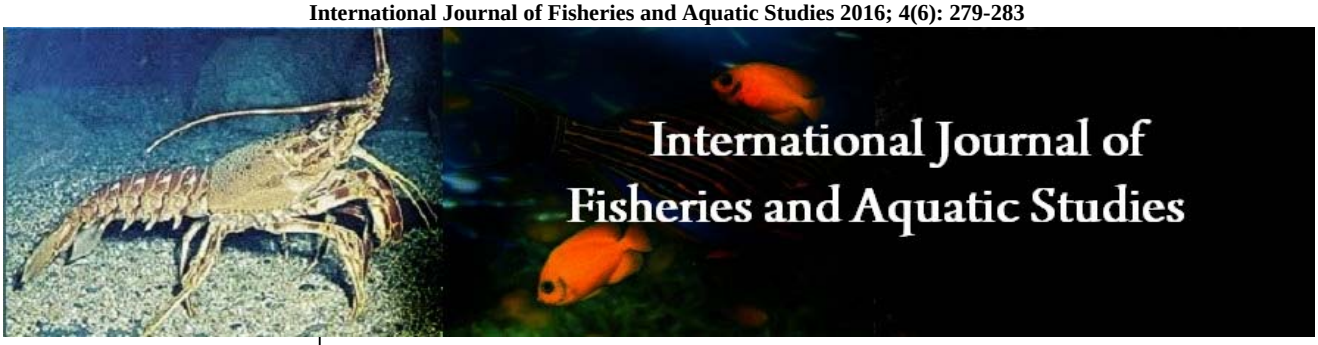
International Journal of Fisheries and Aquatic Studies 2016; 4(6): 279-283



ISSN: 2347-5129
(ICV-Poland) Impact Value: 5.62
(GIF) Impact Factor: 0.549
IJFAS 2016; 4(6): 279-283

www.fisheriesjournal.com
Received: 10-09-2016
Accepted: 11-10-2016

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Effect of salinity levels on growth, feed utilization, body composition and digestive enzymes activities of juvenile silver pompano *Trachinotus blochii*

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Abstract
The present study was conducted to investigate the effects of different salinity levels on growth, feed utilization, body composition and digestive enzyme activity of silver pompano (*Trachinotus blochii).* Three treatment groups (designated as S1, S2 and S3) and a control group (S), each in triplicates, was established. The pompano in the control were kept at salinity of 34 ppt and those in S1, S2 and S3 were kept at salinity of 5, 15 and 25 ppt, respectively. After 56 days, growth performance was significantly higher (P<0.05) at S2 and S3 treatment. Body composition was significantly affected by salinity levels (P<0.05). The specific activities of amylase and total alkaline protease enzymes were significantly higher (P<0.05) for S2 and S3 respectively. The results collectively suggested that intermediate salinity of 15 ppt and 25 ppt improve the growth performance, feed utilization and digestive enzyme activity of pompano.

Keywords: Salinity Growth Body composition Enzyme activity

1. Introduction
Salinity is one among the environmental factors which are extensively affecting fish growth (Lisboa *et al.* 2015) [12]. Understanding of optimum salinity of specific species will contribute in expansion of aquaculture productions by utilizing wide range of culturing environments including the brackish waters). Inadequate rearing salinity levels for both freshwater and marine fish species directly affects fish physiology which results in the reduction of growth, survival, immune respond and disease resistance (Semra, 2013) [19]. Therefore, fish are constantly adjusting to their external surroundings environments and maintaining the proper balance of salt solutions within their bodies (isosmotic) by osmoregulation (Lisboa *et al.* 2015) [12]. Nevertheless, osmoregulation mechanism is energy demanding processes, species with lower metabolic rates utilizing about 20 to 50% of the total energy available (Ramos *et al.* 2010) [18].
In addition the effects of salinity in fish growth performance and survival rates are unlikely to be demonstrated by single factor rather than interaction of multiple factors including digestive enzymes activities. Foods particles are ingested with salt from surrounding environment alter the ionic concentration in the gastric lumen where enzymes are found. The salinity variation in the gastric lumen may alter digestive enzymes activities which affect feed digestibility and fish growth performance by increasing energy demand in continuous ionic regulation (Moutou *et al.* 2004, Tsuzuki *et al.* 2007, Gheisvandi *et al.* 2015, Vargas-Chacoff *et al.* 2015) [16, 20, 7, 21]. It is well known that fish digestive enzyme activities correlate with feeding ecology and diet requirement (Tsuzuki *et al.* 2007) [20]. Therefore, aim of the present study was to quantifying the extent of salinity effects on enzymatic activity and growth performance of silver pompano which will contribute in the improving its aquaculture production.

