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Morpho-Histological Study of Kidney in Farmed Juvenile Beluga, *Huso huso* (Linnaeus, 1758)

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Abstract: In this study, structure, size and distribution of nephron cells on kidney in *Huso huso* were investigated. The head, body and caudal part of kidney in juvenile *Huso huso* were sampled. The kidney of *Huso huso* consisted of glomerulus, proximal, distal and collecting tubule cells. The average area in 1 and 2 year old sturgeon were 2718.07±1387.51 and 2793.89±1348 μm in proximal cells, 2678.80±1249.12 and 2599.98±1428.13 μm in distal cells, 2275.44±1289.52 and 2312.23±1629.58 μm in collecting tubule cells, 4359.8±1573.59 and 5071.04±1916.87 μm in glomerulus and 6019.68±1800.55 and 8307.49±2073.53 μm in Bowman's capsule, respectively. Average long and small diameter in 1 and 2 year were 68.03±17.82 and 49.94±12.73, 63.29±16.15 and 45.58±12.46 μm in proximal cells, 63.25±16.01 and 44.3±15.09, 63.514±15.25 and 45.46±13.3 μm in distal cells, 51.9±13.04 and 40.54±12.21, 57.08±16.7 and 45.53±15.28 μm in the collecting tubule cells, 91.18±17.93 and 68.72±16.22, 98.7±21.85 and 72.24±17.48 μm in glomerulus and 99.32±19.82 and 76.45±1896, 125.44±24.93 and 93.85±24.78 μm in Bowman's capsule, respectively. In all cases no statistically significant difference detected in the measured cells among 1 and 2 year old fishes. Morpho-histological pattern of kidney can be developed on the basis of size, feature and distribution of cells in farmed Sturgeon.

Key words: Chondrostean, sturgeon, morphology, nephron

INTRODUCTION

The vertebrate kidney is the main organ involved in the maintenance of body fluid homeostasis. The morphology and function of the kidney have been modified through evolution to fulfill different physiological requirement and the widest range of kidney types is found in fishes (Hentschel and Elger, 1989). Within the group of chondrostean, the sturgeons constitute a family (Acipenseridae) of relic fish that is phylogenetically very interesting. Sturgeons show great ecological plasticity and are able to thrive in both freshwater and marine environments. While the kidney should play a key role in this process of adaptation (Ojeda *et al*., 2003). Also, attempts are underway to improve and diversify the cultivation of sturgeon aquaculture, including production in brackish and salt water. So, the kidney is important organ for this (Mancera *et al*., 1993; Alltinok *et al*., 1998; Krayushkina *et al*., 2001). Hence the kidney is the important organ for adaptation to different conditions in fishes (Sveltana, 2006).

The structure of the sturgeon kidney is largely unknown. Sturgeons are an endangered species and are also considered to be valuable commercially. Thus, a systematic study of the sturgeon kidney would be of considerable importance from an anatomical, phylogenetic, or environmental standpoint (Ojeda *et al*., 2003).

Also, the kidney is the major and universal site of hemopoiesis in sturgeons (as well as in other bony fishes) but it includes the pronephros, whose renal epithelium completely disappears as animals grow and the mesonephros, which combines the excretory function and hemopoietic function throughout ontogenesis (Fange, 1986). The kidney plays a significant role in the removal of surplus ions from the fish in the early stages of adaptation to sea water.

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In bony fishes a particular relation between the structure of the nephron and the external environment is found. In marine teleosts the nephrons are weekly developed and in some, the glomeruli and distal canaliculi have completely disappeared. In this case, the nephron may consist of several sections of the proximal segments and of the short connecting sections, joining the nephrons with the system of collecting tubules (Hoar and Randall, 1983; Kristinskaya and Manusova, 1974; Krestinskaya *et al*., 1973). Teleostean nephrons exhibit extensive diversity in morphology, probably reflecting differences in their requirement and capability for water excretion and salt retention in various environment (Elger and Hentschel, 1981) but, however, probably posses the most diverse kidney among vertebrates (Hickman and Trump, 1969). The other histological studies on the kidney of sturgeons consist of: *Acipenser persicus* (Bahmani *et al*., 2004) juvenile *Huso huso* (Krayushkina *et al*., 1996), juvenile *Acipenser naccarii* (Cataldi *et al*., 1995), sturgeons of Caspian Sea basin (Gambaryan, 1984). Beluga (*Huso huso*) is a brackish water species with breeding pattern in fresh water (Tortonese, 1989). Krayushkia *et al*. (1996) and Cataldi *et al*. (1995) doing the similar of this project but in the different salinities and not in this extensive. So, increased understanding of the kidney structure and histo-morphological pattern in commercial species will be of great importance in some progressive information about its adaptation and environmental condition effects.

