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Effects of stocking density on mortality, growth and physiology of juvenile Amur sturgeon (*Acipenser schrenckii*)

Meng Ni, Haishen Wen, Jifang Li, Meili Chi, Yan Bu, Yuanyuan Ren, Mo Zhang, Zhifei Song & Houmeng Ding

Fisheries College, Ocean University of China, Qingdao, Shandong, China

Correspondence: H S Wen, Fisheries College, Ocean University of China, 5 Yushan Road, Qingdao 266003, China.
E-mail: wenhaishen@ouc.edu.cn

Abstract

To evaluate effects of stocking density on welfare of Amur sturgeon (*Acipenser schrenckii*), an experiment was designed using three initial stocking densities in flow-through tanks (LSD = 3.7 kg m^{-3}, MSD = 6.9 kg m^{-3}, and HSD = 9.3 kg m^{-3}, respectively) for 60 days. Growth, body composition, and haematological and biochemical parameters were monitored. The mortality and feed conversion rate (FCR) were not affected by stocking density. However, the specific growth rate (SGR), final weight and weight gain in the HSD group were significantly lower than in the LSD and MSD groups. The hepatosomatic (HSI) and viscerosomatic indices (VSI) varied inversely with regard to stocking density. Stocking density did not affect crude protein levels in fish. In contrast, the total lipid level was significantly higher in the LSD group compared to the MSD and HSD groups. The levels of erythrocytes and haemoglobin were positively correlated with stocking density. Serum total bilirubin and urea in HSD group were significantly higher than in the LSD group while serum triglycerides showed opposite tendencies. Differences between treatments were not registered for glucose, total protein and albumin. In conclusion, higher stocking density resulted in increased immunosuppression and enhanced energy mobilization. The latter was necessary to enable Amur sturgeon to cope with crowding.

Keywords: *Acipenser schrenckii*, stocking density, growth, haematological parameters, serum biochemical parameters, body chemical composition

Introduction

Fish welfare has become a growing interest in aquaculture. Stocking density, as one of the main factors in aquaculture, can directly influence survival, growth, health status, water quality, gene expression and production (Gomes, Baldisserotto & Senhorini 2000; Gornati, Terova, Vigetti, Prati, Saroglia & Bernardini 2004; Schram, Van der Heul, Kamstra & Verdegem 2006). Fish could be either positively or negatively affected by stocking density. High density may result in stress (Leatherland & Cho 1985) and impair welfare (North, Turnbull & Ellis 2006; Lupatsch, Santos & Schrama 2010; Yvette, Wunderink & Silke 2011), although with some fish species, positive effects of density have been shown (Jørgensen, Christiansen & Jobling 1993). Such species differences need to be taken into account when designing husbandry systems.

Sturgeons are one of the most remarkable species among other vertebrates due to their unique properties: they are ancient and have relatively low rates of evolution, and all 27 sturgeon species have been listed in CITES (Bemis, Findeis & Grande 1997; Ludwig 2008). The techniques of sturgeon artificial breeding have been developed in many northern hemisphere countries (Asia, North America and Europe) (Williot, Sabeau, Gessner, Arlati, Bronzi, Gulyas & Berni 2001; Wei, He, Yang, Zheng & Li 2004). Amur sturgeon, *Acipenser schrenckii*, is one of the species in the Acipenseridae family distributed throughout the Amur River basin and its tributaries in China and Russia. Nowadays, Amur sturgeon has developed into one of the most popular aquaculture species in China due to its high value (Zhuang,

Kynard, Zhang, Zhang, Zhang & Li 2002). Yang, Zhu, Luo, Zhao and Chen (2011) suggested that both with small and large Amur sturgeon alike, the growth parameters final weight, final condition factor and specific growth rate decreased with increasing stocking density. Li, Liu and Xie (2012) reported that stocking density is inversely related with food consumption and specific growth rate, and that Amur sturgeon exhibits density-dependent internal physiological changes. Rafatnezhad, Falahatkar and Gilani (2008) found that stocking density significantly influenced growth of great sturgeon (*Huso huso*), rather than stress response. In stellate sturgeon (*Acipenser stellatus*) fingerlings, high stocking densities showed no significant effects on growth and physiological parameters except for a decrease in survival (Dicu, Cristea, Maereanu, Dediu & Petrea 2013a). Still little is known about the effect of stocking density of Amur sturgeon on welfare. The aim of this study was to investigate the effect of stocking density on growth, survival, haematological parameters, biochemical parameters and body composition in juvenile Amur sturgeon to determine the optimal stocking density in intensive culture.

