# The impact of captivity on fertilization, cortisol and glucose levels in plasma in kutum broodstock

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**Abstract**

Gravid wild kutum broodstock, *Rutilus frisii kutum,* on their upstream migration to Valiabad River (northern Iran) were kept in captivity to allow them to ovulate (captive ripe). Then the impact of captivity on some reproductive and physiological parameters (*i.e.*, plasma cortisol and glucose levels, fertilization percentage, gonadosomatic index and fecundity) were assessed and results were compared with those obtained from naturally ovulated broodstock (ripe). Plasma cortisol level was not significantly different between gravid and captive ripe broodstock but was significantly higher ($P<0.05$) than that in ripe group. Glucose level was significantly higher ($P<0.05$) in captive ripe compared to that in ripe or gravid groups. Mean gonadosomatic index, fecundity, and fertilization rate did not affect as a result of stress by captivity. Inverse relationship between plasma cortisol levels and fertilization rate was observed.

**Keywords**: Captivity, Cortisol, Fertilization rate, Glucose, Stress, Kutum.

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## Introduction

In teleosts the Hypothalamic-Pituatory-Interenal (HPI) is activated under stress conditions and consists of a hormone cascade culminating in the release of cortisol from the interrenals to the blood stream (Martinez-Porchez et al*.,* 2009). Cortisol is the major corticosteroid among teleosts (Stratholt et al., 1997) and its increase in response to environmental stressors reveal the primary response to acute stress. Secondary responses occur as a consequence of the released stress hormones (Barton and Iwama, 1991), causing changes in the blood and tissue chemistry, e.g. an increase of plasma glucose (Barton, 2002). Adverse effects of stress on fish endocrine system performance and quality of gametes and larvae is recently reviewed by Schreck (2009). Data on the effect of broodstock stress on the subsequent gamete quality are scarce (Bobe and Labbé, 2010) 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 (Campbell et al. 1992, 1994; Foo and lam 1993; Kime and Nash 1999). In aquaculture, fish are typically encountered by stress conditions, such as handling, transport and captivity (Bayunova et al*.,* 2002; Divers, 2006). Although short-term holding of broodstocks in captivity is a way to obtain ovulated eggs (Kestemont and Mellard, 2000), there is still limited information on the physiological responses and gamete quality of kutum, *Rutilus frisii kutum* wild broodstock to stress arising from captivity condition. The objective of this study was to investigate whether short term captivity of kutum broodstocks caught in the river on their upstream migration will affect reproductive and physiological parameters (plasma cortisol and glucose levels, fertilization percentage, gonadosomatic index and fecundity) when compared to naturally ovulated (ripe) fish.

## Materials and methods

Fish were caught at Valiabad River (northern Iran) by cast net of 2 cm mesh size (23-28 March, 2009) and were classified into three groups as described by Patterson et al. (2004): 1- *ripe fish*: these fishes were releasing (ovulating) gametes when caught in the river. 2- *Gravid fish*: in these fishes gametes (oocytes) were attached tightly to the body cavity. 3- *Captive ripe fish*: these were gravid fish after natural ovulation by keeping in the cages. 20 ripe fish (weight: 1024.3±116.3 g, length: 45.2±2 cm) and 45 gravid fish (weight: 969.8±89 g, length: 44.35±1.45 cm) were used in this study.Blood samples from 20 ripe and 15 gravid fish was collected first, then two groups of 15 gravid fish each were held in 1×1×1.3 m wooden cages (the cages were placed in river water) each group per cage. Water temperature was 14.2±1.8°C, water flow was 3.5-4$m^3s^{1-,}$ and the dissolved oxygen content was 7$mgL^{-1}$. Fish were kept in cages for 72 hours and ovulated fish (captive ripe fish) were removed from the cages and their blood was collected.

To minimize the effect of handling stress, blood samples were taken with heparinized syringes within 3 minute of capture from the caudal vein, then were

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kept in ice and followed bycentrifuge at 4000 g for 10 minutes to obtain plasma which was stored at -20ºC until assay (Mingist et al., 2007). The eggs were obtained through a gentle pressure on the abdomen and fertilized by dry fertilization technique. Two minutes after fertilizing, the excess of sperm was rinsed off and fresh water was added. The eggs were then washed with river water (temperature 14ºC) for 20 minutes to remove the stickiness. Then, fertilized eggs were placed in plastic jars and were allowed to water harden for 45 minute and stirred every 5 to 10 minutes before being placed in to the incubators. Eggs were then transferred to Seth green incubators (60×40×25 cm) in river water (temperature 14ºC), maintained in there for 48 hours. Fertilization percentage was determined at 8-cell stage (Razavi Sayyad, 1984).

