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Serum Biochemical Parameters of Caspian Lamprey, *Caspiomyzon wagneri* During Final Spawning Migration

Mohamad Reza Imanpoor and Mehdi Abdollahi

Department of Fisheries, Faculty of Fisheries and Environment,
Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran

Abstract: Serum biochemical parameters are important aspects in the management of endangered species such as *Caspiomyzon wagneri*. The objective of this survey was to determine some serum ionic and metabolic parameters and those relationships in 22 migratory population of Caspian lamprey in shirood river (a river located at the south of Caspian sea). There was no significant ($P<0.05$) difference between level of calcium (8.52 ± 2.9-9.14±0.97 mg/dL), magnesium (2.97±1.04-2.86±0.97 mg/dL), phosphorus (11.23±3.18-13.57±6.61 mg/dL), Iron (0.37±0.19-0.54±0.3 mg/dL), total protein (4.7±2.47-5.81±3.85 g/dL), glucose (93.98±22.89-104.10±32.38 mg/dL) and cholesterol (164.00±59.19-170.99 ±60.77 mg/dL) in male and females. The correlation between magnesium with calcium ($P<0.05$) and cholesterol ($P<0.05$) and glucose ($P<0.01$) was significant. There was a significant correlation between phosphorus and total protein ($P<0.05$). The correlation between cholesterol with glucose ($P<0.01$) was significant. But correlation between total protein with magnesium to phosphorus ratio and calcium to phosphorus ratio ($P<0.05$), was invert. According to influences of ionic and metabolic blood's serum on regulation of reproduction and growth in Caspian lamprey, can use from these results in management of reproduction and culture of this endangered species and our results can help to more understand lampreys physiology in starvation time compare to other higher vertebrates.

Key words: Blood serum · Ionic and Metabolic biochemical · Caspian lamprey

INTRODUCTION

The Caspian lamprey is an Eurasian anadromous non-parasitic species. *Caspiomyzon wagneri* is endemic to the Caspian Sea and rivers in its northern, western and southern watershed [1]. The habitat of adults in the Caspian Sea is unknown. When entering the rivers they migrate close to the bottom or the banks, where the current velocity is 0.4 to 0.6 m.s-1 [2]. During winter to late spring prespawning adults stay on the bottom among stone or beneath the substrate [3]. Like to other species of lampreys, Caspian lamprey do not feed in reproductive migratory time at all [4]. The Caspian lamprey is considered to be a valuable and delicious fish. The edible part amounts to 93% of the total weight. Total annual catches of this species between 1930-1934 ranged between 500 and 850 metric tons and in the period from 1941 through 1945, it decrease to well under 100 tons per year [5]. Construction projects along the rivers entering the Caspian sea have had a deleterious impact on the stock of this species. In the Volga river it has become very rare and its catches in the whole area declined so dramatically that it is no longer among the commercially important fishes, despite some attempt to breed it in hatcheries [1, 6]. The Caspian lamprey has been proposed for inclusion in the "Red Book of the U.S.S.R." which forms the basis for measures to protect species and is listed as "vulnerable" in Europe by Lelek [7] and [8]. It is vulnerable because it migrates into rivers which are polluted and dammed and because of its restricted and declining distribution. These conditions apply particularly in Iran, although there is some evidence for spawning based on captures in the 1990s [9, 10].

Serum biochemical parameters are important aspects in the management of endangered species [11]. In edition haematological parameters are valuable tools for the monitoring of fish health [12] and they are affected by many endogenous and exogenous factors such as water temperature, reproduction cycle, metabolic rate [13-15] management [16], diseases [17] and stress [18].

Corresponding Author: Mohammad Reza Imanpoor; Mail address: Shahid beheshti Avenue, Gorgan University of Agricultural Sciences and Natural Resources, Fisheries department, Golestan province, Gorgan city, Iran,
Tell: 00989111787902; Fax number: 00981712245886; Email address: mrimanpoor@yahoo.com

