Evaluation of salt-incorporated diets on growth, body composition and plasma electrolytes of black sea bass *Centropristis striata* reared in a semi-pilot scale low salinity recirculating aquaculture system

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Running title: Effects of dietary salt on black sea bass reared at low salinity

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Abstract

To investigate the effects of dietary salt supplementation on growth performance, survival, body composition, and plasma electrolytes of black sea bass *Centropristis striata,* a feeding trial was conducted in a semi-pilot scale RAS under low salinity (10.8 ± 0.69 g L^{-1}) rearing conditions. Four iso-lipidic and isonitrogenous test diets were formulated with graded levels of sea salt (99.86% NaCl): 0%, 2.5%, 5%, and 7.5% dry wt. In addition, two control diets (0% salt test diet and a premium commercial diet) were tested for fish raised in full-strength seawater (34 g L^{-1}). Twelve tanks (vol = 2-m^3) of the low-salinity RAS were each stocked with black sea bass (mean wt. = 19.6 g) at a density of 100 fish per tank and at a starting salinity of 34 g L^{-1} and salinity was gradually decreased (0.5 g L^{-1} per day) to 10.1 g L^{-1} in 30 days. In addition, six tanks (2-m^3) of a RAS supplied with full-strength seawater were each stocked with 100 fish from the same cohort, with 3 tanks fed either of the two control diets (0% salt or the commercial diet). After the 8-month feeding trial, fish grown at low salinity were fed their respective diets for an additional 5 weeks under adverse low-salinity conditions in which salinity was further decreased gradually from 10.1 g L^{-1} to 4 g L^{-1}. Survival over the 8 months remained high (93-100%) among treatments. Growth (% body weight gain) among the treatments in low salinity ranged from 766 to 825%, comparable to fish raised in full seawater (788-813%). Plasma osmolality (mOsm kg^{-1}) for fish ranged from 336-357 among all treatments. However, no significant relationships between weight gain, feed conversion ratio, survival, plasma osmolality, and electrolyte concentrations and dietary salt level were observed among fish raised in low salinity. When salinity was further reduced from 10.1 g L^{-1} to 4 g L^{-1}, fish fed 0% salt showed poor survival (29%), whereas fish fed 7.5% salt showed highest survival (67%) , and a significant ($P < 0.05$) linear relationship between dietary salt and survival was observed. The

results suggest that black sea bass juveniles can be raised at a low salinity of 10.1-12.3 g L^{-1} with no negative effects on long-term growth performance. Salt-incorporated diets, however, improved survival under extreme low salinity (~ 4 g L^{-1}) challenge conditions. These findings have important implications for rearing black sea bass in low salinity RAS and for the siting of black sea bass RAS grow-out operations.

Keywords: Black sea bass, dietary salt, low salinity, plasma osmolality, plasma electrolytes

Highlights of the manuscript:

1. Black sea bass juveniles were able to maintain good growth, survival, and normal plasma osmolality when reared at low salinities of 10.1-12.3 g L^{-1} for 8 months in a semi-pilot recirculating aquaculture system and fed diets supplemented with up to 7.5% salt.
2. Fish fed diets supplemented with 2.5 to 7.5% sea salt showed significantly higher survival during extreme hyposalinity (4 g L^{-1}) challenge.
3. These findings have important implications for production of black sea bass in recirculating aquaculture systems sited anywhere low-salinity water may be sourced or prepared.

Relevance with commercial aquaculture:

Results of study suggest that black sea bass juveniles may be raised in marine RAS under low salinity of 10.1-12.3 g L^{-1} with no negative effects on long-term growth performance and that salt-incorporated diets improved survival under extreme low salinity (~ 4 g L^{-1}) challenge conditions. This study has important implications for the siting of black sea bass farming operations in coastal areas.

1. Introduction

Black sea bass *Centropristis striata* is a member of the marine finfish family Serranidae (true sea basses and groupers) and inhabits continental shelf waters of the eastern US from Florida to Maine. With increasingly restrictive fishing regulations and potential for limited future supplies of this highly-sought, premium value species, methods for artificial propagation of black sea bass have been under development since the early 2000's. Techniques for spawning adult black sea bass in captivity (Watanabe et al., 2003; Berlinsky et al., 2005), raising larvae through juvenile stages in a hatchery (Berlinksy et al., 2001; Copeland and Copeland 2006; Rezek et al., 2010; Carrier et al., 2011; Russo et al., 2017), and raising from fingerling to adult stages in recirculating aquaculture system using sustainable, low fish meal-based diets (Watanabe et al., 2003, Watanabe, 2011; Alam et al., 2008, 2012, 2018) have been developed in the eastern US for black sea bass.

In the Eastern US, interest in commercial culture of marine finfish species such as the black sea bass using land-based recirculating aquaculture systems (RAS) is increasing among private fish farmers. For marine finfish production in RAS, the culture of euryhaline finfish species in low salinity brackishwater would be advantageous because as it reduces the salt required to prepare and replace saltwater in inland RAS, mitigates environmental problems related to discharge of salty effluent, and can provide more flexible options for siting for production facilities, which do not need to be located next to a source of natural seawater.

Marine finfish drink seawater and excrete excess salt that they receive through their gills and intestines. In low-salinity brackishwater, however, these fish must actively take up salt through their skin, kidneys, gills and through their food (Gatlin et al., 1992; Karnaky, 1998;

Perry et al., 2006) to compensate for a passive outward loss of ions (e.g. Na and Cl) through the gills, intestine and kidneys.

In European sea bass *Dicentrarchus labrax*, growth of juveniles fed 1-9% dietary NaCl in freshwater over a 50-day period was 19% higher than fish fed 0% salt (Eroldogan et al., 2005). Growth and feed efficiency were greatest at a moderate level (1-5%) of salt supplementation, with an optimum at 3% (Eroldogan et al., 2005). In Asian sea bass (barramundi) *Lates calcarifer* juveniles reared in fresh water, addition of up to 4% NaCl to the diet increased intestinal brush border digestive enzymes activities and improved feed conversion ratio and feed costs (Harpaz et al., 2005). Juvenile gilthead sea bream *Sparus aurata* 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).

Black sea bass are moderately euryhaline as reported by some previous studies and the growth performances at salinities of 20 and 30 g L^{-1} were much higher than the fish raised at 10 g L^{-1} (Atwood et al., 2001, 2003, 2004; Cotton et al., 2003; Young et al., 2006). This suggested that rearing of black sea bass in high-salinity brackish water to full-strength seawater is needed for commercial production of this finfish. However, recently published studies in our laboratory at the University of North Carolina Wilmington (UNCW) have revealed that optimum growth and survival of juveniles at a relatively low salinity of 10 g L^{-1} under controlled laboratory conditions was maintained in fish fed diets supplemented with 5% sea salt (Alam et al., 2015). These findings suggest that other factors (e.g. age or strain) may affect salinity tolerance (Watanabe,2011) and reveal that dietary salt supplementation can be used to meet the requirements for osmoregulation under salinities considered sub-optimal for culture and that the

effects of dietary salt supplementation become more distinct at lower salinities and increasing hypo-osmoregulatory stress. The overall goal of this study was to extend the results of these laboratory studies by evaluating the efficacy of dietary salt incorporation in black sea bass reared in a low-salinity RAS from juvenile to near marketable stages. The specific objectives were to evaluate the effects of three selected levels of dietary salt supplementation on growth, survival, body composition and osmoregulatory ability of juvenile black sea bass reared from small juvenile to sub-adult stages in a pilot-commercial scale RAS operated under low salinity (10 g L^{-1}).

