Comparative Study of Biochemical and Hematological Features in Reared Sturgeons

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Abstract: Suitable physiological condition in fishes for the selection of broodfishes can be achieved through hematological studies. The present study aimed to examin the hematological indices in Juvenile Sturgeons at different stages of growth. Total albumin content in blood serum, hemoglobin concentration, the erythrocyte and leukocyte count of 54 sturgeon specimens (Persian sturgeon *Acipenser persicus* and Great sturgeon *Huso huso*) were determined at the age of 1, 2 and 6 years reared fish.

In the Persian sturgeon specimens studied, total albumin concentration increased from 26.1 to 52.5 g L^{-1}, hemoglobin from 42.2 to 65.8 g L^{-1}, erythrocyte count from 240 to 452.5 thousand cells/μL^{-1}, and leukocyte count from 17.4 to 46.7 thousand cells/μL^{-1}. The total leukocyte was composed of 73.2-82.7 % lymphocytes, 12.3 to 20 % neutrophils, 2.2 to 6.5 % eosinophils and 0.2 to 2.5 % monocytes with the increase of age.

In the great sturgeon specimens studied, total albumin concentrations in blood increased from 20 to 42.4 g L^{-1}, hemoglobin from 46.3 to 78.6 g L^{-1}, and erythrocyte count from 495 to 735 thousand cells μL^{-1}. The total leukocyte was composed of 54.5 to 67.5 % lymphocytes, 6.1 to 26.9 % neutrophils, 6.6 to 13.25 % eosinophils and 0.6 to 2.5 % monocytes with the increase of age.

This research revealed the evolutionary trends of hematological features in the natural growth and development of sturgeons in the South Caspian Sea, reared in artificial conditions and shows that with the increase in age, the hematological indices also increased.

KEY WORDS: Biochemical, Hematological, Juvenile sturgeon, Rearing Condition

Introduction

Sturgeon fishes are anadromous and potomodromous species of the Northern Hemisphere. The history of which goes back to 100 million years regarding evolution and includes 28 species of which 6 species inhabit the Caspian basin (Bahmani, 1998).

Blood is an important index of the physiological condition of organs in diagnosing diseases and controlling the biological trends in living organisms, including fishes. Significance of hematological studies as a valuable tool to achieve suitable physiological condition in fishes for the selection of broodfishes has been emphasised. The study of the quantitative features in blood of fish serves as a reliable index of their physiological conditions. This is especially important clues for sturgeon aquaculture practices (Alyakrinskaya & Dolgova, 1984). Analysis of blood indices is a valuable guide in assessing the condition of aquatic organisms. For example in response to stress, pollution, nutrition and ecological as well as physiological conditions, major changes have been brought on the fish blood composition such as fluctuations in the levels of hormones, proteins, serum glucose, cholesterol and other basic components. Cytological studies have also revealed variations in the levels of red and white blood cells. The foundations of fish haematology were established at the beginning of the 19th century. In 1902 Meinertz studied the characteristics of leukocytes in reptiles, invertebrates and fish. In this work leukocytes were illustrated with 177 dyes and each was given a different name (Stoskopf, 1993a). Hematology in bony fish was in its early stages in 1976 (Williams & Warner, 1976). Hematological studies on sturgeons is a very new science and a few reports in this regard are available (Alyakrinskaya & Dolgova, 1984 ; Amini *et al.*, 1997 ; Bahmani *et al.*, 1997 ; Clementi *et al.*, 1997 ; Domezain *et al.*, 1997 ; Pourkazemi *et al.*,1997 ; Saiedi *et al.*, 1997 and Bahmani *et al.*, 1999). The fact that the surrounding conditions of fish influence the metabolic content of blood indices (Bullivs, 1993), The blood indices of juvenile reared sturgeons (Persian sturgeon *Acipenser persicus,* and Great sturgeon *Huso huso)*, at age 1,2 and 6 years old were studied.

This paper comprises the qualitative assessment of sturgeons of different age groups reared under artificial conditions (at the Shahid Dr. Beheshti fish propagation and rearing center), It was conducted jointly with the researchers of the KaspNIRKH institute, Russia at the International Sturgeon Research Institute in Iran.

Materials and Methods

In this study, 54 sturgeon specimens at age 1 (10 Persian sturgeon, 10 great sturgeon), 2nd (10 Persian sturgeon, 10 great sturgeon) and 6th years old (5 Persian sturgeon, 9 great sturgeon), were analysed hematologically. Two milliliters of blood were collected from the caudal vein of juvenile fish. The studied fish belonged to the batch of fish produced during 1990, 1995 and 1996, which either reared and maintained in earthen ponds or rearing tanks by the late Dr. Yousefpour (for the first time in Iran). Fish were fed with a combination of live and artificial foods (during the early stages of growth) and entirely on artificial foods (during the later stages of growth) in same hydrobiological conditions.

