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A SENTINEL FISH SPECIES TO HUMAN IMPACT ASSESMENT IN THE SYRDARYA WATERSHED

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Abstract

Water ecosystems of the Aral Sea and Syrdarya River watershed bear adverse anthropogenic impact last decades. Sattar snowtrout (*Schizothorax intermedius McClelland*, 1842) is a widespread and well recognized native species of fishes here. Morphological and histological analyses were provided. Decreasing of maximal age and body length as well as several impairments gills and liver were revealed for populations of sattar snowtrout from different inflows of the Syr-Darya River as a result of human activity. Hence, sattar snowtrout can be recommended as a bioenvironmental sentinel organism for human impact assessment in the Syrdarya watershed.

Key words: ecosystem, Syrdarya watershed, sattar snowtrout, fish

1. INTRODACTION

Shortage of fresh water becomes the major crisis of the 21st century. Desire to satisfy the needs of today have always been determined by people's attitude to water and forced them to overcome the desire to preserve something for future use (Pearce F., 2006). Therefore, freshwater ecosystems have been the most vulnerable elements of the biosphere (Revenga et al., 2005; Jenkins, 2003). Loss of species diversity and transformation, or the disappearance of habitats in continental waters is greater than in terrestrial or oceanic systems. Freshwater and ecosystems are under increasing influence from the construction of dams, water withdrawals for irrigation and industrial uses, pollution and biological invasions (Kudersky, 1991; Ricciardi, Rasmussen, 1999). Examining the status of fish is of great interest for the development of early diagnosis of water pollution - up to the level which threatens human well-being (Hoagstrom, Berry, 2008; Walters et al., 2009).

The Syrdarya (Syr-Darya) River is one of the biggest rivers of the Central Asia. This river begins from the Tien Shan Mountains and brings water to the Aral Sea. Three states as Kirghizia, Uzbekistan and Kazakhstan use water from the Syrdarya.

For the long time the Syrdarya river and the Aral Sea functioned as a single whole. This basin had the great importance for fishery. Residential population used fish resource of the Syrdarya river basin since late Stone Age. The largest fish catches (near 30 thousand tons per year) were being gotten since 1930-thes until 1950-thes. Previously ichthyofauna of the Syrdarya river basin consisted from 28 species of fish and another 24 species was introduced in the last century (Mitrofanov, 1992; Reshetnikov, Shakirova, 1993; Baimbetov, Timirkhanov, 1999; Mitrofanov et al., 2003; Karpov, 2005).

The catastrophic decreasing of the level of Aral Sea in the end of 20th century was made by the using of water of the rivers Amudrya and Syrdarya for crops growing - mainly cotton in Uzbekistan and rice in Kazakhstan. In period since 1961 till 1991 the Aral Sea had water shortage about 750-800 km^3 and about 600 km^3 from that because enlargement of extensive agriculture.

The other side of that problem was the water pollution by different kind of fertilizers and pesticides. After catastrophic decreasing of the level in the end of 20th century, the Aral Sea was divided in two parts. The smaller northern part (Small Aral Sea) in connected with the Syrdarya River and is not so salted as the bigger southern part. The dump in the Berg Strait divides both parts of the Sea now.

N.Amirgaliev with colleagues had investigated contents of chlorocarborn and phosphocarbon pesticides in water of the lower reach of the Syrdarya River and the Aral Sea 1991-1997 (Amirgaliev et al., 1993; Amirgaliev, 1998). Total concentration of isomers of chlorocarborn pesticides varied from 0.06 to 0.44 mkg/l for samples taken from different places. 65-70% of samples had metabolic products of DDT in concentration up to 0.502 mkg/l. Akreis, keltan, tiodan, bayleton, metaphos were found in some isolated samples. Average concentration of the chlorocarborn pesticides and DDT in water samples was 0.04 and 0.08 mkg/l accordingly. The rice pesticides as saturn and propanid were found too. The highest concentration of saturn (7.3 mkg/l) was observed

in some water samples from western part of the Aral Sea. The highest concentration of propanid was 0.25 mkg/l (Annual report, 1991).

At the same time B.Bragin and S.Matmuratov (1997) had done their investigation of pesticides and heavy metals contamination. Results obtained by both teams show that water contamination in 1990-thies was rather significant.

Fishes are mostly consumers of high trophic level and so they are good objects for indication of water pollution. The main purpose of the study was to describe modern fish biodiversity, evaluate some fish characteristics (at the morphological and histological levels) describing modern conditions of fish populations and possible environmental impacts.

2. MATERIALS AND METHODS

Gathering material was carried out during the period from April to September 2012 and 2013. Fish were fixed in formalin, and further the processing was performed in the laboratory. Schematic map of the study area is shown in Figure 1.

The biological analysis of fishes was carried out according to the standard schemes in ichthyology (Holcik, 1989). Age of fishes was determined by vertebras as additional registering structure, the branchiate cover was used. Registering structures were studied under binocular (increase x2-x4) (Chugunova, 1959; Byslov, 2002).

Nomenclature names of most fish are in compliance with Fish Base (Froese, Pauly, 2013).