It was the purpose of the present study to conducting the morpho-histological examination of kidney based on size, features and distribution of cells in *Huso huso*. With regard to these, this pattern would be useful for sturgeon biology and make the morpho-cytological pattern in normal conditions.

MATERIALS AND METHODS

Fishes: Six specimens including young farmed beluga *Huso huso* (1 and 2 year old, average length 51.14±0.14 cm and weight 550.23±15.65 g) that reared in freshwater with: pH 6.81, total hardness (as $CaCO_3$): 406 mg L^{-1} and with water temperature 10°C, after transferred from fiber glass tank to laboratory (in International Sturgeon Research Institute) were studied in January. The part of water of each tank was changed every day. Fishes were removed from each tank for biometry and then, killed for sampling.

Sampling: Sampling of the head, body and caudal part of kidney tissue was done. Kidney samples were fixed for light microscopy in Bouin's liquid. Histological samples were dehydrated by routine methods and embedded in paraffin wax (Akhundov and Federove, 1995). The samples were then sectioned to a thickness of 7 μm by microtome (Leitz, 1512, Germany) and stained by H and E (Hung *et al*., 1990) and PAS (Mochiduk and Harada, 2007) stain.

Measurement: The microscope slides were statistically processed with the image analysis software Biocom Visolab and light microscope (Nikon, E-600).

Fifteen scopes were selected in each slide, randomly; and in each scope, 8-10 cells were observed and measured.

The cells of kidney were recognized based on functional histology (Young and Heath, 2000) and Atlas of fish histopathology (Takashima and Hibiya, 2000).

External area (means the outer epithelium area in each cell), long diameter and small diameter of glomerulus, Bowman's capsule, proximal, distal and collecting tubule cells were measured on μm (Wong and Woon, 2006; Krayushkina *et al*., 1996; Cataldi *et al*., 1991, 1995; Gambaryan, 1984; Nash, 1931) cited in Hoar and Randall (1983).

Statistical analysis: For statistical analysis, all data were tested among the different cells sizes (area, long diameter, small diameter) in two different ages, for this purpose data were analysis by SPSS 11 and Excel software and all data were presented as Means±SE. values were subjected to one- way ANOVA followed by a Tukey test using $p<0.05$ to delineate significance.

RESULTS

Kidney structure: The kidney of young *Huso huso* observed slender and long and may be divided into three parts: a head, body and a caudal part. The head of kidney consists of hemopoietic tissue. The hemopoietic tissue (Fig. 1) continues to body and caudal segment. But it has low dispersion in these regions. The high dispersion of nephrons were observed in the caudal part.

We observed in each nephron cell: glomerulus, enclosed by Bowmen's capsule, the proximal, distal and collecting tubules. In both ages, undifferentiated nephron cells can be observed.

Proximal tubule: There were two proximal tubule segments: the first proximal tubule has a well developed brush border and the height of cell is lower (Fig. 3), but the second proximal tubule has an elongate lumen and the brush border was lower and sparser with the high columnar cells (Fig. 1). In the proximal tubule, the nuclei are mainly spherical and situated in the lower part of the cells. Brush borders and situation of nuclei displays obviously in PAS staining (Fig. 2). The measurement of cells is shown in Table 1.

