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Influence of Daily Water Temperature Changes on Chemiluminescent Response of Phagocytes and Mortality in Cultured Gray Mullet (*Mugil cephalus* L.)

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The influence of daily water temperature changes on chemiluminescent (CL) response of phagocytes, plasma glucose level and mortality in cultured gray mullet (*Mugil cephalus*) was investigated. Among three experimental temperature groups, the fish in group I and group II were maintained constantly at 27℃ and 29℃, respectively, and those in group III were suffered daily elevation of water temperature from 27℃ to 29℃ for 5 hours, then restored again to 27℃. After 1 week of maintaining at each experimental tank, the fish in group III showed significantly lower CL peak value ($p < 0.01$) and higher plasma glucose levels ($p < 0.05$) than those in group I and group II. The cumulative mortality of group III was 86.9% within 3 days after being subjected to acute handling stress. On the other hand, the cumulative mortalities of group I and group II were 12.5% and 19.2%, respectively. In conclusion, gray mullet farms, especially, in the vicinity of thermoelectric power plants should avoid stressing the fish during periods of high water temperature.

Key words: Temperature change, Chemiluminescent response, Glucose, Mortality, Gray mullet (*Mugil cephalus*)

Introduction

The health of fish in intensive culture can be affected by both variations and extremes in water temperature in which they live (Wedemeyer, 1996). Although each fish species has its own thermal tolerance range, rapid change of water temperature within the range acts as a stressor, and therefore influences the physiology, including immune functions, of fish.

The effect of temperature on the immune system of fish has been extensively studied (Miller and Clem, 1984; Scott et al., 1985; Bly and Clem, 1988, 1991, 1992; Ainsworth et al., 1991; Collazos et al., 1994; Hardie et al., 1994; Carlson et al., 1995; Le Morvan et al., 1995, 1997, 1998). However, little is known about the effect of daily water temperature change on the non-specific immune response of fish.

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Usually, the water temperature of some gray mullet farms situated on the southern coastal areas in Korea rose to 27℃ during the hottest summer season. Moreover, the gray mullet farms in the vicinity of thermoelectric power plants suffer daily water temperature changes by thermal discharge. In the present study, a laboratory experiment was designed to mimic the water temperature conditions that gray mullet in the vicinity of power plants may experience during the hottest summer periods.

The goal of the present study was to elucidate whether daily water temperature changes influence the chemiluminescent (CL) response of phagocytes, plasma glucose concentration and mortality of cultured gray mullet, *Mugil cephalus*.

Materials and Methods

Fish

Fingerlings of gray mullet weighing 16.67 ± 2.48 g were obtained from a local commercial farm. The

fish were divided into three groups of 30 fish, and were acclimated to 200 ℓ fiberglass tanks at 20℃ for 4 weeks prior to the experiment. They were fed a dry commercial pelleted diet at 1% of body weight, once a day.

Experimental regime

The water temperature of each experimental tank was gradually increased (1℃/day) to 27℃. Then, the fish in group I and group III were maintained at that temperature for an additional week. The water temperature of group II was increased from 27℃ to 29℃ for 2 days and acclimated at that temperature for an additional 5 days. After the acclimation, the temperature of group III was increased daily to 29℃ for 5 hours, then restored again to 27℃. The water temperature of group I and group II was not changed, and were maintained at 27℃ and 29℃, respectively, throughout the experiment (Fig. 1). The dissolved oxygen was measured daily and maintained above 7 mg/ℓ in all tanks throughout the experiment. After a week, 5 fish in each experimental group were selected at random and anaesthetized with 200 mg/ℓ tricaine methanesulfonate (MS-222, Sigma), then blood was withdrawn by caudal vein venipuncture for analysis of glucose concentration. The fish, after blood collection, were dissected and excised spleen aseptically for analysis of CL response of phagocytes. The remaining fish in the tanks (25 fish/group) were then subjected to a 10-min handling stress, which included chasing and scooping them in a net. The water temperature was maintained at each experimental temperature regime for another 15 days. Mortality in each tank was recorded daily.

