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A comparative study of some blood factors in male and female Caspian kutum (*Rutilus frisii kutum*) broodstock from the southern basin of the Caspian Sea

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Abstract: In this study, some serum factors in male and female Caspian kutum (*Rutilus frisii kutum*) broodstocks were investigated. The estimated values were compared between male and female fish of various age groups. A total of 80 mature fish (40 males and 40 females) were randomly caught from the Shirood and Tajen rivers (southern Caspian Sea) during spawning season. Blood samples were then taken from the caudal peduncles and the serum biochemical parameters were measured according to common laboratory methods. Cholesterol levels showed significant differences ($P < 0.05$) between male and female broodstocks from the Tajen River, as well as between male and female kutum of the Tajen and Shirood rivers. Comparison of the estimated serum factors with the fish ages revealed no significant changes ($P > 0.05$). A high correlation was found between total protein and weight in fish from both rivers based on Pearson's correlation. This study suggests that male and female variations in blood factors should be taken into account when these parameters are used to assess sex, age, and spawning sites.

Key words: *Rutilus frisii kutum*, serum factors, broodstock, Caspian Sea

1. Introduction

A number of disturbances including coastal, urban, and rural developments; dam and bridge constructions; other obstacles in the migration path; and river pollution have led to unfavorable river conditions for upstream fish migrations by perturbing their natural spawning. Hence, in order to maintain the natural broodstock, the fish migrate to the estuaries, are caught, and are artificially propagated.

Fish hematological characteristics are considered to be critical records of their physiological stages, reflecting the relation between habitat attributes and fish health status (1). Several studies have demonstrated the influence of environmental conditions upon fish blood factors, implying that fish reveal fluctuating blood factors under diverse circumstances (2,3). Different factors including season (4–6), water contamination (7,8), physicochemical parameters of the water (9,10), stressors (11), age and sex (12,13), and fish species (14) have been shown to affect hematological parameters.

The kutum *Rutilus frisii kutum* (from the family Cyprinidae) is one of the most commercially valuable fish species in the southern coasts of the Caspian Sea that has adapted to inhabit the brackish water of the sea and the surrounding lagoons (15). The foremost distribution of kutum is from the Cora River to the Gomishan region (northeastern Caspian Sea), 90% of which is native to the sea. The Shirood and Tajen rivers are among the zones to which this anadromous species migrates for reproduction in late winter and early spring (16).

Nikoo et al. (13) recently reported changes in blood factors including the number of white and red blood cells, hematocrit, and hemoglobin percentages of both male and female Caspian kutum; Nikoo et al. (11) also studied the effects of environmental stresses. Most recently, Bani et al. (6) investigated the seasonal fluctuations of Caspian kutum blood factors. In addition, earlier studies on some fish species pointed out that serum factors such as cholesterol, triglycerides, and glucose display fluctuations with aging and also with the advent of propagation time (17,18).

There has been no report of changes in Caspian kutum serum factors during reproduction to be compared with their sex, age, and spawning sites. Therefore, the present study was undertaken to compare some serum parameters based on weight, age, and sex in *R. frisii kutum* captured from the Shirood and Tajen rivers in northern Iran.

2. Materials and methods

A total of 80 migrating *R. frisii kutum* (40 males and 40 females) were sampled within 2 weeks from the estuaries of the Shirood and Tajen rivers (southwest and southeast

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Caspian Sea, respectively) during spawning season. The fish were randomly caught using gill nets with a mesh size of 2 cm. The water temperature at the time of sampling averaged 12 ± 0.3 °C and 15 ± 0.2 °C for the Shirood and Tajen rivers, respectively. The fish were anesthetized using clove extract and then underwent biometry. Blood samples were taken from the caudal peduncles using 2-mL syringes and transferred to test tubes without adding anticlotting agents. To separate the serum, the blood samples were kept on ice and centrifuged afterwards at 3000 rpm for 15 min. The separated serum was then poured into plastic tubes and stored at –20 °C until use. For sex determination, undamaged fish scales (6–8) were excised from the lower part of the dorsal fin (above the lateral line) and were analyzed. Serum glucose, cholesterol, and triglycerides were determined by the enzymatic methods; cholesterol oxidase and glycerophosphate dehydrogenase, respectively, and total protein were assessed by the Burette method, for all of which commercial kits (Pars Azmoon, Tehran, Iran) were applied.

2.1. Statistical analysis

The estimated parameters at different ages and diverse populations were compared by one-way analysis of variance. To compare the mean values of the populations, Duncan's test was applied. The regression among length, weight, and age at different populations was expressed using multivariable regression. Pearson's correlation coefficient was applied to show correlation levels among the studied variables and blood biochemical factors. All the statistical analyses were carried out with SPSS 16.

