Thesis for the Degree of Master of Fisheries Science

Effects of Temperature, Salinity and Fish Number on Oxygen Consumption and Blood Property of Young Rockbream *Oplegnathus fasciatus*

by

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KOICA-PKNU International Graduate Program of Fisheries Science

Graduate School of Global Fisheries

Pukyong National University

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어린 돌돔의 산소소비와 혈액성상에 미치는 수온, 염분 및 수용밀도의 영향

Advisor: Prof. Young Jin Chang

by

Veasna Ao

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Abstract

Several factors on oxygen consumption (OC) of rockbream *Oplegnathus fasciatus* of with a body weight of 74.65±19.61 g and a total length of 14.38±0.99 cm was investigated wherein four studies were designed such as: 1) OC and blood property by water temperature change (20-25-30°C); 2) OC and blood property by salinity change (35-25-15 psu); 3) OC and blood property in different fish number in respiratory chamber (1, 3 and 6 fish); and 4) lethal dissolved oxygen (DO) at different water temperatures (20- 25-30°C).

The tendency of OC in temperature experiment increased with temperature, the highest value was found at 30°C. The average of OC during light period was higher than the dark period. Increasing water temperature did not affect the level of hemoglobin (Hb) and hematocrit (Ht). The level of Na+ increased with decreasing water temperature, the highest level was found

at 20°C. Level of K^+, Cl^-, Ca, Mg and osmolality did not affect by increasing water temperature. Changing water temperature had effect on cortisol and glucose level of rockbream, the highest value of cortisol and glucose were at 30°C. The level of total protein did not affect by increasing water temperature.

The tendency of OC in salinity experiment increased with salinity. During light period, rockbream consumed oxygen higher than the dark period. The level of Hb and Ht were not affected by changing salinity. The level of Na^+ and K^+ of rockbream were affected by changing salinity. The highest value of Na^+ was found at 30 psu, while K^+ was at 25 psu. The level of Cl^-, Ca, Mg and osmolality were not affected by changing salinity. The cortisol and glucose level were significantly different by changing salinity. The highest level was found at 15 psu. The level of total protein was not affected by changing salinity.

The average of OC of fish number experiment increased with fish number. The average of OC during light period was higher than the dark period. The level of Hb and Ht were not affected by increasing fish number. The level of Na^+, K^+ and osmolality were not also affected by increasing fish number. Increasing fish number affected on the level of Cl^-, Ca and Mg. The highest value was found at 6 fish. Increasing fish number had strong effect on level of cortisol and glucose. The highest level was reported at 6 fish. The level total protein did not affect by changing salinity.

The lethal DO level of rockbream increased with the temperature (0.8, 0.9 and 1 mg/L). All blood property components during lethal DO experiment increased with the depletion of

DO concentration in every temperature. For recovering experiment, both of fish groups, which was exposed in DO level from 8.6 to 2 mg/L (hypoxia) and 8.6 to 4 mg/L (moderate hypoxia) did not die at the end of experiment. For moderate hypoxia group, it took 24 hours for recovering to normal behavior, while hypoxia group took 1 week to recover. Exposing rockbream in different DO concentration did not effect on the level of Hb and Ht. The plasma of Na^+ K^+, Ca and osmolality did not effect by exposing in different DO concentration, while the plasma of Cl^- and Mg were affected by the depletion of DO concentration. The highest values were in control group. Exposing in low DO concentration had strongly affected on cortisol and glucose. The highest level was observed in hypoxia group. The level total protein was not affected by exposing in low DO concentration.

In conclusion, temperature, salinity and fish number, had strong effect on fish activity OC and blood properties of young rockbream. Moreover, temperature had strong effect on lethal DO level and speed of DO decrease. To avoid hypoxia in culture environment and high mortality, aeration should be increased when rockbream is exposed in high temperature, high stocking density in culture farm. Rockbream is not good in osmoregulation. In order to increase aquaculture production, rockbream should not be reared low salinity.

Introduction

Oxygen is essential for metabolism where it participates in different oxidative activities to release the necessary energy for biological work (Spanopoulos-Hernández et al., 2005). The amount of dissolved oxygen (DO) available in culture environment plays a key role in the success of aquaculture and hatching of fertilized eggs. Low oxygen concentrations cause variations in the energetic systems of organisms, which can affect feeding, reproduction (Spanopoulos-Hernández et al., 2005), larval development as well as growth of other life cycle stages (Liu et al., 2011).

Oxygen consumption (OC) has not only played the important role for the living organism in water, but also the aquaculture activity. Fish are greatly influenced by water environment with effects upon food ingestion and digestibility, metabolism and growth (Requena et al., 1997). In aquaculture, OC is one of the main factors, which was used to evaluate carrying capacity, production (Chang et al., 2005; Tsuzuki et al., 2008) and metabolic rate in fish. It can also be used to determine the effects of salinity changes on energy costs.

There are many factors, which affect the OC rate. Water temperature and salinity are the main abiotic factors influencing OC in aquatic animals. When temperature increases, their metabolic rate will also increase. On the other hand, the temperature increase will give negative effects to amount of DO saturation in the water (Sollid et al., 2005). The amount of energy required for osmoregulation depends on the difference between internal and external concentrations of ions. Differences in the energetic cost of osmoregulation play a significant role in the difference for growth rate between seawater and freshwater-adapted fish (Altinok and Grizzle, 2003)

Various factors effect on OC in fishes has investigated by many researchers. Chang et al. (2005) reported that the OC of black porgy *Acanthopagrus schlegelii* increased with temperature; and Requena et al. (1997) mentioned that the OC of gilthead sea bream *Sparus aurata* increased with the line of temperature. In case of salinity, Liu et al. (2011) reported that OC of juvenile Chinese sturgeon *Acipenser sinensis* increased with increase of salinity; Abdul et al. (2012) mentioned that OC of tilapia fingerlings decreased with increasing of salinity. For stocking density, Szczepkowski et al. (2011) reported that no significant differences were noted in OC in the groups at different stocking densities of Atlantic sturgeon *Acipenser oxyrinchus oxyrinchus.* During light period, black porgy consume oxygen more than during the dark period (Chang et al., 2005).

The rockbream *Oplegnathus fasciatus* is a subtropical and carnivorous species, which widely distributed in the Pacific Ocean, including southern part of Korea, Japan, Taiwan and Hawaii (Kim et al., 2009) and inhabit coastal rocky reefs (Shimada et al., 2009). Rockbream is popular for raw fish slices both Korean and Japanese cuisine and become an important marine fish in East Asia. It has recently been a target species for commercial aquaculture and stock enhancement. The culture scale of rockbream has increased gradually to meet the market demand in the last decade (He et al., 2011).

To develop advance aquaculture technique, changing OC and biochemical blood properties of rockbream exposed at different water temperatures, salinities and fish numbers needs to be investigated. The aim of this study was to find out the effects of temperatures, salinities and fish numbers on OC and blood properties of young rockbream.

Materials and Methods

1. Experimental design

Sixty rockbreams with average body weight 74.65±19.61 g and total length 14.38±0.99 cm was used to investigate in this experiment. Before conducting experiment, fish was reared in 200 L fiber class tanks for 30 days and fed with commercial diet twice per day at 2% of their body weight for nursing. Injured and unhealthy fish was removed.

Photoperiod investigation of OC was done at the same time in different water temperature, salinity and fish number experiment. In each experiment, fish were kept in respiratory chamber (RC) and exposed for 12 hours with dark period (no light) and 12 hours with light period. Fluorescent light were used from 09:00 to 21:00 to assume as the light period. For the dark period, paper box and cloth sheets were used to cover the RC from 21:00 to 09:00 to avoid the light penetration.

2. Measurement of oxygen consumption

Experimental fish were fasted for 48 hours before measurement of OC to ensure the post-absorptive digestive state. In order to stabilize the metabolic rate, fish were acclimated in respiratory chamber for 24 hours before recording DO. In each experiment, water temperature was controlled by using recirculating water bath (JS-WBR-170RP, Johsam, Bucheon, Korea).

Recirculating system (close system), which showed in Fig. 1 was used for measuring the effects of different water temperature, salinity and fish number on OC and blood properties of rockbream. RC was designed with transparent PVC with 31 cm length, 23 cm width and 24 cm depth (volume 17.1 L). During the experiment, water in RC was kept constant. The cycle of water flow pattern was started from the water reservoir I (WRI) to inlet water (IW) to RC to outlet water (OW) to water reservoir II (WRII) to water bath and filter unit (WB & FU) and return to WRI. The flow water from respiratory chamber went to WRII was full aerated to 100% saturation with air. Two oxygen sensors (OS) were used at IW and OW to compare the DO value before and after RC. At the same time, two water temperature sensors (TS) were placed at WRI and WRII to make sure that water temperature between WRI and WRII are same. Water flow rate was measured at the WRII. Data of DO during experiment was

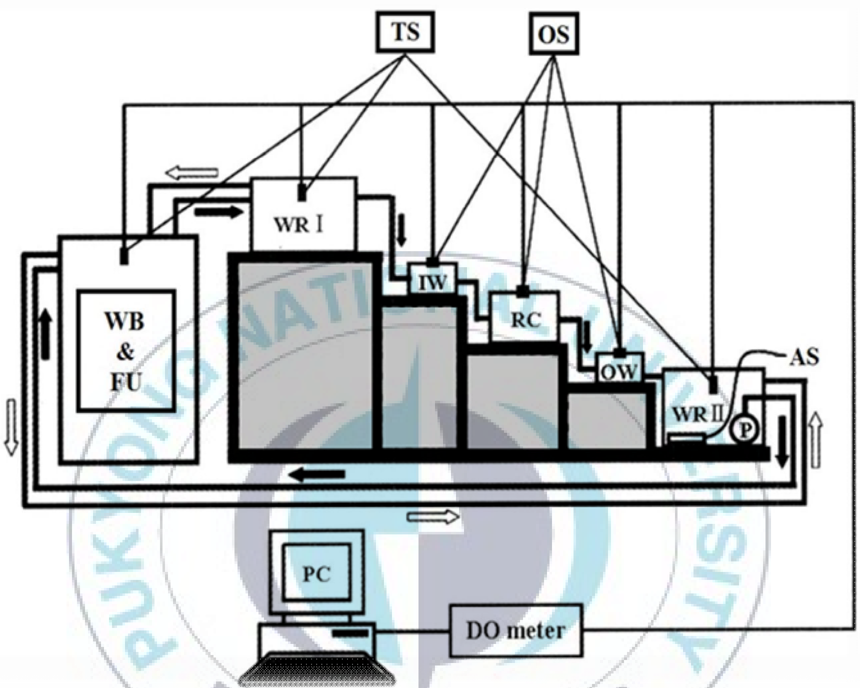

Fig. 1. Digram of OC measurement system. AS: air supply, FU: filter unit, IW: inflow water, OS: oxygen sensor, OW: outflow water, RC: respiratory chamber, TS: temperature sensor, WB: water bath, WR: water reservoir.

automatically recorded every 10 minutes by using Multichannel Monitoring System for DO (OxyGuard 6, OxyGuard International A/S, Birkerd, Denmark). Heater and low temperature circulation bath were used to control water temperature.

