J. Fish. Sci. Tech. 4(3), 112~119, 2001

JOURNAL OF
FISHERIES SCIENCE
AND TECHNOLOGY

Effect of Temperature Shock on Cultured Olive Flounder (*Paralichthys olivaceus*) and Black Rockfish (*Sebastes schlegeli*)

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(Received March 2001, Accepted September 2001)

Aim of this research is to investigate the effect of temperature shocks on the physiological responses of cultured olive flounder (*Paralichthys olivaceus*) and black rockfish (*Sebastes schlegeli*). Olive flounder and black rockfish were suffered with high and low temperature shocks for 4 and 8 h, respectively, in laboratory conditions and then the changes in glucose, lactate, total protein, uric acid, and triglycerides-glycerol in blood plasma were analyzed. We observed that lactate and uric acid increased for up to 4 h and then decreased for up to 8 h by the high and low temperature shocks, and total protein decreased for up to 4 h and then recovered for up to 8 h by the high temperature shock in both fishes. Glucose by the high and low temperature shocks and triglycerides-glycerol by the low temperature shock increased for up to 4 h, and then decreased in olive flounder, but increased for up to 8 h in black rockfish. From the result, we speculated that the two fishes have an interspecific variation in the regulatory systems of glucose and triglycerides-glycerol. Glucose would play important role as an energy source during the temperature shocks and for an intermediate substance for low temperature tolerance, and glycerol of triglycerides-glycerol would play an important role for low temperature tolerance. In olive flounder, the turnover of chemical change by temperature shock took more than 4 h, all chemicals returned almost to the initial level for up to 8 h, but fish death followed only in 8 h with the high temperature shocked group within two days. Therefore, we suggested that fish would be damaged severely by the longer time exposure of high temperature and mortality would occur after a certain time later than the shocked time as a post-effect.

Key words: Acute temperature fluctuation, High temperature shock, Low temperature shock, Aquaculturing marine fishes, Blood plasma

Introduction

Olive flounder (*Paralichthys olivaceus*) and black rockfish (*Sebastes schlegeli*) are very important aquaculturing fishes in Korea, comprising up to 85 % of the total annual fish product in aquaculture. During the summer, there have been a few cases of the fish death in aquaculture, which have been suspected by the sudden exchanges between the high and low temperature seawater currents since Korea is located in the temperate-zone, but there is no clear evidence on it. In summer, it has been reported that the highest sea water temperature goes up to 25~28℃, and the lowest temperature goes down to 10℃ during the current fluctuation in Korea. Therefore, it is presumptive that the acute temperature fluctuation of seawater tide would affect the physiology of fish. On physiological responses of fish to the high or/and low temperatures, several investigations have been made on fresh water fishes including rainbow trout (Connors et al., 1978; Schneider et al., 1981; Wagner et al., 1997), tilapia

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(Kindle and Whitmore, 1986; Sun et al., 1992), striped bass (Davis and Parker, 1990), carp (Albrecht, 1982; Chen et al., 1995), curimata and carp (Fontes-Zuim and Macari, 1985), ayu (Del-Valle and Taniguchi, 1995), and cat-fish (Akela, 1987; Ottolenghi et al., 1995). However, no investigation has been conducted on the aquacultured marine fishes, such as olive flounder and black rockfish, to the acute temperature fluctuation so far.

In this study, to understand the effect of acute temperature fluctuations on the cage cultured marine fishes, we exposed olive flounder and black rockfish to the alternative conditions of the high and low temperatures for a short period of time in the laboratory, and then analyzed the concentrations of glucose, lactate, total protein, uric acid, and triglycerides-glycerol in the blood plasma by the time interval.

Materials and Methods

Fish

Olive flounder (*Paralichthys olivaceus*) and black rockfish (*Sebastes schlegeli*) were conditioned for two months in different tanks. The body length and weight of olive flounder (body length, 26.5±4.28 cm) and black rockfish (20.15±1.63 cm) were used for high and low temperature shocks, respectively.

