

EFFECTS OF SALINITY ON OXYGEN CONSUMPTION AND BLOOD PROPERTIES OF YOUNG GREY MULLET *Mugil cephalus*

Vitas Atmadi Prakoso¹⁾, Ki Tae Kim²⁾, Byung Hwa Min³⁾, Rudhy Gustiano¹⁾, and Young Jin Chang²⁾

¹⁾ Institute for Freshwater Aquaculture Research and Development

²⁾ Department of Marine Bio-materials and Aquaculture, Pukyong National University, Korea

³⁾ National Fisheries Research and Development Institute, Korea

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ABSTRACT

Oxygen consumption (OC) is one of important factors in aquaculture activities, as the oxygen is a vital condition for all the organisms living in the water and having an aerobic type of respiration. OC is the preferred method for measuring and reporting the metabolic rate in fish. The aims of this study were to evaluate the effects of salinity on OC and blood properties of grey mullets. Five experimental groups were conducted to measure OC and blood properties of grey mullets *Mugil cephalus* (BW: 187.9 ± 45.8 g) according to salinity (30→0 psu, 0→30 psu) changes; SDS: fish reared in seawater (SW, 30 psu) directly shifted to SW, SGF: SW fish gradually shifted to freshwater (FW, 0 psu), SDF: SW fish directly shifted to FW, FDF: FW fish directly shifted to FW, and FDS: FW fish directly shifted to SW. The result showed that OC tended to decrease in the groups of SW fish shifted to FW showing 194.5 mg O₂/kg/h at 25°C in SDS to 82.4 mg O₂/kg/h at 15°C in SGF. On the contrary, OC increased in the groups of FW fish shifted to SW showing 80.5 mg O₂/kg/h at 15°C in FDF to 184.0 mg O₂/kg/h at 25°C in FDS. Cortisol levels at the end of experiments were rapidly increased with the lowering salinities in SW fish shifted to FW showing 20.6 ng/mL in SDS to 316.2 ng/mL in SDF, while those were decreased with the increasing salinities in FW fish shifted to SW showing 40.2 ng/mL in FDF to 10.3 ng/mL in FDS. However, glucose levels showed no significant differences among all experimental groups. Based on the information from this study, aquaculture of grey mullet might be applied or developed in freshwater due to its osmotic adaptation ability.

KEYWORDS: grey mullet, salinity, oxygen consumption, blood properties

INTRODUCTION

Oxygen consumption (OC) is one of a vital parameter for all the organisms living in the water and having an aerobic type of respiration. OC is the preferred method for measuring and reporting the metabolic rate in fish. OC rate by fish is useful in determining carrying capacity of fish in culture system and in predicting aeration needs and flow rates in various aquaculture environments (Lovell, 1998).

The researches on OC were continuously developed for aquaculture benefit, not only on freshwater species but also marine species (Kim *et al.*, 1995; Lyytikainen & Jobling, 1998; Jeong *et al.*, 2007). Many authors investigated about the effects of various factors on OC of fish, such as: temperature (Franklin *et*

al., 1995; Wares & Igram, 1979; Requena *et al.*, 1997; Van Maaren *et al.*, 2000; Turker, 2011), salinity (Tsuzuki *et al.*, 2008; Iwama *et al.*, 1997), photoperiod (Chang *et al.*, 2005), and rearing density (Szczepkowski *et al.*, 2011; Bjornsson & Olafsdottir, 2006; Duan *et al.*, 2011).

Related with salinities, grey mullet *Mugil cephalus* is one of interesting species to be observed for aquaculture development, especially for freshwater aquaculture development. Grey mullet is a good osmoregulator species which can be found in coastal marine, brackish waters, and freshwater. They move between marine and freshwater environments of rivers and flooded rice fields (Saleh, 2008). Therefore, basic information on OC and blood properties of grey mullets is needed for development of grey mullets' freshwater aquaculture. The aims of this study were to evaluate the effects of salinity on OC and blood properties of grey mullets.

Correspondence: Institute for Freshwater Aquaculture Research and Development. Jl. Sempur No. 1, Bogor 16154, Indonesia. Phone: + (0251) 8313200
E-mail: vitas.atmadi@gmail.com

MATERIALS AND METHODS

Thirty one grey mullets *Mugil cephalus* (TL: 27.3 ± 2.1 cm, BW: 187.9 ± 45.8 g) which were collected from Suncheon Bay and reared in culture tanks were used for the experiments. Before the experiments, grey mullets were divided and acclimated into two different rearing environments, which were seawater (SW) and freshwater (FW). The fish were reared in recirculating tanks and fed two times a day at 2% of their body weight with commercial feed. No food was given to any experimental fish for 24 hours until experiments.

Five experiments were divided into two groups, the groups of SW fish shifted to FW (SDS, SGF, and SDF), and the groups of FW fish shifted to SW (FDF and FDS). SDS: fish reared in SW directly transferred to SW, SGF: fish reared in SW gradually transferred to FW, SDF: fish reared in SW directly transferred to FW, FDF: fish reared in FW directly transferred to FW, FDS: fish reared in FW directly transferred to SW.