Three water temperatures (20-25-30^{o}C) was determined OC in series pattern experiment. In this experiment, OC was measured two replicates to calculate mean ± SD. In order to minimize thermal shock of high fluctuation during increase of water temperature, fish needs interval time to acclimate from rearing water temperature to experimental water temperature to attain desired water temperature by 0.6^{o}C/hour.

Three salinities was used (15-25-35 psu) to measure the OC. In this experiment OC was measured twice to calculate mean ± SD. To avoid the high fluctuation of salinity, interval time for acclimating was needed. Salinity was adjusted to the experimental levels by 5-10 psu per day (Swanso, 1998).

For investigating, the effect fish numbers on OC, three groups of fish (1, 3 and 6 fish) were measured individually. In each group was repeated twice. The experimental conditions in OC and blood properties were shown in Table 1.

Table 1. Experimental conditions of OC and blood properties

Experiment	Water Temp (°C)	Salinity (psu)	Fish number	L:D (hour)	TL (cm)	BW (g)
OC						
Temperature	20-25-30	35	3	12:12	14.4±1	74.6±19
Salinity	25	15-25-35	3	12:12	14.4±1	74.6±19
Fish number	25	35	1, 3, 6	12:12	14.4±1	74.6±19
Lethal DO						
Temperature	20-25-30	35	3	-	15.5±1	94.1±1
Recovering behavioral response in different DO concentration						
MH (4 mg/L)	20	35	3	-	15.5±1	94.1±1
HP (2 mg/L)	20	35	3	-	15.5±1	94.1±1

B.W: body weight, HP: hypoxia, L:D: light and dark period, MH: moderate hypoxia, Temp: temperature, TL: total length.

To calculating the OC, the bellow formula was used:

$$OC = (DO_{iw} - DO_{ow}) \times F \times 60/W$$

Where DO_{iw} and DO_{ow} are the DO concentration (mg O_2/L) in the respective water of the inflow water and outflow water; F is the flow rate (L/min); OC is the oxygen consumption expressed in miligrams of oxygen per hour per kilogram of fish (mg O_2/kg/h); W is the weight of fish biomass (kg).

3. Measurement of lethal dissolved oxygen

Magnetic stirrer was used to mix the water in RC during the measurement of lethal DO on rockbream (Fig. 2). In order to observe the behavioral respone of fish during the gradual depletion of DO in RC, three rockbreams were used as the subject to determine the effect of different water temperatures (20, 25 and 30°C) on lethal DO and their behavior. Before each experiment, fish was acclimated from rearing condition to experimental condition. During experiment, water temperature was maintained by temperature control carbine. Oxygen sensor (OS) and temperature sensor (TS) were placed at the RC and connected to the computer to measure the depletion of DO in different time.

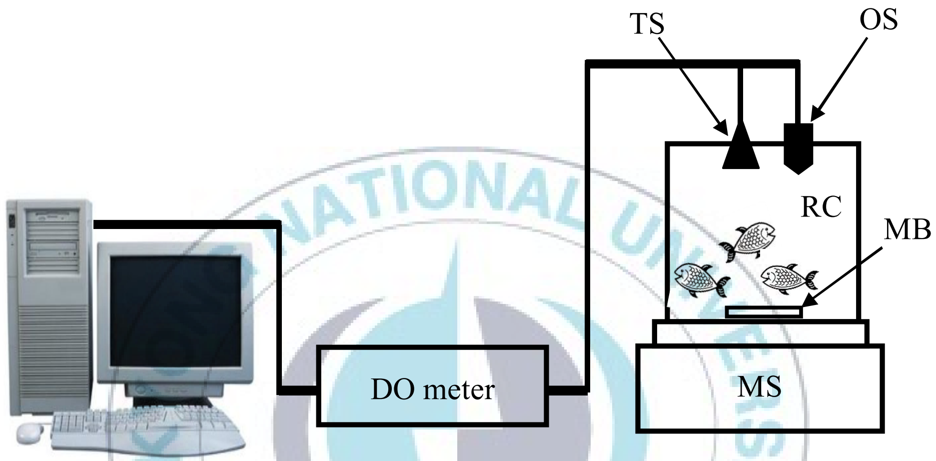

Fig. 2. Diagram of lethal DO measurement system. DO: dissolved oxygen, MB: magnetic bar, MS: magnetic stirrer, OS: oxygen sensor, RC: respiratory chamber, TS: temperature sensor.

Depletion of DO by time in RC will be automatically recorded every 10 min by using Multichannel Monitoring System for DO (OxyGuard 6, OxyGuard International A/S, Birkerd, Denmark).

For calculating the lethal DO the below formula was used:

$$OC = (DO_0 - DO_t) \times (V / (t \times W))$$

Where DO_0 is initial dissolved oxygen (mg/L); DO_t is dissolved oxygen after time; OC is the oxygen consumption (mg O_2/kg/h); t is time passed for fish which consumed oxygen (h); V is the volume of water in respiration chamber (L); W is fish weight (kg).

4. Observation of behavioral response

4.1. Oxygen consumption

Breath frequency (opercula movement) per minute was used as the indicator for fish behavioral response during OC (Table 2). One of three fish was repeated 3 times in different time (09:00, 14:00 and 18:00) to calculate the average of breath frequency per minute.

Table 2. Behavioral index during OC experiment of *Oplegnatus fasciatus* in different temperatures, salinities and fish numbers

Index	Movement	Breath frequency/minute
I	Active swimming	> 130
II	Moderate swimming	110-130
III	Slow swimming	90-109

4.2. Lethal dissolved oxygen

Breath frequency was counted per minute. Swimming behavior, attacking, struggling, and body color were observed at the same time (Table 3).

4.3. Recovery from different low dissolved oxygen

At 20^{o}C, recovering experiment was done at 2 different groups according to DO level, moderate hypoxia (8.6 to 4 mg/L) and hypoxia (8.6 to 2 mg/L). Three rockbreams in each group were exposed in RC to observe the behavioral response during DO deletion. The protocol for measuring the DO depletion form starter to desirable levels was the same to lethal DO (Fig. 2). When DO depleted until the desirable points, fish was taken out from the RC and sampled the blood and then returned to rearing tanks. Fish was observed their behavioral response (swimming, diet consuming behavior, and black strips color) during recovering from hypoxia condition for two weeks.

Table 3. Behavioral index of lethal DO and recovering experiment of *Oplegnatus fasciatus*

Index	Movement	Attacking /min	Struggling /min	Balancing	Black str color
I	Active swimming	> 2	0	Normal	Light blac
II	Moderate swimming	2-1	0	Normal	Light blac
III	Slow swimming	< 1	0	Equilibrium	Brown
IV	Very slow swimming	0	0	Spastic	Brown
V	Lost balance	0	< 2	Upside down	Light bro
VI	Before death	0	2-4.5	Sank and gasping	Light bro
VII	Died	0	0	Laid down	Pale

min: minute

5. Analyses of blood properties

The effect changing of temperature, salinities and fish number on blood properties were done at the same time during the OC experiments. Blood sampling was done after OC experiment (Fig. 3). During lethal DO measurement and recovering experiment at different DO concentration, blood properties study was done. Before experiment, three fish from rearing tank were obtained the blood (control). During experiment, after one of three fish died, blood was sampled from the two alive fish (experiment).

Before blood sampling, fish was anaesthetized with 2-phenoxyethanol (200 ppm). Approximately 1 mL of blood was obtained from fish with heparinized syringe. Blood was divided into two tubes; one tube was used to analyze hemoglobin (Hb) and hematocrit (Ht) concentration immediately (Martínez-Álvarez et al., 2002), while another sample was centrifuged at 1500 rpm for 5 minutes to obtain the plasma and then stored at -80°C up to the day of analyzing Na^+, K^+, Cl^-, Ca, Mg, osmolality, cortisol glucose and total protein (Martínez-Álvarez et al., 2002 and Gholampoor et al., 2011).

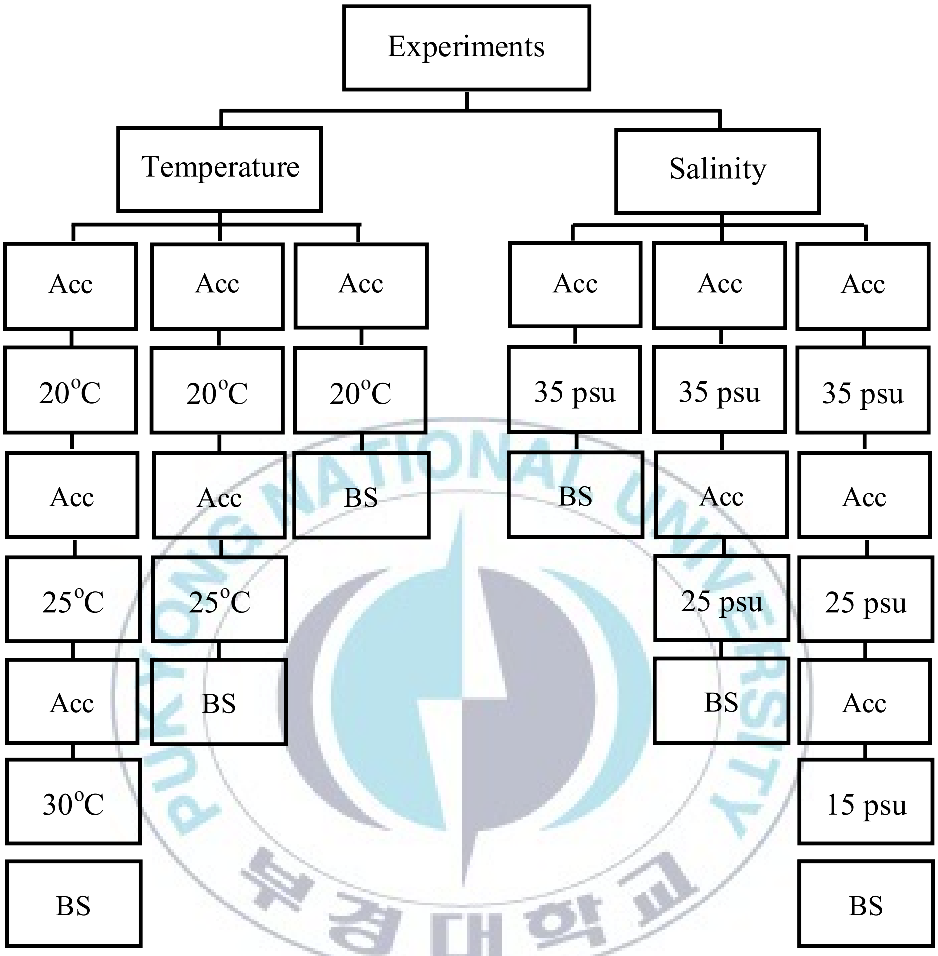

Fig. 3. Flow chart of OC and blood properties in different water temperatures and salinities experiment. Acc: acclimation, BS: blood sampling.

Hb and Ht were bound as the oxygen factor, while Na^+, K^+, Cl^-, Ca, Mg and osmolality were bound as osmoregulation factor. Cortisol glucose and total protein were bound as stress factor.

