한국어병학회지 제 14권 제 1호 (2001)
J. Fish Pathol., 14(1) : 54~58 (2001)

Effects of seasonal variations in temperature and transport stressor on blood protein and glucose concentrations in wild teleosts of marbled sole (*Limanda yokohamae*) and greenling (*Hexagrammos otakii*)

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The seasonal trends of plasma protein and glucose concentrations in marbled soles(*Limanda yokohamae*) and greenlings(*Hexagrammos otakii*), and the influence of transport stressor on those levels were investigated. Total plasma protein levels of marbled soles and greenlings in late spring and summer were significantly higher than those in winter(January). Plasma glucose levels were consistently increased according to elevation of water temperature both in marbled soles and greenlings. Transport stressor gave rise to decrease of plasma protein levels and increase of blood glucose levels.

Key words : Marbled soles (*Limanda yokohamae*), Greenlings (*Hexagrammos otakii*), Plasma protein, Plasma glucose, Temperature, Transport stressor

Introduction

Temperature is the most important nonliving feature of the environment for most marine organisms. Many poikilotherms can shift their physiological responses to changes in seasonal temperature. Various stressors also induce physiological responses addressed to restore or maintain homeostasis (Adams, 1990).

Those physiological or sublethal stress response in fish to endogenous or exogenous changes can be estimated by measuring various blood parameters. Among them, plasma or serum protein concentrations provides important information on health and physiological status. The functions of blood proteins are to maintain osmotic pressure in the blood, to act as a reserve of protein for tissue repair and growth, to act as pH buffers, to act as transport mechanisms, to act as immunological agents, to provide factors necessary for normal blood coagulation, and to provide necessary enzymes in the blood.

The utilization of carbohydrates in fish is poor in relation to higher vertebrates, however, seasonal temperature change influences on the metabolism of sugar (Nace *et al.*, 1964 ; Chavin and Young, 1970 : Ottolenghi *et al.*, 1995). Glycogen is the primary source for plasma glucose, and elevated blood glucose concentration is commonly used as an indicator of secondary stress response to an acute stress in fish (Barton and Iwama, 1991).

Marbled soles(*Limanda yokohamae*) and greenlings(*Hexagrammos otakii*) are commonly caught from coastal areas in Korea, and many fisheries units in Korea attempt to culture greenlings in caged pens. In the present study, the seasonal trends of plasma protein and glucose concentrations in marbled soles and greenlings, and the influence of transport stressor on those levels were investigated.

Materials and Methods

Fish

Marbled soles(*Limanda yokohamae*) and greenlings(*Hexagrammos otakii*) were collected monthly or bimonthly between January and August 1999, from the Bay of Kwangyang(34°54.97′ to 56.23′ N : 127°49.11′ to 50.9′E) in Korea. Surface water temperature was measured at the sampling site(Fig.

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1). Fish were captured by an otter trawl, and tows were of a maximum 30 min duration. Immediately after trawling, blood samples were taken from caudal vein within 10 min of capture. The other unbled fish were transported to the laboratory in plastic buckets supplied with oxygen and sealed for journey. The duration of transport was about 3 hr, and the fish were bled upon arrival to the laboratory. Blood samples were placed on ice immediately after sampling, and the plasma were separated by centrifugation.

Plasma analyses

Plasma glucose was measured using a glucose /peroxidase enzymatic assay kit(Sigma). Total protein level in plasma was measured by the Lowry et al.(1951) method using bovine serum albumin as reference.

Statistical analyses

Data were analysed by one-way analysis of variance(ANOVA), followed by Student's *t*-test. Levels of confidence P<0.05 were considered statistically significant.

Results

Total protein concentration in plasma

In marbled soles(*Limanda yokohamae*), mean plasma protein concentration markedly(P<0.01) increased in March compared to January(Fig. 2), and the elevated levels of plasma protein were maintained throughout August. The plasma protein levels were significantly(P<0.05) decreased by transport stressor in all sampling months except in January (Fig. 2).

In greenlings(*Hexagrammos otakii*), plasma protein level in April was significantly(P<0.01) higher than in March(Fig. 3), but was not significantly different from that in May, July, and August. Transport stress gave rise to significant(P<0.01 or 0.05) decrease of plasma protein levels in all sampling months except in January and March(Fig. 3).