The experiments were conducted to observe the effect of different salinity level on OC of grey mullets, from 0 psu (Practical Salinity Unit) up to 30 psu (Table 1). In each experiment, acclimation to exact salinity level was carried out 2 days before fish OC measurement in respiratory chamber running for six days, except in SDF (direct transfer without acclimation).

To measure the OC according to salinity and temperature changes, OC measurement system and OC calculation methods were adapted to Chang *et al.* (2005). Three grey mullets were put into respiratory chamber (dimension= 20 cm × 30 cm × 20 cm) inside the closed recirculating system with photoper-

iod of 12 hours light (07:00-19:00) : 12 hours dark (19:00-07:00). DO of inflow water was maintained above 7.0 mg/L in each experiment. During experiment, water temperature inside the OC measurement system was increased slowly from 15°C to the target temperature (15°C, 20C, and 25°C) at a rate of 0.5°C/h to minimize any thermal shock to the fish.

In addition to measuring the OC, the behavior of the fish was observed during experiments, including their movements in the water and breathing frequency per minute. Behavioral index was used to evaluate the fish activity in each experiment inside the respiratory chamber (Table 2). At the end of each experiment of OC measurement according to salinity and temperature changes, blood samples were collected from three grey mullets from respiratory chamber (Exp.) and three grey mullets from rearing tank (Con.). In lethal DO experiments, blood samples were collected from three of five fish just after the fish died. Fish were anesthetized using 2-phenoxyethanol and blood samples were collected using heparinized syringes, centrifuged (12,000 rpm, 5 min.), and stored in deep freezer until analyzing blood properties of hemoglobin (Hb) and hematocrit (Ht) as the oxygen binding factor. Na⁺, K⁺, Cl⁻, Ca, Mg, and osmolality as the osmoregulation factor. Furthermore, cortisol, glucose, and total protein were analyzed for the stress factor. Hematocrit (Ht) was analyzed by using micro-hematocrit reader (Micro Hematocrit Reader, Hawksley). Hemoglobin (Hb), Na⁺, K⁺, Cl⁻, Ca, Mg, glucose, and total protein were analyzed by using Chemical Analyzer (Fujifilm Dri-Chem 3500i, Japan). 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).

Table 1. Experimental conditions in OC measurement

| Exp. | Water temperature change (°C) | Salinity change (psu) | Total length (cm) | Body weight (g) | No. of fish |
|------|-------------------------------|-----------------------|-------------------|-----------------|-------------|
| SDS | 15→20→25 | 30→30 | 26.4±1.1 | 176.0±18.4 | 3 |
| SGF | 15→20→25 | 30→10→0 | 25.5±0.9 | 157.3±19.4 | 3 |
| SDF | 15→20→25 | 30→0 | 28.4±1.6 | 228.0±58.6 | 3 |
| FDF | 15→20→25 | 0→0 | 28.3±0.9 | 197.0±14.1 | 3 |
| FDS | 15→20→25 | 0→30 | 29.0±0.9 | 221.3±20.6 | 3 |

Note: SDS: fish reared in SW directly transferred to SW, SGF : fish reared in SW gradually transferred to FW, SDF : fish reared in SW directly transferred to FW, FDF : fish reared in FW directly transferred to FW, FDS : fish reared in FW directly transferred to SW.

Table 2. Behavioral indices of fish in experiments of OC and lethal DO

| Index | Movement | Breath freq./min. |
|-------|---------------------------|-------------------|
| I | Active swimming | > 110 |
| II | Moderate swimming | 81-110 |
| III | Slow swimming | 51-80 |
| IV | Very slow swimming | 31-50 |
| V | Lost balance, no movement | 1-30 |
| VI | Died | 0 |

The data were analyzed by using PASW Statistics 18 software. One-way ANOVA was conducted to analyze OC and physio-chemical properties of blood according to different salinity experiment, two-way ANOVA was conducted to analyze the interaction between salinity and temperature on OC. and *t*-test was conducted to analyze the differences of OC according to different photoperiod and compare the physio-chemical properties of blood between control and experiment.

RESULTS AND DISCUSSION

Oxygen Consumption According To Salinity

The grey mullets from the groups of SW fish shifted to FW and the groups of FW fish shifted to SW showed various rhythm in closed recirculating system at each water temperature from the continuous OC measurements with stepwise rising temperature

from 15°C to 25°C. As shown in Figure 1 and 2, the changes in OC with water temperature for each experiment showed a linear increase in line with water temperature, except the SDF. The OC of grey mullets which determined every hour clearly showed various type of OC fluctuations. Sudden increase of OC occurred in each experiment during the beginning of light period and dark period with the various amount in each experiment.

The OC of grey mullets in SDS during the light period was higher than dark period at each temperature (Figure 1). Same patterns with SDS were also found in SDF and SGF, while grey mullets in FDF showed a reversed day/night OC rhythm and consumed less oxygen during the day period. The behavioral change also occurred in FDS. In FDS, the grey mullets shifted to consume oxygen higher in light period start from 20°C.

