International Journal of Aquatic Biology (2015) 3(3): 129-134
ISSN: 2322-5270; P-ISSN: 2383-0956
Journal homepage: www.NPAJournals.com

Original Article

Hydrocortisone treatment may enhance survival and stocking of Beluga sturgeon (*Huso huso* Linnaeus, 1758) in estuaries of the Caspian Sea

Nastaran Mazloumi*[1], Bagher Mojazi Amiri[2], Mohammadali Nematollahi[2], Gholamreza Rafiee[2]

[1]Southern Seas Ecology Laboratories, School of Biological Sciences, University of Adelaide, South Australia 5005 Australia.
[2]Department of Fisheries, Faculty of Natural Resources, University of Tehran, PO Box 4314-31583, Karaj, Iran.

Abstract: Beluga sturgeon (*Huso huso* Linnaeus, 1758) fingerlings are released into the Caspian Sea for recruiting and enhancing commercial and recreational fishing purposes. These fingerlings are reared in fresh water, but released to the estuaries that may be caused mortalities due to acute osmotic stress. In this study, the fingerlings in whole (*in vivo*) or their gill tissue (*in vitro*) were exposed to three different levels of 'the stress hormone' cortisol (3, 5, 7 mg L^{-1} hydrocortisone sodium phosphate) for 24 hrs. The effects of treatments on blood cortisol levels and the size and numbers of gill chloride cells were monitored. In each case, hormonal treatment significantly increased blood cortisol levels and also the number but decreased the size of the chloride cells. We conclude that bathing in hydrocortisone could promote the survival rate of the fingerlings in brackish water and may be have a positive effect on their osmoregulation potentiality.

Article history:
Received 30 October 2014
Accepted 10 April 2015
Available online 25 June 2015

Keywords:
Cortisol
Chloride cell
Salinity
Acipenseridae
Great Beluga

Introduction

The beluga sturgeon, *Huso huso* is an anadromous acipenserid species in the Caspian Sea basin, and subjected to intensive fishing as a source of caviar. The stock of this species has been declined in recent years (Fakharzadeh et al., 2011), and therefore, listed as 'Critically Endangered' (IUCN, 2012). In March-June of each year, the belugas migrate into inflowing rivers, e.g. the Volga (Russia) and Sefidrood (Iran), where they spawn (Vosoghi and Mostajir, 2002). In early March, the broodstocks are captured from the sea and transferred to the hatchery for artificial breeding, and then produced juveniles (with 2-3 g) released to the estuaries, where the water salinity is about 12 ppt. This releasing may be caused mortality because of insufficient fish osmoregulation capabilities (Abdolhay and Tahori, 2006). Strategies to minimise osmotic stress and promote juvenile survival, therefore, are likely to be commercially and ecologically beneficial.

The steroid hydrocortisone (cortisol) hormone is produced in response to stress, and acts on receptors in the gills, kidneys and intestines of fishes (Van der Salm et al., 2002). This hormone increases salinity tolerance by increasing the size and the number of gill chloride cells, and promotes chloride ion transport through increased Na^+/K^+/ATP–ase enzyme activity (Khodabandeh et al., 2009). As blood cortisol levels are known to increase in sturgeons, salmon, lampreys and other fishes during downstream, post-spawning migrations from fresh to saline water (Tipsmark and Madsen, 2001), it is possible that artificially increase of cortisol levels could help to offset osmotic stress. Therefore, the present study aimed to describe the effects of direct (fingerlings in whole, *in vitro*) and indirect (gill tissue culture, *in vivo*) exposure of Beluga sturgeon to cortisol. We anticipated that hormonal treatment will increase the osmoregulation potency through the blood cortisol level and increase the number and

* Corresponding author: Nastaran Mazloumi
E-mail address: nastaran.mazloumi@adelaide.edu.au

reduce the size of gill chloride cells, preparing the fingerlings for osmotic shock associated with their release to the brackish water.

