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JEB
Journal of Environmental Biology

ISSN: 0254-8704
CODEN: JEBIDP

Blood physiological responses and growth of juvenile starry flounder, *Platichthys stellatus* exposed to different salinities

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Publication Info

Paper received:
09 February 2011

Revised received:
15 December 2011

Re-revised received:
27 July 2012

Accepted:
10 February 2013

Abstract

Blood physiological responses, growth and survial rates were examined in juvenile starry flounder, *Platichthys stellatus* exposed to different salinities (5, 10, 20, 33 ppt) for 90 days. At the end of the experiment, the plasma levels of Na^+ and osmolality were similar at 10, 20, 33 ppt, however, the values were significantly lower at 5 ppt compared to those at other salinities. Stress responses such as plasma levels of cortisol, glucose, hematocrit (Ht) and hemoglobin (Hb) levels in all groups showed no significant difference. Although no differences in growth were observed, body weight at 20 ppt tended to be higher than others. Survival in all groups was greater than 99% with no significant differences. These results suggest that starry flounder is euryhalin species, thus this fish can be reared with normal growth and survival rate at 5-33 ppt salinity without osmoregulatory disturbance and stress.

Key words

Growth, Osmoregulation, *Platichthys stellatus*, Salinity, Starry flounder, Stress responses, Survival

Introduction

Starry flounder, *Platichthys stellatus* (Family pleuronectidae), is widely distributed from East Asia, Okhotsk Sea to North Pacific Ocean as a cold water species. In Korea, the flounder mainly inhabits within 150 m from the shore of the East Sea. Starry flounder has firm muscle and, compared to other flatfish, is evaluated to be more expensive ingredient of higher quality for sliced raw fish dish in Korea (Lim *et al.*, 2007). Therefore, recently, demand for starry flounder is rapidly increasing. However, the demand is not being met only with wild stocks (Lim *et al.*, 2007). As a way to solve this problem, priority must be given to establish farming technology and, accordingly, stable mass-production of this species.

Starry flounder can be found in coastal areas, bays, estuaries, brackish and occasionally in freshwater lagoons (Tomiyama and Omori, 2008). This suggests the outstanding osmoregulatory function of starry flounders as a marine euryhaline with strong tolerance against low salinity (0-25 ppt). Currently, in Korea, culture of starry flounder is dependent on the method of olive flounder, *Paralichthys olivaceus,* culture using seawater. However, using the osmoregulatory characteristic of starry flounder is able to rear the fish in both hyper and hypo-osmotic conditions.

Low salinity culture of starry flounders may produce several advantages to the conventional SW culture. Firstly, in case of rearing in salinity similar to osmolality in the body fluids (10-15 ppt: Jobling, 1994), amount of energy consumed in osmoregulation is smaller than that consumed in seawater (or freshwater) culture. Therefore, energy available in growth becomes relatively larger and this will lead to rapid growth. The growth replacement effect of osmoregulatory energy in the osmotic conditions is displayed in a variety of teleost fish, such as *Scophthalmus maximus*; (Gaumet *et al.*, 1995), silver *Sparus sarba*; (Woo and Kelly, 1995) and *Morone saxatilis*; (Peterson *et al.*, 1996). Secondly, it will be possible to treat diseases, such as extermination of parasites and

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bacteria, by osmotic shock. Min *et al.* (2006) reported the possibility of using osmotic shock therapy to treat diseases occurring in seawater and freshwater culture of euryhaline fish with extremely superior osmoregulatory ability. This method is to treat disease through osmoregulatory competition between fish with strong tolerance against salinity and parasites and bacteria with relatively weaker resistance to salinity. Thirdly, in case of using underground seawater of relatively consistent temperature (15-18ºC) with low salinity (18-25 ppt) throughout the year in Jeju Island, Korea, continuous growth is achieved even during wintertime resulting in reduction of fish culture period.