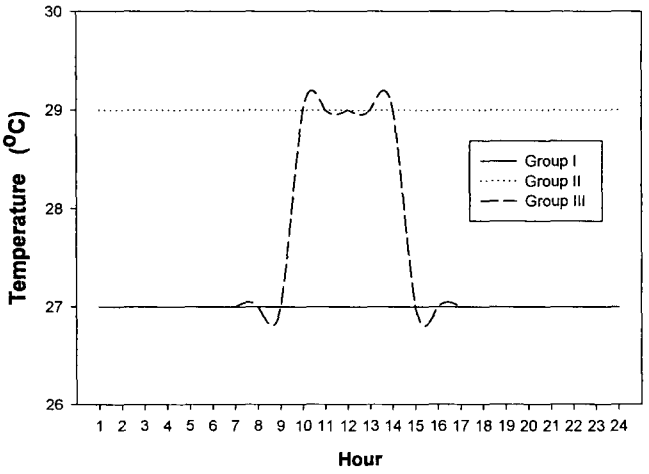

Fig. 1. Daily water temperature regimes of each experimental group.

CL assay

Each aseptically removed spleen was passed through a 100 μm nylon mesh using Hanks' balanced salt solution (HBSS, Sigma) containing heparin (10 units/ml, Sigma), penicillin (100 μg/ml Sigma) and streptomycin (100 U/ml, Sigma). The resulting cell suspension was placed on a 34/51% Percoll (Sigma) density gradient and centrifuged at 400 g for 30 min at 4℃. The interphase was collected and the cells were washed twice at 400 g for 5 min in HBSS containing heparin and antibiotics. The cell viability was examined with trypan blue exclusion and was evaluated to be greater than 95%. The phagocytes were adjusted to 3×10^5 cells/ml HBSS.

Zymosan (Sigma) was mixed with serum, which was prepared previously from a gray mullet weighing about 250 g, and incubated at 30℃ for 30 min. The opsonized zymosan was separated by centrifugation, washed three times and suspended in HBSS.

The reactive oxygen intermediates (ROIs) produced by stimulated phagocytes was quantified using an automatic photoluminometer (Bio-Orbit 1251, Sweden). Each test cuvette contained 0.7 ml luminol (Sigma) made according to the method of Scott and Klesius (1981), 0.5 ml cell suspension, and 0.3 ml opsonized zymosan, which was added just prior to measurement. The measurements were made for 100 min and the light emission was recorded as mV.

Glucose

The collected blood from 5 fish in each group was immediately centrifuged at 700 g at 4℃ for 30 min. Plasma glucose was measured using a glucose oxidase/peroxidase enzymatic assay based upon the method of Werner et al. (1970).

Statistical analysis

The data of CL and glucose were subjected to ANOVA using Statistix 3.1 (Analytical Software, St.

Paul, MN, USA). The criterion for statistical difference was $p < 0.05$.

Results

CL response

The fish in group III, which were subjected to daily water temperature changes, showed the lowest CL peak value ($p < 0.01$) among experimental groups (Fig. 2). The CL response of group II was higher than that of group I ($p < 0.01$).

Glucose

The plasma glucose level was higher in group III than the other experimental groups, but there were no significant differences among experimental groups (Fig. 3).

Mortality

The cumulative mortality of group III was 86.9% within 3 days after being subjected to acute handling stress. On the other hand, the cumulative mortalities of group I and group II were 12.5% and 19.2%, respectively (Fig. 4).

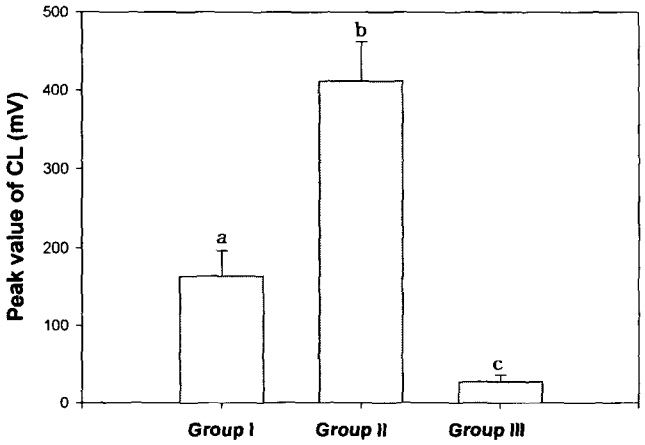

Fig. 2. Peak values of chemiluminescent (CL) response of phagocytes in each experimental group. Values are mean ± S.E. and different letters indicate statistical significance at $P < 0.01$. (group I, maintained constantly at 27℃; group II, maintained constantly at 29 ℃; group III, raised water temperature from 27℃ to 29℃ for 5 hours daily)

