Effect of different stocking densities on hematological parameters and growth performance of great sturgeon (*Huso huso* Linnaeus, 1758) juveniles

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Abstract

A 5-week study was conducted to determine the effects of different stocking density on hematological parameters and growth performance of great sturgeon. Fish were cultured in duplicates of fiberglass tanks under stocking densities of 50, 75, 100 and 125 fish/m3 as T1, T2, T3 and T4 respectively. Based on the results, significant increase ($p<0.05$) in total body weight and total length were related to low density group. The lowest mean body weight (103.26 g) and length (29.65 cm) were recorded in T4. At the end of the experiment, Results of hematological analysis showed changes in the stocking density had non-significant impact on values of WBC and MCH ($p>0.05$). The highest mean corpuscular hemoglobin concentration (MCHC) was also recorded in T4. The highest level of hemoglobin, hematocrit (7.25 g dL^{-1} and 22.2%) were significantly associated with T1 ($p<0.05$).

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The findings suggested that the haematological parameters and growth performance are considered as a benefactor for indicating stress in fish due to different physical or chemical parameters.

Keywords: Great strugeon, Stocking density, Hematological characteristics, Growth performance

Introduction

Acipenseridae as a family of species of sturgeon fish are currently considered to be at risk of extinction, making them more critically endangered than any other group of species. Great sturgeon (Beluga sturgeon), *Huso huso*, is one of the most important species of sturgeon in the Caspian Sea (Jalali, Hosseini & Imanpour 2008). Fast growth rearing sturgeon as a commercially important source of caviar and meat in intensive aquaculture systems is effected by appropriate and sufficient knowledge about impacted limiting factors on growth and improvement of a certain species (Vlasenko 1994). Some biochemical parameters in cultured fish are justified by their importance for evaluating the general health condition of the animals, including the possible effects of food availability, stocking density and presentation to stressors (Kebus, Collins, Brownfield, Amundson, Kayes & Malison 1992; Chen, Wooster & Bowser 2004; Tintos, Miguez, Mancera & Soengas 2006). One of the most vital factors in rearing fish that can be a source of stress for fish is the stocking density (Cristea, Grecu & Ceapa 2002) and that is precisely associated with animal comfort and fish culture productivity (Dicu, Cristea, Maereanu, Dediu & Petrea 2013). Current studies relevant to stocking as a determined factor in the economic return on production is critical to judge the effect of the importance of any density limits on economic stability (Rafatnezhad, Falahatkar & Tolouei Gilani 2008). Most authors also depicted that high stocking density may repress fish growth and it can originate from different factors such as decreasing water quality, social behavior and the change in metabolic rate (Ellis, North, Scott, Bromage, Porter & Gadd 2002; Lupatsch, Santos, Schrama & Verreth 2010; Tolussi, Hilsdorf, Caneppele & Moreira 2010). The stocking density as a stressor factor was researched on many species of fish such as *Orechromis niloticus* (Yousif, 2002), *Acipenser brevirostrum* (Wuertz, Lutz, Gessner, Loeschau, Hogans, Kirschbaum & Kloas 2006) *Oncorhynchus mykiss* (North, Turnbull, Ellis, Porter, Migaud, Bron & Bromage 2006). Haematological parameters and blood biochemistry were known as secondary stress indicators of diseases and physiological condition of fish (Stoskopf 1993). The stocking stress analysis is a requirement to argue hematological variation due to stress (Salah & Wael 2011). After all the identification and control of stress were noticed for ensuring the welfare and optimal growth of fish in captivity, few research have examined these issues and there are little information on species in this field (Rafatnezhad *et al*., 2008), thus, the aim of the present work was to investigate the effects of different stocking densities on hematological parameters and growth performance of great sturgeon juveniles reared in fiberglass tanks.

Materials and Methods

The present study was conducted at Shahid Marjani sturgeon hatchery and culture center, fishery organization of Golestan Province, Gorgan, Iran, for 5 weeks.

