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Stress responses in sub-yearling great sturgeon to the air exposure

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ABSTRACT
This study was conducted the effect of stress on blood glucose and cortisol levels in cultured great sturgeon. Blood sampling was done and then water level decreased to the half in experimental tanks. The fish were exposed in that situation and second blood sampling was taken after 6 hours. Glucose and cortisol concentrations were measured by glucose oxidase method and Radioimmunoassay (RIA), respectively. The results showed that average of cortisol concentration reached from 10.8 ± 3.3 to 14.6 ± 9.0 ng ml^{-1} ($P<0.05$). Indeed, average of glucose concentration was 56.4 ± 12.0 pre-stress exposures and reached to 111.6 ± 17.5 mg dl^{-1} post-stress ($P<0.05$). Stress is most often associated with a negative perspective. We recommend for increasing efficiency in rearing, should avoid of stressors possibly that it needs to right management on rearing practice and this action causes to increase in production, fish welfare, restoration and economic efficiency.

Keywords: Cortisol, Glucose, Handling, Great Sturgeon (*Huso huso*), Stress.

INTRODUCTION
Sturgeon fish have been distributing over of northern hemisphere, north and east in Asia, Northern America and Europe. Caspian Sea is the biggest closed lake in the world which with Lake Basin is the most suitable habitat for sturgeon fish around the world (Keyvan, 2003). Resources of sturgeon fish is declining in the Caspian Sea and for this matter have stated different reasons (Kozlov, 1993). Among of this fish, farmers have noticed to some of them because of quick growth, resistance to less water quality and well-being for aquaculture in rearing systems (Williot *et al*., 1993).Great sturgeon is one of the biggest fish in fresh water that their resources have declined because of human activity and their caviar have high-grade quality. Some researchers believe that their survival related to artificial propagation and rearing, also releasing to the sea completely. Thus, sturgeon fish have cultured in confined spaces in developed and developing countries because of the best quality of meat and caviar, extreme reduction in natural resources and also, being important in side economic (Keyvan, 2003).

Concerning this, rearing great sturgeon have a particular situation among of other sturgeons because of their quick growth (Keyvan, 2003) and in recent years, have been noticed to rear this fish a lot in artificial spaces. Whereas, the situation in farms are different and fluctuant. A farm is an unnatural and stressful space. Stress is a sort of menace for balance or body's homeostasis and response to stress have related to quality, magnitude and duration in the effect of stimuli, constructor and situation of organism. Stress has negative affects on body systems. Schreck (1981), Wedemeyer and McLeay, (1981), Wedemeyer *et al*., (1990) demonstrated that the most suitable indices in variety of stress in fish is studying jointly plasma cortisol and glucose for hyperglycemia property result of gluconeogenesis and glycogenolysis by cortisol (Barton *et al*., 1985; Pickering *et al*., 1987). Therefore, the objective of this study was conducted to determine the biochemical factors in blood

including serum cortisol (as primary response) and glucose (as secondary response) levels in fish subjected to stressors.

MATERIALS AND METHODS

Fish

Sub-yearling great sturgeons with 268 ± 71 g body weight were obtained from International. Sturgeon Research Institute in Guilan province, Iran. 3 tanks were considered which each one containing 30 fish.

Sampling and analysis

Three fish per tank were randomly selected for cortisol and glucose concentration measurements. Blood sampling were taken immediately after fish going out. The blood samples were taken from the caudal vein using a non-heparinized syringe. Then the fish marked by the tag. After taking the blood, water levels were decreased to the half in experimental tanks.

The fish were exposed to this situation about 6 hours. Second blood Samplings were taken. Blood samples were kept in room temperature about 3-4 h. Serum was separated from other component by centrifugation 4500 *g* for 10 min. Blood serum was then separated and stored in 1.5 ml eppendorf tubes at -20°C until cortisol and glucose analysis was performed. Serum glucose was determined by glucose oxidase method. The serum cortisol concentration was quantified using a radioimmunoassay (RIA) (Bayunova *et al*., 2002).

