International Research Journal of Biological Sciences _____ ISSN 2278-3202
Vol. **4(3),** 15-20, March **(2015)** *Int. Res. J. Biological Sci.*

Effects of Different Salinities on Growth, Feeding Performance and Plasma Cortisol Level in Hybrid TGGG (Tiger Grouper, Epinephelus fuscoguttatusx Giant Grouper, Epinephelus lanceolatus) Juveniles

Amni raihan othman*, Gunzo Kawamura, Shigeharu Senoo, Ching Fui Fui
Borneo Marine Research Institute, Universiti Malaysia Sabah, Jalan UMS, 88400 kota kinabalu, Sabah, MALAYSIA

Available online at: www.isca.in, www.isca.me
Received 15th May 2014, revised 16th October 2014, accepted 2nd January 2015

Abstract

The hybrid TGGG, tiger grouper Epinephelus fuscoguttatusx giant grouper, Epinephelus lanceolatus has a high resistance towards extreme conditions due to its genetic improvement. This study investigated the effects of different salinities on growth, feeding performance and plasma cortisol level in TGGG juveniles. The TGGG juveniles were acclimatized and subjected to 7 different water salinities at 5, 10, 15, 20, 25, 30 and 35 part per thousand (ppt) for 30 days. The survival rate, growth rate and feed conversion ratio (FCR) were calculated at 10-day intervals. The optimum salinity is 10 to 20 ppt based on higher growth performance in terms of the final weight (g= gram), average daily growth (ADG= g/f/d,gram/fish/day), and specific growth rate (SGR=%/d ,percentage/day) observed at 10 ppt (19.20±0.63 g, 0.32±0.01 g/f/d and 2.69±0.06%/d), 15 ppt (18.52±1.55 g, 0.31±0.04 g/f/d and 2.80±0.22%/d) and 20 ppt (18.17±1.04 g, 0.30±0.03 g/f/d and 2.74±0.23%/d). Besides, the optimum salinity was also determined based on the lower feed conversion ratio value at 10 ppt (1.24±0.04), 20 ppt (1.26±0.14) and 15 ppt (1.30±0.20). This study shows that high salinity at 35 ppt, (14.38±2.11 g, 0.19±0.02 g/f/d and 1.95±0.40%/d) and 30 ppt, (15.31±0.68 g, 0.21±0.02 g/f/d and 2.06±0.17%/d) leads to poor growth performance of TGGG juveniles. Plasma cortisol levels in 5 ppt (56.50 nmol/L), 15 ppt (19.31 nmol/L) and 35 ppt (33.54 nmol/L) were significantly higher compared to those in 10, 20, 25 and 30 ppt. On a broad scale, this study is very significant in providing useful information for the TGGG to increase mass production and promote economic growth.

Keywords: Salinity, hybrid grouper; cortisol; tiger grouper; giant grouper.

Introduction

The hybrid tiger grouper, Epinephelus fuscoguttatusx giant grouper, Epinephelus lanceolatus (TGGG), was first produced in 2007 by Universiti Malaysia Sabah (UMS)[1]. The parental species of this hybrid grouper, Epinephelus fuscoguttaus, commonly known as a brown marbled grouper or tiger grouper is an Indo-specific species mainly inhabit lagoon pinnacles, channel and coral reef [2]. The Epinephelus lanceolatus or giant grouper with a common name of queensland grouper inhabit marine, brackish and reef associated environment[3].

Both of this parental species are inherently vulnerable to fishing and being widely cultured in the hatchery around Southeast Asia[4]. However, there were constrains exists related to the culture of these species where in tiger grouper, farmer having a difficulties in obtaining high quality sperm while in giant grouper, it is difficult to obtain high quality eggs. This problems lead to the research on improving the seed production through crossbreeding[1]. The hybrid species is highly commercialized in the Asian market with an increasing demand for it around the region. The production of TGGG can be maximized by optimizing the parameters affecting water quality in the rearing environment. Water parameters such as temperature, pH, dissolved oxygen and particularly salinity are key factors of good water[5]. Salinity has been shown to have a high influence on growth capacities of teleost fish[6].

