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ORIGINAL ARTICLE

Comparison of serum enzyme activity in great sturgeon, *Huso huso*, cultured in brackish and freshwater earth ponds in Iran

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Abstract Blood samples were collected from 60 great sturgeons, *Huso huso*, to establish the following serum enzyme activity: aspartate amino-transferase (AST), alanine amino-transferase (ALT), lactic acid dehydrogenase (LDH), creatine kinase (CK), and alkaline phosphatase (ALP) using an autoanalyzer, and acid phosphatase (ACP) by manual method. Thirty 5-year-old cultured fish were caught from each of two sites; a brackish-water earth pond in Bafgh and a freshwater pond in Gorgan in the centre and northeast of Iran, during May 2006. Results of the serum enzymes activity for *H. huso* samples from Bafgh and Gorgan were: AST, 502.9±258.2 and 436.1±186.8; ALT, 104.4±35.1 and 53.1±38.7; LDH, 3094.2±1277.5 and 2486.3±1393.3; CK, 3632.9±2618.7 and 3967±5054.9; ALP, 281.2±112.7 and 762.2±600.2; ACP, 13.3±2.5 and 33±6.8 IU/L. Mean values of ALT, ALP and ACP were significantly different in the fish from the two sites ($p<0.05$). These results may be used to understand some biological (e.g., serum enzyme activity) and ecological characteristics of cultured *H. huso.*

Keywords Sturgeon · *Huso huso* · Culture · Serum enzymes · Iran

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Introduction

Sturgeons are very important commercial fish because of their expensive caviar. The Caspian Sea contains large stocks of these fish but some species are becoming extinct, resulting in the culturing of different species of sturgeons in countries neighbouring the Caspian Sea (Bahmani 1998; Kim et al. 2006). The great sturgeon *Huso huso* (Linnaeus 1758) has the most expensive caviar and has been cultured in Iran since 1991 (Yousof-pour 1992). The population numbers of *H. huso* is dependent on several complex factors, such as: conditions for reproduction, abundance and quality of spawners and food supply. In the 1990s, a decline in the spawning population of the Ural River was recorded and by the year 2000, the population consisted of 2,660 individuals. The population within the Volga River also showed an enormous reduction when compared with previous periods; 1971–1975, 20,700 specimens, 2,000 tons and 1991–1995, 8,000 specimens, 500 tons. In the 1980s, the abundance of great sturgeon in the sea was over 18 million individuals, however, this has dropped to 8 million specimens (Bahmani 1996; Kim et al. 2006).

Many investigations concerning the physiological aspects of sturgeon species have been carried out (Slynko 1976; Metallov and Aksenov 1999; Shahsavani et al. 1999, 2001; Ghorbanianfar 2003; Falahatkar et al. 2005; Shirazian 2005; Taghvaiimoghaddam 2005; Rajabipour 2006; Khoshbavar Rostami et al. 2006; Asadi et al. 2006).

Haematological tests and analyses of serum constituents have proved useful in the detection and diagnosis of metabolic disturbances and disease processes. The lack of physiological values for various blood parameters prevents their use in assessing the condition of the aquatic environments and ecosystems (Çelik 2004).

The purpose of this study was to establish serum enzyme activity to provide baseline data to detect differences between the great sturgeon, *H. huso* cultured in brackish and freshwater as two different ecological conditions in Iran.

Materials and methods

Experimental design Sixty great sturgeon, *H. huso* fish were collected during May 2006 using a gill net from earth ponds at two stations: a fisheries research farm in Bafgh (brackish water) and Shahid Marjani fish aquaculture centre in Gorgan (freshwater), situated in the centre and northeast of Iran. All fish were initially hatched in the same hatchery at Shahid Marjani from eggs from a single individual, but were cultured in two different areas for 5 years. Following the catch, total length and body weight were measured and recorded, as was water temperature, salinity, hardness, pH level and dissolved oxygen.

Blood collection Thirty fish in each site were handled carefully to reduce any possibilities of stress. Blood sampling was performed immediately after the fish were captured. Samples were collected from behind the anal fin using a 5-mL plastic syringe and 22 gauge needle. The blood samples, approximately 2.5 ml were placed into dry plastic tubes and immediately centrifuged at 4,000 rpm for 10 min (Çelik 2004; Casillas et al. 1983).