Water temperature was kept at 13°C and the dissolved oxygen content was 9.85 mg l^{-1}, nitrite 0.06 mg l^{-1}, nitrate 1.25 mg l^{-1}, and pH 7.52 was carried out using a portable water multichecker probe (WTW, Germany) and experimental kits (Machery-Nagel GmbH, Düren, Germany.

Data analysis

Cortisol and glucose data were subjected to two-way analyses of variance (ANOVA) to test for differences among treatments and among tanks for each treatment. Data for the three tanks were analyzed by Student's *t*-test to determine differences between pre-stress and post-stress group means for each feature ($P< 0.05$).

RESULTS

In this study, minimum serum cortisol level was 8.0 ng ml^{-1} pre-stress exposures and maximum was 19.0 ng ml^{-1}. Minimum and maximum levels of serum cortisol were 7.0 and 56.0 ng ml^{-1} post-stress exposure, respectively (Table 1).Minimum and maximum of glucose levels were also shown in table 2.

Average of serum cortisol level reached from 10.8 ± 3.3 to 14.6 ± 9.0 ng ml^{-1} and glucose level was 56.4 ± 12.0 to 111.6 ± 17.5 mg dl^{-1} pre and post stress, respectively (table 3).

The results of analysis showed that statistical difference for average cortisol levels were observed significantly between pre and post stress ($P<0.05$). However significant difference was observed for glucose pre-stress and post-stress exposure to stressor ($P<0.05$).

Table 1. Minimum, maximum, and average (Ave ± SD) of serum cortisol and glucose levels before and after stress (*n*=9).

	Glucose(mg dl^{1})		Cortisol(ng ml^{1})	
	Pre-stress	Post-stress	Pre-stress	Post-stress
Min	33	79	8	7
Max	84	150	19	56
Ave± SD	56.4±12.0	111.6±17.5*	10.8±3.3	14.6±9.0*

Table 2. Minimum and maximum glucose level (n=9).

Glucose level before exposure to stress (mg dl^{-1})		Glucose level after exposure to stress (mg dl^{-1})	
Min	Max	Min	Max
33	84	79	150

Table 3. Average (average ± SD) of serum cortisol and glucose levels before and after stress (n=9).

Average of serum cortisol (ng ml^{-1})		Average of serum glucose (mg dl^{-1})	
Pre-stress	Post-stress	Pre-stress	Post-stress
10.8 ± 3.3	14.6 ± 9.0	56.4 ± 12.0	111.6 ±17.5

DISCUSSION

Results of this study showed that average of serum cortisol level reached from 10.8 ± 3.3 to 14.6 ± 9.0 ng ml^{-1} with significantly differences ($P<0.05$). Plasma cortisol in Pallid (*Scaphirhynchus albus*) and hybrid sturgeon

(Pallid × Shovelnose, *S. albus* × *Platorynchus*) yearling reached from 3 to 13-14 ng ml $^{-1}$ during 6 hours in extreme confinement. In addition, Plasma cortisol level increased 0.22 ng ml min $^{-1}$ in 2 years fish (Barton *et al*., 1999). Floodmark *et al.,* (2002), He observed an increase in plasma cortisol level in brown trout (*Salmo trutta*) that exposed to decline in water current. Effect of acute stress showed an increase in plasma cortisol level in Russian and Stellate sturgeon (Bukovskaya *et al*., 1997).

It's dramatically that the cortisol response may be influenced not only by the nature of the stress, but also by other factors such as the age, sex and state of maturity of the fish (Sumpter, 1997), the environmental temperature (Sumpter *et al*., 1986), the species and strain of fish (Pickering and Pottinger, 1989) and even, under certain circumstances, by the chemical composition of the water (Pickering and Pottinger, 1987). A common problem in comparing stress responses in fish is the determination of basal cortisol levels, as factors such as the method of capture, the stage of sexual maturity, season, time of day and sex can all influence plasma cortisol levels (Barton and Iwama, 1991; Pankhurst and Sharples, 1992; Sumpter, 1997). Even, blood sampling procedures and chasing cause increases in blood levels of the hormone that is not showing basal blood cortisol levels markedly. Schreck (1981), Wedemeyer and McLeay (1981) believe that the most suitable indicators of stress varieties in fish is studying blood plasma cortisol and glucose together (Barton *et al*., 1985). Measuring blood plasma glucose after exposure to stress is similar to cortisol that it is suitable factor for estimating intensity of neuroendocrine responses in fish (Pottinger and Carrick, 1999). In this study, the average of serum glucose level reached from 56.4 ± 12.0 to 111.6 ± 17.5 mg dl $^{-1}$.