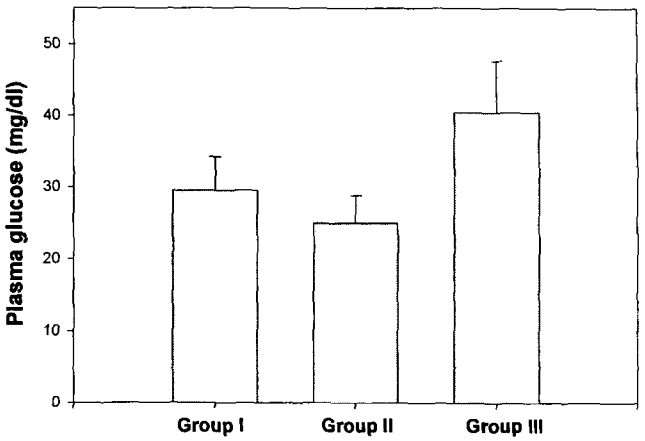

Fig. 3. Plasma glucose levels of each experimental group. Values are mean ± S.E. (group I, maintained constantly at 27℃; group II, maintained constantly at 29℃; group III, raised water temperature from 27℃ to 29℃ for 5 hours daily)

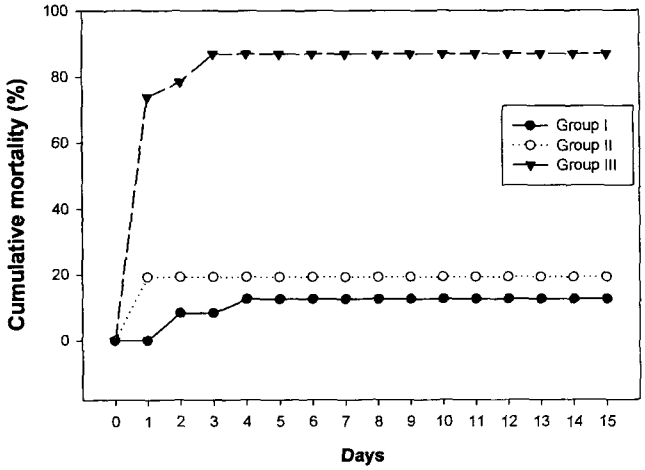

Fig. 4. Cumulative mortality rates (%) of gray mullet in each temperature group during experimental period. (group I, maintained constantly at 27℃; group II, maintained constantly at 29℃; group III, raised water temperature from 27℃ to 29℃ for 5 hours daily)

Discussion

The present results showed that daily water temperature changes acted as a severe stressor, and exerted an negative influence on the CL response of phagocytes of cultured gray mullet. Moreover, the synergistic effect of temperature changes and acute

handling stressor was fatal to gray mullet matained at a high water temperature.

Elevated plasma glucose concentration is commonly used as an indicator of secondary stress response to an acute stress in fish (Barton and Iwama, 1991). The mechanism of stress-mediated suppression of phagocytic activity in fish is not fully understood, but appears to be mediated by the endocrine system (Bayne and Levy, 1991). Angelidis et al. (1987) assumed that the decrease in the CL response in stressed fish might be based on the corticosteroid effects. In the present study, the higher plasma glucose levels and significantly lower CL responses of the fish in group III than the fish in other groups indicated that the respiratory burst activity of gray mullet phagocytes was severely damaged by the stress of temperature changes.

It is known that stress induced mortality increases with increasing temperature (Strange, 1980; Barton and Schreck, 1987). In the present study, the 86.9% mortality of the fish in group III after being subjected to acute handling stress indicated that multiple stressors of water temperature changes and handling at high water temperature affected synergistically on the gray mullet health, and resulted in the high mortality.

In conclusion, gray mullet farms, especially, in the vicinity of thermoelectric power plants should be avoid stressing the fish during periods of high water temperature.

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