Methods and materials

Animals and husbandry

The experiment was performed in Shandong Xunlong Fishery Tech and Development Co. Ltd., China. Prior to the start of the trial, fish was acclimatized for 2 weeks. After this period of adaptation, 11 100 fish (42.0 ± 2.3 g; mean ± SEM, $N = 9$) were randomly distributed into three stocking densities (low stocking density (LSD) = 3.7 kg m^{-3}; medium stocking density (MSD) = 6.9 kg m^{-3} and high stocking density (HSD) = 9.3 kg m^{-3} respectively). Nine square flow-through tanks (4.4 * 4.4 * 0.45 m^3) were used for the experiment, three for each stocking density. The effective water level was about 0.40 meter high. The total experiment duration was 60 days. Ningbo Tech-Bank pelleted feed (42% of crude protein) was fed three times daily during acclimatization and the experiment. The feeding rate was adjusted daily by carefully observing accumulation of uneaten feed in the tanks.

Freshwater, passed through mechanical filters, was supplied at approximately 15 L/min per tank. The quality of the supply water was checked daily. Temperature was determined with a mercurial thermometer. Dissolved oxygen and pH were analysed using a HQ40D dual input digital multi-meter (HACH, Loveland, CO, USA). Ammonium (NH_4-N) was measured by Nessler's reagent colorimetric method (Crosby 1968). Parameters of the flow-through water were: temperature at 18.2 ± 1.8°C, O_2 at 8.5 ± 1.2 mg L^{-1}, pH at 7.84 ± 0.08 (Mean ± SEM) and NH_4-N below 0.001 mg L^{-1}.

Growth and biometric measurements

Body weight was measured every 10 days (50 fish in each tank). Other parameters were obtained per tank at the end of the trial (60 days) including average individual weight (g), average individual total length (cm), weight gain (WG %) = [final weight (g)−initial weight (g)] * 100/initial weight (g), specific growth rate (SGR) = [ln final weight (g)−ln initial weight (g)]/days * 100%, feed conversion ratio (FCR) = [weight of feed (g)/weight gain (g)] and mortality. The biometric indices including condition factor (K) = [wet weight (g)/(total length (cm)]^3 * 100), hepatosomatic index (HSI) = [liver weight (g) / body weight (g)] * 100), viscerosomatic index (VSI) = [visceral weight (g)/body weight (g)] * 100) were also measured.

Haematological and biochemical parameters

At the end of the experiment, 36 fish (four from each tank) were sampled to evaluate physiological parameters. Fish were anesthetized with tricaine methane sulphonate (MS-222, 200 mg L^{-1}). Blood samples were collected from the caudal vein of Amur sturgeon for haematological and biochemical analysis. Per fish, a blood sample of 0.5 mL was immediately transferred into a plastic tube coated with EDTA-K_2 to analyse total leucocyte numbers, erythrocyte numbers and haemoglobin values using a Mindray Automatic Blood Analyzer (BC1800, China) according to Rehulka's method (2000). Per fish, another 2 mL blood sample was directly transferred to a plastic tube without anticoagulant, clot at 4°C for 4–8 h, centrifuged at 3000 *g* for 10 min, then the serum was separated and stored at −20°C for subsequent analysis. Urea (mg L^{-1}), total protein (TP, g L^{-1}), glucose (GLU, mmol L^{-1}), cholesterol (CHOL, mg L^{-1}), triacylglycerol (TGL, mg L^{-1}), and activities of alanine aminotransferase (ALT, U), aspartate aminotransferase (AST, U) and alkaline phosphatase (ALP, U) were determined with a

Mindray Automated Biochemistry Analyzer (BS180, China) according to Rehulka's method (2000).

Proximate analysis and body chemical composition

Another 36 fish (four from each tank) were sampled to evaluate the body chemical composition according to the standard methods of Association of Official Analytical Chemists (AOAC) (1995). Dry matter was determined by oven drying method (105°C). Crude protein was measured by the Kjeldahl method using an Auto Kjeldahl System (FOSS KT260, Switzerland). Crude lipid was measured by Soxhletether extraction method using the Soxtec System HT6 (Tecator, Sweden). Ash was analysed by incineration for 12 h (550°C). Fatty acids were analysed according to the method of Metcalfe (1966) with some modification by Ai, Zhao, Mai, Xu, Tan and Ma (2008).

