Stress responses of olive flounder, *Paralichthys olivaceus* by sudden rise of temperature in low and high water temperature conditions

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Abstract- We examined effects of sudden change in water temperature (WT) on the stress responses of the cultured olive flounder, *Paralichthys olivaceus* in large size (FL, 30.2 cm in TL, 295.6 g in BW) and in small size (FS, 13.7 cm, 22.6 g). In Exp.I, the WT was increased from 14 to 17℃ during a 6 h period (0.5℃/hour) and maintained at 17℃ for 3 h. The temperature was returned to 14℃ for 6 h. In Exp.I, the temperature was increased from 14 to 20 (12 h) and 23℃ (18 h). After increasing the temperature, the fish were maintained at 23℃ for 3 h, and then returned to the original temperature for 12 and 18 h after 3 h, respectively. In Exp.II, the WT increased from 23 to 26, 29 and 32℃ by the same method of Exp.I. During the increase from 14 to 23℃, plasma cortisol levels of FS showed no significant differences in the group of temperature increase of 6 and 9℃, but FL showed significant differences of physiological change. In Exp.II, plasma cortisol levels at 23℃ were 2.4 ng/mL in FL and 0.1 ng/mL in FS. In the increase from 23 to 26℃, the cortisol levels were 20.7 ng/mL in FL and 16.2 ng/mL in FS at 6 h. In the 6℃ increase group, the levels increased to 14.9 ng/mL in FL and 5.5 ng/mL in FS at 12 h. In the 9℃ increase group, the levels increased to 48.5 ng/mL in FL and 10.6 ng/mL in FS at 18 h. The glucose levels of FL in the 9℃ increase group increased from 30.0 (23℃) to 59.0 mg/mL at 18 h and 48.5 mg/mL at 21 h. Lactic acid levels of FL in the 9℃ increase group were increased from 0.7 to 2.6 mmol/L at 18 h to 3.6 mmol/L at 21 h and to 1.7 mmol/L at 51 h. The survival rate was also very high during times of temperature increases and the stress response also occurred for maintenance of homeostasis following, but it was recovered in a short period of time. But there was a significant change in respiration rate by increasing the temperature from 29 to 32℃. In this study, a single stress event of a rise and drop of WT was given. It is necessary to study stress responses to repeated WT changes in future study.

Keywords- olive flounder, *Paralichthys olivaceus*, water temperature, stress, cortisol

I. INTRODUCTION

Physiological stresses of fish in fish farming are mainly induced from artificial factors [1-2] such as changes of water temperature [3-6], salinity [7-8], rearing density, handling, confinement and transport [9].

These stresses on fish induce the releases of catecholamine and cortisol in fish and these cause rapid metabolism of stored energy, harming health by biochemical effect [10-11] and slow growth [12]. Mazeaud et al.

reported that fish showed primary, secondary, and tertiary responses against the stress [13]. The primary response showed rapid exchange of plasma catecholamine and corticosteroid. When this response to stressful condition exceeded normal level, harmful secondary and tertiary responses occurred. Therefore, stress induces decreased energy metabolism, decreased growth rate and distracted reproduction.

WT change, one environmental stress factors, directly affects the metabolism of rearing fish. Barton and Iwama suggested that a rapid change of WT resulted in the changes of *in vivo* metabolism and hematology [10]. Rapid drop of WT such as cold-water mass, often occurring along the coast of eastern waters of Korea in summer, affects the growth and survival of farm fish [3-6]. On the other hand, the increasing of in WT due to heated effluent water from power plant, and high WT during the summer season affects also the health of fish in culture farm. Water at about 7℃ higher than natural seawater is made by the cooling system in power plant. This heated effluent water caused rapid change in WT at an adjacent coast. The rearing fish cannot easily adapt to such temperature changes and the growth and survival of farm fish will be directly influenced by these changes.

To obtain basic data on the stress response of farm fish to heated effluent water, we studied the stress responses of olive flounder, *Paralichthys olivaceus* to sudden changes in WT.

II. MATERIALS AND METHODS

2.1 Preparation of the experimental fish and conditions

Olive flounder (large size, FL, mean length: 30.2±0.5 cm, weight: 295.6±13.0; small size, FS, 13.7±0.1 cm, 22.6±0.6 g) were purchased from Finfish Research Center, National Fisheries Research and Development Institute, Korea, and kept for 3 weeks under a natural photoperiod in the tank supplied with a seawater-running system. Before the initiation of the feeding trial, fish were acclimated to experimental conditions for 2 weeks. The WT and salinity of the seawater during the period ranged from 19 to 21℃ and from 34 to 35 g/L, respectively. During the acclimation and experimental period, fish were fed a commercial extruded pellet (Ewha Oil and Fat Industry Co. Ltd., Korea) containing 56.0% crude protein and 12.0% crude lipid twice a day. Fish in the each group were hand-fed to apparent satiation, 100% of satiation, twice a day at 09:00 and 18:00 h. Uneaten feed was removed 30 min after feeding and deducted from feed consumption calculations.

An experiment tank of 270 L flow-through (water volume: 250 L) was used, and the experiments were conducted by manipulation WT (two types, Fig. 1 in a running seawater culture system. Control of WT was automatically regulated by seawater temperature control system (Hana Com., Korea).

