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Research Article

Effect of different stocking densities on haematological and biochemical parameters of great sturgeon juveniles (*Huso huso* Linnaeus, 1758)

Mazdak Aalimahmoudi[1*], Siamak Salehipour Bavarsad[2] and Saeid Moghdani[3]

[1]Department of Fisheries Sciences, College of Agricultural Sciences and Natural Resources, Ahvaz Branch, Islamic Azad University, Ahvaz, Iran; [2]The Administration Fisheries of Khouzestan Province, Ahvaz, Iran; [3]Young Researchers and Elite Club, Bushehr Branch, Islamic Azad University, Bushehr, Iran

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Abstract

The influence of stocking density (1.5, 3 and 6 kg/m^2) was investigated on weight, haematological and biochemical parameters of great sturgeon (*H. huso*) juveniles for a period of 8 weeks. After 8 weeks of rearing, the mean weight was 527.27±6.82g, 467.91±18.81g and 431.02±24.25g in densities of 1.5, 3 and 6 kg/m^2, respectively with no significant differences among treatments (P>0.05). A significant difference (P<0.05) was observed in haematocrit and eosinophil at treatment 1.5 kg/m^2 on 30th and 60th day of rearing period, but the other haematological parameters including red blood cells (RBC), and lymphocyte, neutrophil and monocyte showed no significant effect with stocking density (P>0.05). WBC count showed significant increase in 1.5, 3 and 6 kg/m^2 at 60 day of the experiment. Cortisol and glucose increased significantly on day 30 at the level of 3 kg/m^2. Our results showed that rearing density had no major effect on the growth and some haematological parameters suggesting that great sturgeon exhibited lower stress responses to high stocking density. However, increasing stocking density exhibited significant effect on eosinophil, haematocrit and WBC count as well as cortisol and glucose.

Keywords: *Huso huso*; haematological parameters; growth; biochemical parameters; stocking density

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Introduction

Great sturgeon (*Huso huso* Linnaeus, 1758), an important aquaculture species in Iran, is a commercially well known source of caviar and meat. This species is suitable for aquaculture due to their fast growth, reproduction capacity and adaptability for unfavourable rearing conditions (Rafatnezhad et al., 2008). Stocking density is one of the key factor in determining the productivity and profitability of commercial fish farms. Many studies have concluded an effect of stocking density on various aspects of the health of farmed fish (Iwama et al., 2011), though the results depend on the species (Jørgensen et al., 1993; Montero et al., 1999; Vazzana et al., 2002). Overcrowding is a common chronic stressor in aquaculture that can induce a prolonged elevation of plasma cortisol levels (Pickering and Pottinger, 1989), which may have damaging consequences (Barton and Iwama, 1991). The evaluation of blood biochemistry and hormones could

***Corresponding author:** Mazdak Aalimahmoudi, Department of Fisheries Sciences, College of Agricultural Sciences and Natural Resources, Ahvaz Branch, Islamic Azad University, Ahvaz, Iran
E-mail: maalimahmoudi@yahoo.com; Tel.: +989163341355

be useful for the diagnosis of diseases and to monitor the physiological status of fish (Stoskopf, 1993). There is little information about the effects of rearing density on this species; therefore, the aim of this study was to evaluate the effect of various stocking density on haematological and biochemical indices in great sturgeon juveniles.