After blood collection, fish weight (±0.01 g), total length (± 0.1cm) and total egg mass (±0.01 g) were measured. Gonadosomatic index (GSI) was calculated as [ovary weight/body weight] ×100. One gram of egg mass was weighed to the nearest 0.0001 g and number of eggs was counted, then fecundity (3 replicates for each fish) was estimated as the number of egg per gram of egg mass × total egg mass of that female.Serum glucose was determined by colorimetric auto analysis using commercial kit (Greiner, Bahlingen, Germany) and expressed as ng $ml^{-1}$. Serum cortisol level was measured by radioimmunoassay (RIA) according to Rottlant et al. (2001) and expressed as ng $ml^{-1}$. All samples were analyzed in duplicate.

Reproductive parameters in ripe and captive ripe broodstock were analyzed by unpaired t-test while difference inphysiological parameters among three groups (ripe, gravid and captive ripe broodstock) were analyzed by one-way analysis of variance (ANOVA) followed by Duncan multiple range test using SPSS statistical software, release 13 (SPSS Inc., Chicago, IL, USA). Correlations between plasma cortisol and fertilization rate was analyzed by Pearson's coefficient for linear regression (r). The differences were considered significant at $P<0.05$. All data were expressed as mean ± S.E.M.

**Results**

Plasma cortisol level in ripe wild broodstock was significantly ($P<0.05$) lower than that in gravid and captive ripe fish. Cortisol levels increased after captivity (in captive ripe fish) compared to gravid fish but this increase was not significant (Fig. 1).

Plasma glucose level between ripe and gravid fish was not significantly different however after captivity (in captive ripe fish) a significant increase was observed ($P<0.05$) (Fig. 2). Mean gonadosomatic index was not significantly different between ripe and captive ripe fish (P value: 0.105; N= 19). Means of total fecundity in ripe and captive ripe fish did not show any significant difference (P value: 0.084; N= 19). Fertilization percentage did not vary between ripe and captive ripe fish (P value: 0.14; N= 19) (Table 1). There was a negative relationship between plasma cortisol level and fertilization rate ($r^2$= -0.746).

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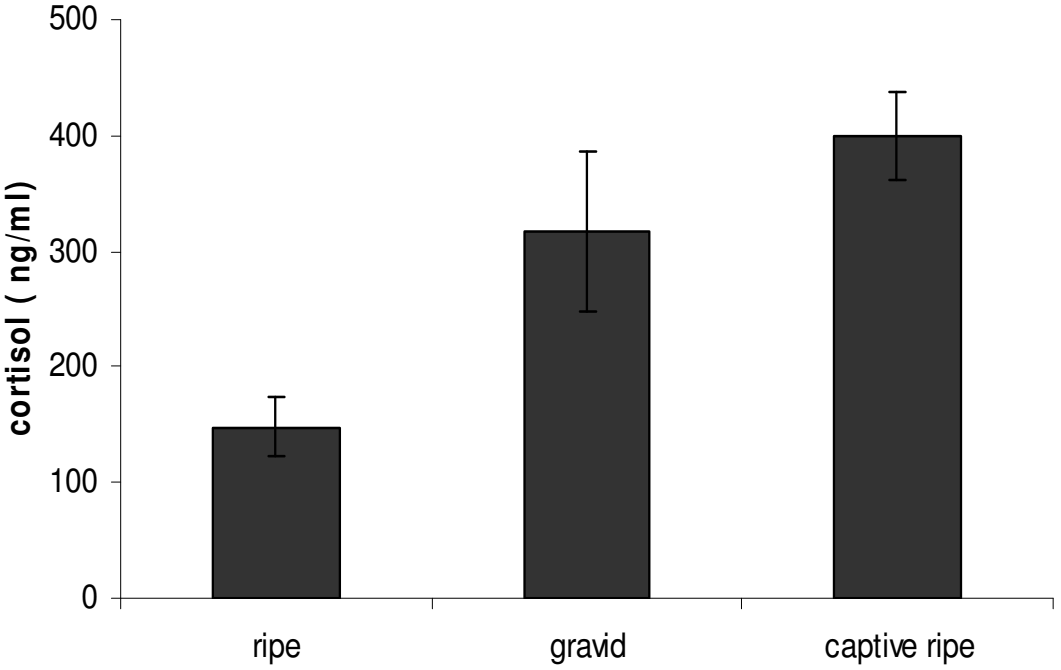



**Figure 1: Mean values for cortisol level (ng ml$^{-1}$) in ripe (n= 20), gravid (n= 15) and captive ripe (n= 19) kutum broodstock.**