To study the WT stress responses, in the case of 3℃ increase in Exp.I, WT increased from 14 (low-WT condition) to 17℃ within 6 h (0.5℃hour^{-1}) and maintained at 17℃ for 3 h. Thereafter, the WT was returned to the original WT within 6 h. In 6 and 9℃ increases of Exp.I, WT was increased to 20℃ within 12 h and 23℃ within 18 h, respectively. The WT maintained at 20 and 23℃ for 3 h, and then returned to the original WT within 12 and 18 h after 3 h, respectively, and maintained for 12 h in the original WT. In Exp. II, the WT increased from 23 (high-WT) to 26 (3℃ higher), 29 (6℃ higher) and 32 (9℃ higher) by the same method of Exp.I. The experiment was performed in duplicate, water exchange rate was 32 times of water volume and dissolved oxygen was maintained over 5 mg/L.

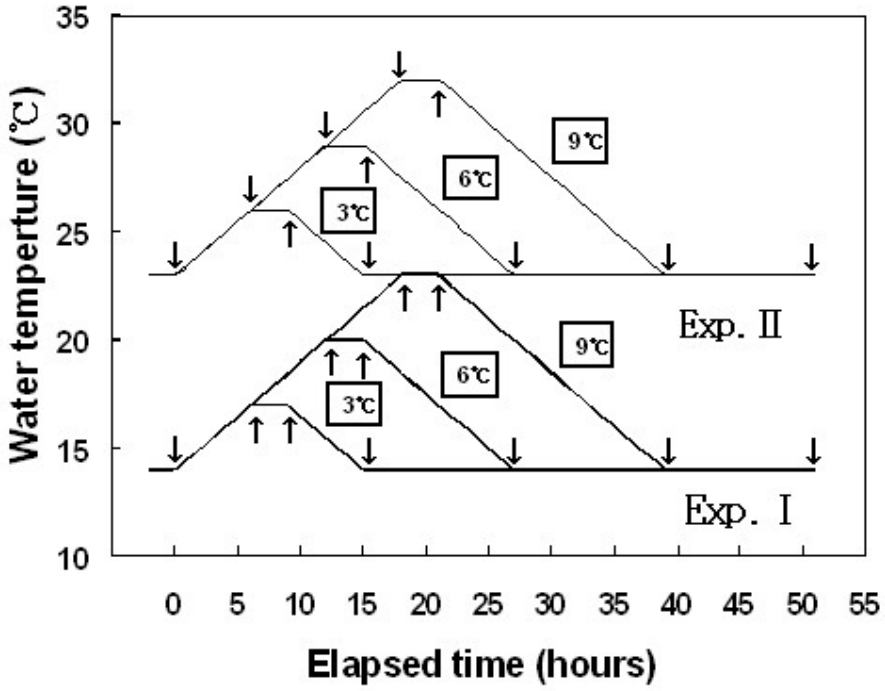

Figure 1. WT changes designed for the Exp. I and II.

2.2 Blood collection and analysis

The bloods of experimental fish were sampled from different tanks just before and after, 3 h after the WT increase and at the time returning to the original WT, and at the end of the experiment. Blood samples were collected from the caudal blood vessel complex using heparinized syringes within 1 min without anesthesia.

Hematocrit, red blood cells, and hemoglobin were analyzed immediately using an automatic blood analyzer (Excell 500, USA). Blood samples were kept in 2-mL vacuum containers treated with sodium fluoride/potassium oxalate (Vacutainer, UK) and in 1.5-mL polypropylene microcentrifuge tubes held on ice for less than 5 min before centrifugation at 5,600 *g* for 5 min. Plasma was then collected and stored in a deep freeze (CLN-500 UW Nihon Freezer; Nihon Co., Japan) at –80℃ until analysis.

Plasma cortisol concentrations were determined in 50-μL samples using radioimmunoassay kits (Coat-A-Count TKCO Cortisol RIA Kit; DPC, USA). Mixtures of samples in 100 mL of antiserum were incubated for 45 min at 37℃, and then 1000 mL of separation reagent was added. The mixtures were placed in a refrigerator at 4℃ for 15 min, then centrifuged at 1,200 *g* for 15 min. The supernatant was assayed for gamma radiation using an automatic gamma counter (Cobra II; Packard Co., USA). Glucose, lactic acid, aspartate aminotransferase (AST), alanine aminotransferase (ALT), Na^+, K^+, Cl^-, and total protein were analyzed using an automatic chemistry analyzer (Hitachi 7180, Hitachi, Japan). Osmolality was determined using a micro-osmometer (Fiske 210; Fiske, USA).

2.3 Statistical analysis

The experiment was performed in triplicate and results are reported as means ± SD unless otherwise stated. Data were analyzed by one-way ANOVA with the SPSS statistical package (SPSS Inc., USA). Means were separated by using Duncan's multiple range test and were considered significantly different at $P < 0.05$.