3. Results

Table 1 shows the results of serum glucose, total protein, triglyceride, and cholesterol measured in the broodstock kutum from the Shirood and Tajen rivers, broken down by sex. The estimated levels of triglyceride and total protein in the broodstocks from both rivers did not reveal significant differences ($P > 0.05$). The male and female broodstock of the Tajen River were significantly different ($P < 0.05$) with respect to cholesterol levels, whereas male and female kutum from the Shirood River did not show a significant difference in cholesterol levels ($P > 0.05$). A comparison of cholesterol values between male kutum from both the Tajen and Shirood rivers revealed significant dissimilarities ($P < 0.05$), whereas female kutum from both rivers were not statistically different in cholesterol estimations ($P > 0.05$).

Female kutum presented the highest glucose levels compared to the male fish (Table 1). The male and female kutum from both the Tajen and Shirood rivers were significantly different ($P < 0.05$) in glucose values, but these levels did not reveal statistical differences among the female fish of both rivers.

The studied male kutum were between the ages of 2 to 3 years and females from 3 to 4 years. No significant changes ($P > 0.05$) were detected from the comparison of estimated serum factors with fish ages (Table 2).

Based on multivariable regression analysis for the biochemical parameters, amounts of cholesterol, total protein, and glucose in the kutum from the Tajen River displayed a high correlation with fish weight, whereas the triglyceride levels had a high correlation with fish age

Table 1. Average values of serum parameters in male and female Caspian kutum samples (n = 80) from the Tajen and Shirood rivers in northern Iran. Different superscript letters in a column denote significant differences ($P < 0.05$).

Population	Sex	Cholesterol	Triglyceride	Total protein	Glucose
Tajen	Male	353.37 ± 96.17[b]	218.7 ± 98.81[a]	7.32 ± 76.3[a]	169.1 ± 33.4[b]
	Female	244 ± 99.73[a]	240.7 ± 138.65[a]	5.34 ± 1.39[a]	197.4 ± 17.3[c]
Shirood	Male	229.1 ± 92.73[a]	149.4 ± 86.39[a]	7.58 ± 1.8[a]	137.8 ± 9.27[a]
	Female	261.9 ± 92.7[a]	149 ± 118.42[a]	7.33 ± 2.73[a]	208.82 ± 15.15[c]

Table 2. Comparison of average values of serum parameters based on age in male and female Caspian kutum samples (n = 80) from the Tajen and Shirood rivers in northern Iran. Different superscript letters denote significant differences ($P < 0.05$).

Population	Age (years)	Cholesterol	Total protein	Triglyceride	Glucose
Tajen	3	179.93 ± 29.4[a]	6.44 ± 3.31[a]	236.6 ± 123.5[a]	307.92 ± 109[a]
	4	185.5 ± 30.4[a]	6.47 ± .602[a]	181.5 ± 72.6[a]	294.25 ± 67.3[a]
Shirood	2	159.8 ± 40.1[a]	8.58 ± .912[a]	172 ± 109.2[a]	277.25 ± 64.8[a]
	3	173.76 ± 39.6[a]	7.3 ± 2.74[a]	113.72 ± 83.19[a]	242.25 ± 97.05[a]
	4	205.66 ± 9.0[a]	6.16 ± 1.3[a]	243.33 ± 142.93[a]	232.66 ± 123.45[a]

($P < 0.05$). In the samples from the Shirood River, the amounts of cholesterol and glucose were highly correlated with weight while significant correlations ($P < 0.05$) were obtained between total protein and age, and triglyceride and both length and weight (Table 3).

Application of the backward method resulted in 4 models to calculate the relation between the 4 independent parameters (length, weight, age, and sex) and the constant variable (cholesterol). The greater the correlation coefficient (R) is in a model, a higher correlation is expected ($P < 0.05$). Pearson's correlation coefficient showed positive relationships of cholesterol levels with length and weight, and total protein with weight in the kutum from the Shirood River (Table 4). Such a correlation was found for total protein with weight, triglyceride, and glucose in the kutum from the Tajen River (Table 5).

Altogether, no statistical differences were observed in the levels of total protein, triglyceride, and cholesterol between male and female *R. frisii kutum* from the Tajen and Shirood rivers. The cholesterol values, however, differed significantly between male and female kutum from both rivers. Diverse groups were also statistically dissimilar in glucose levels.

4. Discussion

Plasma proteins in fish have various functions and are especially important for the regulation of water balance (19). The effect of age on total plasma protein was studied

Table 3. Multivariable regression analysis of some serum biochemical parameters in Caspian kutum (n = 80) against length, weight, and age from the Tajen and Shirood rivers in northern Iran (C = constant; W = weight; A = age; L = length).

River	Dependent variable	Independent variable	Regression coefficient	P-value	Correlation coefficient
Tajen	Cholesterol	C	288.82	0.004	0.038
		W	0.011	0.88	0.038
	Triglyceride	C	389.95	0.039	0.221
		A	−50.91	0.362	0.221
	Total protein	C	6.982	0.011	0.066
		W	0.000	0.782	0.066
	Glucose	C	150.172	0.00	0.324
		W	0.031	0.163	0.324
Shirood	Cholesterol	C	203.67	0.004	0.188
		W	0.065	0.441	0.188
	Triglyceride	C	−1077.827	0.051	0.515
		L	40.62	0.029	0.515
		W	−0.671	0.039	0.515
	Total protein	C	10.98	0.00	0.338
		A	−1.218	0.145	0.338
	Glucose	C	128.45	0.00	0.503
		W	0.072	0.020	0.503

Table 4. Pearson's correlation coefficients for cholesterol, triglyceride, total protein, glucose, length, and weight in Caspian kutum broodstock (n = 80) from the Tajen River in the southeast Caspian Sea.