On the basis of the age group, the sex of the reared fish were determined through histological studies using the hematoxillin-Eosin (H & E) staining method (Hung *et al.*, 1990; Bahmani & Kazemi, 1998). The hemoglobin concentration in the blood was determined by using a CECIL 1010 spectrophotmeter at wave length of 560 nm. The total albumin content in blood was determined using a CECIL 2040 spectrophotometer at wave length of 260-280 nm. The number of erythrocytes was counted using a hemacytometer and finally leukocytes were counted by staining them with Giemsa stain (2 %).

Results

The values of blood indices in juvenile reared sturgeons are shown in tables 2, 3, 4 and 5.

Table 1. Average length, weight and condition factor of fishes under study

No.	Species and age	Weight (g)	Length (cm)	Condition factor
1	*Huso huso*, 1 year old	741.0	58.21	0.40
2	*Huso huso*, 2 year old	2377.0	80.40	0.41
3	*Huso huso*, 6 year old	14135.7	139.07	0.51
4	*Acipenser persicus*, 1 year old	190.0	42.36	0.27
5	*Acipenser persicus*, 2 year old	1196.5	71.18	0.32
6	*Acipenser persicus*, 6 year old	3040.0	87.90	0.44

Persian sturgeons:

The study on the changes in blood composition in the Persian sturgeon was undertaken on the basis of age, for total albumin, haemoglobin concentration, erythrocyte and leukocyte count.

The results obtained for 1, 2 and 6 years old specimens are shown in table 2. These results indicate that the blood indices for 1 year old Persian sturgeon are lower in contrast to those of the 2 and 6 years old specimens. Also the total albumin content in blood and haemoglobin concentration of 6 years old Persian sturgeon specimens maintained in ponds were below those of the same age group reared in tanks (Table 3).

Great Sturgeon:

The results obtained from the haematological study on 1, 2 and 6 years old great sturgeons are shown in table 4.

The data obtained in this study indicate an increase in the values of blood indices in the 6 years old specimens. The study of haematological illustrations of 6 years old great sturgeon maintained at different ecological conditions indicate a tendency for decrease in values of total albumin content by 17 %, haemoglobin concentration by 35 %, erythrocyte count by 30 % and leukocyte count by 45 % in the group reared in ponds in contrast to the group reared in tanks (Table 5). The data obtained also show that the values of blood indices analyzed in 2 years old great sturgeon were 11-12 % lower when compared to those of 6 years old.

The variations in the blood indices depend on age as well as on the ecological and physiological requirements of sturgeons (Fig. 1-10).

Table 2. Values of blood indices in Persian sturgeon *Acipenser persicus* on the basis of age and sex

Blood Index	Unit	1 year old			2 years old		3 years old	
		female	male	undifferentiated	female	male	female	male
Blood albumin	gL^{-1}	28.45	28.65	26.10	42.30	43.20	49.70	49.40
Hemoglobin	gL^{-1}	42.20	44.5	48.75	46.80	51.0	64.40	59.50
Erythrocyte	thousand cells µL^{-1}	240.0	340.0	358.3	355.0	365.0	452.5	398.3
Leukocyte	thousand cellsµL^{-1}	13.43	18.89	18.25	30.90	30.90	46.4	26.6
Neutrophill	percent	17.75	20.0	18.25	18.40	17.58	13.80	16.3
Eosinophil	percent	6.0	6.5	4.42	2.23	3.17	4.0	3.80
Lymphocyte	percent	73.75	73.25	76.33	78.50	78.0	81.0	79.8
Monocyte	percent	2.50	0.25	1.0	0.87	1.25	1.20	0.20

Table 3. Average values of blood indices in reared Persian sturgeon *Acpenser persiecus* at different ages

Blood index	Unit	1 year old	2 years old	6 years old	
				pond	tank
Blood albumin	g L^{-1}	27.73	42.75	46.8	52.5
Hemoglobin	g L^{-1}	45.15	48.90	58.3	65.8
Erythrocyte	thousand cells µL^{-1}	312.77	360.0	451.5	387.5
Neutrophil	percent	18.66	17.99	12.3	16.8
Eosinophil	percent	5.64	2.7	4.7	4.4
Lymphocyte	percent	74.45	78.25	77.5	82.7
Monocyte	percent	1.25	1.06	1.0	0.6