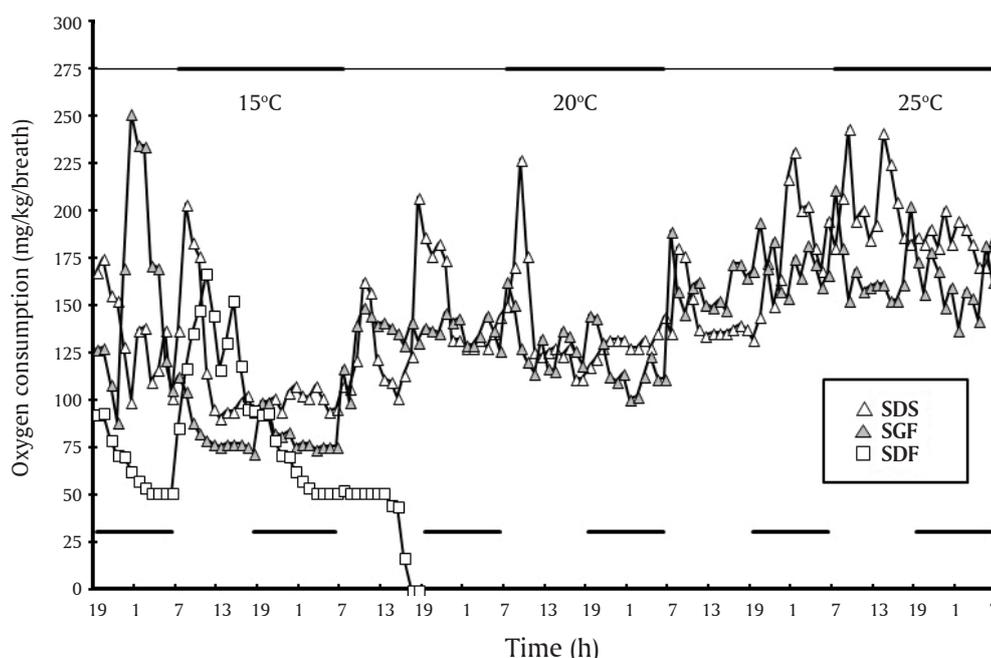


Figure 1. OC of grey mullets *Mugil cephalus* by water temperature in the groups of SW fish shifted to FW; SDS, SGF, and SDF are the same abbreviations as shown in Table 1

In the groups of SW fish shifted to FW, grey mullets consumed 112.4, 136.0, and 194.5 mg O₂/kg/h at 15°C, 20°C, and 25°C, respectively in SDS, showing a linear increase in OC with water temperature, which was significantly different at each temperature (P<0.05). In SGF, grey mullets acclimated in freshwater by gradual salinity change consumed lower amount of oxygen compare to SDS in each temperature. The value was 82.4, 124.1, 164.6 mg O₂/kg/h at 15°C, 20°C, and 25°C, respectively (P<0.05). These values were also lower compare to FDF and FDS, showing clearly different response due to the different salinity. Another result showed that grey mullets consumed 95.5 mg O₂/kg/h at 15°C in SDF. Meanwhile, OC data from

SDF experiment during 20°C and 25°C cannot be observed due to fish mortality during experiment caused by the effect of abrupt salinity changes from SW to FW (Table 3).

OC of fish increased linearly with the temperature rise in this study. The OC of fish reflected the activity of fish itself (Beamish & Mookherjee, 1964). This seems in this present study the activity of fish including their breath frequency increased by the rising of temperature.

The grey mullets in SDS showed that the energy demand of grey mullets reared in seawater elevated on active at the morning. This result was opposite