Experimental design

A total number of 900 great sturgeon juveniles, *H. huso*, with mean body weight of 48.59±8.52 were divided into 12 tanks (1000 L, by using water from the source water pond in this culture center) at 4 treatments with stocking densities of 50, 75, 100 and 125 fish/m3 as T1, T2,T3 and T4, respectively, with three replicates. Fish were randomly distributed (CRD method) at the desired stocking density. During experimental period water quality parameters were measured such as pH, Dissolved Oxygen and water temperature.

Before the initiation of the experiment, fish were adapted to experimental conditions for a week. The feeding of the fishes was done at the rate of 5% of the body weight, three times (at 06:00,14:00 and 22:00) a day.

Sample collection and analysis

At the end of the experimental period, in each tank, 10 fish were randomly sampled in order to evaluate haematological variables. Fish were captured and blood samples were taken from the caudal vein employing a heparinized syringe and were transferred to eppendorf tubes that were kept on ice until centrifugation at 3000 g for 10 min .The count of erythrocytes (RBC) and leukocytes (WBC) were determined as the number of cells/mm^3 in Neubauer hemocytometer. The red blood cell counts were readied by counting the erythrocytes from 5 small squares of Neubauer hemocytometer using Rees–Ecker diluting (Blaxhall & Daisley, 1973; Svobodova, 2001) Hemoglobin concentration (Hb in grams per deciliter) was determined based on cyanomethemoglobin spectrophotometry method (Blood was diluted in a Drabkin solution) Then the concentration determined using standard curve (Noga, 2010). Hematocrit (Ht) was also determined according to the standard microhematocrit method using hematocrit centrifuge at 13000 rpm for 5 min. The erythrocytic indices such as mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were calculated through standard formulas (Coles, 1986).

$$MCV=(Hct\times10)/RBC$$

$$MCH=(Hb\times10)/RBC$$

$$(Hb\times100)/Hct=MCHC$$

Data Analysis

Analyzed in a completely randomized design using SPSS statistical software to compare Duncan procedure was performed at the 5% level.

Results

Growth performance

At the end of the fifth week, changes in the average weight of Beluga sturgeon juveniles reared at different densities were investigated (Table 1). Based on the results, significant increase ($p<0.05$) in total body weight and total length were related to low density group. The lowest mean body weight (103.26 gr) and length (29.65 cm) were recorded in T4.

Table1 Changes in growth parameters of great sturgeon juveniles during the experiment(mean± SD)

	T1	T2	T3	T4
Total body weigt (gr)	125.14±14.78^a	105.32±14.94^b	108.26±13.47^b	103.26±14.05^b
Total length(cm)	31.47±1.37^a	35.04±1.73^b	30.18±1.66^b	29.65±1.78^b

Means with the same letter in the same column for each parameter are not significantly different.

(T1= 50, T2= 75, T3= 100 and T4= 125 fish/m3)

Hematological analyses

Results of hematological analyses are presented in Table 2. According to finding results, changing the stocking density had non-significant impact on values of WBCs and MCH ($p>0.05$). Meanwhile, the highest values of RBC recorded in T1. The Stocking density had a significant effect on haematocrit and haemoglobin concentration and the highest values of Hb and Ht were recorded in T1. MCV increased in high stocking density group (T4) and showed significant difference as compared to T1 and T2. MCH did not significantly differ among treatments. Values of MCHC showed significant increase ($p<0.05$) in T1 and this value had significant effect in T1, T3 as compared to T2 and T4.

Table 2 Changes in hematological Parameters of great sturgeon juveniles during the experiment (mean± SD)