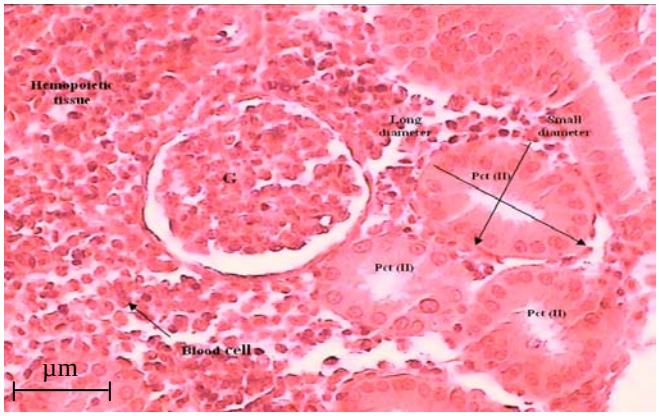

Fig. 1: The kidney of juvenile farmed beluga, *Huso huso*. Pct (II), second proximal convoluted tubule; G: Glomeruls; (H and E, 20X)

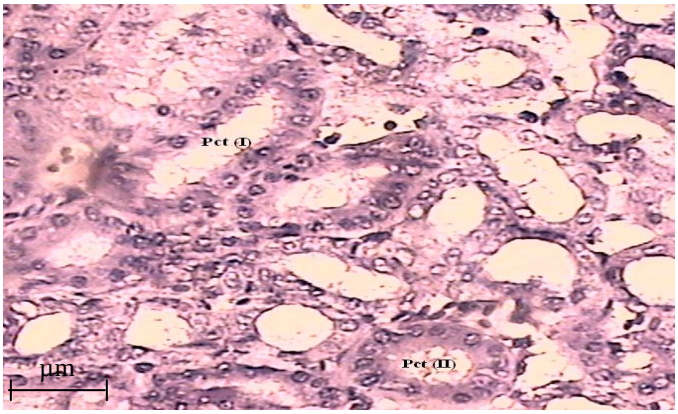

Fig. 2: The kidney of juvenile farmed beluga, *Huso huso*. Pct (II), second proximal convoluted tubule; Pct (I), first proximal convoluted tubule; (PAS, 20X)

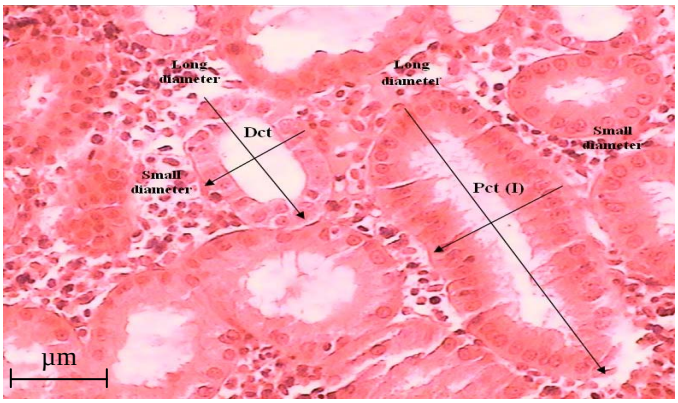

Fig. 3: The kidney of juvenile farmed beluga *Huso huso*. Pct (I), first proximal convoluted tubule; Dct, distal convoluted tubule; (H and E, 20X)

Distal tubule: The height of the distal tubule is not tall and is formed by cuboidal epithelium. The lumen of distal tubule is larger than the proximal tubule (Fig. 3). Some of them has the elongate feature but other ones had the spherical feature. The nuclei are elliptical or spherical that located in the whole of the epithelium. As shown in Fig. 4 the nuclei are represented obviously by PAS staining. Measurements of cells shown in Table 2.

Collecting tubule: The collecting tubules had the regulate feature (Fig. 5). These cells are morphologically distinct from other tubules as they were constructed of tall columnar epithelial cells. The nuclei of collecting tubule are mainly spherical that located in the central part of the cells. Located of nuclei is completely clear in PAS staining (Fig. 5). Measurements of cells shown in Table 3.