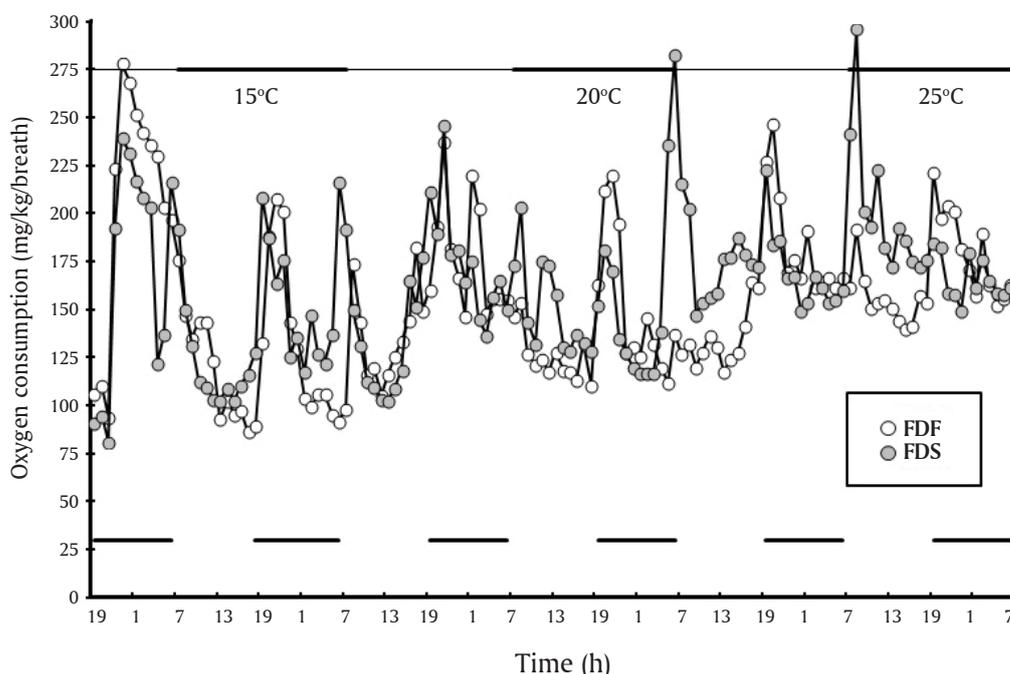


Figure 2. OC of grey mullets *Mugil cephalus* by water temperature in the groups of FW fish shifted to SW; FDF and FDS are the same abbreviations as shown in Table 1

Table 3. Average OC (mg O₂/kg/h) of grey mullets *Mugil cephalus* in each experiment

| Group | Water temperature (°C) | | | b | a | r ² |
|-------|---------------------------|----------------------------|---------------------------|------|--------|----------------|
| | 15 | 20 | 25 | | | |
| SDS | 112.4±31.0 ^{a**} | 136.0±24.8 ^{b**} | 194.5±19.1 ^{c**} | 8.21 | -16.59 | 0.621 |
| SGF | 82.4±10.8 ^{a°} | 124.1±15.6 ^{b°} | 164.6±17.2 ^{c°} | 8.22 | -40.72 | 0.843 |
| SDF | 95.5±37.1 [°] | - | - | - | - | - |
| FDF | 126.8±36.5 ^{a**} | 139.1±30.3 ^{a**} | 167.9±22.2 ^{b*} | 4.11 | 62.43 | 0.239 |
| FDS | 139.2±34.4 ^{a**} | 155.0±40.4 ^{a***} | 184.0±32.0 ^{b**} | 4.48 | 69.78 | 0.213 |

Note: All abbreviations are the same as shown in Table 1. Each values represent means ± SD (n = 24). Different letters indicate significant differences between water temperatures in each experiment, respectively. Asterisks indicate significant differences within groups of seawater to freshwater and within the groups of FW fish shifted to SW (P<0.05, one-way ANOVA)

to FDF where grey mullets reared was active at night. Another pattern occurred in some experimental groups, which was slightly different. The OC behavior shifted from consumed lower amount at dark period to lower amount at light period or the vice versa. Lyytikäinen & Jobling (1998) concluded that daily variations in OC had an influence on the water requirements of fish in aquaculture, water requirements should be estimated according to peak OC rates rather than the daily average OC.

OC tended to decrease in the groups of SW fish shifted to FW showing the highest value of 194.5 mg O₂/kg/h at 25°C in SDS and the lowest value of 82.4 mg O₂/kg/h at 15°C in SGF. On the contrary, OC increased in the groups of FW fish shifted to SW showing the highest value of 184.0 mg O₂/kg/h at 25°C in FDS.

A two-way ANOVA was conducted that examined the effect of salinity and temperature on OC. There was a significant interaction between the effects of salinity and temperature on OC within the groups of FW fish shifted to SW, while the interaction between the effects of salinity and temperature within the groups of SW fish shifted to FW was not significant (P≥0.05) (Table 4).

The fish in each experiment showed various pattern on OC according to salinity in both of group during light and dark period. In the groups of SW fish shifted to FW, the average of OC in SDS during the dark period was 81.2%, 91.5%, and 90.7% lower than light period at 15°C, 20°C, and 25°C, respectively. Same pattern was found in SGF and SDF. In SGF, the average of OC during the dark period was 97.8%, 90.9%, and 95.0% lower than light period at 15°C, 20°C, and 25°C, respectively. Meanwhile, the average of OC in SDF during the dark period was 51.2% lower than light period only at 15°C. There was no record of OC at 20°C and 25°C due to fish mortality.

In the groups of FW fish shifted to SW, another different pattern was found in FDF, the average of OC during the dark period in FDF was 111.9%, 120.6%, and 115.7% higher than light period at 15C, 20C, and 25°C, respectively. Which means the OC pattern was all higher in dark period than light period. Meanwhile,

the average of OC in FDS during the dark period was 127.0% and 104.1% higher than light period at 15°C and 20°C, and it was 82.7% lower than light period at 25°C. The change of tendency on OC due to salinity changes during light and dark period was found in these experiments (Table 5).

The slope (b) of linear regression in the groups of SW fish shifted to FW during the light period was significantly higher than that of the groups of FW fish shifted to SW. Same pattern was also occurred during the dark period. The slope (b) of linear regression in the groups of SW fish shifted to FW during the dark period was significantly higher than that of the groups of FW fish shifted to SW. These conditions indicated that increment of OC of grey mullets were faster in the groups of SW fish shifted to FW than the groups of FW fish shifted to SW (Table 5).