For analyzing Ht, blood was transferred into capillary tube and centrifuged with 1500 rpm for 5 minutes by using micro-hematocrit reader (Micro-Hematocrit Reader, Hawksley) to determine the percentage of Ht. For analyzing Hb, whole blood was used. Blood was diluted with Asan set reagent (20µl of blood and 5 mL of Asan set reagent) and analyzed by Tecan infinite 200 with 450 nm measuring wave length. Na^+, K^+, Cl^-, Ca, Mg, glucose and total protein were analyzed by using Chemical Analyzer (Fujifilm Dri-Chem 4000i, Japan). Enzyme Immunoassay (EIA) was used to analyze plasma cortisol by using Cortisol EIA kit (Oxford Biochemical Research). Plasma osmolality was analyzed by using Vapor Pressure Osmometer (Vapor 5520; Wescor Co., USA).

6. Data analyses

All data represented as mean ± SD, and analyzed by one-way ANOVA and two-way ANOVA multiple comparisons (LSD) with SAS program (9.3).

Results

1. Effect of temperature on oxygen consumption

1.1. Oxygen consumption

As shown in Fig. 4, the OC of young rockbream which was exposed in three different water temperatures (20, 25 and 30°C) showed the clear rhythm every hour. The tendency of OC increased with water temperature and showed significant difference. In both photo period, the average of OC comsunption also increased at the same time with water temperature. The value of OC during light period was higher than the dark period and significant difference was observed in every water temperature. In both photo period, the highest OC was reported at 30°C (Table 4). During light period, the abrupt changes of OC occured at 10:00, 1 hour after starting the light period and sharply decreased until 11:00. From 12:00 to 20:00, the OC slightly changed in every temperature. During the dark period, the OC gradually decreased from 21:00 until 05:00. From 05:00 to 8:00, OC gradually increased. As shown in Table 4, the average OC at 30°C during light was higher 1.6 times than 20°C (b=12.56). During the

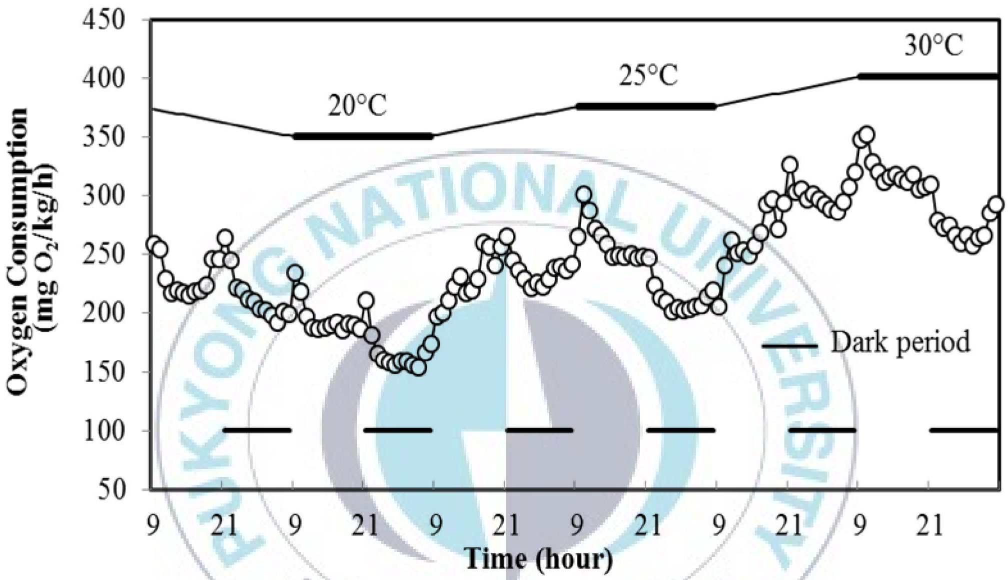

Fig. 4. OC of *Oplegnathus fasciatus* in different water temperatures.

Table 4. Average OC (mg O_2/kg/h) of *Oplegnathus fasciatus* during light and dark period in different water temperatures

L:D	Water temperature (°C)			b	a	r^2
	20	25	30			
L	195.6 ± 18.5^{c}	261.9 ± 34.1^{b}	321.2 ± 10.5^{a}	12.6	-54.4	0.92
D	$166.7 \pm 6.8^{c*}$	$212.9 \pm 35.4^{b*}$	$274.3 \pm 28.9^{a*}$	10.8	-50.9	0.9

Each value represents mean ± SD (n = 6). Different letter indicate significant difference in each water temperature, respectively. Asterisk shows significant difference between light and dark period ($P<0.05$, two-way ANOVA multiple comparison (LSD)). D: dark period, L: light period.

dark period, the OC at 30^{o}C was also higher 1.6 times than 20^{o}C (b=10.76).

1.2. Breath frequency and oxygen consumption per breath

The breath frequency of rockbream at the different water temperatures showed significant difference. The linear regression between breath frequency and water temperature showed strong relationship (Fig. 5). The average of breath frequency was 98.2 at 20^{o}C, 124.7 at 25^{o}C and 152.6 at 30^{o}C.

The OC per breath of experimental fish increased with temperature, but it was not significantly differrent. The average of OC per breath was 1.86 at 20^{o}C, 1.91 at 25^{o}C and 1.96 mg O_2/kg/h at 30^{o}C.

1.3 Behavioral response

The activity of rockream increased as the same time with water temperature. In 20^{o}C, the behavior of fish showed in index III, 25^{o}C was in index II, while the 30^{o}C was in index I (Table 5).

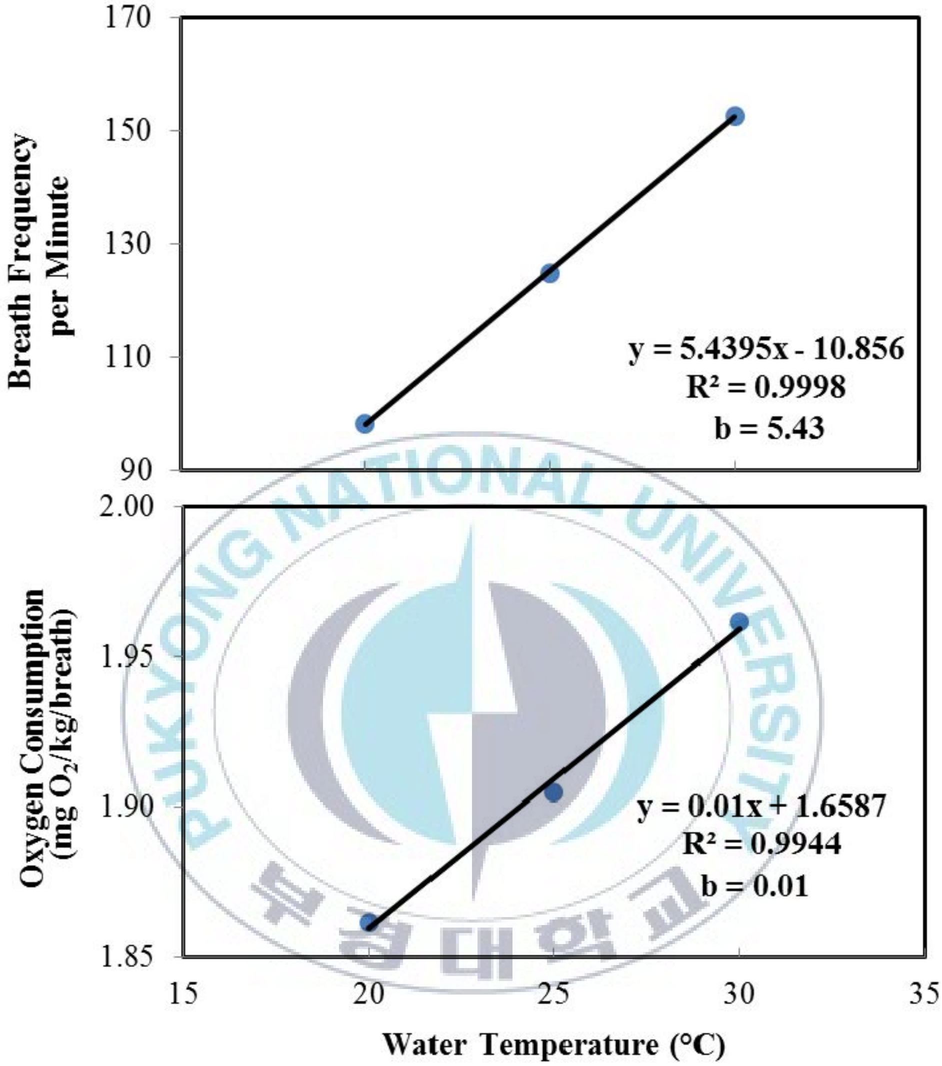

Fig. 5. Breath frequency per minute and OC per breath of *Oplegnathus fasciatus* in different water temperatures.

Table 5. Behavioral index in OC experiment of *Oplegnathus fasciatus* in different temperatures, salinities and fish numbers

Temperature (^oC)			Salinity (psu)			Fish number		
20	25	30	35	25	15	1	3	6
III	II	I	I	II	II	II	II	I

1.4. Blood properties

Oxygen factors: The level of hemoglobin (Hb) and hematocrit (Ht) were not significantly different by increasing water temperature, but highest level was reported at 30°C (Table 6).

Osmoregulation factors: As shown in Table 6, the plasma of Na^+ level showed significant difference. The highest value was found at 20°C, while the lowest was observed at 25°C. The plasma level of K^+, Cl^-, Ca, Mg and osmolality did not show significant difference by increasing water temperature, but the highest values of plasma K^+ and Mg were observed at 20°C, while plasma of Cl^-, Ca and osmolality were found at 30°C.

Stress factors: Increasing water temperature induced stress to rockbream. Based on the result of study, the plasma level of glucose and cortisol showed significant difference by increasing water temperature. The highest level was found at 30°C, while the lowest level was found at 25°C. In case of total protein was not significantly different, but the highest level was found at 30°C (Table 6).

Table 6. Physio-chemical properties of blood *Oplegnathus fasciatus* exposed in different water temperatures

Component	Temperature (°C)		
	20	25	30
Hemoglobin (g/dL)	7.1±1.3 [a]	7.7±1.0 [a]	9.9±0.6[a]
Hematocrit (%)	27.2±1.6[a]	27.0±2.8 [a]	33.3±1.8[a]
Na^+ (mEq/L)	184±1.4[a]	174.0±0.0[b]	177±1.4[b]
K^+ (mEq/L)	4.7±0.0 [a]	4.3±04 [a]	4.3±06 [a]
Cl^- (mEq/L)	147.5±07 [a]	149.5±2.1[a]	154.5±10.6 [a]
Ca (mg/dL)	10.7±0.4 [a]	10.5±0.1[a]	10.9±0.9 [a]
Mg (mEq/L)	2.3±0.0 [a]	2.2 ±1.1 [a]	1.8±04 [a]
Osmolality (mmol/kg)	319.0±4.2[a]	327.5±12 [a]	336.0±5.7[a]
Cortisol (ng/dL)	29.3±5.6[b]	7.3±1.7[c]	63.7±6.4[a]
Glucose (mg/dL)	86.5±10.6[ba]	69.5±9.2[b]	113±4.2[a]
Total protein (g/dL)	2.7±0.3[a]	2.7±0.1[a]	3.3±0.2[a]

Each value represents mean ± SD (n = 6). Different letter indicate significant difference in each water temperature, respectively ($P<0.05$, one-way ANOVA multiple comparison (LSD)).

