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Effects of Stocking Density on Blood Cortisol, Glucose and Cholesterol Levels of Immature Siberian Sturgeon (*Acipenser baerii* Brandt, 1869)

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Abstract

Siberian sturgeon (*Acipenser baerii* Brandt, 1869) is a suitable species for aquacultural purposes. This study was conducted to investigate the influence of stocking density on blood parameters of cultured immature Siberian sturgeon introduced to Iranian aquaculture systems, in order to monitor crowding stress. Two-year old juvenile fish (weight 607.7±21.49 g, total length: 68±9.46 cm [mean ± SE]) were reared at low, moderate and high densities (6, 12 and 18 fish 250 L^{-1}, respectively) for 5 months. Cortisol, glucose and cholesterol concentration of blood plasma were measured four times during the experiment (July, August, October and November 2008). The present study indicated that (1) density had no significant effect of blood cortisol, glucose and cholesterol concentration in *A. baerii* (cortisol: 7.8-13.1, cholesterol: 115.6-133.9, glucose: 41.4-46.8 ng ml^{-1}[range]) (2) a significant increase in cortisol concentration of all densities was found in August which was related to water temperature rise (23.75±2.69 in August *vs* 7.09±1.16 ng ml^{-1} for other months [mean ± SE]). Therefore, it seems that the Siberian sturgeon is not sensitive species against rearing density in aquaculture systems. Further investigation on critical density and factors that change tolerability to high density are needed to be addressed.

Keywords: Siberian sturgeon, stress, rearing density, growth.

Introduction

Sturgeons (Family: Acipenseridae) are the most valuable fishes. Over-exploitation for caviar and habitat destruction have caused declines of many sturgeon populations (Doukakis *et al*., 1999). The Iranian government has tried to restore natural stocks of the sturgeons through artificial reproduction and release of fingerlings into rivers flowing into the Caspian Sea (Bartley and Rana, 1998). In addition, culture of sturgeons has been encouraged, which may decrease the fishing pressure and subsequently assist to restore the wild stocks.

Siberian sturgeon (*Acipenser baerii*) is a non-anadromous migratory species, living in deep parts of large rivers, spawning over stone-gravel or gravel-sand substrates with moderate to swift current (Kottelat and Freyhof, 2007). Adults live essentially in freshwater although some fish frequently occur in estuaries. Males are sexually mature between 9-15 years; females between 16-20 years (Kottelat and Freyhof, 2007). *Acipenser baerii* can tolerate low temperatures, lives in freshwater and consumes a wide range of food items (Holcik, 1989; Koksal *et al*., 2000), which make it a suitable species for aquacultural purposes.

Density is a key factor in determining profitability and economic sustainability of fish farm (Rafatnezhad *et al*., 2008). Farmers often increase rearing densities to intense the production (Iguchi *et al*., 2003). Suboptimal conditions may result in chronic stress in fish culture (Ramsay *et al*., 2006). Three types of stress indicators can be detected in fishes: release of corticosteroid hormones (*e.g.* cortisol) into blood circulation (Bolasina *et al*., 2006), changes in hematological parameters (Valenzuela *et al*., 2008), and the whole animal performance like growth and survival rates (Barton, 2002). Hormonal and blood parameters have frequently been used as indicators of stress in sturgeons (Falahatkar *et al*., 2009; Zarejabad *et al*., 2009). Also, these indicators have often been utilized to find if the culture condition is optimized for sturgeons (Cech *et al*., 1984; Ghomi *et al*., 2010; Jarvis *et al*., 2001; Ruchin, 2007). As an stressor, stocking density has been studied in many bony fishes (North *et al*., 2006; Ramsay *et al*., 2006; Yousif, 2002), there is however relatively few studies on sturgeons. This may partly be related to the difficulty in obtaining sturgeons as experimental animals (Doroshov *et al*., 1997). The present study thus aimed to investigate the influence of stocking density on blood parameters of immature *A. baerii*, *i.e.* cortisol, glucose and cholesterol. This study will provide useful information on the most suitable stress indicator and also optimal density for rearing Siberian sturgeon.

