Hematological characteristics and reproduction indices of wild beluga (*Huso huso*) broodstocks from the southeast of the Caspian Sea

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Abstract:

BACKGROUND: Hematological studies are valuable diagnostic techniques in the medical care of fish, reflecting the health condition and the effects of environmental factors on fish. **OBJECTIVES:** Due to the high value of sturgeon fish and necessity to recover the brood stock, this study was done to provide data about hematological parameters and reproduction normative of wild brood stock beluga, *Huso huso*, from the southeast of the Caspian Sea. **METHODS:** Hematological parameters including RBC, WBC, hematocrit, hemoglobin, MCV, MCH, MCHC, and differential leukocyte count were determined. In this regard, 7 male and 7 female wild broods were studied. Differences in mentioned parameters between genders were evaluated. The correlations among parameters were also determined. RESULTS: According to the results, the male fish had significantly higher WBC and lymphocyte and lower neutrophil compared to the females. Also, functional fecundity was 447000 ± 157210 and weight of extracted oocytes was 35.4 ± 3.78 mg/cell. The oocytes' long diameter was 4.17 ± 0.21mm and width diameter was 3.75 ± 0.19 mm. Egg fertilization rate was 46.7 ± 24.33%, and larvae weight was 25.48 ± 1.56 mg. **CONCLUSIONS:** Hematological parameters of juvenile and fingerling reared beluga showed wide ranges, and there are differences in hematological parameters between reared and wild broodstock belugas. These findings highlight the importance of hematological study in wild beluga sturgeon.

Introduction

Sturgeons are anadromous and potamodromous species, living in the northern hemisphere. Their evolutionary history goes back to 100 million years ago. There are six sturgeon species inhabiting in the Caspian Sea basin, and beluga, *Huso huso*, is one of the most valuable species in the Caspian Sea. This species is included as endangered species in IUCN Red List and the CIIES (1997) appendices. Thus, to restore its population in the Caspian Sea, Iranian Fisheries Organization has tried to re- lease beluga fingerlings to the sea. The fingerlings are from artificial propagation of both wild- caught and reared broods. The reared broods are cultured under artificial conditions that are different from that of the wild. They are fed by artificial diet, which is different quantitatively and qualitatively from natural foods. Therefore, it is supposed that wild fish have different physiological characteristics compared to reared ones.

Because of the high value of caviar and meat of sturgeons, conservation of this species is necessary; therefore, information on clinical and preclinical conditions can be used for health management purposes. Hematologic data are not easy to use in fish health monitoring because of the difficulty of obtaining samples, the challenges involved in evaluating hemograms, and the lack of meaningful reference intervals to aid in interpretation (Clauss et al., 2008). Such problems are more pronounced in sturgeons because of large body size and low number of available specimens. Therefore, hematological studies are valuable diagnostic techniques in the medical care of these fish; they can reflect health condition of the fish and the effect of environmental factors on them (Bahmani et al., 2001). These parameters can be used to evaluate the effect of different stressors such as hypercapnia, hypoxia, and water pollutants (ammonia, nitrite and heavy metals) on the fish (Barton, 2002; Wendelaar Bonga, 1997). On the other hand, hematological parameters may be sex-dependent (Hickey, 1982; Murray, 1984). Thus, these parameters should be studied in each sex, separately. There are few studies on broodstock and premature beluga hematological reference interval, and just reared fish at different ages (most in juvenile ages) were studied (Ahmdifar et al., 2001; Bahmani et al., 2001; Ghomi et al., 2011; Khajepour et al., 2011; Mohammadi Zarajabad et al., 2009; Yousefi et al., 2012; Akrami et al., 2013). Hence, it is necessary to monitor hematological parameters of beluga for artificial propagation and the restocking program. The present study aimed to investigate the hematological characteristics of wild-caught beluga, separately for each gender along with their artificial reproduction indices.

Materials and Methods

Subjects: In this study, 7 wild male and 7 wild female beluga (broodstock) were caught from the southeastern part of the Caspian Sea by gill net (it was difficult to collect more samples because of low quantity of wild sturgeon in the Caspian Sea). The fish were transferred to propagation center and were kept in circular concrete pool for 3 days. Water dissolved oxygen was 5.8 ± 1 ppm. The fish were monitored for abnormality, and healthy fish were selected for examination (all fish were ready to spawning) and sampling was done before propagation activity.