III. Results

3.1 Plasma cortisol, glucose and lactic acid

In the control group of low-WT condition, plasma cortisol levels ranged 2.7±0.1-3.1±0.1 ng/mL (FL) and 3.0±0.3-3.6±0.8 ng/mL (FS) during the experimental period (Fig. 2). The levels of the control group showed no significant differences to 3.0±0.4 ng/mL in FL and 2.8±0.8 ng/mL in FS at the beginning of the experiments of the experiment group. In the increase of 3℃, in FS showed no significant differences at 6 (1.3±0.2 ng/mL) and 9 h (3.7±0.2 ng/mL), but in the decrease from 17 to 14℃, it was significantly increased to 5.2±1.8 ng/mL at 15 h. In high-WT conditions, the cortisol levels from increasing from 23 to 26℃ were significantly increased in all FL (20.7±0.6 ng/mL) and FS (16.2±2.6 ng/mL) at 6 h. In the increase of 6℃, the cortisol levels of FL and FS was significantly increased to 14.9±0.5 and 5.5±1.5 ng/mL at 12 h, respectively. In the increase of 9℃ the cortisol levels were significantly increased to 48.5±2.5 ng/mL in FL and 10.6±6.2 ng/mL in FS at 18 h.

Plasma glucose levels were shown in Fig. 3. In increase of 6 and 9℃ in low-WT conditions, the levels of FS was significantly increased from 26.5±0.7 to 31.0±1.4 mg/mL at 12 h and to 32.0±0.0 mg/mL at 18 h. In the case of FL, the levels were significantly increased from 30.0±0.0 (at the beginning of experiments) to 59.0±1.4 mg/mL at 18 h and 48.5±5.0 mg/mL at 21 h by increasing 9℃.

In low-WT conditions, the lactic acid levels from increasing 3℃ were significantly increased from 0.6±0.0 (FL and FS) to 0.9±0.0 (FL), 0.9±0.1 mmol/L (FS) at 15 h, respectively (Fig. 4). In high-WT condition, the levels of FL from an increase of 9℃ were significantly increased from 0.7±0.0 to 2.6±0.5 (18 h), 3.6±0.1 (21 h) and 1.7±0.1 mmol/L (51 h).

3.2 Hematocrit, hemoglobin and osmolality

Hematocrit changes of fish in the WT increase of low (14℃) and high-WT (23℃) conditions were shown in Table 1. In the control group of FL, low (11.5±0.4-12.9±1.2%) and high-WT (15.4±0.9-16.4±1.0%) during experimental period were shown. By increasing by 3℃ in low and high-WT, hematocrit of FL significantly increased from 11.6±0.3 (at the beginning of experiments) to 27.0±10.0, 24.0±1.3% at 9 h, respectively. By increasing by 6℃, Hematocrit of FL and FS were significantly raised at 12 h. In the case of increasing by 9℃ was similar to those of 3 and 6℃. However, hematocrit of FL at 18 h was higher than 3 (30.8±1.2%) and 6℃ (31.0±1.1%).

