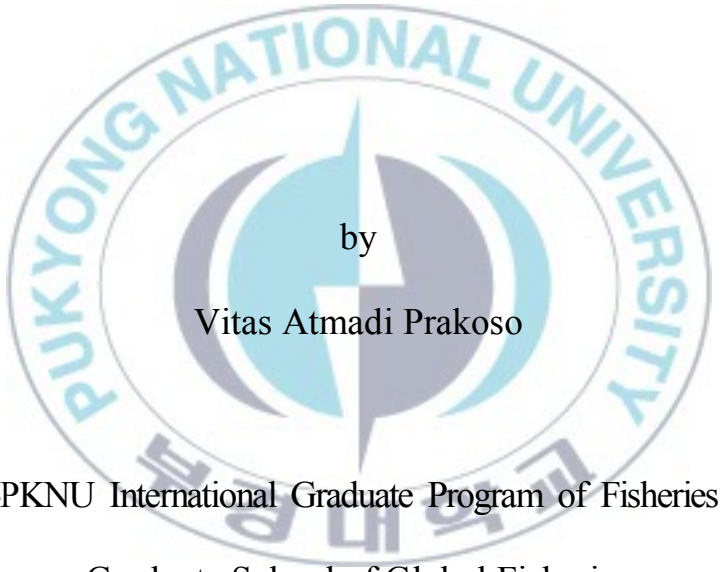
Thesis for the Degree of Master of Fisheries Science

Effects of Salinity and Temperature on Oxygen Consumption and Blood Properties of Young Grey Mullets *Mugil cephalus*



by

Vitas Atmadi Prakoso

KOICA-PKNU International Graduate Program of Fisheries Science

Graduate School of Global Fisheries

Pukyong National University

February 2014

Effects of Salinity and Temperature on Oxygen Consumption and Blood Properties of Young Grey Mullets *Mugil cephalus*

어린 숭어의 산소소비 및 혈액성상에 미치는 염분과 온도의 영향

Advisor: Prof. Young Jin Chang

by

Vitas Atmadi Prakoso

A thesis submitted in partial fulfilment of the requirements for the degree of

Master of Fisheries Science

in KOICA-PKNU International Graduate Program of Fisheries Science

Graduate School of Global Fisheries

Pukyong National University

February, 2014

Effects of Salinity and Temperature on Oxygen Consumption and Blood Properties of Young Grey Mullets *Mugil cephalus*

A dissertation

by

Vitas Atmadi Prakoso

Approved by :

(Chairman) Prof. Chang-Hoon Kim

(Member) Dr. Byung Hwa Min

(Member) Prof. Young Jin Chang

February 21, 2014

Contents

List of Figures

List of Tables

Effects of Salinity and Temperature on Oxygen Consumption and Blood Properties of Young Grey Mullets *Mugil cephalus*

Vitas Atmadi Prakoso

KOICA-PKNU International Graduate Program of Fisheries Science

Graduate School of Global Fisheries

Pukyong National University

Abstract

The effects of salinity and temperature on oxygen consumption (OC) and blood properties of grey mullets *Mugil cephalus* (TL: 27.3±2.1 cm, BW: 187.9±45.8 g) were studied by using respiratory chamber.

Seven experimental groups were conducted to measure OC according to salinity (30→15→0 psu, 0→15→30 psu) and temperature changes (15→20→25°C), and two groups were conducted to measure the lethal dissolved oxygen (DO); SDS: fish reared in seawater (SW, 30 psu) directly shifted to SW, SDB: SW fish directly shifted to brackish water (BW, 15 psu), SGF: SW fish gradually shifted to freshwater (FW, 0 psu), SDF: SW fish directly shifted to FW, FDF: FW fish directly shifted to FW, FDB: FW fish directly shifted to BW, FDS: FW fish directly shifted to SW, LOS: lethal DO in SW, and LOF: lethal DO in FW.

OC was tended to decrease in the groups of SW fish shifted to FW showing 194.5 mg O_2/kg/h at 25°C in SDS to 82.4 mg O_2/kg/h at 15°C in SGF. On the contrary, OC was increased in the groups of FW fish shifted to SW showing 80.5 mg O_2/kg/h at 15°C in FDB to 184.0 mg O_2/kg/h at 25°C in FDS. Likely, hematocrit (Ht) and hemoglobin (Hb) were shown with the highest values (30.6% and 7.4 g/dL) in SDS and SDB, while those were shown with the lowest values (16.2% and 4.2 g/dL) in SDF. However, Ht and Hb were shown with the lowest values (26.8% and 5.8 g/dL) in FDF, while those were shown with the highest values (47.9% and 8.5 g/dL) 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.

Lethal DO in SW and FW showed the same DO of 0.3 mg/L in both environments. The blood factors including Ht, Hb, osmolality, cortisol, and glucose in LOS were totally higher than those in LOF.

In conclusion, salinity and temperature clearly affected the OC, behavior, and blood property of grey mullets. Grey mullet must be an excellent osmoregulator species which can be applied for increasing aquaculture productivity by culturing this species in freshwater.

Introduction

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. 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 OC of fish had been constantly studied since the research started by Gardner and King (1922**).** Furthermore**,** other researches were established to get information about the relation between temperature and OC of fish (Wells, 1935; Fry and Hart, 1948). Another study was also found that OC is variable and depends on many factors (Chadwick et al., 2010). Because of the importance of OC study for aquaculture, the researches on OC were continuously developed, not only on freshwater species but also marine species (Kim et al., 1995; Lyytikainen and Jobling, 1998; Jeong et al., 2007).

Many authors investigate about the effects of various factors on the OC of fish, such as : temperature (Franklin et al., 1995; Wares and 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 and Olafsdottir, 2006; Duan et al., 2011).

Grey mullet *Mugil cephalus* (Fig. 1) is distributed around the world, mainly in tropical and subtropical countries. Most mullets are found in coastal marine and brackish waters, but they are also euryhaline and move between marine and freshwater environments of rivers and flooded rice fields (Saleh, 2008). Grey mullets are truly cosmopolitan and considered as a commercial important food (Oren, 1981). Grey mullet is a very important aquaculture species in the Mediterranean, Southeast Asia, Taiwan, Japan and Hawaii (Saleh, 2008). Grey mullets also have a good market in some countries including Korea.

Because of their wide salinity tolerance, the freshwater aquaculture for grey mullets is now developing. According to those facts, basic information about OC and blood properties of grey mullets is needed and could be beneficial for development of grey mullets' freshwater aquaculture. The aims of this study were to evaluate the effects of salinity and temperature on OC and blood properties of grey mullets.

Fig. 1. The external shape of grey mullet *Mugil cephalus*.

Materials and Methods

Experimental design

Thirty one grey mullets *Mugil cephalus* (TL: 27.3±2.1 cm, BW: 187.9 ±45.8 g) were used for the experiments. Before the experiments, grey mullets were divided and acclimated into 2 different rearing environments, which were seawater (SW) and freshwater (FW). The fish were reared in recirculating tanks and fed 2 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.

As shown in Table 1, seven experimental groups (SDS, SDB, SGF, SDF, FDF, FDB, and FDS) were conducted in the OC measurements and blood properties of the experimental fish. These seven experiments were divided into 2 groups, the groups of SW fish shifted to FW (SDS, SDB, SGF, and SDF), and the groups of FW fish shifted to SW (FDF, FDB, and FDS). The experiments were conducted to measure the OC according to salinity changes, which was from 0 psu (FW), 15 psu (BW), and to 30 psu (SW), and water temperature (15^{o}C, 20^{o}C, and 25^{o}C). Lethal DO of grey mullets (LOS and LOF) was also measured in seawater and freshwater (Table 1).

Table 1. Experimental conditions in OC and lethal DO measurement

Exp.	Water temp. 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
SDB	15→20→25	30→15	29.6 ± 1.8	234.0 ± 31.2	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
FDB	15→20→25	0→15	27.1 ± 0.5	181.3 ± 18.0	3
FDS	15→20→25	0→30	29.0 ± 0.9	221.3 ± 20.6	3
LOS	18	30	27.7 ± 2.7	182.7 ± 42.8	5
LOF	18	0	27.4 ± 1.1	199.0 ± 28.5	5

SDS : fish reared in SW directly shifted to SW, SDB : fish reared in SW directly shifted to BW, SGF : fish reared in SW gradually shifted to FW, SDF : fish reared in SW directly shifted to FW, FDF : fish reared in FW directly shifted to FW, FDB : fish reared in FW directly shifted to BW, FDS : fish reared in FW directly shifted to SW, LOS : lethal DO of fish reared in SW, LOF : lethal DO of fish reared in FW.