Materials and methods

A total of 500 beluga fingerlings (3.00 ± 0.5 g) were obtained from the Hyrkan Sturgeon Culture Farm (Ramsar, Mazandaran Province, Northern Iran), and transferred to the Aquaculture Laboratory at the University of Tehran. Fish were acclimatized for seven days to laboratory conditions in circular 150L tanks with continuously aerated, filtered and recycled tap water (water temperature 20 ± 2°C, dissolved oxygen >5 mg L^{-1}, pH 7, 12/12h photoperiod). Throughout the acclimatization and experimental periods, the fish were fed twice daily with a dry commercial pellet diet (45% protein, 12% lipid, 3.5% carbohydrate, 10% ash; Behparvar Co., Iran), at 1.5% of tank biomass. Uneaten food and faeces were removed from the tanks twice a day. Water quality parameters were checked daily, and salinity was adjusted, if necessary, using de-chlorinated tap water. All dissecting equipment (scalpels, scissors, and forceps) was autoclaved for 24 hrs before use.

In the *in vivo* experiment, three treatments (each of 20 fingerlings) were exposed to 3, 5 and 7 mg L^{-1} of the hydrocortisone sodium phosphate, respectively, along with a control group without hormonal exposure, each with three replicates. After 24 hrs, fish were anaesthetized using clove oil solution (150 ppm) and killed by spinal section. The blood samples were randomly taken from 20 specimens in Eppendorf® tubes with a drop of EDTA anticoagulant, and transferred to a glass jar containing ice (without direct contact with the ice). The blood cortisol levels were determined in the blood serum, using the ELIZA method (Diagnostics Biochemistry Canada, Ontario; sensitivity 0.4 μg dL^{-1}) (Lucía et al., 2011). In addition, the second left gill arch of the specimens were removed in sterile conditions, and fixed in 4% formalin for 24 hrs (Merck®), maintained in 70% alcohol for one week (Eagderi et al., 2013). The tissues were processed and 5 μm histological section prepared and stained with haematoxylin and eosin based on Eagderi et al. (2013). Furthermore, the chloride cells on the prepared gill sections were counted, and their sizes measured along the bases of five gill lamella according to Khatooni et al. (2011) using an optical micrometer.

In the *in vitro* experiment, the second left gill arch of the fingerlings were removed, divided by scalpel into 20 mm pieces and washed three times for 15 min in phosphate-buffered saline solution (PBS: 80 g NaCl, 2 g KCl, 26.8 g $Na_2HPO_4.7H_2O$, 7.4 g K_2HPO_4 and 1 L distilled water) (McCormick, 1995). Then, the samples were transferred to the 24-chamber cell culture-plate (BD Falcon®, cat. 3043) with each chamber containing 1 mL Ringer's solution (Mojazi Amiri et al., 1999). Furthermore, three concentrations of hydrocortisone, i.e. 3, 5 and 7 mg L^{-1}, were added to three treatments with three replicates. Meanwhile, a control group was left without adding hormone with three replicates. The plates were kept over ice before transferring to an incubator with 15°C (Wood and Pärt, 1997). After 24 hrs, the samples were fixed in Bowen's solution (75 mL picric acid, 25 mL formalin, 5 mL acetic acid), and 5 μm histological section prepared and stained based on Eagderi et al. (2013). The chloride cells were counted, and their sizes measured as mentioned for the *in vivo* experiment.

Statistical analyses were performed by the packages SPSS 19 and SAS 9. One-way ANOVA was applied to compare treatment means ($\alpha = 0.05$); the LSD Test was applied to compare the treatments with controls and Duncan's Test to pairwise combinations of treatments.

Results

The results showed significant differences in blood cortisol between the controls and treatments ($P<0.01$). Based on the results, the blood cortisol levels increased by increasing the levels of the hormonal exposure (Fig. 1). This correlation is proportional with high sensitivity (Fig. 2). The results revealed that the *in vivo* hormonal treatment

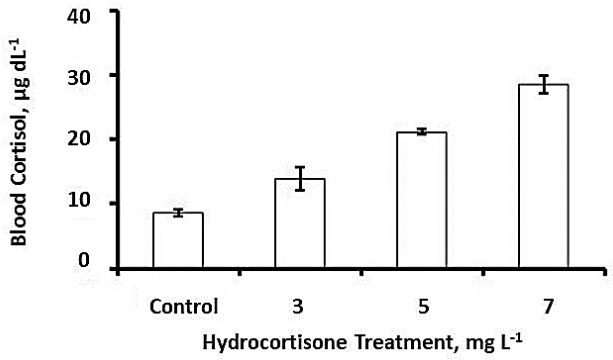

Figure 1. Effects of three levels of hydrocortisone on blood cortisol levels in beluga fingerlings, Data are mean ± SD, $n = 20$.