Data analysis

All data were analysed in SPSS. One-way Analysis of Variance (ANOVA) was used to detect differences between stocking densities, followed by Duncan's test. Differences were considered significant at $P < 0.05$. Results are displayed as means ± SEM.

Results

Water quality

There was no significant difference ($P > 0.05$) in the mean temperature between treatments. The dissolved oxygen content decreased with increasing stocking density. The pH fluctuated between 8.06 and 8.09. NH_4-N concentrations increased with increasing stocking density (Table 1).

Growth and biometric parameters

Stocking density significantly influenced the body weight of Amur sturgeon from 30–60 days (Fig. 1). No significant differences in mortality were found among different stocking densities. The final total length in the MSD and HSD groups was significantly lower than in the LSD group. The final weight, weight gain and SGR showed a significant decrease with increasing stocking density. The FCR was 1.18, 1.17 and 1.15 in the LSD, MSD and HSD group, respectively, and equal among the treatments (Table 2). At the end of the experiment, HSI, VSI and K decreased with increasing stocking density ($P < 0.05$) (Table 2).

Table 1 Water quality values from Amur sturgeon in different stocking densities at the end of a 60-day growth trial

	Stocking densities		
Parameter	**LSD**	**MSD**	**HSD**
T (°C)	16.13 ± 0.033	16.13 ± 0.033	16.17 ± 0.033
DO (mg L^{-1})	7.06 ± 0.189^a	6.19 ± 0.064^b	5.62 ± 0.123^c
pH	8.09 ± 0.006^a	8.07 ± 0.003^b	8.06 ± 0.003^c
NH_4-N (mg L^{-1})	0.05 ± 0.004^a	0.16 ± 0.011^b	0.21 ± 0.032^c

The following abbreviations were used: LSD, low stocking density; MSD, medium stocking density; HSD, high stocking density; T, temperature; DO, dissolved oxygen; NH_4-N, ammonia nitrogen.

Data are presented as mean ± SEM, $N = 3$. Different letter in a row indicates significant differences between the stocking densities ($P < 0.05$).

Body composition

At the end of the experiment, body moisture content was not different between the three stocking

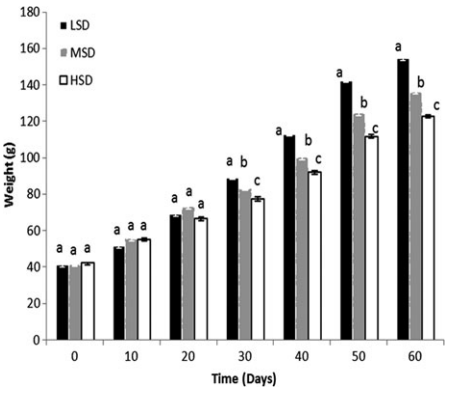

Figure 1 Body weight (g) over time of Amur sturgeon reared in different initial stocking densities (LSD = 3.7 kg m^{-3}, MSD = 6.9 kg m^{-3} and HSD = 9.3 kg m^{-3}). Data are presented as mean ± SEM, $N = 3$. Different letters within one sample time indicate significant difference ($P < 0.05$, one-way ANOVA, followed by Duncan's multiple range test).

Table 2 Mortality and growth parameters from Amur sturgeon reared in different stocking densities