	Length	Weight	Cholesterol	Triglyceride	Total protein	Glucose
Length	–					
Weight	0.860	–				
Cholesterol	0.027	0.038	–			
Triglyceride	0.205	0.252	− 0.419	–		
Total protein	0.088	−0.027	0.450	−0.295	–	
Glucose	0.188	0.282	−0.270	0.311	−0.487	–

Table 5. Pearson's correlation coefficients for cholesterol, triglyceride, total protein, glucose, length, and weight in Caspian kutum broodstock (n = 80) from the Shirood River in the southwest Caspian Sea.

	Length	Weight	Cholesterol	Triglyceride	Total protein	Glucose
Length	–					
Weight	0.955	–				
Cholesterol	0.211	0.300	–			
Triglyceride	0.311	0.141	−0.336	–		
Total protein	0.087	−0.004	0.673	−0.040	–	
Glucose	0.461	0.533	0.286	0.177	−0.057	–

in hybrid striped bass by Hrubec et al. (10), who reported the highest total protein in fish aged 4 months compared to age groups of 6, 9, 15, and 19 months. It had previously been found that levels of total protein elevate with fish aging (20). The present study also detected a descending pattern of plasma total protein with kutum aging, though the changes were insignificant. Khajevand et al. (21) did not observe significant differences between male and female fish in 2 species, *Barbus sharpei* and farmed *Ctenopharyngodon idella*, with respect to total protein. Similarly, a study on the Persian sturgeon, *Acipenser persicus*, revealed no differences in total protein between males and females (22). Similar to the results on kutum in this investigation, decreased total protein levels were also observed in kutum (6), *Esox lucius* (23), and *Silurus glanis* (24) during reproduction time. Previous studies, on the other hand, reported increased total protein concentrations in the blood plasma of *Tinca tinca* broodstock before reproduction time (25,26).

Blood glucose is a highly variable parameter that is strongly influenced by handling and environmental stresses as well as seasonal variations, nutritional status, and sexual maturity, among other stressors (27). Glucose levels in the female broodstock were significantly lower than levels in the male broodstock determined by this study. This finding may have resulted from the fact that *R. frisii kutum* as an anadromous species endures long periods of starvation during spawning migrations and allocates more energy for egg production leading to reduced glucose levels. Low plasma glucose levels in the winter could be indicative of reduced food intake and increases in tissue uptake mediated by pancreatic hormones (28). Decreased plasma glucose levels during the prespawning period in kutum may be due to the higher energy demand needed for gonad development, the combined reproductive effects, or the prevailing food shortages observed in winter (6). In addition, lipid and protein levels are recognized as the main energy sources to assist fish migration upriver during spawning season (29,30); therefore, glucose is not normally utilized during the migration period (spring). This result is in accordance with previous plasma glucose findings in tench, *Tinca tinca* (31). Some studies, on the other hand, reported slight changes in glucose levels when comparing male and female broodstock of *A. persicus* (22), *B. sharpei*, and farmed *C. idella* (21).

Cholesterol and triglycerides account for 2 important compounds used during ovary development and vitellogenesis as well as for biosynthesis of sex hormones, and hence their plasma levels fluctuate accordingly. Hemre et al. (32) suggested that in the Atlantic cod, *Gadus morhua*, only cholesterol amongst the plasma nutrients was affected by gonadal development. The role of cholesterol is well known in developing eggs at the time of fish reproduction (25). Additionally, cholesterol is utilized as a precursor for steroid hormone synthesis (33) during prespawning in late winter and spawning in early spring. Nevertheless, triglycerides are potentially considered as a source of energy for reproduction (34). Subtle cholesterol rise with age and decreased triglyceride levels were observed in the current study; however, neither level was significant. In the same way, Khajevand et al. (21) and Shasavani et al. (22) found no significant fluctuations in cholesterol and triglyceride levels in the species mentioned above.

Cholesterol variations follow different conditions, one of which is changes in sex steroids during the reproduction season (35). The relationship between triglycerides and total protein may be because triglycerides are combined with some lipoproteins to be translocated in the plasma. An increase in lipoprotein synthesis may, therefore, have an effect on total protein levels. Furthermore, total cholesterol shows fluctuations with age, i.e. levels initially decline with fish aging and rise afterwards (20). Barnhart (36) indicated that age variations in trout (*Oncorhynchus mykiss*) led to changes in cholesterol and triglyceride levels. Overall, the outcome of this study implies that male and female variations in blood factors of Caspian kutum should be considered when these parameters are used for the assessment of sex, age, and spawning sites.

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