Table 1: External area, long diameter and small diameter in proximal convoluted tubules of juvenile farmed beluga *Huso huso*

		Diameter (μm)	
Age (year)	Area (mμ)	Small	Long
1	2718.07±1387.51	12.73±49.94	68.03±17.82
2	2793.89±1348	12.46±45.58	16.15±63.29

$p<0.05$

Table 2: External area, long diameter and small diameter in distal convoluted tubules of juvenile farmed beluga *Huso huso*

		Diameter (μm)	
Age (year)	Area (mμ)	Small	Long
1	2678.80±1249.12	44.36±15.09	63.25±16.01
2	2599.98±1428.13	45.46±13.3	63.51±15.25

$p<0.05$

Table 3: External area, long diameter and small diameter in collecting convoluted tubules of juvenile farmed beluga *Huso huso*

		Diameter (μm)	
Age (year)	Area (mμ)	Small	Long
1	2275.44±1289.52	40.54±12.21	51.9±13.04
2	2312.23±1629.58	45.53±15.28	57.08±16.7

$p<0.05$

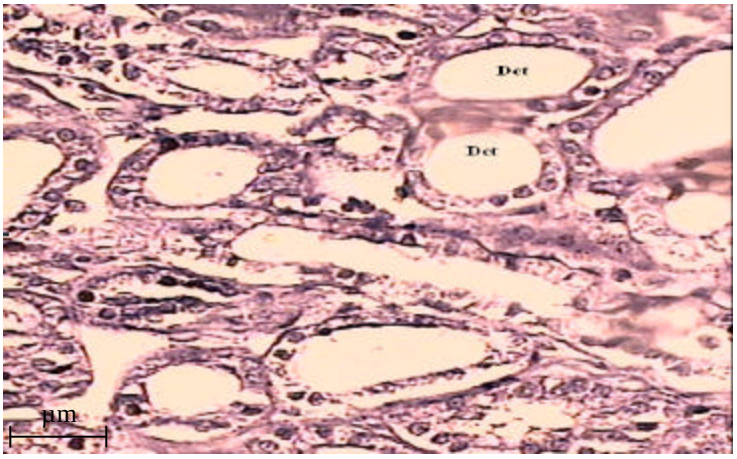

Fig. 4: The kidney of juvenile farmed beluga *Huso huso*. Dct: Distal convoluted tubule; (PAS, 20 X)

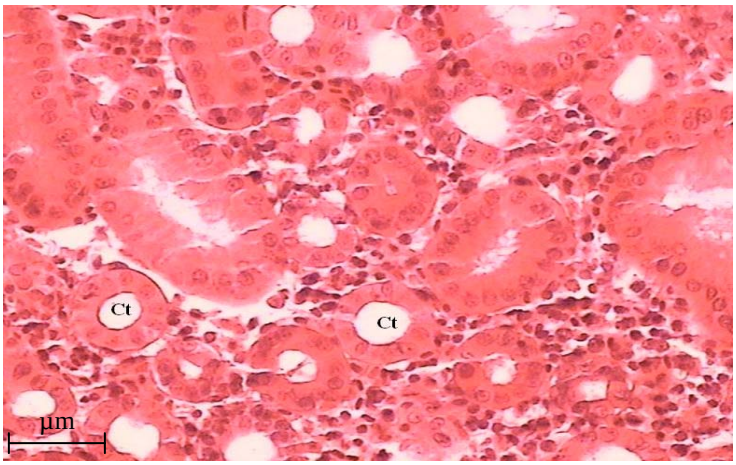

Fig. 5: The kidney of juvenile farmed beluga, *Huso huso*. CT: Collecting tubule; (H and E, 20X)

Glomerulus: The glomerulus surrounded by Bowman's capsule and the space between glomerulus and capsule is bowman's space (Fig. 7, 8). Measurements of cells shown in Table 4 and 5.

Dispersion of cells: Dispersion of nephron cells in the head of kidney was low, but, proximal in the body part and collecting tubules in caudal part of kidney showed maximum dispersion.