Materials and Methods

Experimental design and maintenance of fish

Using great sturgeon juveniles (*Huso huso*), with an initial weight of 143± 0.29 g were artificially propagated at the same hatchery and adapted to an artificial diet. Fish were randomly distributed into nine small ponds reinforced concrete at three different densities including 1.5 (n=11), 3 (n=22) and 6 (n=43) kg/m^2 with three replicates. The dimensions of the rearing small pond were (2×1×1 m), with a water flow rate of 36.2± 1.0 l/min (using water from the deep well). Water depth in each pond was 70 cm. The water supply of each pond was independent of the others. Before the initiation of the experiment, fish were acclimatized to experimental conditions for two weeks. Feed was offered four times (08:00, 12:00, 16:00 and 20:00 h) daily using a commercial diet (BioMar, no.3 Nersac, France; 50% crude protein, 18% crude fat, 10% ash, 1.3% fiber) for 8 weeks. Food was supplied at 2% of body weight (BW) on daily basis during the experimental period (Rafatnezhad et al., 2008). Uneaten pellets were drained off and counted every day to calculate the total feed consumption per pond using a mean dry pellet weight. There was no fighting among fish for obtaining food. Dissolved oxygen, pH and temperature were measured daily and CO_2, NH_3, NO_3 and NO_2 were measured fortnightly during the experiment (Table 1).

Sampling protocol and blood analysis

Body weight was measured on biweekly basis. In each pond, 30% of the fish were randomly sampled at the start of trial (30^{th} day) and the end of the experimental period (60^{th} day) in order to evaluate haematological and biochemical parameters. Animals were not anaesthetized before blood sampling since anaesthesia has been considered as a stressful and unsuitable for cortisol measurement. Fish were quickly captured and 3 ml blood samples were taken from the caudal vein using a non-heparinized 5ml syringe (Trenzado et al., 2003). Blood samples were stored in ice and transferred to the laboratory, haematocrit was determined by centrifuging whole blood in heparinized micro hematocrit capillary tubes at 3500 g for 10 minutes (Trenzado et al., 2003). Blood plasma was separated by centrifugation at 3000 g for 10 min (Rotllant et al., 2001).

A 100 µl blood plasma sample from each fish (67 blood plasma samples per sampling) was transferred to an eppendorf tube and stored at -20°C for next analyses. Cortisol was measured by radioimmunoassay (Rotllant et al., 2001) and glucose was determined by enzymatic method (Weil et al., 2001; Yousefi et al., 2012).

We also calculated number of white blood cells (WBCs), number of red blood cells (RBCs), percent of lymphocyte, neutrophil, eosinophil and monocyte as differential WBC count (Ranzani-Paiva et al., 2004).

Statistical analysis

Results were analyzed by analysis of variance using One-way ANOVA, and comparisons among treatment means were made by Tukey test as a post-hoc test using SPSS®19 software (Chicago, IL, USA). Statistical significance was accepted at the $P<0.05$ level. All data in the text are presented as Mean±SE.

Results

Changes in the average weight of great sturgeon juveniles reared at different densities are presented in Figure 1. Final weight was not significantly different among treatments ($P>0.05$). In this investigation, haematological parameters including RBC, lymphocyte, neutrophil and monocyte did not differ among rearing densities ($P>0.05$; Tables 2&3). A significant increase ($P<0.05$) was observed in eosinophil at treatment 1.5 kg/m^2 on 30^{th} and 60^{th} day of rearing. A significant decrease in Hct concentration was found in 1.5 kg/m^2 on the sampling day. Similarly, WBC showed significant increase in 1.5, 3 and 6 kg/m^2 at 60 day of the experiment.

The plasma cortisol and glucose concentration showed significant difference ($P<0.05$; Table 4). Cortisol and glucose increased significantly on day 30 at the level of 3 kg/m^2.

Discussion

Our data showed that high stocking density had no significant effects on some haematological parameters including RBC count (erythrocyte), lymphocyte, neutrophil, and monocyte in the great sturgeon juveniles, but there was a significant differences in the percentage of haematocrit, eosinophil and WBCs values. These results suggested some sensitivity to high density stress in juveniles. Many researchers reported effect on haematological parameters as a common effect of fish crowding (Kjartansson et al., 1988; Mazur and Iwama, 1993; Caldwell and Hinshaw, 1994; Martinez et al., 1994; Montero et al., 1999 & 2001). This response may be the cause of increasing the oxygen carrying capacity of blood under high-energy

