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EFFECTS OF DIFFERENT SALINITY LEVELS ON PHYSIOLOGICAL AND HEMATOLOGICAL RESPONSE OF ROCK BREAM *Oplegnathus fasciatus*

Vitas Atmadi Prakoso[*)#], Jun Hyung Ryu[**)], Byung Hwa Min[***)], Rudhy Gustiano[*)], and Young Jin Chang[**)]

[*)] Institute for Freshwater Aquaculture Research and Development

[**)] Department of Marine Bio-materials and Aquaculture, Pukyong National University, Korea

[***)] National Fisheries Research and Development Institute, Korea

ABSTRACT

Rock bream *Oplegnathus fasciatus* is one of economically important marine fish species in East Asia. However, lack of information about the salinity tolerance of rock bream related to its physiological response made this issue were needed to be studied. The present study was conducted to determine the effects of different salinity levels on physiological and hematological response of rock bream in order to obtain its salinity tolerance. Twelve rock breams (total length: 26.9 ± 0.6 cm, body weight: 477.3 ± 61.9 g) were used for experiments. Four experimental groups with three replications were conducted to measure the effects of salinity (5, 15, 25, and 35 practical salinity unit (psu)) on physiological and hematological response of rock bream. Fish were stocked into the chamber inside the closed recirculation system. At the end of each experiment, blood samples were collected. The study revealed that lower salinity exposure had tendency to decrease the physical and chemical properties of blood in rock bream. The value of Na^+, Cl^-, Ca, Mg, and osmolality showed tendency to decrease with lowering salinity, while cortisol and glucose showed tendency to increase from 35 psu to low salinity environment, indicating the enhancement of fish stress and resulted in fish mortality at 5 psu. The lowest cortisol value was 76.3 ng/mL in 25 psu, and the highest value was 188.8 ng/mL in 5 psu. Meanwhile, the lowest glucose value was 35.3 mg/dL and the highest value was 166.7 mg/dL ($P < 0.05$). Results indicate that rock bream could tolerate lower salinity up to 15 psu.

KEYWORDS: ***Oplegnathus fasciatus*; salinity; physiological response; hematology**

INTRODUCTION

Stress can be defined as a state of threatened homeostasis that is reestablished by a complex suite of adaptive responses (Chrousos, 1998). Stress response of fish is an adaptive mechanism which provides fish to overcome real or perceived stressors for keeping it in normal or homeostatic state. Environmental stressors had been studied by researchers because of its effects on metabolism and growth, disease resistance, reproductive capacity, health, condition, and survival of fish populations (Barton *et al*., 2002). Blood parameters can be one of the methods to determine the stress response of fish.

Several studies have observed the effects of stressors to the stress of fish, such as temperature (Houston & Rupert, 1976), salinity (Chang & Hur, 1999; Choi *et al*., 2007), and high stocking density (Tapia *et al*., 2012). According to several studies, the popular methods to evaluate fish physiological responses related to environmental disturbances are by blood properties measurements (Barton *et al*., 2002).

The effect of salinity on stress response have already investigated in several fish species, such as pejerrey *Odontesthes bonariensis* (Tsuzuki *et al*., 2001), euryhaline flounder *Paralichthys orbignyanus* (Sampaio & Bianchini, 2002), sturgeon *Acipenser naccarii* (Martinez-Alvarez *et al*., 2002), and chinook salmon *Oncorhynchus tshawytscha* (Morgan & Iwama, 1991; Stewart *et al*., 2016). The metabolic responses of fish to salinity changes showed inconsistent patterns, and the optimal salinities for growth are different within species, life stage, and season (Morgan & Iwama, 1991). Other species need to be observed their physiological response related to salinity changes.

[#] Correspondence: Institute for Freshwater Aquaculture Research and Development. Jl. Sempur No.1, Bogor 16154, West Java, Indonesian.
Phone: + (0251) 8313200
E-mail: *vitas.atmadi@gmail.com*

Rock bream *Oplegnathus fasciatus* is one of high commercial fish species in East Asia (Lipton & Kim, 2009; Biswas *et al*., 2010). This species have high metabolism activity (Liu *et al*., 2011) and normally live at salinity range of 25-35 psu (Liu *et al*., 2012). The growth potential of rock bream under intermediate salinity conditions is one of interesting topics to be observed for understanding their osmoregulation ability. However, there are limited information on low salinity tolerance of rock bream related to their stress response. According to that reason, the aim of this study was to evaluate the effects of various level of salinity on physiological and hematological response of rock bream.

