.5	0.5	0.5
Total	100	100	100	100	100	100
Analyzed proximate composition						
Protein %	46.6	45.5	46.3	46.7	45.1	45.8
Lipid %	10.3	10.5	9.8	9.7	10.0	10.3
Ash %	13.4	15.4	17.6	20.5	22.6	25.1
Energy kJ/g diet[j] (calculated)	16.5	16.2	16.0	15.7	15.5	15.4

[a] Southern States, Wallace, NC, USA (solvent extracted, crude protein 47.5%).
[b] Omega protein, Houston, TX, USA (crude protein 59%, lipid 11%).
[c] Southern States, Wallace, NC, USA (crude protein 65%, lipid 12%).
[d] VWR International, Radnor, PA, USA.
[e] Virginia Prime Silver, Omega Protein, Hammond, LA, USA.
[f] ADM, IL, USA.
[g] (g kg^{-1} diet) p-amino benzoic acid, 0.26; biotin, 0.01; inositol. 2.57; nicotinic acid, 0.51; ca-pantothenate, 0.18;pyridoxine-HC1 (B 6), 0.03; riboflavin (B 2), 0.13; thiamin-HCL (B 1), 0.04; menadione (K3), 0.03; vitamin A-palmitate, 0.02; a-tocopherol (E), 0.26; cyanocoalamine (B 12), 0.001; calciferol (D3), 258 IU; ascorbyl-2-phosphate-Mg, 0.01; folic acid, 0.01 and choline chloride, 5.25 and cellulose, 2.57. Tomita Pharmaceutical Company, Kagoshima, Japan.
[h] (g kg^{-1} diet) $MgSO_4$, 3.17; Na_2HPO_4, 2.02; K_2HPO_4, 5.54; $Ca(HPO_4)$,3.14; Fe-citrate, 0.68; Ca-lactate, 7.56; $Al(OH)_3$, 0.01; $ZnSO_4$, 0.08; $Cu(SO_4)$, 0.002, $MnSO_4$, 0.02; $Ca(IO_3)_2$, 0.003 and $CoSO_4$, 0.02. Tomita Pharmaceutical Company, Kagoshima, Japan.
[i] Cargill Salt, Minneapolis, MN.
[j] Calculated based on carbohydrates, proteins and lipids are 17.2, 23.6, and 39.5 kJ g^{-1}, respectively (Blaxter, 1989).

2.1.2. Experiment 2 (utilizing rearing water at a salinity of 10 g L^{-1})

Experiment 2 was designed to determine the effects of dietary salt enhancement on growth and feed utilization in black sea bass reared at 10 g L^{-1}, six graded levels of sea salt-based diets as formulated for experiment 1 and an additional diet was formulated with 0% sea salt, but no Kadai mineral mix (Table 2) and prepared as described in experiment 1. All other nutrients were similar to experiment 1, except wheat starch

Table 2
Composition of diets (%) (experiment 2 at 10 g L^{-1} salinity). All ingredients sources are same as Table 1. All diets contained a standard mineral premix, except Diet 1 (0%—mineral premix).

Diets	1	2	3	4	5	6	7
(% Sea salt)	0-Min.	0	2.5	5.0	7.5	10	12.5
Basal mixture[a]	85.0	85.0	85.0	85.0	85.0	85.0	85.0
Mineral premix	0	2.5	2.5	2.5	2.5	2.5	2.5
Wheat starch	9	6.5	5	3.5	2	0.5	0
Cellulose	6	6	5	4	3	2	0
Sea salt	0	0	2.5	5	7.5	10	12.5
Total	100	100	100	100	100	100	100

[a] Contains menhaden fish oil, poultry meal, soybean meal, wheat gluten, menhaden fish oil, soybean lecithin, L-methionine, L-lysine and vitamin pre-mix as Table 1.

and cellulose those were used to adjust the salt levels in diet. The crude protein and lipid levels in the diets were similar to experiment 1.

2.2. Feeding trials

2.2.1. Experiment 1 (rearing water salinity level of 15 g L^{-1})

The feeding trial was conducted at the UNCW-Aquaculture Facility, Harbor Island (Wrightsville Beach, NC). Juvenile black sea bass originated from eggs spawned at the UNCW aquaculture facility and raised to juvenile stages in 300-L larval rearing tanks. The experimental system consisted of 18 rectangular tanks (75-L) supported by a recirculating aquaculture system. Water salinity was kept at 15 g L^{-1} salinity in a controlled-environment indoor laboratory under 14 L: 10 D photoperiod conditions. Seawater (32 g L^{-1}) was supplied to the system by pumping from a natural source adjacent to the laboratory and was diluted with municipal freshwater in a 1890-L reservoir to the required salinity for the experiment. The brackish water culture medium was de-chlorinated by vigorous aeration for at least 48 h before use.

Tanks were stocked with early juveniles (mean initial weight = 8 g) at a density of 15 per tank (N = 270 total). Before feeding the experimental diets, salinity of water was decreased by 1–2 g L^{-1} daily by adding freshwater until the target salinity of 15 g L^{-1} was reached by day 15. After this, the six experimental diets were fed twice per day (9:00 and 16:00 h) to apparent satiation to triplicate groups of fish per diet. The amount of diet fed was recorded and uneaten diets (if any) were collected after 1 h of feeding, freeze dried and were considered when calculating actual feed intake. Tanks were siphoned daily or as needed. A 10 L: 14 D photoperiod was maintained. Water quality was verified twice weekly. During the feeding period, water temperature ranged from 21.8 to 24.0 °C and dissolved oxygen ranged from 6.20 to 7.82 mg L^{-1}. The ranges of other water quality parameters in the experimental tanks during the experimental periods were as follows: pH 7.7–8.2, total ammonia-N 0.37–0.45 mg L^{-1} and nitrite 0.18–0.23 mg L^{-1}. To monitor growth, all fish were sampled and weighed every two weeks for the duration of the 8-week experiment.

2.2.2. Experiment 2 (rearing water salinity level of 10 g L^{-1})

On the basis of the results of experiment 1, a second feeding trial (experiment 2) was conducted in which rearing salinity was reduced to 10 g L^{-1}. Experimental conditions and collection of data were as described for experiment 1. The experimental system consisted of 24 rectangular tanks (75-L) supported by a recirculating aquaculture system at 10 g L^{-1} salinity in a controlled environment laboratory under 14 L: 10 D photoperiod conditions. Tanks were stocked with early juveniles (mean initial weight = 9.37 g) at a density of 15 per tank (N = 360 total). Before feeding the experimental diets, salinity of water was decreased by 1–2 g L^{-1} daily by adding freshwater until the target salinity of 10 g L^{-1} was reached by day 15. After this, the seven experimental diets were fed to triplicate groups of fish per diet two times a day to apparent satiation for 10 weeks. During the feeding period, water temperature ranged from 21.6–24.4 °C and dissolved oxygen ranged from 6.32–8.36 mg L^{-1}. The ranges of other water quality parameters in the experimental tanks during the experimental periods were as follows: pH 7.7–8.3, total ammonia-N 0.19–0.30 mg L^{-1} and nitrite 0.07–0.13 mg L^{-1}.

