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Plasma Cortisol Changes and Body Composition in *Stizostedion lucioperca* Exposed to Handling Stress

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Abstract: *Stizostedion lucioperca* aquaculture/stoking, remains a restrained industry due to several factors such as the paucity of freshwater resources and studies on the physiological responses of this species under environmental changes. The fish were subjected to handling stress by holding them out of the water in a hand-held dip net for 30 sec and netting the fish from the rearing tanks and transferring them to a small confinement tank. Sufficient aeration was supplied to the confinement tank to revert additional stress from oxygen depletion. Then measured changes in plasma cortisol levels and the growth ability (body composition) in *Stizostedion lucioperca* subjected to handling stress. Blood samples were collected from the fish after exposure to the handling stress. Crude protein (N×6.25) was determined according to the Kjeldahl method, moisture content was determined by oven drying at 105±2°C to constant weight and ash by heating in a muffle furnace at 550°C to constant weight. Total lipids were extracted according to the Bligh and Dyer method. The results indicated that, handling stress significantly increased the plasma levels of cortisol 59.04 ng mL^{-1} versus 40.83 ng mL^{-1} in control group. Also the decrease of the level of protein and lipid concentrations show a significant difference between treatment and control ($p<0.05$). As protein and lipid decreased, moisture increased from 78.19% in control to 80.40% in treatment groups. According to the results, there was no significant change in ash content in control and treatment groups which was about 9%. In other words, it could be emphasized that nutrition-related behavior of *Stizostedion lucioperca* resulting from the activation of the hypothalamic/inter-renal axis in response to stress despite of different reactions bear resemblance to that of other fishes. Present data indicate that cortisol appears to be adequate to assess stress in *Stizostedion lucioperca*.

Key words: Cortisol, handling stress, body component, *Stizostedion lucioperca*

INTRODUCTION

Stress in teleosts is characterized by the immediate release of catecholamines and cortisol; both hormones are concerned with energy reallocation from anabolic activities such as growth toward activities to restore homeostasis (Wendelaar Bonga, 1997). Fish are sensitive to acute and chronic environmental changes and show a stress response (Wedemeyer *et al*., 1990; Barton and Iwama, 1991; Iwama *et al*., 1995; Wendelaar Bonga, 1997; Barton *et al*., 1997; Barton, 2000). In aquaculture, fish are frequently exposed to stressful situations such as handling and confinement (Van der Salm *et al*., 2006). Acute stresses from different origins are reported to change the hematological parameters in different species (Demers and Bayne, 1997; Jeney *et al*., 1997; Barcellos *et al*., 1999; Kubokawa *et al*., 1999; Lohner *et al*., 2001; Biswas *et al*., 2004; Geslin and Auperin, 2004; Wang *et al*., 2004). Exposure of fish to stressors can elicit physiological changes at multiple levels of animal organization, these alterations are collectively known as the stress response. The initial response to stress represents the perception of an altered state and is characterized by a neuroendocrine response, which includes the release of stress hormones, such as cortisol (Hosoya *et al*., 2007). Cortisol is the major corticosteroid produced during stress-induced activation of the Hypothalamic-Pituitary-Interrenal (HPI) axis and is considered a principal component of the primary stress response (Donaldson, 1981) cortisol is a major stress-related hormone and its plasma level increases in response to stress (Billard and Gillet, 1981; Pickering *et al*., 1982; Sumpter and Donaldson, 1986).

The purpose of this study was to investigate the effects of an acute handling and confinement stress on circulating levels of cortisol in *Stizostedion lucioperca*. In addition, determine protein, lipid, moisture and ash concentration and physiological measurements were made to verify and expand upon.

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MATERIALS AND METHODS

Fish: There were two experimental groups: control and stressed fishes (two replicates in each). Twenty eight specimens of *Stizostedion lucioperca* were obtained from the volga river and transported to one of the culture ponds of the Research Institute of Aquaculture of the Shahid Rajaii, Rasht City, Iran, were used for the stress experiment. Their body weights (means±SEM) were 750±47 g, respectively.

Experiment 1: Acute handling and confinement stress: After one week of acclimation, a group of fourteen was subjected to acute stress according to Biswas *et al.* (2004). After 48 h, Approximately 1 mL of blood was collected from the caudal vein using a heparinized syringe equipped with a 25 G needle and maintained on ice until centrifugation. Anaesthesia, measurement and blood withdrawal took less than 3 min for five fish. Plasma was separated by 3000 rpm centrifugation at 4°C for 15 min and stored at -80°C for subsequent analysis of different parameters.

Radioimmunoassays: Concentrations of cortisol in the plasma samples were determined by radioimmunoassays as described by Takahashi *et al.* (1985) and Lou *et al.* (1985) for cortisol. All samples were measured in duplicate determinations.