Moreover basic ecological factors, such as feeding regime and stocking density, also have a direct influence on certain biochemistry parameters [19]. Since during the winter the lampreys are subjected to marked morphological and biochemical changes, most of them due to the cessation of feeding: a decrease in the body mass and length (the only organs which are not reduced are the heart and kidney), the intestinal atrophy, the regressive changes in the alimentary canal. The sexual maturation of the lamprey starts in the beginning of the migration. The life activity and gonadal growth are provided exclusively by mobilization of lipids and proteins (mainly from the body skeletal muscle) accumulated during the sea period of active feeding. Being monocyclic, these animals die after the spawning in Spring. As Larsen [20] states ''...it may be wise to avoid the term starvation for the final phase in the lampreys life, because this word indicates a stressful situation in which the animal is prevented from obtaining food [21] so all of these changes in this stage of lampreys life can influence on their serum parameters. In the other hand establishing reference intervals for various haematological parameters of fish is important for evaluating the effects of various environmental changes on the health of populations in the wild [15].

At the other side the analysis of blood parameters is one of the most valuable modern methods because it has been shown that their physiological values are species-specific and sex dependent [11, 22] Since any data on Caspian lamprey blood parameters have not been published, Thus, this study was performed to determine some serum ionic and metabolic parameters and those relationships and compare these parameters in Caspian lamprey males and females to obtain basic information to improve management of this worthwhile and endangered species.

MATERIAL AND METHODS

Caspian lamprey were caught in the Shirood river in Tonekabon, Iran (36°44′-36°51′ N, 50°48′-50°49′ E), in the last of April 2009. Sampling was done with hand and in reproductive migratory glen of fish at night [1]. Blood sample were collected from the caudal vein and was left for 1 h on ice and then centrifuged at 3000 rpm for 10 min to isolate the serum, which was collected as a supernatant and stored in liquid nitrogen prior to further analysis. Biochemical analyses for all parameters were determined spectrophotometrically with commercial reagents kits (Pars azmoon., Iran).

The quantitative determination of serum glucose was carried out using commercially available diagnostic Experimental Protocols kits Pars Azmoon, Iran (1 500 0178), at 546 nm and 37 °C by the glucose oxidase method. The limit of detection (LOD) of the procedure was 5 mg/dl. Intra-assay and Inter-assay Mean ± SD were 64.2 ± 1.12 and 92.5 ± 1.10 mg/dl respectively.Glucose was measured photometrically based on the quantification of NADH after a glucose oxidation catalyzed by glucosedehydrogenase. The quantity of NADH formed is proportional to the glucose concentration.

Serum total protein levels were determined using Pars Azmoon, Iran (1 500 028) kit, with bovine serum albumin serving as standard at 546 nm and 37C. The limit of detection (LOD) of the procedure was 5 mg/dl. Intra-assay and Inter-assay coefficients of variation were of 0.91% and 1.06% respectively. Intra-assay and Inter-assay Mean ± SD were 5.27 ± 0.05 and 5.24 ± 0.06 g/dl respectively.

Sex of the fish was determined by surgery and saw gonad morphology of male and females. The levels of different serum parameters in females (N=10) and males (N=12) were compared by means of student t test and The correlation between Serum biochemical parameters were analysed using either a linear or a multiple regression (SPSS, ver. 18.05; SPSS, Chicago, IL).

RESULTS

In this survey mean of male and female total length were 40.40±2.01 and 38.72±2.66 cm respectively and mean of male and female weight were 1056.64±164.74 and 965.83±181.81 respectively and there is no significant ($P<0.05$) deference in total length and weight in male and female of Caspian lamprey.

Serum levels of calcium, magnesium, phosphorus, iron, total protein, glucose and cholesterol in females and males of Caspian lamprey are shown in Table 1. There was no significant ($P<0.05$) difference between the groups in any of parameters.

The statistical correlation between Serum biochemical parameters in *C. wagneri* are presented in Table 2. The correlation between magnesium with calcium ($P<0.05$), cholesterol ($P<0.05$) and glucose ($P<0.01$) was significant. There was a significant correlation between phosphorus and total protein ($P<0.05$).

The correlation between cholesterol with glucose ($P<0.01$) was significant, but correlation between total protein with magnesium to phosphorus ratio and calcium to phosphorus ratio ($P<0.05$), was invert.