2. Material and Methods
2.1 Experiment set up
Juveniles of *T. blochii* caught from the sea off Nungwi located at 5.7^0 S and 39.3^0 E of Zanzibar, Tanzania were used for the experiments. Experiments were conducted in triplicate in 1000 L tanks with 10 juveniles (average initial length: 5.52 ± 0.23 cm, average initial weight: 2.64 ± 0.31g) per tank. During this period of 28 days of acclimatization to different salinity

fish were fed to apparent satiation with a 40% crude protein and 9% crude fat commercial diet (EXTR 400, Rangen Inc). During the experiment, three salinity reduction protocols were followed. In the first set, salinity was reduced from 34 ± 1 ppt by 10 every two days up to 25 ppt (S3). In the second set, salinity was reduced by 5 ppt every two days up to 15 ppt (S2). In the third set, salinity was reduced by 5 ppt every two days up to 5 ppt (S1) and control groups were maintained at 34 ± 1 ppt (S). After acclimatization period fish was feed 5% of body weight, three times daily with artificial diet of appropriate pellet size. Uneaten feed and fecal matter were removed periodically. Dead fish, if any, were removed and recorded. Fish from each tank were counted and weighed every two weeks (14th day) and feed percentage was adjusted, until the end of 56 days to monitor survival and growth. Water exchange (50%) was done daily and care was taken to maintain the treatment salinity with reverse osmosis water and salinity levels were obtained by mixing dechlorinated tap water with natural seawater, and measured with optical refractometer Model F3000. Fish were fed with the same diet as previously described.

2.2 Data and statistical analysis

At the end of the experiment, different parameters were calculated: Specific Growth Rate (SGR; %/d) = 100 × [Ln (Final Body Weight (g)) - Ln (Initial Body Weight (g))] / Duration (days) of the experiment, Survival Rate (SR, %) = 100 x FN/IN, Protein Efficiency Ratio (PER) = (FB - IB) / (FD x Dietary Protein), Weight Gain (WG%)= 100 × [(Final Body Weight (g)) - (Initial Body Weight (g))] / Initial Body Weight (g).With: IB: Initial Biomasses (g), FB: Final Biomasses (g). The data were analyzed using a one-way analysis of variance (ANOVA) with the facilities of STATVIEW version 5.01 software, after the verification of variance homogeneity, using Hartley's test. Significant differences among means were determined using Fisher's test p= 0.05 significance level