	Stocking densities		
Parameter	**LSD**	**MSD**	**HSD**
Initial densities (kg m^{-3})	3.7	6.9	9.3
Final densities (kg m^{-3})	14.1	22.1	27.0
Mortality (%)	0.24 ± 0.052	0.31 ± 0.039	0.33 ± 0.018
Initial body weight (g)	41.12 ± 1.032	41.21 ± 0.368	42.31 ± 0.722
Final body weight (g)	155.99 ± 0.803^{a}	131.38 ± 1.203^{b}	123.04 ± 1.784^{c}
CV of body weight (%)	2.25	2.92	3.82
Initial total length (cm)	20.53 ± 0.602	20.68 ± 0.773	22.17 ± 0.853
Final total length (cm)	29.54 ± 0.320^{a}	28.01 ± 0.372^{b}	27.76 ± 0.271^{b}
CV of total length (%)	4.06	5.03	3.87
Weight gain (%)	279.37 ± 2.183^{a}	218.85 ± 2.776^{b}	188.51 ± 3.983^{c}
Specific growth rate (%/day)	2.22 ± 0.047^{a}	1.93 ± 0.014^{b}	1.78 ± 0.005^{c}
Feed conversion ratio	1.18 ± 0.015	1.17 ± 0.009	1.15 ± 0.007
K	0.60 ± 0.011^{a}	0.57 ± 0.009^{b}	0.56 ± 0.012^{b}
HSI (%)	3.42 ± 0.137^{a}	2.37 ± 0.287^{b}	2.41 ± 0.121^{b}
VSI (%)	19.75 ± 0.506^{a}	19.21 ± 0.533^{ab}	18.19 ± 0.461^{b}

The following abbreviations were used: LSD, low stocking density; MSD, medium stocking density; HSD, high stocking density; CV, coefficient of variation; K, condition factor = total weight/total length^{3} × 100; HSI, hepatosomatic index = (liver weight/body weight) × 100; VSI, viscerosomatic index = (visceral weight/body weight) × 100.
Except for Initial number of fish, data are presented as mean ± SEM, $N = 3$. Different letter in a row indicates significant differences between the stocking densities ($P < 0.05$).

density groups ($P > 0.05$). Stocking density also did not affect the crude protein content ($P > 0.05$). But ash content was significantly higher in the MSD and HSD groups than in the LSD group ($P < 0.05$). In contrast, total lipid was lower in the MSD and HSD groups than in the LSD group ($P < 0.05$). (Table 3).

With increasing stocking density, contents of C14:1, C18:2n-6 and C20:4n-6 increased while C18:3n-6 and docosahexaenoic (DHA) contents decreased ($P < 0.05$). Whole-body saturated fatty acid (SFA) and mono-unsaturated fatty acids (MUFA) contents were equal between stocking densities ($P > 0.05$). However, the n-6 poly-unsaturated fatty acid (n-6 PUFA) contents in the HSD and MSD groups were higher than in the LSD group ($P < 0.05$). Though docosahexaenoic (DHA) contents were lower in the MSD and HSD group than in the LSD group ($P < 0.05$), the whole-body n-3 poly-unsaturated fatty acids (n-3 PUFA) contents were not affected by stocking density ($P > 0.05$) (Table 4).

Table 3 Body composition from Amur sturgeon in different stocking densities at the end of a 60-day growth trial

	Stocking densities		
Parameter	**LSD**	**MSD**	**HSD**
Moisture (%)	75.48 ± 0.782	77.40 ± 0.895	77.21 ± 0.773
Total lipid (%)	7.17 ± 0.248^{a}	5.38 ± 0.313^{b}	5.79 ± 0.501^{b}
Ash (%)	2.62 ± 0.022^{b}	3.56 ± 0.256^{a}	3.11 ± 0.133^{a}
Crude protein (%)	13.86 ± 0.645	12.68 ± 0.478	12.68 ± 0.252

The following abbreviations were used: LSD, low stocking density; MSD, medium stocking density; HSD, high stocking density; the same below.
Data are presented as mean ± SEM, $N = 3$. Different letters indicate statistical differences ($P < 0.05$) between groups.

Haematological parameters

Similar numbers of leucocytes were found at all stocking densities ($P > 0.05$). However, erythrocyte levels were higher in the HSD and MSD groups. Similarly, haemoglobin values in the HSD and MSD groups were higher than in the LSD group ($P < 0.05$) (Table 5).

Biochemical parameters

The lowest serum total cholesterol and triglyceride concentrations were found in the HSD group ($P < 0.05$), while total bilirubin in the HSD group was higher than in the LSD and MSD groups ($P < 0.05$). The urea concentrations in the HSD

Table 4 Fatty acid profile (%) of Amur sturgeon reared in different stocking densities at the end of a 60-day growth trial