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Table 4. Values of blood indices in Great sturgeon *Huso huso* on the basis of age and sex

Blood Index	Unit	1 year old			2 years old		3 years old	
		female	male	undifferentiated	female	male	female	male
Blood albumin	gL^{-1}	31.95	31.15	38.40	31.4	20.05	38.7	36.1
Hemoglobin	gL^{-1}	57.00	55.1	47.8	46.3	53.5	57.9	70.2
Erythrocyte	thousand cells μL^{-1}	643.3	603.6	495.0	530.0	518.8	574.0	655.0
Leukocyte	thousand cells μL^{-1}	66.74	55.32	43.46	57.77	41.37	31.60	32.20
Neutrophill	percent	30.33	33.86	28.85	26.0	22.63	22.8	27.1
Eosinophil	percent	13.17	11.0	8.8	10.0	7.88	9.1	6.75
Lymphocyte	percent	55.67	54.55	60.3	61.5	67.75	67.2	64.90
Monocyte	percent	0.83	0.64	2.1	2.5	1.74	0.90	1.25

Table 5. Average values of blood indices in reared Great sturgeon *Huso huso* at different ages

Blood index	Unit	1 year old	2 years old	6 years old	
				pond	tank
Blood albumin	g L^{-1}	31.50	29.95	33.8	42.2
Hemoglobin	g L^{-1}	56.05	49.2	51.2	78.6
Erythrocyte	thousand cells μL^{-1}	623.45	514.6	510.0	735.0
Neutrophil	percent	32.09	25.81	26.9	22.2
Eosinophil	percent	12.08	8.92	6.6	9.9
Lymphocyte	percent	55.08	63.15	65.1	67.5
Monocyte	percent	0.75	2.12	1.4	0.6

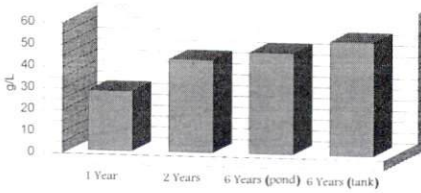**Fig. 1** Average values of albumin content in reared Persian sturgeon

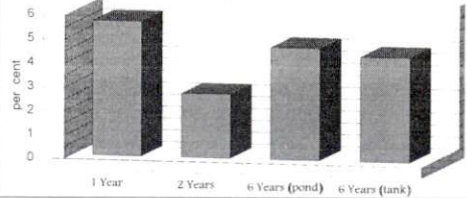**Fig. 5** Average values of eosinophil in reared Persian sturgeon

Fig. 2 Average values hemoglobin cocentration in reared Persian sturgeon
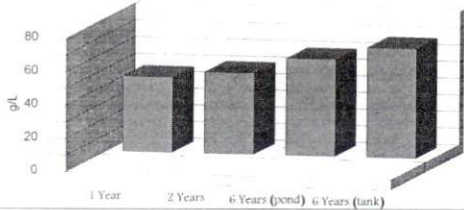
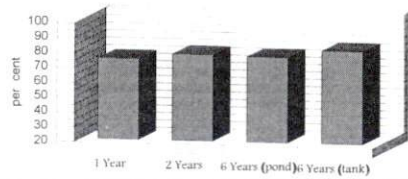**Fig. 6** Average values of lymphocyte in reared Persian sturgeon

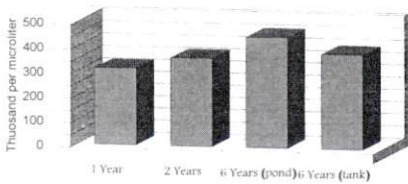**Fig. 3** Average values of erythrocyte count in reared Persian sturgeon
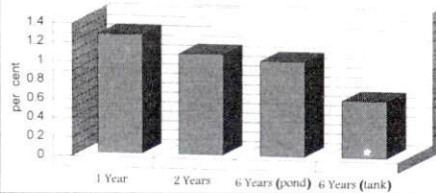
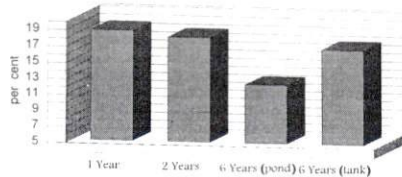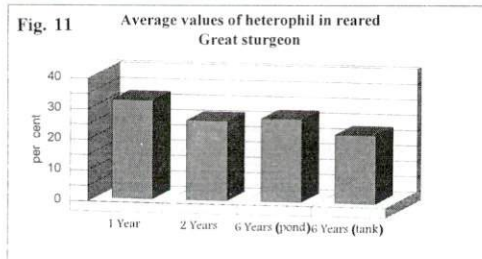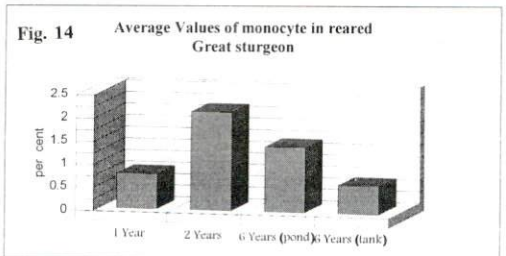**Fig. 7** Average values of monocyte in reared Persian sturgeon