2.3. Plasma osmotic pressure and electrolyte analysis

At final sampling in both experiments 1 and 2, blood samples were collected from three fish from each tank using a hypodermic syringe inserted into the caudle artery, and plasma samples were separated using a centrifuge. Fish plasma osmotic pressure was measured using a vapor pressure osmometer (Wescor Vapor Pressure Osmometer 5520, Logan, Utah, U.S.A.), and plasma electrolytes (Na, K, Cl, Ca, P and Mg) were analyzed at Cornell University College of Veterinary Medicine (Ithaca, NY).

2.4. Na^+/K^+ ATPase (NKA) activity

In experiment 1, NKA activity of black sea bass gill and intestinal tissues after the feeding trial were analyzed at the UNCW-Center for Marine Science. After collecting the blood, gill and intestinal tissues of the fish were collected and stored at −80 °C for NKA analysis. The methodology for measuring NKA activities in fish gill and intestine was established as described by Penefsky and Bruist (1984) and McCormick and Bern (1989), and modified for 96-well microplates (McCormick, 1993; Russo, 2013).

2.5. Diet and whole body electrolyte analysis

Mineral composition of the diets and fish whole body tissues were analyzed at the UNCW Department of Chemistry and Biochemistry using ICP-spectrometry (experiment 1) and at the Animal Health Diagnostic Center (AHDC), College of Veterinary Medicine, Cornell University (experiment 2), while chloride analysis of diets and whole body samples was conducted at the Department of Soil Science, North Carolina State University.

2.6. Whole body proximate analysis

After each experiment, five fish from each tank were collected for whole body proximate and mineral composition analysis. The fish were freeze-dried, and proximate analysis was conducted at the UNCW Aquaculture Research Laboratory (AOAC, Association of Official Analytical Chemists, 2000).

2.7. Hyposalinity challenge (experiment 2)

After the 70-day feeding trial for fish grown at 10 g L^{-1} in experiment 2, fish tissues and blood samples were collected for biochemical analysis, and the remaining fish (5 fish per tank) were fed their respective diets in triplicate tanks for an additional two weeks under adverse low-salinity conditions. Starting from 10 g L^{-1}, the salinity of the water was decreased by 0.5 g L^{-1} daily until a salinity of 4 g L^{-1} was reached after 14 days. During this hyposalinity challenge, fish mortalities in each tank were recorded daily for a period of 14 days when the experiment was terminated.

2.8. Statistical analysis

All data were subjected to statistical verification using one-way analysis of variance (ANOVA) (JMP, version 7.0, SAS Institute, Cary, North Carolina). Multiple comparisons among treatment means were made by Tukey–Kramer test (Kramer, 1956). Probabilities of $P < 0.05$ were considered significant. In addition, pairwise comparisons (Student's t-test) between each treatment mean and the control (0% sea salt) value was conducted for the body weight gain data of experiment 2.

3. Results

3.1. Diet electrolyte concentrations

As prescribed by experimental design, sodium and chloride concentrations in the diets increased incrementally as the level of salt supplementation to the diets was increased (Table 3). In experiment 1, as sea salt was increased from 0% to 12.5%, sodium increased from 0.38 to 5.64%, while chloride increased from 0.64 to 8.15%, respectively (Table 3) in the diets. In contrast, the concentrations of other major electrolytes (K = 0.89–1.29%, Mg = 0.21–0.27%, Ca = 2.51–3.72% and P = 1.86–2.54%) were similar among diets (Table 3). In experiment 2, sodium increased from 0.36 to 5.41%, while chloride increased from 0.60 to 7.64% in the diets. The other electrolytes concentrations were more or less similar in all diets for experiment 2 (Table 3).

Table 3
Analyzed electrolytes (% sample) in experimental diets. Values are average of triplicate analysis.

Diet	% Sea salt	Na	K	Mg	Ca	P	Cl
Experiment 1 at 15 g L^{-1}							
1	0	0.38	1.00	0.21	2.89	2.07	0.64
2	2.5	1.23	0.89	0.21	2.89	2.12	2.27
3	5	2.14	1.05	0.21	2.56	1.86	3.77
4	7.5	3.28	1.07	0.23	2.51	2.01	5.2
5	10	4.57	1.17	0.23	2.56	2.11	6.35
6	12.5	5.64	1.29	0.23	2.73	2.19	8.15
Experiment 2 at 10 g L^{-1}							
1	0-Mineral	0.36	0.85	0.21	3.61	2.36	0.60
2	0	0.47	1.05	0.27	3.71	2.48	0.65
3	2.5	1.45	1.07	0.27	3.69	2.54	2.07
4	5	2.47	1.11	0.27	3.69	2.53	3.56
5	7.5%	3.45	1.16	0.27	3.67	2.51	4.90
6	10	4.39	1.07	0.26	3.65	2.39	6.38
7	12.5	5.41	1.09	0.25	3.65	2.42	7.64

3.2. Growth performance and feed utilization at water rearing salinity level of 15 g L^{-1} (experiment 1)

After 8 weeks of feeding in experiment 1, survival ranged from 95.3 to 97.7%, with no significant differences ($P > 0.05$) among treatments (Table 4). The highest body weight gain was found for the fish fed 7.5% salt diet (249%), whereas the lowest body weight gain was found for the fish fed 0% salt (217%) (Table 4). Body weight gain of fish fed 7.5% salt appeared to be higher than for fish fed the diets with lower or higher levels of salt; however, body weight gain was not significantly different among treatments.

In experiment 1, feed intake (g fish^{-1} day^{-1}) was not significantly different among fish fed diets containing 0 to 10% salt (0.51–0.52) (Table 4). However, feed intake was significantly ($P < 0.05$) lower in fish fed 12.5% salt (0.47) compared to the other diet treatments. Feed conversion ratio (1.49–1.66) was not significantly different among treatments (Table 4).