Increasing or decreasing of OC clearly related with salinity changes due to stress response reflecting in immune-related parameters. It caused physiological disturbances and change photoperiodical activity and metabolism of fish. Fish mortality could be occurred by the abrupt salinity changes from seawater to freshwater. In contrary, Arnason *et al.* (2013) on Atlantic cod *Gadus morhua* that were reared in salinity and abrupt salinity changes have limited or no effects on stress and immune-related parameters, and there were no indications of ion regulatory disturbances at low salinities. Meanwhile, the effect of salinity change from freshwater to seawater will not cause the mortality due to grey mullets' osmoregulation ability to tolerate seawater as its natural habitat.

Fish Behavior and Breath Frequency

As shown in Table 6, the fish in each experiment showed six subsequent responses based on their swimming activity and breath frequency. All experimental groups were in normal behavior during experiments, showing stable activities with behavioral index from I to III, except for SDF. SDF showed abnormal behavior with index from IV to VI because of abrupt salinity change. The highest index was found in SDS, SGF, and FDS, while the lowest index was found in SDF.

Table 4. P-values from two-way ANOVA of OC in grey mullets *Mugil cephalus* at the end of experiments

| Group | Temperature (°C) | Salinity (%) | Temperature (°C) x Salinity (%) |
|-----------------------|------------------|--------------|---------------------------------|
| SW fish shifted to FW | < 0.001 | < 0.001 | 0.064 |
| FW fish shifted to SW | < 0.001 | < 0.001 | 0.006 |

Note: SW = seawater; FW = freshwater

Table 5. Average OC (mg O₂/kg/h) of grey mullets *Mugil cephalus* during light and dark periods in each experiment

| Group | L : D | Water temperature (°C) | | | b | a | r ² |
|-------|-------|---------------------------|---------------------------|---------------------------|------|--------|----------------|
| | | 15 | 20 | 25 | | | |
| SDS | L | 124.1±41.1 ^a | 142.0±34.1 ^a | 204.0±22.0 ^b | 7.99 | -3.21 | 0.486 |
| | D | 100.8±4.7 ^{a**} | 130.0±6.7 ^{b**} | 185.1±8.9 ^{c**} | 8.43 | -29.97 | 0.936 |
| SGF | L | 83.3±12.8 ^a | 130.0±14.9 ^b | 168.8±19.8 ^c | 8.55 | -43.59 | 0.835 |
| | D | 81.5±8.8 ^a | 118.2±14.6 ^b | 160.4±13.9 ^c | 7.89 | -37.84 | 0.875 |
| SDF | L | 125.6±25.3 ^a | - | - | - | - | - |
| | D | 65.4±15.9 ^{a***} | - | - | - | - | - |
| FDF | L | 119.7±29.4 ^{a**} | 126.1±13.0 ^{a**} | 155.7±13.8 ^{b**} | 3.61 | 61.70 | 0.350 |
| | D | 134.0±42.6 ^a | 152.1±37.2 ^{ab} | 180.1±22.8 ^b | 4.61 | 63.17 | 0.237 |
| FDS | L | 122.6±26.1 ^a | 151.8±24.7 ^b | 201.4±36.7 ^c | 7.88 | 1.08 | 0.555 |
| | D | 155.7±34.6 ^a | 158.1±52.8 ^a | 166.5±11.6 ^a | 1.08 | 138.49 | 0.015 |

Note: All abbreviations are the same as shown in Table 1. Each values represent means ± SD (n = 12). Different letters indicate significant difference between water temperature in each experiment, respectively (P<0.05, one-way ANOVA). Asterisk indicates significant difference between light and dark in each experiment, respectively (*: P<0.05, **: P<0.01, ***: P<0.001, t-test)

The breath frequency of grey mullets from whole experiments at 15°C, 20°C, and 25°C were shown in Figure 3. The slope of linear regression of breath frequency according to different water temperature in grey mullets from the groups of SW fish shifted to FW which consisting of SDS and SGF was 3.07 and 1.81, respectively, while the slope of linear regression of breath frequency according to different water temperature in grey mullets from the groups of FW fish shifted to SW which consisting of FDF and FDS was 0.99 and 2.18, respectively. These values were indicating the breath frequency was higher at SW condition. The slope of linear regression of breath frequency from the results might be related with fish metabolism. Grey mullets had higher metabolic rates during SW rearing condition, while the result was vice versa during FW rearing condition. Morgan &

Iwama (1991) suggested that low metabolic rates were the most often associated to the water salinity, which was in species that was commonly found and adapted at a particular life stage. This condition seems to be related with the natural environment of grey mullets. Grey mullets occurred in seawater as their natural habitat. The grey mullets *Mugil cephalus*, migrates to feed, moving from seawater to brackish as it grew from fry to adult (Kim *et al.*, 2004).