To assess the diversity of communities the following parameters have been used: S - the total number of species in the community (species richness), D - Simpson's diversity index, E - uniform distribution according to Simpson, H - Shannon index, J - uniform distribution according to Shannon (Bigon et al., 1989). Natural logarithm and the logarithm with the base were to calculate Shannon indices 2.

Calculation of the index of unfavorable state (IUS) is a method of expert assessment of individuals, populations and communities of the whole fish pond based on patalogo-anatomical and environmental performance of fish. The ranking system of pathologies by scores is performed the same way. IUS is defined as the sum of all the scores for all bodies, including infestation and fat accumulation. Males and females on this indicator do not differ, but it is higher in older fish, as with age there is an accumulation in fish harmful substances and various anomalies (Reshetnikov et al., 1999). IUS values allow us to determine the state of the habitat for fish of different ecological groups: the values from 0 to 4 and 0 to 7, respectively, for the peace and predatory fish indicate dwelling in the zone of relative environmental well-being, values 5-7 for peaceful fish and 8-13 for predators correspond to the ecological disaster zone, the above values correspond to a zone of ecological crisis.

Fish gills, livers and kidneys were collected and prepared according the recommendations of J.D. Bancroft and M.Gamble (2002) and S.Mumford et al. (2007). The choice of internal organs as object of study was dictated by the fact that usually all water contaminating xenobiotics is mostly concentrated in the internal organs. To elucidate the pathological changes in the above organs, material processing was carried out by conventional histological methods with filling in paraffin and hematoxylin and eosin (Horobin, Kevill-Davies, 1971). Micrographs were done with histological preparations with a microscope Leica DMLB2, equipped program Biovision 4,0 and camera Leica - DFC 320 , with the increasing x200 , X400.

3. RESULTS AND DISCUSSION

There are 23 native and 12 alien species of fish in the surveyed part of the basin of the Syrdarya River. Native species are: northen pike *Esox lucius* Linnaeus, 1758; Aral roach *Rutilus rutilus aralensis* (Berg, 1916); rudd *Scardinius erythrophthalmus* (Linnaeus, 1758); Turkestan gudgeon *Gobio gobio lepidolaemus* (Kessler, 1872); dace *Leuciscus sp*.; Aral shemaya *Alburus (Chalcalburnus) chalcoides aralensis* (Berg, 1923); Tashkent bystranka *Alburnoides oblongus (*Bulgakov, 1923); asp *Aspius aspius* (Linnaeus, 1758); eastern bream *Abramis brama orientalis* (Berg, 1949); Aral white-eye *Abramis sapa aralensis* (Tiapkin, 1939); sabrefish *Pelecus cultratus* (Linnaeus, 1758); goldfish *Carassius gibelio* (Bloch, 1782), common carp *Cyprinus carpio aralensis* (Spitczakov, 1935); sattar snowtrout *Schizothorax intermedius* (MacClelland, 1842) = *Schizopyge curvifrons* (Heckel, 1838); spotted thicklip loach *Triplophysa strauchii* (Kessler, 1874); Tibetian stone loach *Triplophysa stoliczkai elegans* (Kessler, 1874); Ters stone loach *Triplophysa conipterus* (Turdakov, 1954); Kushakewitz's stone loach *Iskandaria kuschakewitschii* (Herzenstein, 1890); Aral spiny loach *Sabanejewia aurata aralensis*

(Kessler, 1877); wells *Silurus glanis* (Linnaeus, 1758); perch *Perca fluviatilis* (Linnaeus, 1759); sander *Sander lucioperca* (Linnaeus, 1758); sculpin *Cottus sp.*



Fig. 1. Modern distribution of sattar snowtrout *Schizothorax intermedius McClelland*, 1842 in the Syrdarya watershed. Enumerated circles pointed to isolated populations: 1 – Arys river, 2 – Badam River, 3 – Keles River, 4 – Tasatak River, 5 – Bugun River; 6 – Arystandy River, 7 Karashyk River. Arrows without number pointed to water bodies where sattar snowtrout was not found 2002-2013.

Alien fish species are: grass carp *Ctenopharyngodon idella* (Valenciennes, 1844); silver carp *Hypophthalmichthys molitrix* (Valenciennes, 1844); Amur bitterling *Rhodeus sericeus sericeus* (Pallas, 1776); ocellated bitterling *Rhodeus ocellatus* (Kner, 1865); three-lips *Opsariichthys uncirostris amurensis* (Berg, 1932); topmouth gudgeon *Pseudorasbora parva* (Temminck et Schlegel, 1846); Chinese false gudgeon *Abbottina rivularis* (Basilewsky, 1855); Eastern mosquitofish *Gambusia holbrooki* (Girard, 1859); Japanese rice fish *Oryzias latipes* (Temminck et Schlegel, 1846); beautiful sleeper *Micropercops (Hypseleotris) cintus* (Dabry de Thiersant, 1872); snakehead *Channa argus* (Cantor, 1842); Amur goby *Rhinogobius sp.*

Only alien species of fish live in Small Aral's northern part: Transcaucasian goby *Knipowitschia caucasica*, Caspian monkey goby *Neogobius fluviatilis*, gorlap *Neogobius gorlap* and Black Sea flounder *Platichthys flesus.*

In general, variety of fish communities was much poorer than that which would be expected according to published data (about 48 species) (Nikolsky, 1940; Berg, 1949; Dukravets, Mitrofanov, 1992). Indicators of diversity of communities are presented in Table 1. The greatest diversity of fish is noted in Arystandy, most of the Syrdarya, r.Karashik. However, there are alien species in these rivers, indicating the disruption of homeostasis of the native fish community.