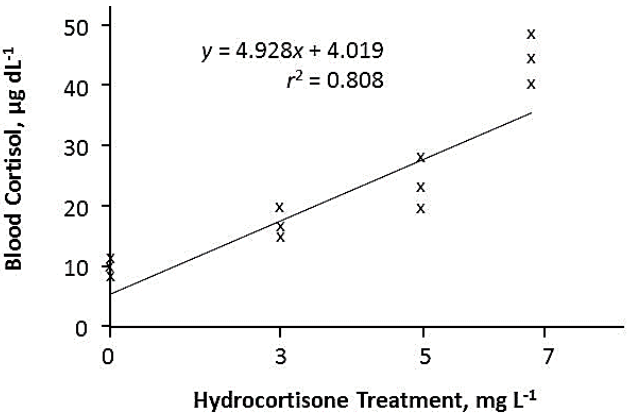

Figure 2. Relationship between blood cortisol levels and exposure to hydrocortisone treatments in beluga fingerlings.

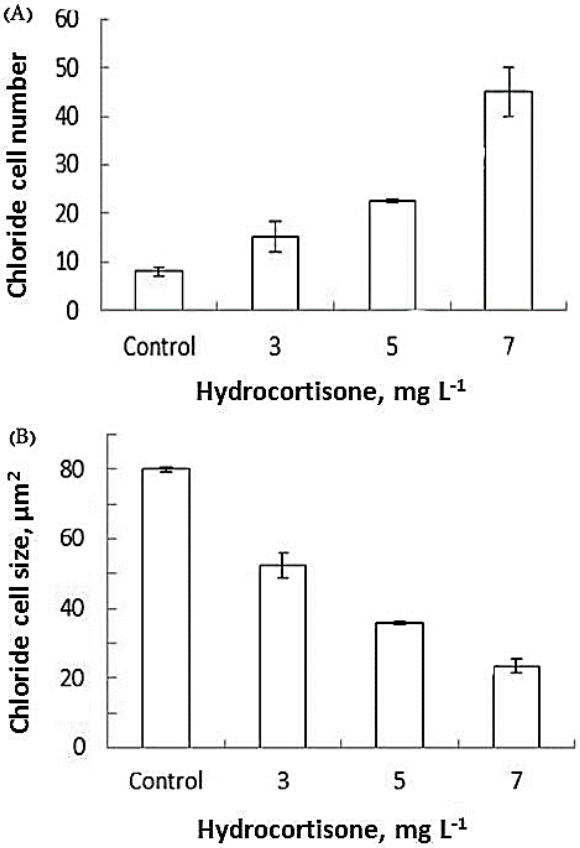

Figure 3. Effects of three levels of the hydrocortisone on the number (A) and size (B) of gill chloride cells in beluga fingerlings in the *in vivo* experiment. Data are mean ± SD, $n = 20$.

Table 1. The effect of hydrocortisone treatment (mg L^{-1}) on numbers and sizes (μm^2) of gill chloride cells of beluga fingerlings *in vivo* and *in vitro* experiments. Each cell shows the mean ± SD for 3 replicates. The trend lines estimate the effect (y) from the treatment (x); r^2 is the coefficient of determination.

	In vivo		***In vitro***	
Treatment	**Number**	**Size, µm²**	**Number**	**Size, µm²**
Control*	9 ± 1	68.37 ± 0.93	12 ± 2	80.37 ± 0.75
3 mg L^{-1}	14 ± 3.21	38.21 ± 1.10	20 ± 5	51.21 ± 3.5
5 mg L^{-1}	21 ± 4.04	26.04 ± 2.9	35.3 ± 6.4	35.42 ± 0.32
7 mg L^{-1}	45 ± 5	19.69 ± 1.03	45 ± 5.1	24.69 ± 1.8
Trend, y =	11.833x–6.833	–18.645x+94.447	13.333x–4.667	–17.376x+75.808
r^2	0.912	0.965	0.993	0.991

have significantly affected the numbers and size of the gill chloride cells in 7 mg L^{-1} treatments (Figs. 3 and 4). The similar results obtained in the in vitro experiment (Figs. 5 and 6).