Blood Properties

The salinity change from SW to FW tended to decrease Ht and Hb values in the groups of SW fish shifted to FW. The highest value of Ht and Hb was 30.6% and 7.3 g/dL in SDS, respectively. Whereas, Ht and Hb tended to increase in the groups of FW fish shifted to SW. The highest value of Ht and Hb was

Table 6. Behavioral indices of grey mullets *Mugil cephalus* in OC experiments

| Exp. | Beginning | 15°C | 20°C | 25°C | End |
|------|-----------|------|------|------|-----|
| SDS | I | II | II | I | I |
| SGF | II | II | II | I | I |
| SDF | IV | V | VI | - | - |
| FDF | II | II | II | II | II |
| FDS | II | II | I | I | I |

Note: All abbreviations are the same as in Table 1

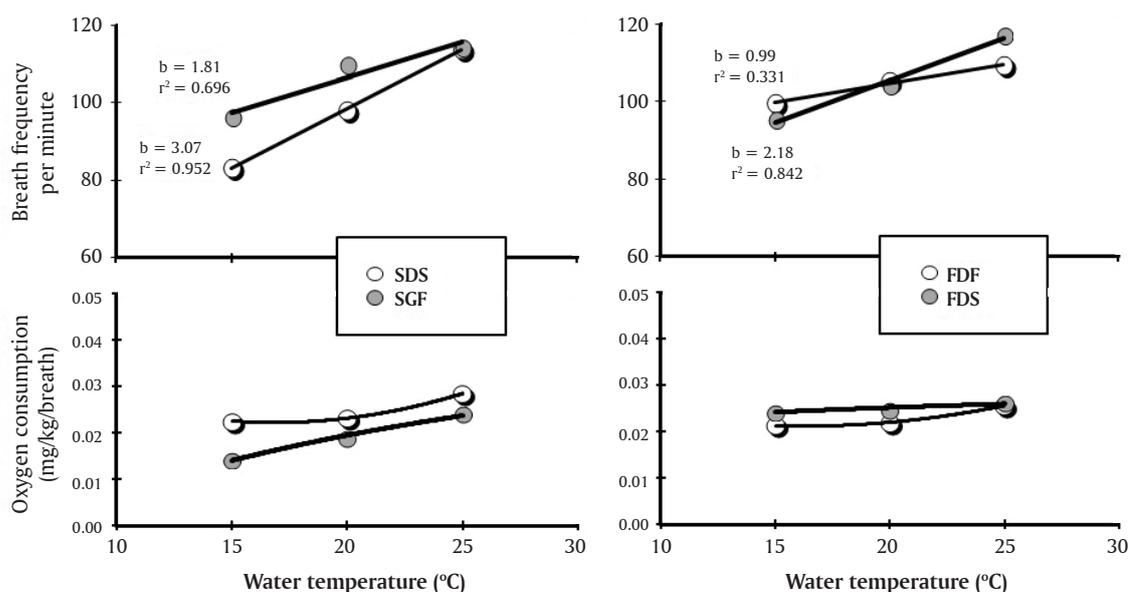


Figure 3. Breath frequency per minute and OC per breath in grey mullets *Mugil cephalus* from the groups of SW fish shifted to FW and the groups of FW fish shifted to SW; All abbreviations are the same as shown in Table 1

47.9 ± 0.2% and 8.5 ± 0.6 in FDS, respectively (Table 7).

Na⁺ values were decreased within the groups of SW fish shifted to FW from 163.5 mEq/L in SDS to

134.5 mEq/L in SDF, while those values were increased within groups of FW fish shifted to SW from 136.5 mEq/L in FDF to 162.0 mEq/L in FDS. Cl⁻ and Ca values also showed the same pattern. Cl⁻ values decreased showing 155.5 mEq/L in SDS to 134.5 mEq/L in SDF,

Table 7. Physical properties of blood in grey mullets *Mugil cephalus* at the end of experiments

| Group | Component | Component | |
|-------|-----------|-------------------------|------------------------|
| | | Ht (%) | Hb (g/dL) |
| SDS | Con. | 33.8±5.5 | 8.5±0.5 |
| | Exp. | 30.6±10.2 ^b | 7.3±0.9 ^b |
| SGF | Con. | 37.1±3.0 | 8.2±0.8 |
| | Exp. | 26.2±1.1 ^{b**} | 6.7±0.6 ^{b*} |
| SDF | Con. | 30.2±4.9 | 6.9±0.4 |
| | Exp. | 16.2±1.2 ^{a**} | 4.2±1.0 ^{a**} |
| FDF | Con. | 18.9±5.4 | 4.4±0.8 |
| | Exp. | 26.8±0.2 ^a | 5.8±1.1 ^a |
| FDS | Con. | 22.8±5.4 ^{***} | 5.9±0.9 [*] |
| | Exp. | 47.9±0.2 ^c | 8.5±0.6 ^b |