In general, when marine fish are exposed to low salinity environment, osmoregulatory confusion is induced to result in lowering of osmolality and ion content in blood and this directly leads to stress. When fish are exposed to stress factors, primarily the activity in hypothalamus-pituitary gland-interrenal axis is increased and, as a result, cortisol is excreted in blood (Wendelaar Bonga, 1997; Chang *et al.*, 2007). This hormone promotes glucose synthesis mainly in the liver. Glucose is used as energy source necessary to maintain homeostasis in fish. If stress continues for a longer period, it leads to lowering of health, delay in growth, reproduction and reduction of survival (Singley and Chavin, 1971; Wendelaar Bonga, 1997).

Therefore, in this study, we investigated the osmoregulatory ability and stress responses of juvenile starry flounder in different salinities (5-33 ppt).

Materials and Methods

Fish and blood analysis : The experiment was conducted at East Sea Mariculture Research Center of National Fisheries Research and Development Institute, Uljin, Korea. 1,200 juveniles (average body length of 9.9±0.8 cm and body weight of 26.4±0.7 g) adapted to natural seawater (NSW, 33 ppt) were divided into four different groups (3 tanks per group) and transferred directly to 12 tanks of 300 l with flow system (100 fish per tank). Salinity of experiment groups was set with 5, 10, 20 and 33 ppt. Fish were exposed to a specific environmental salinity for 90 days. Different salinities were obtained by mixing NSW with underground FW, and measured with an optical refractometer (ATAGO^{CS}, ATAGO Co., Japan). During the rearing period, water temperature was kept at 14ºC by thermostat (AQUATRON^{CS}, YOUWON ELECTRIC, INC., Korea) and natural photoperiod was maintained. Fish were fed satiation twice daily with a commercial extrude pellets and were fasted for 24 hr before sampling. Blood sampling and growth investigation were conducted at 0, 30, 60 and 90 days.

Fish were anaesthetized with 150 mg l^{-1} tricaine methanesulfonate (MS-222) prior to collection. Blood was collected from the caudal vasculature in a 3 ml syringe coated with heparin. Plasma samples were separated by centrifugation for 5 min at 9800×g and 4ºC and were stored at -80ºC until analysis. Hematocrit (Ht) was immediately determined after sampling by the microhematocrit method. Hemoglobin (Hb) was measured spectrophotometrically (at 540 nm) using cyanmethemoglobin method (with HemoCue^{CS}, Sweden).

Plasma cortisol was analyzed using a commercially available competitive radioimmunoassay (Coat-a-count, Diagnostics Product Corp., California) and an automatic gamma counter (1470 Wizard Automatic Gamma Counter, PerkinElmer, Finland). Plasma glucose, Na^+ and Cl^- were analyzed with a Biochemistry Auto analyzer (Fuji dry-chem 3500, Fujifilm Co., Japan). Plasma osmolality was examined with a Vapor Pressure Osmometer (Vapro 5520; Wescor Co., Logan, UT, USA). Growth parameters such as weight gain (WG), specific growth rate (SGR) and feed efficiency (FE) were calculated.

All data were analyzed with the SPSS statistical package (version 10.0; SPSS Inc., Chicago, IL, USA). One-way ANOVA followed by a *post hoc* multiple comparison test (Tukey test) was used to compare differences between treatments. Results were considered significantly different at $P < 0.05$.

Results and Discussion

Osmoregulatory ability : Plasma Na^+ levels in 5 and 10 ppt groups were lower than that of 33 ppt group at 30 days. 20 ppt and 10 ppt groups did not show significant difference from 33 ppt group at the end of the experiment. However, 5 ppt group indicated significantly lower level in comparison to 33 ppt group (Fig. 1). Plasma Cl^- level of 5 ppt group was significantly lower than those of other groups at 60 days. However, no difference was observed among all groups at the end of the experiment (Fig. 1). Plasma osmolality level of 5 and 10 ppt group were significantly lower than that of 33 ppt group at 30 days. However, the level of 10 ppt group started increasing at 60 days and showed similarity to NSW group at the end of the experiment (Fig. 1).