The effects of *in vivo* and *in vitro* exposures are presented in Table 1. In general, the exposure to the hydrocortisone increased the number of chloride cells and decreased their size. The changes were slightly greater in vitro, that can be due to the effect of the Ringer's solution or PBS buffer on tissues.

Discussion

We exposed beluga fingerlings to three levels of the hydrocortisone, anticipating increase of the osmoregulation potency through the changes of their physiological indices. The results support this hypothesis and the levels of the cortisol in the blood plasma increased and also, the number of the gill

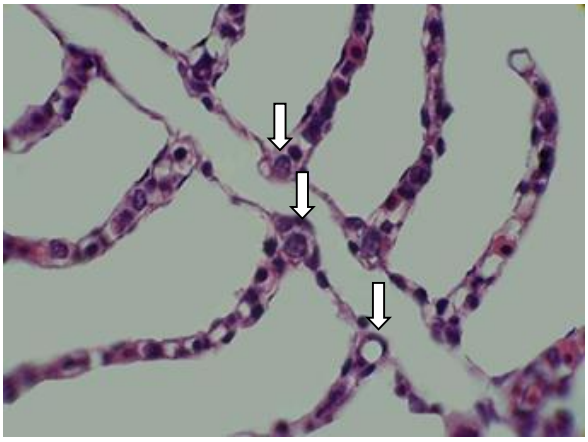

Figure 4. Gill chloride cells (arrows) of a beluga fingerling exposed to 7 mg L^{-1} hydrocortisone in the in vivo experiment (H&E, 100X).

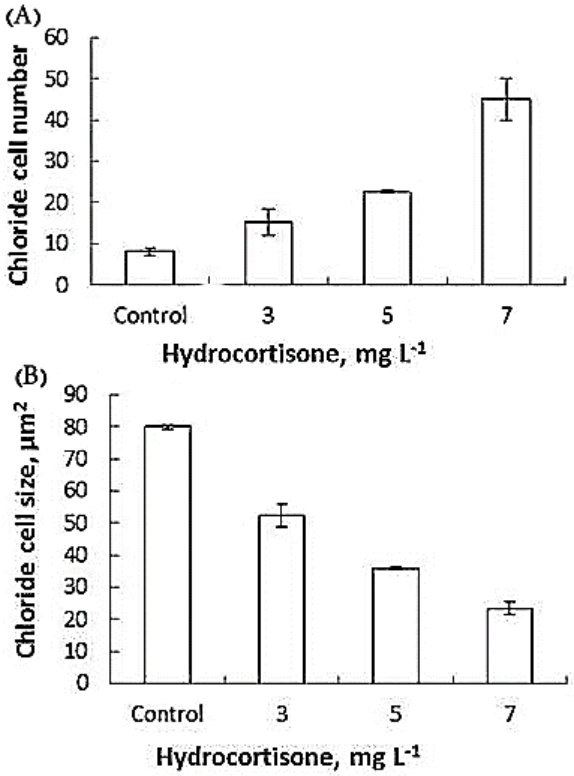

Figure 5. Effects of three levels of the hydrocortisone on the number (A) and size (B) of gill chloride cells of beluga fingerlings *in vitro*. Data are mean ± SD, $n = 20$.

chloride cells increased, whereas their size reduced. Plasma cortisol levels in fish are known to increase as an adaptive response to osmotic stress, implying that an artificial increase in plasma cortisol could increase the tolerance of fingerlings to saline water (Swallow and Fleming, 1970). Khodabandeh et al. (2009) pointed out that the blood cortisol level of Persian sturgeon fingerlings, *Acipenser persicus*, is

