

Stress responses of Persian sturgeon, *Acipenser persicus* to repetition of a management stressor (handstripping of milt)

Saeed HAJIRAZAEE

Gholam Reza RAFIEE

Department of Fisheries and Environmental Sciences, Faculty of Natural Resources, University of Tehran, Karaj, IRAN

*Corresponding Author

e-mail: S.Hajirzaee@gmail.com

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Abstract

To determine the dynamics of stress responses in the Persian sturgeon, *Acipenser persicus*, two most indicators of stress (i.e. cortisol and glucose) were measured after pituitary preparation injection and during repeated handstrippings of milt. The serum values of cortisol and glucose increased after pituitary preparation injection with maximum levels in the first and second handstripping respectively. Afterward, the cortisol decreased to initial values while glucose levels remained in their maximum values during further handstrippings. Also, a significant negative relationship was recorded between serum levels of cortisol and glucose. In conclusion, our results show a dissimilarity between Persian sturgeon (as a chondrosteian fish) and teleost fish in terms of physiological responses to a stressor, however, the more studies are invited for confirmation of this claim in sturgeons.

INTRODUCTION

Under culture condition, adult fish are exposed to various types of stressors such as confinement, crowding, handling, biopsy, transportation and hormonally induced spawning. Understanding of the stress responses in fish may help to improve management, production, and animal welfare.

Sturgeons are economically and culturally important in the world and the investigation of their biological aspects could provide a key to successful management of their populations in nature and aquaculture. The Persian sturgeon, *Acipenser persicus* is one of valuable species of sturgeons (figure1) which has been considered for a biological conservation program in the southern basin of the Caspian Sea. At now, each male brooder of Persian sturgeon is stripped more than once during the spermiation period due to the shortage of stock. Therefore, the fish are exposed to a repeated management stressor i.e. handstrippings during spermiation period.

In the teleosts, it is well recognized that the stressor stimulates the hypothalamic-pituitary-interrenal (HPI) axis to release cortisol from the interrenal tissue as a primary response to stress [1,7,8,9,14]. Afterward, cortisol mediates the stress-induced hyperglycemia (increasing of glucose concentration in blood) that is thought to be crucial for supporting the increased energy

demand associated with stress [8,10,13]. Therefore, the serum changes of cortisol and glucose are used as two most indicators of stress in the teleost fish.

Compared to teleosts, little is known about the stress response in chondrosteians including sturgeons. Thus, in this study, we investigated the changes of two most indicators of stress i.e. cortisol and glucose in response to repeated handlings of fish for milt collection. Such study could provide more information about the dynamics of stress responses in the Persian sturgeon.

MATERIALS and METHODS

Nine Persian sturgeon males (Total length= 120-148cm, Total weight = 19.5-21 Kg) were captured in the southern basin of the Caspian Sea during March to June 2008 and transported to Shahid Beheshti Artificial Sturgeon Propagation and Rearing Center (SAPRC), Iran, Rasht. After delivery to SAPRC, the males were kept in pond (1m×4m×8m) with follow of river water (about 1.5 m³/min). During the experiment, the water temperature was 16-18°C, dissolved oxygen was 8 -8.3 mg/L and pH: 7.3-7.5.

Just before experiment, to induce the spermiation, the males were injected intramuscularly with pituitary preparation (PP) at doses of 50-70 mg [6]. Approximately after 23h, 6 males were spermiated and the rest did not

respond to injection. The blood samples were taken from the caudal vein of males using heparinized syringe in different times as follow: at the time of the PP treatment; at the beginning of spermiation (first stripping of milt) and 12h (second stripping of milt), 24h (third stripping of milt) and 36h (fourth stripping of milt) after the first stripping (figure1). The blood samples were centrifuged (D-78532 centrifuge, Tuttlingen, Zentrifugen, Germany; 5000 rpm for 10 min) to separate the serum. Then, the serum samples were stored at -20°C until further analyses.

Cortisol concentrations (ng/mL serum) were measured by enzyme-linked immunosorbent assay (ELISA) according to Bayunova et al. (2002) [2] and Semenkova et al. (2002) [11]. Also, glucose (mMol/L serum) concentrations were measured with colorimetric method using an Auto-analyser Technican (RA 1000, Technicon-Swords, Dublin, Ireland).

The SPSS software was used for data analysis. The plasma values of cortisol and glucose were normal according to Kolmogorov Smirnov test. One-way analysis of variance (ANOVA) was employed to compare the means of cortisol and glucose among different times of handstripping. When significant F-ratios were calculated by ANOVA, the Tukey test was applied to identify which means were different. The correlations between glucose and cortisol were using the bivariate correlation coefficients of Pearson. Then, Linear and non-linear regression models were investigated using regression fits.