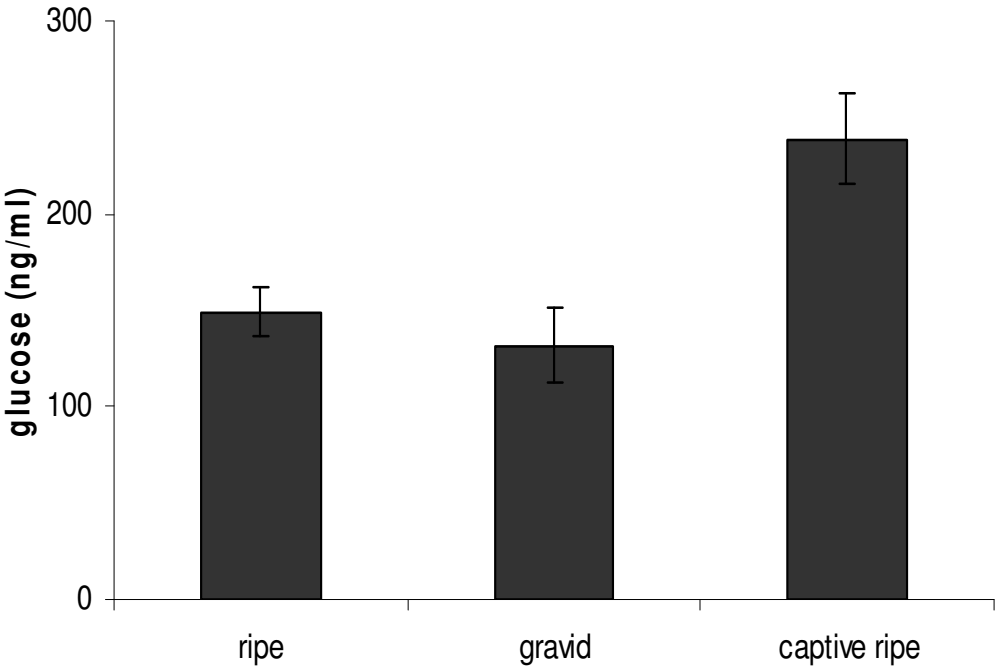



**Figure 2: Mean values for glucose (ng ml$^{-1}$) in ripe (n= 20), gravid (n= 15) and captive ripe (n= 19) kutum broodstock.**

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**Table 1: Mean values for the reproductive variables in ripe and captive ripe kutum broodstock**

| variable | broodstock | n | mean±SEM | range | P-value |
|---|---|---|---|---|---|
| GSI (%) | Ripe | 20 | 13.26±0.8 | 7.33-17.25 | 0.105 |
| | Captive Ripe | 19 | 14.28±0.64 | 9.84-18.95 | |
| Fecundity (thousand) | Ripe | 20 | 32639.5±4124.3 | 11465-58859.4 | 0.084 |
| | Captive Ripe | 19 | 33919.3±2769 | 13499-63314 | |
| Fertilization rate (%) | Ripe | 20 | 92.6±1.1 | 82-100 | 0.14 |
| | Captive Ripe | 19 | 89±2 | 75-96 | |

## Discussion

In this study about 1.3-fold increase in plasma cortisol levels after captivity was observed but the increase was not significant. Similarly, in Coho salmon, *Oncorhynchus kisutch,* subjected to stressor, plasma cortisol levels increased while egg viability and gonadosomatic index were not affected (Stratholt et al., 1997).

In rainbow trout, *Oncorhynchus mykiss* repeated acute stress during prolonged period prior to spawning negatively affected gamete quality (lower survival of eyed stage, hatching and swim-up) (Campbell et al., 1992) and chronic confinement stress reduced gamete quality in rainbow and brown trouts (Campbell et al.,1994).

In the present study, captivity did not affect egg fertilization percentage and there was no significant difference between ripe and captive ripe fish. Similarly Contreras-Sanchez et al. (1998) found that stressor applied during late vitellogenesis-final maturation did not affect progeny survival.

In agreement with Stratholt et al. (1997) gonadosomatic index was not affected as a result of captivity. Mean fecundity in captive ripe fishes was not different from the fecundity of ripe groups. Similarly, Patterson et al. (2004) was also found no significant difference in fecundity between natal (non feeding natural migratory) and captive fishes. According to Tyler et al. (1994) fecundity is set month prior to freshwater re- entry, therefore stress may not affect fecundity at spawning time.

In this study plasma glucose levels did not show significant difference between ripe and gravid fish but significantly increased after captivity. It is reported that hyperglycemia after stress results from the release of catecholamines (Anderson et al., 1991) which is used by the fish to satisfy the increasing demand for energy during active swimming and continuous disturbances (Umminger, 1977). In the present study, negative relationship between serum cortisol levels and fertilization was observed. Similarly negative relationship between serum

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cortisol and eyed-egg percentage was found in Masu salmon *Oncorhynchus masou* (Mingist et al., 2007).

In conclusion, slight increase in plasma cortisol and 1.8-fold increase in glucose levels occurred in kutum broodstock subjected to captivity indicate that this species is responsive to stress. Short-term stress due to captivity did not affect the gamete quality parameters. Similarly Bayunova et al. (2002) observed that short term and low severity associated stress did not affect gamete quality of Stellate, *Acipenser stellatus* and Russian sturgeon, *A. guldenstaedtii*. Further experiments is suggest to be undertaken to assess the effect of crowding (low and high density) of gravid fishes under captivity condition on the physiological responses and on the more advanced egg developmental stages, e.g., hatching success and yolk-sac resorption stage.

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