Table 1: Serum parameters of male (N=12) Caspian lamprey

parameter	mean± SD	Range	P-value
Calcium(mg/dL)	9.14±0.97	8.14-11.01	198
Magnesium(mg/dL)	2.79±1.04	1.08-5.01	655
Phosphorus(mg/dL)	11.23±3.18	3.52-16.43	232
Iron(mg/dL)	0.54±0.10	0.12-1.06	532
Total protein(g/dL)	5.81±3.85	3.12-17.06	681
Glucose(mg/dL)	104.10±32.38	70.68-175.94	614
Cholesterol(mg/dL)	164.00±59.19	56.92-266.15	504

Table 2: Serum parameters of female (N=9) Caspian lamprey

parameter	mean± SD	Range	P-value
Calcium(mg/dL)	10.12±0.97	5.89-14.36	198
Magnesium(mg/dL)	2.86±0.97	1.83-4.70	655
Phosphorus(mg/dL)	13.57±6.06	1.83-4.70	232
Iron(mg/dL)	0.37±0.09	0.18-0.76	532
Total protein(g/dL)	4.70±2.47	1.24-8.28	681
Glucose(mg/dL)	93.98±22.89	60.15-127.82	614
Cholesterol(mg/dL)	170.99±60.75	100.0-264.62	504

Table 3: The statistical Pearson's correlation between Serum biochemical parameters in *C. wagneri*

Variables	Calcium	Magnesium	Phosphorus	iron
Magnesium	0.873*	-	0.664	-0.615
Phosphorus	0.478	0.664	-	-0.185
Iron	-0.633	-0.615	-0.185	-
Total protein	0.121	0.296	0.773*	0.323
Cholesterol	0.774	0.943*	0.667	0.582
Glucose	0.583	0.947**	0.397	-0.586
Magnesium/Calcium	0.292	-0.208	-.399	-0.053
Phosphorus/Calcium	0.097	-0.278	0.765*	-0.047
Phosphorus/Magnesium	0.032	-0.183	-0.841**	-0.166

Table 4: The statistical Pearson's correlation between Serum biochemical parameters in *C. wagneri*

Variables	Total protein	Cholesterol	Glucose	Calcium/ Magnesium	Calcium/ phosphorus
Cholesterol	0.310	-	-	-	-
Glucose	0.388	0.788*	-	-	-
Magnesium/Calcium	0.428	-0.412	-0.458	-	-
Phosphorus/Calcium	-0.738*	-0.376	-0.309	0.851*	-
Phosphorus/Magnesium	-0.796*	-0.297	-0.279	0.498	0.873

*P<0.05, **P<0.01

DISCUSSION

To understand the physiology of the lampreys during starvation it is important to know as much as possible about the metabolism of protein, lipid and carbohydrate. It is characteristic of lampreys that they do not feed during the period of sexual maturation. Gonadal growth during this period, especially the ovaries, depends on the energy reserves. The parasitic species accumulate these reserves during the juvenile feeding stage, whereas the nonparasitic species utilize reserves accumulated by suspension feeding before metamorphosis. This phase of natural starvation for the comparable phase in salmon; the word means "Liquidation" and refers to mobilization of muscle substance for use in ovarian growth is characterized by a dramatic remodeling of the body, drawing on fat and protein reserves, mainly in muscles and skin. This phase end in death after spawning; in most cases death occurs within a few days or weeks after spawning [20].

Caspian lamprey total protein level was similar to reports about other lamprey species in migratory time. For example Baltic lampreys protein level when they enter the river are about 5.0-5.5 g/dL [20, 23]. By the time of spawning and at the end of the nonfeeding period the values drop to 19-20 mg/ml [23]. Sex differences were not investigated in these studies. But Sower *et al*. [24] found same trends in circulating protein profiles in P. marinus, during the upstream spawning migration in addition they found substantial differences in plasma protein concentrations between male and female lampreys. We agree with their idea that the lower plasma protein levels in females probably reflect the higher metabolic demands of gonad development relative to that of males in this life stage. similar findings have been reported for Pacific lampreys by Mesa *et al*. [25].

The blood serum Ca2+ and Mg2+ in this survey was similar to results of Logan *et al*. [26] and Vicar and Rankin [27] about river Lamprey (*Lamptera fluviatilis*). In fish, as in all vertebrates, magnesium is found mineralized in bony tissues and is ionised (Mg2+), complexed or protein-bound in all tissues. The total magnesium concentration of blood plasma in most cases does not exceed 2 mmol/L and the ionic concentration is normally less than 1 mmol/L. It is thought that the level of free, ionic magnesium (Mg2+) is kept at a relatively stable and low value because it is an important determinant of cellular function through its control over catalytic reactions [28].