MATERIALS AND METHODS

Twelve rock breams with a total length of 26.9 ± 0.6 cm and body weight of 477.3 ± 61.9 g were used for experiments. Four experimental conditions with three replicates for each condition were conducted to measure the effects of salinity (35, 25, 15, and 5 psu) on the physiological and hematological response of rock bream (Table 1). The fish were fed twice a day at 2% of its body weight with commercial feed. Fish were deprived for 24 hours before experiment.

Fish were stocked into the chamber (dimension = 20 cm x 30 cm x 20 cm) inside the closed recirculation system. To observe the fish with lower salinity exposure (5, 15, and 25 psu), water salinity inside the closed recirculation system was declined slowly into the target salinity by adding some amount of freshwater on the day before experiment. Water temperature was maintained at 20°C ± 0.5°C with 12:12 hour light : dark cycle. Each experiment lasted for 24 hours. At the end of each experiment, blood samples were collected, and analyzed to determine the physiological and hematological response of rock bream.

Fish were anesthetized using 2-phenoxyethanol and blood samples were collected from caudal part of fish using heparinized syringes (1 mL each samples). Blood samples were centrifuged (12,000 rpm, 5 minute). The physical and chemical properties were analyzed. The physical blood properties analyzed in this study were hematocrit (Ht) and hemoglobin (Hb). On the other hand, the chemical blood properties obtained in this study were sodium (Na^+), potassium (K^+), chloride (Cl^-), calcium (Ca), magnesium (Mg), osmolality, cortisol, glucose, and total protein. Hematocrit (Ht) was analyzed by using micro-hematocrit reader (Micro Hematocrit Reader, Hawksley). Hemoglobin (Hb), glucose, sodium (Na^+), potassium (K^+), chloride (Cl^-), calcium (Ca), and magnesium (Mg) were analyzed by using Chemical Analyzer (Fujifilm Dri-Chem 3500i, Japan). Meanwhile, plasma cortisol was analyzed by Enzyme Immunoassay (EIA) using Cortisol EIA kit (Oxford Biomedical Research, USA). Plasma osmolality was examined with Vapor Pressure Osmometer (Vapro 5520; Wescor Co., USA). The collected data were analyzed by one-way ANOVA.

RESULTS AND DISCUSSIONS

Salinity changes affected the value of Ht and Hb. There were a tendency of decreasing the value of Ht and Hb in relation with the changes of salinity level from 35 psu to 5 psu. The highest value of Ht and Hb were found in salinity level of 35 psu (43.3% and 12.5 g/dL, respectively). On the other hand, the lowest value of Ht and Hb was 28.0% and 7.1 g/dL in salinity of 5 psu. Hematocrit (Ht) and hemoglobin (Hb) levels among experimental conditions were significantly different ($P<0.05$), except the hematocrit level on the experiments of 15 and 5 psu ($P>0.05$) (Table 2).

At this present study, the results of physical blood properties indicated that salinity affected the hematological parameters of rock bream. The changes of salinity had a tendency to decrease the hematocrit and hemoglobin levels. Based on the observation, rock bream exposured in low salinity showed slower opercular movement due to the stress condition. It might be lethal if the exposure is in a long-term. Hematocrit (Ht) and hemoglobin (Hb) values decreased from 35 psu to a lower salinity. These results indicated that low salinity exposure (as the environmental stressor) affected hematocrit and hemoglobin of rock bream, which will have an impact on metabolism. Barton (1997) stated that temperature, salinity,

Table 1. Four experimental conditions in physiological and hematological response measurement according to different salinity levels

Experiment	Water temperature (°C)	Salinity (psu)	Number of fish	Replication
I	20	35	1	3
II	20	25	1	3
III	20	15	1	3
IV	20	5	1	3

Table 2. Physical properties of blood in rock bream *Oplegnathus fasciatus* at the end of experiments

Salinity (psu)	Component	
	Ht (%)	Hb (g/dL)
35	43.3 ± 0.6[c]	12.5 ± 0.4[d]
25	32.3 ± 0.6[b]	9.7 ± 0.1[c]
15	28.3 ± 0.6[a]	9.4 ± 0.1[b]
5	28.0 ± 1.0[a]	7.1 ± 0.1[a]

HB: hemoglobin, Ht: hematocrit. Values are the mean ± SD (n = 3). Different letters within the same column indicate significant differences between experimental conditions ($P < 0.05$, one-way ANOVA)

nutritional state, water quality, time of day, light, and fish density were the environmental factors that affected the stress response of fish.