RESULTS

The values of cortisol increased significantly after PP treatment (Figure2, P<0.05) with a maximum level in the first stripping. Afterward, these values decreased (Figure2, P<0.05) to initial levels during further handstrippings. The concentration of serum glucose elevated significantly after PP treatment (Figure3, P<0.05) with a maximum range of 0.41-0.63 (mMol/L serum) during second, third and fourth handstripping.

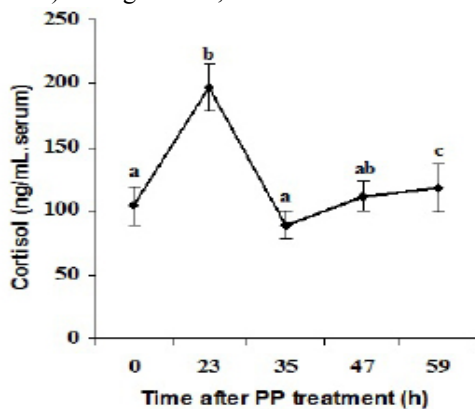


Figure2. Serum cortisol levels (mean±sd) of Persian sturgeon males after PP treatment and during repeated hanhstrippings. Means with same superscripts are not significantly different (P>0.05).

Family: Acipenseridae
Common name: Persian sturgeon
Scientific name: *Acipenser persicus* (Borodin, 1897)



(Collection of Persian sturgeon males from the southern basin of the Caspian Sea)

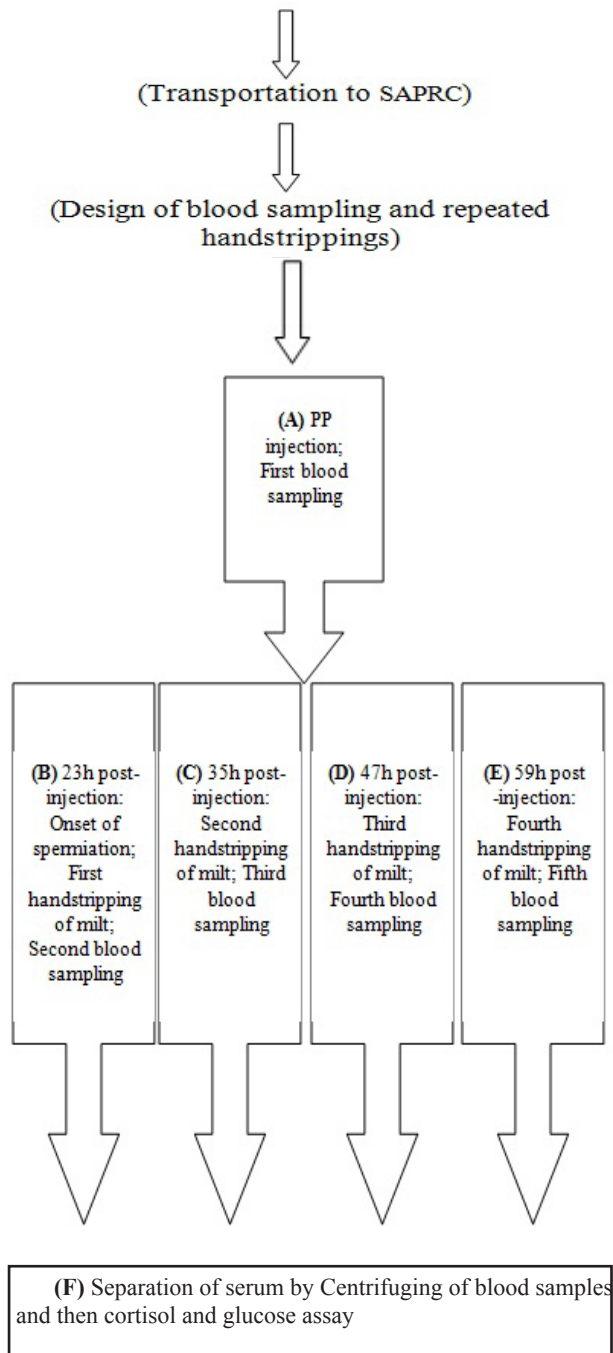


Figure1. Schematic of the procedure used in the present publication for, pituitary preparation (PP) injection, blood sampling and handstrippings of milt.

In the present experiment, a significant negative relationship was found between plasma levels of cortisol and glucose (Figure4, $P < 0.05$).