Fatty acid	Stocking Densities		
	LSD	MSD	HSD
14:0	3.18 ± 0.291	3.36 ± 0.090	3.52 ± 0.184
16:0	21.71 ± 1.432	23.03 ± 0.255	23.40 ± 0.373
18:0	6.45 ± 0.162	4.30 ± 0.858	4.22 ± 1.002
20:0	1.18 ± 0.203	0.79 ± 0.154	1.12 ± 0.012
∑SFA	32.52 ± 1.324	31.48 ± 1.041	32.26 ± 1.042
14:1	0.12 ± 0.008^{b}	0.14 ± 0.004^{b}	0.20 ± 0.023^{a}
16:1	4.13 ± 0.432	4.33 ± 0.146	4.68 ± 0.289
18:1	32.35 ± 1.833	35.31 ± 1.104	32.49 ± 0.611
∑MUFA	36.60 ± 2.253	39.78 ± 1.014	37.37 ± 0.607
18:2n-6	4.22 ± 0.422^{b}	6.92 ± 0.604^{a}	6.53 ± 0.109^{a}
18:3n-6	0.22 ± 0.002^{a}	0.09 ± 0.021^{b}	0.13 ± 0.026^{b}
20:4n-6	0.46 ± 0.131^{b}	0.54 ± 0.112^{ab}	0.83 ± 0.023^{a}
∑n-6 PUFA	4.57 ± 0.467^{b}	7.55 ± 0.685^{a}	7.48 ± 0.156^{a}
18:3n-3	0.23 ± 0.011	0.22 ± 0.020	0.26 ± 0.022
EPA (20:5n-3)	0.44 ± 0.051	0.49 ± 0.073	0.57 ± 0.234
DHA (22:6n-3)	0.43 ± 0.014^{a}	0.32 ± 0.008^{b}	0.32 ± 0.016^{b}
∑n-3 PUFA	1.10 ± 0.072	1.18 ± 0.105	1.28 ± 0.033
n-3/n-6 PUFA	0.23 ± 0.020	0.16 ± 0.028	0.17 ± 0.012
DHA/ EPA	1.00 ± 0.094	0.68 ± 0.107	0.98 ± 0.186

SFA, saturated fatty acids; MUFA, mono-unsaturated fatty acids; n-6 PUFA, n-6 poly-unsaturated fatty acids; n-3 PUFA, n-3 poly-unsaturated fatty acids; n-3 HUFA, n-3 highly unsaturated fatty acids; DHA/EPA, Docosahexaenoic acid (22:6n-3)/ Eicosapentaenoic acid (20:5n-3).

Some fatty acids, of which the contents are minor, trace amount or not detected, such as C22:0, C24:0, C20:1n-9, C22:1n-11, C20:2n-6, C20:3n-6, C22:5n-3, were not listed in the table. Data are presented as mean ± SEM, $N = 3$. Different superscript letters in each row show significant differences among stocking densities by Duncan's test ($P < 0.05$).

and MSD groups were higher than in the LSD group. Differences between stocking densities were not detected for glucose, total protein and albumin, as well as in the enzymes of ALT, AST and ALP ($P > 0.05$) (Table 6).

Discussion

A high stocking density reduced welfare in juvenile stellate sturgeon and European seabass (*Dicentrarchus labrax*) (Santos, Schrama, Mamauag, Rombout & Verreth 2010; Dicu, Cristea, Dediu, Docan, Grecu & Vasilean 2013b). Kebus, Collins, Brownfield, Amundson, Kayes and Malison (1992) reported that in rainbow trout (*Oncorhynchus mykiss*) culture, at high stocking density the water quality deteriorated. Shi, Zhuang, Zhang and Nie (2006) found that high stocking density could deteriorate water quality. In our study, all the water quality parameters except for the water temperature deteriorated with increasing stocking density suggesting that the decrease in growth at high stocking density is in part caused by deterioration in water quality.

The growth of Amur sturgeon in our study was affected by stocking density after 60 days. In Atlantic salmon (*Salmo salar*), no direct correlation between specific growth rate and stocking density was found, but the stocking densities studied were lower than in our study (Soderberg & Krise 1986; Soderberg & Meade 1987). There were no changes found in the feed conversion ratio among different stocking densities in the present study. Similar results have been described in sea bass and brill (*Scophthalmus rhombus*) (Sammouth, Roque, Gasset, Lemarié, Breuil, Marino, Coeurdacier, Fivelstad & Blancheton 2009; Herrera, Ruĭz-Jarabo, Hachero, Vargas-Chacoff, Amo & Mancera 2012). The condition factor (K) is a good indicator of fish health (George, Sharma, Venkateshvaran, Sinha, Venugopal & Birader 1985). Similar to our results, Rafatnezhad *et al.* (2008) observed a better condition factor (K) in great sturgeon kept at low density. In general, these studies suggest that fish grown at low density perform better than at higher densities.