Material and Methods

This study was performed in the International Sturgeon Research Institute (Rasht, Iran) from July to November 2008. Two-years old, one hundred and six farmed Siberian sturgeons of both sexes with a similar size (body weight 607.7±21.49 g, total length 68±9.46 cm [mean±S.E.]) were selected for the experiment. They were randomly stocked in 300 L round fiberglass tanks (diameter: ≈ 0.8 m height: ≈ 0.6 m) containing 250 L aerated water with three replicates at three densities of 6, 12, 18 fish per tank (low, moderate and high densities). Tanks were supplied with untreated water from a reservoir being fed from the Sefidrood River (37°06′29.78″ N, 49°43′15.89″ E), at a constant flow of 1 L S^{-1}. Water was constantly drained using a drainage valve installed on the bottom of the tanks. Tanks were kept under a 14:10 (light:dark) photoperiod throughout the experiment. Fish were fed a pellet food (Chineh Co., Tehran, Iran; 36% protein, 14% fat, 10% ash, 4% fibre, 11% moisture) at rate of 1-2% of live weight, four times a day (7 am, 12, 5 and 10 pm). Dissolved oxygen and temperature were measured daily during the experiment. Specimens were allowed to acclimate for two weeks.

Animal's blood was sampled four times during the experiment: July, August, October, and November. Feeding was stopped 24 hours before the sampling. Animals were not anesthetized before blood sampling as anesthesia has been considered as a stressful action and unsuitable for cortisol measurement (Barton *et al*., 1998; Marino *et al*., 2001; Papoutsoglou *et al*., 2007). At each sampling time, three or four animals were selected from each tank and one ml blood was taken from the caudal vein of each tagged fish using a heparinized 2-ml syringe (Trenzado *et al*., 2003). Blood samples were stored in ice and transferred to the laboratory where plasma was separated by centrifugation at 7000 rpm during 7 minutes based on Rotllant *et al.* (2001). A 100-µl blood sample from each specimen was transferred to an eppendorf tube and stored at -20°C for later analyses. Cortisol was measured by radioimmunoassay (Rotllant *et al*., 2001), glucose by enzymatic method (Weil *et al*., 2001) and cholesterol using a commercial kit (Pars Azmun Co. Ltd., Tehran, Iran) (Yousefi *et al*., 2011).

Data analysis: Effects of various densities, time and tank on biochemical composition of the blood were examined using a split-split plot in time, with all three factors considered random (Quinn and Keough, 2002). Tank was not an important factor for the aim of the present study. However, it was included it in the analysis to deduct its variance from total variance and increase the precision of the analysis. Where there was an interaction between factors, levels of a factor was examined for a significant difference at each level of other factors separately (Underwood, 1997) using ANOVA. The ANOVA assumptions of normality and homogeneity of variance (Little and Hills, 1978) were examined using the Kolmogorov-Smirnov goodness-of-fit-test (Zar, 1999) and the Levene test (Glass, 1966), respectively. All statistical analyses were performed using SPSS 15.0 (SPSS Inc.) with a 0.05 probability of Type I error.

Results

Cortisol concentration of the blood had a lot of fluctuation over times and tanks (range: 0.1-51.00; Figure 1). The highest cortisol concentration was measured in August and November. In contrast,

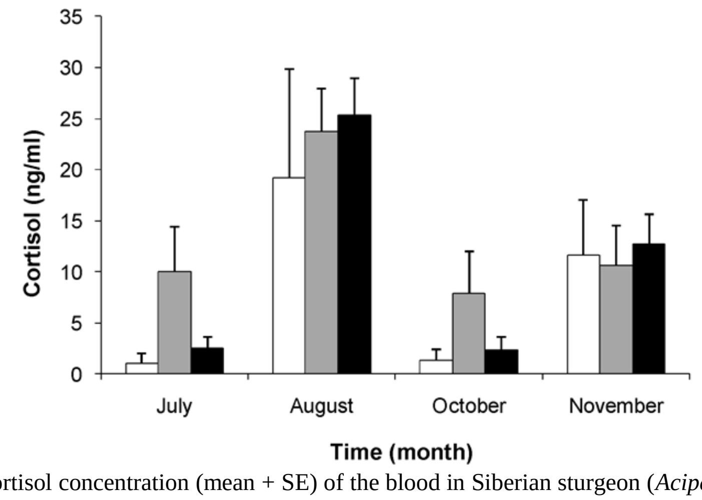

Figure 1. Changes of cortisol concentration (mean + SE) of the blood in Siberian sturgeon (*Acipenser baerii*) reared under different densities and time. Data are pooled over tanks.

glucose concentration of the blood had a lower fluctuation over density, time and tank ranging from 10 to 89 mg/dl (Figure 2). Similar concentration of glucose was found among different densities and tanks at various times. Cholesterol concentration of blood increased slightly from July until August then decreased after than at all densities (Figure 3).