3.3. Growth performance and feed utilization at water rearing salinity of 10 g L^{-1} (experiment 2)

After 10 weeks of the feeding trial at 10 g L^{-1}, survival ranged from 77.7 to 91.0%, with no significant differences among treatments (Table 4). Survival at 10 g L^{-1} was lower than for fish reared at 15 g L^{-1} in experiment 1 (95.3–97.7) (Table 4). The highest body weight gain was found for fish fed 5% salt (250%), whereas the lowest body weight gain was found for the fish fed 0% salt without mineral premix (177%) (Table 4). The relationship between the salt level in diet and the percent body weight is shown in Fig. 1. Tukey–Kramer test with multiple comparisons among treatment means showed that body weight gain was not significantly different among treatments. However, pairwise comparisons of each treatment with the control diets (0% salt) showed that body weight gain was significantly ($P < 0.05$, ANOVA) higher in fish fed 5% salt (Diet 4) than in fish fed the diets 0% salt (Diet 1), which showed the poorest growth performance.

In experiment 2, feed intake (g fish^{-1} day^{-1}) was significantly lower in fish fed the 0% salt (Diets 1 and 2) (0.29–0.32) than in fish fed 12.5% sea salt (0.44) (Diet 7) (Table 4), and there was a trend toward higher feed intake with increasing dietary salt. FCRs for fish fed 10% salt (1.46) and 12.5% salt (1.53) were significantly higher than in fish fed 5% salt (1.14). However, FCRs were not significantly different among fish fed 0 to 7.5% salt (1.13–1.36) (Table 4).

3.4. Plasma osmolality (experiments 1 and 2)

Plasma osmolality (mOsm L^{-1}) for fish raised at salinity level of 15 g L^{-1} in experiment 1 ranged from 330–350 (Table 4) and was significantly higher for fish fed 10% salt (350) than in fish fed 2.5% salt (330). Plasma osmolality was not significantly different among fish fed 0%, 2.5%, 5%, 7.5% and 12.5% salt (330–347).

Plasma osmolality of fish raised at 10 g L^{-1} in experiment 2 ranged from 237–285 mOsm L^{-1}, markedly lower than in fish raised at 15 g L^{-1} in experiment 1 (330–350). However, plasma osmolalities were not significantly different among fish fed 0 to 12.5% sea salt (Table 4).

3.5. Hyposalinity challenge (experiment 2)

At the end of experiment 2, when juvenile black sea bass raised at 10 g L^{-1} were challenged with a further reduction in salinity from 10 to 4 g L^{-1} within a period of 12 days, fish fed diets without both sea salt and mineral premix (0% salt–mineral mix) showed poor survival (22%), not significantly different from fish fed diets with 0% salt + premix (64%), or 2.5% salt which showed only moderate survival (65%). However, fish fed diets without both sea salt and mineral premix (0% salt–mineral mix) showed significantly lower survival (22%) than in fish fed 5–12.5% salt which maintained high survival (82–95%) (Fig. 2A). The relationship between the salt level in diet (excluding diet 1, 0% salt–mineral mix) and survival of fish at 4 ppt is shown in Fig. 2B, which indicates an improvement in survival with increasing dietary salt to a maximum at 7.5% salt.

Table 4
Effects of different test diets on percent body weight gain (BWG) feed intake, feed conversion efficiency (FCR) and plasma osmolality. Values are mean ± SEM ($N = 3$). Tukey–Kramer HSD was used for multiple comparisons among means. Means not sharing a common letter are significantly ($P < 0.05$) different.

Diets (% salt)	BWG (%)	Feed intake (g fish^{-1} day^{-1})	FCR	Survival (%)	Osmolality (mOsm l^{-1})
Experiment 1 (15 g L^{-1})					
1 (0)	217 ± 16.0	0.51 ± 0.01a	1.66 ± 0.14	97.8 ± 2.23	342 ± 1.0ab
2 (2.5)	220 ± 14.8	0.51 ± 0.01a	1.62 ± 0.12	95.5 ± 2.23	330 ± 2.3b
3 (5.0)	224 ± 14.3	0.51 ± 0.01a	1.62 ± 0.09	95.5 ± 2.23	347 ± 0.9ab
4 (7.5)	249 ± 10.7	0.52 ± 0.00a	1.44 ± 0.07	97.8 ± 2.23	341 ± 4.2ab
5 (10.0)	235 ± 16.1	0.51 ± 0.01a	1.52 ± 0.06	95.5 ± 2.23	350 ± 6.9a
6 (12.5)	221 ± 5.7	0.47 ± 0.01b	1.49 ± 0.04	97.8 ± 2.23	339 ± 3.8ab
Experiment 2 (10 g L^{-1})					
1 (0-Mineral)	177 ± 6.8	0.32 ± 0.02bc	1.36 ± 0.07ab	82.3 ± 9.59	271 ± 4.5
2 (0)	193 ± 8.4	0.29 ± 0.02c	1.13 ± 0.06b	91.0 ± 2.00	285 ± 6.3
3 (2.5)	207 ± 23.1	0.38 ± 0.05abc	1.36 ± 0.06ab	78.0 ± 5.85	260 ± 18.2
4 (5.0)	250 ± 31.2*	0.39 ± 0.07abc	1.14 ± 0.06b	82.3 ± 7.85	237 ± 9.0
5 (7.5)	220 ± 8.3	0.39 ± 0.0abc	1.31 ± 0.07ab	82.3 ± 2.33	268 ± 2.0
6 (10.0)	213 ± 16.5	0.42 ± 0.04ab	1.46 ± 0.03a	77.7 ± 2.33	281 ± 11.7
7 (12.5)	217 ± 18.1	0.44 ± 0.03a	1.53 ± 0.08a	80.0 ± 4.04	259 ± 4.5

BWG: {(Final weight − Initial fish wet) / Initial weight} × 100. FCR: Feed conversion ratio (feed intake (g) / wet weight gain (g)).
*For experiment 2 in body weight gain, asterisks indicate means that were significantly different from the control (*t*-test).

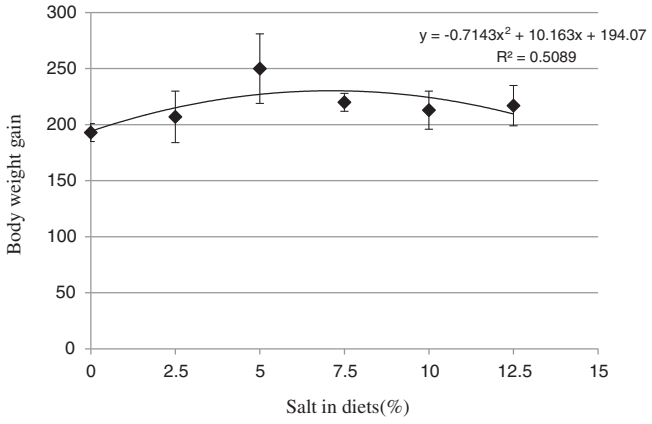

Fig. 1. The relationship between dietary salt level and the percent body weight gain of juvenile black sea bass after 10 weeks of feeding at a salinity level of 10 g L^{-1}.