	T1	T2	T3	T4
WBC (n/ml)	7850.00±52.00^{a}	8700.00±200.00^{a}	11000.00±300.0^{a}	11000.00±200.00^{a}
RBC (10^6/ml)	0.880±0.001^{a}	0.858±0.009^{a}	0.802±0.005^{b}	0.818±0.007^{b}
Hb (g dl^{-1})	7.250±0.06^{a}	6.850±0.05^{b}	6.70±0.09^{c}	6.75±0.05^{bc}
Ht (%)	22.20±0.3^{a}	21.45±0.05^{b}	20.45±0.15^{c}	21.45±0.18^{b}
MCV (fl)	252.42±3.84^{b}	250.40±9.26^{b}	255.00±1.02^{ab}	262.16±1.42^{a}
MCH (pg)	82.43±0.43^{a}	79.94±9.19^{a}	83.55±0.15^{a}	82.50±0.09^{a}
MCHC (%)	32.67±0.67^{a}	31.94±0.30^{b}	32.77±0.07^{a}	31.41±0.13^{b}

Values in the same column with different superscripts for each species are significantly different ($p<0.05$).
(T1= 50, T2= 75, T3= 100 and T4= 125 fish/m^3)

Discussion

According to finding, growth parameters such as total body weight and total length were significantly affected by rearing density, although all of them were feed at the same level (5% of body weight per day). Growth is likely the most describable physiological parameter in relevance to social interactions (Sloman & Armstrong 2002) which can be estimated readily and could be applied as a sign of social stress (Bolasina, Tagawa, Yamashita & Tanaka 2006). Fish production function can improve by providing environmental condition. The physiological system of fish can be extremely affected by a diversity of biological, chemical, physical factors. Stocking density as the main variable factor can effect on the hematological and biochemical parameters of fish (Salah & Wael 2011).

According to the researches done by scientists, increasing stocking density have negative impact on the growth of fish and this result can root from the change of water quality parameters, physical space, social interaction, food availability, so heavily high loading rates can end in an accumulation of un-ionized NH_3, which can affect the health and finally the growth of fish (Wedemeyer Barton & McLeay 1990; Ellis *et al*., 2002; Wedemeyer, 1997; Hastein *et al*., 2005; Sloman, 2002). Rafatnezhad *et al* (2008) demonstrated the influence of stocking density on growth of great sturgeon (*Huso huso*) juveniles although reportes at all densities examined that great sturgeon juveniles can bear high densities, but with reducing impacts on fish growth, then they claimed the lack of mortality refers to strong nature of great sturgeon during the high stocking density period. Rafatnezhad & Falahatkar (2011) reported high survival of juvenile great sturgeon at all densities but the best growth (SGR, ADG, RWG) appeared at the lowest density.

Similar results which were obtained by Nafsika, Sofronios & Georgios (2007) that also determined a low stocking density has the best general performance on growth, food utilization, body protein content of juvenile white seabream (*Diplodus sargus L*.), this result may be true that the change of water physical parameters ends in increasing growth indicators as an effect of density improvement and decreasing stress.

Measurement of secondary biochemical indicators, such as hematological characteristics can assess the secondary reactions of fish to the stressors. Fluctuation of fish blood biochemistry can monitor improper environmental conditions and the presence of stressing factors (Wedemeyer *et al*., 1990; Barcellos, Kreutz, Souza, Rodrigues, Fioreze, Quevedo, Cericato, Soso., Fagundes, Conrad J, Lacerda & Terra 2004). According to Hrubec, Cardinale & Smith (2000), fish hematology devoted great attention in fish culture to itself, because of its influential role in indicating the health condition of fish.

Regarding to the findings of our research, the evaluation of RBC, Hb and Ht values indicated significant difference in T1 compared to the other treatments which shown that increasing the stocking density over 50 fish/m3 effected subsequently these hematological parameters of fish. The reason attributed to these changes could be the physical or environmental stress that leads to increasing the concentration of hemoglobin. The reason refers to collect erythrocytes from the spleen and the hemoconcentration because of the loss of plasmic water (Nicula 2004).

It was concluded by many authors that hemoglobin concentration was used to distinguish the effect of stocking density on fish health (Salah & Wael 2011; Kjartansson, Fivelstad, Thomassen & Smith 1988; Caldwell & Hinshaw 1994; Montero, Tort, Robaina,Vergara & Izquierdo 2001; Kopp *et al*. 2010).