Fish exposed to environmental stressor will suffer physiological disturbances in order to preserve the consistence and body stability. Blood hematocrit will change due to the differences between the internal and external environment ions concentrations. The decrease in hematocrit that related with low salinity exposure could be attributed to changes in the water content in the blood, caused by the change in environmental salinity (Plaut, 1998; Martinez-Alvarez *et al*., 2002). Wurts (1998) stated that in order to cope with low salinity exposure, fish need to replace salts lost through diffusion to the water and eliminate excess water absorbed from the environment. This condition affected the percentage of hematocrit. On the other hand, hemoglobin concentrations has also been considered as indicator for environmental change (Bani & Vayghan, 2009). Hemoglobin as protein was carried by red blood cell and having a role in respiration. The change in hemoglobin leads to change the number of red blood cell which have an important role to transfer the oxygen (Al-Hilali & Al-Khshali, 2016). Low salinity exposure had clear impact on their modification of energy demands and oxygen consumption (Morgan & Iwama, 1991). Therefore, lack of hemoglobin tended to decrease the oxygen consumption rate of fish and lead to increase the stress of fish. The statement was also strengthened by the results from low salinity exposure observations on oxygen consumption rate and blood properties of grey mullets (*Mugil cephalus*) (Prakoso *et al*., 2015). Similar results about the effects of environmental stressor to hematological features was also reported by Houston & Rupert (1976), Dheer *et al*. (1986), and Chang & Hur (1999).

The results of chemical properties of blood showed a tendency of decreases by low salinity exposure, except for cortisol, glucose, and total protein. The blood factors including Na^+, Cl^-, Ca, Mg, and osmolality decreased with lowering salinity. Meanwhile, cortisol and glucose have tendency to increase from 35 psu to 5 psu. The lowest cortisol value was 76.3 ng/mL in 25 psu, and the highest value was 188.8 ng/mL in 5 psu, while the lowest glucose value was 35.3 mg/dL and the highest value was 166.7 mg/dL ($P < 0.05$). In other parameter, total protein did not have any special tendency since the values were fluctuated compared among experimental conditions (Table 3).

The results of biochemical properties of rock bream also showed a clear impact of salinity to their physiological and hematological response, which clearly indicating the stress of fish by low salinity exposure. Stress will have impact to the metabolism and growth, immune systems, reproduction, and behavior of fish. It can be recognized by loss of amount of sodium, chloride, and total osmolality inside the blood. Blood ions and plasma osmolality were the indicator of hydromineral imbalance which disturbed the fish osmoregulatory system. In marine fish species, stress might increase the osmolality, ion influx, and water loss across the gills (Wendelaar Bonga, 1997; Cech, 2000; Barton *et al*., 2002).

Present study showed that cortisol and glucose level had tendency to increase by lowering salinity, and it was associated with their low activity during low salinity rearing as the impact of stress increase. This results was similar to those of Tsuzuki *et al*. (2001) and Choi *et al*. (2007) which reported the stress increase of fish by low salinity exposure. Moreover, Sampaio & Bianchini (2002) investigated *Paralichthys orbignyanus* under low salinity exposure, and suggest

Table 3. Biochemical properties of blood in rock bream *Oplegnathus fasciatus* at the end of experiments

Salinity (psu)	Osmoregulation factors						Stress factors		
	Na^+ (mEq/L)	K^+ (mEq/L)	Cl^- (mEq/L)	Ca (mg/dL)	Mg (mg/dL)	Osmolality (mOsm/kg)	Cortisol (ng/mL)	Glucose (mg/dL)	Total protein (g/dL)
35	196.3 ± 4.0^d	4.0 ± 0.2^b	171.3 ± 3.5^d	13.1 ± 0.1^c	2.7 ± 0.2^b	462.3 ± 13.2^b	94.7 ± 7.7^a	35.3 ± 4.2^a	3.7 ± 0.3^a
25	182.7 ± 2.1^c	8.1 ± 0.1^d	167.5 ± 2.1^c	10.4 ± 0.3^b	1.8 ± 0.1^a	349.3 ± 7.1^a	76.3 ± 17.1^a	36.3 ± 1.5^a	3.4 ± 0.2^a
15	173.3 ± 3.1^b	5.2 ± 0.2^c	158.0 ± 3.5^b	9.1 ± 0.2^a	2.2 ± 0.2^a	366.0 ± 6.1^a	183.6 ± 56.3^b	37.7 ± 1.5^a	4.2 ± 0.2^a
5	154.0 ± 6.2^a	1.7 ± 0.4^a	135.7 ± 4.2^a	9.2 ± 0.2^a	1.5 ± 0.3^a	359.0 ± 22.5^a	188.8 ± 48.0^b	166.7 ± 18.4^b	3.4 ± 0.3^a