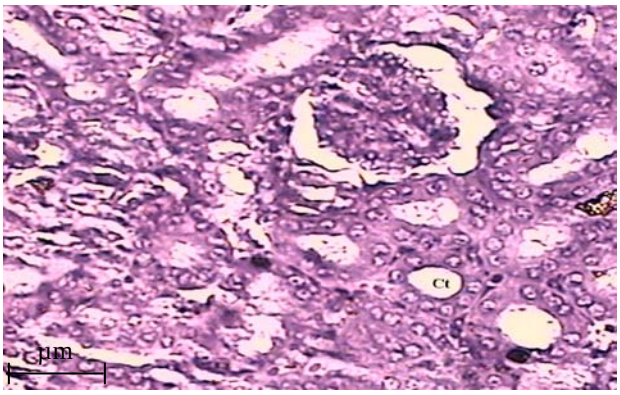

Fig. 6: The kidney of juvenile farmed beluga, *Huso huso*. CT: Collecting tubule; (PAS, 20X)

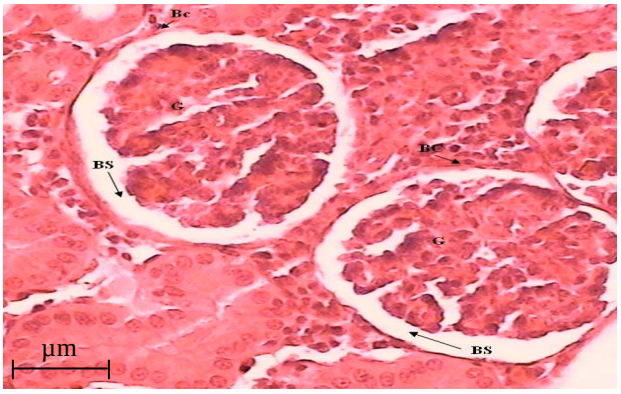

Fig. 7: The kidney of juvenile farmed beluga, *Huso huso*; G: Glomerulus, Bc: Bowman's capsule, BS: Bowman's Space; (H and E; 20X)

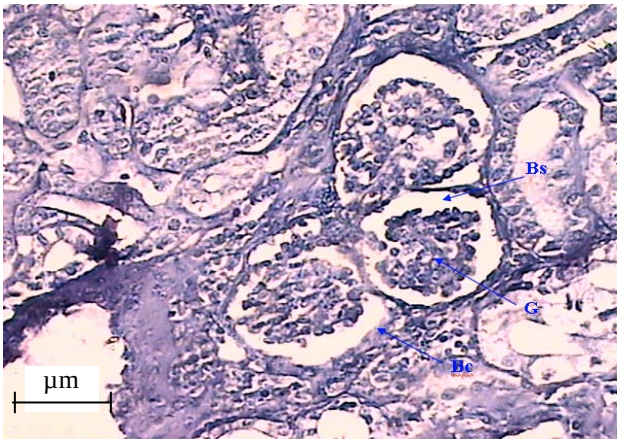

Fig. 8: The kidney of juvenile farmed beluga, *Huso huso*, G: Glomerulus; Bc: Bowman's capsule, BS: Bowman's Space; (PAS; 20X)

Table 4: External area, long diameter and small diameter in the glomerulus of juvenile *Huso huso* in farmed conditions

		Diameter (μm)	
Age (year)	Area (mμ)	Small	Long
1	4359.80±1573.59	68.72±16.22	91.18±17.93
2	5071.04±1916.87	72.24±17.48	98.72±21.85

$p<0.05$

Table 5: Exterual area, long diameter and small diameter in the Bowman's capsule of juvenile *Huso huso* in farmed conditions

		Diameter (μm)	
Age (year)	Area (mμ)	Small	Long
1	6019.68±1800.55	76.45±1896	99.32±19.82
2	8307.49±2073.53	93.85±24.78	125.44±24.93

$p<0.05$

The kidney of *Huso huso* consist of dispersed glomeruli and these cells are not grouped in one region.