2.3 Digestive enzyme activity

During the 56 days of the experiment, fish were sampled and the activity of acid protease and amylase in the stomach were measured in order to evaluate the effect of salinity on the digestive enzymes activity of silver pompano. Three juveniles were sampled from each salinity levels replicate (9 fish per treatment) at the end of the experiment and immediately frozen at -20 ^{0}C until the preparation of the homogenate for enzyme activity analysis. The fish stomach were extracted and washed in distilled water, and sliced into small pieces. Collected tissues samples were homogenized in ice-cold distilled water using a van Potter homogenizer for 2.5 min and centrifuged at 27,167 x g for 15 min at 4 °C. The supernatant was used for enzyme and soluble protein assays. Total amylase activity was estimated using soluble starch as substrate as described by Aguillar-Quaresma and Sugai (2005) [1]. The released reducing sugar was assayed using maltose as the standard. The total amylase activity was expressed as specific activity (µmol reducing sugars/min/ml/mg protein in the extract, U/mg protein) at 37 °C. Total alkaline proteinase activity of the extract was measured using the azocasein hydrolysis method described by Garcia-Carreño *et al.* (1997) [6]. The enzymatic system was incubated for 10 min at 22 – 25 °C, and the absorbance at 366 nm for the released dye was estimated. The control (blank) was assayed by adding 20% trichloroacetic acid to the reaction system before substrate addition. The total alkaline proteinase was expressed as specific activity, the difference in the absorption at 366 nm between sample and blank per minute, per ml, and per mg protein in the extract (Δ absorbance 366 nm/min/ml/mg protein). Soluble protein of crude enzyme extracts was quantified (Lowry *et al.* 1951) [13] using bovine serum albumin as standard. The enzymatic assays were performed in duplicate for each homogenate per replicate.

2.4 Biochemical analysis

Temperature, pH and dissolved oxygen were monitored twice a day 9:00 am and 16:00 pm by multi-parameter 340i model no. 08391120. Using the Association of Analytical Chemist methods (AOAC, 2000) [2], the proximate composition of the diets was analyzed for crude protein, crude lipid and gross energy. Fish carcasses were analyzed for crude protein before and after experiment at the Department of Animal Science and Production of Sokoine University of Agriculture (SUA) in Morogoro, Tanzania.

3. Results

The overall mean water quality parameters: temperature = 29.8 ± 0.2 ^{0}C, DO = 6.21 ± 0.02 mg/L, total ammonia nitrogen = 0.29 ± 0.01 mg/L, nitrite-nitrogen = 0.25 ± 0.03 mg/L and pH = 7.71 ± 0.01, were typical for these systems. The values of all water quality parameters were consistent and within acceptable ranges for pompano production (Watanabe, 1995) [22].

Table 1: Survival rate, growth performance and food conversion ratio of juveniles *T. blochii* at different salinities for 56 days.

	Salinity levels			
Parameters	**5 ppt**	**15 ppt**	**25 ppt**	**34 ppt**
Initial weight (g)	2.60 ± 0.32^a	2.73 ± 0.40^a	2.73 ± 0.2^a	2.56 ± 0.32^a
Final weight (g)	6.8 ± 0.79^a	28.90 ± 1.08^b	19.76 ± 1.09^c	12.63 ± 0.90^d
WG%	62.013 ± 2.33^a	893.12 ± 11.48^b	529.93 ± 36^b	298. ± 95^c
FCR	2.26 ± 0.01^a	1.62 ± 0.01^b	1.54 ± 0.01^b	1.75 ± 0.02^c
PER	0.981 ± 0.005^a	1.43 ± 0.017^b	1.36 ± 0.01^b	1.26 ± 0.01^c
SGR (% day^{-1})	2.91 ± 0.01^a	3.31 ± 0.01^b	3.70 ± 0.03^b	3.42 ± 0.02^c
HI	1.16 ± 0.01^a	1.14 ± 0.04^a	1.12 ± 0.01^a	1.15 ± 0.03^a
SR (%)	96	100	97	99

Initial Body weight, Final Body weight, Percentage weight gain (%WG), Feed conversion ratio (FCR), Protein efficiency ratio (PER), Specific growth rate (SGR) and Hepatosomatic index in juvenile *T. blochii* maintained in different salinities for 8 weeks. Data are expressed as mean ± standard error (n = 30). Different letters denote significant difference among treatments for the same parameter (P<0.05).