Values are the mean ± SD (n= 3). Different letters within the same column of each experiment indicate significant differences between experimental conditions ($P<0.05$, one-way ANOVA)

that long-term exposure to freshwater reduced the growth rate, possibly due to an increase in energy expenditure associated to the osmoregulation and ionoregulation under low salinity exposure. Morgan & Iwama (1991) reported that such kind of response could indicate that fish was subjected to a stressing salinity condition. In this present study, low salinity exposure as the stressor triggered the hypothalamus-pituitary-interrenal activity. This condition resulted in cortisol secretion (Perry & Reid, 1993). Cortisol had been shown to increase glucose concentration and liver gluconeogenic capacity (Mommsen *et al*., 1999). As a result, the circulating level of cortisol is commonly used as a stress indicator of fish (Barton & Iwama, 1991; Wendelaar Bonga, 1997). The physiological response of fish may be compromised or influenced their health and well-being, or may become maladaptive by long-term environmental stressors exposure (Barton & Iwama, 1991). Liu *et al*. (2012) stated that the optimal salinity range for rock bream growth is 25-30 psu. Based on that information, the present study strengthened the statement that optimal rearing condition for rock bream was 25 to 35 psu. However, rock bream can survive and grow in 15 psu rearing condition.

CONCLUSION

Low salinity exposure increased the stress of rock bream *Oplegnathus fasciatus*. Salinity tolerance for rock bream was 15 psu or higher. Meanwhile, the optimal condition for rock bream rearing condition was in 25-35 psu.

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REFERENCES

Al-Hilali, H.A., & Al-Khshali, M.S. (2016). Effect of water salinity on some blood parameters of common carp (*Cyprinus carpio*). *International Journal of Applied Agricultural Sciences*, 2(1), 17-20.

Bani, A., & Vayghan, A.H. (2009). Temporal variation in haematological and biochemical indices of the Caspian Kutum *Rutilus frisii kutum*. *Ichthyological Research*, 58(2), 126-133.

Barton, B.A. (1997). Stress in finfish: past, present and future: a historical perspective. *In* Iwama *et al*. (Eds.). *Fish stress and health in aquaculture*. p. 1-33. Cambridge University Press. Cambridge.

Barton, B.A., & Iwama, G.K. (1991). Physiological changes in fish from stress in aquaculture with emphasis on the response and effects of corticosteroids. *Annual Review of Fish Diseases*, 1, 3-26.

Barton, B.A., Morgan, J.D., & Vijayan, M.M. (2002). Physiological and condition-related indicators of environmental stress in fish. *In* Adams S.M. (Ed.). *Biological indicators of ecosystem stress*. p. 111-148. Bethesda, MD. American Fisheries Society.

Biswas, A., Kazushige, I., & Takii, K. (2010). Feeding interval and photoperiod influence the growth performance of striped knifejaw, *Oplegnathus fasciatus*. *Aquaculture Research*, 41, 517-523.

Cech, Jr., J.J. (2000). Osmoregulation in bony fishes. *In* Stickney, R.R. (Ed.). *Encyclopedia of aquaculture*. p. 614-622. John Wiley and Sons. New York.

Chang, Y.J., & Hur, J.W. (1999). Physiological responses of grey mullets (*Mugil cephalus*) and nile tilapia (*Oreochromis niloticus*) by rapid changes in salinity of rearing water. *Journal of Korean Fisheries Society*, 32, 310-316.

Choi, C.Y., Min, B.H., Jo, P.G., & Chang, Y.J. (2007). Molecular cloning of PEPCK and stress response of black porgy (*Acanthopagrus schlegeli*) to increased temperature in freshwater and seawater. *General and Comparative Endocrinology*, 152(1), 47-53.

Chrousos, G.P. (1998). Stressors, stress, and neuroendocrine integration of the adaptive response. *Annuals of the New York Academy of Sciences*, 851, 311-335.

Dheer, J.M.S., Dheer, T.R., & Mahajan, C.L. (1986). Haematological and haematopoitic response to sodium chloride stress in a fresh water *Channus punctatus*. *Journal of Fish Biology*, 28(1), 119-128.