Salinity affects various physiological processes in aquatic animals such as metabolism, osmoregulation and biorhythm[7]. Most study shows that sea water fish show a higher growth rate at lower water salinity levels; whereas, freshwater fish show a higher developmental rate at higher salinity[6]. It has been demonstrated at optimum salinity, energy can be saved to improve the growth rates of fish[8].

In general, on the other hand, fish at the extremes of their salinity tolerance range often exceed their ability to osmoregulate. The changes in salinity that are outside of optimal salinity range cause disturbance in physiological processes in fish. This leads to the higher production of cortisol levels in the blood[9]. The plasma cortisol level was also determined in the juveniles reared in different salinities.

Information on the effects of salinity on TGGG is very important to maximize and improve its production. Besides that, TGGG may have a wide range of salinity as one of its parental species (giant grouper) inhabits estuarine areas. Thus, this study

aims to investigate the production of the hybrid TGGG by manipulating salinity. In the present study, the experiment was carried out to determine the effects of salinity on the survival rates, growth, feeding performance and cortisol level on the hybrid TGGG grouper.

Material and Methods

Animal care and handling was carried out according to the guidelines set by the World Health Organization (WHO) in Geneva, Switzerland, the Malaysia Animal Handling Code of Conduct, and the National Research Council guide for the care and use of laboratory animals (National Research Council).

This study was conducted at the fish hatchery of Borneo Marine Research Institute, Universiti Malaysia Sabah from January 2012 to July 2013. Duration of experiment taken account for preliminary experiment and repetition. Feed-trained, hatchery-reared juvenile (60-d post-hatch) fish were used in this experiment. The culture was done in 1 tonnes round tank (150 cm in diameter) in sea water with the original salinity of 31±0.23 ppt. At the early stage of larval rearing, Nannochloropsis sp were added to the rearing tank with 10^6 cells/mL as a supplement. Starting from 5 days after hatching (dAH), the bottom of tank was cleaned every day while water changing was done up to 30% to control the water quality. The temperature of rearing water was maintained using heater (29.0 – 30.2 C) and water aerated with 500 – 700 mL/min of air bubbles. Several feeds were given according to the life stage of TGGG. Rotifer Brachionus sp. (150 µm) as a first feeding, brine shrimp Artemia salina nauplii (350-540 µm) from 12 dAH up to 30 dAH and artificial formulated feed, Otohime starting from 28 dAH up to juvenile.

Acclimatization was done before subjecting the juvenile into experimental salinity by changing the salinity gradually with the rate of salinity reduction of 5 ppt/hour. Twenty one plastic aquaria (18 x 26 x 17 cm) were prepared and divided into three groups for triplicates and were arranged randomly in a 700 L water bath. Water level was filled up to 6L/aquaria and equipped with aeration with the rate of 850ml/min of air bubbles. A heater and aeration was inserted into the water bath to ensure uniform temperature throughout the water bath and maintain the temperature within 28±0.5 C. Seven treatments were prepared (5, 10, 15, 20, 25, 30 and 35 ppt) with the salinity of each treatment controlled by mixing filtered sea water and fresh water. Commercial marine salt that specifically made for making artificial sea water was used to prepare 35 ppt of water. The original salinity of filtered sea water was 31±0.23 ppt. The desired salinity was determined using a hand refractometer (ATAGO, 2442-W05, Japan) precalibrated using distilled water. Two hundred and ten individuals of TGGG fish with the initial size of 6.34±0.42 g was transferred from the acclimatization tank to the experiment tank. The total number of fish was 10 individuals/aquaria.