Proximate analysis of muscle tissue: Crude protein (N×6.25) was determined according to the Kjeldahl method (AOAC, 1996), moisture content was determined by oven drying at 105±2°C to constant weight and ash by heating in a muffle furnace at 550°C to constant weight. Total lipids were extracted according to the Bligh and Dyer method (1959), evaporated, weighted and then redissolved in chloroform methanol 9:1, v/v; finally they were stored at a temperature of 0°C.

Statistical analyses: The data were subjected to one -way ANOVA to test the effects of feeding frequency and dietary moisture content. Where significant $p<0.05$ differences were found in the one-way ANOVA test.

RESULTS AND DISCUSSION

Plasma cortisol levels in fish exposed to acute handling and confinement stress rapidly to maximum value and circulating levels of cortisol were significantly ($p<0.05$) different between treatments at the time of control (from 40.82 ng mL^{-1} in control group to 59.04 ng mL^{-1} in treatment group) (Fig. 1).

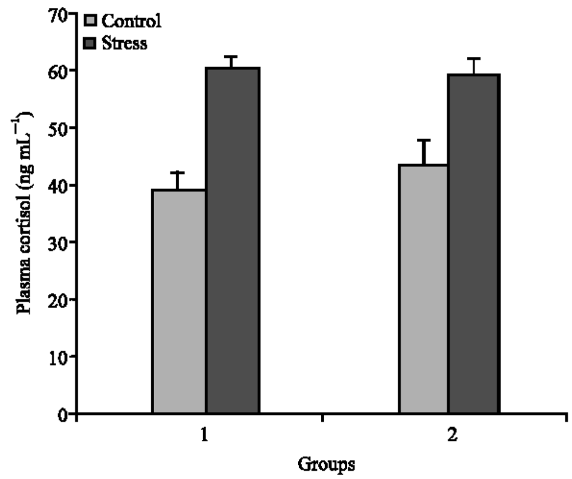

Fig. 1: Changes in mean plasma cortisol levels with the standard error (a vertical line on each column) in *Stizostedion lucioperca* exposed to the acute stress during and after 48 h **: Significantly different at $p<0.05$

Protein and lipid concentrations show significantly decrease between treatment and control ($p<0.05$). Protein from 20.82% in control to 18.74% in treatment and lipid from 39% in control to 22% in treatment decreased. Moisture from 78.19% in control to 80.40% in treatment increased (Fig. 2).

Characteristic cortisol elevations of fishes in response to acute stressors tend to range within about 30 and 300 ng mL^{-1} (Wedemeyer *et al.*, 1990; Barton and Iwama, 1991) but there are notable exceptions. Barton *et al.* (1997) and Barton (2000) observed that peak levels in cortisol following an acute handling stressor were low in scaphirhynchid sturgeons (*Scaphirhynchus* sp.) and paddlefish (*Polyodon spathula*). Their results suggest a trend toward lower stress responses in those chondrosteans compared with teleosts.

Response differences to stressors are clearly evident among closely related fish species and such differences appear to be consistent. Barton (2000) showed that brown trout (*Salmo trutta*) exhibited greater cortisol increases after brief handling and short-term confinement, respectively, than did rainbow trout (*Oncorhynchus mykiss*). This difference was also consistent with glucose responses between these two species. Similarly, Barton (2000) found that lake trout (*Salvelinus namaycush*) were more sensitive to a transport stressor than brook trout (*Salvelinus fontinalis*), a closely related char species.

The acute handling and confinement stress used in these experiments evoked a clear and similar stress response in *Stizostedion lucioperca*, confirming and