Measurement of oxygen consumption

The experimental fish were acclimated into the respiratory chamber for several hours before running the experiment in order to stabilize the metabolic rate. OC rate were measured by using closed recirculating system with a respiratory chamber (Fig. 2). The experimental fish were exposed to 12 hours light (07:00 - 19:00) : 12 hours dark (19:00 - 07:00) periods.

The water temperature in each experiment was controlled using a circulating water bath (JS-WBP-170P, Johnsam, Bucheon, Korea). A closed recirculating system with a respiratory chamber was used to measure OC (Fig. 2). The respiratory chamber (RC, dimension = 20 × 30 × 20 cm) was made from transparent PVC with 10 mm thickness. The water volume and a flow rate were kept constant in each experiment.

In water reservoir 1 (WR 1), a constant water flow (solid arrow) was maintained by controlling the circulating water which overflowed (open arrow) upon reaching a certain height, thereby maintaining a constant volume of water flow in the respiratory chamber.

The outflow water from the respiratory chamber went to water reservoir II (WR II), which was fully aerated to 100% saturation with air. In the water bath (WB) and filtering unit (FU), temperature was controlled and the water

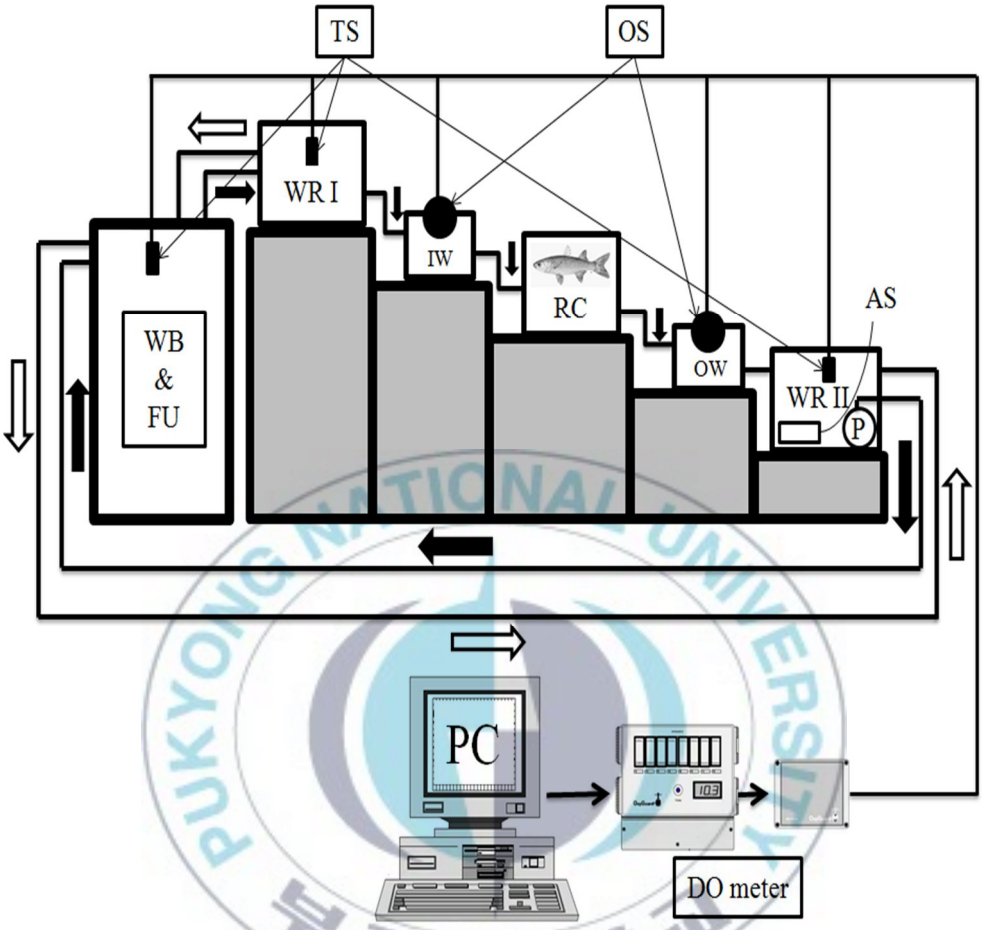


Fig. 2. Diagram of OC measurement system. Solid and open arrows indicate circulating and overflowing water, respectively. AS : air supply, FU : filtering unit, IW : inflow water, OS : oxygen sensor, OW : outflow water, P : pump, PC : personal computer, RC : respiratory chamber, TS : temperature sensor, WB : water bath for temperature control, WR I and II : water reservoir I and II.

was cleaned before reentering the respiratory chamber. DO of inflow water (IW) was maintained above 7.0 mg/L in each experiment, and each oxygen sensor (OS) automatically measured the DO of the inflow water (IW) and outflow water (OW) every 10 minutes by using a multichannel monitoring system for DO and other parameters (Oxyguard 6, Oxyguard International A/S, Birkerd, Denmark). The data were logged into a personal computer (PC). Water temperature inside the respiratory chamber was increased slowly from 15^{o}C to the target temperature at a rate of 0.5^{o}C/h to minimize any thermal shock to the fish. In all experimental groups, corrections for any bacterial consumption were unnecessary as there was a negligible change in oxygen concentration over the hour.

Breath frequency was counted using opercular cover movements (Wares and Igram, 1979). The opercular cover movements were counted for 1 minute interval and expressed as the average from 10 records for each fish. The OC was calculated by using formula: $OC = (DO_{in} - DO_{out}) \times F/W$, where OC is the oxygen consumption expressed in miligrams of oxygen per hour per kilogram of fish (mg O_2/kg/h). DO_{in} and DO_{out} are the dissolved oxygen (mg O_2/L) in the respective water of the inflow or outflow. F is the water flow (L/min). W is the fish weight (kg).

Measurement of lethal dissolved oxygen

In order to determine lethal DO, closed rectangular chamber with 14 L volume and magnetic stirrer were used for mixing the experimental water (Fig. 3). Five grey mullets were put into closed rectangular chamber and observed their behavioral response due to depletion of DO inside the chamber and time when the fish dying was observed. The oxygen sensor in chamber was connected to personal computer to collect the DO data inside the chamber every 10 minutes, and water temperature inside the rectangular chamber was kept constant at 18^{o}C. In both experiments, corrections for any bacterial consumption were unnecessary as there was a negligible change in oxygen concentration over the hour.

During lethal DO experiments, the OC of fish was also measured. The OC was calculated by using formula: $OC = (DO_0 - DO_t) \times (V / (t \times W))$, where OC is the oxygen consumption expressed in miligrams of oxygen per hour per kilogram of fish (mg O_2/kg/h). DO_0 and DO_t are dissolved oxygen of initial and after t hours (mg O_2/L). While t is time passed for fish which consumed oxygen (h**).** V is the water volume of respiratory chamber (L), and W is the fish weight (kg).

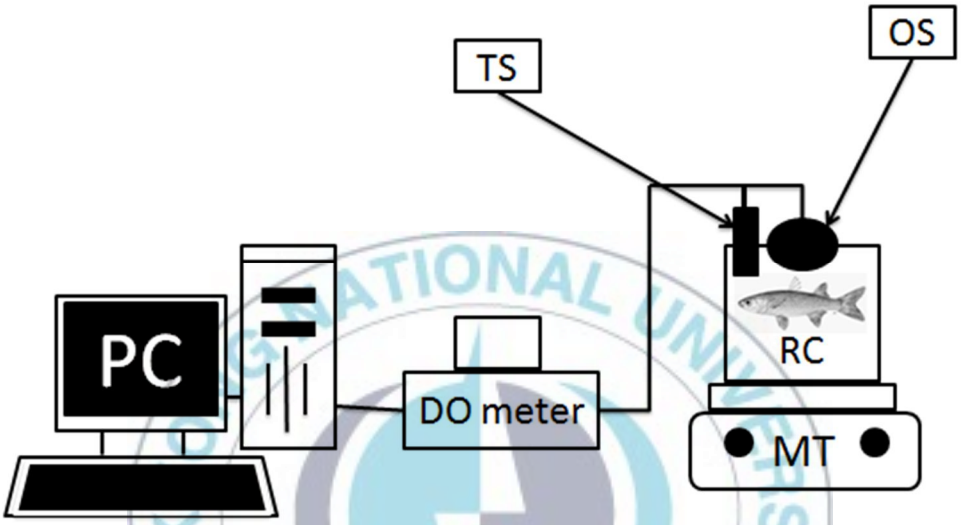


Fig. 3. Diagram of lethal DO measurement system. MT : magnetic stirrer, OS : oxygen sensor, PC : personal computer, RC : respiratory chamber, TS : temperature sensor.