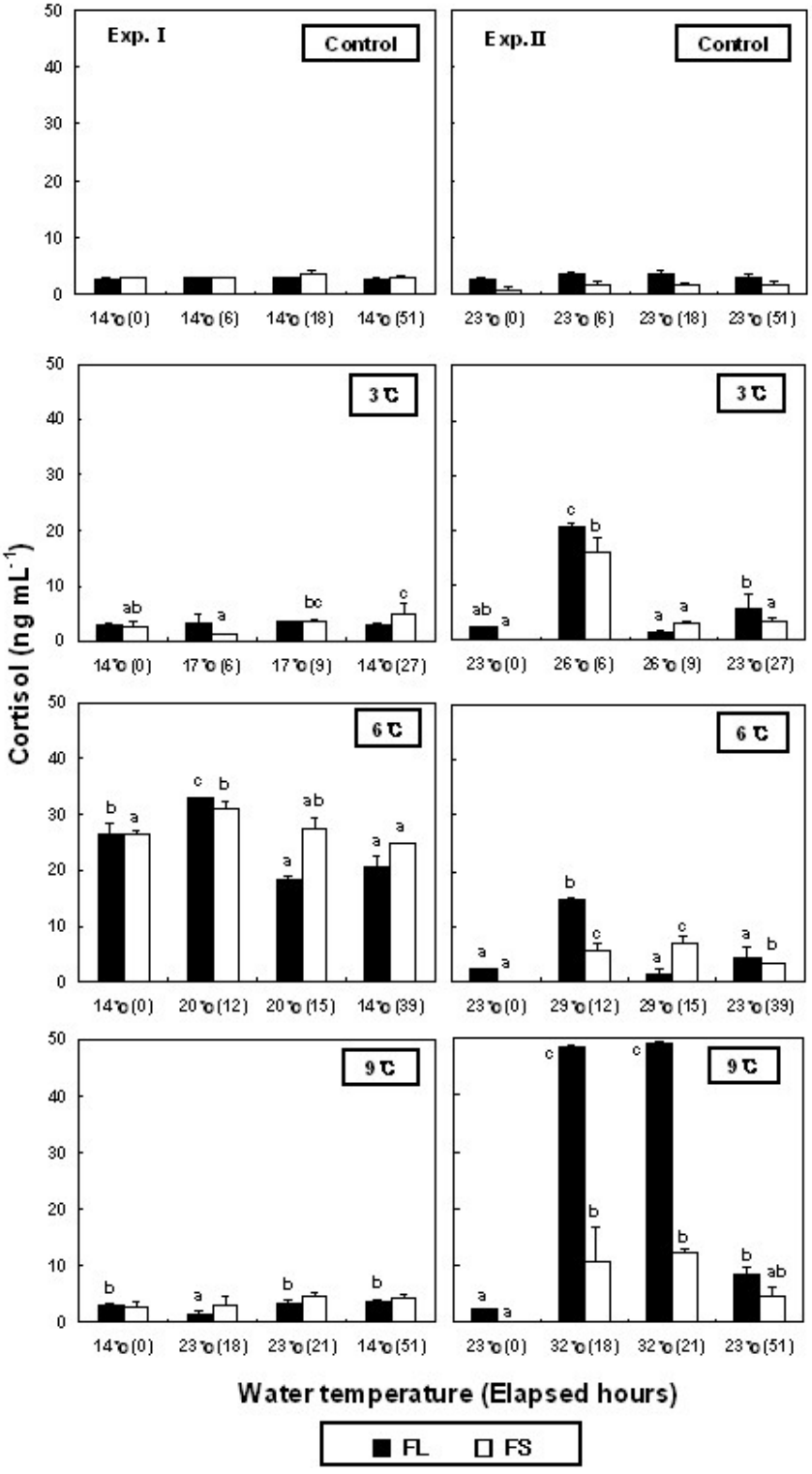

Figure 2. Variations of plasma cortisol in olive flounder, *Paralichthys olivaceus* after WT changes. Values are means ± SD (FL; *n*=6, FS; *n*=8) for experiments run on two occasions. Shared alphabetic letters on shaded bars indicate lack of significant difference (Duncan's multiple range test P **>** 0.05). FL: olive flounder in large group, FS: olive flounder in small group.

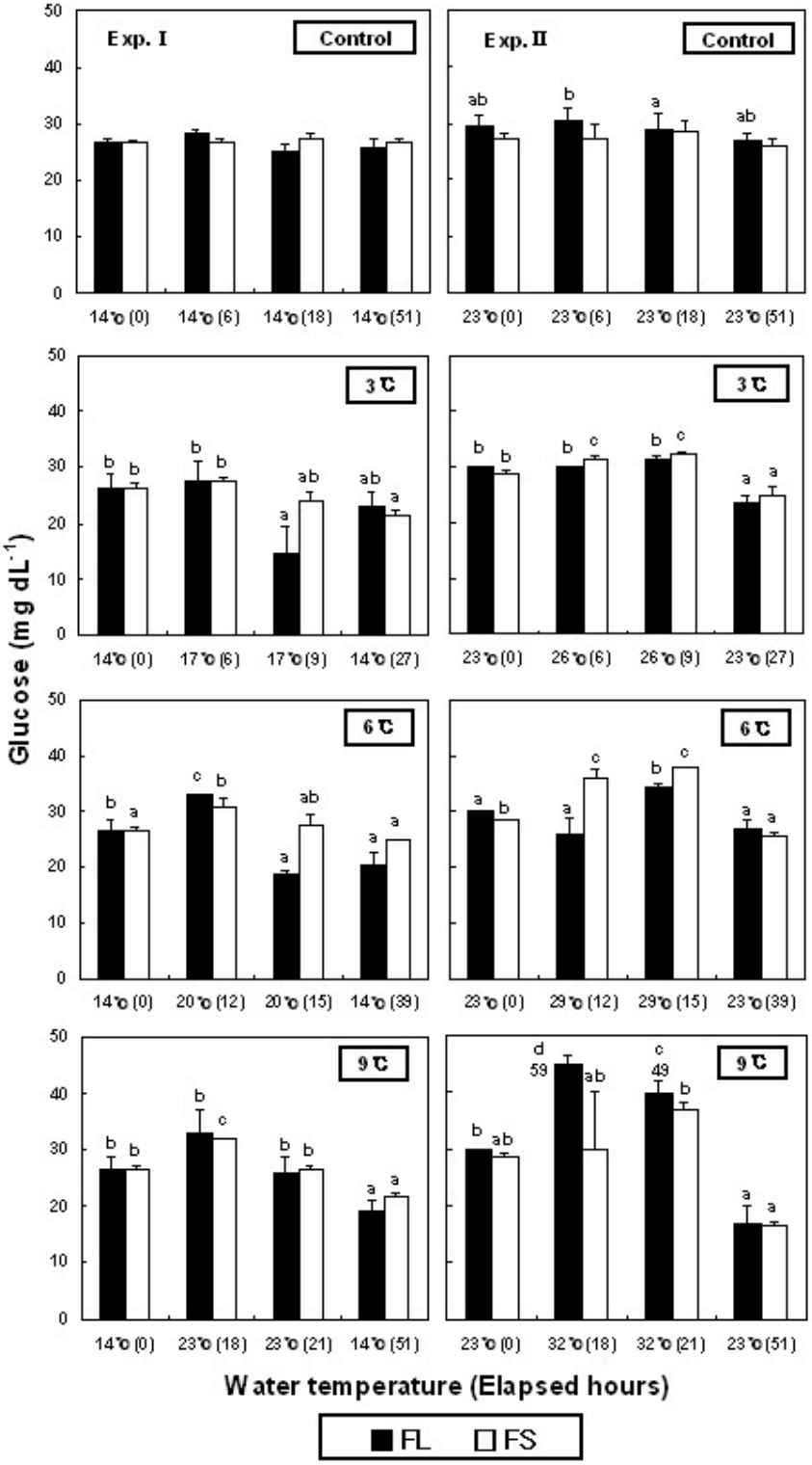

Figure 3. Variations of plasma glucose in olive flounder, *Paralichthys olivaceus* after WT changes. Values are means ± SD (FL; *n*=6, FS; *n*=8) for experiments run on two occasions. Shared alphabetic letters on shaded bars indicate lack of significant difference (Duncan's multiple range test P **>** 0.05). FL: olive flounder in large group, FS: olive flounder in small group.