2. Materials and Methods

2.1. *Experimental System*

2.1.1. Low salinity reservoir system

A 25-m^3 automated reservoir system was assembled to prepare and store water of prescribed salinities for the low-salinity rearing trials. The automated system (Point Four™ Monitors & Controllers, Pentair Aquatic Eco-Systems, Inc. Apopka, FL) was designed to allow the targeted salinity to be entered on a key pad to control electronic solenoid valves that metered the required volumes of both seawater and municipal freshwater into the reservoir where water was mixed by vigorous aeration. The low-salinity reservoir system enabled salinity in the pilot scale RAS (see 2.1.2 below) to be efficiently lowered from full strength seawater at 34 g L^{-1} to the desired experimental salinity of 10 g L^{-1} at a desired rate of salinity decrease. The reservoir and RAS were filled with seawater and conditioned for 2 weeks before experimental fish were stocked for the feeding trial with salt-incorporated diets.

2.1.2. Semi-pilot scale low-salinity RAS

The low salinity experimental units consisted of twelve, 1.8-m diameter (vol. = 2,660 L, depth = 0.91 m) insulated fiberglass tanks supported by a RAS. The RAS consisted of a bubble wash bead filter, fluidized bed biofilter, foam fractionator and UV sterilizer. Temperature was controlled using a heat pump, and aeration was supplemented with pure oxygen via diffusers. Each tank was covered with a conical lid to prevent fish from jumping out. The tanks were supplied with water of prescribed salinity from the low salinity reservoir system.

2.1.3. Semi-pilot scale seawater RAS

The full-strength sea water experimental units consisted of six 1.8-m diameter (vol. = 2,660 L, depth = 0.91 m) insulated fiberglass tanks supported by a RAS similar to that used for the low-salinity RAS described above. The tanks were supplied with filtered, full strength sea water pumped from Bank's Channel, a protected channel near the laboratory that is continually flushed from a natural ocean inlet.

2.2 Experimental fish

This study was conducted at the UNCW Aquaculture Facility (Wrightsville Beach, North Carolina) under the UNCW IACUC protocol #A14-14-021. Juvenile black sea bass were cultured from eggs produced by adult broodstock held in 2.6-m^3 tanks under controlled daylength and temperature regimes. Mature (post-vitellogenic stage) females were implanted with pelleted luteinizing hormone-releasing hormone analog to induce ovulation and volitional spawning (Watanabe et al., 2003), and fertilized eggs were hatched and larvae reared through the juvenile stages using protocols established at UNCW (Copeland and Copeland 2006; Rezek et al., 2010; Carrier et al., 2011; Watanabe, 2011, Russo et al., 2017).

2.3 Experimental diets and design

Based on previous studies on black sea bass, a basal diet containing fish meal and alternative proteins (soybean and poultry by product meal) was formulated to contain 52% crude protein and 13% crude lipid (Alam et al., 2008; 2009; 2015) and a 2.5% mineral mix formulated for marine finfish (Alam et al., 2000). To determine the effects of additional dietary salt enhancement on growth and feed utilization in black sea bass reared at sub-optimal salinity (10 g L^{-1}), four test diets were formulated to supplement graded levels (2.5% increments) of salt: 0% (control, D1-A), 2.5% (D2), 5.0% (D3), and 7.5% (D4) of a premium food grade natural sea salt (Cargill Hi-Grade Evaporated Salt, 99.86% NaCl) (Cargill Salt, Minneapolis, MN) (Table 1).

As salt levels were increased in the test diets, wheat starch and alpha cellulose were decreased proportionally, while the percentage of other ingredients used in the diets were constant (Alam et al., 2012; 2018). Diets (1.5 mm and 3.5 mm pellets) were prepared at the UNCW Aquaculture Facility feed preparation laboratory. As control groups, fish were also raised in full-strength seawater (33-34 g L^{-1}) RAS on the 0% salt test diet (control D1-B) and on a premium commercial diet (3-5 mm pellet size Europa, Skretting, D5) containing 50% crude protein (analyzed value 52.5%) and 15% lipid (analyzed value 16.3%) (Table 1).

2.4. Feeding trial

Juvenile black sea bass (N = 1,800; age = approximately 4.5 mos. post-hatching, mean wt. = 19.6 $\pm$ 0.32 g) were used for the experiment. To begin the experiment, fish to be acclimated to low salinity brackishwater (N = 1,200) were stocked into twelve tanks of the low salinity RAS in seawater (34 g L^{-1}) at a density of 100 fish per tank. Three replicate tanks were assigned to each of the four test diet treatments containing different levels of salt (0%- D1-A, 2.5%-D2, 5%-D3, and 7.5%-D4).

Control fish to be maintained in full strength seawater ($N = 600$) were stocked into six tanks of the seawater RAS at 34 g L^{-1} and at a density of 100 fish per tank. Three replicate tanks were assigned to the first control group fed the 0% salt diet (D1-B), while the remaining three tanks were assigned to the second control group fed the premium commercial diet (D5). For fish raised in the low-salinity RAS, salinity was gradually decreased from 34 to 10.1 g L^{-1} over a period of four weeks at a rate of approximately 0.5 g L^{-1} per day. Fish were initially fed their respective treatment diets two times a day (AM and PM) to apparent satiation (i.e., as much as they could consume with little or no wastage) for 30 mins. After 4 months of feeding, the fish were fed once a day (AM) to avoid excess feed in the water, as fish were satiated by a single feeding. During the feeding trial, water quality was monitored every day. Water temperature was maintained at 21-23 ^{o}C and dissolved oxygen from 7.5 - 8.5 mg/L. The ranges of other water quality parameters in the experimental tanks during the experimental periods were maintained at suitable levels for black sea bass as follows: pH 7.7 - 8.3, total ammonia-N 0.00 - 0.30 mg/L and nitrite-N 0.00 - 0.13 mg/L (Watanabe, 2011). Salinity, pH, temperature and DO were monitored daily, while total ammonia-N, and nitrite-N were monitored twice a week.