Fish behavior

In addition to measuring the OC and lethal DO, 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).

Blood analysis

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),

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

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 Immonoassay (EIA) using Cortisol EIA kit (Oxford Biomedical Research, USA). Plasma osmolality was examined with Vapor Pressure Osmometer (Vapro 5520; Wescor Co., USA).

Statistical analysis

Mean ± SD were calculated, and all statistical analysis were analyzed by one-way ANOVA, two-way ANOVA, and *t*-test using PASW Statistics 18 software.

Results

Oxygen consumption according to salinity and temperature

1. Oxygen consumption

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 Figs. 4 and 5, the changes in OC with water temperature for each experiment showed a linear increase in line with water temperature. The OC of grey mullets which determined every hour clearly showed various type of OC fluctuations. Sudden increase of OC occured 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 (Fig. 4). Same patterns with SDS were also found in SDF and SGF. However, SDB showed slightly different result, the grey mullets were changed to consume slightly higher amount of oxygen in dark period at 20°C and 25°C after they consumed higher amount of

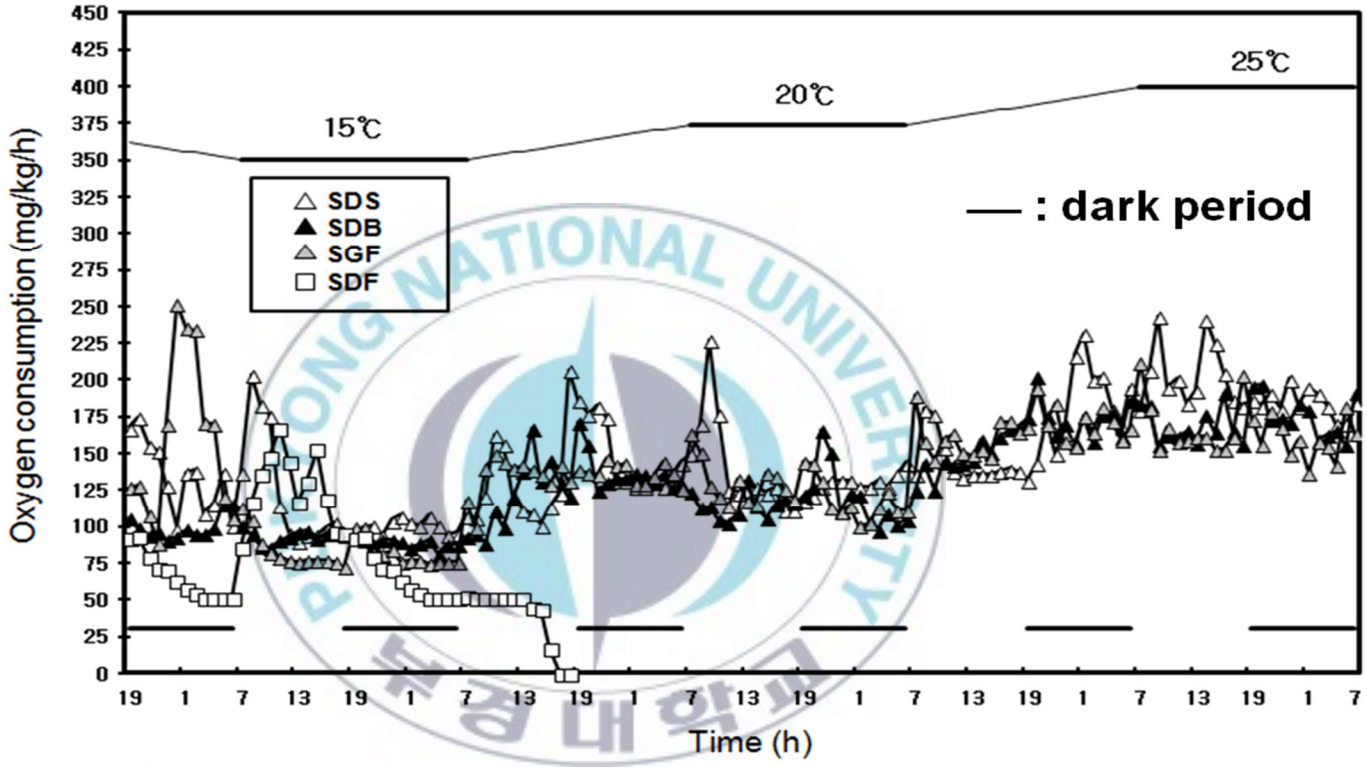


Fig. 4. OC of grey mullets *Mugil cephalus* by water temperature in the groups of SW fish shifted to FW. SDS, SDB, SGF, and SDF are the same abbreviations as shown in Table 1.

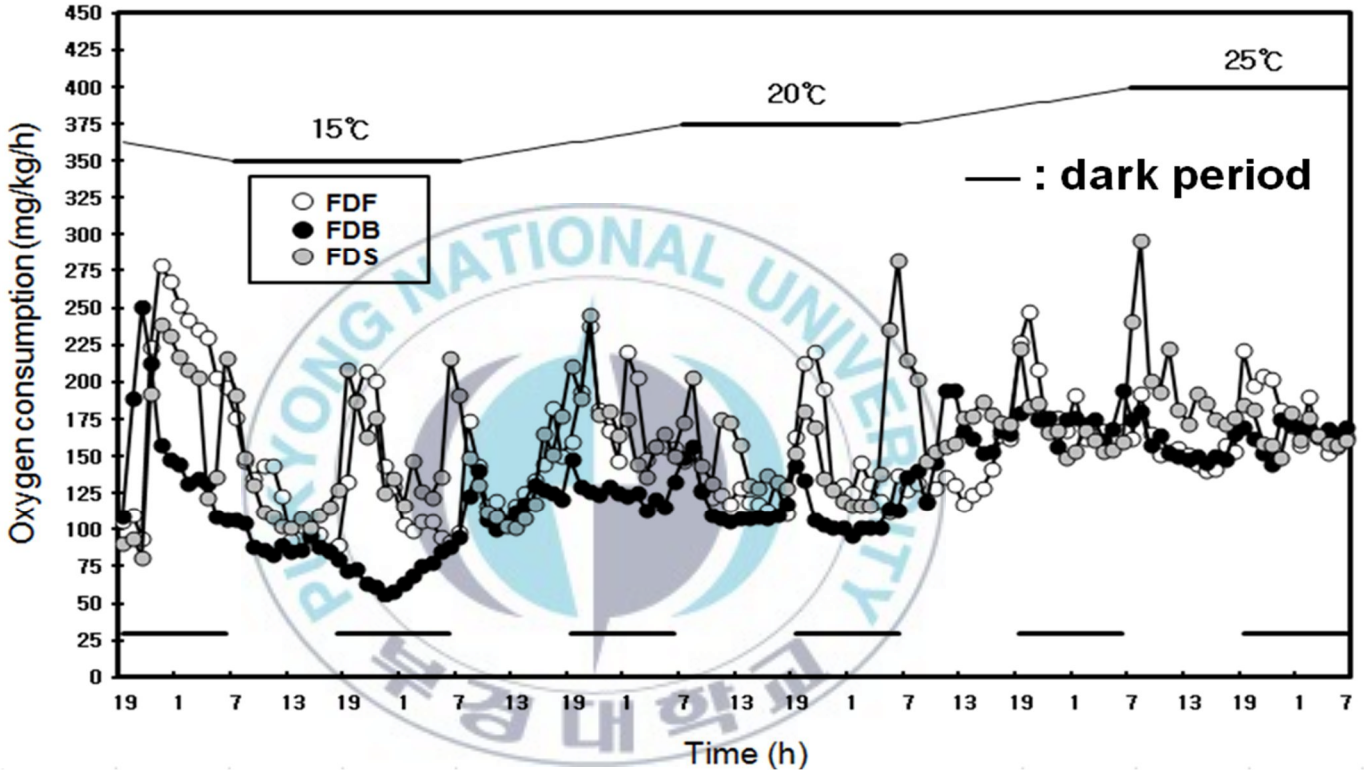


Fig. 5. OC of grey mullets *Mugil cephalus* by water temperature in the groups of FW fish shifted to SW. FDF, FDB, and FDS are the same abbreviations as shown in Table 1.

oxygen in light period at 15°C, while grey mullets in FDF showed a reversed day/night OC rhythm and consumed less oxygen during the day period.