High and low temperature shock treatment

The two fishes were acclimatized at 20±1.5℃ for two weeks from October 29 through November 6, 1998, starved for 18 h, and were directly transferred to the sub-lethal temperature of 28℃ and 10℃ for high and low temperature shocks, respectively. Less than 10 fish for each group (Table 1) were used and kept for 4 and 8 h, respectively in the 100 L of polyethylene tanks filled with 80 L of sea water with a strong aeration. After temperature shock, the fish were bled immediately and acclimatized to the natural seawater temperature of 20±1.5℃ from the treated temperature by increasing or decreasing 1℃/10 min.

Table 1. Kinds and numbers of fishes used for the temperature shock experiment

Fishes	High temperature shock			Low temperature shock		
	0	4	8 h	0	4	8 h
Olive flounder (n)	8	8	8*	7	8	8
Black rockfish (n)	8	8	6	7	7	8

*All fish were dead within two days after shock.

Blood plasma collection

After the high and low temperature shocks, the fishes were immediately anesthetized in a bath of 0.4 mL/L of 2-phenoxyethanol (Sigma) and then 1 mL of blood was collected from the caudal vein of each individual using a heparinized tube. The collected blood was kept on ice and immediately centrifuged 3,000 rpm for 10 min at 4℃, and the supernatant was collected, kept on ice, and used for assay directly.

Chemical measurement

We determined the concentrations of glucose, lactate, total protein, and uric acid in the blood plasma with Sigma reagents (No. 16, 735, 541, and 685, respectively). Sigma reagent 336 was used to measure triglycerides-glycerol level, in which triglycerides were converted to glycerol and fatty acid by lipase, so that triglycerides and glycerol in the blood plasma could be assayed together as triglycerides-glycerol. All assays were conducted at 20℃.

Data analysis

Analysis of variance was performed at a significance level of 0.05 in all tests. Determination of statistical significance among the experimental groups was made utilizing Tukey test (Zar, 1984).

Results and Discussion

Effects of the temperature shocks on the concentrations of glucose, lactate, total protein, uric acid and triglycerides-glycerol in the blood plasma of olive flounder and black rockfish are shown in Figs. 1A, 2A, 3A, 4A and 5A for high temperature shock, and Figs. 1B, 2B, 3B, 4B, and 5B for low temperature shock, respectively.

Glucose

The concentration of glucose increased for up to 4 h by the high temperature shock in both fishes ($p<0.05$), after then gradually decreased in olive flounder, but increased in black rockfish for up to

8 h (Fig. 1A). By the low temperature shock, the glucose concentration was increased in both fishes for up to 4 h continually ($p<0.05$), after then decreased in olive flounder, but increased for up to 8 h in black rockfish (Fig. 1B). Glucose is the principal substrate for glycolytic pathway. Up to 4 h of temperature shock, the concentration of glucose increased similarly in two fishes, but a little bit higher by the low temperature shock than by the high temperature shock (Figs. 1A, 1B). However, after 4 h, there were differences in the pattern of glucose changes between the two fishes. Glucose was increased continually in black rockfish for up to 8 h of the conducted high and low temperature shocks, but glucose was fallen in olive flounder after 4 h of the high and low temperature shocks. The increase of glucose in fishes by high or/and low temperature shock was reported in many investigations (Connors et al., 1978; Schneider et al., 1981; Albrecht, 1982; Akela, 1987; Sun et al., 1992; Chen et al., 1995; Del-Valle and Taniguchi, 1995; Ottolenghi et al., 1995; Wagner et al., 1997). At high temperatures, Plesofsky-Vig and Brambl (1995) explained that increased glucose is likely to be required as an energy source for glycolysis. Therefore, we suggested that the increased glucose in this study has a strong relation with glycolysis for energy generation during the high and low temperature shocks for up to 4 h. From the change of glucose in olive flounder, we guessed that, after 4 h, glycolysis is not the main source of energy generation any more and that the acclimatization of mitochondrial oxdative phosporylation begins after 4 h gradually. However, we have no idea why glucose was continually increased in black rockfish after 4 h in the high and low temperature shocks (see the section for triglycerides-glycerol).