The juvenile rearing was done for 30 days. The stock water for each treatment was prepared every 10 days in a 700 L fibreglass tank. Water exchanged was done up to 50% every 2 days to maintain the water quality. Fish were fed manually twice daily at 0900 and 1500 at apparent satiation level using extruded pellet of marine sinking pellet (Crude protein: 48.0%, Crude lipid: 14.5%). The excess feed was removed after 2 hours feeding was done. The total feed intake in each treatment was recorded daily. Survivors were counted to calculate survival and total length (to 0.1 cm) and body weight (to 0.01 g) were measured at 10-day intervals. Measurements were done after anaesthetizing all juveniles in each treatment using the anaesthetic, amethylquinoline with a concentration at 25 ppm. Growth performance and feed efficiency were evaluated based on the weight gain (g), survival (%), average daily growth rate (ADG), specific growth rate (SGR), and feed conversion ratio (FCR).

At the end of the experiment, fish blood was taken to determine the plasma cortisol level. Samples of blood were drawn from three individual fish in each treatment. Blood was taken from the caudal fin following anaesthetizing the fish using anaesthetic, amethylquinoline, and using a 24 G needle with a 10 ml syringe. Approximately 3 ml of blood was drawn from three individuals in each treatment and kept in a centrifuged tube. The blood was then kept at room temperature (28-29 C) for 2 hours before it was centrifuged (100g) at 4200 rpm for 15 minutes. The blood serum was taken and kept at a temperature of -18 C for further analysis of blood cortisol levels. Analysis was done using the Elecsys Cortisol Assay (Roche, Cobas E411, Switzerland).

A one-way ANOVA (Fisher) and Tukey's test of multiple comparisons were used for statistical evaluation of the growth, feed conversion ratio, and plasma cortisol level among fish kept under the seven salinities. The null hypothesis is accepted or rejected, significance is declared at $P<0.05$.

Results and Discussion

Growth performance: The survival rate of TGGG showed no significant difference ($P>0.05$) in the different treatments as survival was 100% in all treatments. The growth performance of juvenile, however, was affected by salinity (table-1). The growth performance (final weight, ADG) was observed to be relatively higher ($P<0.05$) in 10 to 20 ppt compared to 30 and 35 ppt. There was no significant difference ($P>0.05$) in 5 ppt for all treatments.

In terms of SGR, 15 ppt yielded the higher rate; there was no significant difference ($P>0.05$) at 20 ppt, 10 ppt, 25 ppt and 5 ppt. Growth performance in 35 ppt was significantly lower compared to 10 to 25 ppt.

Table-1

Growth performance and survival rates in hybrid TGGG juveniles kept under different salinities treatments. Data presented as mean ±SD. Different letters indicate statistically significant differences at P<0.05. Final weight (g= gram), average daily growth (ADG= $gf^{-1}d^{-1}$,gram/fish/day), and specific growth rate (SGR=$\%d^{-1}$, percentage/day)

Salinity (ppt)	Growth	Performance		
	Final Weight (g)	ADG (g/f/d)	SGR (%/d)	SR (%)
5	16.85±1.70^{abc}	0.25±0.04^{ab}	2.30±0.30^{abc}	100
10	19.20±0.63^{c}	0.32±0.01^{b}	2.69±0.06^{c}	100
15	18.52±1.55^{bc}	0.31±0.04^{b}	2.80±0.22^{c}	100
20	18.17±1.04^{bc}	0.30±0.03^{b}	2.74±0.23^{c}	100
25	15.87±0.57^{ab}	0.25±0.07^{ab}	2.52±0.53^{bc}	100
30	15.31±0.68^{a}	0.21±0.02^{a}	2.06±0.17^{ab}	100
35	14.38±2.11^{a}	0.19±0.02^{a}	1.95±0.40^{a}	100

The increasing weights of TGGG in a duration of 30 days in different salinities is shown in figure-1. On the first 10 days, mean weight gain was higher in 5 and 10 ppt; there was no significant difference (P>0.05) in 15 and 20 ppt. Lower mean weight gain was observed in 30 and 35 ppt, while there was no significant difference (P>0.05) in 20 and 25 ppt. However, at the end of 30 days, the graph shows that mean weight gain was significantly higher in 10 ppt while there was no significant difference (P>0.05) in 15, 20, 25 and 5 ppt. However, mean weight was significantly lower in 30 and 35 ppt compared to the others. Salinity of 5 ppt was not significantly different (P>0.05) from any of the other treatments.