The behavioral change was also occured in FDB and FDS. In FDB, the grey mullets shifted to consume oxygen higher in dark period at 25°C after previously consumed oxygen higher in light period during 15 and 20°C, while on FDS the grey mullets shifted to consume oxygen higher in light period start from 20°C.

In the groups of SW fish shifted to FW, grey mullets consumed 112.4, 136.0, and 194.5 mg O_2/kg/h at 15, 20, 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 SDB, the grey mullets consumed 90.9, 116.8, and 172.1 mg O_2/kg/h at 15, 20, and 25°C, respectively, which was also significant different at each temperature (P<0.05). In SGF, grey mullets consumed 82.4, 124.1, 164.6 mg O_2/kg/h at 15, 20, and 25°C, respectively (P<0.05). However, grey mullets consumed 95.5 mg O_2/kg/h at 15°C in SDF. The OC at 20°C and 25°C can not be measured due to the death of fish during experiment which was caused by the effect of sudden salinity changes from SW to FW (Table 3).

Table 3. Average OC (mg O_2/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
SDB	90.9 ± 4.4^{a}*	116.8 ± 15.5^{b}*	172.1 ± 13.3^{c}*	8.12	-35.84	0.854
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
FDB	80.5 ± 13.6^{a}*	114.4 ± 16.1^{b}*	161.5 ± 10.8^{c}*	8.12	-43.26	0.853
FDS	139.2 ± 34.4^{a}**	155.0 ± 40.4^{a}***	184.0 ± 32.0^{b}**	4.48	69.78	0.213

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).

In the groups of FW fish shifted to SW, grey mullets consumed 126.8, 139.1, and 167.9 mg O_2/kg/h at 15, 20, and 25°C, respectively in FDF, showing an increase in OC with water temperature. The OC was significantly different only at 25°C (P<0.05). In FDB, the grey mullets consumed 80.5, 114.4, 161.5 mg O_2/kg/h at 15, 20, and 25°C respectively, while the grey mullets in FDS consumed 139.2, 155.0, 184.0 mg O_2/kg/h at 15, 20, and 25°C, respectively. No significant differences were found between 15°C and 20°C at FDS. Furthermore, OC was tended to decrease in the groups of SW fish shifted to FW showing the highest value of 194.5 mg O_2/kg/h at 25°C in SDS and the lowest value of 82.4 mg O_2/kg/h at 15°C in SGF. On the contrary, OC was increased in the groups of FW fish shifted to SW showing the lowest value of 80.5 mg O_2/kg/h at 15°C in FDB and the highest value of 184.0 mg O_2/kg/h at 25°C in FDS.

As shown in Table 4, 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 (P<0.05), while the interaction between the effects of salinity and temperature within the groups of SW fish shifted to FW was not significant.

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

Group	Temp.	Salinity	Temp. x salinity
SW fish shifted to FW	<0.001	<0.001	0.064
FW fish shifted to SW	<0.001	<0.001	0.006

Temp: temperature

The fish in each experiment showed various pattern on OC according to salinity and temperature 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% than light period at 15, 20, and 25°C, respectively. Same pattern was seen in SGF and SDF. In SGF, the average of OC during the dark period was 97.8, 90.9, and 95.0% than light period at 15, 20, and 25°C, respectively. While in SDF, the average of OC during the dark period than light period was 51.2% only at 15°C, there was no record of OC at 20 and 25°C due to the death of fish. However, in SDB, the average of OC during the dark period was 94.8, 104.9, and 104.4% than light period at 15, 20, and 25°C, respectively. Which means in SDB at 20 and 25°C grey mullets consumed higher amount of oxygen at dark period than light period (P<0.05).

In the groups of FW fish shifted to SW, another different pattern was found on FDF, the average of OC during the dark period on FDF was 111.9, 120.6, and 115.7% than light period at 15, 20, and 25°C, respectively. Which means the OC pattern was all higher in dark period than light period. In FDB, the average of OC during the dark period was 78.2, 93.2, and 105.0% than light period at 15, 20, and 25°C, respectively, while in FDS the

average of OC during the dark period was 127.0, 104.1, and 82.7% than light period at 15, 20, and 25°C, respectively. The change of tendency on OC due to salinity changes during light and dark period was also found in this experiment (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 the groups of FW fish shifted to SW. Same pattern was also occured 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 the groups of FW fish shifted to SW, except for FDB. These conditions indicated that increment of OC of grey mullets were faster in the groups of SW fish shifted to FW (Table 5).

2. Breath frequency and oxygen consumption per breath

The breath frequency of grey mullets from whole experiments at 15, 20, and 25°C were shown in Figs. 6 and 7. 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, SDB, and SGF was 3.07, 2.96, and 1.81, respectively, while the slope of linear regression of breath frequency according to different water temperature in

Table 5. Average OC (mg O_2/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
SDB	L	93.3 ± 4.3[a]	114.0 ± 8.2[b]	168.4 ± 12.0[c]	7.51	-24.97	0.876
	D	88.5 ± 3.0[a]	119.6 ± 20.4[b]	175.8 ± 14.1[c]	8.73	-46.70	0.849
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
FDB	L	90.3 ± 8.4[a]	118.4 ± 17.1[b]	157.5 ± 11.5[c]	6.72	-12.39	0.827
	D	70.6 ± 10.3[a]*	110.3 ± 14.5[b]*	165.4 ± 8.7[c]	9.48	-74.13	0.918
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

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).