3.6. Whole body proximate composition (experiments 1 and 2)

The crude protein and moisture contents of fish whole bodies in experiments 1 and 2 are presented in Table 5. For fish raised at 15 g L^{-1} in experiment 1, whole body moisture (66.7–67.9%), crude protein (17.1–17.8%), lipid (10.5–11.4%) and ash (4.09–4.41%) contents for fish fed different diets containing graded levels of salt were not significantly different among treatments (Table 5).

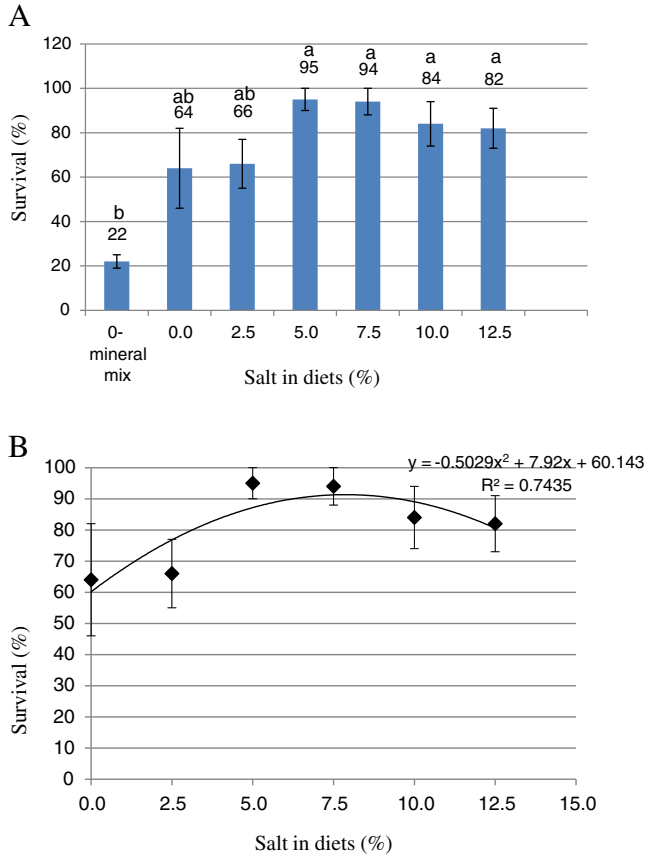

Fig. 2. A. Effects of dietary salt levels on survival of black sea bass raised at 10 g L^{-1} for 10 weeks (experiment 2) following exposure to a reduction in salinity from 10 g L^{-1} to 4 g L^{-1} over 12 days. Values represent means ± SEM ($N = 3$). Means not sharing a letter in common are significantly ($P < 0.05$) different. **B:** The relationship between dietary salt level and percent survival of juvenile black sea bass raised at 10 g L^{-1} and then challenged with a reduction of the rearing water salinity from 10 g L^{-1} to 4 g L^{-1} over 12 days.

For fish raised at 10 g L^{-1} in experiment 2, whole body moisture content (range = 66.8–68.8%) showed minor differences among diet treatments (Table 5). Whole body lipid content was significantly higher in fish fed 5% salt (11.0%) than in the other diets (8.15–9.68%) (Table 5). Whole body protein (15.6–16.8%) and ash content (4.07–4.53%) were not significantly different (Table 5).

3.7. Plasma electrolytes (experiments 1 and 2)

Plasma electrolyte concentrations for fish reared at 15 g L^{-1} in experiment 1 are presented in Table 6. No significant differences were observed in plasma electrolytes concentrations among fish fed the various treatments diets. For fish reared at 10 g L^{-1} in experiment 2, no significant differences were observed in plasma electrolytes among fish fed the treatments diets, although phosphorus and magnesium levels appeared to be lower in fish fed 0% salt with no mineral premix (experiment 2, Diet 1) (Table 6). Overall Na and Cl concentrations in plasma were slightly higher in fish raised at 15 g L^{-1} (experiment 1) than at 10 g L^{-1} (experiment 2) irrespective of dietary salt supplementation (Table 6).

3.8. Whole body electrolytes (experiments 1 and 2)

In experiment 1 at 15 g L^{-1}, whole body tissues electrolytes (Ca, P, Mg, K, Cu, Fe, Mn and Zn) fell within narrow ranges and were not significantly different among fish fed the treatment diets containing different levels of supplemental salt (Table 7). However, whole body chloride level was significantly higher in fish fed 7.5% salt (0.39 mg g^{-1}) than in the other diets (0.28–0.32 mg g^{-1}).

Whole body tissues electrolytes for fish raised at 10 g L^{-1} in experiment 2 are presented in Table 8. The concentrations of Ca, P, Mg, K, Fe, Mn and Cl in whole body tissues were not significantly different among treatments groups. Sodium levels in fish fed 7.5% salt (Diet 5, 0.38%) were significantly higher than in fish fed 5% salt (Diet 4, 0.34%). The highest level of Zn was found for the fish fed 0% salt–mineral mix (Diet 1, 48.32 ppm) and ranged from 42.6–47.5 ppm among the other diets (Table 8).

3.9. Na^+/K^+ ATPase (NKA) activities at water rearing salinity of 15 g L^{-1} (experiment 1)

For fish raised at 15 g L^{-1} in experiment 1, the NKA activities in gills ranged from 80 to 148 μmol-ADP mg^{-1} protein $hour^{-1}$ among fish fed diets supplemented with 0 to 12.5% sea salt (Fig. 3). Gill NKA activities appeared to be higher in fish fed the diet with highest level (12.5%) of supplemental salt compared to the fish fed diets with 0%, 2.5%, 5.0%

Table 5
Whole body proximate composition (% wet basis) in fish fed different test diets. Values are mean ± SEM ($N = 3$).