2. Effect of salinity on oxygen consumption

2.1. oxygen consumption

During the experiment of different salinities, the OC of rockbream showed various changes in every hour (Fig. 6). The average of OC increased with the salinity and significant difference in each given salinity was observed. In both photoperiods, the highest values of OC were found at 35 psu, while 15 and 25 psu did not show significant difference (Table 7). The OC during light period was higher than the dark period. The average OC at 35 psu during light was higher 1.7 times than 15 psu (b=6.6), while dark period was higher than 1.6 times (b=4.9). The abrupt increase of OC in each experimental salinity occured when light and dark period were started. During light period, the highest OC occured at 10:00, 1 hour after starting the light, while the dark period was found at 22:00, 1 hour after turning off the light. The tendency of OC at 35 and 25 psu were similar, but the tendency of OC at 15 psu was slightly different. During light period, OC sharply increased at 17:00 and slightly changed until 20:00. At 21:00, OC sharply decreased (Fig. 6). During dark period, OC gradually increased from 7:00 to 8:00 in every salinity experiment.

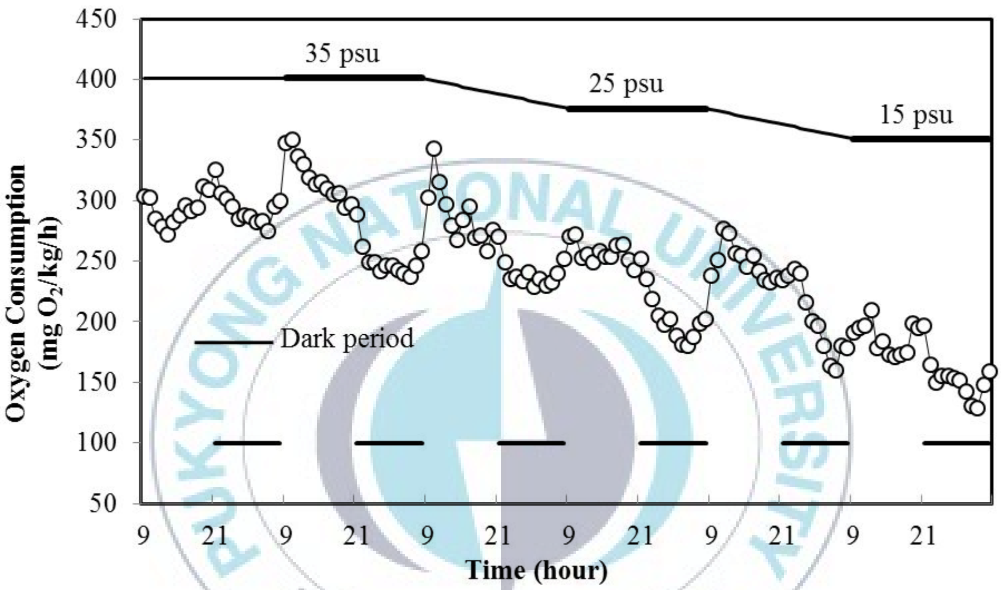

Fig. 6. OC of *Oplegnathus fasciatus* in different salinities.

Table 7. Average OC (mg O_2/kg/h) of *Oplegnathus fasciatus* during light and dark period in different salinities

L:D	Salinity (psu)			b	a	r^2
	35	25	15			
L	318.8±48.4^{a}	257.0±5.0^{b}	186.5±16.7^{b}	6.6	88.8	0.94
D	250.8±11.6^{a*}	204.0±0.1^{b*}	153.0±27.4^{b*}	4.9	80.4	0.84

Each value represents mean ± SD (n = 6). Different letter indicate significant difference in different salinities. Asterisk shows significant difference between light and dark period (P<0.05, two-way ANOVA multiple comparison (LSD)). D: dark period, L: light period.

2.2. Breath frequency and oxygen consumption per breath

The breath frequency of experimental subjects in three different salinities (15, 25 and 35 psu) was shown in Fig. 7. The breath frequency showed a linear relationship with salinity (b=0.88). The average of breath frequency increased with salinity (115.2 at 15 psu, 118.8 at 25 psu and 132.9 times at 35 psu), but it was not significantly different.

The OC per breath of experimental fish, which showed in Fig. 7 increased at the same time with salinity and showed significant differrence. The average of OC per breath at 15 and 25 psu (1.32 and 1.94 times/min) did not show significant difference, while 35 psu showed significant difference (2.15 times/min).

2.3. Behavioral response

Changing salinity had strong effect on the activity of rockbream. As shown in Table 5, the activity of rockbream inreased at the same time with salinity. At 15 and 25 psu, rockbream showed their activity in index II, but at 35 psu showed in index I.

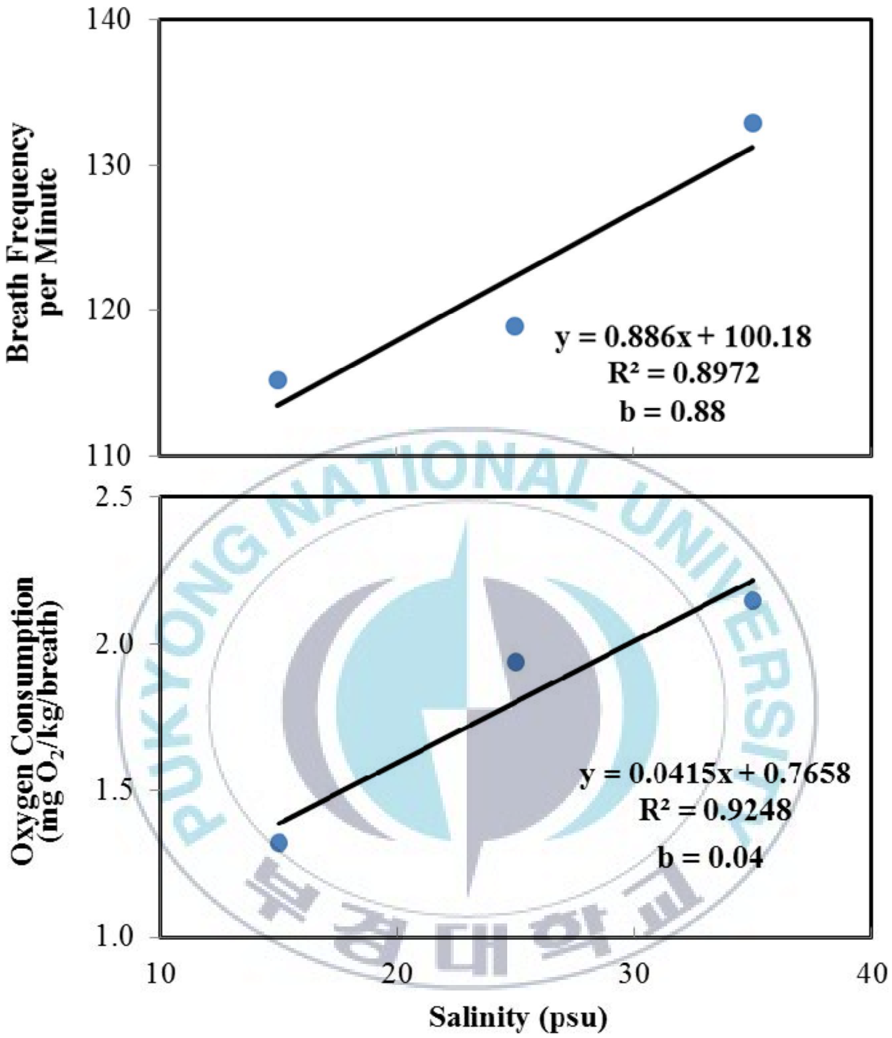

Fig. 7. Breath frequency per minute and OC per breath of *Oplegnathus fasciatus* in different salinities.

2.4. Blood properties

Oxygen factors: The levels of Hb and Ht of rockbream did not vary at different salinities, but highest level of Hb was at 15 psu, while the highest level of Ht was observed at 25 psu (Table 8).

Osmoregulation factors: As shown in Table 8, the plsma levels of Na^+ and K^+ of rockbream showed significant difference by changing salinity. The highest level of Na^+ was observed at 35 psu, while 25 and 15 psu did not show significant difference. The plasma level of highest level K^+ was found at 25 psu, while the lowest was observed at 15 psu. The plasma of osmolality level did not show significant difference, but the highest was observed at 35 psu. The plasma level of Cl^-, Ca, and Mg did not vary by changing salinity.

Stress factors: Changing salinity had strong effect on stress factor on rockbream. The tendency of plasma glucose and cortisol increased with decreasing salinity. The highest level was found at 15 psu, while 20 and 35 psu did not show significant difference. The level of total protein plasma did not show significant difference ($P>0.05$), but the highest level was found at 15 psu (Table 8).

Table 8. Physio-chemical properties of blood of *Oplegnathus fasciatus* exposed in different salinities

Component	Salinity (psu)		
	35	25	15
Hemoglobin (g/dL)	8.2±1.3^{a}	8.3±1.3^{a}	8.3±3.0^{a}
Hematocrit (%)	27.5±4.9^{a}	29.8±4.0^{a}	28.8±9.7^{a}
Na^{+} (mEq/L)	175.5±0.7^{a}	168.0±2.8^{b}	166.5±0.7^{b}
K^{+} (mEq/L)	4.3±0.4^{b}	5.1±0.2^{a}	3.6±0.1^{b}
Cl^{-} (mEq/L)	147.5±0.7^{a}	143.0±14.1^{a}	139.0±1.4^{a}
Ca (mg/dL)	8.9±0.5^{a}	10.5±2.1^{a}	10.2±0.0^{a}
Mg (mEq/L)	1.5±0.1^{a}	1.4±0.1^{a}	1.3±0.3^{a}
Osmolality (mmol/kg)	386.0±8.5^{a}	340.0±5.7^{a}	370.0±28.3^{a}
Cortisol (ng/dL)	8.1±0.5^{b}	7.8±0.7^{b}	37.3±3.0^{a}
Glucose (mg/dL)	52.0±2.8^{b}	39.5±0.7^{b}	71.5±9.2^{a}
Total protein (g/dL)	2.6±0.1^{a}	2.7±0.6^{a}	3.1±0.1^{a}

Each value represents mean ± SD (n = 6). Different letter indicate significant difference in each salinity, respectively (P<0.05, one-way ANOVA multiple comparison (LSD)).