Density had no significant effect on cortisol ($F_{2, 2.647}$ = 0.169, P=0.853), glucose ($F_{2, 1.012}$ = 2.476, P=0.408) and cholesterol concentration ($F_{2, 3.591}$=0.664, P=0.569). Time had only a significant effect on cortisol concentration of blood ($F_{3, 4.977}$=5.957, P=0.042; glucose: $F_{3, 0.902}$=16.089, P=0.205; cholesterol: $F_{3, 3.945}$=5.807, P=0.062). Tank had no significant effects on biochemical composition of the blood (cortisol: $F_{2, 5.995}$=0.368, P=0.707; glucose: $F_{2, 0.052}$=10.035, P=0.857; cholesterol: $F_{2, 3.747}$=0.243, P=0.796). There was a significant interaction between time and tank on cortisol concentration of blood ($F_{6, 9.827}$ = 3.659, P=0.035) and thus further ANOVA at each level of tank was used and indicated that, at all three tanks, time has a significant effect on cortisol concentration. However, ANOVA at each level of time confirmed that tank has no significant effect on cortisol concentration of blood. No other significant interaction was found between the three factors for any composition of the blood.

Discussion

Density had no significant effect on biochemical parameters of blood in immature Siberian sturgeon. Our results are consistent with those of Cataldi *et al*. (1998) for *Acipenser naccarii* and Rafatnezhad *et al*. (2008) for *Huso huso*. In the present study, lack of significant difference in blood parameters among specimens reared at dissimilar densities may be

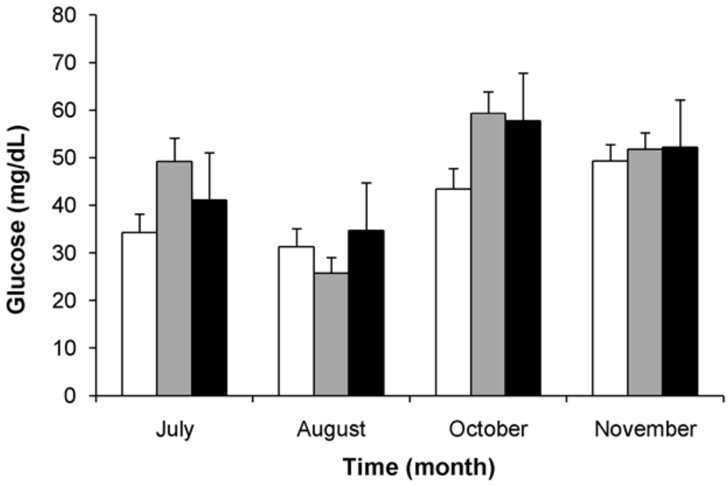

Figure 2. Changes of glucose concentration (mean + SE) of the blood in Siberian sturgeon (Acipenser baerii) reared under different densities and times. Data are pooled over tank.