Feed Conversion Ratio: Feeding efficiency was observed based on the feed conversion ratio (FCR). As shown in figure-2, a significant difference (P<0.05) was observed between treatments in brackish water (10, 15 and 20 ppt) and high salinity (30 and 35 ppt) except those in 5 and 25 ppt which showed no significance in all treatments (figure-2). The lowest feed conversion ratio was shown at 10 ppt, followed by 20 ppt and 15 ppt. The highest FCR was observed in high salinity treatments at 30 ppt and 35 ppt.

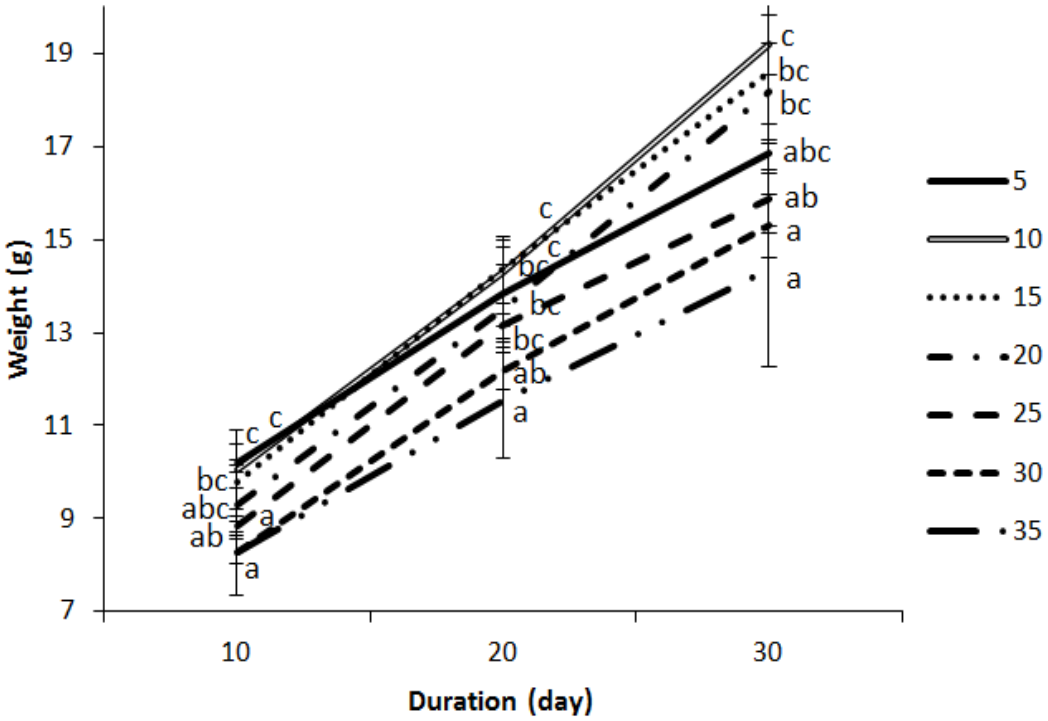

Figure-1

Changes in body weight in hybrid TGGG juveniles kept under different salinities treatments. Data presented as mean ± SD. Different letters indicate statistically significant differences at P<0.05

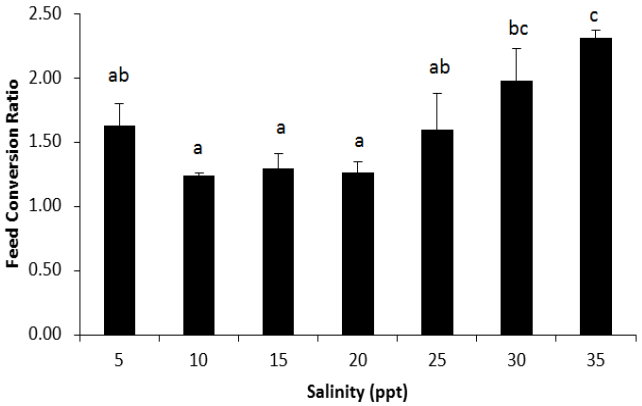

Figure-2

Feed conversion ratios in hybrid TGGG juveniles kept under different salinities treatments. Data presented as mean ±SD. Different letters indicate statistically significant differences at P<0.05.