The reduction of hemoglobin concentration can have effect on the oxygen quantity from tissues then cause to decrease metabolic action and finally result in decreasing production of energy (Montero *et al*. 2001). Nicula (2004) stated the reduction of hemoglobin concentration can effect on the cardiac action because the circulating requirement and the cardiac rhythm are essential for releasing O_2 to tissues. Salah & Wael (2011) during their research about the effect of different stocking densities on hematological and biochemical Parameters of Silver Carp, *Hypophthalmichthys molitrix* Fingerlings, reported that increasing the stocking density caused significant increase in values of RBCs, Hb and Ht. Haematological changes also in jundia (Rhamdia quelen, Quoy and Gaimard) under chronic stress condition demonstrated to increase the RBC count (Barcellos *et al*., 2004).

The erythrocyte constants (MCV, MCH and MCHC) were calculated by using the values of Hb, RBC with applying the standard formulas. The results of this present study indicated that mean corpuscular volume (MCV) significantly increased in high stocking density treatment as compared to the low stocking density. Conversely, high stocking density did not have a significant effect on some haematological parameters such as WBC and MCHC. This is in agreement with the findings of salah & Wael (2011), Charoo, Chalkoo & Qureshi (2013). The observed non-significant differences in values of MCHC and WBC confirm that increasing the stocking density did not put challenge to the erythrocytes and did not monitor pathological condition in the studied fish. Likewise, erythropoiesis and hemoglobin synthesis need long time to complete and can merely be affected in long-term adaptation (Weber & Jensen 1988).

Rafatnezhad *et al* (2008) investigated the effects of stocking density on haematological parameters of great sturgeon (*Huso huso*) juveniles and reported significant difference was observed in haematocrit, but the other haematological parameterssuch as RBC, WBC, Hb concentration, MCV and MCHC did not show significant difference.

Based on the presented results it can be concluded that the stocking density have effects on growth and haematological parameters. In this condition the most efficient result was observed in lowest stocking density group. In case of high stocking density group, a significant ($p<0.05$) increase in value of RBC, hematocrit, MCV, MCH, the hemoglobin concentration and the lowest mean body weight and length were recorded. Haematological parameters are considered as a benefactor for indicating stress in fish due to different physical or chemical factors.

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تأثیر تراکم‌های مختلف بر پارامترهای خونی و عملکرد رشد فیل ماهی‌های ***Huso huso*** **(Linnaeus, 1758)** جوان

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چکیده

مطالعه‌ای ۵ هفته‌ای با هدف بررسی اثر تراکم‌های مختلف بر پارامترهای خونی و عملکرد رشد فیل‌ماهی‌های جوان صورت گرفت. ماهی‌ها در تانک‌های فایبرگلاس در ۴ تیمار و هر یک با ۳ تکرار با تراکم ۵۰، ۷۵، ۱۰۰ و ۱۲۵ ماهی در هر متر مکعب تحت عنوان T1، T2،T3 و T4پرورش یافتند. با توجه به نتایج افزایش معنی‌داری در وزن کل و طول کل در گروه با کمترین تراکم مشاهده شد ($p<0.05$). کمترین وزن (۱۰۳/۲۶ گرم) و طول (۲۹/۶۵ سانتی متر) در T4 مشاهده شد. در انتهای آزمایش نتایج بررسی پارامترهای خونی نشان داد که تفاوت در تراکم، اثر معنی‌داری بر WBC و MCH نداشت ($p>0.05$). بالاترین غلظت متوسط هموگلوبین‌های گلبول قرمز (MCHC) در T4 گزارش شد. بیشترین میزان هموگلوبین و هماتوکریت (۷/۲۵ گرم در دسی لیتر و ۲۲/۲ درصد) به ظور معنی‌داری با تیمار T1 مرتبط بود ($p<0.05$). طبق یافته‌ها پارامترهای خونی و عملکرد رشد، فاکتورهای مفیدی به منظور نشان دادن استرس ناشی از پارامترهای فیزیکی و شیمیایی مختلف می‌باشند.

کلمات کلیدی: فیل ماهی، چگالی تراکم، مشخصات خونی، عملکرد رشد

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