In general, if osmolality of ambient water is higher than body fluid of fish, inflow of ion is induced while water is discharged due to osmoregulation. Therefore, marine fish, in order to maintain consistent osmolality, are equipped with the hypo-osmoregulatory function to emit ion and absorb water. On the contrary, osmolality of ambient water is lower than body fluid of freshwater fish. Therefore, ion is discharged while water flows into the body of fish. Accordingly, in order to maintain homeostasis, freshwater

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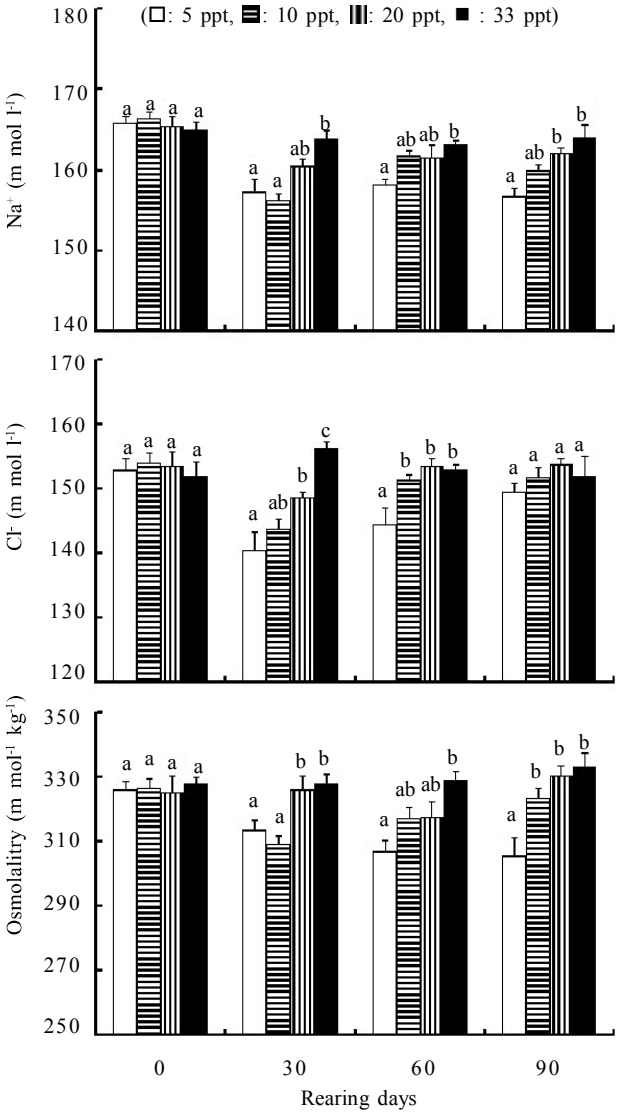

Fig. 1 : Levels of plasma Na^+, Cl^- and osmolality of starry flounder reared in different salinities. Different letters indicate significant difference (P>0.05). Each value represents the mean±S.E. (n=6)

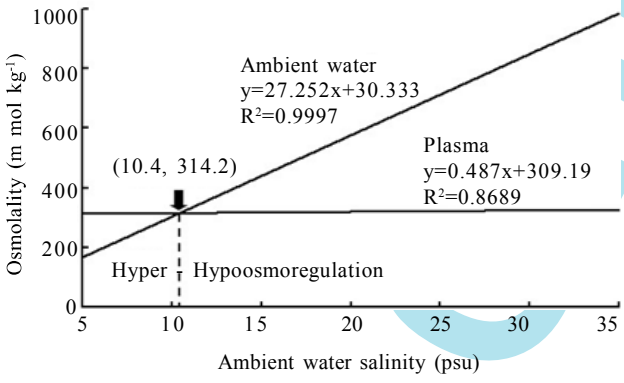

Fig. 2 : Osmolality of ambient water and plasma of starry flounder. The arrow indicates the isosmotic point