DISCUSSION

The kidney of young *Huso huso*, as in other species of Acipenseridae, may be divided into three parts: a head, body and a caudal part (Krayushkina *et al*., 1996). According to the other results by other researchers, the kidney of *Acipenser persicus* (Bahmani *el al*., 2004) juvenile *Huso huso* (Krayushkina *et al*., 1996), juvenile *Acipenser naccarii* (Cataldi *et al*., 1995) and the other species of sturgeons (Gambaryan, 1984), generally, the freshwater nephrons (Reimschuessel, 2001) consists of glomeruli, Bowman's capsule, proximal, distal and collecting convoluted tubules. As a results of statistical analysis in this and the other studies on the kidney cells of sturgeons (Gambaryan, 1984; Cataldi *et al*., 1995; Krayushkina *et al*., 1996) there is not considerable changes in the size (area, long diameter and small diameter), feature and distribution of cells in different ages of juvenile fishes, although, it seems that the structure and size of nephron cells is not dependent on the age or size of juvenile sturgeons. As a result of statistical analysis, the significant difference ($p>0.05$) was not detected in measured cells of kidney among 1 and 2 year old *Huso huso*.

While, measurements of glomerular size and filtration surface (Marshall and Smith, 1930; Ogawa, 1962) strongly indicated that the filtration surface expressed per square micro meter of body surface is significantly greater in freshwater forms. In addition, Nash (1931) observed that in both marine and freshwater teleosts the size of the glomeruli and filtration area tend to increase with the size of the fish, but not proportionately. The results showed, there were not any differences in the size of glomeruli in two different ages. It is possible characteristics of species, culturing conditions and enclosed environment, caused to these differences. Until 1983, no data on the structure or function of kidney units in sturgeons was available (Hoar and Radall, 1983). Fang (1986) displayed, the head of kidney in sturgeons is a universal hemopoietic organ, which is in agreement with the results but the most part of caudal tissue of kidney contains most of the nephrons (Krayushkina, 1996), the caudal kidney is composed of nephrons surrounded by hematopoietic tissue dispersed throughout the organ (Edwards and Schnitter, 1933; Endo and Kimura, 1982).

The results showed, the hemopoietic tissue cover from head of kidney to the body of kidney, largely, in 2 year old farmed *Huso huso*. But, this tissue in 1 year specimens had a little distribution. Variable amounts of hemopoietic are distributed among the tubules and vascular spaces in body of kidney, in teleosts (Hoar and Radall, 1983).

The results showed that the average long diameter of proximal tubules in 1 and 2 year old fishes were 63.29±16.15 and 68.3±17.82 μm, respectively, but another experiment on juvenile *Huso huso* (Krayushkina *et al*., 1996) shown that the average diameter of proximal tubules in fresh water was 28.1±0.5 μm. Keeping the fishes in various conditions such as: salinity, temperature and pH, may cause to differences.

In this study the surface of glomerular was 4359.8±1573.59 μm (1 year old beluga) and 5071.04±1916.87 μm (2 year old beluga) but, Cataldi *et al*. (1995) displayed the surface of glomerular of farmed *Acipenser naccari* in fresh water was 174.85±98.38 μm (14 months fishes) and 137.56±62.59 μm (20 months fishes), This differences may be related to the composition of medium and different condition of farming or different species were studied. The dispersion of the glomerulus were accumulate and were not grouped in some points of kidney. As reported by Anderson and Loewen (1975) many teleostean kidneys consist of dispersed glomeruli, but in silver sea bream *Sparus sarba*, glomeruli were grouped as small clusters, situated in the peripheral regions of the kidney (Wong and Woo, 2006).

Histological structure of kidney in juvenile farmed beluga *Huso huso* is similar to tilapia, *Oreochromis mossambicus* and *Oreochromis niloticus* (Cataldi *et al*., 1991) and Silver sea bream, *Sparus sarba* (Wong and Woo, 2006).

The most important role of proximal tubules is transportation and reabsorbing of ions (Junqueira and Carneiro, 2005). So, the high dispersion of proximal tubules in the body of kidney demonstrated that the most transportation and reabsorbing of ions occur in this region.

Also, the high dispersion of collecting tubules in the caudal part of the kidney shows that, the most of the reabsorbing and secretion of water occur in this part (Junqueira and Carneiro, 2005).

The results show that the structure and size of kidney cells is not dependent on the age or size of fish, in juvenile farmed *Huso huso*.

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