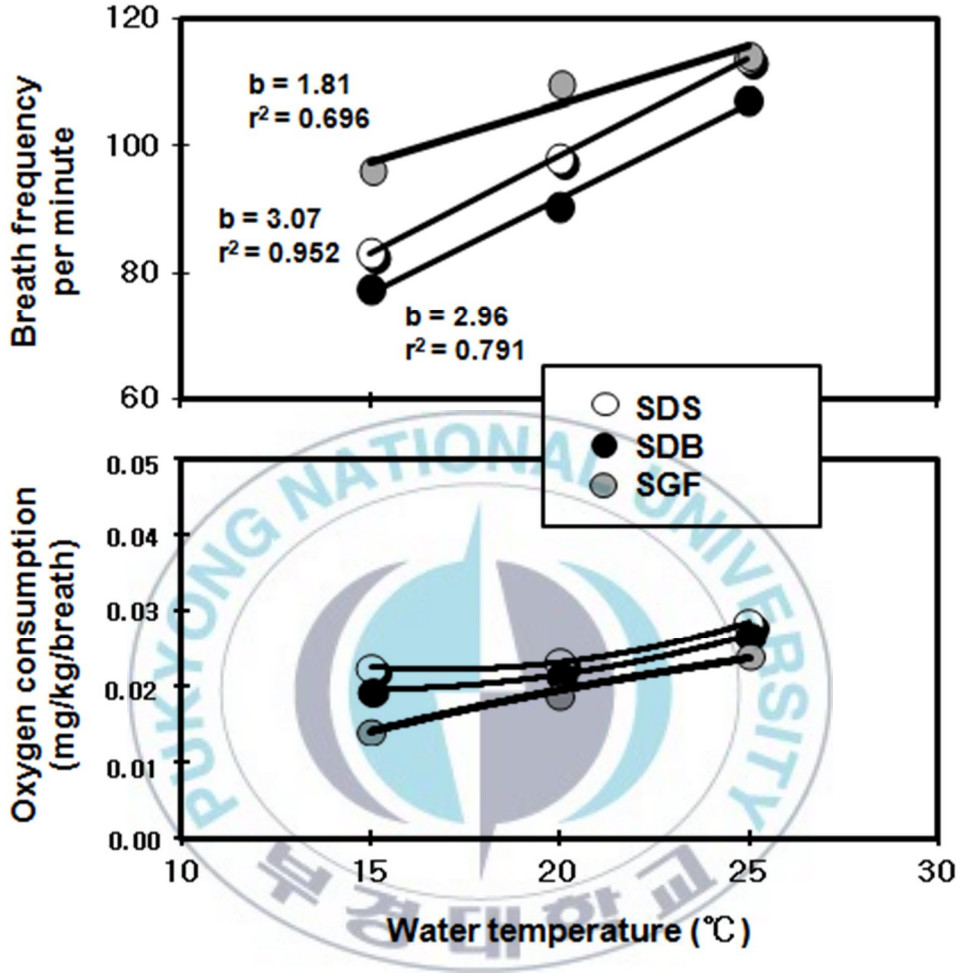


Fig. 6. Breath frequency per minute and OC per breath in grey mullets *Mugil cephalus* from the groups of SW fish shifted to FW. All abbreviations are the same as shown in Table 1.

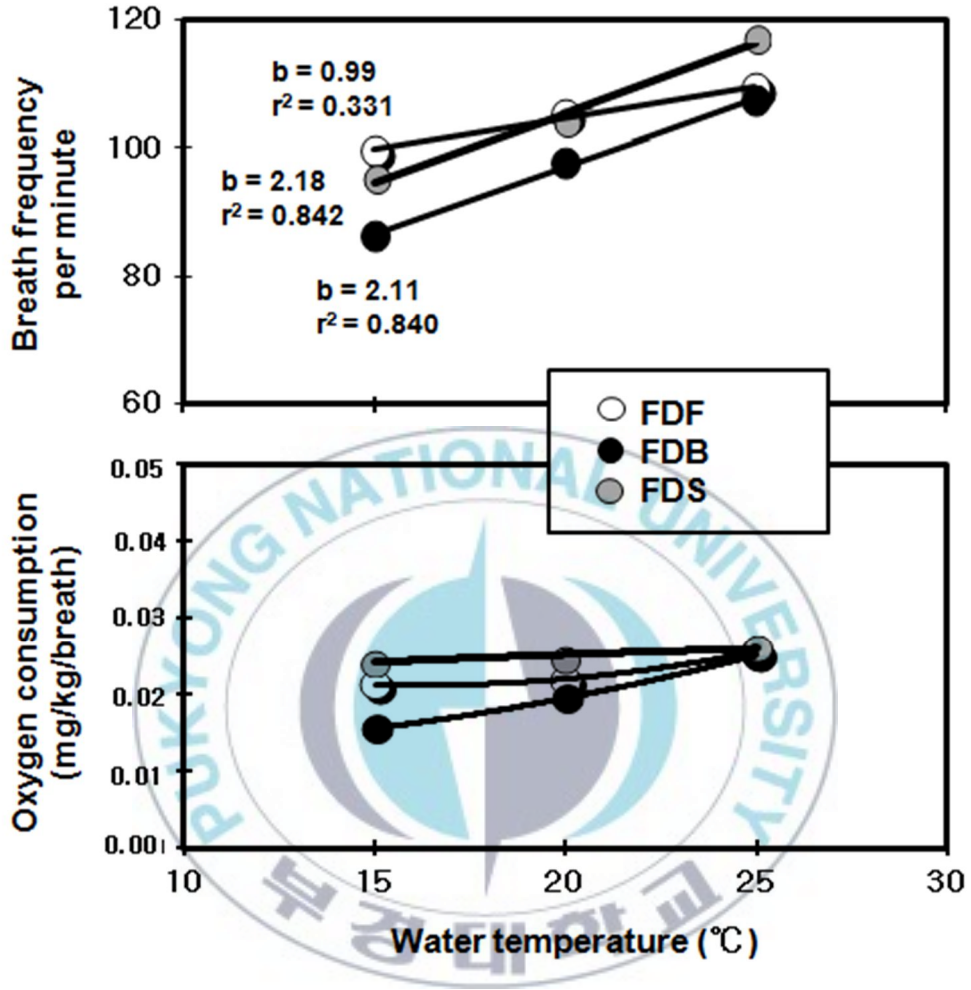


Fig. 7. Breath frequency per minute and OC per breath in grey mullets *Mugil cephalus* from the groups of FW fish shifted to SW. All abbreviations are the same as shown in Table 1.

grey mullets from the groups of FW fish shifted to SW which consisting of FDF, FDB, and FDS was 0.99, 2.11, and 2.18, respectively. These values were indicating the breath frequency rapidly increasing in grey mullets in the groups of SW fish shifted to FW than the grey mullets in the groups of FW fish shifted to SW.

The OC per breath in the groups of SW fish shifted to FW and the groups of FW fish shifted to SW were showing linear relationship with water temperature. The OC per breath in grey mullets from the groups of SW fish shifted to FW was higher than grey mullets from the groups of FW fish shifted to SW, but not significantly different.

3. Behavioral response

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 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
SDB	II	III	II	II	II
SGF	II	II	II	I	I
SDF	IV	V	VI	-	-
FDF	II	II	II	II	II
FDB	II	II	II	II	II
FDS	II	II	I	I	I

All abbreviations are the same as shown in Table 1.

4. Blood property

Table 7 showed that most of experimental groups revealed the significant differences in hematocrit (Ht) and hemoglobin (Hb) levels between the experimental fish and control, except for SDS (P<0.05). The salinity change from SW to FW was 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% in SDS and 7.4 g/dL in SDB, respectively. Whereas, Ht and Hb was tended to increase in the groups of FW fish shifted to SW. The highest value of Ht and Hb was 47.9% and 8.5 g/dL in FDS, respectively.

As shown in Table 8, 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 were also showed the same pattern. Cl^- values were decreased showing 155.5 mEq/L in SDS to 134.5 mEq/L in SDF, while those were increased from 152.0 mEq/L in FDF to 158.0 mEq/L in FDS. Furthermore, Ca values were found decreased from 12.6 mg/dL in SDS to 8.4 mg/dL in SDF. Those were found 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.

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

Group		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}
SDB	Con.	29.2 ± 3.9	7.1 ± 0.5
	Exp.	24.2 ± 7.3^{b}*	7.4 ± 0.6^{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}
FDB	Con.	16.7 ± 1.5***	4.4 ± 0.4**
	Exp.	31.1 ± 1.8^{b}	8.2 ± 1.3^{b}
FDS	Con.	22.8 ± 5.4***	5.9 ± 0.9*
	Exp.	47.9 ± 0.2^{c}	8.5 ± 0.6^{b}

SDS, SDB, SGF, SDF, FDF, FDB, 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 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)
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}
SDB	Con.	139.5 ± 3.5	3.5 ± 0.4	142.0 ± 4.2	9.5 ± 0.9	1.2 ± 0.1*	323.5 ± 2.1	0.4 ± 0.1***	35.0 ± 2.8*	2.8 ± 0.1
	Exp.	135.0 ± 2.8^{a}	3.1 ± 0.6^{a}	134.5 ± 0.7^{b}	8.1 ± 1.6^{a}	2.2 ± 0.2^{b}	303.5 ± 7.8^{a}	32.8 ± 1.5^{a}	47.5 ± 2.1^{a}	2.7 ± 0.1^{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}
FDB	Con.	163.0 ± 35.4	2.6 ± 0.6	162.5 ± 6.4	7.2 ± 0.6	1.1 ± 0.1**	357.5 ± 37.5	5.1 ± 0.3	21.5 ± 7.8*	1.8 ± 0.2*
	Exp.	154.0 ± 4.2^{b}	2.5 ± 0.6^{a}	145.5 ± 3.5^{a}	7.4 ± 0.5^{a}	2.5 ± 0.1^{b}	360.0 ± 0.0^{a}	17.7 ± 7.2^{a}	56.0 ± 8.5^{b}	3.0 ± 0.3^{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}

SDS, SDB, SGF, SDF, FDF, FDB, 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).