Plasma Cortisol Level: The concentration of cortisol levels was significantly higher (P<0.05) in 5 ppt followed by 35 ppt and 15 ppt. (figure-3). However, plasma cortisol levels remained low without significant difference (P>0.05) at 10 ppt, 20 ppt, 25 ppt and 30 ppt.

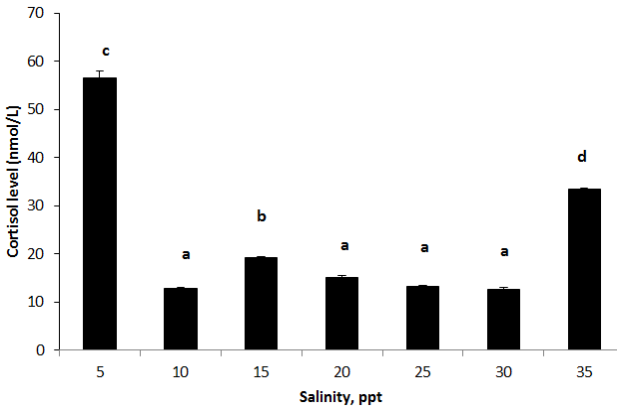

Figure-3

Cortisol level in hybrid TGGG juveniles kept under different salinities treatments. Data presented as mean ±SD. Different letters indicate statistically significant differences at P<0.05.

Discussion: The findings shows TGGG were able to survive in all treatment. Results from table 1 show that TGGG can tolerate a wide range of salinity. The hybrid grouper has been reported to have a high resistance towards stress, disease and extreme conditions due to genetic improvement [1]. According to Stickeney (1979), the ability of body fluid to tolerate changes of osmolality and ion concentrations will affect the survival rate of fish[21]. This is in relation with the larger size of the exchange layer especially on the gill, skin and intestine which are responsible for greater uptake of water[10].

Besides that, the acclimatization process for the juvenile fish was done to reduce the stress level of the fish during exposure to the different salinity treatments. Juvenile fish were not directly introduced to the various salinity treatments. Indeed, the salinity was decreased or increased slowly to the targeted level using a dropper to avoid startling the fish. Thus, the juvenile can survive in a wide range of salinity without affecting its mortality rate.

Contradically, growth performance was shows significant affect where it was observed that there was a significant difference within treatments in terms of specific growth rate (SGR), and average daily gain (ADG). The growth performance was observed to be the best in low salinity ranging from 5 ppt to 20 ppt. The range of salinity in which the optimum growth can be achieved has been reported as having an isotonic condition [11]. Isotonic salinity has an effect on metabolic rates and growth of fish [11]. A number of studies have shown that marine species have a better growth in lower salinity while freshwater species have a better growth in higher salinity[10].

Growth performance of the juvenile TGGG in water with a high salinity (35 and 30 ppt) was poor. Rearing fish in high salinity conditions has the potential to supress the appetite of the fish. Previous study suggested that decreasing growth in increased salinity was related to the decrease in food consumption[12]. It was reported that in high salinity, the energy was used to compensate for osmoregulation and ionic regulations[10]. Thus, in high salinity, the juvenile of TGGG may need more energy to regulate the concentration gradient between the blood and media. In optimum salinity, less energy spend for osmoregulation and thus, itcan be conserved for the metabolic process, thus increasing the growth of fish[8]. Any salinity range that is out of the optimal level will lead to an increase in energy costs for osmoregulation and a decrease in the growth performance of fish. Besides that, the juvenile may has a high possibility to follow the trait of its paternal parents, giant grouper that inhabit brackish water. However, further analysis on genetic study should be conducted for the confirmation. Based on this study, the optimum salinity for TGGG rearing was in the range of 10 ppt to 20 ppt.