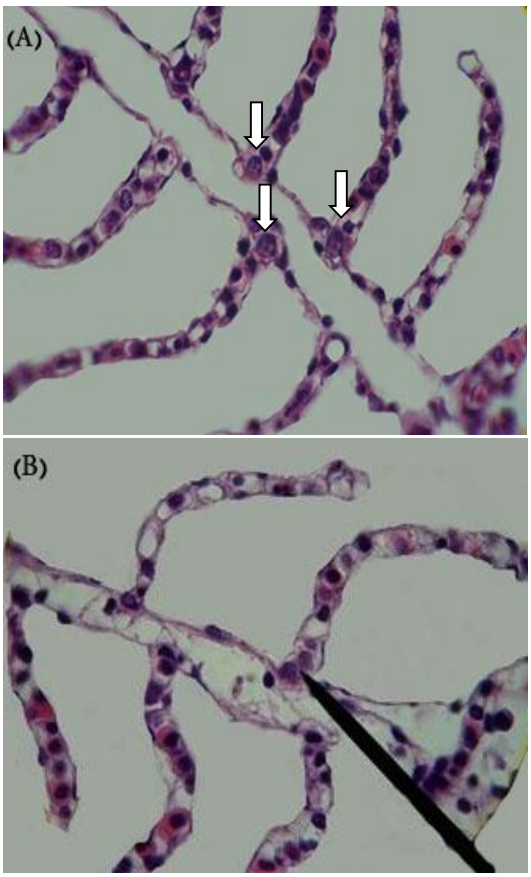

Figure 6. Gill chloride cells (arrows) of the beluga fingerlings in the control group (A) and after exposure to 7 mg L 1 hydrocortisone in the vitro experiment (B) (H&E, 100X).

increased by exposing to hydrocortisone. The present study showed similar results in beluga fingerlings by increasing the blood cortisol, up to about 40 µg dL^{-1}, with those of Khodabandeh et al. (2009).

In the present study, the numbers of gill chloride cells in beluga fingerlings increased with increasing the concentration of the hydrocortisone, so that, the 7 mg L^{-1} treatment showed a fourfold increase in compare to those of control group, in both *in vitro* and *in vivo* experiments. The *in vitro* results suggest that this was due to mitotic divisions of the chloride cells. Furthermore, the sizes of the cells decreased more than threefold, both *in vitro* and *in vivo*. This response may be different if the hormonal treatment was extended for more than 24 hrs (cf. Kazemi et al., 2003). An increase in the plasma cortisol generally is accompanied by changes in gill chloride cells, implicated in osmoregulation (e.g. Adams, 1990),

although the nature of the response differs between bony and cartilaginous fish. In bony fish like rainbow trout (*Oncorhynchus mykiss*: Van der Salm et al., 2002) and Atlantic salmon (*Salmo salar*: Madsen et al., 1997), cortisol increases the size of the chloride cells, but in cartilaginous species the opposite is true (McCormick, 2001). In Persian sturgeon fingerlings, Khoshnood et al. (2010) showed that treatment with 5 mg L^{-1} hydrocortisone increased the number of gill chloride cells. Carmona et al. (2004) and Farabi et al. (2009) demonstrated similar responses in Adriatic sturgeon (*A. naccarii*) and ship sturgeon (*A. nudiventris*).

Although 7 mg L^{-1} hydrocortisone treatment was most effective in juvenile beluga, this could be lethal for other fish, including Persian sturgeon (cf. Fakharzadeh et al., 2011), and the optimal dose should be established for each species. For beluga fingerlings, we conclude that 24 hrs exposures to 7 mg L^{-1} hydrocortisone could be a practical measure to forestall mortality after transfer from fresh to brackish water. We suggest that our observations warrant further testing under field conditions.

Acknowledgements

We are grateful for assistance from Nourallah Ghorbani, Director of the Hyrkankesht Sturgeon Culture Farm, Mehdi Hedayati, Institute of Endocrinology, Shahid Beheshti University, and Mohammad Ebrahimi, BS Fisheries Laboratory and Faculty of Veterinary Histology, University of Tehran. Our thanks also to Keith Walker, School of Earth and Environmental Sciences, The University of Adelaide, for help in preparing this work for publication.

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