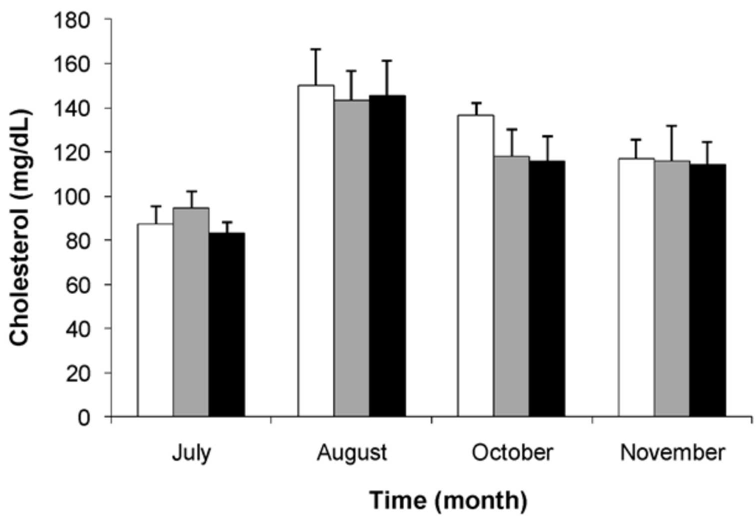

Figure 3. Changes of cholesterol concentration (mean + SE) of the blood in Siberian sturgeon (Acipenser baerii) reared under different densities and times. Data are pooled over tank.

explained by the following scenarios: firstly, response to stress may be species-specific. Barton (2002) found the magnitude of post-disturbance cortisol concentrations in pallid sturgeon, common carp and rainbow trout as 1, 10 and 25 times, respectively. Barton (2000) found various species of salmonids responded differently to handling stress in terms of cortisol and glucose of plasma. Also, Pottinger and Pickering (1987a) pointed out the importance of fish species in response to density-related stress. Our study may show the hardy nature and tolerability of Siberian Sturgeon to changes in density.

Secondly, the specimens used for the present study were relatively large. It has been shown that size is an important factor in showing physiological response to environmental stress under the rearing condition (Biswas *et al*., 2006; Hallaraker *et al*., 1995). Therefore, smaller individuals of Siberian sturgeon may be more prone to get stressed by crowding. Thirdly, Rafatnezhad *et al*. (2008) found that hematological parameters of the great sturgeon did not change in response to changes of density although growth rate changed. Similarly, Pickering and Stewart (1984) discovered that brown trout kept crowded showed elevated level of plasma cortisol for a short period (up to 25-39 days) while growth rate decreased. This suggests that biochemical composition of the blood may not be a reliable indicator of chronic stress and general performance should be monitored too. Fourthly, influence of density-driven stress on fishes can be mediated by deterioration of water chemistry (Pickering and Pottinger, 1987b) and/or social hierarchy of some specimens in the stock (Øverli *et al*., 1999). Although we did not monitor water chemistry of the tanks but our flow-through rate was almost twice those of other studies on sturgeons (Ghomi *et al*., 2010; Jodun *et al.*, 2002, Rafatnezhad *et al*., 2008) suggesting good water quality. In the present study, the specimens were selected to have a similar size and social hierarchy could not thus be an influencing factor.

Time had a significant effect on cortisol concentration of the blood with maximum values found in August. This effect can be related to increase of water temperature. Such an effect on cortisol level has also been found in other sturgeons, *i.e. Acipenser naccarri* (Cataldi *et al.*, 1998) and *Acipenser medirostris* (Lankford *et al*., 2003). An increase of water temperature decreases DO and may result in elevation of cortisol concentration as in *Acipenser baerii* (Maxime *et al*., 1995). While we had sufficient water exchange to provide enough oxygen to the specimens it is probable that cumulative effects of temperature increase and oxygen decrease induced a 5.5-fold increase in plasma cortisol concentration in August.

Our results for cholesterol were two-fold of those of Asadi et al. (2009) and Gessner *et al*. (2009). In the present study, mean values of cortisol, glucose and cholesterol in specimens that experienced moderate and high densities were comparable to those of other studies reared in low density or were well adapted to rearing condition, *i.e.* for cortisol and glucose (Barton, 2000; Rafatnezhad *et al*., 2008), and for cholesterol (Shahsavani *et al*., 2010), showing that animals were not stressed. Therefore, a high concentration of blood cholesterol may suggest the dietary lipid imbalance (Wedemeyer *et al*., 1990).

Acipenser baerii was first introduced to Iran in 2007 and information on its rearing condition is still scanty. Our study was the first experiment attempted to evaluate the compatibility of this species to the Southern Caspian Sea aquaculture systems. The sturgeon did not seem susceptible to crowding since plasma cortisol, glucose and cholesterol were not affected by this stressor. In fact, the compatability to rearing condition have occurred, therefore, this species is suitable for aquaculture helping to reduce current fishing pressure on endangered wild stocks. Further investigation on critical density and factors that change tolerability to high density are needed to be referred.

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