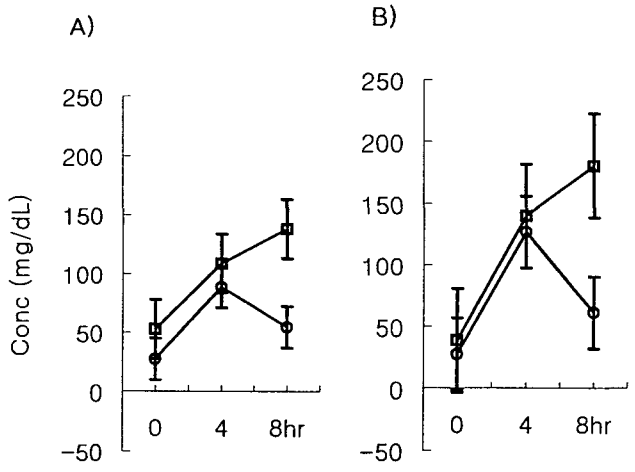

Fig. 1. Change of glucose level in the plasma of olive flounder and black rockfish after temperature shock. The 20 ± 1.5℃ acclimatized fish were directly transferred to the experimental temperature of 10 or 28℃, and maintained for 8 h. All values are expressed as the mean ± SEM.
Symbols are A: High temperature shock (28℃); B: Low temperature shock (10℃); —○—: Olive flounder; —□—: Black rockfish. The 20 ± 1.5℃ acclimatized fishes were used as control.

Lactate

The concentration of lactate increased for up to 4 h by the high temperature shock in both fishes ($p>0.05$), and then decreased in both fishes (Fig. 2A). By the low temperature shock, the concentration of lactate increased for up to 4 h in black rockfish ($p<0.05$) and in olive flounder ($p>0.05$), and than decreased in both fishes, in which the concentration was higher in black rockfish than in olive flounder (Fig. 2B). Lactate is generally recognized as an end product of glycolytic pathway. At 4 h, the concentration of the accumulated lactate was a little bit higher by the high temperature shock than by the low temperature shock (Figs. 2A, 2B). The accumulation of lactate by the high temperature shock has been reported by Schneider

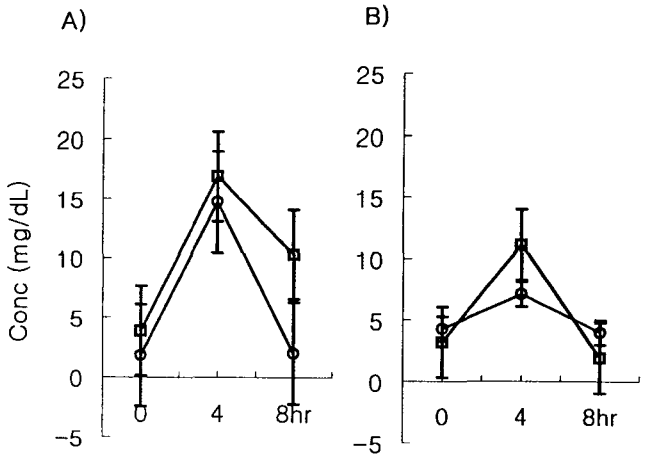

Fig. 2. Change of lactate level in the plasma after temperature shock. Temperature shocks and symbols are same as in Figure 1.

et al. (1981). In this study, the concentration of lactate increased during the first 4 h of temperature shock in which we suggested that the function of lactate dehydrogenase (LDH) would be reduced (or inhibited) by the high and low temperature shocks. However, after 4 h temperature shock, in the two fishes, lactate would be converted to pyruvate by a new type of the alternatively acclimatized LDH, as such an isozyme of M4-LDH, which was well known in adapting to the temperatures between approximately −1.86℃ and 35~47℃ in vertebrate, including an endotherm and ectotherms (Somero et al., 1983), and then pyruvate entered the mitochondrial oxidative phosphorylation systems, so that gradually lactate would be reduced as shown in Figs. 2A, 2B.