Fig. 4 Average values of heterophil in reared Persian sturgeon

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Fig. 8 — Average values of albumin content in blood in reared Great sturgeon

Fig. 12 — Average values of eosinophil in reared Great sturgeon

Fig. 9 — Average values of hemoglbin concentration in reared Great sturgeon

Fig. 13 — Average values of lymphocyte in reared Great sturgeon

Fig. 10 — Average values of erythrocyte count in reared Great sturgeon

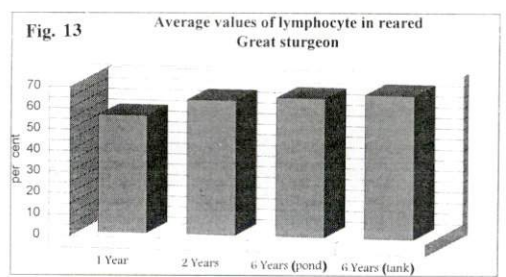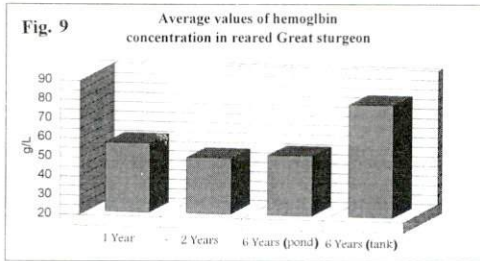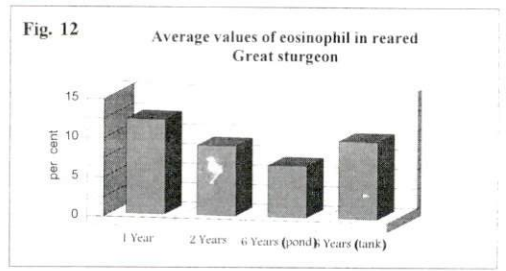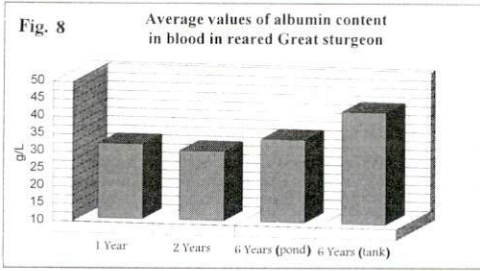**Fig. 14** — Average Values of monocyte in reared Great sturgeon

Fig. 11 — Average values of heterophil in reared Great sturgeon

Discussion

Sakalov and Geribova (1972) were of the opinion that hematological factors are the best means to study the equilibrium state of living organisms with their surrounding environment. In order to understand the hematological condition of fish in a particular habitat, it is necessary to examine the diversity of blood cells (red and white) in different rearing conditions on the basis of species, age, season and changes in the blood indices during the outcome of diseases (Glovina and Trombitski. 1989). The living environment of fish and the conditions governing them such as temperature, nutrition, pollution and fishery activities strongly influence the content of metabolites and blood cells (Bullis, 1993). Considering that hematology is a valuable tool to diagnose diseases in fish (Stoskopf, 1993b), and keeping in view the significance of such studies on sturgeons particularly the Persian and Great sturgeon in different rearing conditions and age groups for the production of broodstocks and marketing purposes, the present study was undertaken. Alyakrinskyaya and Dolgova (1984) pointed out the importance of hematological studies on sturgeons from the evolutionary point of view, and also as an important factor in determining and controlling the physiological condition of juvenile fish in rearing conditions. They are of the opinion that the characteristics of blood in juvenile sturgeons are fairly close to one another. In the first year of life a large percent of blood is composed of hemoglobin. With the increase in age this percent first shows a tendency to decrease and then increases again. These variations also depend on the ecological and physiological requirements of sturgeons (Clementi et al., 1997).The present study also supports this view. Our data show that the total albumin content of blood, hemoglobin concentration and erythrocyte count in the great sturgeons under study decreased in the 2 years old individuals as compared to those in the 1 years old and again increased in the 6 years old individuals. It's said that the total protein and haemoglobin concentration in blood increased with age and also the erythrocyte count in fish blood decreases in response to stress (Martem'Yanov, 1995).