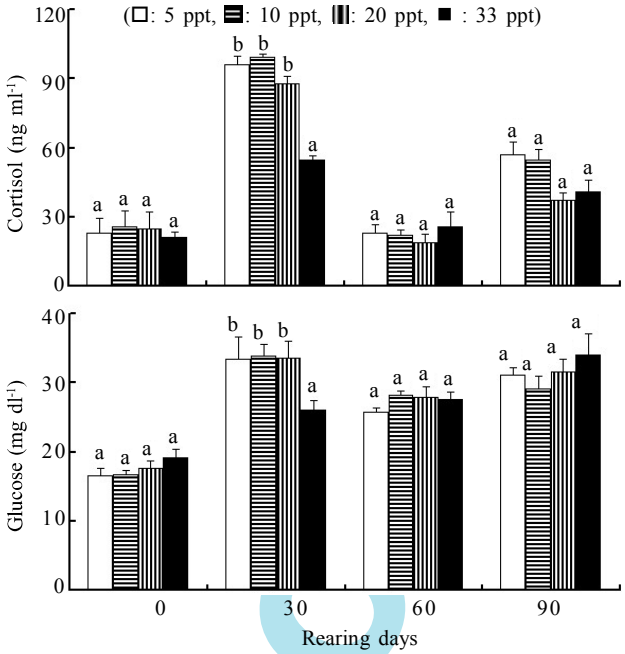

Fig. 3. Levels of plasma cortisol and glucose of starry flounder reared in different salinities. Different letters indicate significant difference (P>0.05). Each value represents the mean±S.E. (n=6)

fish have hyper-osmoregulatory function to absorb ion and emit water outside the body (Min *et al.*, 2005). In this study, flows the osmolality of ambient water corresponding to body fluid of fish was 10.4 ppt. Therefore, starry flounder underwent hypo-osmoregulation in NSW and 20 ppt, hyper-osmoregulation in 5 ppt and isomotic-osmoregulation similar to isotonic solution in 10 ppt (Fig. 2), respectively.

Rearing marine evryhaline telcost in hypo-osmotic environment by using hyper-osmoregulatory function has been earlier reported by various workers. Rearing black porgy, *Acanthopagrus shlegeli*, in freshwater and seawater for 90 days, no difference was found in osmolality (Min *et al.*, 2005). Also, on rearing sea bass, *Lateolabrax japonicus*, for 6 months in 2 ppt and 30 ppt, no significant difference was found in osmolalities (Han *et al.*, 2003). In freshwater rearing of mullet, *Mugil cephalus*, Na^+ level and osmolality were not different from those of mullets reared in SW (Hur and Chang, 1999).

Stress responses : Plasma cortisol levels in 5, 10 and 20 ppt groups were significantly higher than that of 33 ppt group at 30 days. However, no significant difference was observed in all groups at the end of the experiment (Fig. 3). Plasma glucose levels showed similar changing patterns as cortisol (Fig. 3). Although Ht and Hb in 5 ppt group were significantly higher than that of 33 ppt group at 30 days, there were no differences among all groups from 60 days (Table 1).

Table 1 : Levels of hematocrit (Ht) and hemoglobin (Hb) of starry flounder reared in different salinities

Rearing days	5 ppt		10 ppt		20 ppt		33 ppt	
	Ht (%)	Hb (g·dl^{-1})	Ht (%)	Hb (g·dl^{-1})	Ht (%)	Hb (g·dl^{-1})	Ht (%)	Hb (g·dl^{-1})
0	22.7±1.4^{a}	3.4±0.4^{a}	23.0±0.7	3.5±0.3	21.8±1.2	3.6±0.4	22.3±1.2	3.7±0.3
30	27.9±1.1^{b}	4.3±0.2^{b}	21.0±0.3	3.8±0.2	21.5±0.4	3.5±0.2	21.6±0.4	3.9±0.1
60	17.2±0.5^{a}	3.3±0.1^{a}	18.5±0.9	3.5±0.1	21.4±1.5	3.6±0.3	18.9±0.1	3.3±0.2
90	18.9±0.7^{a}	3.6±0.3^{a}	20.0±1.2	3.6±0.5	19.3±0.6	3.5±0.2	21.1±0.7	3.6±0.2

Different letters indicate significant difference (P>0.05). Each value represents the mean±S.E. (n=6)