Mg value in FDB was significantly higher in the groups of FW fish shifted to SW with the highest value of 2.5 mg/dL in FDB and the lowest value of 1.1 mg/dL in FDF (P<0.05), while it was not significantly different in the groups of SW fish shifted to FW.

Cortisol levels at the end of experiments were 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, 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).

Lethal dissolved oxygen

1. Lethal dissolved oxygen and oxygen consumption

Lethal DO in SW and FW showed the same DO of 0.3 mg/L in both environments. The OC of grey mullets was decreased in line with the depletion of DO inside the chamber showing rapid changes from 313.7 mg

O_2/kg/h to 0 mg O_2/kg/h within 60 minutes in LOS, while the OC in LOF was decreased from 295.5 mg O_2/kg/h to 0 mg O_2/kg/h within 70 minutes. There was no significant difference between these lethal DO experiments (Fig. 8).

2. Breath frequency

Breath frequency in both experiments showed the relationship with the OC and depletion of DO. Those were decreased in line with the declining of OC. Breath frequency was decreased from 116 times per minute to 0 within 60 minutes in LOS, while it was decreased from 113 times per minute to 0 within 70 minutes in LOF. These results were indicating the grey mullets consumed more oxygen in SW environment.

3. Behavioral response

The behaviors of grey mullets during lethal DO were observed based on their swimming activity and breath frequency. The lethal DO was 0.3 mg/L for both experiments (LOS and LOF). In both experiments, the grey mullets showed normal behavior within 20 minutes, and then their behavior became unstable after DO levels were below 2 mg/L.

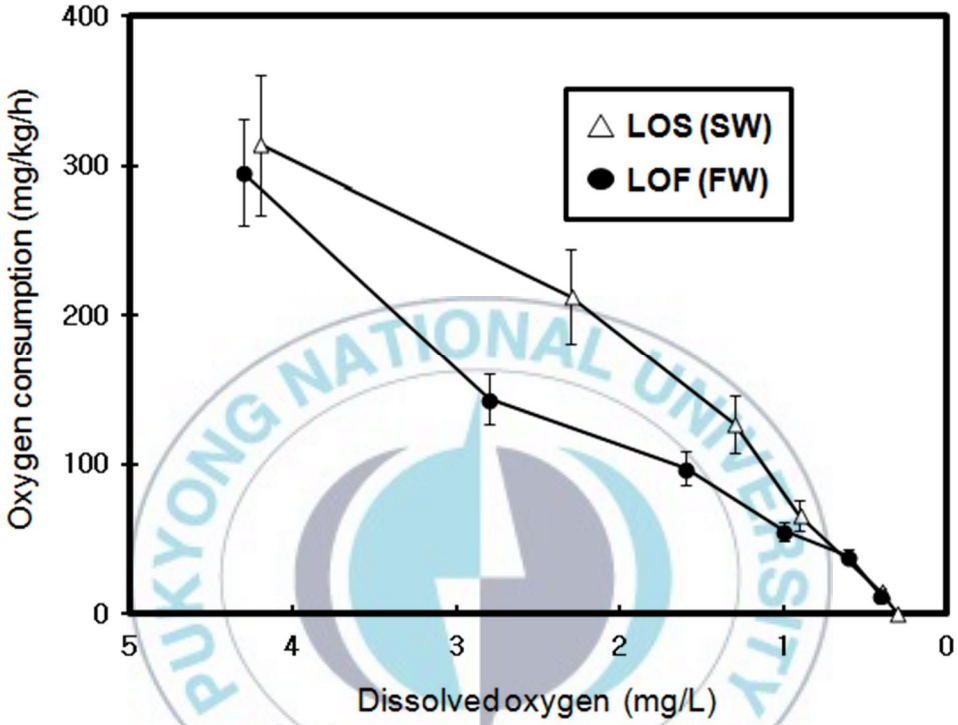


Fig. 8. Relationship between DO and OC of grey mullets reared in SW and FW due to oxygen depletion. LOS and LOF are the same abbreviations as shown in Table 1.

As the DO levels declined to 1 mg/L, their behavioral index was IV to VI, which means their activities became lower and totally died at 0.3 mg/L (Table 9). The grey mullets in LOS and LOF showed the same behavior under the low level of DO, showing unstable behavior, unsteady, hit the chamber, and slower opercular movement due to the stress condition. At the lethal level, the dying fish showing red spots in their bodies, change of fins color, and severe condition in opercular parts.

4. Blood property

As shown in Table 10, the results of blood properties investigation showed that most of parameter on grey mullets in LOS was significantly higher than LOF. The blood factors including Ht, Hb, Na^+, Cl^-, Ca, Mg, osmolality, cortisol, and glucose in LOS were significantly higher than those in LOF. However, the values of K^+ and total protein in LOS were not significantly different than LOF ($P<0.05$).

Table 9. Behavioral indices of fish with time course of lethal DO experiments

Time elapsed	LOS			LOF		
	DO (mg/L)	Behavioral index	Dead fish	DO (mg/L)	Behavioral index	Dead fish
1	7.6	I	0	8.1	I	0
10	4.2	II	0	4.3	II	0
20	2.3	III	0	2.8	II	0
30	1.3	IV	0	1.6	III	0
40	0.9	V	2	1.0	IV	2
50	0.4	V	3	0.6	V	2
60	0.3	VI	5	0.4	V	3
70	-	-	-	0.3	VI	5

LOS and LOF are the same abbreviations as shown in Table 1.

Table 10. Physio-chemical properties of grey mullets' blood before dying in lethal DO experiments

Component	Experiment	
	LOS	LOF
Hematocrit (%)	37.0 ± 6.1	20.7 ± 2.1*
Hemoglobin (g/dL)	7.4 ± 0.4	4.6 ± 0.1***
Na^+ (mEq/L)	181.0 ± 9.9	139.0 ± 2.8**
K^+ (mEq/L)	3.6 ± 0.5	3.6 ± 0.5
Cl^- (mEq/L)	162.5 ± 12.0	129.0 ± 4.2**
Ca (mg/dL)	13.3 ± 1.1	10.5 ± 0.0**
Mg (mg/dL)	4.9 ± 1.5	1.7 ± 0.1**
Osmolality (mOsm/kg)	455.0 ± 12.7	361.0 ± 50.9*
Cortisol (ng/dL)	90.7 ± 11.3	56.4 ± 28.5*
Glucose (mg/dL)	169.0 ± 39.6	71.5 ± 17.7**
Total protein (g/dL)	2.5 ± 0.2	2.6 ± 0.1

LOS and LOF are the same abbreviations as shown in Table 1. Values are the mean ± SD (n = 3). The mean values with different superscript are significantly different (* : $P<0.05$, ** : $P<0.01$, *** : $P<0.001$, *t*-test).

Discussion

In this study, OC of fish increased linearly with the temperature rise. The OC of fish reflected the activity of fish itself (Beamish and Mookherjii, 1964). This seems in this present study the activity of fish including the their breath frequency was increased by the rising of temperature. Generally the OC of fish increased directly within the optimal temperature, it was reported by Gardner and King (1922).

Various type of OC fluctuations occured during the experiments. Sudden increase of OC occured during the beginning of light period and dark period with the various amount in each experiment. These results were almost similar with Marais (1978) which reported that maximum OC of grey mullets *Mugil cephalus* was occured during the first 2 hours after sunrise and just after sunset. These results suggested that the grey mullets had high sensitivity according to the light changes, similar condition had reported in black porgy (Chang et al., 2005). The rate of OC of grey mullet in SDS increased during the day time and lower at night time, while SDF and SGF showed same pattern with SDS. It was similar like black porgy (Chang et al., 2005) and tilapia (Turker, 2011). The OC was related to the fish activity

level, it was reported from many fish species. In this study, OC was measured every hour. These experiments suggested that the fluctuation of OC was potentially the results of grey mullets activity during the light and dark period. Several studies on fish activity (Verheijen and De Groot, 1967; Gibson, 1973; Muller, 1978) showed that low activity is to be expected at night. However, interestingly grey mullets in FDF showed the opposite, their OC were lower at day time. This pattern was similar with *Paralichthys olivaceus* (Liu et al., 1997).