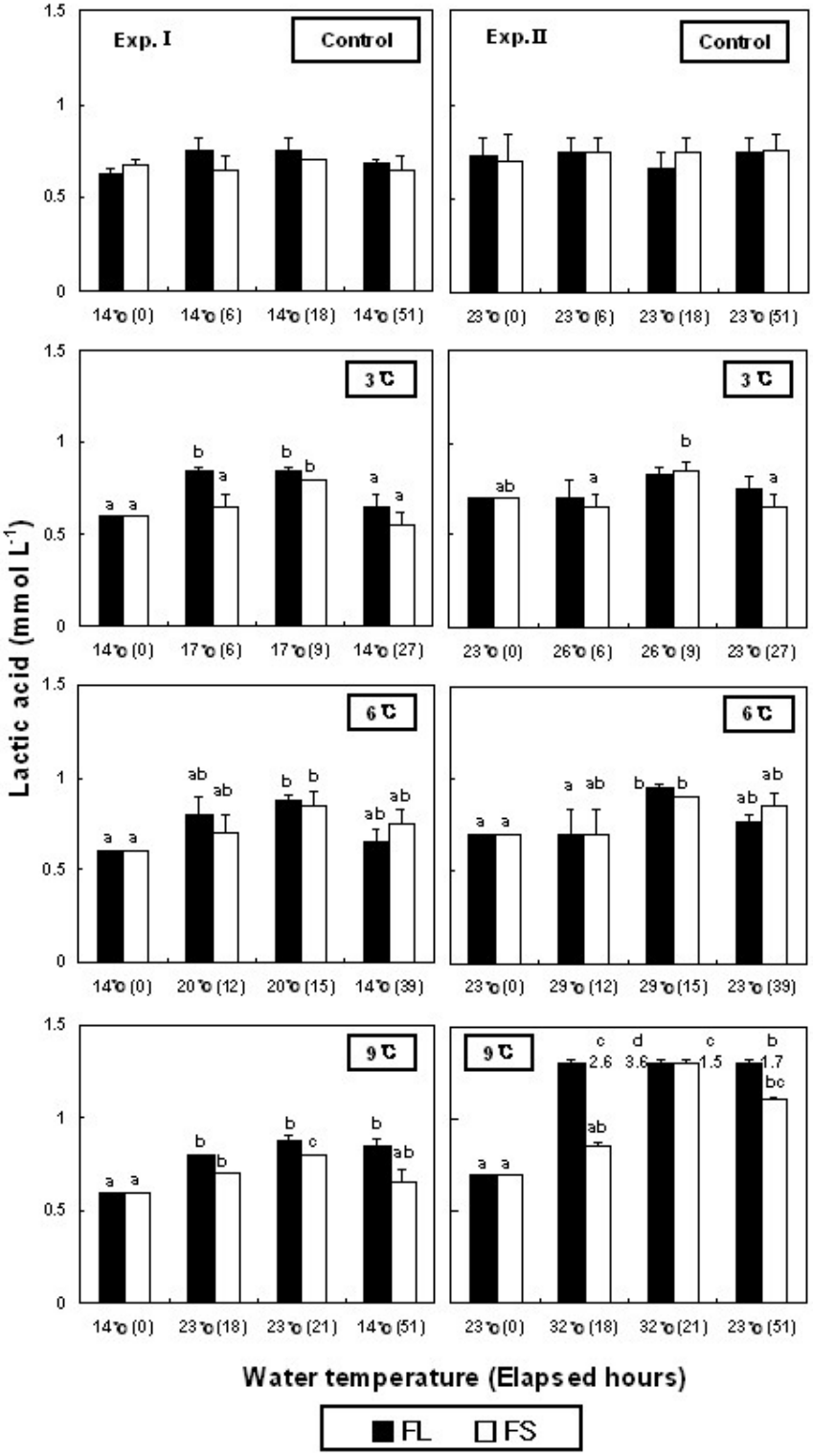

Figure 4. Variations of plasma lactic acid in olive flounder, *Paralichthys olivaceus* after WT changes. Values are means ± SD (FL; *n*=6, FS; *n*=8) for experiments run on two occasions. Shared alphabetic letters on shaded bars indicate lack of significant difference (Duncan's multiple range test $P > 0.05$). FL: olive flounder in large group, FS: olive flounder in small group.