3. Effect of fish number in respiratory chamber on oxygen consumption

3.1. Oxygen consumption

The average OC of fish number experiment inceased with increasing fish number and was significantly different. In every fish number experiment, the peak of OC was observed at 10:00, 1 hour after starting light. At 3 and 6 fish experiment, the pattern of OC change did not show clear rhythm in every hour in both light and dark period, while in 1 fish experiment showed clear rhythm in both light in and dark period. During light period of 1 fish experiment (09:00 to 10:00), OC abruptly increased, and then abruptly decreased at 11:00. At 15:00 and 18:00, while the OC slightly increased. During the dark period, from 21:00 to 01:00, OC gradually decreased. At 3:00, OC slightly increased and gradually decreased from 4:00 to 6:00. From 6:00 to 8:00, OC gradually increased (Fig. 8). As shown in Table 9, the average of OC of rockbream during light period was higher than the dark period ($P<0.05$). During light period, the OC average of 6 fish was higher 1.5 times than 1 fish (b=20.9), while dark period was higher than 2.2 times (b=28.8).

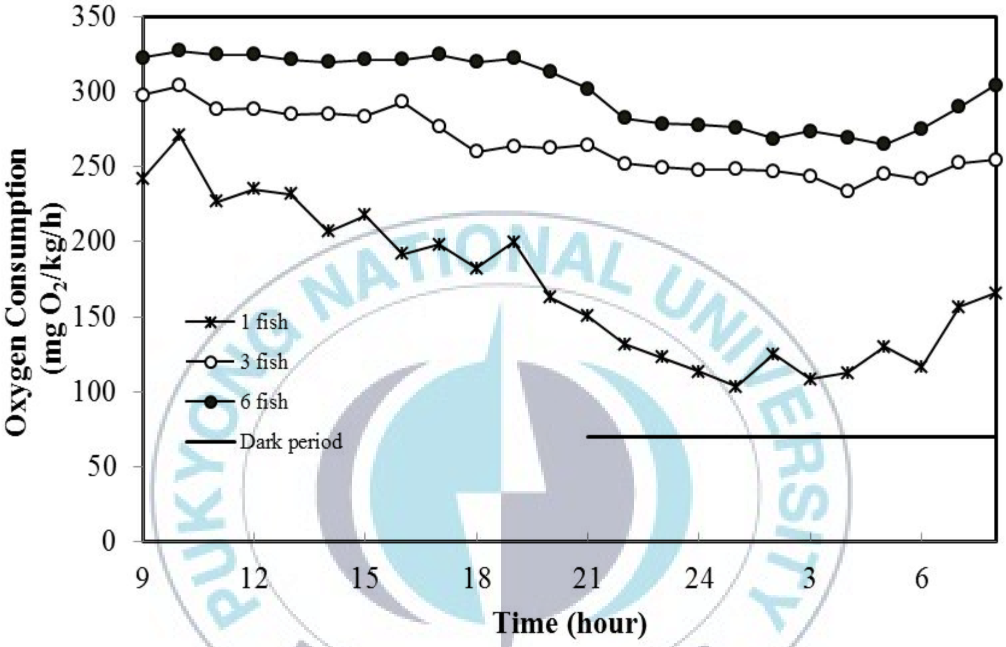

Fig. 8. OC of *Oplegnathus fasciatus* in different fish numbers.

Table 9. Average OC (mg O_2/kg/h) of *Oplegnathus fasciatus* during light and dark period in different fish numbers

L:D	Fish number (individual)			b	a	r^2
	1	3	6			
L	213.8 ± 24.9^{c}	282.1 ± 1.5^{b}	321.8 ± 16.8^{a}	20.9	202.8	0.79
D	$128.2 \pm 7.5^{c*}$	$248.3 \pm 5.1^{b*}$	$280.2 \pm 12.7^{a*}$	28.8	122.7	0.78

Each value represents mean ± SD (n = 6). Different letter indicate significant difference. Asterisk shows significant difference between light and dark period ($P<0.05$, two-way ANOVA multiple comparison (LSD)). D: dark period, L: light period.

3.2. Breath frequency and oxygen consumption per breath

As shown in Fig. 9, the breath frequency of young rockbream in different groups of fish number (1, 3 and 6 fish) showed a linear realtionship and significant difference. The average of breath frequency was 119.8 at 1 fish, 132.4 at 3 fish and 151.5 times per minute at 6 fish experiment.

Oxygen consumption per breath did not show a linear relationship with the different fish numbers, but it showed significant difference. The highest OC per breath occured at 3 fish (3 fish and 6 fish were not significatly different). The average of OC per breath was 1.43 at 1 fish, 2.01 at 3 fish and 1.99 mg O_2/kg/h at 6 fish experiment (Fig. 9).

3.3. Behavioral response

The activity of rockbream during OC experiment increased with increasing fish number. The activity of 1 fish and 3 fish groups showed in index II, while 6 fish group showed in index I (Table 5).

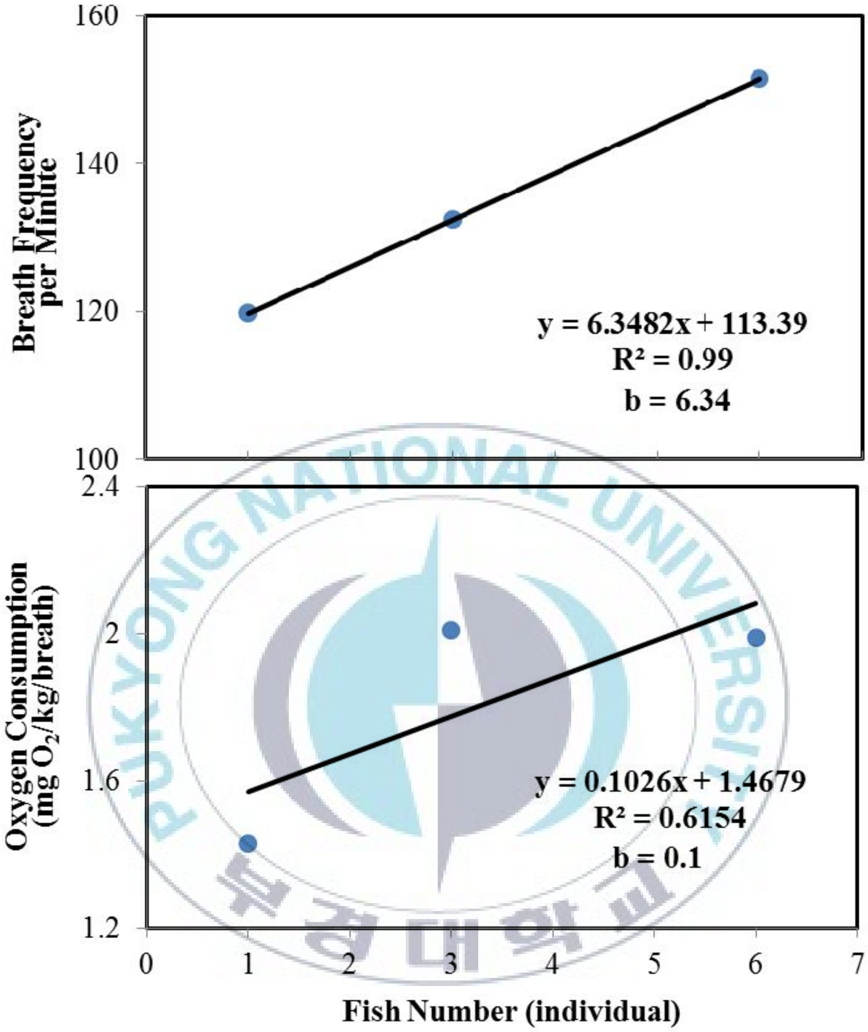

Fig. 9. Breath frequency per minute and OC per breath of *Oplegnathus fasciatus* in different fish numbers.

3.4. Blood properties

Oxygen factors: The levels of Hb and Ht did not show significant difference by increasing fish number. The highest level of Hb was observed at 1 fish , while that of Ht was observed at 3 fish (Table 10).

Osmoregulation factors: The plasma level of Na^{+} and K^{+} did not show significant difference by increasing fish number. Plasma level of Cl^{-} , Ca, and Mg showed significant difference. The highest level was found at 6 fish. The plasma of osmolality did not show significant difference, but the value was increased by increasing with fish number (Table 10).

Stress factors: As shown in Table 10, increasing fish number in respiratory chamber induced stress to rockbream. The tendency of cortisol, glucose and total protein level increased at the same time with fish number and showed significant difference ($P<0.05$). The highest level was found at 6 fish, while the lowest level was found at 1 fish.

Table 10. Physio-chmical properties of blood of *Oplegnathus fasciatus* exposed in different fish numbers

Component	Fish number (individual)		
	1	3	6
Hemoglobin (g/dL)	8.6±2.3[a]	8.3±0.8[a]	7.6±0.0[a]
Hematocrit (%)	28.8±5.3[a]	32.0±3.5[a]	30.3±3.3 [a]
Na^+ (mEq/L)	178.0±4.2[a]	175.0±2.8[a]	180.5±0.7[a]
K^+ (mEq/L)	4.1±0.2[a]	3.8±0.2[a]	4.0±0.2[a]
Cl^- (mEq/L)	143.5±3.5[b]	143.0±1.4[b]	153.5±0.7[a]
Ca (mg/dL)	8.0±1.7[b]	10.7±0.3[ab]	11.5±0.2[a]
Mg (mEq/L)	1.2±0.3[b]	1.5±0.2[ab]	2.0±0.1[a]
Osmolality (mmol/kg)	315.5±31.8[a]	318.5±2.1[a]	326.0±4.2[a]
Cortisol (ng/dL)	2.3±0.7[c]	27.2±1.7[b]	56.8±4.5[a]
Glucose (mg/dL)	53.5±16.3[b]	73.5±4.9[ab]	153.5±47.3[a]
Total protein (g/dL)	2.1±0.1[b]	2.7±0.2[ab]	3.0±0.3[a]

Each value represents mean ± SD (n = 6). Different letter indicate significant difference in each fish number group, respectively ($P<0.05$, one-way ANOVA multiple comparison (LSD)).

4. Lethal dissolved oxygen in different temperatures and recovery behavior

4.1. Lethal dissolved oxygen and oxygen consumption

Lethal DO in three different water temperatures (20, 25 and 30^{o}C), which showed in Fig. 9 increased with water temperature, but did not show significant difference ($P>0.05$). In 20^{o}C, the initial DO was 7.45 mg/L (101% saturated); one fish died after DO reached 0.8 mg/L (10.84% saturated), 25^{o}C was 7 mg/L (104% saturated); one fish died after DO reached 0.9 mg/L (13.33% saturated), while 30^{o}C was 6.6 mg/L (106% saturated) and one fish died after DO reached 1 mg/L (16.08 % saturated). In three different water temperatures, The OC of rockbream decreased at the same time with the DO depletion. At 20^{o}C, rockbream consumed oxygen from 260.47 to 41.02 mg O_2/kg/h within 135 minutes, 25^{o}C was 120 minutes (573.15 to 22.59 mg O_2/kg/h) and 30^{o}C was 60 minutes (645.63 to 19.54 mg O_2/kg/h).

