Int Aquat Res (2010) 2: 55-60
ISSN 2008-4935
Available online at www.intelaquares.com

International
Aquatic Research

Physiological stress responses in kutum *Rutilus frisii kutum* subjected to captivity

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Received: 10 December 2009; Accepted: 5 March 2010

Abstract

Kutum, *Rutilus frisii kutum* wild broodstocks (970 ± 90 g) were subjected to captivity during their upstream migration and their physiological responses to the stressful condition was assessed. No changes in cortisol levels by was observed after captivity ($P > 0.05$). An increase in glucose levels (1.8 fold) was observed after captivity ($P < 0.05$), but plasma lactate concentration was not changed ($P > 0.05$). Chloride and sodium levels decreased following captivity ($P < 0.05$), while no change in calcium levels was obtained in response to stress. Hematological parameters including red blood cells (RBC), hematocrit (Hct), mean corpuscular volume (MCV), and white blood cells (WBC) were not significantly affected by captivity ($P > 0.05$), whereas hemoglobin (Hb), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) decreased ($P < 0.05$). In addition, the decrease in the number of circulating total lymphocytes and the increase in circulating neutrophiles were observed after captivity ($P < 0.05$).

Keywords: Captivity, Stress, Hematology, Cortisol, Glucose, *Rutilus frisii kutum*

Introduction

Under stress conditions, body of animal responds immediately to the stress recognized as primary and secondary response. The primary responses is the perception of an altered state by central nervous system and the release of stress hormones, cortisol and catecholamines into the blood circulation by endocrine system (Martinez-Porchas et al. 2009). Primary stress responses trigger the sequential secondary response (e.g. increase in plasma glucose, lactate and hematocrit and decrease in chloride, sodium and potassium) in teleosts (Mommsen et al. 1999; Barton 2002). Changes in blood biochemistry and hematology have been studied extensively in different fish species subjected to

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the stressful conditions (Ellsaesser and Clem, 1986; Carragher and Rees, 1994; Cech et al. 1996; Carneiro et al. 2002; Patterson et al. 2004; Barcellos et al. 2004; Gbore et al. 2006; Caipang et al. 2009). The evaluation of blood cells, blood biochemistry and hormones could be useful for the diagnosis of fish disease and to monitor the physiological status of fish (Stoskopf 1993). In aquaculture, fish are typically encountered by stress conditions such as handling, transport and captivity (Bayunova et al. 2002). Kutum, *Rutilus frisii kutum* aquaculture is aimed at restocking the natural environment (southern Caspian Sea) and every year up to 200 millions of 1 g juveniles are released into the natural environment (Abdoli and Naderi 2009). Physiological responses of kutum broodstocks to captivity condition are not well known. Study on the physiological responses of fish species to stressful conditions could lead to better understanding of breeders' management. The aim of the present study was to investigate the physiological response to stress arising from captivity in kutum, *Rutilus frisii kutum*.

Materials and methods

Experimental fish and captivity

Fish were caught from the mouth of Valiabad River (northern Iran) by cast net with 2 cm mesh size. Fourty-five gravid females (which had oocytes attached tightly to the body cavity) (Patterson et al. 2004) were used in this study and divided into three groups of 15 fish each. Fish weight and total length were 970 ± 90 g and 44.4 ± 1.5 cm respectively. To minimize the effect of handling stress, blood samples from first group (including 15 gravid fish) were taken using heparinized syringes within 3 minutes of capture from the caudal vein, then kept in ice. The samples were centrifuged at 4000 g for 10 minutes to obtain plasma which was stored at -20 ºC until assay (Mingist et al. 2007). Thereafter the remained two groups (each with 15 gravid fish) was held in 1×1×1.3 m wooden cages (the cages were placed in river water). Each group was placed in one cage. Water temperature was 14.2±1.8 °C; water flow was 3.5-4 m^3/s and the dissolved oxygen content was 7 mg/l. Fish kept in cages for 72 hours and ovulated fish (captive ripe fish) were removed from the cages and their blood was collected. Their weight (±0.01 g) and total length (± 0.1 cm) were also estimated.