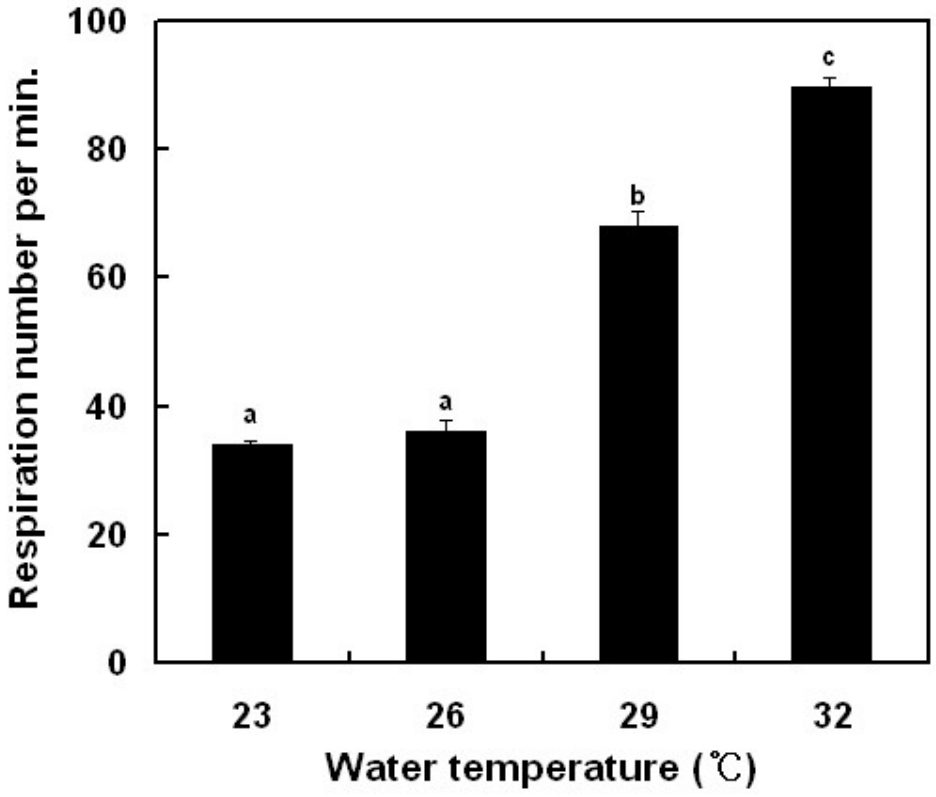

Figure 5. Variations of respiration number in olive flounder, *Paralichthys olivaceus* after WT changes.

Hemoglobin levels in low and high-WT conditions were shown in Table 3. Hemoglobin levels in low-WT during the experimental period showed no significant differences from the control groups. However, by increasing WT from 23 to 26℃ of FL, hemoglobin was significantly increased from 10.0±1.0 g/dL at the beginning of experiments to 12.0±0.9 g/dL at 6 h. In the increase of 9℃ of FL, hemoglobin was increased from 11.0±1.6 to 15.0±1.1 g/dL at 21 h. In the increase of 9℃ of FS, it was increased from 8.9±0.8 to 13.0±0.5 g/dL at 21 h.

Plasma osmolality levels in low-WT and high-WT conditions are shown in Table 4. The levels of FL and FS in low-WT during experimental period showed no significant differences, ranging from 433.5±7.8-443.0±9.9 mOsm/kg (FL) and 390.0±7.1-394.5±7.8 mOsm/kg (FS), respectively. The levels showed no differences in increasing from 14 to 17, 20 and 23℃, but the levels from increasing by 9℃ in high-WT were increased from 441.5±0.7 mOsm/kg at the beginning of experiments to 474.0±2.8 mOsm/kg at 18 h.

3.3 Respiration number and survival

Before increasing WT (23℃), respiration numbers of FL were increased from 34.0±2.4 to 36.1±5.0 time/min at 26℃, 68.0±7.2 time/min in 29℃ and 89.6±5.1 time/min at 32℃ (Figure 5). Survivals of FL and FS were 100%

until the end of the experiment in low-WT condition. In high-WT condition, they were 100% (FL and FS) by increasing 3 and 6℃, but in increasing 9℃, survival was 93.3% in FL (no data).

Table 1. Variations of blood hematocrit (%) in olive flounder, *Paralichthys olivaceus* after WT changes

Elapsed time (hours)	WT (℃)	Exp.I		WT (℃)	Exp.II	
		FL	FS		FL	FS
0	14	11.5±0.4^{a}	11.8±0.5^{b}	23	15.5±0.8	15.8±1.3
6	14	12.6±1.2^{b}	10.6±1.1^{a}	23	15.4±0.9	16.1±1.2
12	14	12.0±0.9^{ab}	11.7±0.8^{ab}	23	16.4±1.0	16.1±2.2
18	14	12.6±0.9^{b}	10.7±0.7^{ab}	23	15.8±0.8	15.0±1.6
51	14	12.9±1.2^{b}	11.0±1.2^{ab}	23	15.9±1.4	15.9±1.3
0	14	11.6±0.3^{a}	12.1±0.9^{b}	23	16.0±3.7^{b}	15.2±4.9^{b}
6	17	21.0±10.0^{ab}	13.2±3.5^{b}	26	20.0±5.3^{c}	17.4±1.4^{b}
9	17	27.0±10.0^{b}	11.9±0.5^{b}	26	24.0±1.3^{c}	16.5±2.4^{b}
15	14	13.0±2.6^{a}	9.0±0.8^{a}	23	21.0±1.1^{c}	9.6±2.2^{a}
27	14	19.0±7.6^{ab}	12.6±3.0^{b}	23	12.0±0.4^{a}	9.1±1.4^{a}
0	14	12.0±0.3^{a}	12.3±4.0^{a}	23	17.0±2.7^{a}	12.4±1.1^{a}
12	20	23.0±8.6^{b}	18.4±5.1^{c}	29	25.0±4.6^{b}	19.3±4.3^{d}
15	20	12.0±1.2^{a}	17.3±3.6^{bc}	29	20.0±1.5^{a}	16.9±1.7^{cd}
27	14	18.0±6.9^{ab}	11.0±1.7^{a}	23	20.0±1.5^{a}	15.3±0.4^{bc}
39	14	17.0±4.5^{ab}	14.2±1.4^{ab}	23	18.0±2.1^{a}	13.2±1.3^{ab}
0	14	11.1±0.4^{a}	10.9±0.4^{b}	23	17.0±3.3^{a}	10.9±1.6^{a}
18	23	30.8±1.2^{c}	15.6±3.0^{c}	32	31.0±1.1^{c}	14.2±0.7^{b}
21	23	23.6±8.6^{b}	15.9±1.5^{c}	32	22.0±3.6^{b}	17.0±2.3^{c}
39	14	14.9±4.2^{a}	14.4±3.5c	23	19.0±2.4^{a}	13.3±0.8^{b}
51	14	21.6±4.6^{b}	8.5±1.3^{a}	23	18.0±1.9^{a}	13.2±1.3^{b}