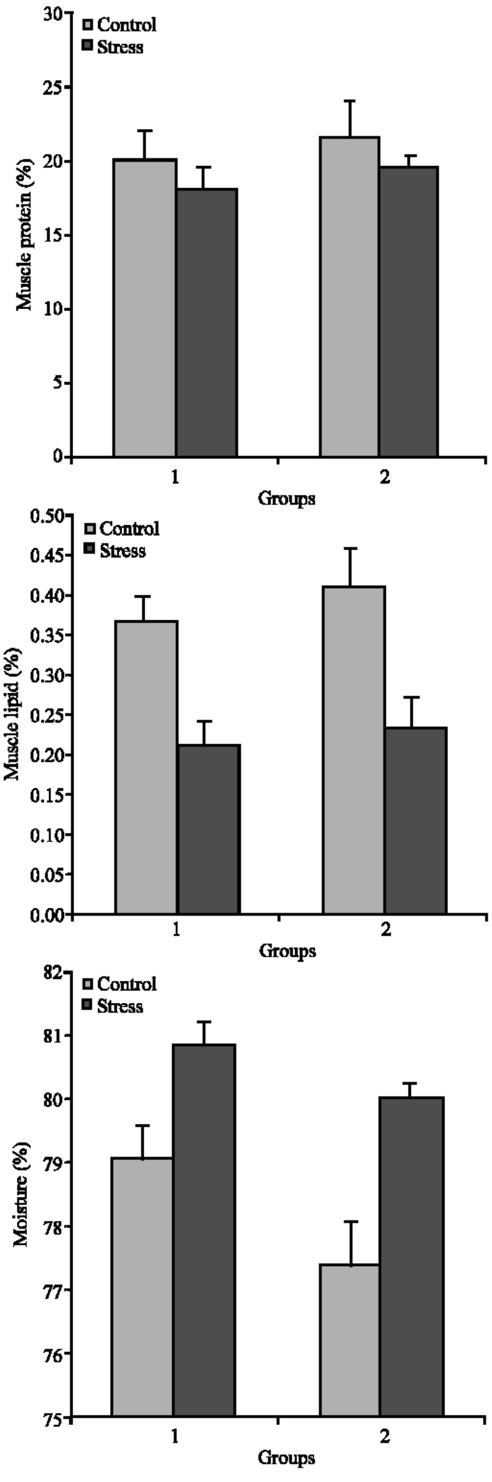

Fig. 2: Changes in mean SE protein, lipid and moisture concentration with the standard error (a vertical line on each column) in *Stizostedion lucioperca* exposed to the acute stress during and after 48 h
**: Significantly different at $p<0.05$

expanding upon the results of Biron and Benfey (1994). This includes rapid elevation of plasma cortisol levels, indicative of activation of the HPI axis and increased plasma glucose levels, due to stress-induced mobilization of energy reserves.

The present results in the stress experiment clearly show that *Stizostedion lucioperca* responded to the acute stress caused by capture, confinement by increasing plasma cortisol levels. Similar results have been reported already in many teleost species including diploid rainbow trout (Dick and Dixon, 1985; Angelidis *et al*., 1987; Pickering *et al*., 1987), brown trout (*Salmo trutta*) (Pickering *et al*., 1982, 1987), Atlantic salmon (*S. salar*) (Pickering *et al*., 1987) and salmon (Fagerlund, 1967; Billard and Gillet, 1981; Pickering *et al*., 1982; Pickering and Pottinger, 1987; Carragher *et al*., 1989; Carragher and Sumpter, 1990; Foo and Lam, 1993; Chopin *et al*., 1996; Jardine *et al*., 1996; Waring *et al*., 1996; Clearwater and Pankhurst, 1997).

Present results show that *Stizostedion lucioperca* responded to acute stress by increasing cortisol. As for the mechanism that determines the maximal level of cortisol in plasma, we suspect the presence of a negative feedback mechanism in the hypothalamus-pituitary-interrenal axes. Suppression of ACTH secretion by elevated cortisol level might determine the maximal level of cortisol as suggested by Pickering and Pottinger (1987). The cortisol in plasma in *Stizostedion lucioperca* captured in the lake were measured to confirm whether the cultured *Stizostedion lucioperca* used in the stress experiment are physiologically equivalent to wild individuals. The values of the physiological parameters observed at the beginning of the experiment in cultured fish were comparable to the values observed in wild individuals captured. Accordingly, the initial condition of the cultured fish can be regarded as equivalent to the wild fish after ovulation but before spawning under the present experimental conditions. This allows us to further generalize the results of the stress experiment.

In the present study, transporting the fish, keeping the fish in the pond and handling the fish for bleeding in all likelihood caused stress and affected plasma hormone and body composition.

Studies of protein concentration of control group in autumn and winter show that protein concentration is increased (from 20.82% in control to 18.74% in treatment) which is due to growth of physiology and tissue substitution against to moisture. Therefore structural role and shaping body tissue by protein is important. In this species, increase in cortisol level causes increased in lipid metabolism, lipid decomposed and therefore a release of FFA. Increasing oxidation of lipid acids lead to intensification of metabolite. Sadovy (1993), also has studied about body composition in different phase of reproductive and found that spawning decrease tissue reserve such as lipid. Steven *et al*. (2000) have studied body composition biochemistry in the season changes as a stress stimulus in fish. In this research we found that lipid in control group 39% is decreased to 22% in treatment group. That is statistically significant. These results consist of data and the difference is due to cortisol role as a lipitic factor in the treatment. As protein and lipid decreased, moisture from 78.19% in control to 80.40% in treatment increased. Therefore protein and lipid tissue substitution with moisture tissue increase. Moisture group in autumn and winter shows that, in winter is decreased in control. According to the results, there is no significant change in ash (control and treatment) it is about 9%.

However primary and secondary physiological response to season changing stress in *Stizostedion lucioperca* is completely normal, but this species is as an active and hunter species tertiary response (low active changing behavior).

In conclusion, present results have shown that *Stizostedion lucioperca* exhibit the typical physiological responses to acute stress induced by handling and brief confinement and acute stress increased the plasma levels of cortisol in this species. Similarly, the acute stress induced decrease in total protein level parallels the findings in other species.

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