Laboratory analysis

Plasma cortisol levels were measured by radioimmunoassay (RIA) according to Rottlant et al (2001) and expressed as ng/ml. Plasma glucose and lactate levels were determined by enzymatic colorimetric method using the commercial kit (Greiner, Bahlingen, Germany) and expressed as mg/dl and ng/ml, respectively. Plasma sodium concentration was determined by flame photometry (Fc 180 Clem, Sao Paulo, Brazil). Plasma chloride and calcium were measured using a colorimetric method using Technicon autoanalyser (Technicon Instruments Corporation, New York, USA). Number of red blood cells (RBC) was counted by Neubar hemocytometer using Dacie and Lewis solution as diluting fluid (3 g sodium citrate, 99 ml distilled water and 1ml formalin). The counting was performed with four corner and the center fields. For hematocrit (Hct) determination, the sample was collected in glass capillary tubes and centrifuged for 3 min at 4500 × g and expressed as percent packed cell volume (Rehulka 2000). Hemoglobin (Hb) concentration was determined spectrophotometrically at 540 nm (Cyanmethemoglobin method). Erythrocyte indices including mean corpuscular hemoglobin (MCH) mean corpuscular hemoglobin concentration (MCHC) and mean corpuscular volume (MCV) were calculated according to Dacie and Lewis (1984). Differential leukocyte count was performed with blood smears stained with May-Grunwald/Giemsa solution. The smears (two slides per fish) were examined by light microscopy (Olympus, Tokyo, Japan) under oil immersion at 100 × magnification.

Statistical analysis

Normality and homogeneity of data were checked. Then differences in physiological variables before and after captivity were compared with unpaired *t*-test at level of $P < 0.05$. All procedure was done with using SPSS software (SPSS version 13, Chicago, IL, USA). Data into the text and tables are presented as mean ± SEM (standard error). All samples were analyzed in duplicate.

Results

Cortisol concentrations were not changed after holding the fish in captivity ($P > 0.05$). The plasma glucose level increased after captivity ($P < 0.05$) and showed a 1.8-fold increase, compared to pre captivity values. Plasma lactate on the other hand increased slightly following captivity but the increase was not significant ($P > 0.05$) (Table 1). The levels of plasma ions are shown in Table 1. Levels of chloride and sodium decreased following captivity ($P < 0.05$). Moreover plasma levels of calcium showed reduction after captivity compared to the pre captivity values but this reduction was not significant ($P > 0.05$).

Following captivity, blood indices (RBC, Hct, and MCV) were not altered ($P < 0.05$) (Table 2). However values of Hb, MCH and MCHC were decreased compare to the pre captivity values ($P < 0.05$) (Table 2). Mean values of white blood cells were increased slightly after captivity although this increase was not significant ($P > 0.05$). Differential white blood cell counts indicated a significant decrease on the number of circulating lymphocytes as well as the increase in the number of circulating neutrophils (Table 3).

Table 1. Changes in biochemical parameters in kutum subjected to captivity. Values are expressed as mean ± SEM. Different letters in the same row indicate significant difference at $P < 0.05$

Variable	Pre captivity	Post captivity
Cortisol (ng/ml)	308 ± 61.2^{a}	378.5 ± 30.3^{a}
Glucose (ng/ml)	126.5 ± 19.5^{a}	230 ± 23.7^{b}
Lactate (mg/dl)	60.3 ± 1.5^{a}	64.3 ± 1.9^{a}
Chloride (mg/dl)	109.6 ± 4.0^{a}	91.0 ± 3.4^{b}
Sodium (mg/dl)	146.4 ± 3.0^{a}	138.6 ± 2.1^{b}
Calcium (mg/dl)	13.5 ± 0.6^{a}	12.7 ± 0.3^{a}

Table 2. Blood cell parameters in kutum subjected to captivity. Values are expressed as mean ± SEM. Different letters in the same row indicate significant difference at $P < 0.05$

variable	Pre captivity	Post captivity
RBC ($\times 10^4$/mm)	127.0 ± 7.0^{a}	129.0 ± 12.0^{a}
Hct (%)	39.7 ± 2.3^{a}	36.6 ± 2.4^{a}
Hb (g/dl)	8.0 ± 0.4^{a}	5.6 ± 0.4^{b}
MCV (fl)	311 ± 17.0^{a}	287.8 ± 12.6^{a}
MCH (pg)	64.7 ± 5.0^{a}	44.3 ± 1.9^{b}
MCHC (%)	19.9 ± 0.6^{a}	15.4 ± 0.2^{b}

Table 3. White blood cell and differential counts in kutum subjected to captivity. Values are expressed as mean ± SEM. Different letters in the same row indicate significant difference at $P < 0.05$

Variable	Pre captivity	Post captivity
WBC (/mm^3)	7277.7 ± 613.0^{a}	7520.0 ± 430.5^{a}
Lymphocyte (%)	88.5 ± 5.1^{a}	65.0 ± 9.0^{b}
Neutrophil (%)	8.5 ± 4.0^{a}	34.6 ± 8.9^{b}

Discussion

In present study cortisol levels increased 1.3 fold after captivity with no significant changes. In striped bass *Morone Saxatilis*, 3.5-fold increase in cortisol up to 400 ng/ml was observed after handling stress (Cech et al. 1996). In Eurasian perch, *Perca fluviatilis*, 3-fold increase in cortisol levels was observed following stress (Acerete et al.