Table 5 Haematological values of Amur sturgeon blood in different stocking densities at the end of a 60-day growth trial

Parameter	Stocking Densities		
	LSD	MSD	HSD
Leucocytes ($\times 10^{9}$ L^{-1})	225.26 ± 8.063	245.11 ± 10.661	250.40 ± 8.118
Erythrocytes ($\times 10^{12}$ L^{-1})	0.14 ± 0.013^{b}	0.32 ± 0.027^{a}	0.26 ± 0.038^{a}
Haemoglobin (g L^{-1})	68.75 ± 2.093^{b}	78.50 ± 1.305^{a}	79.78 ± 3.413^{a}

Data are presented as mean ± SEM, $N = 3$. Different letters indicate statistical differences ($P < 0.05$) between groups.

Table 6 Biochemical values of Amur sturgeon blood serum in different stocking densities at the end of a 60-day growth trial

	Stocking Densities		
Parameter	**LSD**	**MSD**	**HSD**
Metabolites			
TP (g L^{-1})	18.62 ± 0.36	18.23 ± 1.50	21.20 ± 3.00
GLU (mmol L^{-1})	1.96 ± 0.14	2.19 ± 0.17	2.17 ± 0.12
TGL (mmol L^{-1})	7.66 ± 1.18^{a}	7.28 ± 0.98^{a}	5.11 ± 0.61^{b}
CHOL (mmol L^{-1})	2.74 ± 0.48^{a}	2.52 ± 0.20^{a}	1.72 ± 0.13^{b}
Urea (mmol L^{-1})	2.35 ± 0.03^{b}	3.52 ± 0.25^{a}	3.57 ± 0.31^{a}
T.BILI (μmol L^{-1})	4.67 ± 0.63^{b}	3.90 ± 0.60^{b}	9.33 ± 1.39^{a}
Enzymes			
ALT (U L^{-1})	3.2 ± 0.18	4.4 ± 0.31	4.4 ± 0.80
AST (U L^{-1})	11.6 ± 6.32	14.8 ± 1.47	19.0 ± 5.00
ALP (U L^{-1})	266.1 ± 23.06	266.7 ± 2.84	251.7 ± 36.03

The following abbreviations were used: GLU, glucose; TP, total protein; ALB, albumin; TGL, triglyceride; CHOL, Total cholesterol; T.BILI, total bilirubin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase.
Data are presented as mean ± SEM, $N = 3$. Different letters indicate statistical differences ($P < 0.05$) between groups.

In our study, mortality showed no significant difference among different stocking densities. Similarly, many studies have not found any significant effects of density on survival (Mazzola, Favaloro & Sarà 2000; Gomes, Chagas, Martins–Junior, Roubach, Ono & Lourenco 2006; Rafatnezhad *et al.* 2008). Low mortality of Amur sturgeon at high densities reveals the amenability of this species to crowding. HSI is related to the nutritional state and growth rate of the fish (Luckenbach, Murashige, Daniels, Godwin & Borski 2007). Previous studies reported that HSI was significantly lower at high stocking density (Leatherland & Cho 1985). This reduction was probably due to a worse nutritional state and an increased lipid mobilization at high stocking density.

Previous studies revealed that differences in stocking density may cause differences in body composition. Piccolo, Marono, Bovera, Tudisco, Caricato and Nizza (2008) found that the moisture, crude protein and ash contents of sole muscle were not influenced by stocking densities while the lipid content was higher in the low density group. In gilthead sea bream (*Sparus aurata*), lipid contents was significantly influenced by stocking density (Montero, Izquierdo, Tort, Robaina & Vergara 1999). In our research, the total lipid was significantly lower in HSD and MSD groups than that in LSD group. This reduction seems to be related to an increasing lipid mobilization to cope with the increasing energy demand caused by the crowding environment. This result was further backed by the reduction in HSI. Fatty acids play an important role in fish growth and development. A higher level of arachidonic acid (ARA; 20:4n−6) improved fish survival and development (Koven, Barr, Lutzky, Ben-Atia, Weiss, Harel, Behrens & Tandler 2001). In this study, ARA increased in fish in the HSD group. The result was in accordance with the good survival rate of the HSD group. EPA (20:5n-3) and DHA (22:6n-3) are essential fatty acids (EFA) (Sargent, Bell, Bell, Henderson & Tocher 1995; Sargent, Tocher & Bell 2002). DHA deficiency could directly reduce vision development and growth (Mourente, Rodrĩguez, Tocher & Sargent 1993; Wu, Ting & Chen 2002; Glencross, Rutherford & Jones 2011). DHA contents in our research showed a decline in the MSD and HSD groups, the result indicated relatively higher energy utilization at high stocking density.