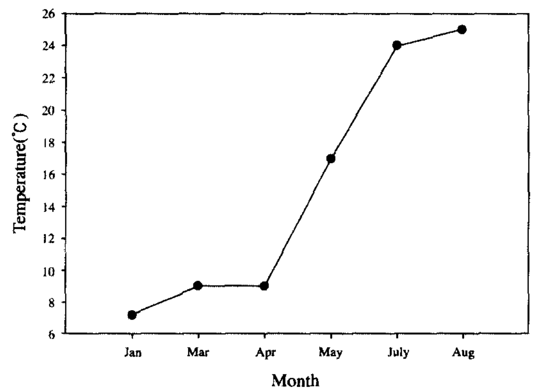

Fig. 1. Changes in water temperature(℃) at sampling site of Kwangyang Bay in Korea from January to August 1999.

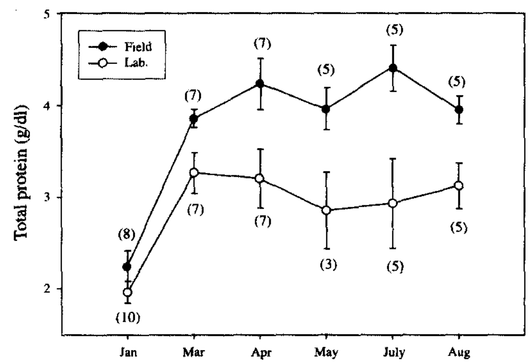

Fig. 2. Total protein concentrations in blood collected on the board (Field, -●-) and collected after transport(Lab., -○-) in marbled soles(*Limanda yokohamae*). Values as mean ± standard error. The number in parenthesis indicates examined number of fish.

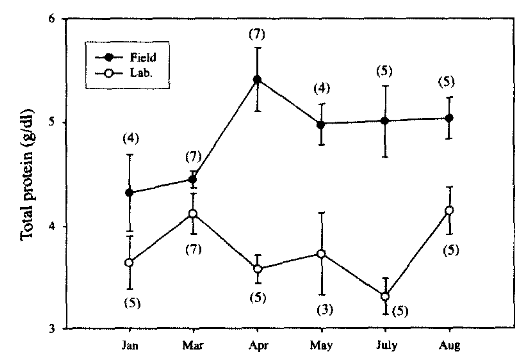

Fig. 3. Total protein concentrations in blood collected on the board(Field, -●-) and collected after transport(Lab., -○-) in greenlings(*Hexagrammos otakii*). Values as mean ± standard error. The number in parenthesis indicates examined number of fish.

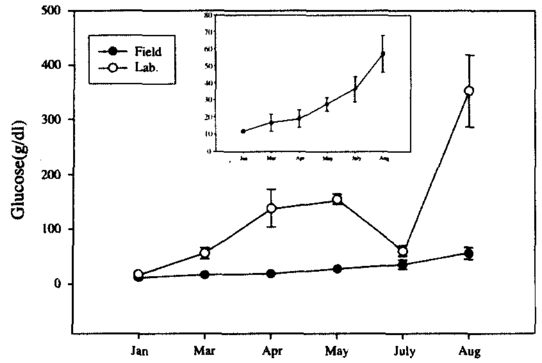

Fig. 4. Glucose concentrations in blood collected on the board(Field, - ● -) and collected after transport(Lab., - ○ -) in marbled soles(*Limanda yokohamae*). Values as mean ± standard error. The number of examined fish were identical with that in Fig. 2. The internal small graph indicates glucose levels in blood collected on the board to show the changing patten more clearly.

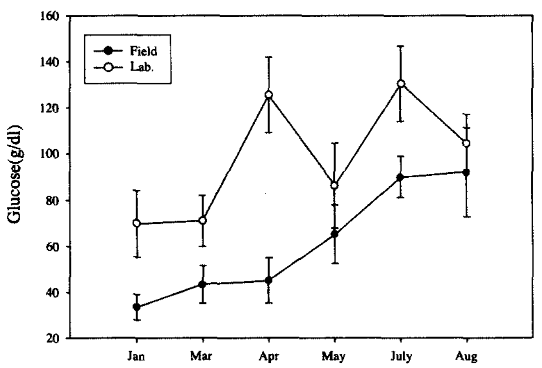

Fig. 5. Glucose concentrations in blood collected on the board(Field, - ● -) and collected after transport(Lab., - ○ -) in greenlings(*Hexagrammos otakii*). Values as mean ± standard error. The number of examined fish were identical with that in Fig. 3.