Juvenile sturgeon on the whole have a higher amount of hemoglobin in contrast to other fish. Comparative analysis of the data in hand shows a significant variability in certain values with respect to different rearing conditions. It is known that unusual high or low temperatures, insufficient food or decrease in dissolved oxygen concentrations in water have adverse effects on the values of blood indices (Alyakrinskyaya & Dolgova, 1984 ; Wedemeyer & Yasutake, 1994).

For example the erythrocyte count is clearly influenced by fluctuations in water temperature, whereas low oxygen concentrations in water results in the increase in the number of erythrocytes (Bonnet, 1929; Dewilde & Houton, 1967 cited in: Williams and Warner, 1976). Food quality (Domezain *et al.*, 1997) also strongly affects the morphological characteristics of blood and the qualitative and quantitative properties of hemoglobin in juvenile sturgeons. Anemia was caused in juvenile sturgeons given a diet of oligochaete (Alyakrinskyaya & Dolgova, 1984).

Studies conducted by Amini *et al.*, (1997) on persian sturgeon broodfish show that average values of albumin concentration in blood was 32.1 g L^{-1} in females and 29.1 g L^{-1} in males. Average values of hemoglobin in females was 84.6 g L^{-1} and in males 110.5 g L^{-1}. The erythrocyte count in females and males was 479.8 and 485 thousand cells/μL^{-1} and leukocyte count was 22.6 and 19.5 thousand cells/μL^{-1} in females and males, respectively. Results obtained from the studies conducted by Bahmani *et al.* (1997) also indicate that under artificial rearing conditions, the evolutionary trends of the hematological system exhibited by the sturgeons of the southern basin of the Caspian Sea, follow a natural course of growth and development. The investigations conducted by Pourkazemi *et al.*, (1997) on stellate sturgeon (*A. stellatus*) broodfish show average values of blood albumin and hemoglobin as 24 g L^{-1} and 69 g L^{-1}, respectively. Saeidi *et al.*, (1998) in their studies on Persian sturgeon showed that the values of hematological indices of juveniles at different temperatures were similar, whereas in adult specimens these values were higher. Lymphocytes and neutrophils constituted the maximum percent of leukocytes in these fish. This has been demonstrated for other fish too (Williams and Warner, 1976). Fluctuations in lymphocyte levels in fish in response to stress and corticosteroid hormones is similar to that in mammals (Ellis, 1989). The neutrophil levels also increase in response to stress, bacterial and protozoal infections and inflammations (Stoskopf, 1993a). According to the early investigations conducted by Jakowska, the eosinophil content also increases during bacterial infections. In fish, the normal eosinophil content is 2 - 3 % and maximum 10 % of the total leukocyte count (Stoskopf, 1993a). Our data show eosinophil values of 2.23-13.17 %. The presence of 13.17 % eosinophil in 1 year old female great sturgeons revealed in this study is beyond the range mentioned above. Besides eosinophil content also increases as a result of environmental stress (Gardner & Yevich, 1969, cited in: Williams and Warner, 1976). The decrease in neutrophil and eosinophil values with increase in

age observed in this study are indicative of normal rearing conditions and the absence of disease and stress in fish. Monocytes have not been observed in most of the hematological studies conducted on fish (Catton, 1951, Watson et al., 1956; Sabnis & Rangnekar, 1962; McKnight, 1966; Saunders, 1988; and Blaxhall & Daisly, 1973 cited in Williams & Warner, 1976). However, Gluckman and Gardon and also Lieb et al., have documented the presence of monocytes in fish blood. Monocytes are the largest granules in blood. Their values reported in the present study are negligible and range between 0.2-2.5 % of the total leukocyte.

Results obtained from the studies conducted by Bahmani et al. (1999) also indicate that in Persian sturgeon broodfish caught in the southern region of the Caspian Sea, lymphocyte, neutrophil and eosinophil values fluctuated from 5-88%, 8-95% and 0-24%, respectively during catch, transport, handling and confinement of these fishes for artificial propagation.

Sturgeons have a broad ecological niche and exhibit a wide range in the composition of blood (Alyakriskyaya and Dolgova, 1984). Thus fish in this study were reared under identical rearing conditions, showed similar variations in blood indices. However decrease in values of blood indices in 6 years old sturgeons reared in ponds in contrast to those reared in tanks, indicate suitable rearing conditions in respect to food availability, sufficient oxygen and control of sanitary conditions in the fiber glass tanks.

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