Sampling and hematological study: The fish were anesthetized with 150 ppm clove oil before sampling. Blood samples were taken by heparin-coated 10 ml syringe (21 gauge needle) from caudal vein for hematological analysis. Blood samples were transferred to lab on ice bag for hematological analysis.

Red blood cell (RBC) and white blood cell (WBC) were counted using Neubaeur hemocytometer in standard techniques. RBC (×106/mm3) and leukocytes (in 1mm3) were enumerated with 1:100 dilution in Dacie’s fluid (Blaxhall and Daisley 1973; Dacie and Lewis, 2001). Hematocrit (Hct) was determined by the standard microhematocrit method and expressed as percentages (Sniesz-ko 1960). The amount of hemoglobin (Hb) was determined according to cyano_methemoglobin procedure using available commercial kit (Pars Azmun Co. Tehran, Iran). Hb content was expressed as milligram per deciliter (Drabkin and Austin, 1935). Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) were calculated based on the method described by Dacie and Lewis (2001). MCV was expressed in femtoliters (fl), MCH value was given in picogram (pg), and MCHC was expressed in grams per deciliter. Blood smears were dried at the room temperature, fixed with absolute methanol and stained with Wright–Giemsa solution. Afterward, 200 leukocytes were determined and differential leukocyte counts were recorded as percentage. Cells were identified on the basis of morphology and cell structure (Rowley 1990).

Reproduction normative: After biometric activity on breeders, functional fecundity was estimated by gravimetric method as explained by Biswas (1993). Fertilization rate of spawn in each female was determined by visual counting of the number of fertilized10 grams of eggs in Petri dish under microscope. To determine spawns' diameter, 100 spawns were sampled and the largest and smallest diameter of the eggs were measured. To evaluate the weight of spawn and hatched larvae, 10 grams of spawn or larvae were sampled and the numbers of specimens were counted.

Statistical analyses: Data were analyzed using SPSS software version No. 16. The normality of distribution was tested with the Kolmogorov– Smirnov test, and t-test was used to determine the presumptive differences between the sexes depending on the mentioned variables. Pearson's correlation coefficient was applied to show correlation between the variables. $p<0.05$ was considered significant. Data were presented as mean ± SD.

Results

Results of the hematological parameters of the wild great sturgeon for males and females are presented in Table1. According to the results, the females had significantly lower WBC (24900 ± 547 vs. 23042 ± 1375), lymphocyte percentage (72.80 ± 1.10 vs. 68.40 ± 1.14) and higher neutrophil percentage (21.40 ± 1.14 vs. 18.60 ± 0.55) compared to the males. There was no significant difference in the other hematological parameters (Hb, Hct, RBC, MCV, MCH, MCHC, eosinophil and neutrophil) between the wild male and female great sturgeon broodstocks (Table 1). Furthermore, regarding the correlation between hematological parameters, illustrated in Table 2, there was significant correlation between MCV and MCH, hematocrit and monocyte, Hct and eosinophil ($0.01 \leq p \leq 0.05$), Hb and eosinophil, lymphocyte and neutrophil ($p<0.01$) (Table 2).

Artificial reproduction indices in the studied wild beluga broodstocks are shown in Table 3. According to the results, functional fecundity for female beluga was 447000 ± 157210 and weight of extracted spawn was 35.4 ± 3.78 mg/cell. The oocyte long diameter was 4.17 ± 0.21 mm and width diameter was 3.75 ± 0.19 mm. Egg fertilization rate was 46.7 ± 24.33%. Larvae weight was 25.48 ± 1.56 mg.

Discussion

Hematologic evaluation can be useful in monitoring the health status of fish, especially if there are standard values of blood parameters. However, there are a lot of factors affecting these parameters such as stress from capture and sample collections, genetic variation, age, gender, water quality, season, and physiological stages (such as spawning) (Knowles et al., 2006). Unfortunately, there is no study reporting the hematological values of wild broodstock beluga. On the other hand, there are some studies on the hematology of beluga, but all of these studies focused on juvenile and fingerling fish and reported wide variation in hematological parameters (Bahmani et al., 2001; Mohammadi Zarejabad et al., 2009; Ahmadifar et al., 2011; Khajepour et al., 2009). These finding more and less are different compared to wild fish, for example Akrami et al. (2013) evaluated the hematological parameters of 4, 6, 7, and 8 years old reared great sturgeon and recorded MCV and MCH values for reared fish, significantly less than this study records.Today, propagation and restocks centers of sturgeon in Iran are going to save and reuse sturgeons' broodstocks by micro-caesar-

Table 1. Difference in hematological characteristics of wild male and female *H. huso*. ($p<0.05$; t-test; n=7 male and 7 female). (*) Significant at $0.01 \leq p \leq 0.05$ level (2- tailed). (**) Significant at 0.01 level (2 -tailed).