2.5. *Growth performance*

To monitor growth all the fish in each replicate tank were weighed after 5 mos. post-stocking. Fish were lot weighed (approximately 10 fish per lot) using an anesthetic (MS-222, 60 ppm). Mortalities (if any) were recorded daily during the feeding trial. A final sampling was conducted after 8 months of feeding and all fish were weighed to complete the feeding trial. Samples (3 fish from each tank) were collected for proximate composition analyses.

2.6. Plasma osmotic pressure and electrolyte analysis

After the feeding trial, the fish were fed their respective diets once daily and the next morning three fish were harvested from each tank and euthanized on ice with fresh water and blood samples were immediately collected using a hypodermic syringe inserted into the caudle artery, and the plasma was immediately separated using a centrifuge. Plasma osmotic pressure of fish was measured immediately upon sampling using a vapor pressure osmometer (Wescor Vapor Pressure Osmometer 5520, Logan, Utah, U.S.A.). Plasma was stored in 1 ml plastic vials and at -80 ^{o}C and the samples were sent on dry ice to Cornell University (Animal Health Diagnostic Center, College of Veterinary Medicine, Ithaca, NY) for analysis of plasma electrolytes (Na, K, Cl, Ca, P, Mg and HCO_3).

2.7. Diet electrolyte analysis

Mineral composition of the diets was also analyzed at Cornell University (Animal Health Diagnostic Center), while chloride analysis of diets samples was conducted at, North Carolina State University (Department of Soil Science, Raleigh, NC).

2.8. Hyposalinity challenge

After collecting fish for whole body biochemical analysis and blood samples from the feeding trial at 10.1-12.3 g L^{-1}, remaining fish (15 fish per tank) were fed the respective diets under acute low salinity conditions for another 5 weeks. Starting from 10.1 g L^{-1}, the salinity of the water was decreased gradually (0.5-0.25 g L^{-1} per day until a salinity of 4 g L^{-1} was reached in 30 days. Fish mortalities in each rearing tank were recorded daily during this 5-week hyposalinity challenge.

2.9. Whole body proximate analysis

At the end of the 8-month feeding trial, three fish from each tank were collected freeze-dried, ground and part of the proximate analysis was conducted at the UNCW Aquaculture Research Laboratory (AOAC, 2000). Due to renovation of the UNCW Aquaculture Research Lab after hurricane Florence, some of the analysis were conducted at New Jersey Feed Lab (Trenton, NJ, USA). Fish whole bodies were freeze dried to determine moisture content (Labconco Freeze Dryer, Kansas City, MO, USA), and ash contents were determined using a muffle furnace (BARNSTAD Thermolyne Muffle Furnace, IA, USA). Crude protein and total lipid of all experimental diets and body tissues were analyzed (AOAC, 2000) at New Jersey Feed Lab (Trenton, New Jersey, USA). Moisture contents in the diets were analyzed by standard methods (AOAC, 2000) using a Fisher Scientific Isotemp oven (Pittsburgh, PA, USA).

2.10. Statistical analysis

The results of growth performance, biochemical analysis and the concentration of plasma electrolytes are presented as mean ± SE (standard error of the mean). All statistical analyses were performed using JMP Pro 13.0 for windows. All evaluated variables were subjected to analysis of variance to determine if the levels of salt supplementation significantly ($P < .05$) affected the observed responses in low-salinity water. In addition, to determine if the effect was linear and/or quadratic, a follow-up trend analysis using orthogonal polynomial contrasts was performed (Burns and Gatlin, 2019; Rosales et al., 2017). Analysis of variance was also used to compare treatment means between fish fed the control diet in low salinity and full strength seawater and between fish fed the formulated control (0% salt) diet and commercial diet in full strength seawater.

3. Results:

3.1. Diet electrolyte concentrations

As salt level was increased in the diets, the analyzed levels of sodium and chloride in the test diets increased incrementally (Table 2). In the test diets, sodium level increased from 0.52 to 2.99% and the chloride level increased from 0.53 to 5.19% as salt levels was increased from 0-7.5%. However, the concentrations of other major electrolytes (K = 0.1.24-1.28%, Mg = 0.22-0.24%, Ca = 2.52-2.74% and P = 2.11 -2.23%) were similar among diets (Table 2), except in the commercial diet (D5), in which all electrolyte concentrations were lower than in the UNCW formulated diets, including the 0% salt diet (D1) (Table 2). The Na and Cl concentrations in the commercial diet D5 were approximately 57.7% and 81.1%, respectively, of those in the UNCW formulated 0% salt diet (D1).

3.2. Effects on growth performance, survival and feed conversion ratio (FCR) after 8 months of the feeding trial

3.2.1. In low-salinity water on UNCW formulated diets

The initial body weight of the fish among fish reared in the low salinity RAS fed the salt-incorporated diet treatments ranged from 19.4 to 19. 8 g with no significant ($P > 0.05$) differences (Table 3). After 8 months of the feeding the fish, final body weights (171- 182 g) and body weight gains (766-825%) were not significantly different among treatments (Table 3). For fish raised in low-salinity water, the highest percent weight gain (825%) was found for fish fed 2.5% salt (D2) , whereas the lowest percent weight gain (766%) was found for fish fed 5% salt (D3) (Table 3). However, differences in percent weight gain among fish fed the experimental diets in low salinity were not significant. Survival of fish raised in low-salinity after 8-months remained high (87.7-

96.7%), with no treatment differences (Table 3). FCR among treatment groups ranged from 1.41 to 1.61, with no significant differences. No significant linear or quadratic trends were found between dietary salt level and final weight, body weight gain, survival, or FCR (Table 3).

3.2.2. In low salinity vs seawater on 0% salt UNCW formulated diet

Initial body weight (19.5-19.7 g) was not significantly different between fish fed the UNCW formulated control (0% salt –D1) diet under low salinity (D1-A) and in full-strength sea water (D1-B) (Table 4). After 8 months of the feeding trial, there were no significant differences in final body weight (176-182 g), body weight gain (813-821%), survival (89.3-99.6%) and FCR (1.46-1.52) between fish fed the UNCW formulated control diet (0% salt –D1) under low salinity and in full-strength sea water (Table 4).

3.2.3. In seawater on 0% salt UNCW formulated vs commercial diet

Initial body weight (19.5-19.6 g) was not significantly different between fish fed the UNCW formulated control diet (0% salt, D1-B) and the commercial diet (D5) in full strength seawater (Table 5). After 8 months of the feeding trial in seawater, the survival was over 99% for both treatments. There were no significant differences in final body weight (173-178 g), percent weight gain (788-813%), and FCR (1.41-1.46) between fish fed the UNCW formulated control diet (0% salt, D1-B) and the premium commercial diet (D5) (Table 5).