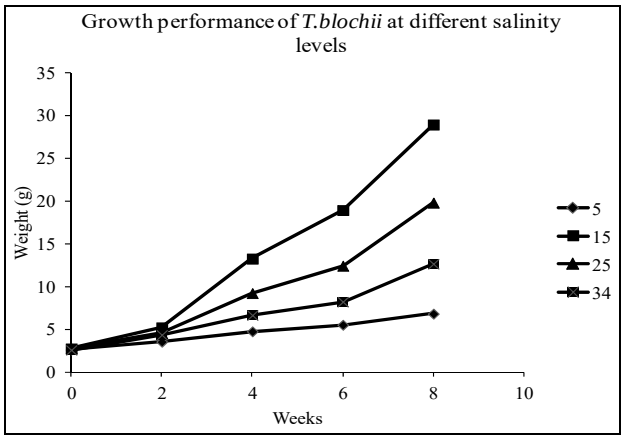

Fig 1: Growth performance of *T. blochii* at different salinity for 56 days

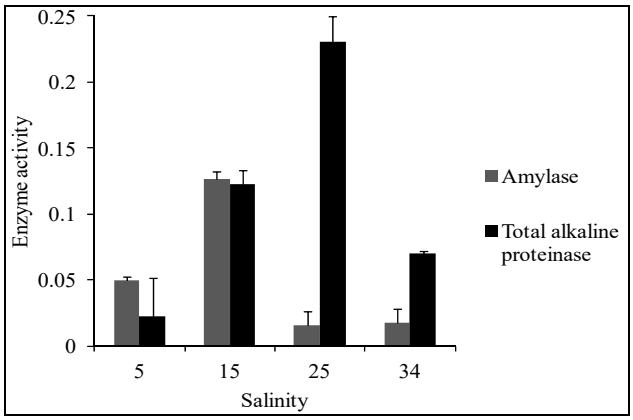

Fig 2: Activity of total amylase and total alkaline proteinase in the digestive tract of juvenile *T. blochii* at different salinity levels

Table 2: Carcass proximate composition of *T. blochii* in the 56 days feeding trials (N=6)

	Salinity levels				
Parameters	**5 ppt**	**15 ppt**	**25 ppt**	**34 ppt**	**p. value**
Moisture %	67.33 ± 0.001[a]	68.97 ± 0.04[b]	67.25 ± 0.1[c]	67.86 ± 0.02[d]	0.006
Crude protein %	15.54 ± 0.01[a]	16.31 ± 0.003[b]	16.41 ± 0.008[c]	16.19 ± 0.005[d]	0.001
Crude Lipid %	13.47 ± 0.01[a]	14.43 ± 0.03[b]	14.62 ± 0.12[c]	14.75 ± 0.04[d]	0.001
Ash %	2.02 ± 0.13[a]	1.93 ± 0.1[b]	1.72 ± 0.21[c]	1.2 ± 16[d]	0.001

After, 56 days of salinity trial, the highest growth was observed in fish cultured in salinity of 15 ppt followed by salinity of 25 ppt (28.90 ± 1.08 and 19.76 ± 1.09) respectively (Figure. 1). Fish weight, Feed conversion ratio, protein efficiency ratio, and specific growth rate increased significantly (P<0.05) over the course of the 56 days with increasing salinity levels (Table. 1). Pompano cultured in high salinity levels accumulated significantly more amount of lipid within the body and had an associated decrease in moisture and ash content while protein content significantly increased at intermediate salinity (Table. 2). The activity of digestive enzymes of juvenile silver pompano varied with salinity levels (Figure. 2). The specific activity of total amylase was significantly higher in fish reared at salinity of 5 and 15 ppt than the activity of those found in fish reared at salinity of 25 and 34 ppt (P<0.05). The total alkaline proteinase activity was significantly different in all treatments, and the highest peak was noticed in salinity of 25 and 15 ppt while the lowest at salinity of 5 ppt (Figure.2).