To some extent, haematological parameters might provide useful information in assessing the pathologies and physiological status (Stoskopf 1993). Leucocyte plays vital roles in the immune response during stress. Leucocyte concentrations increased in common carp (*Cyprinus carpio*) after *Aeromonas hydrophila* infection (Harikrishnan, Nisha-Rani & Balasundaram 2003). In our study, leucocyte count showed no significant alteration under different stocking densities. This suggests Amur sturgeon remains healthy under different stocking densities. However, the erythrocytes and haemoglobin showed a significant increase in the MSD and HSD groups, which was also observed by Barcellos, Luiz, Souza, Rodrigues, Fioreze, Rosmari, Cericato, Soso, Fagundes, Conrad, Lacerda and Terra (2004) in jundiá (*Rhamdia quelen*) subjected to 10 days crowding stress. Dicu, Cristea, Dediu *et al.* (2013b) reported that the haemoglobin level in juvenile stellate sturgeon increased with increasing stocking density. This response may be related to the increase in energy demand imposed by high stocking density.

It has been reported that levels of plasma glucose fluctuated with glycogenolysis (Vijayan & Moon 1992). Amlashi, Falahatkar, Sattari and

Tolouei (2011) observed that the levels of glucose were different before and after stress in great sturgeon (*Huso huso*). In our experiment, glucose showed no significant alteration in the HSD group. Rafatnezhad *et al.* (2008) found that stocking density had no significant effect on glucose concentration in great sturgeon juveniles. This result could be related to the capability of Amur sturgeon to adapt to intensive culture. Serum protein reflects health state and susceptibility to disease (Yin, Lam & Sin 1995). However, we could not detect a significant difference among different stocking densities. It has been reported that high stocking density may lead to an enhanced mobilization of triglycerides, to cope with the stressful condition (Montero *et al.* 1999). Similarly, triglycerides in our study declined in the HSD group suggesting that triglycerides seem to be another energy substrate under stress due to high stocking density. ALT, AST and ALP could reflect liver impairment indirectly (Lemaire, Drai, Mathieu, Lemarie, Carrière, Guidicelli & Lafaurie 1991). Many studies have been reported that stress influenced the activities of enzymes related to cellular energy metabolism (Chatterjee, Pal, Das, Manush, Sarma, Venkateshwarlu & Mukherjee 2006; Sarma, Pal, Sahu, Ayyappan & Baruah 2009). However, unlike other parameters in this study, no significant differences were observed in the activity of these enzymes. This result suggests that these enzymes may not serve as good markers of stress in this species. Urea was related to damage of gill or kidney (Folmar, Gardner, Hickey, Bonomelli & Moody 1993; Adams, Ham, Greeley, LeHew, Hinton & Saylor 1996). Fish could excrete more ammonia as urea by enhancing the ornithine urea cycle (Rasmussen & Korsgaard 1998; Ip, Chew & Randall 2001; Ruyet, Lamers, Roux, Sévére, Boeuf & Mayer-Gostan 2003). In this study, urea in the HSD and MSD groups was significantly higher than that in the LSD group. The increase in blood urea was also in accordance with the ambient ammonia concentration (data not shown).

In conclusion, a range of welfare indicators, consisting of growth performance, body composition, and haematological and biochemical parameters, were used to assess the effects of stocking density on fish welfare. Stocking density influenced performance and welfare of Amur sturgeon. Considering welfare, a low stocking density (initial stocking density = 3.7 kg m^{-3}) resulted in better culture performance. If this also would translate in better profitability still needs to be checked. Further research will be necessary to understand the influence of stocking density on other physiological aspects (genetics, reproduction, etc.) and other size classes of Amur sturgeon.

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