3.3. Plasma osmolality and electrolyte after 8 months of the feeding trial

3.3.1. In low-salinity water on UNCW formulated diets

Plasma osmolality (mOsm kg^{-1}) of fish raised at a salinity level of 10.1-12.3 g L^{-1} ranged from 344-357 (Table 3) and was not significantly different among the different treatment levels

of dietary salt. No significant linear or quadratic trends were found between dietary salt level and plasma osmolality. No significant differences were observed in plasma electrolyte concentrations among fish fed the formulated diets incorporating different levels of salt (0%-D1, 2.5%-D2, 5%-D3, and 7.5%-D4) (Table 6). No significant linear or quadratic trends were also found between the levels of dietary salt and the concentrations of plasma electrolytes (Table 6).

3.3.2. In low salinity vs seawater on 0% UNCW formulated diet

After 8 months of the feeding trial, there were no significant differences in plasma osmolality (336-353 mOsm kg^{-1}) between fish fed the UNCW formulated control (0% salt –D1-A and D1-B) diet under low salinity and in full-strength sea water (Table 4). No significant differences in plasma electrolyte concentrations (mEq L^{-1}) were found for Na, K, Cl, Ca, and Mg between fish fed the control diet (0% salt – D1-A) under low salinity and in full-strength sea water (D1-B), except for P (Table 4) . Plasma P (mg dL^{-1}) was significantly ($P < 0.05$) lower in fish reared in low salinity (12.0) vs seawater (15).

3.3.3. In seawater on 0% UNCW formulated vs commercial diets

There were no significant differences in plasma osmolality (336-344 mOsm kg^{-1}) between fish fed the UNCW formulated control diet (0% salt, D1-B) and a premium commercial diet (D5) (Table 5). No significant differences in plasma electrolyte concentrations (mEq L^{-1}) were found for Na, K, Cl, Ca, and Mg between fish fed the control diet (0% salt – D1-B) and commercial diet (D5) in full-strength sea water, except for P and bicarbonate concentrations (Table 5) . Plasma P (mg dL^{-1}) was significantly higher in fish fed 0% UNCW diets (D -B) (15.0) vs seawater (10.7). On the other hand, HCO_3^- (mEq L^{-1}) was significantly lower in fish reared with 0% UNCW diets (9.3) vs commercial diet (12.0) (Table 5).

3.4. Whole body proximate composition after 8 months of the feeding trial in low salinity and full strength sea water

The whole-body proximate composition of fish fed in low salinity with UNCW formulated diets is shown in Table 7. No significant effects of dietary salt supplementation on whole body moisture, crude protein, total lipid and ash content were found. However, a significant ($P < 0.05$) linear trend was found between the level of dietary salt and crude protein content in the fish whole body (Table 7), with crude protein decreasing at higher levels of salt supplementation. After 8 months of the feeding trial, there were no significant differences in whole body proximate compositions between fish fed the UNCW formulated control (0% salt –D1-A and D1-B) diet under low salinity and in full-strength sea water (Table 4). There were no significant differences in whole body proximate between fish fed the UNCW formulated control diet (0% salt, D1-B) and a premium commercial diet (D5) (Table 5) in full strength sea water.

3.5. Under acute hyposalinity challenge

After 8 months of the feeding trial, when juvenile black sea bass raised at 10.1-12.3 g L^{-1} were challenged with a further reduction in salinity from 10.1 to 4 g L^{-1} within a period of 5 weeks. A clear trend ($P < 0.05$) toward higher survival (Y) with increasing dietary salt level (X) was observed from 29% survival for fish fed 0% salt to 67% survival for those fed 7.5% salt. Linear regression defined this relationship as follows: $Y = 13.2X + 16.5$, $R^2 = 0.9756$, $P < 0.05$) (Fig. 1).

4. Discussion

4.1. Growth performance

In the present study, dietary salt supplementation up to 7.5% did not impair growth of juvenile black sea bass reared at a suboptimal salinity of 10.1-12.3 g L^{-1} over a period of 8 months, and the overall growth (weight gain = 766 to 825%) of the fish reared at low salinity (Table 3) at all levels of dietary salt supplementation was similar to the growth of fish (weight gain 813%) reared in full strength sea water (Table 4) when fed a UNCW formulated control diet with 0% salt. These results indicate that following gradual acclimation of juvenile black sea bass from full strength seawater to a relatively low rearing salinity of 10.1-12.3 g L^{-1}, produced by diluting natural seawater with freshwater, the UNCW formulated diet satisfied the physiological needs of these juveniles reared to near harvestable sizes. These results are in accord with and extend our previous findings that growth of black sea bass juveniles fed diets supplemented with 0 to 7.5% salt under low-salinity (10.1-12.3 g L^{-1}) was not impaired after 10-weeks of rearing in laboratory aquaria (Alam et al., 2015). For fish raised in full strength seawater in the present study, the UNCW formulated diet with 0% salt (D1), produced similar growth to a premium grade commercial diet for marine finfish (Skretting, Canada) (Table 5). As this well-established commercial diet has been routinely used to raise black sea bass from juvenile to marketable stages in full strength seawater RAS in our laboratory (Watanabe, 2011), we infer that the UNCW formulated diet is of comparable quality and that it provided sufficient nutrients and minerals for rearing under sustained low salinity conditions (10.1-12.3 g $L^{-1}$of approximately 6.5 months (excluding the acclimation period). Growth performance of black sea bass after eight months of the feeding trial in both low salinity and full strength sea water in a pilot scale RAS was comparable to what was reported in previous studies of wild-caught (Copeland et al., 2002)

and hatchery-raised (Watanabe, 2011) juvenile black sea bass reared in RAS in full strength seawater (34 g L^{-1}) and fed commercial diets over a comparable size range (Copeland et al., 2002, Watanabe, 2011).

The supplemental effects of dietary salt have been studied on growth and survival of several freshwater and marine fish reared in fresh or brackish water. In the freshwater tilapia *Oreochromis niloticus* L., adding 1-1.5% salt to the diet improved growth performance in fresh water (Fontaınhas-Fernandes et al., 2001; Cnaani et al., 2009; Mzengereza and Kang'ombe, 2015; Debnath et al., 2017).

For marine finfish species, however, the benefits of salt supplementation under suboptimal salinity conditions are varied. For example, in the marine finfish cobia *Rachycentron canadum* reared at 5 g L^{-1} (Santos et al., 2014) and in the anadromous Atlantic salmon *Salmo salar* (Shaw et al., 1979) raised in freshwater, the effects of dietary salt (at 0-10% and 2-12%, respectively) on growth, feed efficiency and feed intake were negligible. On the other hand, in the marine finfish red drum reared in freshwater, juveniles fed a NaCl-supplemented diet at 2% showed improved growth and feed efficiency (Gatlin et al., 1992). However, in brackish water (6 g L^{-1}) or seawater (35 g L^{-1}), the NaCl supplemented diet did not influence growth performance of these red drum juveniles (Gatlin et al., 1992). These authors suggested that the salt requirements for normal physiological functions of red drum juveniles were fulfilled in brackish or seawater but not in freshwater (Gatlin et al., 1992). In the present study, body weight gain and survival of black sea bass reared in low salinity 10.1-12.2 g L^{-1} over an extended study period of 8 months were not different among dietary salt treatments ranging from 0 to 7.5%, also suggesting that the amount of salt available under these brackish salinities was sufficient to satisfy its physiological needs.