Diets	% Sea salt	Moisture	Protein	Lipid	Ash
Experiment 1 at 15 g L^{-1}					
1	0	67.2 ± 1.02	17.8 ± 0.58	10.9 ± 0.16	4.41 ± 0.18
2	2.5	67.2 ± 0.14	17.6 ± 0.32	10.9 ± 0.16	4.09 ± 0.09
3	5.0	66.8 ± 0.49	17.1 ± 0.05	10.6 ± 0.08	4.18 ± 0.12
4	7.5	67.0 ± 0.27	17.4 ± 0.12	11.4 ± 0.21	4.21 ± 0.06
5	10.0	66.7 ± 0.38	17.4 ± 0.80	11.2 ± 0.31	4.37 ± 0.14
6	12.5	67.9 ± 0.29	17.5 ± 0.48	10.7 ± 0.21	4.28 ± 0.03
Experiment 2 (10 g L^{-1})					
1	0-Mineral	68.8 ± 0.21a	16.6 ± 0.13	8.15 ± 0.14c	4.35 ± 0.10
2	0	68.1 ± 0.52ab	16.2 ± 0.53	9.27 ± 0.15b	4.45 ± 0.04
3	2.5	68.7 ± 0.14a	16.0 ± 0.37	9.00 ± 0.11b	4.38 ± 0.09
4	5.0	66.8 ± 0.30b	15.7 ± 0.26	11.0 ± 0.09a	4.07 ± 0.12
5	7.5	68.2 ± 0.10ab	16.8 ± 0.19	9.64 ± 0.24b	4.20 ± 0.11
6	10.0	68.2 ± 0.40ab	15.6 ± 0.34	8.98 ± 0.12b	4.44 ± 0.11
7	12.5	68.1 ± 0.29ab	15.7 ± 0.32	9.68 ± 0.17b	4.53 ± 0.04

Table 6
Plasma electrolytes values (mEq L^{-1}), except Ca and P (mg d L^{-1}). Values are mean ± SEM of triplicate tanks ($N = 3$).

Diets (sea salt)	Na	K	Cl	Ca	P	Mg
Experiment 1 (15 g L^{-1})						
1 (0%)	169 ± 1.5	4.8 ± 0.2	138 ± 4.0	12.7 ± 0.2	17.8 ± 2.2	2.8 ± 0.3
2 (2.5%)	163 ± 1.9	4.9 ± 0.5	132 ± 4.3	13.1 ± 0.4	17.5 ± 1.0	2.8 ± 0.1
3 (5%)	169 ± 2.0	5.3 ± 1.0	140 ± 2.0	12.8 ± 0.5	18.2 ± 1.1	2.8 ± 0.1
4 (7.5%)	166 ± 2.3	5.7 ± 1.5	140 ± 1.5	12.5 ± 0.1	16.3 ± 0.5	2.5 ± 0.1
5 (10%)	167 ± 7.5	5.5 ± 0.6	141 ± 6.0	12.0 ± 0.2	17.0 ± 1.3	2.6 ± 0.2
6 (12.5%)	162 ± 1.5	5.3 ± 0.1	134 ± 3.8	12.7 ± 0.2	14.3 ± 0.1	2.2 ± 0.1
Experiment 2 (10 g L^{-1})						
1 (0%-Min)	148 ± 0	4.7 ± 0.82	125 ± 1.76	11.3 ± 0.66	14.3 ± 0.66	1.9 ± 0.03
2 (0%)	156 ± 3.18	4.7 ± 0.47	130 ± 2.72	12.0 ± 0.03	16.1 ± 0.23	2.3 ± 0.15
3 (2.5%)	145 ± 12.7	6.9 ± 3.4	123 ± 9.07	10.7 ± 1.4	17.4 ± 1.34	2.4 ± 0.18
4 (5%)	135 ± 2.91	6.7 ± 0.21	113 ± 2.72	9.4 ± 0.56	16.7 ± 0.97	2.3 ± 0.18
5 (7.5%)	146 ± 1.45	7.4 ± 0.58	121 ± 0.33	11.2 ± 0.38	18.2 ± 0.69	2.6 ± 0.12
6 (10%)	153 ± 5.03	5.2 ± 0.81	128 ± 5.13	12.1 ± 0.26	17.7 ± 1.40	2.6 ± 0.12
7 (12.5%)	140 ± 3.51	7.8 ± 0.61	117 ± 3.28	11.2 ± 0.64	18.5 ± 1.31	2.6 ± 0.20

and 7.5% salt (Fig. 3). The gill NKA activity was significantly ($P < 0.05$) higher in fish fed 12.5% salt than in those fed 0% or 2.5% salt (Fig. 3).

In contrast to gill tissue, NKA activities in intestinal tissue were lower, ranging from 35 to 78 μmol-ADP mg $protein^{-1}$ h^{-1} among all diet treatments (Fig. 4). Intestine NKA activity appeared to be maximal at lower levels of dietary salt, and was significantly higher in fish fed 2.5% salt than in fish fed 12.5% salt (Fig. 4).

4. Discussion

4.1. Growth performance

The overall growth of the fish was satisfactory for fish reared for 56 d at 15 g L^{-1} in experiment 1 (initial wt. 8 g and final wt. 23–30 g) and for 56 d at 10 g L^{-1} in experiment 2 (initial wt. 9 g and final wt. 25–39 g) for 70 d. Growth performance was comparable to what was observed in previous studies of juvenile black sea bass of similar size fed formulated diets in full strength seawater (34 g L^{-1}) over similar durations (Alam et al., 2008, 2012). In the present study, dietary salt supplementation within the range of 0 to 12.5% did not significantly improve or impair growth of juvenile black sea bass reared at a suboptimal salinity of 15 g L^{-1} over a period of 8 weeks (Table 4). These results indicate that a rearing salinity of 15 g L^{-1}, produced by diluting natural seawater with freshwater, was sufficient to satisfy the physiological needs of black sea bass, at least through the advanced juvenile stages. Hence, supplementing dietary salt to a complete, pelleted diet produced only minor effects on fish growth performance at 15 g L^{-1} salinity.

In contrast to experiment 1 at 15 g L^{-1} where negligible effects of dietary salt on growth were observed, the results of experiment 2 showed that, for fish raised at a lower salinity of 10 g L^{-1}, diets with moderate to high levels of salt (5.0–12.5%) produced better growth than diets with low (0% or 2.5%) salt, and growth was optimized at dietary salt level of 5% (Table 4, Fig. 1). This suggests that supplementation of 5% sea salt enhanced growth performance of fish raised at 10 g L^{-1}. This also suggests that effects of salt supplementation on black sea bass were more clearly manifested at the lower salinity, which produced lower plasma osmolality and hence, greater osmoregulatory stress than at 15 g L^{-1}. Gatlin et al. (1992) found that while a NaCl-supplemented diet had no effect on growth of juvenile red drum reared in 6 g L^{-1} brackish water, this diet improved growth and feed efficiency of fish reared in freshwater, also consistent with the idea that the effects of dietary salt supplementation become more distinct under increasing osmoregulatory stress.