Change in salinity at low salinity culture of starry flounder may act as a stress factor to the fish. When fish are exposed to stress factors, glucose level increases together with plasma cortisol level. Here, cortisol amplifies gluconeogenic capacity of phosphoenolpyruvate carbixykinase (PEPCK), gluconeogenic enzyme of liver. Therefore, it is known that cortisol intercede with hyperglycemia caused by stress (Choi *et al*., 2007) and hyperglycemia supplements the required amount of energy increased by stress (Vijayan *et al.*, 1997). In this study, plasma cortisol levels in low salinity group were significantly higher than that of NSW group at 30 days. Also, glucose levels were found to increase as of cortisol level. The co-increase of cortisol and glucose level by stressors has been reported in various teleost fish (Wendelarr Bonga, 1997). However, from 60 days, stress hormone in low salinity group did not display any difference from that of NSW group to indicate that the initial hormonal level was restored due to acclimation of the fish to new environment. The restoration of initial hormonal level after acclimation to new salinity has been reported in *Acanthopagrus schlegeli* (Min *et al.*, 2005; Mancera *et al.*, 1993) and *Plectropomus leopardus* (Frisch and Anderson, 2005). When stress continues, resistance against virus decreases to result in increased rate of disease occurrence (Johansen *et al*., 2011). This resultantly affects growth and propagation (Santos *et al*., 2010; Schreck, 2010). In this study, low salinity acted as a stress factor in comparison to NSW until 30 days. However, growth and survival rate of starry flounder in low salinity did not have difference from those of NSW group and no pathological symptoms were displayed, it is assumed that low salinity rearing did not exert significant impact on physiological state of starry flounder, the euryhaline fish. On the contrary, there is a possibility that low salinity environment, as 'eustress' (daily repeating stress with low stimulation to increase vitality) among the types of stress proposed by Selye (1974), acted as a positive stimulant to the fish. At the end of 90 days, body weight, growth parameters as well as survival rate were not significantly affected by salinity (Table 2).

Salinity in ambient water changes the amount of energy required in growth of fish by changing energy cost for ion level control and osmoregulation (Morgan and Iwama, 1996).

Table 2 : Growth parameters and survival rate of starry flounder reared in different salinities for 90 days

Salinity (ppt)	BW (g)	WGR (%)	SGRW (%)	FE (%)	Survival (%)
5	83.5±1.3	200.0±4.5	1.2±0.1	121.3±2.5	99.9±0.3
10	82.6±5.5	200.7±5.2	1.2±0.2	121.8±0.4	100
20	92.7±5.0	203.4±2.2	1.2±0.2	122.9±2.5	100
33	82.6±1.0	202.0±6.1	1.2±0.1	125.8±4.1	100

Each value represents the mean±S.E. (n=3). There was no difference among different salinities. BW: body weight, FE: feed efficiency, FI : daily feed intake, SGRW: specific growth rate, WGR: weight gain rate

Brett (1979) hypothesizes that the salinity of ambient water of which growth of euryhaline teleost fish can be maximized in general is 10±2 ppt (close to isotonic solution). When salinity of ambient water is of isotonic solution that is similar to osmolality of fish (osmotic gradient between blood and water minimized), energy cost for osmoregulation in the fish would be low and energy can be sufficiently reserved for growth increase. (Morgan and Iwana, 1991; Altinok and Grizzle, 2001; Boeuf and Payan, 2001). Based on this hypothesis, we anticipated the highest growth rate in 10 ppt group. However, although no significant difference was found, increase in weight was the greatest in 20 ppt group. Tsuzuki *et al.* (2007) reported that, when rearing the young fat snook, *Centropomus parallelus*, for 50 days at 5, 15 and 35 ppt, total length was highest at 15 ppt. Also, growth rate of the fry of Atlantic cod, *Gadus morhua*, was higher at 14 ppt than at 7.28 ppt, respectively (Lambert *et al.*, 1994). As such, while there are fish of which the growth is maximized in the range that corresponds to medium salinity of seawater (14-17 ppt), other fish, such as Arctic cisco, *Coregonus autumnalis*, did not display difference in growth at inhabitable salinity (6-30 ppt) (Fechhelm *et al.*, 1993). In case of milk fish, *Chanos chanos*, reared at 15, 35 and 55 ppt, the best growth was observed at 55 ppt (Swanson, 1998). Considering this, it can be concluded that the effect of salinity on growth of fish is species-specific.