Analysis of data showed a significant differences ($P<0.05$). Barton *et al*., (1999) performed an experiment on Pallid and hybrid sturgeon that results of it showed that plasma glucose increased 20 % in hybrid sturgeon that kept in extreme confinement. They stated that glucose concentration changed a bit in yearling Pallid sturgeon, too. Raising glucose level was reported in Tropical labrids from 2 to 4 mmol L^{-1} by Grutter and Pankhurst (2000). Plasma glucose level was elevated in common carp (*Cyprinus carpio*) during confinement (Ruane *et al*., 2001). Pottinger (1998) demonstrated that glucose levels increased in common carp after handling plus confinement stressor.

Stress is an unavoidable component in intensive aquaculture. Fish culture in intensive systems and artificial farms cause the fish expose to different stressful factors that there are not similar of this situation in natural position (Donaldson, 1981; Faulkner and Moberg, 1997; Fevolden *et al*., 2002). Therefore, intensive aquacultural practice requires the careful management of the rearing environment and is assisted by a thorough understanding of the physiology of fish to minimize the deleterious effects of stress.

Stress is imposed to fish via water, food, microorganisms and other factors that cause to changes in osmoregulation, ability of blood in transferring oxygen, digestion and absorption, hormonal and neural systems which finally, cause to reduce resistance in fish against infectious factors and mortality. Generally, stress can affect on growth, behavior, reproduction, mortality rates (disease resistance and defense systems) and product quality.

Continual appraisal and improvement of current farming practices is the most important way forward some approaches for preservation of fish against stressor such as reducing the time-course of netting, grading or hauling, handling and so on, performing practice at lower water temperatures, avoiding of repeated stresses, the withdrawal of food 24-36 h prior to any operation, distinguishing optimum of natural environment for fish, and rearing selective breeding of fish that are less sensitive to stressors. Reducing sturgeon resources in their original habitat and improving knowledge in their artificial reproduction have made necessity and development possibility of rearing in many countries. Great sturgeon rearing was noticed by farmers because of high growth coefficient compare to other sturgeon fish. We recommend for increasing efficiency in rearing, should avoid of stressor possibly that it needs to right management on rearing practice and this action cause to increase in production, fish welfare, restoration and economic efficiency.

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REFERENCES

Barton, B.A. and Iwama, G.K. (1991) Physiological changes in fish from stress in aquaculture with emphasis on the response and effects of corticosteroids. Ann. *Rev. Fish. Dis*. **1,** 3-26.

Barton, B.A., Weiner, G. S. and Schreck, C. B. (1985) Effect of prior acid exposure on physiological responses of juvenile rainbow trout (*Salmo gairdneri*) to acute handling stress. *Can. J. Fish. Aquat. Sci.* **42,** 710-717.

Barton, B.A., Jansen, C.R., Hauskins, B.L. and Bollig, H. (1999) Juvenile scaphirhynchid sturgeons exhibit low physiological responses to aquaculture-related stressors. *Aquaculture Canada* **99,** *October* 26-29, *Victoria.*

Bayunova, L., Barannikova, I., Semenkova, T. (2002) Sturgeon stress reactions in aquaculture. *J. Appl. Ichthyol.***18,** 397-404.

Bukovskaya, O., Lambert, J.G.D. and Kime, D.E. (1997) *In vitro* steroidogenesis by gonads of the Russian sturgeon, *Acipenser gueldenstaedti* Brandt. *Fish Physiol. Biochem.* **4,** 345-353.

Donaldson, E.M. (1981) *The Pituitary-interrenal axis as an indicator of stress in fish.* In: Pickering, A.D. (Ed.), Stress in fish. Academic Press, London. pp. 11-47.