5. Discussion

The growth performance of acclimatized *T. blochii* was best in intermediate salinity ranging from 15 to 25 ppt and decrease in very low salinity of 5pp and sea water salinity of 34 ppt (Figure.1). These findings recounts to what have been reported in a number of studies which indicate the increase in growth performance of juvenile pompano and Atlantic cod in intermediate salinities range 10 -25 ppt (Imsland *et al.* 2011, Kalidas *et al.* 2012) [9, 10]. Moreover, previous studies suggested that decreasing fish growth in very low or high salinity conditions has potential to suppress the appetite and related to the decrease in food consumption with high energy demand for osmoregulation (Imsland *et al.* 2008, Evans, 2010, Mahmudul *et al.* 2014) [8, 4, 14]. Thus, in salinity of 5 ppt and 34 ppt the juveniles *T. bochii* may utilize more energy to regulate the concentration gradient than conserved for growth. Salinities have multidimensional effects on fish physiology, the finding from this study revealed that the activity of total amylase increased significantly from salinity of 5 to 15 ppt (0.05 ± 0.003 and 0.126 ± 0.006, µmol reducing sugars/min/ml/mg protein at pH 6.8 and 37 °C) respectively, and decrease a higher salinity of 25 and 34 ppt (P<0.02). While specific activity of total alkaline proteinase increased significantly with increasing salinities (P<0.01), the maximum peak at salinity is of 15 and 25 ppt. Similar observations were reported by Tsuzuki *et al.* (2007) [20] where higher level of total alkaline proteinase recorded in fish reared at salinity of 15 ppt. Furthermore, Vargas-Chacoff *et al.* (2015) [21] have reported that activities of total proteinase increased with increasing salinity. However, the present study observation slightly differs from what have been reported by Moutou *et al*. (2004) [16] where total alkaline proteinase activity was higher in salinity between 7 and 15 ppt.

Despite, the growth parameter and enzymatic activities, also salinity level affects the fish carcasses composition. The results from the present study indicate that moisture content decreased significantly (P<0.05) from 68.97 ± 0.04 to 67.25 ± 0.1 with increasing salinity from 5 to 34 ppt. This change is also reported on milkfish carcasses composition whereby moisture content decreased from 79.93 ± 0.01 to 78.47 ± 0.01 with increased salinity from 0 – 15 ppt (Barman, 2012, Kumar *et al.* 2016) [3, 11]. Nevertheless, the percentage protein and lipids increased significantly from 15.54 ± 0.08 to 16.41 ± 0.03 and 13.4 7 ± 0.01 to 14.75 ± 0.04 with increasing salinity respectively. The variation in fish growth performance and carcasses compositions attributed by effect of different level of salinity on digestive enzymes, oxygen consumption, osmoregulation and other metabolic factors have been covered extensively by Imsland *et al.* (2008) [9], Evans, (2010) [4] and Mahmudul *et al.* (2014) [14]. These studies support the hypothesis that the energetic cost for osmoregulation is lower at an isosmotic medium, in which gradients between the blood and water are minimal, and the energy saved is directed for increasing growth. Moreover, It has been reported that the higher the salinity the lower feed uptake and poor digestibility which result in poor growth performance and affect the fish

carcasses compositions due to the depression of enzymes in gastric digestion by the sea water surrounding the solid meal (Evans, 2010) [4]. The poor digestibility results in the increase in feed consumption rate due to the less feed retention time in the fish intestine and contributes to low nutrients absorptions thus affecting growth performance and carcasses compositions (Pérez-Robles *et al.* 2012; Fazio *et al.* 2013; Mahon *et al.* 2014; Lisboa *et al.* 2015) [17, 5, 15, 12]. The baseline information generated in this study clearly indicates that the growth performance and body composition of *T. blochii* vary with the increase in salinity

6. Conclusion and recommendation

These findings clearly addressed the effect of different salinities levels on *T. blochii* growth performance in relation to carcasses composition and digestive enzyme activities. The juvenile pompano tolerate wide range of salinity, and mostly collected from sand shore of saline and brackish water. However, *T. blochii* survive in wide range of salinities the recommended salinity for maximizing its aquaculture production ranges from 15 to 25 ppt.

7. Acknowledgement

We would like to express our sincere gratitude to the staff of Institute of Marine Sciences University of Dar es salaam, Zanzibar Ministry of Fisheries and Livestock, and WIOMSA. We also acknowledge the University of Dodoma for contributions to the work. The study was fully financed by the Sida through Institute of Marine Sciences, who is highly acknowledged.

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