Values are means ± SD (FL; n=6, FS; n=8) for experiments run on two occasions. Means sharing the same superscripted letter are not significantly different (Duncan's multiple range test P **>** 0.05). FL: olive flounder in large group, FS: olive flounder in small group.

Table 2. Variations of blood hemoglobin (gdL^{-1}) in olive flounder, *Paralichthys olivaceus* after WT changes

Elapsed time (hours)	WT (℃)	Exp.I		WT (℃)	Exp.II	
		FL	FS		FL	FS
0	14	10.1±0.5	10.3±1.0	23	10.5±0.8^{a}	10.9±0.7
6	14	10.9±0.8	10.8±0.7	23	11.3±0.9^{ab}	11.0±1.1
12	14	10.8±1.0	10.4±1.4	23	11.9±0.6^{b}	11.5±1.0
18	14	10.9±0.8	10.7±2.7	23	11.6±1.1^{b}	11.2±0.7
51	14	10.8±0.9	10.4±0.6	23	11.8±1.0^{b}	11.3±1.1
0	14	9.8±0.5^{a}	10.5±1.2^{ab}	23	10.0±1.0^{a}	11.0±0.8^{ab}
6	17	12.0±2.6^{a}	12.4±2.1^{b}	26	12.0±0.9^{b}	12.0±1.2^{b}
9	17	12.9±2.8^{a}	10.2±2.7^{ab}	26	12.0±1.0^{b}	11.0±1.3^{ab}
15	14	10.6±3.0^{a}	9.5±3.3^{a}	23	12.0±2.8^{b}	10.0±1.7^{a}
27	14	10.3±1.4^{a}	9.1±1.3^{a}	23	12.0±1.1^{ab}	12.0±1.4^{ab}
0	14	10.1±0.5^{a}	11.2±1.4^{ab}	23	12.0±1.4^{ab}	10.0±2.0^{a}
12	20	12.3±2.0^{b}	14.6±2.5^{c}	29	12.0±2.0^{a}	9.9±1.8^{a}
15	20	9.9±1.6^{a}	13.2±2.7^{bc}	29	14.0±1.7^{bc}	12.0±1.3^{b}
27	14	11.4±2.4^{ab}	12.0±2.3^{ab}	23	13.0±2.5^{abc}	9.4±1.1^{a}
39	14	10.4±1.3^{ab}	10.6±0.8^{a}	23	14.0±1.0^{c}	11.0±1.4^{ab}
0	14	10.9±0.6^{a}	11.0±0.4^{bc}	23	11.0±1.6^{a}	8.9±0.8^{a}
18	23	11.5±0.4^{a}	12.0±0.3^{cd}	32	14.0±0.5^{b}	13.0±0.5^{c}
21	23	12.4±1.9^{a}	13.0±0.7^{d}	32	15.0±1.1^{c}	12.0±0.5^{c}
39	14	11.2±1.2a	11.0±1.1^{b}	23	12.0±1.0^{a}	10.0±0.9^{b}
51	14	11.7±2.9^{a}	7.0±1.2^{a}	23	12.0±0.6^{a}	11.0±0.2^{b}

Values are means ± SD (FL; n=6, FS; n=8) for experiments run on two occasions. Means sharing the same superscripted letter are not significantly different (Duncan's multiple range test P **>** 0.05). FL: olive flounder in large group, FS: olive flounder in small group.

Table 3. Variations of plasma osmolality (mOsm/kg) in olive flounder, *Paralichthys olivaceus* after WT changes

Elapsed time (hours)	WT (℃)	Exp.I		WT (℃)	Exp.II	
		FL	FS		FL	FS
0	14	443.0±9.9	393.5±6.4	23	420.5±29.0	425.0±21.2
6	14	434.5±7.8	394.5±7.8	23	437.0±24.0	408.0±4.2
18	14	433.5±7.8	390.0±7.1	23	425.5±13.4	407.5±10.6
51	14	435.0±5.7	393.0±5.7	23	420.0±14.1	399.5±14.8
0	14	423.5±33.2	404.0±17.0^{ab}	23	441.5±0.7^{bc}	449.5±13.4^{ab}
6	17	444.0±38.2	418.0±5.7^{b}	26	467.0±18.4^{c}	411.5±0.7^{c}
9	14	420.0±28.3	395.0±7.1^{ab}	23	437.5±3.5^{b}	417.5±3.5^{b}
27	14	397.5±3.5	382.5±3.5^{a}	23	405.0±7.1^{a}	396.5±2.1^{a}
0	14	423.5±33.2	404.0±17.0	23	441.5±0.7	449.5±13.4
12	20	449.0±5.7	417.5±21.9	29	457.0±29.7	477.5±3.5
15	14	444.5±0.7	433.0±4.2	23	454.5±6.4	468.0±4.2
39	14	425.5±6.4	421.0±1.4	23	445.5±7.8	460.0±2.8
0	14	423.5±33.2	404.0±17.0	23	441.5±0.7^{ab}	449.5±13.4
18	23	429.0±12.7	418.0±25.5	32	474.0±2.8^{c}	433.5±13.4
21	14	433.5±3.5	423.0±2.8	23	446.0±7.1^{b}	432.5±3.5
51	14	405.5±7.8	392.5±5.0	23	431.5±0.7^{a}	433.0±2.8