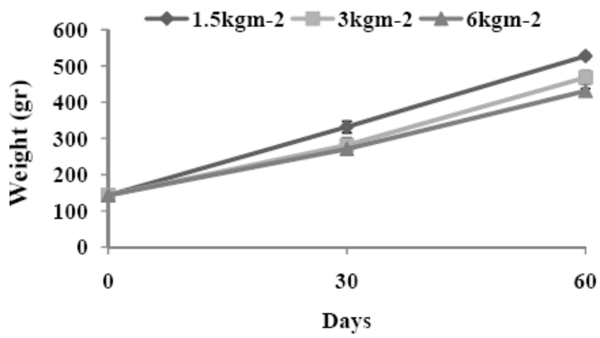

Fig. 1: Changes in average weight of great sturgeon juveniles reared at different densities during 8 weeks (Mean±SE)

Table 1: Physico-chemical parameters of pond water inflow during the culture of great sturgeon juveniles (Mean±SE)

Parameter	(Mean±SE)	Parameter	(Mean±SE)
Temperature ºC	23.3±1.48	NH_3 (mg/l)	0.35±0.26
O_2 (mg/l)	6.81±0.98	PO_4 (mg/l)	0.32±0.11
pH	8.06±0.09	BOD_5 (mg/l)	5.34±0.99
CO_2 (mg/l)	0.75±0.21	NO_3 (mg/l)	14.66±2.64
NO_2 (mg/l)	1.07±0.66		

Table 2: Effect of different density on values of blood indices (RBC, WBC, and Haematocrit) in great sturgeon juvenile (Mean±SE)

Density (kg/m^2)	Day	RBC (10^3/mm^3)	WBC (10^3/mm^3)	Hct (%)
control	0	836±16.64^a	40.51±1.14^a	20.5±2.21^a
1.5	30	847.5±32.01^a	20.71±1.77^b	17.7±3.42^b
3	30	751.66±38.29^a	20.98±1.77^b	20.3±3.32^a
6	30	872.77±17.08^a	22.37±1.84^b	19.8±1.72^a
1.5	60	1268.57±72.04^a	27.60±5.67^c	17.9±2.84^b
3	60	924.44±28.30^a	35.43±8.84^c	19.8±2.53^a
6	60	965.55±67.34^a	33.26±6.98^c	19.9±2.81^a

Means values bearing different superscripts in a column differ significantly (P<0.05).

demand situations such as chronic stress (Montero et al., 2001). Rafatnezhad et al. (2008) reported that Hct in great sturgeon under higher densities (8 kg/m^2) increased and that is similar with present study.

Falahatkar and Barton (2007) reported that after 8 weeks of rearing at low (2 kg/m^2) and high (7 kg/m^2) density had no significant effect on hematocrit in juvenile. Changes in hematocrit in fish subjected to acute disturbance may be due to changes in erythrocyte number, erythrocyte swelling or haemodilution from osmo regulatory disturbance (Wedemeyer et al., 1990; Iwama et al., 1995). Nilson and Grove (1984) demonstrated that adrenergic and cholinergic innervations cause contract of the spleen which increases hemoglobin and hematocrit. These changes occurs as a possible strategy to increased oxygen-carrying capacity of blood or a consequence of increased swimming activity during periods of high energy demand (Caruso et al., 2005).

In the present study, the number of RBC, lymphocyte, neutrophil, and monocyte had no significant different among stocking densities, that agreed to the study of Rafatnezhad et al. (2008) in great sturgeon juveniles, and study of Mazur and Iwama (1993) in wild and hatchery-reared Chinook salmon (*Oncorhynchus tshawytscha*). A drop in WBC principally leucocytes at 30th day of rearing as compared to control group, is a typical response to acute stress in fishes (Wedemeyer et al., 1990) and may be mediated by cortisol (McLeay, 1983).

During our experiment, fish showed stocking density had dramatic effect on plasma glucose and cortisol concentration in great sturgeon juveniles. Rafatnezhad et al. (2008) found that stocking density did not affect at plasma glucose and cortisol in great sturgeon juveniles at different densities (1-8 kg/m^2). Ruane et al. (2002) found that density does not affect plasma glucose in common carp (*Cyprinus carpio*). Increased blood glucose is a characteristic response of fish subjected to an acute or chronic stressor.