Fish are hypo-osmotic to sea water and hyperosmotic to freshwater. Osmo regulation is the regulation of water and ion concentration in the body. Osmoregulation requires large amount of energy. It was estimated that osmo regulation might consume as much as 54-68% of the non-swimming metabolic output in skipjack tuna Katsu won us pelam is and yellow fin tuna Thunnus albacares[13]. Osmo regulation seems to use a high amount of the available energy even though it is in species with lower metabolic rate which within 20 to >50% of the total energy expenditure [8]. It is expected that in anisosmotic medium, energetic cost of osmo regulation is lower. This is the situation of minimum gradients between water and blood and that these energy savings are substantial enough to increase growth. Indeed, most of the fish species has been tested and it is proved that level of salinity influences growth of true marine species or

freshwater species[8]. It was observed that marine fish shows higher growth rates as it approaches the condition of iso-osmotic.

Feed Conversion Ratio (FCR) was also affected by salinity, with results showing that FCR in salinity levels of 5 ppt to 25 ppt was significantly lower than that in high salinity, 30 ppt and 35 ppt. However, 5 ppt did not produce any significant difference in all treatments. This indicates that salinities of 30 ppt and 35 ppt are not suitable for culturing TGGG. High salinity may supress stress, lowering the feed intake and resulting in poor growth[14]. The juvenile TGGG at high salinity conditions needs to compensate for osmoregulation and ionic regulations [15]. Therefore at 35 ppt and 30 ppt, the juvenile need higher energy and hence will reduce the energy available for growth, leading to poor growth performance.

The range of plasma cortisol levels observed in all treatments in figure-3 was within 12.65 to 56.50 nmol / L. In teleost fish, plasma cortisol levels is normal if the range observed was within 20.549 and 102.747 nmol/L. In the present study, plasma cortisol levels were relatively low in all treatments except those in 5 and 35 ppt. In 5 and 35 ppt, the higher production of plasma cortisol level is caused by an osmotic imbalance in the fish. This is in agreement with the study carried out by Pickering and Pottinger showing that levels of plasma cortisol were high due to the fish being exposed to hypertonic and hypotonic environments[16]. This shows that 5 and 35 ppt are respectively considered as hypotonic and hypertonic environments to TGGG juvenile.

A similar result was obtained in research conducted on Epinephelus malabaricus where low levels of plasma cortisol were found in fish maintained in salinity controlled at 24 ppt[17]. Plasma cortisol has direct effects on environmental stressors. Similar study has shown that high salinity can lead to higher cortisol levels[18]. The overall results show that the juvenile has a good growth and feeding performance in salinity within 10 to 20 ppt. This lead to the new information showing that TGGG can be successfully cultured in low salinity as previous culture practices were done in seawater environment with an average salinity of 30 ppt[1]. The culture of marine fish in low salinity is considered as a low cost technique without considering the limited availability of coastal land and water resources[19]. Trials were successfully done on the Florida Pompano fish by culturing the fish in low salinity ponds (19 ppt)[20].

Conclusion

The juvenile TGGG has a wide range of salinity tolerance. The optimum salinity is suggested to be within 10 ppt to 20 ppt due to iso-osmotic conditions, best growth performance, better feed conversion ratios and low levels of cortisol. The results of this study will be very useful to provide proper rearing technique that enables to maximize the production of TGGG and promotes economic growth to the aquaculture industry.

Acknowledgement

This study was funded by Higher Centre of Excellence (hi-COE) programme through the Higher Ministry of Malaysia Education.

References

1. Ch'ng C. L. and Senoo S., Egg and larval development of a new hybrid grouper, tiger grouper, Epinephelus fuscoguttatus x giant grouper, E. lanceolatus. Aquacult, *Sci.*, **56(4),** 505-512 **(2008)**

2. Pears R.J., Choat J.H., Mapstone B.D. and Begg G.A., Demography of a large grouper,Epinephelus fuscoguttatus, from Australia's Great Barrier Reef : Implications for fishery management*, Marine Ecology Progress Series,***307,** 259–272 **(2006)**

3. Primefacts., Giant Queensland Grouper, NSW department of Primary Industries, New South Wales, Cornish, A., IUCN Red List of Threatened Species, 1. In: IUCN 2013. **(2013)**