Through in this study, it was found that in case of low salinity rearing of starry flounder, osmoregulation took place normally in the fish. Also, since stress responses were

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not continuous, no impact was exerted on the growth and survival of the fish. However, it must not be concluded that the same result will be produced in the actual sites of farming. This is because of the difference in rearing conditions, such as quality of water, rearing density and environmental conditions, etc. Therefore, verification through direct onsite rearing will be necessary in order to achieve stable low salinity culture of starry flounder.

The results of this study, conclude that starry flounder capable of normal osmoregulation in a wide range of low salinities (converting to hyper-osmoregulation in 10 ppt or less) and stress responses to exert impact on survival and growth were not observed. Therefore, low salinity culture of starry flounder is sufficiently possible and, in particular, the highest growth rate is anticipated at 20 ppt, salinity.

Acknowledgments

This research was supported by Fisheries Research and Development Program of the Ministry for Food, Agriculture, Forestry and Fisheries (F106018-06A2200-00110, RP-2009-AQ-026).

References

Altinok, I. and J.M. Grizzle: Effects of brackish water on growth, feed conversion and energy absorption efficiency by juveniles euryhaline and freshwater stenohaline fishes. *J. Fish Biol*., **59**, 1142-1152 (2001).

Boeuf, G. and P. Payan: How should salinity influence fish growth. *Comp. Biochem. Physio*l., *C*, **130**, 411-423 (2001).

Brett, J.R.: Environmental factors and growth. In: Fish Physiology (Eds.: W.S. Hoar, D.J. Randall and J.R. Brett). Academic Press, New York, pp. 599-675 (1979).

Campbell, P.M., T.G. Pottinger and J.P. Sumpter: Preliminary evidence that chronic confinement stress reduces the quality of gametes produced by brown and rainbow trout. *Aquaculture*, **120**, 151-169 (1994).

Chang, Y.J., B.H. Min and C.Y. Choi: Black porgy (*Acanthopagrus schlegeli*) prolactin cDNA sequence: mRNA expression and blood physiological responses during freshwater acclimation. *Comp. Biochem. Physiol., B*, **147**, 122-128 (2007).

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

Fechhelm, R.G., P.S. Fitzgerald, J.D. Bryan and B.J. Gallaway: Effect of salinity and temperature on the growth of yearling Arctic cisco (*Coregonus autumnalis*) of the Alaskan Beaufort Sea. *J. Fish Biol*., **43**, 463-474 (1993).

Frisch, A. and T. Anderson : Physiological stress responses of two species of coral trout (*Plectropomus leopardus* and *Plectropomus maculatus*). *Comp. Biochem. Physiol., A*, **140**, 317-327 (2005).

Gaumet, F., G. Boeuf, A. Severe, A. Le Roux and N. Mayer-Gostan: Effects of salinity on the ionic balance and growth of juvenile turbot. *J. Fish Biol.*, **47**, 865-876 (1995).

Han, H.K., D.Y. Kang, C.Y. Jun and Y.J. Chang: Effects of salinity changes on physiological response and growth of yearling sea bass, *Lateolabrax japonicus*. *J. Aquaculture*, **16**, 31-36 (2003).

Hanson, R.W. and L. Reshef: Regulation of phosphoenol pyruvate carboxykinase (PEPCK) gene expression. *Ann. Rev. Biochem*., **66**, 581-611 (1997).

Hur, J.W. and Y.J. Chang: Physiological responses of grey mullet (*Mugil cephalus*) and nile tilapia (*Oreochromis niloticus*) by gradual change in salinity of rearing water. *J. Aquaculture*, **12**, 283-292 (1999).

Johansen, L.H., I. Jensen, H. Mikkelsen, P.A. Bjørn, P.A. Jansen and Ø. Bergh: Disease interaction and pathogens exchange between wild and farmed fish populations with special reference to Norway. *Aquaculture*, **315**, 167-186 (2011).

Lambert, Y., J.D. Dutil and J. Munro: Effect of intermediate and low salinity conditions on growth rate and food conversion of Atlantic cod *Gadus morhua*. *Can. J. Fish Aquat. Sci*., **51**, 1569-1576 (1994).