In an earlier study conducted in laboratory aquaria under controlled conditions at 10 g L^{-1} salinity growth of small juvenile black sea bass from an initial weight of 9 g to a final weight of 25-39 g over a relatively short study period of 10 weeks was faster on a 5% salt diet compared to a 0% salt control diet (Alam et al., 2015). In the present study, however, dietary salt supplementation from 0 to 7.5% had no effects on growth performance of larger juveniles raised from an initial weight of 19.7 g, to a final weight 171-182 g in a semi pilot scale RAS over an extended period of 8 months. The lack of a significant effect of dietary salt supplementation on growth in the present study could be due to the use of older and larger fish than in the previous laboratory study. It is known, for example, that in euryhaline finfish such as tilapia (Watanabe et al., 1990) and salmonids (Shaw et al., 1979), osmoregulatory ability varies ontogenetically, increasing from relatively low levels in early juveniles and reaching a maximum at the late juvenile stages (Watanabe et al., 1990, Gatlin et al., 1992). It is possible, therefore, that larger juveniles used in the present study had already attained a maximum level of hypoosmoregulatory ability (i.e., low salinity tolerance) at stocking so that dietary salt effects on growth were minimized. Salman and Eddy (1988) likewise showed different growth responses between small- and large-sized rainbow trout to salt feeding in dechlorinated tap water, with fish of 12.5 g initial weight showing higher weight gain with 9.2- 11.6% salt supplementation to both commercial and formulated diets, whereas larger fish of 28 and 33 g initial weight showed no differences in weight gain at these levels of dietary salt (Salman and Eddy, 1988).

4.2 Feed Utilization

The results of the present study suggest a greater euryhaline ability of black sea bass without affecting the feed utilization than was anticipated based on previous reports in which

growth at 10 g L^{-1} was much slower than at 20 or 30 g L-1 (Atwood et al., 2001, 2003, 2004; Cotton et al., 2003; Young et al., 2006). The results also suggest that all of the test diets, including the 0% salt basal diet, provided sufficient minerals to the fish over the relatively long 8-month study period under suboptimal salinity conditions of 10.1-12.3 g L^{-1}.

Haung et al. (2017) concluded that high soybean meal-based diets need to be supplemented with appropriate minerals to compensate for the negative effects of their low mineral composition on *L. vannamei* cultured in low-salinity water. In the present study, the basal diet included significant levels of soybean and poultry byproduct meal; however, a mineral premix formulated for marine fish (Alam et al., 2000) was included in each diet in addition to the supplemental sea salt. Therefore, except for the Na and Cl concentrations, the electrolyte concentrations (Table 2) were similar among the test diets fed in low salinity water. Based on comparable growth performance at all levels of dietary salt, we infer that the dietary minerals were balanced in all treatment diets and were sufficient for the normal osmoregulation of black sea bass raised in low saline water from juvenile to near marketable stages over an extended time period.

4.3. Survival under acute hyposalinity challenge conditions

In the present study, following an 8-month period of acclimation and rearing of black sea bass at 10.1-12.2 g L^{-1}, a further and gradual reduction of salinity to 4 g L^{-1} proved lethal to these fish. This is consistent with a previous 7-day study in which black sea bass juveniles survived abrupt transfer from 15 g L^{-1} to 8 g L^{-1}, but not to a slightly lower salinity of 6 g L^{-1} (Young et al., 2006). Atwood et al. (2001) reported that at 21°C, black sea bass survived for 7 d following abruptly exposure to salinities of 10, 20, or 35 g L^{-1}. However, fish exposed to a salinity of 5 g

L^{-1} died within 3 d. All of these studies demonstrate that 4 g L^{-1} is a lethal salinity for the black sea bass.

In the present study, when fish raised for 8 months at 10.1-12.3 g L^{-1} were challenged with acute hypo-osmoregulatory conditions by gradually decreasing salinity from 10.1 g L^{-1} to 4 g L^{-1} over a period of 30 days, clear 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 (0%) showed much lower survival (29%) than fish fed 2.5% salt (42%), while survival remained much higher among fish fed diets with 5.0 - 7.5% supplemental salt (60-67-%) (Fig. 1). Hence, definitive beneficial effects of dietary salt supplementation on hyposalinity tolerance and survival became evident under acute hyposalinity conditions (4 g L^{-1}). This is in accord with our previous short term laboratory study where juvenile black sea bass fed 5 to 12.5% salt showed higher survival than those fed 0 to 2.5% salt under acute low salinity (4 g L^{-1}) challenge conditions (Alam et al., 2015). This finding also suggests that when a coastal fish farmer is faced with adversely low saline water in estuarine conditions, dietary salt supplementation could be used to reduce the mortality of fish.

In the present study, higher survival of fish fed a high level (7.5%) of dietary salt under acute low salinity challenge conditions may be related to increased absorption of amino acids to satisfy the fish's metabolic requirements during rearing under low salinities (Davis and Gatlin, 1996). Many researchers have reported that gill and intestinal Na^+ K^+ATPase (NKA) activity is the driving force behind ionic exchanges in seawater and freshwater fishes (Marshall and Bryson, 1998; Lin et al. 2003; Varsamos et al., 2005; Alam et al., 2015). In the present study, the higher survival of fish fed a high level of dietary salt (7.5%) under hyposaline (4 g L^{-1}) challenge conditions could be due to enhanced branchial enzyme activity that helped to maintain

tissue homeostasis and function. Although branchial NKA activity was not measured in the present study, this possibility is supported by our previous laboratory findings in which black sea bass juveniles fed 12.5% salt under hyposalinity (4 g L^{-1}) challenge conditions showed a marked elevation of branchial NKA activity (Alam et al., 2015).