	Males	Females	P value
WBC (cell/µl)	24900 ± 547	23042 ± 1375	0.023
RBC (× 10^6 cell/µl)	0.50 ± 0.02	0.49 ± 0.06	0.745
Hemoglobin (mg/dl)	11.12 ± 1.26	12.18 ± 1.03	0.184
Hematocrit (%)	32.3 ± 5.20	35.4 ± 3.36	0.301
MCH (pg)	221.9 ± 35.2	249.6 ± 35.4	0.250
MCV (fl)	645.5 ± 133.4	723.9 ± 98.4	0.321
MCHC (g/dl)	34.63 ± 1.59	34.58 ± 3.59	0.976
Monocyte (%)	4.00 ± 2.73	3.20 ± 0.84	0.561
Neutrophil (%)	18.60 ± 0.55	21.40 ± 1.14	0.001*
Eosinophil (%)	4.60 ± 2.19	7.00 ± 1.22	0.074
Lymphocyte (%)	72.80 ± 1.10	68.40 ± 1.14	0.0001**

Table 2. The correlation coefficient between blood parameters of wild beluga sturgeon. (*) Correlation is significant at the $0.01 \leq p \leq 0.05$ level (2- tailed). (**) Correlation is significant at the 0.01 level (2 -tailed); Lym=lymphocyte, Neut=neutrophil, Eos=eosinophil, Mon=monocyte.

	RBC	Hct	Hb	MCH	MCV	MCHC	Mon	Eos	Neut	Lym
WBC	0.69	-0.29	-0.13	-0.61	-0.72	0.22	0.10	-0.36	-0.08	0.26
RBC	1	0.093	0.047	-0.75	-0.64	-0.013	0.008	-0.56	0.30	0.14
Hct		1	0.68	0.36	0.70	-0.59	0.83*	-0.83*	0.10	-0.14
Hb			1	0.62	0.49	0.18	-0.54	0.89**	0.22	-0.46
MCH				1	0.81*	0.25	-0.33	0.67	-0.14	-0.18
MCV					1	-0.04	-0.60	0.71	-0.21	0.02
MCHC						1	0.51	-0.11	0.08	-0.30
Mon							1	-0.74	-0.07	-0.06
Eos								1	0.34	-0.48
Neut									1	-0.89**

Table3. Artificial reproduction normative of wild great sturgeon broodstocks.

	Standard deviation	Average	Minimum	Maximum
Total length (Cm)	30.96	227.2	200	276
Body weight (kg)	53.75	136.2	89	223
Total weight of extracted ovum (kg)	4.9	13.2	6.8	18
Oocyte long (mm)	0.21	4.17	3.94	4.38
Oocyte width (mm)	0.19	3.75	3.44	3.9
Weight of spawn (mg)	3.78	35.4	33	42
Functional fecundity (number)	157210	447600	224000	612000
Egg fertilization (%)	24.33	46.7	27.1	78.8
Larvae weight after hatch (mg)	1.56	25.48	25	27

ean operation; thus, every preclinical finding can be useful for health monitoring and healing management. Hematological parameters of juvenile and fingerling beluga have been recorded in a wide range by Hoseinifar et al. (2011), Mohammadi Zarejabad et al. (2009), Ghomi et al. (2011) and Yousefi et al. (2012). The results of mentioned studies were higher than the results of our study on wild beluga sturgeon. In the present study, there were significant differences between males and females in the quantity of WBC, neutrophil, and lymphocyte. In the other reports, differences between sexes in quantity of some blood parameters were recorded in reared sturgeons; for example, Bahmani et al. (2001) reported that there were significant differences in Hb, RBC, WBC, neutrophil, lymphocyte and eosinophil between reared male and female beluga. The reason of such contradictory results is not clear; however, it could be related to methodological differences or different response of males and females to stress. Studies on other species reported different effects of fish sex on hematological and biochemical variables too; there was significant difference in WBC between male and female Persian sturgeons, *Acipenser persicus*; although Hb, RBC and WBC differential count showed no variation between the sexes (Bahmani et al., 2001). In a similar study, there were significant differences in some blood biochemical parameters between genders (Asadi et al., 2006). Murray (1984) showed that Hb, RBC, MCV, MCHC and eosinophil values were significantly affected by the fish sexes in bluegill, *Lepomis macrochirus*; whereas, Hct, MCH, lymphocyte, and neutrophil were not. Matsche et al. (2013) reported similarity of Hct, WBC, total protein, albumin, globulin, and urea values in short nose sturgeon, *Acipenser brevirostrum*, between genders; however, serum calcium and glucose levels were sex-dependent. None the less, in ship sturgeon, *Acipenser nuventris* broodstocks, there was no significant difference in blood parameters between males and females (Mazandarani et al., 2013). The mentioned studies showed that the effect of fish sex on hematological parameters is affected by other factors such as fish species, sampling, and life stage, which should be taken into account in the studies.