Note: SDS, SGF, SDF, FDF, and FDS are the same abbreviations as shown in Table 1. Con: control, Exp: experimental fish, Hb: hemoglobin, Ht: hematocrit, Values are the mean ± SD (n = 3). Different letters in each experiment indicate significant differences within the groups of SW fish shifted to FW and within the groups of FW fish shifted to SW (P<0.05, one-way ANOVA). Asterisks indicate significant differences between Con. and Exp. in each experiment (*: P<0.05, **: P<0.01, ***: P<0.001, t-test)

Table 8. Biochemical properties of blood plasma in grey mullets *Mugil cephalus* at the end of experiments

| Group | Osmoregulation factor | | | | | | | | | | Stress factor | | |
|-------|----------------------------|---------------------------|----------------------------|-------------------------|-----------------------|-------------------------|-------------------------|-------------------------|-------------------------|----------------------|---------------|--|--|
| | 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) | | | | |
| SDS | Con. | 158.5±6.4 | 2.9±0.1 | 151.5±9.2 | 10.9±0.7 | 1.5±0.1 [*] | 331.5±23.3 | 5.5±2.3 ^{**} | 23.0±7.1 | 3.2±0.6 | | | |
| | Exp. | 163.5±6.4 ^b | 2.5±0.3 ^a | 155.5±7.8 ^c | 12.6±0.6 ^b | 2.6±0.1 ^b | 302.0±14.1 ^a | 20.6±5.4 ^a | 65.0±18.4 ^{ab} | 2.8±0.5 ^a | | | |
| SGF | Con. | 145.0±2.8 | 4.4±1.1 | 141.0±1.4 | 8.8±0.1 | 1.0±0.1 | 368.0±12.7 | 5.3±3.9 [°] | 36.5±0.7 | 2.7±0.2 | | | |
| | Exp. | 141.0±2.8 ^a | 2.5±0.5 ^a | 144.0±5.7 ^c | 9.6±0.4 ^a | 1.3±0.1 ^a | 323.5±17.7 ^a | 154.6±44.6 ^b | 42.0±2.8 ^a | 2.6±0.4 ^a | | | |
| SDF | Con. | 138.0±5.7 | 4.3±0.2 | 139.5±2.1 | 9.2±0.6 | 1.2±0.1 | 332.5±67.2 | 4.2±0.1 ^{**} | 34.5±2.1 [°] | 2.3±0.0 | | | |
| | Exp. | 134.5±4.9 ^a | 4.1±1.1 ^a | 124.0±4.2 ^a | 8.4±0.1 ^a | 3.2±0.8 ^b | 311.5±9.2 ^a | 316.2±21.1 ^c | 62.0±4.2 ^b | 2.7±0.6 ^a | | | |
| FDF | Con. | 142.5±17.7 | 3.1±0.8 | 129.0±4.2 | 8.3±0.5 | 1.2±0.0 [°] | 299.5±9.2 | 3.8±2.1 ^{**} | 31.0±2.8 | 2.6±0.0 | | | |
| | Exp. | 136.5±10.6 ^a | 2.5±0.3 ^a | 152.0±17.0 ^a | 9.4±0.8 ^b | 1.7±0.1 ^a | 314.5±40.3 ^a | 40.2±21.1 ^b | 46.5±4.9 ^{ab} | 2.7±0.2 ^a | | | |
| FDS | Con. | 143.0±5.7 | 2.3±0.3 | 138.5±9.2 | 9.1±0.4 [*] | 1.3±0.1 | 371.0±28.3 | 0.9±0.8 [°] | 34.0±0.0 | 2.3±0.6 | | | |
| | Exp. | 162.0±11.3 ^b | 3.4±0.2 ^a | 158.0±11.3 ^a | 12.0±0.4 ^c | 1.8±0.4 ^a | 379.0±8.5 ^a | 10.3±1.3 ^a | 31.5±10.6 ^a | 3.6±0.1 ^b | | | |

Note: SDS, SGF, SDF, FDF, and FDS are the same abbreviations as shown in Table 1. Con: control, Exp: experimental fish. Values are the mean ± SD (n = 3). Different letters in each experiment indicate significant differences within the groups of SW fish shifted to FW and FW fish shifted to SW (P < 0.05, one-way ANOVA). Asterisks indicate significant differences between Con. and Exp. in each experiment, respectively (*: P < 0.05, **: P < 0.01, ***: P < 0.001, t-test)

while those increased from 152.0 mEq/L in FDF to 158.0 mEq/L in FDS. Furthermore, Ca values decreased from 12.6 mg/dL in SDS to 8.4 mg/dL in SDF. Those increased from 9.4 mg/dL in FDF to 12.0 mg/dL in FDS. Other factors such as K⁺ and osmolality were not significantly different. The highest value of Mg was found at 3.2 mg/dL in SDF, and the lowest value was found at 1.3 mg/dL in SGF (P<0.05) (Table 8).

Cortisol levels at the end of experiments increased with the lowering salinities in SW fish shifted to FW showing 20.6 ng/mL in SDS to 316.2 ng/mL in SDF, while those decreased with the increasing salinities in FW fish shifted to SW showing 40.2 ng/mL in FDF to 10.3 ng/mL in FDS. However, the glucose levels among all the experimental groups were not significantly different, while total protein values were different only in the groups of FW fish to SW with the highest value of 3.6 g/dL in FDS, while the lowest value was 2.7 g/dL in FDF (P<0.05).