Total protein

Total protein concentration decreased for up to 4 h after high temperature shock in both fishes ($p<0.05$), and then gradually recovered for up to 8 h at the control level (Fig. 3A). We guessed that the reduction and recovery of total protein concentration caused by the reduction and recovery of protein synthesis during the high temperature shock. In fact, there have been few reports on the reduction and recovery of protein synthesis in thermotolerant cells after heat shock. Carper et al., (1997) reported that thermotolerant CHO cells had a 50% reduction in protein synthesis, which recovered within 7 h following the heat shock. Exposure of mammalian cells to a nonlethal heat shock treatment, protein synthesis was completely inhibited for as long as 5 h. Upon resumption of translational activity, there was a marked induction of heat shock (or stress) protein synthesis, which continued for several hours (Mizzen and Welch, 1988). Panniers et al. (1985) also reported that almost all living organisms studied responded to elevated temperature with a marked inhibition of overall protein synthesis but increased synthesis of specific set of proteins, the so called heat shock proteins (hsps). Therefore we believe that the reduction and recovery of total protein concentration by the high temperature shock in this study depended upon the similar reduction and recovery of protein synthesis shown by heat shock. However, there was little change in total protein by the low temperature shock in both fishes ($p>0.05$) (Fig. 3B). In both fishes, after 4 h of the high temperature shock, the concentration of total protein recovered for up to 8 h gradually (Fig. 3A). Therefore we guessed that the recovery from the high temperature shock would probably have a relation with the acclimatization of the cellular mechanism of thermotolerance after 4 h of the high temperature shock. It has been known that several hsps are required for cellular thermotolerance, which have been shown to function as chaperones facilitating the folding or membrane translocation of the target proteins (Gething and Sambrook, 1992). We knew that several hsps were expressed in fish cell lines within few hours after heat shock at 28℃ (Heikkila et al., 1982; Kothary and Candido, 1982; Gedamu et al., 1983; Mosser et al., 1986). In this study, the hsps expressible temperature of 28℃ was used. Therefore, we guessed that the expressible hsps in the two fishes by the high temperature shock may be involved in the recovery of the total protein concentration gradually after 4 h of the high temperature shock, but we did not check presence of any hsp in fish plasma.

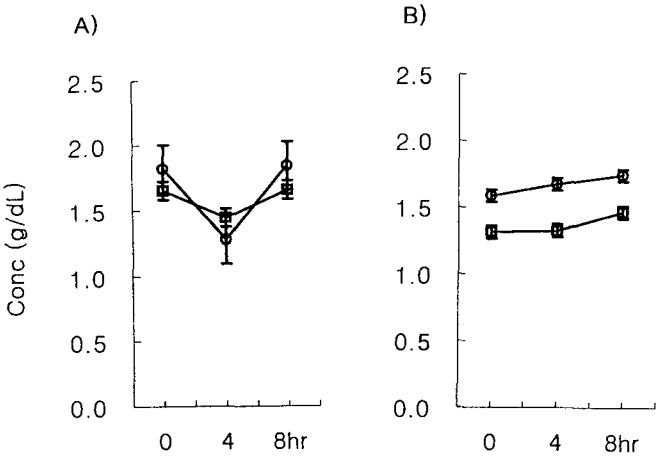

Fig. 3. Change of total protein level in the plasma after temperature shock. Temperature shocks and symbols are same as in Figure 1.

Uric acid

The concentration of uric acid increased for up to 4 h in black rockfish ($p<0.05$) and olive flounder ($p>0.05$) by the high temperature shock, and then gradually decreased toward the initial level in both fishes (Fig. 4A). By the low temperature shock, the

concentration of uric acid increased more for up to 4 h in black rockfish ($p<0.05$) than in olive flounder ($p>0.05$), and then gradually decreased in black rockfish, however, there was little change in olive flounder (Fig. 4B). Uric acid is the excretion form of NH_4^+ derived from protein. Therefore, we suggested that in the two fishes, the excretion of NH_4^+ reduced for up to 4 h by high and low temperature shocks, after then recovered for up to 8 h gradually.

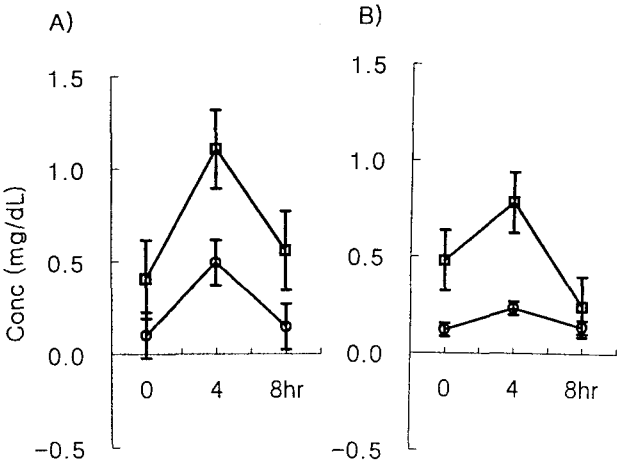

Fig. 4. Change of uric acid level in the plasma after temperature shock. Temperature shocks and symbols are same as in Figure 1.