2004). Regarding secondary stress response, plasma glucose levels were increased in Caspian Kutum after captivity. Increase in blood glucose or hyperglycemia after stress results from the release of catecholamines (Anderson et al. 1991) in order to satisfy the increasing demand for energy during active swimming and continuous disturbances (Umminger 1977). Cortisol and glucose are two of the most common stress indicators but due to their high variability, they must be complemented with other stress indicators (e.g. lactate) in order to have a more complete profile about the stress status of any fish (Martinez-Porchas et al. 2009) and its levels are enhanced in under adverse situations (Grutter and Pankhurst 2000). In this study, plasma lactate slightly increased in fish after captivity, which was in accordance with other studies (Haux et al. 1985; Carragher and Rees 1994; Cech et al. 1996; Vijayan et al. 1997; Arends et al. 1999). However, the levels of *Perca fluviatilis* were not significantly changed after stressor (Acerete et al. 2004).

Decrease in the levels of chloride and sodium are considered as indices of secondary stress response (Barton 2002). This decrease could be due to diffusive loss of small ions across gill membrane associated with the changes in gill mechanisms (an increase in branchial blood flow and gill permeability) as a consequence of the action of primary stress response (Wendelaar-Bonga 1997). In our study, chloride and sodium concentrations decreased following captivity. Moreover plasma levels of calcium were also reduced after captivity compared to the pre captivity values, but this reduction was not significant ($P > 0.05$). Decrease in chloride and sodium levels in fish subjected to stressors was reported in some species such as Smallmouth bass (Carmichael et al. 1983) and Matrinxa *Brycon cephalus* (Urbinati et al. 2004; Carneiro and Urbinati 2002). However in some species e.g. *Perca fluviatilis* (Haux et al. 1985) *Salmo salar* (Einarsdottir and Nilssen 1996) and Jundia *Rhamdia quelen* (Carneiro et al. 2009), their levels were not affected as a result of stress.

In kutum, hematocrit values did not show significant difference (in spite of slight decrease) after captivity and similar result was found in Jundia, *Rhamdia quelen* when subjected to transport stress (Carneiro et al. 2009). Decrease in hematocrit values after stress was also reported in other species such as *Tilapia zilli* and *Clarias gariepinus* (Gbore et al. 2006). Moreover mean values of MCH and MCHC were declined following captivity and a similar result was reported in *Tilapia zilli* (Gbore et al. 2006). However in other species e.g. Eurasian perch, *Perca fluviatilis* plasma MCV, MCH and MCHC showed no difference following acute handling (Acerete et al. 2004). Erythrocyte numbers in Kutum did not change significantly after captivity. This was in agreement with the findings of Urbinati et al. (2004) on Matrinxa, *Brycon cephalus* after loading and transport stress. Significant reduction of Hb in Kutum following captivity was comparable to *Clarias gariepinus* after handling and transport stress (Gbore et al. 2006).

A non-significant increase in WBC numbers was observed in kutum after captivity. Similar finding was reported in *Limanda limanda* (Pulsford et al. 1994) and *Rhamdia quelen* (Barcellos et al. 2004). Increase in WBC following stress may be caused by migration of WBC from the spleen to blood circulation (Barcellos et al. 2004). Reduction of circulating lymphocytes in kutum after captivity was similar to other telesots such as *Salmo trutta* (Pickering 1984), *Icthalurus punctatus* (Ellsaessar and Clem 1987), *Oncorhynchus mykiss* (Pottinger et al. 1994) and *Rhamdia quelen* (Barcellos et al. 2004). It has been reported that lymphopenia after stress may be associated by re-trafficking of cells to lymphoid tissues (Harris and Bird 2000) and to cortisol-induced apoptosis in B cells and consequent clearance from the blood following stress (Weyts et al. 1997). Circulating neutrophile which is known to increase in fish following stress (Ellsaessar and Clem 1987) showed a marked increase in kutum after captivity. Increases in the numbers of these cells were also reported in *Perca fluviatilis* (Haux et al. 1985), *Oncorhynchus mykiss* (Pottinger et al. 1994) and *Rhamdia quelen* (Barcellos et al. 2004).

Decrease in plasma chloride, sodium and lymphocyte and increase in the values of glucose and neutrophils were the main changes observed in kutum following captivity which may indicative of the responsiveness of broodstocks to stress. Adverse effects of stress on fish endocrine system performance and quality of gametes and larvae have been recently reviewed by Schreck (2009). Data on the effect of broodstock stress on the subsequent gamete quality are scare (Bobe and Labbé 2009) and depends primarily on when in the life cycle it is experienced and the severity and duration of the stress. Elevated plasma cortisol has caused in declines in body size, gonado-somatic index, egg size and gamete quality (Foo and lam 1993; Kime and Nash 1999; Campbell et al. 1992, 1994). Therefore further study on the effect of captivity and crowding on gamete quality (fertilization and hatching success) should be carried out.

Acknowledgments

The authors would like to thank to Mr. Chopani for assisting with fish sampling and to Mr. Maleki for helping in laboratory assay.

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