4.4. Plasma osmolality and electrolytes

Under hyper- or hypo-osmotic conditions, the ability to control blood osmolality of aquatic animals is species-dependent (Varsamos et al., 2005). There were no differences observed in blood osmolality (range = 398-435 mOsm L^{-1}) in marine rabbit fish *S. rivulatus* reared at salinity ranges 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 10.1-12.3 g L^{-1} ranged from 344 - 357 mOsm kg^{-1} (Table 3), slightly higher than plasma osmolality for black sea bass raised in full strength seawater on the UNCW formulated diet (D1-B) or on the commercial diet (D5) (336-344 mOsm kg-1) (Table 5). Blood osmolality of fish raised at 10.1-12.3 g L-1 was also similar to what was observed in our previous study in juvenile black sea bass fed salt supplemented diets at 15 g L^{-1} (330-350 mOsm kg^{-1}) (Alam et al., 2015). These values are lower than the values reported for some marine finfish (380-450 mOsm kg^{-1}) (Barton, 2007). As evidenced by inability to maintain stable blood osmotic pressure (i.e, osmotic imbalance), osmoregulatory ability in fish is reduced as the salinity tolerance limit is reached (Iwata and Shigueno, 1980; Morgan and Iwama, 1996; Sampaio and Bianchini, 2002; Gong et al., 2004). The close blood osmolality values of black sea bass raised at a rearing salinity of 10.1-12.3 g L^{-1} vs full strength seawater in the present study suggests that a rearing salinity of 10.1-12.3 g L^{-1} did not pose a significant osmoregulatory challenge for juvenile black sea bass and is consistent with the

negligible effects of dietary salt supplementation on growth at this salinity. Plasma osmolality was not statistically different among fish fed test diets containing graded levels of salts from 0-7.5% under a low rearing salinity of 10.1-12.3 g L^{-1}, suggesting that dietary salt supplementation did not produce significant osmoregulatory imbalances throughout the feeding trial. Based on the slightly higher plasma osmolality observed under low salinity rearing conditions (344 - 357 mOsm kg^{-1}) compared to the fish raised in full strength sea water (336-344 mOsm kg^{-1}), we infer that, while black sea bass were able to survive and grow well at 10.1-12.3 g L^{-1}, this salinity is approaching the lower limit of tolerance, below which fish would be unable to adapt and maintain osmotic balance.

At 10.1 -12.3 g L^{-1} fish plasma electrolyte concentrations of Ca, K, Na, Mg, and Cl were not influenced by the salt supplementation in the diets from 0 to 7.5% (Table 6). Similar results have been reported for Pacific white shrimp *Litopenaeus vannamei* fed different levels of mineral-supplemented diets in low salinity (4 g L^{-1}) water (Haung et al., 2017; Roy et al., 2007) and for juvenile black sea bass fed salt-supplemented diets ranging from 0 -12.5% in low salinity (10 g L^{-1}) water (Alam et al., 2015). Plasma electrolytes (K, Na, Mg and Cl) concentrations were similar between fish fed the UNCW formulated control diet (D1-A - 0% salt) in low salinity and in full strength seawater (D1-B – 0% salt), indicating that the basal diet provided sufficient electrolytes irrespective of rearing salinity.

Interestingly, for fish reared in full seawater, significantly lower phosphorous and higher level of bicarbonate concentrations in the plasma were found in fish fed the commercial diet compared to fish fed the UNCW formulated control diet (D1-A, D1-B) (Table 4, 5). The reasons for these differences are not easily understood as the formulation of the commercial diet is proprietary. However, it is speculated that the use of poultry byproduct meal and high amount

of fish meal in the UNCW formulated diet provided a significant source of phosphrous which was reflected in blood concentrations.

4.5. Whole body proximate composition

In low salinity (10.1-12.3 g L^{-1}), no significant effects of dietary salt supplementation were observed on the whole body proximate composition (moisture, crude lipid, and ash), irrespective of level of salt supplementation. In cobia reared in low salinity (5 g L-1), salt supplementation in the test diets also did not affect the proximate composition of muscle (Santos et al., 2014). In European sea bass the dry matter and ash content of muscle rose slightly with increasing dietary salt (Eroldoğan et al., 2005). The body composition of the Asian sea bass, on the other hand, was not affected by salt feeding, although there was less fat accumulation in the fish fed diets containing additional salt (Harpaz et al., 2005). In rainbow trout reared in freshwater muscle water content was not affected by test diets containing either 0% or 10% salt (Duston, 1993). In black sea bass (present study), salinity of rearing water (low salinity vs full strength seawater) (Table 4), or type of diet used (UNCW formulated control diet vs commercial diet in full strength sea water) (Table 5) also did not affect whole body proximate composition. In contrast, juvenile black sea bass raised at 10 g L^{-1} under controlled laboratory conditions for 10 weeks showed a higher whole body lipid content when fed a diet with 5% salt compared to diets with 0, 2.5 or 7.5% salt (Alam et al., 2015). However, the lack of effects of dietary salt on whole body lipid content could be due to the larger fish used in the present long-term study. The lipid level in all salt-supplemented formulated diets (D1-D4) were very similar (13.1-13.4%), while the lipid level in the commercial diet was slightly higher (16.3%). However, the higher lipid level did not produce any differences in whole body lipid content, growth performance, survival, or other evaluated parameter compared to the formulated diets. This was probably

because the lipid levels in the formulated diets were sufficient to fulfill the lipid requirements of black sea bass as reported in a previous study (Alam et al., 2009).

In the present study, a trend toward lower whole body protein with higher dietary salt was observed in black sea bass reared in low salinity water (Table 7). Higher levels of salt (more than4 %) caused lower nutrient and energy digestibility as they interfered with the acid secretion in rainbow trout (Salman, 1987) and could likewise have reduced protein assimilation in muscle tissue in the present study. In addition to reducing the apparent protein digestibility of salty diets, it is possible that lower assimilation of protein in muscle was related to the dilution of nutrients in the salt-enriched diets, or to their faster evacuation from the digestive system (Jobling, 1986). In rainbow trout, high salt levels increased the rate of passage of digesta and therefore reduced the time available for the digestive enzymes to attack their substrate with subsequent reductions in digestibility (Jobling, 1986).

5. Conclusions

Growth performance and biochemical analysis of body tissues suggest that, irrespective of dietary salt supplementation, juvenile black sea bass can be acclimated to and raised at a low salinity of 10.1-12.3 g L^{-1}, with no adverse effects on long-term growth performance, plasma osmolality, or fish plasma electrolytes profiles compared to fish raised in full-strength 34 g L^{-1} seawater. Salt-incorporated diets up to a 7.5% level of supplementation, however, significantly improved fish survival under extreme low salinity (~ 4 g L^{-1}) challenge conditions. These findings have potentially important implications for rearing black sea bass and potentially other marine finfish in low salinity RAS and for expanding siting options for RAS grow-out operations, which

may consider locations where brackish water of 10.1-12.3 g L^{-1} or higher may be sourced or prepared.

Ethical approval

All national and institutional guidelines for animal care and welfare were followed by all authors. This study was conducted under the UNCW IACUC protocol #A14-14-021.

Declaration of conflict of interest: None

Author contributions:

M.S. Alam: Methodology, Resources, Supervision, Investigation, Formal analysis, Data curation, Writing – original draft, Funding acquisition (co-Principal Investigator).

W.O. Watanabe: Methodology, Resources, Supervision, Writing – review & editing, Project Management and Administration, Funding acquisition (Principal Investigator).

P.M. Carroll: Resources, Investigation, Supervision, Funding acquisition (co-Principal Investigator).