4.2. Breath frequency

The average of breath frequency during lethal DO experiment increased with temperature (127.7±195, 134.3±27.8, and 158.031.1). In each temperature,

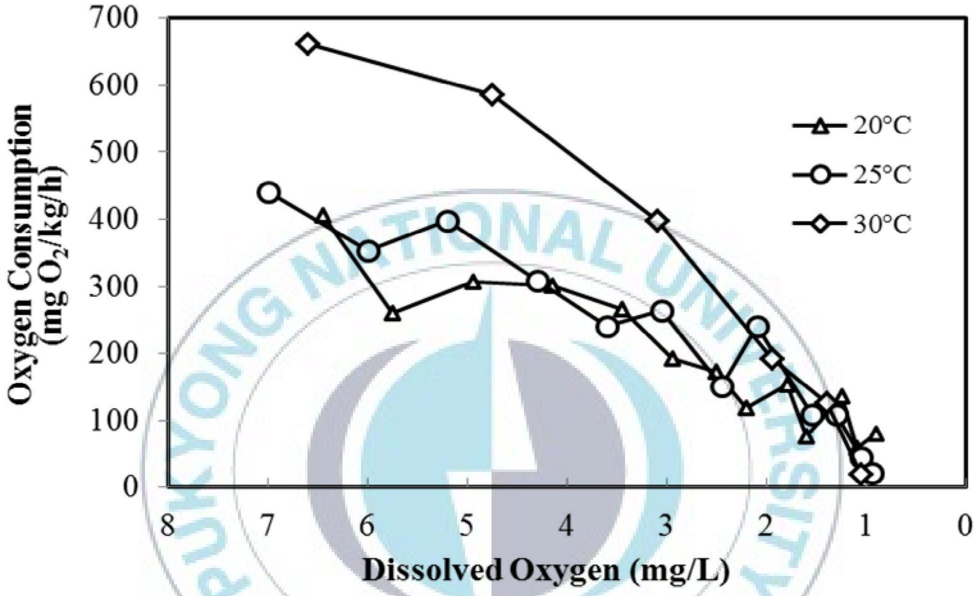

Fig. 10. Lethal DO level of *Oplegnathus fasciatus* at different water temperatures.

breath frequency showed significant difference ($P<0.05$), but it did not decrease with the DO. In every temperature experiment, breath frequency sharply decreased at the lethal DO level and stop breathing immediately (Table 11).

4.3. Behavioral response during dissolved oxygen depletion

For lethal DO of rockbream, swimming performance, body color changes were proper indicators for fish behavioral response observation. In 20°C, rockbream showed normal behavior within 70 minutes with 2.5 mg/L (33.88% saturated); their behavior became unstable when the DO was 2.2 mg/L (29.81% saturated). In 25°C, the normal behavior of fish was observed within 60 minutes with 2.45 mg/L of DO (36.3% saturated); the unstable behavior was observed at 2.1 mg/L of D.O (31.1% saturated). In 30°C, the normal behavior was investigated within 20 minutes with 3.1 mg/L (49.84% saturated), while the unstable behavior was found in 1.95 mg/L (30.55% saturated). In three water temperatures of lethal DO experiment, rockbream showed similar abnormal behavior such as swimming with head up, swimming to top, then sinking to bottom, upside down swimming, sinking down to the bottom of RC, struggling

Table 11. Relationship between breath frequency and DO at different temperatures

Time (min)	20°C		25°C		30°C	
	DO (mg/L)	Breath/min	DO (mg/L)	Breath/min	DO (mg/L)	Breath/min
10	6.5±0.2	124.5±16.7 ba	6±0.0	145.0±14.0 ba	4.8±0.2	180.3±5.2 a
20	5.8±0.2	122.7±2.4 ba	5.2±0.3	143.0±12.3 ba	3.1±0.3	163.7±9.4 b
30	5.0±0.2	128.7±12.3 ba	4.3±0.4	142.8±13.4 ba	2.0±0.1	173.3±3.3 ba
40	4.2±0.5	132.7±17.0 ba	3.6±0.4	140.5±9.7 ba	1.4±0.1	172.2±0.7 ba
50	3.5±0.6	133.8±13.0 ba	3.1±0.4	145.0±5.7 ba	1.1±0.1	169.3±0.5 b
60	3.0±0.6	134.3±5.7 ba	2.5±0.5	147.0±1.4 ba	1±0.0	89.2±0.7 c
70	2.5±0.5	138.0±7.1 a	2.1±0.3	155.5±6.4 a	-	-
80	2.2±0.4	139.0±0.0 a	1.6±0.1	137.8±11.1 ba	-	-
90	1.8±0.4	141.5±1.2 a	1.3±0.0	141.7±17.0 ba	-	-
100	1.6±0.4	140.7±0.0 a	1.1±0.1	137.8±15.8 ba	-	-
110	1.3±0.4	135.0±7.1 ba	0.1±0.1	127.7±14.4 b	-	-
120	1.1±0.3	134.3±6.6 ba	0.9±0.0	48.2±2.6 c		
130	0.9±0.1	117.8±6.8 b			-	-
135	0.8±0.0	64.3±8.0 c	-	-	-	-

Each value represents mean ± SD. Different letter indicate significant difference in each water temperature, respectively ($P<0.05$, one-way ANOVA multiple comparison (LSD)). DO: dissolved oxygen, min: minute.

behavior by hitting the wall of RC, laid down and gasping on bottom of RC and then stopped breathing. The black stripe color of dying fish changed into light brown and appeared white color at the nape, around eyes, and gill juncture, when the fish died, their body color changed into pale (Table 12).

4.4. Behavioral response during recovery

Moderate hypoxia: After blood sampling, all fish swam with normal body balance, but their black stripe color was still brown and they lost their appetite. After six hours, all fish recovered their appetite, but their black stripe color was still brown. After 24 hours, all the fish returned to normal black stripe color. Death of fish did not occur until the end of the experiment (two week).

Hypoxia: After blood sampling, one of the three fish swam with abnormal body balance (45°), while other two fish swam with the normal body balance, but their black stripe color was still brown and lost their appetite. After 6 hours, all fish return to normal swimming behavior. One of three fish was kept losing appetite and isolated from the group, while two other recovered their appetite and swam in group. After one week, all the fish returned to normal

Table 12. Behavioral index of *Oplegnathus fasciatus* until lethal DO at different water temperatu

Time (min)	20°C DO (mg/L)	Behavioral index	Dead fish	25°C DO (mg/L)	Behavioral index	Dead fish	DO (mg/L)
10	6.5±0.2	I	0	6.0±0.0	I	0	4.8±0.2
20	5.8±0.2	I	0	5.2±0.3	I	0	3.1±0.3
30	5.0±0.2	II	0	4.3±0.4	II	0	2.0±0.1
40	4.2±0.5	II	0	3.6±0.4	II	0	1.4±0.1
50	3.5±0.6	II	0	3.1±0.4	II	0	1.1±0.1
60	3.0±0.6	III	0	2.5±0.5	IV	0	1±0.0
70	2.5±0.5	IV	0	2.1±0.3	IV	0	-
80	2.2±0.4	IV	0	1.6±0.1	IV	0	-
90	1.8±0.4	IV	0	1.3±0.0	IV	0	-
100	1.6±0.4	V	0	1.1±0.1	V	0	-
110	1.3±0.4	V	0	0.1±0.1	VI	0	-
120	1.1±0.3	VI	0	0.9±0.0	VII	1	-
130	0.9±0.1	VI	0	-	-	-	-
135	0.8±0.0	VII	1	-	-	-	-

Each value represents mean ± SD. min: minute.

appetite and normal swimming. Death of fish did not occur until the end of the experiment (two week).

4.5. Blood properties

4.5.1. Lethal dissolved oxygen

Oxygen factors: As shown in Table 13, the percentage of Ht at control and experiment groups increased with water temperature and showed significant difference. In every temperature, the percentage of Ht level in experiment was significantly different from the control group. The levels of Hb in control and experiment group were significantly different. However, it did not show significant difference by increasing temperature.

Osmoregulation factors: As shown in Table 14, the level of plasma Na^+ between experiment and control group showed significant difference. In experiment group, the highest level was at 25°C while 20 and 30°C did not show significant difference. The plasma of Na^+ in experiment group showed significant difference from the control group in every temperature. The plasma K^+ in experiment group showed significant difference from control group

Table 13. Whole blood properties of *Oplegnathus fasciatus* in different water temperatures of lethal DO experiment

Water temperature (℃)		Ht (%)	Hb (g/dL)
20	Con	26.0 ± 1.4^{b}	7.0 ± 0.7
	Exp	$28.8 \pm 0.2^{b*}$	$7.4 \pm 0.5^{*}$
25	Con	27.0 ± 2.8^{ba}	7.7 ± 1.0
	Exp	$35.5 \pm 5.4^{ba*}$	$9.6 \pm 2.4^{*}$
30	Con	31.5 ± 3.5^{a}	9.8 ± 1.5
	Exp	$39.1 \pm 0.8^{a*}$	$10.2 \pm 0.4^{*}$

Each value represent as mean ± SD (n = 16). Different letter indicate significant difference in each water temperature, respectively. Asterisk indicate significant difference between control and experiment in each temperature ($P<0.05$, two-way ANOVA multiple comparison (LSD)). Con: control, Exp: experiment, Hb: hemoglobin, Ht: hematocrit and showed significant difference by changing temperature. The highest level was at 25°C, while 20 and 30°C did not show significant difference. The plasma level of Cl^-, Ca and osmolality in experiment showed significant difference between control and experiment group, but did not show significant difference by changing temperature. The plasma of Mg in experiment group showed significant difference by changing temperature. The highest of plasma Mg was 25°C, while the 20 and 30°C did not show significant difference. The concentration of Mg in plasma in this experiment showed significant difference from the control group.

Stress factors: The concentration of glucose in control and experiment group showed significant difference by increasing water temperature. The highest level was observed at 25°C, while the 20 and 30°C were not significantly different. The glucose level in experimental group showed significant difference from the control group in every temperature. Plasma of total protein in control and experiment group was not significantly different by increasing temperature, but showed significant difference between control and experiment group in every change of temperature. The concentration of plasma

Table 14. Biochemical properties of *Oplegnathus fasciatus* blood in different water temperatures of l

Water temperature (°C)		Na^+ (mEq/L)	K^+ (mEq/L)	Cl^- (mEq/L)	Ca (mg/dL)	Mg (mg/dL)	Osmolality (mmol/kg)	Cortisol (ng/mL)
20	Con	178.5±3.5	4.8±0.1^{b}	147.5±6.3	10.0±0.0	1.0±0.1	318.0±0.0	1.9±0.3
	Exp	193.5±4.9^{b*}	4.8±0.5^{b*}	155.5±3.5*	13.5±0.0*	2.7±0.7^{b*}	369.5±7.8*	41.6±2.6^{c*}
25	Con	180.5±0.7	4.4±0.1^{a}	142.5±2.1	9.5±0.6	1.5±0.0	314.5±3.5	2.2±0.1
	Exp	203.0±1.4^{a*}	7.7±1.4^{a*}	170.5±4.9*	14.9±0.5*	5.2±0.5^{a*}	384.5±4.9*	64.7±11.4^{b*}
30	Con	179.5±3.5	4.4±0.4^{b}	152.0±0.0	10.9±0.2	1.6±0.1	320.5±0.7	2.7±0.3
	Exp	192.0±1.4^{b*}	4.6±0.0^{b*}	164.0±4.2*	12.9±0.8*	2.6±0.6^{b*}	372.5±2.1*	87.8±3.5^{a*}

Each value represent as mean ± SD (n = 16). Different letter indicate significant difference in each water Asterisk indicate significant difference between control and experiment in each temperature ($P<0.05$, tw comparison (LSD)). Con: control, Exp: experiment.

cortisol level in experiment group showed significant difference by increasing temperature and was significantly different between control and experiment group in every temperature (Table 14).