The benefits of salt supplementation are not universal for euryhaline finfish species, as some studies have documented the lack of effects on growth, feed efficiency and feed intake in some species such as in cobia (Santos et al., 2014) and Atlantic salmon (*Salmo salar*) (Shaw et al., 1979) when raised under suboptimal salinities. In contrast to these findings, dietary salt supplementation was shown to enhance growth rates in other freshwater fish such as the tilapia *Oreochromis niloticus*, L. (Cnaani et al., 2009; Fontainhas-Fernandes et al., 2001) and the rohu *Labeo rohita* reared in freshwater (Gangadhara et al., 2004). The growth performance of several species of marine fish has been evaluated in fresh- or low-salinity water using salt-supplemented diets, including Asian sea bass *L. calcarifer*, Bloch (Harpaz et al., 2005), juvenile red drum *S. ocellatus*, L. (Gatlin et al., 1992) (2.9 g L^{-1}), and gilthead sea bream (Appelbaum and Arockiaraj, 2009). Contrary to the positive results obtained in freshwater, under salt water (20 g L^{-1}) rearing conditions, dietary salt supplementation at a level of 4% had no effect on growth or FCR in Asian sea bass (Harpaz et al., 2005). Gatlin et al. (1992) found that red drum reared in freshwater showed improved growth and feed efficiency when fed a NaCl-supplemented diet. However, the same NaCl-supplemented diet had no effect on growth of juvenile red drum reared in brackish water (6 g L^{-1}) or seawater (35 g L^{-1}) (Gatlin et al., 1992). These authors suggested that the amount of salt available in seawater and brackish water was sufficient to satisfy the physiological requirements of red drum juveniles.

The supplementation of high levels (4.5–11.6%) of NaCl to the diet has been reported to reduce feed efficiency and inhibit growth in rainbow trout (Salman and Eddy, 1988). However, the results of the present

Table 7
Electrolyte concentrations in whole body tissues (experiment 1, 15 g L^{-1}). Values are means ± SEM of triplicate tanks ($N = 3$). Means not sharing the same letter are significantly ($P < 0.05$) different.

Diets (% salt)	Ca (%)	P (%)	Mg (%)	Na (%)	K (%)	Cu (ppm)	Fe (ppm)	Mn (ppm)	Zn (ppm)	Cl (mg/g)
1 (0%)	3.86 ± 0.34	2.45 ± 0.16	0.13 ± 0.005	0.33 ± 0.001	0.85 ± 0.05	1.79 ± 0.14	32.67 ± 3.24	9.44 ± 0.63	43.36 ± 1.10	0.28 ± 0.01b
2 (2.5%)	4.18 ± 0.07	2.58 ± 0.04	0.13 ± 0.003	0.33 ± 0.003	0.93 ± 0.02	1.49 ± 0.06	36.52 ± 1.88	10.44 ± 0.18	44.09 ± 0.32	0.32 ± 0.01b
3 (5%)	4.11 ± 0.08	2.49 ± 0.03	0.13 ± 0.001	0.33 ± 0.004	0.91 ± 0.01	1.74 ± 0.09	32.01 ± 0.65	11.33 ± 0.42	45.77 ± 0.59	0.29 ± 0.01b
4 (7.5%)	3.90 ± 0.20	2.39 ± 0.06	0.13 ± 0.003	0.33 ± 0.005	0.93 ± 0.01	1.74 ± 0.46c	31.57 ± 3.90	10.54 ± 0.41	44.68 ± 1.41	0.39 ± 0.02a
5 (10%)	4.02 ± 0.33	2.41 ± 0.14	0.13 ± 0.005	0.33 ± 0.008	0.91 ± 0.01	1.56 ± 0.03	30.03 ± 0.93	11.22 ± 0.69	43.33 ± 1.09	0.33 ± 0.01b
6 (12.5%)	4.11 ± 0.09	2.49 ± 0.05	0.14 ± 0.003	0.31 ± 0.007	0.91 ± 0.01	1.81 ± 0.02	38.27 ± 1.87	10.17 ± 0.60	45.23 ± 1.13	0.32 ± 0.01b

Table 8
Electrolyte concentrations in whole body tissues (experiment 2, 10 g L^{-1}). Values are means ± SEM of triplicate tanks ($N = 3$). All diets contained a standard mineral premix, except Diet 1. Means within a column not sharing the same letter are significantly ($P < 0.05$) different.

Diet (% salt)	Ca (%)	P (%)	Mg (%)	Na (%)	K (%)	Cu (ppm)	Fe (ppm)	Mn (ppm)	Zn (ppm)	Cl mg g^{-1}
1 (0%-Mineral)	4.89 ± 0.28	3.02 ± 0.15	0.14 ± 0.002	0.37 ± 0.002ab	0.99 ± 0.02	2.58 ± 0.48a	32.75 ± 1.38ab	9.18 ± 0.66	48.32 ± 0.80a	0.38 ± 0.01a
2 (0%)	4.41 ± 0.11	2.75 ± 0.04	0.14 ± 0.002	0.37 ± 0.001ab	0.98 ± 0.02	2.51 ± 0.31ab	39.82 ± 0.57ab	10.86 ± 0.77	47.59 ± 0.50ab	0.37 ± 0.01ab
3 (2.5%)	4.39 ± 0.07	2.73 ± 0.06	0.14 ± 0.001	0.36 ± 0.001ab	1.05 ± 0.02	1.95 ± 0.17ab	43.67 ± 4.27a	11.09 ± 0.17	44.44 ± 0.40ab	0.36 ± 0.01ab
4 (5%)	3.75 ± 0.35	2.48 ± 0.12	0.13a ± 0.001	0.34 ± 0.005b	0.92 ± 0.04	2.22 ± 0.16ab	33.66 ± 0.72ab	10.82 ± 0.22	42.60 ± 0.52b	0.34 ± 0.01ab
5 (7.5%)	4.57 ± 0.08	2.81 ± 0.02	0.14 ± 0.001	0.38 ± 0.01a	0.95 ± 0.02	1.95 ± 0.03ab	34.72 ± 2.91ab	10.38 ± 0.18	46.74 ± 1.20ab	0.37 ± 0.01ab
6 (10%)	4.49 ± 0.26	2.82 ± 0.13	0.14 ± 0.005	0.37 ± 0.01ab	0.95 ± 0.04	1.76 ± 0.07ab	34.73 ± 2.91ab	9.18 ± 0.26	44.38 ± 0.49ab	0.34 ± 0.01ab
7 (12.5%)	4.56 ± 0.48	2.73 ± 0.18	0.14 ± 0.006	0.35 ± 0.01ab	0.91 ± 0.01	1.89 ± 0.25ab	34.24 ± 2.74ab	10.99 ± 1.14	44.99 ± 1.57ab	0.34 ± 0.02ab

study with black sea bass demonstrated that increasing dietary salt up to a level of 10% had no effects on growth and feed utilization in black sea bass raised at 15 g L^{-1}, a salinity assumed to be suboptimal for this species (Atwood et al., 2001, 2003, 2004).