In many studies, fishes reared at high densities had no significant difference in plasma cortisol level compared with those reared at lower densities (Kebus et al., 1992; Rotllant and Tort, 1997; Procarione et al., 1999; Martins da Rocha et al., 2004; Falahatkar and Barton, 2007). A previous exposure to a chronic stressor, such as low water quality or high stocking density, however, can alter the cortisol response to a subsequent acute disturbance (Pickering and Pottinger, 1987; Ruane et al., 2002; Barton et al., 2005).

Bayunova et al. (2002) documented that high loading density of *Acipenser stellatus* during holding in the transport tank showed increased cortisol and glucose concentrations. Similarly, Montero et al. (1999) found that high stocking density in juvenile (*Sparus aurata*) resulted in plasma cortisol concentration four-fold higher than those in fish held under lower density. Falahatkar and Barton (2007) reported significant elevation in serum cortisol from 10.8 to 14.6 ng/ml when juvenile (*H. huso*) were subjected to handling and confinement. In contrast, teleosts responded to a single acute stressor with cortisol elevations that can exceed 200 ng/ml (Barton, 2002) and to multiple and chronic stressors with cortisol responses much higher (Maule et al., 1988; Mazik et al., 1991; Noga et al., 1994). The reasons for low corticosteroid response of some sturgeon species to stress compared with teleosts may result from a variety of factors. These include differences in neuroendocrine events that occur in the brain between sensory perception of the stressor and activation of the HPI axis, anatomical differences in internal tissue (adrenal homologue) and differences in internal sensitivity and physiological response capacity (Barton et al., 2000).

Table 3: Effect of different density on values of blood indices lymphocyte, neutrophil, eosinophil and monocyte in great sturgeon juvenile (Mean±SE)

Density (kg/m^2)	Day	Lymphocyte (%)	Neutrophil (%)	Eosinophil (%)	Monocyte (%)
Control	0	66.9±6.6[a]	18.9±1.5[a]	15.4±1.6[ab]	1.1±0.2[a]
1.5	30	67.9±4.6[a]	17.2±2.7[a]	14.4±3.2[a]	1.3±0.5[a]
3	30	74.1±5.9[a]	15.3±3.7[a]	10.6±2.8[b]	1.2±0.4[a]
6	30	72.8±3.5[a]	15.5±2.9[a]	11.5±2.1[b]	1.2±0.4[a]
1.5	60	78.8±6.6[a]	19.5±3.3[a]	16.2±4[a]	1.3±0.5[a]
3	60	72.5±4.9[a]	19.6±3.3[a]	13.8±6.4[b]	1.7±0.4[a]
6	60	75.5±5.2[a]	17.3±3.1[a]	11.7±3.5[b]	1.2±0.4[a]

Means values bearing different superscripts in a column differ significantly ($P<0.05$)

Table 4: Concentration of cortisol and glucose in blood plasma in great sturgeon juveniles (Mean±SE)

Density (kg/m^2)	Day	Cortisol (ng/ml)	Glucose (mg/dl)
Control	0	8.57±1.1[b]	70.12±3.6[ab]
1.5	30	9.65±0.76[b]	73.77±2.93[ab]
3	30	12.77±1.88[a]	85.00±6.0[a]
6	30	9.77±0.88[b]	63.33±3.89[b]
1.5	60	9.84 ±0.89[ab]	75.66±3.65[ab]
3	60	11.71±1.49[ab]	70.77±3.24[ab]
6	60	10.11±0.78[ab]	66.55±1.97[ab]

Means values bearing different superscripts in a column differ significantly ($P<0.05$)

Conclusion

The results showed that unlike many other fish, great sturgeon (*H. huso*) exhibited significant effect in some parameters during stress when confined at high stocking density.

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