Plasma glucose level

Plasma glucose levels were consistently increased according to elevation of water temperature both in marbled soles and greenlings(Figs. 4 and 5). Marbled soles suffered transport stress showed significantly increased plasma glucose levels in all sampling months(Fig. 4). Plasma glucose levels of greenlings were increased by transport in all sampling months, but were not statistically significant except in April(Fig. 5).

Discussion

The present study showed that total plasma protein levels of marbled soles and greenlings in late spring and summer were significantly higher than those in winter(January). Collazos et al.(1998) also reported that total protein contents in male *Tinca tinca* significantly decreased in autumn and winter compared to spring and summer, and in females protein output significantly decreased in winter compared to the other seasons. According to the results of Hutchinson and Manning(1996), serum total protein concentrations in dab(*Limanda limanda*) sampled during high temperature period were higher than those in fish sampled during low temperature period.

In the present study, results indicated that elevation of water temperature did not affect directly the increase of blood protein levels. The levels markedly increased at a specific period(March in marbled soles and April in greenlings) before sharp increase of water temperature. The spawning period of marbled soles and greenlings ranges from December to Febraury and from October to January, respectively (Kim et al., 1994). Therefore, plasma protein levels might be affected not only by water temperature but also by an intrinsic annual pattern(spawning) of both fish species.

The plasma or serum of fish contains various protective proteins such as hydrolases(e.g. lysozyme and complement), immunoglobulins, transferrin, caeruloplasmin, C-reactive protein, metallothionein, and interferon together with a series of enzyme inhibitors. It can be suggested that changes in the amounts and activities of serum proteins may reflect susceptibility to diseases(Yin et al., 1995). In the present study, total plasma protein levels were significantly decreased by the transport stressor. Plasma protein reduction could be a result of the increase of protein catabolism induced by stress(Mazeaud et al., 1977 ; Vijayan et al., 1990 ; Pags et al., 1995), in order to obtain supplementary energy.

The present results showed that blood glucose levels were positively correlated with water temperature in both marbled soles and greenlings, indicating that the seasonal temperature affects blood glucose levels. Although stress induced hyperglycaemia has been demonstrated in fish by many authors(Barton and Iwama, 1991 ; Wendelaar Bonga, 1997), the post-stress rise in glucose levels is more delayed than that in cortisol or adrenaline levels. Strange(1980) found that plasma glucose concentrations in channel catfish(*Ictalurus punctatus*) did not increase over 30 min period but did rise subsequently. Rainbow trout (*Oncorhynchus mykiss*) held out of the water for 1 min had cortisol levels that peaked at 1 h post-stress, whereas plasma glucose level reached its maximum deviation from baseline level at 6 h post-stress (Kebus et al., 1992 : Barry et al., 1993). Therefore, in the present study, the effect of a catching stressor on the glucose levels of plasma collected on the board would be little as fish at a site were sampled within a 30 min. Nace et al.(1964) found a higher glycemia in winter than in summer in toadfish, and Ottolenghi et al. (1995) reported a higher blood glucose concentration in winter than in summer in starved catfish(*Ictalurus melas*). However, in the present results, the blood glucose levels of marbled soles and greenlings were significantly higher in summer than in winter. According to the results of Chavin and Young(1970), *Ictalurus melas*, *Cyprinus carpio* and *Pamoxis annularis* became hyperglycemic, *Lepomis macrochirus* became hypoglycemic, and *Micropterus salmoides* remained unchanged at low temperatures. Therefore, the differences in seasonal pattern of blood glucose levels according to fish species might be due to specific physiological factors involved in the metabolism of the sugar.

Blood glucose level increase in response to most types of stressors, and is used commonly as an indicator of stress in fish(Barton and Iwama, 1991). In the present study, plasma glucose levels in marbled soles and greenlings increased by the transport stressor. The alteration in glucose metabolism enabled the fish to cope with the maladaptive effects of the stressor(Mazeaud and Mazeaud, 1981 ; Vijayan et al., 1991).

Acknowledgement

This study was supported in part by the academic research fund of Korea Science & Engineering Foundation (981-0614-073-2), Republic of Korea.

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