The results relating to the blood properties showed that salinity changes affected to the oxygen binding factors, and its clearly related to the OC rate. Changes in Ht and Hb according to salinity found somewhat similarity in Ht from other study (Chang & Hur, 1999; Chen *et al.*, 1995; Houston & Rupert, 1976). Ht and Hb values from this study were also compared to grey mullets (30.5 ± 2.9% and 8.0 ± 1.5 g/dL) which was reported by Min *et al.* (2010). Some experimental groups had somewhat similar in Ht and Hb factors, but some experimental groups had lower results. Changes in blood factors caused by salinity on fish *Channa punctatus* was also reported by Dheer *et al.* (1986).

The results of biochemical properties of grey mullets' blood plasma also indicated that salinity changes in grey mullets clearly affected their blood factors. Some ion contents on grey mullets in FW environments were lower amount than that of grey

mullets in SW. Almost 77% of the salts in blood were sodium and chloride. The remainder was made up primarily of bicarbonate, potassium, and calcium. Sodium (Na⁺), potassium (K⁺), and calcium (Ca) salts were critical for the normal function of heart, nerve, and muscle tissue (Wurts, 1998).

Salinity changes from SW to BW or FW which was lower amount of ions than that of SW caused the grey mullets became weaker and lower in their metabolism than before. Khodabandeh *et al.* (2009) observed golden grey mullets and concluded that the main ion content such as Na⁺, Cl⁻, and K⁺ were lower in line with the decreasing salinity. This result could be the main reason of the difference behavior in activity and OC according to salinity changes. To inhabit at low salinity or freshwater, fish need to replace salts lost through diffusion to the water and eliminate excess water absorbed from the environment (Wurts, 1998).

The results of OC and blood properties also showed relationship between OC and other blood properties parameters such as osmolality, cortisol, and glucose. The correlation can be found between OC and cortisol, while the osmolality and glucose were not clearly correlated to OC since the values were not significantly different (Table 9).

Salinity changes from SW to FW tended to decrease the OC of grey mullet and increased the cortisol levels inside the blood. However, salinity changes from FW to SW tended to increase the OC and decreased the cortisol levels. These results suggested that stress factors clearly affected to OC of grey mullets. At the stress conditions by salinity changes, these salinity differences result in modifications in OC and energy demands (Morgan & Iwama, 1991). Cortisol was assigned to stimulate the liver gluconeogenesis and elevating blood sugar levels (Wedemeyer *et al.*, 1990), which was related to the

Table 9. OC, osmolality, cortisol, and glucose levels of grey mullets *Mugil cephalus* in each experiment

| Group | OC (mg O ₂ /kg/h) | Osmolality (mOsm/kg) | Cortisol (ng/dL) | Glucose (mg/dL) |
|-------|---------------------------------|---------------------------|---------------------------|---------------------------|
| SDS | 194.5 ± 19.1 ^c | 302.0 ± 14.1 ^a | 20.6 ± 5.4 ^a | 65.0 ± 18.4 ^{ab} |
| SGF | 164.6 ± 17.2 ^b | 323.5 ± 17.7 ^a | 154.6 ± 44.6 ^b | 42.0 ± 2.8 ^a |
| SDF | 95.5 ± 37.1 ^a | 311.5 ± 9.2 ^a | 316.2 ± 21.1 ^c | 62.0 ± 4.2 ^b |
| FDF | 167.9 ± 22.2 ^a | 314.5 ± 40.3 ^a | 40.2 ± 21.1 ^b | 46.5 ± 4.9 ^{ab} |
| FDS | 184.0 ± 32.0 ^b | 379.0 ± 8.5 ^a | 10.3 ± 1.3 ^a | 31.5 ± 10.6 ^a |

Note: SDS, SGF, SDF, FDF, and FDS are the same abbreviations as in Table 1. Letters indicate significant differences between each experiment (P<0.05, one-way ANOVA).

fish osmoregulation ability. In other results, grey mullets exposed in low salinity did not change the osmolality levels, indicating that grey mullets was a good osmoregulator species. These results were similar with gulf killifish (Boily *et al.*, 2007) and genus *Fundulus* (Griffith, 1974).

CONCLUSION

Salinity clearly affected the oxygen consumption and blood properties of grey mullets *Mugil cephalus*. Lowering salinity from seawater to freshwater decreased the oxygen consumption and causing mortality if the grey mullets transferred directly from seawater to freshwater without gradual salinity changes. Meanwhile, salinity change from freshwater to seawater increased the oxygen consumption of grey mullets. Furthermore, lowering salinity tend to increase the stress level of grey mullets. It mainly identified by the increase of cortisol levels during low salinity environment. Meanwhile, the stress level of grey mullets decreased with the increase of salinity. According to the information from this study, grey mullet rearing in freshwater might be applied for aquaculture development due to its osmotic adaptation ability.

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