Triglycerides-glycerol

The concentration of triglycerides-glycerol was not much affected by the high temperature shock in both fishes for up to 4 and 8 h ($p>0.05$) (Fig. 5A). However, the concentration of triglycerides-glycerol increased for up to 4 h by the low temperature shock in both fishes ($p<0.05$), after then gradually decreased to the initial level in olive flounder, but increased gradually for up to 8 h in black rockfish (Fig. 5B). Therefore the change of triglycerides-glycerol is very different between the two fishes. After the high temperature shock, the concentration of triglycerides-glycerol was not changed much, which means that triglycerides-glycerol has little relation with the high temperature shock. However, by the low temperature shock, triglycerides-glycerol increased very high, which would be required to recover or to sustain from the low temperature. In olive flounder, triglycerides-glycerol was reduced

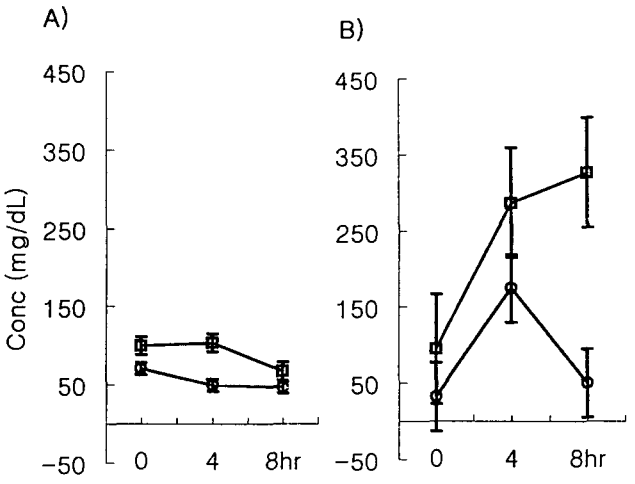

Fig. 5. Change of triglycerides-glycerol level in the plasma after temperature shock. Temperature shocks and symbols are same as in Figure 1.

after 4 h of the low temperature shock, which means that triglycerides-glycerol will not be required for low temperature tolerance continually at the treated temperature. Therefore, after 4 h, we postulated that glycerol of triglycerides-glycerol would be replaced by another substance for low temperature tolerance or would not be required any more. The presence of an alternative material of glycerol of triglycerides-glycerol was known in Pleuronectides as antifreeze agent (Duman and DeVries, 1976; Hew and Yip, 1976; Fletcher et al., 1985). Driedzic et al. (1998) also suggested that glycerol could not serve as an antifreeze agent in smooth flounder. We did not check presence of any antifreeze protein (AFP) after low temperature shock (10℃) in olive flounder, but we could not exclude the presence of AFP, because in winter flounder the low levels of AFP were found in plasma after being exposed to 10~11 ℃ (Fletcher, 1981). However, we strongly believe that glycerol of triglycerides-glycerol would play an important role for the initial stage of low temperature tolerance in olive flounder. The increment of triglycerides-glycerol for up to 4 h and then the reduction of triglycerides-glycerol for up to 8 h by the low temperature shock (10℃) in olive flounder is probably the first investigation of the disappearance of triglycerides-glycerol by the time. Therefore, we postulated that, in olive flounder, glycerol of triglycerides-glycerol would be synthesized (or released) for a short term of low temperature shock,

but not for a longer time of low temperature tolerance.