Young et al. (2006) reported that black sea bass juveniles fed diets without supplemental salt survived abrupt transfer from 15 g L^{-1} salinity to 8 g L^{-1}, but not to 6 g L^{-1} salinity. However, in our present study, when fish raised for 10 weeks at 10 g L^{-1} were subjected to an acute hypo-osmoregulatory challenge by decreasing salinity from 10 g L^{-1} to 4 g L^{-1} over a period of 14 days, pronounced beneficial effects of dietary salt supplementation on low-salinity tolerance and survival were evident. Under these adverse hyposaline conditions, juveniles fed diets without supplemental salt or mineral mix showed much lower survival (22%) than fish fed 0%–2.5% salt (64–66%), while survival remained high among fish fed diets with 5.0–12.5% supplemental salt (82–95%) (Fig. 2A, B). Hence, definitive treatment effects of dietary salt supplementation on survival were evident under acute hyposalinity conditions (4 g L^{-1}), when fish fed diets containing from 5.0 to 12.5% supplemental salt showed excellent hyposalinity tolerance and survival, whereas those fed lower levels showed much lower tolerance and survival.

4.2. Plasma osmolality and electrolytes

The ability to regulate blood osmolality under stressful (i.e., hyper- or hypoosmotic) conditions is species-dependent (Varsamos et al., 2005). Strong osmoregulators such as the marine rabbitfish *Siganus rivulatus* show no significant variation in blood osmolality (range = 398–435 mOsm L^{-1}) when reared in seawater ranging from 10 g L^{-1} to 40 g L^{-1} (Saoud et al., 2007). In the present study, blood plasma osmolality for black sea bass raised at 15 g L^{-1} in experiment 1 ranged from 330–350 mOsm L^{-1} (Table 4), which is only slightly lower than those typically reported for marine teleosts (380–450 mOsm L^{-1}) (Barton, 2007). This suggests that a rearing salinity of 15 g L^{-1} did not pose a significant osmoregulatory challenge for juvenile black sea bass and explains the negligible effects of dietary salt supplementation on growth at this salinity, which showed minimal improvement in fish fed a diet with 5% salt.

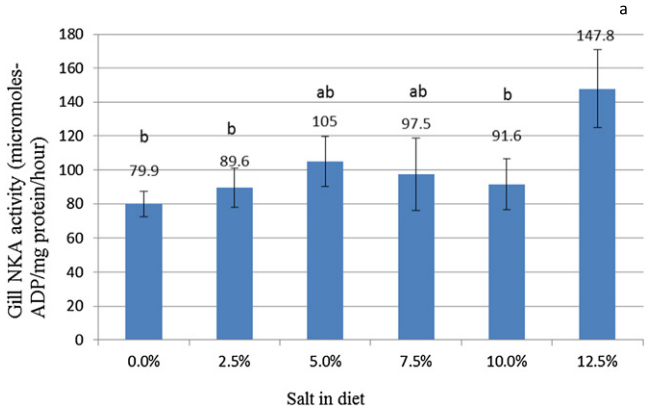

Fig. 3. Effects of graded levels of dietary salt on gill Na^+/K^+ ATPAse (NKA) activity of juvenile black sea bass raised at 15 g L^{-1} salinity. Values are means ± SEM of triplicate tanks ($N = 3$). Means not sharing a letter in common are significantly different ($P < 0.05$).

In contrast to fish grown at 15 g L^{-1} in experiment 1, plasma osmolality values for fish grown at a lower salinity of 10 g L^{-1} in experiment 2 (range = 233–285 mOsm L^{-1}) (Table 4) were noticeably lower than for fish raised at 15 g L^{-1} in experiment 1 (range = 341–350 mOsm L^{-1}). In fact, osmolality values at 10 g L^{-1} were more comparable to values characterizing freshwater teleosts (265–325 mOsm L^{-1}) (Barton, 2007). This conspicuous lowering of blood osmolality in fish raised at 10 g L^{-1}, suggests that fish were experiencing much greater osmoregulatory stress at 10 g L^{-1}. This is similar to what Young et al. (2006) observed in black sea bass where plasma osmolality of juveniles subjected to reduced salinities remained stable at 15 g L^{-1}, but decreased at lower salinities in a manner that approximated osmoconformation. A number of studies have suggested that the ability of fish to osmoregulate declines at the limits of their salinity tolerance range, and that a significant departure from normal ranges under different rearing salinities indicates osmotic imbalance under stressful salinity conditions (Gong et al., 2004; Iwata and Shigueno, 1980; Morgan and Iwama, 1996; Sampaio and Bianchini, 2002). Based on the noticeable decline in plasma osmolality observed in experiment 2, we infer that, while black sea bass were able to survive and grow well at 10 g L^{-1}, this salinity is approaching the lower limit of tolerance, below which fish would be unable to adapt.

In experiment 2, plasma osmolality was not significantly different among fish fed treatment diets containing different levels of supplemental salt. The results of this study suggested that, while a rearing salinity of 10 g L^{-1} produced lower plasma osmolality and was near the lower limits for effective osmoregulation in black sea bass, blood osmolality was still relatively unaffected by the different dietary salt levels tested in experiment 2. This further suggested that plasma osmolality was more greatly influence by rearing salinity than by dietary salt levels under the rearing salinity conditions tested in these experiments.

In this study, levels of electrolytes K, Na, Mg and Ca and chloride in the plasma were not affected by dietary salt supplementation in fish reared at 15 g L^{-1} in experiment 1 (Table 6). Similar results have been reported for Pacific white shrimp, *Litopenaeus vannamei*, fed salt-supplemented diets in low salinity (4 g L^{-1}) water (Roy et al., 2007). It is likely that, at a rearing salinity of 15 g L^{-1}, the levels of these ions in the external medium and in the diet were sufficient to support normal osmoregulatory processes (Sulikowski and Maginniss, 2001). For fish raised at 10 g L^{-1} in experiment 2, however, plasma concentrations (mEq L^{-1}) of Na (135–156) and chloride (113–130) were notably lower than those measured in fish raised at 15 g L^{-1} in experiment 1 (Na = 162–169, chloride = 132–141, respectively), whereas the plasma levels of other elements were comparable between these rearing salinities. These results are consistent with a lower than normal plasma osmolality (and hence, greater hypoosmoregulatory stress) under a rearing salinity of 10 g L^{-1}.