4.5.2. Recovery from different low dissolved

Oxygen factors: As shown in Table 15, the Ht and Hb level of rockbream in control, moderate hypoxia and hypoxia group did not show significant difference, but the highest level was in hypoxia group.

Osmoregulation factors: As shown in Table 16, the plasma level of Na^+, Cl^-, Ca and osmolality did not show significant difference between the control group, moderate hypoxia and hypoxia group. The concentrations of Cl^- and Ca in control group were higher than moderate hypoxia and hypoxia group, but for the Na^+ and osmolality, the highest level was found in hypoxia group. The level of K^+ showed significant difference and the highest was found in control group. On the contrary, the plasma of Mg also showed significant difference, but the highest level was found in moderate hypoxia group.

Table 15. Whole blood properties in recovering behavioral response experiment at different DO concentrations

Group	Ht (%)	Hb (g/dL)
Con	26.0 ± 1.4	7.0 ± 0.7
MH	26.0 ± 0.9	7.0 ± 0.1
HP	27.5 ± 0.7	8.0 ± 0.7

Each value represent as mean ± SD (n = 6) ($P<0.05$, one-way ANOVA multiple comparison (LSD)). Con: control, HP: hypoxia, Hb: hemoglobin, Ht: hematocrit, MH: moderate hypoxia.

Stress factors: Exposing rockbream at low DO concentration induced stress to rockbream. Plasma of cortisol and glucose increased with depletion of DO concentration. The highest levels of both component were found in hypoxia group. The plasma of total protein did not show significant difference, but the highest was found in hypoxia group (Table 16).

Table 16. Biochemical properties of *Oplegnathus fasciatus* blood in recovering behavioral respo
different DO concentrations

Group	Na^{+} (mEq/L)	K^{+} (mEq/L)	Cl^{-} (mEq/L)	Ca (mg/dL)	Mg (mg/dL)	Osmolality (mmol/kg)	Cortisol (ng/mL)	
Con	174.0±9.9^{a}	4.8±0.1^{a}	147.5±6.3^{a}	10.0±0.1^{a}	1.0±0.0^{b}	318.0±0.0^{a}	1.9±0.3^{c}	5
MH	175.5±4.9^{a}	3.7±0.1^{b}	135.0±2.8^{a}	8.7±0.1^{a}	2.0±0.3^{a}	314.0±5.7^{a}	18.6±1.1^{b}	10
HP	176.5±3.5^{a}	4.1±03^{b}	141.0±4.2^{a}	9.5±1.1^{a}	1.7±0.0^{a}	319.0±5.7^{a}	41.6±2.6^{a}	12

Each value represent as mean ± SD (n = 6). Different letter indicate significant difference in differ
(P<0.05, one-way ANOVA multiple comparison (LSD)). Con: control, HP: hypoxia, MH: moderate hyp

Discussion

Changing water temperature had strong affect to OC of rockbream. The tendency of OC of rockbream in this study increased at the same time with water temperature. Fish is an ectothermic animal; therefore, temperature is one of the most important factors that influence the respiratory metabolism. The rate of metabolism increased as the environmental temperature increases. This rise occurs because of the reactants in the cell have greater thermal energy and the activity level of all biochemical processes in organs and tissues, particularly the enzyme activities. The similar tendency of increasing OC with regards to the water temperature of rockbream was observed in many fish species such as juvenile rockbream (Oh et al., 2006), gilthead sea bream *Sparus aurata* (Requena et al., 1997), toadfish *Opsanus tau* (Haschemeyer, 1969), Chinese sturgeon *Acipenser sinensis* (Liu et al., 2011), pacific beakfish *Oplegnathus insigni* (Segovia et al., 2012); and olive flounder *Paralichthys olivaceus*, seabass *Dicentrarchus labrax*, red seabram *Pagrus major,* and black seabream *Spondyliosoma cantharus* (Kim et al., 1995).

The breath frequency and their activity of rockbream in this study increased at the same time with water temperature. Increasing the metabolic rate induced muscles work harder and consumed more energy. The muscles needed more oxygen to sustain this increased level of activity. Heart needed to pump blood faster to get more oxygen to support muscles during high temperature, so fish needed to increase breath frequency to uptake more oxygen from the ambient water (Farrel and Steffensen, 1987), diffuse in blood, dissolve into plasma, and chemically bind with Hb contained in red blood cell. On the other hand, the breath frequency also increased in line with oxygen consumption. Farrel and Steffensen (1987) pointed out that, branchial pumps of trout used 10-15% of total oxygen uptake. Swimming bass *Morone saxatilis* and bluefish *Pomatomus saltatrix* the cost was estimated to be 8.1 and 8.4%, respectively (Freadman, 1981). The relationship between breath frequency, activity and oxygen consumption pattern of rockbream, which was found in present study was similar to silver perch *Bidyanus bidyanus,* rainbow fish *Melanotaeniidae* and rainbow trout *Oncorhynchus mykiss* (Patra et al., 2009); and largemouth bass *Micropterus salmoides*, spotfin shiner *Cyprinella* *spiloptera*, and channel catfish *Ictalurus punctatus* (Hocutt, 1973).

As to the result of this study, the temperature had effect on some parameters of the blood properties of rockbream. For the oxygen factors, Hb value increased with water temperature, did not show significant difference among the three water temperatures, but the highest value occurred at 30°C. The importance of reduction of hemoglobin can modify the oxygen quantity from tissues and can lead to the slowing down of the metabolic activity and the meager production of energy (Atamanalp et al., 2002). The tendency of Hb and Ht were parallel with the OC and activity tendency. Increasing Hb with water temperature in rockbream was similar to snakehead fish *Chana punctatus*, which was exposed in 15°C, 20°C, 30°C and 35°C for 96 hours. The level of hemoglobin content was found to be decreasing at 15 and 20°C and increased at 30 and 35°C (Ravichandra, 2012). Hb level of large olive flounder increased with water temperature (Chang et al., 2001). Imanpoor et al. (2011) exposed gold fish *Carassius auratus* in 23, 27 and 31°C for 45 days, the highest of Ht was found in 31°C but it was not significantly different from the other temperatures. Mirea et al. (2013) stated that the tendency of Hb of Nile tilapia

Oreochromis niloticus decreased by decreasing the water temperature, while the highest value Ht was recorded at low temperature. For the osmoregulation factors, the highest concentration of plasma Na^+, K^+ and Mg were, recorded at 20°C, while Cl^-, Ca and Osmolality were recorded at 30°C. Mackay (1974) acclimated gold fish at 10, 20 and 30°C, the highest plasma of Na^+, Cl^- and osmolality was found at 30°C, while the K^+ was at 10°C. Only plasma of Osmolality and K^+ were similar to rockbream, while Na^+ and Cl^- were opposite. However, Prosser (1970) stated that, in freshwater fish was opposite to marine fish. Freshwater fish had either reduced or unaltered plasma Na^+ and Cl^- concentration when fish was acclimated to low temperature. Houston and Koss (1982) acclimated gold fish at 20, 25 and 30°C, The highest level of Na^+ and K^+ occurred at 20°C and Cl^- was at 25°C, while Ca and Mg was found at 30°C. Environmental stressors such as changes in water temperature acted on the nervous system, causing the release of corticoid stress hormones via humoral responses through the hypothalamus pituitary-internal axis (Choi, 2010). When the fish exposed in stress condition, level of glucose increased together with cortisol. When fish was acclimated to a new environment, it resulted to increased metabolic activity, which was correlated in changing quality and quantity of certain enzymes involved in energy metabolism and compensate with the modifications in the rate of protein synthesis (Kamal and Omar, 2011). With regards to the result of this study, the highest level of cortisol, glucose and total protein were observed at high temperature (30°C), because the optimum temperature of rockbream was ranged from 20 to 25°C. The similar tendency was observed in olive flounder and fat cod, the plasma cortisol and glucose increased with water temperature (Chang et al., 2001). Hur et al. (2008) abrupt increased and decreased in temperature repetitively observed from 24 to 26, 24 to 29 and 24 to 33°C for 21 days in olive flounder, the highest plasma level of cortisol and glucose were reported at 33°C.

Salinity is also one of the most important factors that affect osmoregulation in fish. Some fish species can live in a wide range of salinity (euryhaline) such as starry flounder *Platichthys stellatus* (Lim et al, 2013) and black porgy *Acanthopagrus schlegeli* (Tomy et al., 2009); and some fish species cannot tolerate in wide fluctuation of salinity (stenohaline) such as gold fish (Luz et al., 2008). When fish was exposed in hypo-osmotic environment, fish acted as hyper-osmoregulatory function to maintain their body fluid. On the contrary, when fish was exposed in hyper-osmotic environment, fish acted as hypo-osmoregulatory function (Lim et al., 2013). Swanson (1998) stated that when the fish was exposed in salinities, which differed from the internal osmotic concentration, fish must impose energetic regulatory costs for active ion transport. Lim et al. (2013) reported that amount of energy consumed in osmoregulation was smaller when the fish was reared in salinity similar to osmolality in the body fluid. In this study, OC, activity and breath frequency of rockbream increased at the same time with salinity. So, the OC of rockbream was clearly related to salinity changes. The result found in this study was also similar to Yan et al. (2008), which stated that OC of rockbream increased with salinity. This tendency was not only occurred in rockbream, but also on other species such as *Phoxinus erythrogaster* and *Fundulus catenatus* (Toepfer and Barton, 1992), and Chinese sturgeon (Liu et al., 2011). In the case of milkfish *Chanos chanos*, Swanson (1998) investigated OC at 15, 35, and 55 psu, but the peak of OC and activities were found at 35 psu. However, in case of tilapia, OC decreased with the increase of salinity (Abdul et al., 2012)

Fluctuation of salinity in the environment can cause stress in fish and will lead to the increase or decrease in some of the blood parameters (Salati et al., 2010). However, the level of Hb and Ht of rockbream did not show significant difference by changing salinity, but the similar tendency of rockbream was reported in gold fish, which was exposed in 6 salinity levels: 0, 2, 4, 6, 8 and 10 psu (Luz et al., 2008). In this study, changing salinity effected to some ion in the blood of rockbream. Na^+, K^+ and Ca were considered as the core of heart, nerve, muscle and tissue function. Prakoso (2014) stated concentration of Na^+, K^+ and Ca affected on the activities and metabolic process at low salinity of gray mullet *Mugil cephalus*. In present study, the low concentrations of Na^+, K^+ and Cl^- were also reported in the low salinity. On the other hand, the low metabolic activity and OC of rockbream were also observed at low salinity. As to the result of this study, the lowest plasma osmolality was found at 25 psu, while highest level was found at 35 psu. For the level of cortisol and glucose in this study increased with decreasing of salinity and showed significant difference. The lowest level was observed at 25 psu while the highest was at 15 psu. Glucose and cortisol plsama were considered as the sensitive indicator of environmental stress (Hattingh, 1976). In general, when marine fish were exposed to low salinity environment, osmoregulation confusion can induce in lowering of osmolality and ion content in blood, which directly induce to stress. When fish was exposed in the stress condition, the activity of hypothalamus-pituitary gland-internal axis increased, which induce cortisol excretion in the blood (Wendelaar Bonga, 1997). Salati et al. (2010) stated that stress induced hyperglycemia is caused by catecholamine that affect the liver to release glucose in blood stream The increased glucose provides energy to maintain plasma osmolality range. Stress hormone implicated in osmoregulatory processes. The tendency of glucose in rockbream was similar to winter flounder *Pseudopleuronectes americanus* (Plante, and Audet, 2002). Luz et al. (2008) reported that plasma of cortisol in gold fish increased with salinity. However, gold fish is stenohaline like rockbream, but it is freshwater fish, when it was exposed in hyper-osmotic, it acted as hypo-osmoregulatory function to absorb ion and emits water to maintain its body fluid. The plasma of total protein on rockbream did not also change by changing salinity. The result of this study was similar to Salati et al. (2010), which suggested that plasma of total protein did not involve in osmoregulation response with the increasing of salinity in common carp *Cyprinus carpio*.