4. Cornish A., IUCN Red List of Threatened Species. Version 2013.1. In: IUCN, **(2013)**

5. Thomas B.L., Fundemantal of aquaculture engineering, Kluwer Academic Publisher, **2,** 12-40 **(2002)**

6. Boeuf G., Payan P., How should salinity influence fish growth ?, *Comp. Biochem. Phys*., CBP, **130(4),** 411-23 **(2001)**

7. Varsamos S., Nebel C. and Charmantier G., Ontogeny of osmoregulation in postembryonic fish : A review, *Comp. Biochem. Physiol.,***141,** 401–429 **(2005)**

8. Webster J. and Dill L.M., The energetic equivalence of changing salinity and temperature to juvenile salmon, *Funct. Ecol.*, **20,** 621–629 **(2006)**

9. Harris J. and Bird D.J., Modulation of the fish immune system by hormones, *Vet. Immunol. Immunop*., 77, **163–176** (2000)

10. Hidayat A.S., Effect of salinity on osmoregulation capacity, feed consumption, feed efficiency and growth of juvenile sea bass (Lates calcarifer Bloch), Msc Thesis, Kasetsart University **(2004)**

11. Morgan J.D., Iwana G.K., Effects of salinity on growth, metabolism, and ion regulation in juvenile rainbow and steelhead trout (Oncorhynchus mykiss) and fall chinook salmon (Oncorhynchus tshawytscha), *Can.J.Fish.Aquatic.Sci.*, **48,** 2083-2094 **(1991)**

12. Imsland A.B., Gunnarson S., Foss A. and Stefansson S.0., Gill Na+, K+- ATPase activity, plasma chloride and osmolalilty in juvenile turbot (Scophthalmus maximus) reared at different temperatures and salinities, *Aquaculture,* **218,** 671-683 **(2002)**

13. Bushnell P.G. and Brill R.W., Oxygen transport and cardiovascular responses in skipjack (Katsuwonus pelamis) and yellowfin tuna (Thunnus albacares) exposed to acute hypoxia, *J. comp. Physiol*., **162**, 131–143 **(1992)**

14. Pandley K. and Shukla J.P., Fish and Fisheries, second ed. Rastogi Publication, New Delhi, 261-275 **(2007)**

15. Sampaio L.A. and Bianchini A., Salinity effects on osmoregulation and growth of the euryhaline flounder, Paralichthys orbigyanus., *J. Exp. Mar. Biol. Ecol.***, 269**, 187-196 **(2002)**

16. Pickering A.D. and Pottinger T.G., Biochemical effects of stress. In : Hochachka P.W., Mommsen T.P., (Eds) Biochemistry and Molecular Biology of Fishes, Elsevier, *Amsterdam,* 349- 379 **(1995)**

17. Wen C.T., Jiann C.C. and Sha Y.C., The effects of a sudden salinity change on cortisol, glucose, lactate, and osmolality levels in grouper Epinephelus malabaricus, *Fish. Physiol. Biochem.,***38,** 1323–1329 **(2012)**

18. Ron B., Zohar Y., Borski R., Young G. and Grau E.G., Effects of dorsal aorta cannulation on cortisol and other stress parameters in the euryhaline tilapia, Oreochromis mossambicus, *Aquaculture,***135,** 213–218 **(1995)**

19. Riche M.A., Timothy J.P., Paul S.W., Jon J.A. and Maria S.S., 2012. Inland marine fish culture in low salinity recirculating aquaculture systems, *Bull. Fish. Res. Agen.*, **35,** 65-75 **(2006)**

20. McMaster M.F., Thomas C.K. and John F.C., Pompano mariculture in low salinity ponds, 2nd International Sustainable Marine Fish Culture Conference and Workshop, October 19-21, 2005 Harbor Branch Oceanographic Institution Fort Pierce, *Florida*, **(2005)**

21. Stickeney R.R., Principles of warmwater aquaculture. John Willey and Sons, *New York,* 262-314 **(1979)**