Values are means ± SD (FL; n=6, FS; n=8) for experiments run on two occasions. Means sharing the same superscripted letter are not significantly different (Duncan's multiple range test P **>** 0.05). FL: olive flounder in large group, FS: olive flounder in small group.

IV. DISCUSSION

Recently, many studies have been conducted out on stress response, reproduction ability, and metabolic physiology of fish through the hypothalamus-pituitary-interrnal (HPI) axis response. Barton and Iwama suggested that fish could adequately cope with stress from external environment changes but the stress above a threshold decreased physiological activity of fish and harmed health [10]. Fish with frequent stress events have difficulty in maintaining homeostasis and demands energy to overcome it. Because the energy that should be used for body growth and vital maintenance is consumed, growth retardation occurs and mortality rate increase [6,10,14].

The reports of Chang et al. and Park et al. are the only studies related to the stress response of fish by rapid WT change in Korea [4-5]. In those reports, flounder are known to cope with single stress event very well and have good adaptation ability. Wedemeyer et al. reported that the plasma cortisol level of salmonids in rest and non-

stress conditions was 30-40 ng/mL [15] and Pickering and Pottinger reported less than 5 ng/mL in the best condition [16]. Cortisol levels at the beginning of this study were 2.8-3.0 (14℃), 0.1-2.4 ng/mL (23℃) similar to two other report values. Chang et al. reported that cortisol levels of the same species were 3.6 ng/mL at 18℃, 1.5 ng/mL at 20℃ and 2.0 ng/mL at 23℃ [4]. Park et al. reported that the levels were 2.5 (18℃) and 2.6 ng/mL (20℃), respectively that are very similar to our reports [5]. The reason for the lower cortisol level in rest state than other salmon families are considered to be species specific to flounder as its activity against stress, living habit, and low motor activity, as suggesting in the reports of Chang et al. and Park et al. [4-5].

No change was observed in cortisol level from WT increase in Exp.I of this study. But glucose and lactic acid levels increased with rising WT and decreasing WT at the end of the experiment. In Exp.II, cortisol, glucose, and lactic acid levels increased with increased WT. In Exp.I, cortisol levels did not increase, but glucose and lactic acid levels increased. It would be the appropriate temperature for the best growth in flounder. The WT for the ideal growth of flounder is around 18℃. When WT increased from 14℃, the temperature was similar to WT for the ideal growth of flounder causing cortisol levels to recover to beginning levels (from increase of WT to first blood collect). But in Exp.II, the increased cortisol levels due to rising WT remained at the level it was that just after increasing the WT. And the increases in glucose and lactic acid levels were shown from typical stress response.

Barton and Iwama reported that the increased rate and time of cortisol level differed by fish species in a stressed condition [10]. For example, a peak level of cortisol in Atlantic salmon, *Salmo salar* due to shallow water depth was shown within less than 1 h. After 2 h, the measured value was shown to be similar to the values at the beginning of the experiment. Robertson et al. reported that red drum, *Sciaenops ocellatus* showed a peak in cortisol levels within 1 h by the handling the stress and the value recovered to stable 3 h later [17]. Like these studies, cortisol levels increased to a peak level in 1-3 h and it recovered in 6 h when acute stress was given [16,18]. But Waring et al. reported that cortisol levels of flounder, *P. flesus* and Atlantic salmon increased after 1 h following the handling of the stress and recovered to the starting value after 48 h following to the handling of the stress [19]. And cortisol levels of rainbow trout, *Oncorhynchus mykiss* [20], sea raven, *Hemitripterus americanus* [21] and turbot, *Scophthalamus maximus* [19] recovered after 24 h following stress. And the cortisol level of brown trout, *S. trutta* [16] recovered in 8 h. This suggests that recovery time of cortisol levels show differences according to fish species. It is possible to explain why cortisol did not increase in Exp.I in this study. Barton and Iwama reported that the time of plasma cortisol levels, returning back to the starting value, showed difference according to fish species, and amount and type of stress [10]. And the reason that the peak reaching time of cortisol levels differed to the type of stress in this study can be explained by above report.

In Korea, the olive flounder is known to cope with one stress event very well [3-6,9,12,22-25]. In this study flounder coped with the stress very well. The survival rate was also very high during times of temperature increases and the stress response also occurred for maintenance of homeostasis following, but it was recovered in a short period of time. But there was a significant change in respiration rate by increasing the temperature from 29 to 32℃. In this study, a single stress event of a rise and drop of WT was given. It is necessary to study stress responses to repeated WT changes in future study.

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