The grey mullets in SDS showed a clear diel rhythm in OC with higher values during the day and lower values at night. In these fish, the highest OC was mostly occured in the beginning of light period. This results suggested that the energy demand of grey mullets reared in seawater is elevated on active at the morning. However, the grey mullets in FDF showed higher values during the night time and lower values at day time. This opposite result suggested that the energy demand of grey mullets reared in freshwater was elevated on active at the night. Another pattern occured in some experimental groups, which was slightly different. The OC behavior was shifted from consumed lower amount at dark period to lower amount at light period or the vice versa. Lyytikainen and Jobling (1998)

concluded that daily variations in OC will have 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 was tended to decrease in the groups of SW fish shifted to FW, while on the contrary OC was increased in the groups of FW fish shifted to SW. Those conditions were clearly related with salinity changes. Those were suggested to be the stress response related to salinity. The study showed that salinity changes have effects on stress and immune-related parameters, which caused physiological disturbances and change photoperiodical activity and metabolism of fish. Furthermore, fish mortality can be occured by the abrupt salinity changes from seawater to freshwater. This results were different with Arnason et al. (2013) which observed on Atlantic cod *Gadus morhua* and reported that rearing salinity and abrupt salinity changes have limited or no effects on stress and immune-related parameters, and there are no indications of ion regulatory disturbances at low salinities. Morgan and Iwama (1991) suggested that low metabolic rates are most often associated to the water salinity, which was in species that commonly found and adapted at a particular life stage. This condition seems to be related with the natural environment of grey mullets. Grey mullets

occured in seawater as their natural habitat. The grey mullets *Mugil cephalus*, migrates to feed, moving from seawater to brackish as it grows from fry to adult (Kim et al, 2004).

At this present study, the results of blood properties indicated that salinity and temperature affected to the blood factors of grey mullets, especially salinity. Hematocrit (Ht) and hemoglobin (Hb) were decreased in the groups of SW fish to FW, while those were increased in the groups of FW fish to SW. These results were shown that salinity changes affected to 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 and Hur, 1999; Chen et al., 1995; Houston and 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, while some others had lower results. Changes in blood factors caused by salinity on fish *Channa punctatus* was also reported by Dheer et al. (1986). In this study, the results of biochemical properties were lower in osmolality, Na^+, K^+, and Cl^- than Sampaio and Bianchini (2002) which used flounder *Paralichthys orbignyanus* as experimental fish. The lower results of grey

mullets' biochemical properties was possibly caused by the difference of species.

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 shown lower amount than grey mullets in SW. Almost 77 % of the salts in blood are sodium and chloride. The remainder is made up primarily of bicarbonate, potassium and calcium. Sodium (Na^+), potassium (K^+), and calcium (Ca) salts are 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 SW will cause 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 lowering salinity. These results can be the main reason of the difference behavior in activity and OC according to salinity changes. To inhabit 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 11).

Salinity changes from SW to FW was tended to decrease the OC of grey mullet and increased the cortisol levels inside the blood. However, salinity changes from FW to SW was tended to increase the OC and decrease 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 and Iwama, 1991). Cortisol was assigned to stimulate the liver gluconeogenesis and elevating blood sugar levels (Wedemeyer et al., 1990), which was related to the 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). Those species were able to survive the salinity exposure from freshwater to hypersaline (Boily et al., 2007).

Table 11. 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}
SDB	172.1 ± 13.3^{b}	303.5 ± 7.8^{a}	32.8 ± 1.5^{a}	47.5 ± 2.1^{a}
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}
FDB	161.5 ± 10.8^{a}	360.0 ± 0.0^{a}	17.7 ± 7.2^{a}	56.0 ± 8.5^{b}
FDS	184.0 ± 32.0^{b}	379.0 ± 8.5^{a}	10.3 ± 1.3^{a}	31.5 ± 10.6^{a}

SDS, SDB, SGF, SDF, FDF, FDB, and FDS are the same abbreviations as shown in Table 1. Letters indicate significant differences among the experiments.

Further observations were required to determine the lethal DO for grey mullets in an larger scale aquaculture farm. According to the results, lethal DO for grey mullets was 0.3 mg/L in SW and FW environments. It means no significant difference between grey mullets reared in SW and grey mullets reared in FW according to their lethal DO. There was less information which reported about the lethal DO of grey mullets and oxygen requirements for adult grey mullets. However, another study reported that at larval stage mullets 1arvae apparently can not survive in DO below 4 mg/L (Sylvester et al., 1975).

In this study, when DO less than 1 mg/L, grey mullets started suffering stress and then die either in SW or FW. This results were similar with Kutty and Mohamed (1975) which reported that *Rhinomugil corsula* lose equilibrium at 0.8 mg/L in freshwater at 30^{o}C. Another study (Itazawa, 1959) also reported that *Mugil cephalus* showed symptoms of dyspnoea at oxygen concentration of 20 mmHg in 12-19^{o}C. Grey mullets require a quite high of DO for their normal activities, and based on observations it was normal when DO is 2 mg/L or more.

The blood properties from lethal DO experiments revealed the significant differences between grey mullets in LOS and LOF. The blood

factors including Ht, Hb, osmolality, cortisol, and glucose in LOS were significantly higher than LOF. Those differences were caused by different environmental condition which made the grey mullets reared in FW were lower on those blood factors. Kucuk et al. (2013) reported that salinity influences sodium, potassium, chloride, glucose, total protein, triglycerides as well, while total protein and glucose in plasma are related to fish metabolism. According to the depletion of DO, these results showed high levels of cortisol and glucose. Porchas et al. (2009) reported that cortisol and glucose were the stressor parameters to indicate the stress response of fish. Likewise, Zaki et al. (2009) indicated that the blood glucose level was affected by the rate of carbohydrate metabolism under hypoxia and stress conditions.

In conclusion, salinity and temperature clearly affected the OC, behavior, and blood property of grey mullets *Mugil cephalus*. Grey mullet must be an excellent osmoregulator species which can survive and grow well in freshwater with a good management, and it can be applied for increasing aquaculture productivity by culturing this species in freshwater. Further studies were needed to apply grey mullets' freshwater aquaculture in large scale farm and observe the growth performance in freshwater rearing system.

Acknowledgement

First and foremost, I would like to thank to God, Allah SWT that gave me power, strength, and health during I stayed in Korea and until I complete wrote this thesis. I also wish to thank to KOICA and PKNU for providing this master degree scholarship.

I would like to express my thankful and gratitude to Prof. Young Jin Chang as my supervisor, he gave me so many good advice and new knowledge during I stayed in Korea, so that I can learn a lot of things from him.

I also want to thank to Prof. Chang-Hoon Kim and Dr. Byung Hwa Min as my thesis committee members, for their comments, helps, and supports until I finish this master thesis.

For Dr. Min Hwan Jeong and Mr. Yong Hyun Do, as the staff of National Fisheries Research and Development Institute (NFRDI), thank you for being kindly support my experiments.

To my lab mates (Jun Hyung Ryu and Ki Tae Kim), thanks for being patiently helping and guiding me during I stayed in laboratory. Thank you

very much for your helps. Without your accompany, I will not complete my experiments and this thesis as well.

To my parents, my sister, my wife, and my family. I deeply say thanks from the bottom of my heart for your moral support during I stayed in Korea.

I also give thanks to Dr. Kyoung Mi Kang and Mr. Ki Hyun Kim for their cooperation and tireless helps during my study at Pukyong National University (PKNU).

My special thanks also go to all of Indonesian students in PKNU and all my classmates, thanks for supporting, made a good friendship, and being cooperative with me.

References

Arnason, T., B. Magnadottir, B. Bjornsson, A. Steinarsson and B.T. Bjornsson. 2013. Effects of salinity and temperature on growth, plasma ions, cortisol and immune parameters of juvenile Atlantic cod (*Gadus morhua*). Journal of Aquaculture 380-383, 70-79.

Beamish, F.W.H. and P.S. Mookherjii. 1964. Respiration of fishes with special emphasis on standard oxygen consumption. II. Influence of weight and temperature on respiration of goldfish, *Carassius auratus* L. Canadian Journal of Zoology 42, 161-175.

Boily, P., B.B. Rees and L.A.C. Williamson. 2007. Vertebrate osmoregulation: a student laboratory exercise using teleost fish. Advances in Physiology Education 31, 352-357.

Bjornsson, B. and S.R. Olafsdottir. 2006. Effects of water quality and stocking density on growth performance of juvenile cod (*Gadus morhua* L.). ICES Journal of Marine Science 63, 326-334.