However, in black rockfish triglycerides-glycerol appears very important in low temperature response because it increased continually after 4 h of low temperature shock. Glycerol has been reported to be recognized as antifreeze agent in some insects (Joanisse and Storey, 1994) and in rainbow smelt (Raymond, 1992; Raymond, 1993). Raymond (1995) showed that the intramuscular injection of [^{14}C] glucose leads to the production of labeled glycerol in blood and liver. In this study, in black rockfish, the concentrations of triglycerides-glycerol and glucose increased after the low temperature shock for up to 8 h continually (Figs. 5B, 1B). Therefore, we considered that the increased glucose after 4 h would be used to synthesize glycerol of triglycerides-glycerol continually for low temperature tolerance as mentioned above in rainbow smelt or to produce for the required energy for low temperature tolerance. However, we could not explain the requirement of glucose after 4 h of the high temperature shock in black rockfish continually, except the source of energy for high temperature tolerance.

Summary

In this study, we investigated the biochemical changes of blood plasma in marine fishes of olive flounder and black rockfish by applying high and low temperature shocks. The two fishes were similarly affected in lactate, total protein and uric acid by temperature shock for the first 4 h, in which it seems like that energy metabolism depended upon glycolysis because the concentrations of glucose and lactate in olive flounder and black rockfish increased (Figs. 1A, 1B, 2A, 2B). After 4 h, energy metabolism was seemingly recovered by mitochondrial respiration because the concentration of glucose in olive flounder and lactate in olive flounder and black rockfish decreased, but the two fishes showed different responding patterns in glucose and triglycerides-glycerol to the treated temperature for up to 8 h. From the differences in chemical changes, we suggested that two fishes have an interspecific variation in the regulatory systems of glucose and triglycerides-glycerol to the treated temperature shocks. However, up to 4 h, both fishes had increased glucose in the blood commonly during the high and low temperature shocks. The result indicates that glucose would play important roles for energy source and an intermediate substance for temperature tolerance during the early time of the temperature shocks in two fishes, which could be supplied as the best candidate for nutrient externally when the physical condition of fish was worsened by the temperature fluctuations.

After conducting the statistical test on the variation values of five chemicals for up to 4 h of the temperature shock in the two fishes, we knew that glucose was significant in high and low temperature shocks, total protein was significant in high temperature shock only, and triglycerides-glycerol was significant in low temperature shock only. Lactate and uric acid were not significant in high and low temperature shocks in olive flounder, while in black rockfish lactate was significant in low temperature shock and uric acid were significant in high and low temperature shocks. Therefore, the evaluation data up to 4 h showed that glucose could be used as high and low temperature shock indicators, total protein could be used as a high temperature shock indicator, and triglycerides-glycerol could be used as a low temperature shock indicator in the two fishes. However, up to 8 h, glucose in the high and low temperature shocks and triglycerides-glycerol in the low temperature shock could be used as indicators for black rockfish only.

The experiment was conducted only on a shift of temperature fluctuation from 20 to 28℃ and from 20 to 10℃, acutely up to 8 h. The result indicated that the turnover of new metabolic change in some chemicals by sudden temperature shock took more than 4 h and the recovery to the initial level took more than 8 h at least. We do not know exactly how long it would take for the fishes to recover fully toward the initial level after temperature shock except the tested five chemicals. If any different kind of shock comes within 8 h or before fully recovering from the previous shock, there will be a physiological distrubance in acclimatization, which cause a critical problem in fish health. During the one shift of temperature experiment, we did not observe any fish death, but all olive flounder treated with the 8 h high temperature shock were only dead within two days after the shock. The result showed that the high temperature shock proved to be more

severe than the low temperature shock for fish health.

From the result it was shown that, in olive flounder, all the chemicals were nearly returning toward the initial level after 8 h of the high temperature shock. It means that the metabolic conditions of five chemicals were stabilized almost to the initial level, but fish death was followed after stopping temperature shock only in 8 h with the high temperature shocked group, but not in 4 h. The result indicated that fish death has a strong relation with the shocked time at high temperature and therefore, it is possible to postulate that at least a certain factor for thermotolerance should be required continually to overcome the high temperature during the high temperature shock. It has been known that hsps are involved in protecting cells during heat shock. Therefore, it is possible to postulate that below the tolerable high temperature any shortage of certain level of hsp related substance during the high temperature shock could not protect cells, which would cause fish death slowly a little later than the high temperature shocked time. We consider conducting a further study on fish death in terms of the duration of high temperature shock and the range and frequency of temperature fluctuations with more accurate measurement in the near future.

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