Lim, H.K., S.G. Byun, J.H. Lee, S.U. Park, Y.C. Kim, H.K. Han, B.H. Min and B.Y. Lee: Sexual maturity and reproductive cycle of starry flounder, *Platichthys stellatus* cultured in door tank. *J. Aquaculture*, **20**, 212-218 (2007).

Mancera, J.M., J.M. Pérez-Fígares and P. Fernández-Llebrez: Osmoregulatory responses to abrupt salinity changes in the euryhaline gilthead sea bream (*Sparus aurata*). *Comp. Biochem. Physiol*., *A*, **106**, 245-250 (1993).

Min, B.H., C.Y. Choi and Y.J. Chang: Comparison of physiological conditions on black porgy, *Acanthopagrus schlegeli* acclimated and reared in freshwater and seawater. *J. Aquaculture*, **18**, 37-44 (2005).

Min, B.H., M.H. Jeong, G.A. Noh, H.K. Lim, C.Y. Choi, and Y.J. Chang: Hyposmotic treatment for control of the parasitic copepod, *Alella macrotrachelus* on the gill of cultured black porgy. *J. Aquaculture,* **19**, 19-24 (2006).

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

Morgan, J.D. and G.K. Iwana: Cortisol-induced changes in oxygen consumption and ionic regulation in coastal cutthroat trout (*Oncorhynchus clarki*) parr. *Fish Physiol. Biochem*., **15**, 385–394 (1996).

Pankhurst, N.W. and G. van der Kraak: Effects of stress on reproduction and growth of fish. In: Fish Stress and Health in Aquaculture (Eds.: G.K. Iwama, A.D. Pickering, J.P. Sumpter and C.B. Schreck). Cambridge University Press, Cambridge, pp. 73-93 (1997).

Peterson, R.H., D.J. Martin-Robichaud and Å. Berge: Influence of temperature and salinity on length and York utilization of striped bass larvae. *Aquacult. Int*., **4**, 89-103 (1996).

Pickering, A.D. and T.G. Pottinger: Stress responses and disease resistance in salmonid fish: effect of chronic elevation of plasma cortisol. *Fish. Physiol. Biochem*., **7**, 253-258 (1989).

Santos, G.A., J.W. Schrama, R.E. Mamauag, J.H. Rombout and J.A. Verreth: Chronic stress impairs performance, energy metabolism and welfare indicators in European seabass (*Dicentrachus labrax*): The combined effects of fish crowding and water quality deterioration. *Aquaculture*, **299**, 73-80 (2010).

Schreck, C.B.: Stress and fish reproduction: The roles of allostasis and hormesis. *Gen. comp. Endocrinol*., **165**, 549-556 (2010).

Selye, H.: Stress without distress. Philadelphia, PA: Lippincott (1974).

Singley, J.A. and W. Chavin: Cortisol levels of normal goldfish, *Carassius auratus* L., and response to osmotic change. *Ame. Zool.,* **11**, 653 (1971).

Swanson, C.: Interactive effects of salinity on metabolic rate, activity, growth and osmoregulation in the euryhaline milkfish (*Chanos*

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chanos). *J. Exp. Biol*., **201**, 3355-3366 (1998).
Tomiyama, T. and M. Omori: Habitat selection of stone and starry flounders in an estuary in relation to feeding and survival. *Estuar. Coast. Shelf Sci.,* **79**, 475-482 (2008).
Tsuzuki, M.Y., J.K. Sugai, J.C. Maciel, C.J. Francisco and V.R. Cerqueira: Survival, growth and digestive enzyme activity of juveniles of the fat snook (*Centropomus parallelus*) reared at different salinities. *Aquaculture*, **271**, 319-325 (2007).
Vijayan, M.M., C.E. Pereira, G. Grau and G.K. Iwama: Metabolic responses associated with confinement stress in tilapia: The role of cortisol. *Comp. Biochem. Physiol., C*, ***116***, 89-95 (1997).
Wendelaar Bonga, S.E.: The stress response in fish. *Physiol. Rev*., **77**, 591-625 (1997).
Woo, N.Y.S. and S.P. Kelly: Effects of salinity and nutritional status on growth and metabolism of *Sparus sarba* in a closed seawater system. *Aquaculture*, **135**, 229-238 (1995).

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