In addition in present survey there was a significant correlation between magnesium and calcium of Caspian lamprey serum, several studies showed that magnesium deficiency in fish is accompanied by disturbances in the balance of other important minerals such as calcium, potassium and sodium [28-32]. It is plausible that this relationship reflects the dependence of cellular ion transport mechanisms, such as the Na+/K+-ATPase [33]. Mg2+ affects the permeability of the intestinal epithelium to ions [34, 35] and a low luminal Mg2+ concentration will therefore increase the epithelial permeability to ions, possibly stimulating paracellular Ca2+ absorption [28, 36]. But when adult anadromous lamprey return to fresh water, they stop their feeding to end of life and lose the ability to osmoregulate in salt water [37], Cessation of feeding, or starvation, is accompanied by rapid atrophy of the intestine and further assimilation of exogenous reserves is stopped, at least by intestinal absorption, is impossible to end of life [37], Thus it is plausible that this relationship reflects the dependence of cellular ion transport mechanisms, such as the Na+/K+-ATPase [33] and Mg++/K+-ATPase albeit gill (Na-K)-ATPase activity declined with age of adult lampreys, being highest in feeding adults and effectively absent from those individuals which had entered the river on their spawning migration. However, at no stage was the enzyme activity as high as values found

for teleosts [38, 39]. Another point that we must attention to it about lampreys osmoregulation is their osmolarity fell precipitously during the spawning phase of the life cycle that this may be caused by an increase in skin permeability and a degeneration of the kidney [37]. These factors along with the degeneration of the gut [40] and the loss of "chloride secretory cells" undoubtedly account for the reduced ability of spawning lampreys to osmoregulate in salt water [41].

Also the correlation between magnesium cholesterol ($P<0.05$) and glucose ($P<0.01$) was significant. Mg2+ activates numerous enzymes and also modifies specific enzyme substrates [42].

In this capacity, Mg2+ controls, among other systems, energy metabolism and protein synthesis [28], since fish use lipids as a basic source of energy as well as glucose as a source of energy is requirement for all body cells permanently too and glucose in serum is a major metabolite of carbohydrate metabolism [43, 44], so this correlation seem to be related to magnesium contribute in energy metabolism that be related to its role in metabolic interaction as a cofactor [45].

The significant correlation between phosphorus with total protein seem to be related to Phosphate important role in amino acid metabolism and in muscle and nervous tissue metabolism, as well as various metabolic processes involving buffers in body fluids [46]. Since the plasma amino acid levels increase in lampreys in this life stage because lamprey tissues proteolysis predominates over biosynthetic and energy processes during the winter months [47]. So seem high level of phosphorus in Caspian lamprey in this time of year be related to its role in amino acid metabolism too.

To answer the question how proteolysis is controlled in the tissues of the animals under prolonged starvation and its relation to phosphorus; the proteolysis of protein in the animals under prolonged starvation is thoroughly controlled and lampreys demonstrate much more intensive proteolysis as compared with that in other species such as frogs. Why it is the level of branched amino acids in blood plasma that makes it possible to estimate the intensity of proteolysis was elucidated by Czech scientists on starved rats [48]. If the amount of branched amino acids in tissues is insignificant (low proteolysis), the protein constituent of the dehydrogenase complex of a-keto acids, which arise from branched amino acids, is phosphorylated by protein kinase and, thus, becomes inactivated. Catabolism of branched amino acids stops and they enter the circulation, thereby increasing their levels in blood. If the amount of branched amino acids in tissues is considerable (intensive proteolysis), the dehydrogenase complex is dephosphorylated by phosphatase and a-keto acids produced from branched amino acids are metabolized in the tissues themselves [47].

This study is the first to characterize and provide a comparative physiological account of Caspian lamprey. To summarize, there is an urgent need to make reliable normal databases available for this species of economic importance for its preservation and more understand its physiology in starvation time in compare to other higher vertebrates. We established serum parameters values for the C. wagneri, which can be used as interpretative data obtained from this species kept in similar environmental conditions. However, other studies will have to be do for knowledge of the hematological values of this species in other time of years.

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