Houston, A.H., & Rupert, R. (1976). Immediate response of hemoglobin system of the goldfish, *Cyprinus auratus*, to temperature change. *Canadian Journal of Zoology*, 54, 1737-1741.

Lipton, D.W., & Kim, D.H. (2009). Accounting for economic risk and uncertainty in offshore aquaculture: a case study of Korean rock bream production. *Bulletin of National Fisheries Research and Development Agency*, 29, 93-102.

Liu Wei-Cheng, Ji De-Wei, Shan Le-Zhou, Zhou Zhi-Ming, Zheng Chun-Fang, Chen Shao-Bo, Li Shang-Lu, Yan Mao-Cang, Wu Jin-Gu, & Xie Qi-Lang. (2011). Effects of salinity and temperature on energy budget of juvenile *Oplegnathus fasciatus*. *Acta Agriculturae Zhejiangensis*, 23(5), 894-899.

Liu Min-Hai, Peng Zhi-Lan, Zhang Feng-Ping, Luo Hai-Zhong, Fu Rong-Bing, Li Lai-Guo, & Qin Xiang-Yue. (2012). Effects of salinity on feeding, growth and muscular biochemical composition in striped knifejaw *Oplegnathus fasciatus*. *Journal of Dalian Fisheries University*, 27(5), 392-397.

Martínez-Alvarez, R.M., Hidalgo, M.C., Domezain, A., Morales, A.E., García-Gallego, M., & Sanz, A. (2002). Physiological changes of sturgeon (*Acipenser naccarii*) caused by increasing environmental salinity. *Journal of Experimental Biology*, 202, 3699-3706.

Mommsen, T.P., Vijayan, M.M., & Moon, T.W. (1999). Cortisol in teleosts: dynamics, mechanisms of action, and metabolic regulation. *Reviews in Fish Biology and Fisheries*, 9(3), 211-268.

Morgan, J.D., & Iwama, G.K. (1991). Effects of salinity on growth, metabolism, and ion regulation in juvenile rainbow and steelhead trout (*Oncorhynchus mykiss*) and fall chinook salmon (*Oncorhynchus tshawytscha*). *Canadian Journal of Fisheries and Aquatic Sciences*, 48(11), 2083-2094.

Perry, S.F., & Reid, S.D. (1993). β-Andrenergic signa l transduction in fish: interactive effects of catecholamines and cortisol. *Fish Physiology and Biochemistry*, 11, 195-203.

Plaut, I. (1998). Comparison of salinity tolerance and osmoregulation in two closely related species of blennies from different habitats. *Fish Physiology and Biochemistry*, 19(2), 181-188.

Prakoso, V.A., Kim, K.T., Min, B.H., Gustiano, R., & Chang, Y.J. (2015). Effects of salinity on oxygen consumption and blood properties of young grey mullets *Mugil cephalus*. *Indonesian Aquaculture Journal*, 10(2), 143-153.

Sampaio, L.A., & Bianchini, A. (2002). Salinity effects on osmoregulation and growth of the euryhaline flounder *Paralichthys orbignyanus*. *Journal of Experimental Marine Biology and Ecology*, 269(2), 187-196.

Stewart, H.A., Noakes, D.L.G., Cogliati, K.M., Peterson, J.T., Iversen, M.H., & Schreck, C.B. (2016). Salinity effects on plasma ion levels, cortisol, and osmolality in Chinook salmon following lethal sampling. *Comparative Biochemistry and Physiology, Part A*, 192, 38-43.

Tapia, P.J., Puebla, M.C., Muñoz, A., Rojas, E., Marchant, C.M., Cornejo, M.A., Futagawa, M., & Small, B.C. (2012). Evaluation of the cortisol stress response in a marine Perciform fish, the San Pedro *Oplegnathus insignis*. *North American Journal of Aquaculture*, 74(3), 438-442.

Tsuzuki, M.Y., Ogawa, K., Strüssmann, C.A., Maita, M., & Takashima, F. (2001). Physiological responses during stress and subsequent recovery at different salinities in adult pejerrey *Odontesthes bonariensis*. *Aquaculture*, 200, 349-362.

Wendelaar Bonga, S.E. (1997). The stress response in fish. *Physiological Reviews*, 77(3), 591-625.

Wurts, W.A. (1998). Why can some fish live in freshwater, some in salt water, and some in both?. *World Aquaculture*, 29, 65.