Biochemical analyses Separated serum samples were analyzed for aspartate amino transferase (AST), alanine amino transferase (ALT), lactic acid dehydrogenase (LDH), creatine kinase (CK) and alkaline phosphatase (ALP) by Cobas Mira 27-6764 auto-analyzer using enzymatic procedures with a diagnostic kit (Pars Azmoon Chemical Co.). Serum enzyme activity for acid phosphatase (ACP) was undertaken using the manual method. Biochemical measurements were carried out 1 h after sample collection. Sample collection and biochemical analyses were performed using the methods derived from several different researchers (Anderson and Anderson 2002; Çelik 2004; Cech et al. 2000; Shalaby 2005; Pepys et al. 1978).

Statistical methods Mean±SD, variance, standard error, median, range, quartile, minimum and maximum values were determined for biometric indices and each of the serum enzyme activities using a SPSS ver.11.5/2002 system statistical package. Leven's test was used for scaling the equality of variances. Mean values were compared by *t* test. Two-tailed Pearson's correlation between each biometric and biochemical serum parameter against each other were measured. Differences were considered to be significant for p values less than 0.05.

Results

The water quality of the culture ponds in Bafgh and Gorgan were as follows: temperature 26°C and 23°C, salinity 10 ppm and 1 ppm, pH 8.2 and pH 8.0, dissolved oxygen 6.3 ppm and 6 ppm and hardness 15,530 μm/cm and 100 μm/cm, respectively. Mean values of the total length for the great sturgeon, *H. huso* from Bafgh and Gorgan was 121.7±9.3 (96–139)cm and 125.7±13.2 (100–150)cm, respectively, their average body weight was 10,952.8±4,880.4 g (4,200–20,380) and 12,350±3,436.9 g (8,000–19,000). There was no significant difference in either total length (p=0.184) or body weight (p=0.211) between the two sites.

Mean±S.D values of the serum enzyme activity for *H. huso* samples from Bafgh and Gorgan were as follows:

AST, 502.9±258.2 (99–1,214) IU/L and 436.1±186.8 (183.9–800.7) IU/L; ALT, 104.4±35.1 (39–192) IU/L and 53.1±38.7 (8.1–163.8) IU/L; LDH, 3,094.2±1277.5 (1,106–5,773) IU/L and 2,486.3±1,393.3 (724–5,001) IU/L; CK, 3,632.9±2,618.7 (470–10,589) IU/L and 3,967±5,054.9 (531–23,060) IU/L; ALP, 281.2±112.7 (100–535) IU/L and 762.2±600.2 (117–2097) IU/L; ACP, 13.3±2.5 (8–20.5) IU/L and 33±6.8 (21–49.2) IU/L (see Table 1).

Mean values for ALT, ALP and ACP were significantly different in the fish from the two sites (p<0.0001), but there were no significant differences between AST (p=0.225), LDH (p=0.083) and CK (p=0.749) between the two sites.

Two-tailed Pearson-correlation showed significant direct correlation between total length with body weight of the *H. huso* (p=0.0005); between ALT with AST (p=0.04), LDH (p=0.0005) and CK (p=0.0005); between LDH with CK (p=0.006) and ALP (p=0.003); CK with ALP (p=0.0005) and ALP with ACP (p=0.0005). Significant indirect correlation was obtained between total fish length and ALT (p=0.001), LDH (p=0.02), CK (p=0.0005) and ALP (p=0.04); between body weight and ALT (p=0.0005), LDH (p=0.003) and CK (p=0.0005); and between ALT with ALP (p=0.0005) as well (Table 1).

Discussion

The mean level of ALT in blood serum in *H. huso* samples from Bafgh showed a significant twofold increase when compared with samples from Gorgan (p<0.0001), suggest-

Table 1 Pearson correlation and *p* value between total length, body weight and serum enzymes of *H. huso*

		TL	BW	AST	ALT	LDH	CK	ALP
ACP	Cor.	0.14	0.22	-0.05	*-0.56*	-0.16	0.03	*0.45*
	p	0.27	0.10	0.69	<0.01	0.24	0.82	<0.01
ALP	Cor.	-0.27	-0.21	-0.01	0.18	*0.38*	*0.47*	
	p	0.04	0.10	0.97	0.17	<0.01	<0.01	
CK	Cor.	*-0.52*	*-0.47*	0.01	*0.48*	*0.35*		
	p	<0.01	<0.01	0.92	<0.01	0.01		
LDH	Cor.	-0.31	*-0.38*	0.19	*0.63*			
	p	0.02	<0.01	0.14	<0.01			
ALT	Cor.	*-0.41*	*-0.48*	0.27				
	p	<0.01	<0.01	0.04				
AST	Cor.	0.22	0.23					
	p	0.09	0.08					
BW	Cor.	*0.76*						
	p	<0.01						