Itazawa and Hanyu (1991) stated that, the total OC of multiple fish was higher than single fish, but the mass-specific OC of the multiple fish was lower than the single fish. Bowen (1932) showed that catfish *Ameiurus melas* in groups of four have greater rate of OC per fish than isolated fish, but there was no significant difference. Joeng et al. (2007) stated that black porgy in 3 fish numbers consumed oxygen more than single fish in both seawater and fresh water. The tendency of OC of rockbream in present study also increased with the number of fish in the same water temperature, salinity, and water volume. On the contrary, the OC of olive flounder, tiger puffer, rockfish, seabream, red seabream, and black seabream increased with the decreasing number of fish (Kim et al., 1995). In cast of juvenile Atlantic sturgeon *Acipenser oxyrinchus*, the number of fish did not effect on OC (Szczepokowsk et al., 2011)

High stocking density has been shown to affect some metabolic pathways, such as those related to lipid metabolism. Vijayan et al. (1990) reported a mobilization of triglycerides in brook charr *Salvelinus fontinalis* to help meet the increased energy demand associated with high rearing density, suggesting an enhancement of gluconeogenic capacity from glycerol. High stocking density can be considered as the chronic stress in aquaculture. Stress of fish can be evaluated by the measurement of biochemical indicators, such as changes in hematology and plasma chemistry (Kamal and Omar, 2011). A hematological characteristic is an important tool, which can be used as an index to monitor physiological and pathological changes in fish (Kori-Siakpere et al., 2005). The result of present study showed that the value of Ht and Hb did not show significant difference by increasing fish number, but the highest value of Hb was observed at 1 fish number, while the highest value of Ht was found at 3 fish. Kamal and Omar (2011) reported that Hb and Ht values of silver carp fingerling increased with decreased number of fish. Charoo et al. (2013) stated that the high value of Hb and Ht of rainbow trout were observed in high density. The result of Hb rockbream was similar to silver carp fingerling but different from rainbow trout trout, while Ht was similar to rainbow trout, but different from silver carp. However, Sommouth et al. (2009) stated that Ht value of seabass did not affect by increasing stocking density. The alternative fish blood biochemistry may be indicative of unsuitable environmental conditions or the presence of stressing factors (Barcellos et al., 2004). Plasma of cortisol levels indicated stress indicators in fish. An elevated of blood cortisol level results in gluconeogenesis. Plasma of glucose level has been used as an indicator of environmental stress to reflect changes in carbohydrate metabolism under stress conditions (Kamal and Omar, 2011). The level of plasma cortisol and glucose in this study increased with increasing of fish number. The similar result of glucose was observed in European sea bass (Lupastsch et al., 2010), silver carp *Hypophthalmichthys molitrix* (Kamal and Omar, 2011) and gilthead sea bream (Barton et al, 2005), however, Sammouth et al. (2009) reported that the plasma of glucose of seabass was not affected by changing stocking density. The similar result of cortisol was reported in gilthead sea bream (Montero, 1999). Plasma of total protein was considered diagnostic value in fish which is also related to general nutritional status as well as the integrity of the vascular system and liver functions; and stress factor. In present study, however, total protein did not show significant difference by increasing fish number, but the highest level was observed at 6 fish numbers, the similar tendency was observed in silver carp (Kamal and Omar, 2011).

As to the relationship between OC and fish activity in freshwater fish, they were categorized into three; diurnal, nocturnal and arrhythmic (Chang et al., 2005). Bone and Marshall (1982) stated that oxygen demand varies depending on the activity of fish. The high value of OC of rockbream in this study was found during the light period, while the low value was found during the dark period. The result of this study was similar to previous study, which reported that the OC rates of rockbream during light period was higher than that of dark period (Oh et al., 2006). Rockbream was sensitive with light, which can induce more activity. As to its sensitivity to light, rockbream can be assumed as diurnal species. The highest OC of rockbream showed at 1 hour after starting the light. This result was similar to black porgy (Chang et al., 2005), while gray mullet occurred at 2 hours after starting the light period (Prakoso, 2014).

Kim et al. (1995) stated that the level of lethal DO deepened on the water temperature, population density and body weight. Burdick et al. (1954) reported that the lethal oxygen concentration of smallmouth bass *Micropterus dolomieu* increased at the same with water temperature (0.6 mg/L at 11.5°C, 0.9 mg/L at 21.3°C and 1.1 mg/L at 26.9°C). The level of lethal DO, investigated in this study also increased with water temperature (0.8 mg/L at 20°C, 0.9 mg/L at 25°C and 1 mg/L at 30°C).

The activity of rockbream decreased with DO depletion. Increasing water temperature affected on DO concentration level in the water. For ectothermic animals, temperature is main factor, which induce the metabolic rate and lead to hypoxia. Leveelahti et al. (2011) stated that hypoxia induced abnormal behavior of threespine stickleback. In this study, during hypoxia condition, fish almost lost their body balance, showed struggling behavior, sank down to the RC bottom of RC, laid on the RC bottom until dies. The behavior response of rockbream during hypoxia was similar to the behavior of pigface bream fish *Lethrinus lentjan* during exposed in hypoxia, which was reported by El-Badawi (2014).

The breath frequency of rockbream did not show relationship with the depletion of DO and OC, even fish lost the body balance or laid down on the bottom of RC, but the breath frequency of rockbream was not so far different from the beginning of experiment. The sharp decreased of breath frequency occurred at the level of lethal DO. Brauner et al. (1998) reported that, exposing fish in hypoxia condition would increase its ventilation rate to minimize hypoxia stress both by increasing oxygen uptake and enhancing convective condition for CO_2 removal. Woo, et al. (1984) reported that fish increased the number of perfused gill lamellae to increase water flow over gill and enhanced gill diffusion capacity for maintaining oxygen delivery. The similar tendency was recorded in some fishes such as brown bullheads *Ameiurus nebulosus* and carp (Saunders, 1962); and flounder *Plutichthysflesus* (Steffensen et al., 1982). On the contrary, Prakoso (2014) stated that the breath frequency of grey mullets showed relationship with OC and depletion of DO.

Acclimation of fish to new environment tends to increase the metabolic activity, it also induces the change of quality and quantity of certain enzymes involved in the energy metabolism (Kamal and Omar, 2011). Exposing fish in hypoxia induces various harmful effects on hematological, biological and physiological parameters of fish (Žikić et al., 2002). Decreasing oxygen concentration in water increased Hb and Ht concentration in the fish blood

(Žikić et al., 2002). When fish was exposed in the stress condition, the activity of hypothalamus-pituitary gland-internal axis increased, which induce cortisol excretion in the blood. Moreover, this hormone promotes glucose synthesis in liver and it was being used as energy to maintain homeostasis in fish (Lim et al., 2013). Genetic mechanisms, such as the up-regulation of hypoxia-inducible factor 1α (HIF-1 α), can trigger several physiological responses to improve oxygen transport and carbohydrate metabolism that can allow an individual to cope with hypoxic conditions (Soitamo et al. 2001). In present result, all the parameters in experiment group was significantly different higher than control group in every temperature. The similar tendency was found in flowerhorn fish *Vieja synspilus* (Kupittayanant and Kinchareon, 2011).

In the experiment of recovering response of hypoxia condition, the abnormal behavior of fish was not found during recovering experiment in moderate hypoxia group (8.6 to 4 mg/L). However, hypoxia group (8.6 to 2 mg/L), the abnormal behavior of fish was found when DO was 2.8 mg/L. The occurrence of abnormal behavior at 2.8 mg/L was similar to Diaz and Rosenberg (1995), which stated that the hypoxia level was at the level of DO less than 2.8 mg/L. After blood sampling and returning to the rearing tanks, moderate hypoxia group took only 6 hours to recover their appetite and 24 hours to bring back the normal color, because fish only responded from blood sampling. However, for Hypoxia group, fish took one week to recover their normal behavior, because fish responded to two stress factors: blood sampling and hypoxia. At the end of experiment, death of fish was not observed in both groups. El-Badawi (2014) reported that death of pigface bream fish was not found when it was exposed in 1.4 and 2.8 mg/L at 25.9 ^{0}C and 35.8 psu for 3, 6 and 24 hours.

The Level of Hb and Ht of rockbream, which was exposed in different DO concentration, did not show significant difference. This tendency was contrasting to Žikić et al., 2002), which stated that decreasing oxygen in water, the concentration of Hb and Ht increase at the same time. However, result of the present study was similar to Queensland lungfish *Neoceratodus forsteri* (Kind et al, 2002). On the other hand, Gaulke et al. (2014) acclimated largemouth bass in 9 mg/L and 3.3 mg/L of DO concentration, only Hb and Ht showed significant difference. Meanwhile, other plasma such as glucose, cortisol, Na^+, K^+ and Cl^- did not show significant difference. In present study, however, Na^+, K^+ and Cl^- did not show significant difference, but cortisol, osmolality and glucose showed significant difference. Porchas et al, (2009) reported that cortisol and glucose indicated the stress factors of the fish. Blood glucose level was affected by the rate of carbohydrate metabolism when fish was under stress or hypoxia conditions.

In conclusion, temperature, salinity and fish number, have strong effect to fish activity, OC and blood properties of young rockbream. On the other hand, temperature had strong effect on the lethal DO level, speed of DO decrease and blood properties of young rockbream. To avoid hypoxia in the culture environment and high mortality, aeration should be increased, when the fish was exposed in high temperature and high stocking density in rockbream culture farm. Rockbream is not good in osmoregulation. In order to increase aquaculture production, rockbream should not be reared in low salinity.

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