In the present study, there was correlation be- tween MCV and MCH that was expected because of the relation between Hb and RBC and hematocrit. The correlation between lymphocyte and neutrophil can be due to the fact that lymphocyte and neutrophil comprise of more than 90% of leukocytes. The correlation between hematocrit and monocyte, Hct and eosinophil, and Hb and eosinophil for broodstocks can be due to the low numbers of specimens (sturgeon population in the Caspian Sea is going to vanish; therefore, it is not possible to collect enough samples) or might be because of the physiological stage of spawning and stress. For more accurate judgment about correlations of hematological parameters in broodstocks, larger sample size is needed. Reproduction normative is revealed in Table 3. In this study, relative fecundity was near 10% and was in standard range; however, the percent of egg fertilization was lower than standard in comparison with other reports (Kireeva et al., 2013). This can be due to numerous reasons such as low number of specimens, high stress during capture and storage, different situation, and other conditions.

The present results are valuable, because hematological studies on beluga are rare, especially in wild broodstocks . In other words, knowledge on health and physiological condition of beluga broodstocks can help to protect and conserve this endangered species.

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شاخص‌های خونشناسی وتولید مثلی در مولدین فیل ماهیان وحشی جنوب شرقی دریای خزر

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چکیده

زمینه مطالعه: مطالعات خونشناسی تکنیک تشخیصی ارزشمندی در بررسی وضعیت سلامت ماهیان، درمان و نیز اثرات فاکتورهای محیطی بر آنهاست. **هدف:** بدلیل ارزش بالای ماهیان خاویاری و ضرورت بازیابی مولدین آنها پارامترهای خونشناسی و نرماتیوهای تکثیر مولدین وحشی فیل ماهی (*Huso huso*) دریای خزر در این بررسی مورد مطالعه قرار گرفت. **روش کار:** به این منظور ۷مولد وحشی ماده و ۷ مولد وحشی نر مورد بررسی قرار گرفته و برخی پارامترهی خون شناسی از قبیل RBC، WBC، هماتوکریت، هموگلوبین، MCV، MCH، MCHC و درصد تفریقی گلبول‌های سفید تعیین و ثبت گردید. **نتایج:** بر اساس نتایج این مطالعه درفیل ماهیان وحشی میزان گلبول‌های سفید خون و لنفوسیت در ماهیان نر بیشتر از ماهیان ماده بوده، همچنین میزان نوتروفیل در ماهیان نر کمتر از ماهیان ماده اندازه‌گیری شد. همچنین در این مطالعه هم آوری کاربردی برای ماهیان ماده ۱۵۷۲۱۰±۴۴۷۰۰۰ ثبت گردید. تخمک‌های استحصالی به وزن ۳/۷۸ mg±۳۵/۴ با قطر طولی ۰/۲۱ mm±۴/۱۷ و قطر عرضی ۰/۱۹ mm±۳/۷۵ اندازه‌گیری شدند. درصد لقاح برای تخم ماهیان مورد مطالعه ۲۴/۳۳±۴۶/۷ ٪ و وزن لارو های بلافاصله پس از تخم گشایی ۱/۵۶mg±۲۵/۴۸ ثبت گردید. **نتیجه‌گیری نهایی:** رنج گسترده‌ای از مقادیر پارامترهای خونشناسی در بچه ماهیان و ماهیان جوان فیل ماهی گزارش گردیده است. به نظر می‌رسد مقادیر یاد شده در بررسی‌های گوناگون در ماهیان پرورشی با مولدین فیل ماهیان وحشی متفاوت باشد، این امر نشانگر اهمیت تعیین مقادیر خون‌شناسی مولدین فیل ماهی وحشی است.

واژه‌های کلیدی: مولدین، دریای خزر، خون‌شناسی، فیل ماهی، نرماتیو تکثیر

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