Chadwick, E.M.P., G.J. Parsons and B. Sayavong. 2010. Evaluation of closed-containment technologies for saltwater salmon. National Research Council of Canada. Ottawa, 160 pp.

Chang, Y.J. and J.W. Hur. 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.

Chang, Y.J., M.H. Jeong, B.H. Min, W.H. Neill and L.P. Fontaine. 2005. Effect of photoperiod, temperature, and fish size on oxygen consumption in the black porgy *Acanthopagrus schlegeli*. Journal of Fish Science and Technology 8, 142-150.

Chen, G.R., L.T. Sun, Y.H. Lee and C.F. Chang. 1995. Characteristics of blood in common carp, *Cyprinus carpio*, exposed to low temperature. Journal of Applied Aquaculture 5, 21-31.

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

Duan Y., X. Dong, X. Zhang and Z. Miao. 2011. Effects of dissolved oxygen concentration and stocking density on the growth, energy budget and body composition of juvenile Japanese flounder, *Paralichthys olivaceus* (Temminck et Schlegel). Aquaculture Research 42, 407-416.

Franklin, C.E., I.A. Johnston, T. Crockford and C. Kamunde. 1995. Scaling of oxygen consumption of Lake Magadi tilapia, a fish living at 37°C. Journal of Fish Biology 46, 829-834.

Fry, F.E.J. and J.S. Hart. 1948. The relation of temperature to oxygen consumption in the goldfish. Biology Bulletin 94, 66-77.

Gardner, J.A. and G. King. 1922. Respiratory exchange in freshwater fish, further comparison of goldfish and trout. Biochemical Journal 16, 729-735.

Gibson, R.N. 1973. Tidal and circadian activity rhythms in juvenile plaice, *Pleuronectes platessa*. Marine Biology 22, 379-386.

Griffith, R.W. 1974. Environment and salinity tolerance in the genus *Fundulus*. Copeia 1974, 319-331.

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

Itazawa, Y. 1959. Gas content of the blood in response to that of medium water in fish. II. Comparison of the response in several species. Bulletin of Japan Society Fisheries 25, 301-306.

Iwama, G.K., A. Takemura and K. Takano. 1997. Oxygen consumption rates of tilapia in freshwater, seawater, and hypersaline seawater. Journal of Fish Biology 51, 886-894.

Jeong, M.H., Y.S. Kim, B.H. Min and Y.J. Chang. 2007. Effect of fish number in respiratory chamber on routine oxygen consumption of black porgy *Acanthopagrus schlegeli* reared in seawater or freshwater. Journal of Aquaculture 20, 121-126.

Khodabandeh, S., M.S. Moghaddam and B. Abtahi. 2009. Changes in chloride cell abundance, Na^+, K^+-ATPase immunolocalization and activity in the gills of golden grey mullets, *Liza aurata*, fry during adaptation to different salinities. Yakhteh Medical Journal 11, 49-54.

Kim, I.N., Y.J. Chang and J.Y. Kwon. 1995. Pattern of oxygen consumption in six species of marine fish. Journal of Korean Fisheries Society 28, 373-381.

Kim, S.J., Y.D. Lee, I.K. Yeo, H.J. Baek, H.B. Kim, M. Nagae, K. Soyano and A. Hara. 2004. Reproductive cycle of the female grey mullets, *Mugil cephalus,* on the coast of Jeju Island, Korea. Journal of Environmental Toxicology 19, 73-80.

Kucuk, S., A. Karul, S. Yildirim and K. Gamsiz. 2013. Effects of salinity on growth and metabolism in blue tilapia (*Oreochromis aureus*). African Journal of Biotechnology 12, 2715-2721.

Kutty, M.N. and M.P. Mohamed. 1975. Metabolic adaptations of mullets *Rhinomugil corsula* (Hamilton) with special reference to energy utilization. Aquaculture 5, 253-270.

Liu H., Y. Sakurai, H. Munehara and K. Shimazaki. 1997. Diel rhythms of oxygen consumption and activity level of juvenile flounder *Paralichthys olivaceus*. Fisheries Science 63, 655-658.

Lovell, T. 1998. Nutrition and feeding of fish. Second Edition. Kluwer Academic Publications. Massachusetts, 271 pp.

Lyytikainen, T. and M. Jobling. 1998. The effect of temperature fluctuations on oxygen consumption and ammonia excretion in underyearling Lake Inari Arctic charr. Journal of Fish Biology 52, 1186-1198.

Marais, J.F.K. 1978. Routine oxygen consumption of *Mugil cephalus, Liza dumerili* and *L. richardsoni* at different temperatures and salinities. Marine Biology 50, 9-16.

Min, E.Y., Y.S. Kim and J.C. Kang. 2010. Dietary effects of lactic acid bacteria on growth, hematological and immune response of grey mullets, *Mugil cephalus*. Journal of Fish Pathology 23, 343-355.

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

Muller, K. 1978. Locomotor activity of fish and environmental oscillations. In: J.E. Thorpe. Rhythmic activity of fishes. Academic Press, London. 1-19.

Oren, O.H. 1981. Aquaculture of grey mullets. Cambridge University Press. Cambridge, 507 pp.

Porchas, M.M., L.R.M. Cordova and R.R. Enriquez. 2009. Cortisol and glucose: reliable indicators of fish stress? Pan-American Journal of Aquatic Sciences 4, 158-178.

Requena, A., J. Fernandez-Borras and J. Planas. 1997. The effects of a temperature rise on oxygen consumption and energy budget in gilthead seabream. Aquaculture International 5, 415-426.

Saleh, M. 2008. Capture-based aquaculture of mullets in Egypt. In A. Lovatelli and P.F. Holthus (eds). Capture-based aquaculture. Global overview. FAO Fisheries Technical Paper 508, 109-126 pp.

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

Sylvester, J.R. 1975. Critical thermal maxima of three species of Hawaiian estuarine fish : a comparative study. Journal of Fisheries Biology 7, 257-262.

Szczepkowski M., B. Szczepkowska and I. Piotrowska. 2011. Impact of higher stocking density of juvenile Atlantic sturgeon, *Acipenser oxyrinchus* Mitchill, on fish growth, oxygen consumption, and ammonia excretion. Research Article Archives of Polish Fisheries 19, 59-67.

Tsuzuki, M.Y., C.A. Strussmann and F. Takashima. 2008. Effect of salinity on the oxygen consumption of larvae of the silversides *Odontesthes hatcheri* and *O. bonariensis* (Osteichthyes, Atherinopsidae). Brazilian Archives of Biology and Technology 51, 563-567.

Turker, H. 2011. The effect of water temperature on standard and routine metabolic rate in two different sizes of Nile tilapia. Kafkas Universitesi Veteriner Fakultesi Dergisi 17, 575-580.

Van Maaren, C.C., J. Kita and H.V. Daniels. 2000. Temperature tolerance and oxygen consumption rates for juvenile southern flounder *Paralichthys lethostigma* acclimated to five different temperatures. UJNR Technical Report 28, 135-140.

Verheijen, F.J. and S.J. De Groot. 1967. Diurnal activity pattern of plaice and flounder (*Pleuronectidae*) in aquaria. Netherlands Journal of Sea Resources 3, 383-390.

Wares, W.D. and R. Igram. 1979. Oxygen consumption in the fathead minnow (*Pimephales promelas* Rafinesque), effects of weight, temperature, group size, oxygen level, and opercular movement rate as a function of temperature. Comparative Biochemistry and Physiology 62, 351-356.

Wedemeyer, G.A., B.A. Barton and D.J. McLeay. 1990. Stress and acclimation. In : Schreck, C.B. and P.B. Moyle. Methods for Fish Biology. American Fisheries Society, 491-527.

Wells, N.A. 1935. Variation in the respiratory metabolism of the Pacific killifish due to size, season, and continued constant temperature. Physiological and Biochemical Zoology 8, 318-336.

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

Zaki, M.S., S.O. Mostafa, O.M. Fawzi, M. Khafagy and F.S. Bayumi. 2009. Clinicopathological, biochemical and microbiological change on grey mullets exposed to cadmium chloride. American-Eurasian Journal of Agriculture and Environmental Science 5, 20-23.