Faulkner, I.N. and Moberg, G.P. (1997) Effects of short term management stress on the ability of GnRHa to induce gonadotropin secretion in male white sturgeon, *Acipenser transmontanus*. *Aquaculture.* **159,** 159-168.

Fevolden, S.E., Røed, K.H., Fjalestad, K.T. and Stien, J. (2002) Selection response of cortisol and lysozyme in rainbow trout and correlation to growth. *Aquaculture.* **205,** 61-75.

Floodmark, L.E.W., Urke, H.A., Halleraker, J.H., Arnekleiv, J.V., Vøllestad, L.A. and Pole'o, A.B.S. (2002) Cortisol and glucose response in juvenile brown trout subjected to a fluctuating flow regime in an artificial stream. *Journal of fish biology*. **60,** 238-248.

Grutter, A.S. and Pankhurst, N.W. (2000) The effect of capture, handling, confinement and ectoparasite load on plasma levels of cortisol, glucose and lactate in the coral reef fish (*Hemigymnus melapterus*). *Journal of Fish Biology*. **57,** 391-401.

Keyvan, A. (2003) *Iranian sturgeons in the Caspian Sea. Naghsh Mehr Publication.* pp. 400.

Kozlov, V.I. (1993) *Sturgeon farming*. VNIRO, Moscow. pp. 63.

Pankhurst, N.W. and Sharples, D.F. (1992) Effects of capture and confinement on plasma cortisol concentrations in the snapper, *Pagrus auratus*. *Aust. J. Mar. Freshwater Res.* **43,** 345-356.

Pickering, A.D. and Pottinger, T.G. (1987) Poor water quality suppresses the cortisol response of salmonid fish to handling and confinement. *Journal of Fish Biology*. **30,** 363-374.

Pickering, A.D., Pottinger, T.G., Carragher, J. and Sumpter, J.P. (1987) The effects of acute and chronic stress on the levels of reproductive hormones in the plasma of mature male brown trout, *Salmo trutta* L. Gen. *Comp. Endorinol*. **68,** 249-259.

Pottinger, T.G. (1998) Changes in blood cortisol, glucose and lactate in carp retained in anglers keep nets. *Journal of fish biology*. **53,** 728-742.

Pottinger, T.G. and Carrick, T.R. (1999) A comparison of plasma glucose and plasma cortisol as selection markers for high and low stress-responsiveness in female rainbow trout. *Aquaculture*. **175,** 351-363.

Ruane, N.M., Huisman, E.A. and Komen, J. (2001) Plasma cortisol and metabolite level profiles in two isogenic strains of common carp during confinement. *Journal of Fish Biology*. **59,** 1-12.

Schreck, C.B. (1981) *Stress and compensation in teleostean fishes: responses to social and physical factors.* In: Pickering, A. D. (Ed.), Stress in fish. Academic Press, London. pp. 295-321.

Sumpter, J.P., Dye, H.M. and Benfey, T.J. (1986) The effects of stress on plasma ACTH, α-MSH, and cortisol levels in salmonid fishes. *General and Comparative Endocrinology*. **62,** 377-385.

Sumpter, J.P. (1997) *The endocrinology of stress.* In: Iwama, G.K., Pickering, J.P., Sumpter, J.P. and Schreck, C.B. (Eds.), Fish stress and health in aquaculture. Cambridge, Cambridge University Press.

Wedemeyer, G.A. and McLeay, D.J. (1981) *Methods for determining the tolerance of fishes*

to environmental stressors. In: Pickering, A.D. (Ed.), Stress in fish. Academic Press, London. pp. 247-275.

Wedemeyer, G.A., Barton, B.A. and McLeay, D.J. (1990) *Stress and acclimation.* In: Schreck, C.B. and Moyle, P.B. (Eds.), Methods for fish biology. American Fisheries Society, Bethesda, Maryland. pp. 451-489.

Williot, P., Bronzi, P. and Arlati, G. (1993) A very brief survey of status and prospects of freshwater sturgeon farming in Europe (EEC). In: Ketesmont, P., Billard, R. (Eds.), Workshop on aquaculture of freshwater species (except salmonids), *Ghent, Belgium, EAS Special Publication*. **20,** 32-36.