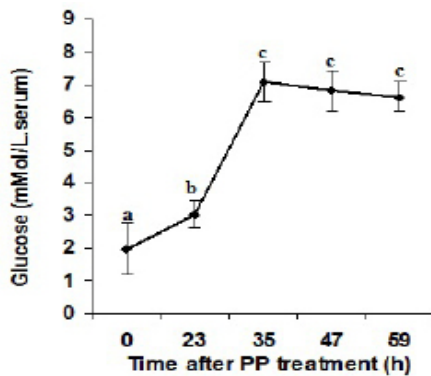


Figure3. Serum glucose levels (mean±sd) of Persian sturgeon males after PP treatment and during repeated handstrippings. Means with same superscripts are not significantly different ($P > 0.05$).

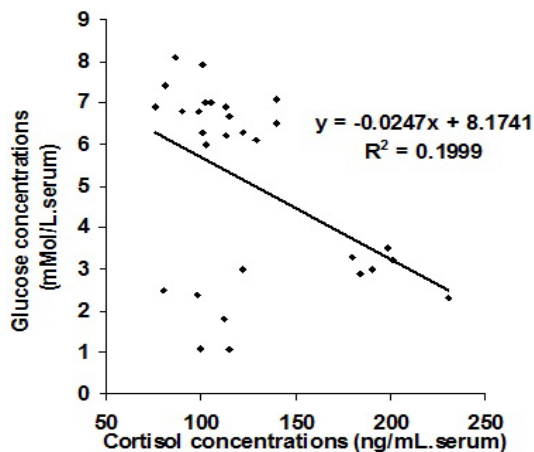


Figure4. The relationship between serum levels of glucose and cortisol in the Persian sturgeon males.

DISCUSSION

After pituitary preparation (PP) treatment, the serum levels of cortisol elevated to a maximum level of 197.16 ± 17.96 (ng/mL serum) in the first stripping and then decreased over further handstrippings. Serum cortisol is the most commonly measured indicator of stress in fish that usually provides a good reflection of the severity and duration of the stress response. The milt collection involves the handling (as an unavoidable management stressor) of fish that mostly is in company with elevations in serum cortisol levels. But, there was not any elevation in cortisol levels of blood after first milt collection practice from Persian sturgeon. This problem may be due to the long intervals (12h) between blood samplings, so that probably the cortisol levels have increased and returned to resting levels before each handstripping, thus, we could not record any elevation in serum cortisol with repetition of handstripping of milt.

It was observed that usually the cortisol levels return to resting level within 2.5-4h after the onset of short-term management stressors in white sturgeon, *Acipenser transmontanus* [4].

However, a maximum level of cortisol was recorded 23h after PP treatment which this time gap is longer than time intervals between handstrippings. On the other hand, the injection stress involves only water reduction and refilling while handstripping stress is composed of water reduction, air exposure, capture, transportation and handling. Therefore, it seems that the severity of injection stress to be less than handstripping. In addition to mediatory role of cortisol in stressful conditions, this steroid has been considered as an endocrine component of the reproductive system in teleost fish [5] and sturgeons as its levels increased after pituitary preparation (PP) treatment [2,11]. Thus, the considerable increases of cortisol after a long gap (Approximately 23h) between the PP injection and the first stripping (beginning of spermiation) emphasis the probable role of this steroid in final maturation of sturgeon spermatozoa as same results have been reported for stellate [3]. These authors recorded the maximum levels of cortisol 8h after spawning induction of stellate males.

In the teleosts, it is well recognized that cortisol play a key role in elevated glucose response that is crucial for coping with the increased energy demand associated with stress [8,13]. During stressful condition, cortisol act directly on the liver to stimulate glycogenolysis, which results in the mobilization of glucose and subsequently hyperglycemia.

In the present experiment, the values of serum glucose increased after PP injection, with a maximum range during repeated handstrippings. It seems that the glucose to be more durable indicator of stress than cortisol since glucose levels did not return to initial levels after removing of stressor (i.e. handstripping) during long interval between two handstrippings.

Such trend of glucose is different than cortisol where their levels decreased to initial levels after first handstripping. As well as, a negative correlation was found between plasma levels of cortisol and glucose. Such relationship shows a dissimilarity between teleost fish and Persian sturgeon (as a chondrosteian fish) in terms of physiological responses to a stressor. Probably, the metabolism rate of glucose is slower in the Persian sturgeon than in a teleost fish, although we did not examine this hypothesis in the present study. In conclusion, it seems that only cortisol could be used as selection markers for stress responsiveness of Persian sturgeon, although the measurement of blood glucose can be carried out rapidly using portable equipment, in contrast to the more sophisticated facilities required for the determination of cortisol.

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