4.3. Whole body mineral composition

Whole body mineral composition (Na, K, Cl, Mn, Mg, Cu, Zn, Ca, P, and Fe) of fish raised at both 15 g L^{-1} (experiment 1) and 10 g L^{-1}

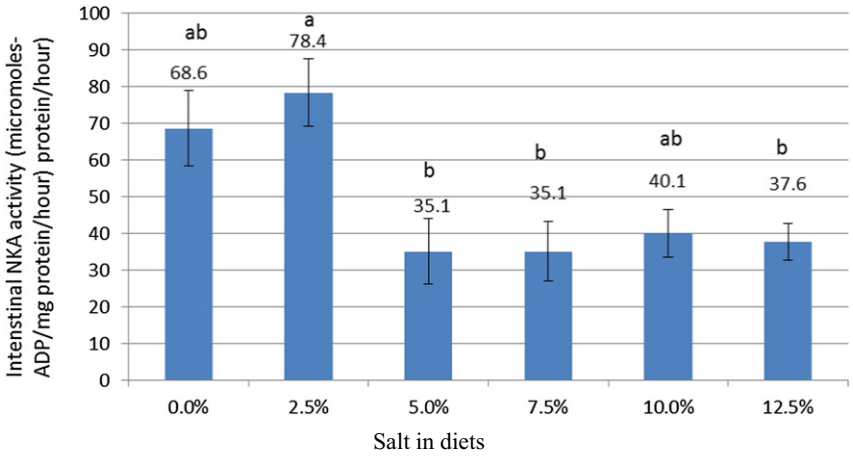

Fig. 4. Effects of graded levels of dietary sea salt on intestinal Na^+/K^+ ATPAse (NKA) activities of juvenile black sea bass raised at 15 g L^{-1} salinity. Values are means ± SEM of triplicate tanks ($N = 3$). Means not sharing a letter in common are significantly ($P < 0.05$) different.

(experiment 2) showed relatively minor differences, similar to what has been observed in Pacific white shrimp *L. vannamei* raised in low salinities (Roy et al., 2007). The similarity in whole body mineral concentrations for fish raised at 15 g L^{-1} and 10 g L^{-1}, despite lower plasma osmolality and electrolyte concentrations (especially Na and Cl) at 10 g L^{-1}, suggests that the incorporation into the body tissues of essential minerals from dietary and waterborne sources is closely regulated within narrow ranges needed to sustain tissue function and viability. This also suggests that more osmoregulatory work was needed to maintain internal homeostasis at 10 g L^{-1} than at 15 g L^{-1} salinity. Smith et al. (1995) also found no differences in muscle Na content in rainbow trout fed diets containing from 0% to 12% salt. Shearer et al. (1992) also reported that salmon fed diets with 10.5–17.5% ash showed no differences in whole body Ca, P, K and Na levels.

4.4. Whole body proximate composition

Diets containing different levels of supplemental salt did not affect the proximate composition of fish reared at 15 g L^{-1} (experiment 1). Duston (1993) found that muscle water content in trout reared in freshwater was not significantly affected by the experimental diets containing either 0% or 10% salt. For fish raised at 10 g L^{-1} in experiment 2, whole body lipid content in fish fed a diet with 5% salt was significantly higher than in fish fed the other diets. Increasing muscle lipid content with increasing dietary salt was observed in common carp (Nandeesha et al., 2000; Tacon et al., 1984). However, dietary salt supplementation did not affect muscle proximate composition in cobia reared in low salinity (5 g L^{-1}) water (Santos et al., 2014).

4.5. Gill and intestinal Na^+/K^+ ATPase (NKA) activity

NKA activity is a key enzyme involved in osmoregulation in fish and is responsible for establishing and maintaining the intracellular electrochemical gradient and is a component of the plasma membranes of chloride cells, primarily in the gills, kidneys and livers of teleosts (Lin et al., 2003; Scheiner-Bobis, 2002). Many researchers have looked at NKA activity as the driving force behind ionic exchanges in freshwater and saltwater fishes (Marshall and Bryson, 1998; Varsamos et al., 2005). For black sea bass raised at 15 g L^{-1} in the present study, no significant differences in gill NKA activity were found among fish fed diets with 0 to 10% salt (Fig. 3), suggesting that these juveniles had already developed sufficient branchial pump activity to allow them to tolerate a decrease in salinity to 15 g L^{-1} without a need to boost NKA activity (Varsamos et al., 2005). Some investigators have shown a positive relationship between gill NKA activity and dietary salt level in freshwater teleosts (Fontainhas-Fernandes et al., 2001; Salman and Eddy, 1987; Zaugg et al., 1983). For example, freshwater rainbow trout fed a high level of salt (12%) showed an increase in numbers of chloride cells and gill NKA activity (Salman and Eddy, 1987). In the present study, whereas little or no change in gill NKA activity was observed in black sea bass fed diets containing from 0% to 10% salt, gill NKA was markedly increased in juveniles fed diets supplemented with 12.5% salt. This suggests that a very high level of dietary salt stimulated a boost in branchial enzyme activity, possibly to help extrude excess salt acquired through the diet and to maintain tissue homeostasis and function.

In contrast to branchial NKA activity, which was highest in black sea bass fed a 12.5% salt-supplemented diet, intestinal NKA activities were significantly higher for fish fed low (0% and 2.5%) salt than in fish fed moderate to high levels (5 to 12.5% salt). This may indicate that, under these low salinity (15 g L^{-1}) rearing conditions, intestinal NKA activity was up-regulated to increase intestinal uptake of salt in fish fed diets containing low salt concentrations, but was down-regulated in fish fed diets with ample salt. These results further suggest that the effects of dietary salt on hyperosmoregulation at reduced environmental salinities may involve a differential regulation of the NKA enzyme in gill and intestinal tissue. Since NKA activity is an energy consuming process, the results also suggest that dietary salt levels of 5% and 7.5% produced the minimum increase in NKA activity in both gill and intestinal tissue of black sea bass raised at 15 ppt and would be optimal for maximum growth performance at this salinity.

5. Conclusions

The results of this study demonstrated under controlled laboratory conditions that black sea bass juveniles were able to maintain good growth, survival and normal whole body proximate and electrolyte concentrations at relatively low rearing salinities of 10 to 15 g L^{-1} over study periods of 8 to 10 weeks. Despite differences is plasma osmolality of fish raised at 15 or 10 g L^{-1}, diets containing different levels of supplemental salt showed little or no effects on the proximate or mineral composition of fish, suggesting that the incorporation into the body tissues of essential minerals from dietary and waterborne sources is closely regulated to sustain tissue function and viability. However, a dietary salt level of 5% improved growth (compared to fish fed 0% salt) in fish raised at 10 g L^{-1}, under which conditions the fish were exposed to greater hypoosmoregulatory stress as evidenced by overall lower whole body osmolality at this salinity. These results indicate that dietary salt supplementation may be used to avert mortality when a producer is faced with adversely low salinity conditions due to weather and tides as

may occur in coastal black sea bass fish farms sourcing brackish water from tidal creeks.

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