ing that liver disorders may be more noticeable in cultured fish from this area. Imprecise feeding management may affect the activity of ALT in serum (Cech et al. 2000), also higher water salinity of pond culture in the Bafgh area may lead to an increase in the serum ALT level as previously shown by Shalaby (2005) in the Nile tilapia, *Oreochromis niloticus*. Androgen hormone levels may be higher in great sturgeons in Bafgh, as shown by Casillas et al. (1983) in the English sole, *Parophrys notopterus notopterus*. Increased maturation is highly favoured in commercial fisheries for the acquisition of caviar. Indirect significant correlations were observed between ALT and total length and body weight for *H. huso* samples. Low ALT activity was recorded in large *H. huso* sturgeon from the Caspian Sea (Taghvaiimoghaddam 2005). ALT is the optimum serum factor for the prognoses of liver disorders (Shalaby 2005).

High AST activity in samples from Bafgh and Gorgan, suggests more investigation into the concentration of heavy metals in the earth ponds water is required. Many metals, whether organically complex or not, are known to accumulate in plant and animal tissues to a very high level, posing a potential toxic hazard to the organisms themselves, or organisms higher in the food chain, including humans (Abel 1998). Earth ponds for culturing sturgeon are close to different mines in Bafgh and near to rice fields and habitable areas in Gorgan, indicating that the viral infections of the fish may be similar at the two fish culture centres (Kaneko1980) due to the similarity of AST activity between the two sites.

The serum activity of ALT and AST obviously varies depending on fish species (Shalaby 2005). The increase in plasma AST and ALT may be connected to stress conditions, hepatocellular damages or cellular degradation due to heavy metals, perhaps in the liver, heart or muscle (Yokoyama et al. 2003; Alter and Tokars 2001; Yamawaki et al. 1986).

Infections cause an increase in both ALT and LDH (Smith 1987). ALT was significantly different in the examined fish from the two areas, however, LDH activity in the fish from Bafgh was not much higher than Gorgan ($p>0.05$), therefore, infection may not be present in these samples. LDH levels correlate directly with growth rate (Khajali and Qujeq 2005; Hart and Reynolds 2002). The activity of this enzyme in muscle and liver tissue of *ascipenserids* were recorded as being similar to other teleosts (Metallov and Aksenov 1999).

Muscle density and damage are probably similar at the two centres since no significant difference in CK levels ($p>0.05$) was found but levels are probably less than the large great sturgeons from the Caspian Sea (Taghvaiimoghaddam 2005). Together, the increase in CK and ALT/AST show a probable decrease in protein catabolism (Cech et al. 2000).

There were significantly higher levels of ALP in fish from both sites compared to fish from the sea (Taghvaiimoghaddam 2005) and with values close to the common carp (Peyghan et al. 2003). The activity of this enzyme is normally higher in young vertebrates (Cech et al. 2000), suggesting that ALP activity may differ in fish by age and size (Trivedi et al. 2001; Das 1966). Although the examined fish were of the same age, an indirect significant correlation was observed between ALP activity and the size of the fish. Increase in ALP may be caused by the obstruction of the biliary canal or osteoblast activity and feeding conditions (Moraes et al. 2005; Cech et al. 2000). Higher levels of ALP and similar levels of AST, LDH and CK were observed in *H. huso* samples from Gorgan compared with Bafgh. Similar findings have been previously reported in canal catfish compared with pond-cultured samples (Warner and Williams 1977).

ACP was significantly higher in fish from Gorgan ($p<0.05$), indicating that phosphatase esters may be hydrolyzed

faster in these samples. Heavy metal pollution especially, cadmium (Shalaby 2006) and mercury (Hossain and Dutta 1986) may be noticeable as well as some viral infections, for example *Myxobolus cerebralis*. Lysosomal proliferation and cellular damage (Moraes et al. 2005), plus disorders of calcium and calcitonin (Suzuki 2005) may also be present. Increased water hardness in Bafgh (Mashaii 2006) may cause a decrease in ACP and ALP following an increase in plasma calcitonin levels (Mukerjee et al. 2004; Chen 2003).

Although high variances are expected in serum enzyme measurements (Folmar et al. 1992, 1993), variations between blood chemistry parameters among fish species depend on the sampling technique, analysis methods, age, habitat and diet (Çelik 2004). This study gives valuable information on the health of an economically important fish species; the great sturgeon. The result of this study may allow us to understand biological and ecological characteristics of cultured *H. huso*.

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