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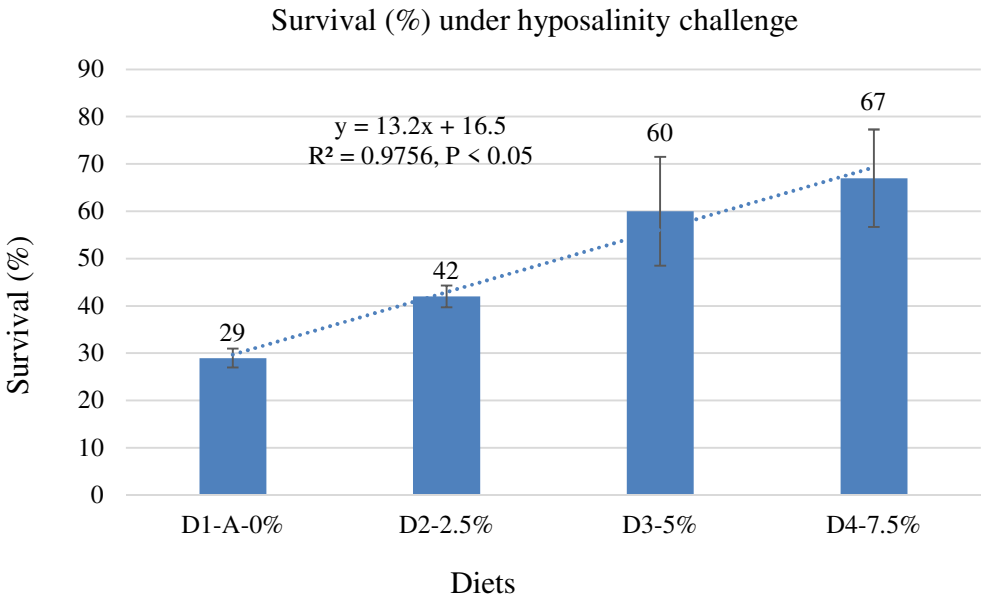

Fig.1. Survival (%) of black sea bass under hyposalinity challenge after the 8-month feeding trial. Fish were fed four treatment diets containing different percentages of salt: 0% (D1-A), 2.5% (D2), 5.0% (D3), and 7.5% (D4) for 5 weeks while gradually decreasing salinity from 10.1 g L^{-1} to 4 g L^{-1}. Regression analysis showed a significant ($P < 0.05$) linear relationship between dietary salt level (X) and survival (Y) following acute salinity challenge.

Table 1**.** Composition of diets (g/100 g).

Diets	D1-A	D-2	D-3	D-4	D1-B	D-5	Commercial*
	Low salinity (10.1-12.3 g L^{-1})				Full strength seawater (34 g L^{-1})		
(% sea salt in diets)	0	2.5	5.0	7.5	0	0	
Soybean meal [a]	17	17	17	17	17		Proprietary
Menhaden meal [b]	42	42	42	42	42		
Poultry meal [c]	16	16	16	16	16		
Wheat starch [d]	4.5	3	1.5	0	4.5		
Wheat gluten [d]	4	4	4	4	4		
Menhaden fish oil [e]	5.5	5.5	5.5	5.5	5.5		
Soybean lecithin [f]	1	1	1	1	1		
Vitamin premix [g]	2.92	2.92	2.92	2.92	2.92		
Stay C (Vit C) [g]	0.08	0.08	0.08	0.08	0.08		
Mineral premix [h]	2.5	2.5	2.5	2.5	2.5		
Cellulose	3.5	2.5	1.5	0.5	3.5		
Sea salt [i]	0	2.5	5	7.5	0		
L-Methionine	0.5	0.5	0.5	0.5	0.5		
L-Lysine	0.5	0.5	0.5	0.5	0.5		
Total	100	100	100	100	100		
Proximate composition							
Protein % (analyzed)	52.4	52.6	53.3	52.9	52.5		52.5
Lipid % (analyzed)	13.4	13.1	13.0	13.3	13.3		16.3
Energy kj/g diet [j] (calculated)	18.4	18.1	17.7	17.7	17.6		18.8

[a] Southern States, Wallace, NC, USA (solvent extracted, crude protein 47.5%).

[b] Omega protein Corporation, Houston, TX, USA (crude protein 59%, lipid 11%).

[c]Scoular Company InC, (crude protein 65%, lipid 12%)

[d] VWR International, Radnor, PA, USA (crude protein 78%).

[e] Virginia Prime Silver, Omega Protein, Hammond, LA, USA.

[f] ADM, IL,USA.

[g] DSM, Canada.

[h] as Alam et al. (2000) (g/ kg diet) $MgS0_4$, 3.17; Na_2HP0_4, 2.02; K_2HP0_4, 5.54; $Ca(HP0_4)$,3.14; Fe-citrate, 0.68; Ca-lactate, 7.56; AI $(OH)_3$, 0.01; $ZnS0_4$, 0.08; $Cu(SO_4)$, 0.002, $MnS0_4$, 0.02; $Ca(IO_3)_2$, 0.003 and $CoSO_4$, 0.02.

[i]Cargill Salt, Minneapolis, MN.

[j] Calculated based on carbohydrates, proteins and lipids are 17.2, 23.6, and 39.5 kJ/ g, respectively (Blaxter, 1989).

[*] premium commercial diet (Europa, Skretting, D5) containing 50% crude protein (analyzed value 52.5%) and 15% lipid (analyzed value 16.3%).

Table 2. Electrolyte concentrations in diets (%). Values are average of duplicate analysis (N = 2).

% salt in diets (Diet No.)	Na	Ca	Mg	K	P	Fe	Zn	Cl
0 (D1)	0.52	2.52	0.23	1.24	2.11	0.06	0.03	0.53
2.5 (D2)	1.41	2.74	0.24	1.26	2.23	0.06	0.03	2.05
5 (D3)	2.29	2.70	0.23	1.28	2.22	0.06	0.03	3.56
7.5 (D4)	2.99	2.67	0.22	1.26	2.20	0.05	0.03	5.19
Commercial (D5)	0.30	2.08	0.16	0.56	1.61	0.02	0.02	0.43

Table 3: Growth performance, survival, FCR and plasma osmolality of fish fed in low salinity water with graded levels of dietary salt after 8 months of the feeding trial. Fish were stocked in seawater (34 g $L^{-1}$and gradually acclimated to 10.1-12.3 g L^{-1}over a period of 3 weeks. Values are mean ± SEM (*N* = 3).

Parameters/Response	Diets designation				Pr > F[1]		
	0% (D1)	2.5% (D2)	5% (D3)	7.5% (D4)	ANOVA	Linear trend	Quadratic trend
Initial wt (g)	19.7 ± 0.21	19.4 ± 0.35	19.7 ± 0.06	19.8 ± 0.18			
Final wt (g)	182 ± 5.6	179 ± 2.3	171 ± 2.1	177 ± 3.8	0.2661	0.1874	0.2506
Weight gain (%)	821 ± 18.3	825 ± 27.9	766 ±12.4	793 ± 11.1	0.1635	0.1393	0.3053
Survival (%)	89.3 ± 7.7	96.7±1.2	87.7±6.8	93.0 ± 1.5	0.6408	0.9328	0.9800
FCR	1.52 ± 0.11	1.41± 0.06	1.50 ± 0.01	1.61 ± 0.05	0.2997	0.2837	0.1724
Osmolality (mOsm kg^{-1})	353 ± 5.7	357 ± 10.7	348 ± 7.2	344 ± 1.8	0.6163	0.2751	0.4754

[1] Significance probability associated with the F-statistic.

Table 4: Growth performance, survival, FCR, plasma osmolality and plasma electrolytes of fish fed the UNCW formulated control diet (0% salt) under low salinity (10.1-12.3 g L^{-1}) (D1-A) and full-strength sea water (34 g L^{-1}) (D1-B) after 8 months of the feeding trial. Values are mean ± SEM (N = 3).

Response	Low salinity (D1-A)	Full seawater (D1-B)	P-value
Initial wt (g)	19.7 ± 0.21	19.5 ± 0.18	0.4950
Final wt (g)	182 ± 5.6	176 ± 1.5	0.5265
Weight gain (%)	821±18.3	813 ± 3.5	0.6906
Survival (%)	89.3 ± 7.7	99.6 ± 0.88	0.2526
FCR	1.52 ± 0.11	1.46 ± 0.05	0.6714
Osmolality (mOsm kg^{-1})	353 ± 5.7	336 ± 1.7	0.0513
Plasma electrolytes (mEq L^{-1}, except Ca and P (mg dL^{-1})			
Na	175 ± 1.9	172 ± 2.33	0.3636
K	5.33 ± 0.46	5.8 ± 0.12	0.5766
Cl	134 ± 1.2	135 ± 0.33	0.8593
Ca	15.6 ± 0.15	15.1 ± 0.72	0.9662
P	12.0 ± 0.43	15.0 ± 0.61	0.0166
Mg	2.7 ± 0.1	3 ± 0.2	0.2020
HCO_3^-	9.6 ± 0.3	9.3 ± 0.03	0.5185
Proximate compositions of whole bodies (% wet basis)			
Moisture (%)	60.9 ± 0.45	61.2 ± 0.49	0.6989
Crude protein (%)	18.1 ± 0.15	17.8 ± 0.34	0.3654
Total lipid (%)	15.08 ± 0.43	15.3 ± 0.36	0.6822
Ash (%)	4.41 ± 0.19	6.32 ± 0.17	0.7568

Table 5: Growth performance of fish fed the UNCW formulated control diet (0% salt D1) and the commercial (Skretting) diet (D5) in full strength sea water (34 g L^{-1}) after 8 months of the feeding trial. Values are mean ± SEM (*N* = 3).

Response	UNCW (0% salt) D1-B	Commercial D5	P-value
Initial wt. (g)	19.5 ± 0.18	19.6 ± 0.12	0.7239
Final wt. (g)	178 ± 1.5	173 ± 5.5	0.3955
Weight gain (%)	813 ± 3.5	788 ± 37.5	0.4289
Survival (%)	99.0 ± 0.5	99.5 ± 0.5	0.5908
FCR	1.46 ± 0.05	1.41 ± 0.00	0.4674
Osmolality (mOsm kg^{-1})	336 ± 2.1	344 ± 2.6	0.0946
Plasma electrolytes (mEq L^{-1}, except Ca and P (mg dL^{-1})			
Na	172 ± 2.3	177 ± 3.0	0.3019
K	5.8 ± 0.72	4.05 ± 0.05	0.1570
Cl	134 ± 3.3	132 ± 2.0	0.5993
Ca	15.1 ± 0.72	14.4 ± 0.1	0.5080
P	15.0 ± 0.62	10.7 ± 1.1	0.0322
Mg	3.0 ± 0.2-	2.45 ± 0.05	0.1256
HCO_3	9.33 ± 0.33	12.0 ±0	0.0085
Proximate composition of whole bodies (% wet basis)			
Moisture (%)	61.2 ± 0.48	59.9 ± 1.63	0.4038
Crude protein (%)	17.7 ± 0.34	17.7 ± 0.94	0.9838
Total lipid (%)	15.3 ± 0.36	15.7 ± 0.5	0.5262
Ash (%)	4.32 ± 0.17	4.79 ± 0.27	0.2128

Table 6. Plasma electrolytes (mEq L^{-1}, except Ca and P (mg dL^{-1}) of fish fed graded levels of dietary salt in low salinity (10.1-12.3g L^{-1}) water after the 8-month feeding trial. Values are means ± SEM of triplicate tanks (*N* = 3).

Parameters/Response	Diets designation				Pr > F[1]		
	0%	2.5%	5%	7.5%	ANOVA	Linear trend	Quadratic trend
	D1-A	D2	D3	D4			
Na	175 ± 1.2	176 ± 1.2	174 ± 0.7	173 ± 1.3	0.2327	0.0844	0.1648
K	5.33 ± 0.3	5.20 ± 0.2	5.10 ± 0.5	5.33 ± 0.1	0.9256	0.9350	0.8029
Cl	134 ± 1.2	135 ± 0.7	131 ± 0.7	131 ± 2.4	0.1926	0.0924	0.2382
Ca	15.1 ± 0.2	13.9 ± 0.2	13.9 ± 0.2	14.2 ± 0.8	0.2179	0.2303	0.1059
P	12.03 ± 0.4	11.3 ± 0.5	11.4 ±1.0	11.1 ± 0.4	0.7931	0.3541	0.6418
Mg	2.7 ± 0.1	2.4 ± 0.1	2.5 ± 0.1	2.5 ± 0.2	0.5122	0.4843	0.3311
HCO_3-	9.66 ± 0.3	8.67 ± 0.3	9.33 ± 0.3	9.33 ± 0.3	0.2679	0.8467	0.4080

[1] Significance probability associated with the F-statistic.

Table 7. Whole body proximate composition (% wet basis) in fish fed different test diets in low salinity (10.1-12.3 g L^{-1}) after the 8-month feeding trial. Values are mean ± SEM (N = 3)

Parameters/Response	D1-A	D3	D3	D4	Pr > F[1]		
	0%	2.5%	5%	7.5%	ANOVA	Linear trend	Quadratic trend
Moisture	60.9 ± 0.45	61.8 ± 0.56	60.5 ± 0.44	60.9 ± 0.87	0.9008	0.8200	0.9162
Crude protein	18.1 ± 0.15	18.2 ± 0.36	17.6 ± 0.16	17.6 ± 0.02	0.1137	0.0419	0.1387
Total lipid	15.08 ± 0.43	14.80 ± 0.80	16.04 ± 0.45	15.46 ± 0.89	0.6115	0.4342	0.7318
Ash	4.41 ± 0.19	4.63 ± 0.27	4.45 ± 0.22	5.07 ± 0.22	0.2080	0.1001	0.1887

[1] Significance probability associated with the F-statistic.