Table 1. Indicators of the variety communities of fishes and share of the sattar snowtrout in July, 2013

Indicators	The Arys River- top, 2013	The Arys River middle area	The Mashat River	The Badam River	The Keles River,up	The Keles River	The Bogen River
S (number of species)	5	8	4	4	7	6	4
D	3.24	2.80	2.86	1.77	3.90	3.55	2.60
E	0.65	0.35	0.72	0.44	0.56	0.59	0.65
H (log2)	1.94	2.09	1.73	1.12	2.23	2.18	1.57
H (ln)	1.35	1.45	1.20	0.78	1.55	1.51	1.09
J (log2)	0.84	0.70	0.86	0.56	0.79	0.84	0.79

Share of the sattar snowtrout	0.03	0.07	0.20	0.01	0.07	0.13	0

Continuation of the Table 1

Indicators	The Shayan River	The Arystandy River	The anonymous River	The Karashik river	The Syrdarya River	The Syrdarya River, grows old in the wildlife area	The irrigational channel near Chiili's village
S (number of species)	4	11	2	9	9	9	4
D	2.84	4.83	1.24	3.97	3.08	3.90	2.18
E	0.71	0.44	0.62	0.44	0.34	0.43	0.55
H (log2)	1.68	2.71	0.49	2.40	2.12	2.30	1.41
H (ln)	1.16	1.88	0.34	1.66	1.47	1.59	0.97
J (log2)	0.84	0.78	0.49	0.76	0.67	0.72	0.70
Chare of the sattar snowtrout	0	0.07	0.89	0.01	0	0	0

In the fish fauna of the Syrdarya River and its tributaries significant differences were revealed. Common species are roach, common carp, asp, silver carp, topmouth gudgeon and Amur bitterling. Currently the Syrdarya River lacks many species of fish, leading bottom lifestyle - gudgeon, loaches, sattar snowtrout disappeared. This may be due to changes in the hydrological regime of the river caused by the construction of several dams.

Water resources are fully utilized by the population and most of the tributaries do not reach the Syrdarya. Therefore, the fish population of tributaries is mainly concentrated in the foothill of semi-arid areas. Probably, this largely explains the differences we observed in the fish fauna of the Syrdarya River and its tributaries.

Sattar snowtrout is currently one of the most common species of native fish in tributaries of Syrdarya in the Republic of Kazakhstan (Figure 2, Table 1). This type meets the requirements necessary for indicator species (Karr et al., 1986; Jia, Chen, 2013): it is widespread, fairly plentiful, is benthophage and has sufficient life expectancy, well distinguished from other species of fish.



Fig. 2. The sattar snowtrout

The main characteristics of the studied selections of the sattar snowtrout are presented in table 2.

Table 2. Main biological indicators of the sattar snowtrout selections

Indicatirs		Rivers		
		The Arystandy, 2012, n=12	The Arystandy, 2013, n=19	The Tastak, 2013, n=10
Total length, mm	min-max	105-168	62-151	76-97
	M±s	122.6±18.04	101.0±31,34	82.6±6.83
Standard length, mm	min-max	85-141	50-122	58-78
	M±s	99.3±16.29	81.5±26,57	64.5±6.55
Total weight, g	min-max	13.78-54.67	2,35-32,16	4.47-8.87
	M±s	23.51±11.914	13.34±11.189	5.74±1.372
Weight without interns, g	min-max	10.76-42.26	1,88-27,08	3.24-4.76
	M±s	17.70±9.220	10,32±9,838	3.85±0.497
Condition factor by Fulton	min-max	1.95-2.58	1,70-2,26	1.83-2.76
	M±s	2.28±0.198	1,98±0,176	2.14±0.292
Condition factor by Clark	min-max	1.51-1.85	1,47-1,67	1.57-1.96
	M±s	1.71±0.104	1,58±0,064	1.68±0.150
Age groups		2	4	2
Maximum age		5	5	2
Minimum size/age of sexual maturity approach		85 mm/2 years	77 mm/2 years	61 mm/1year

Continuation of the Table 2

Indicators		Rivers		
		The Keles, 2012 г, n=16	The Mashat, 2013, n=13	The Arys, 2012 г., n=18
Total length, mm	min-max	84-169	28-115	45-259
	M±s	136.2±30.11	67.4±23.78	116.9±72.24
Standard length, mm	min-max	67-137	20-95	36-225
	M±s	111.0±25.35	51.7±18.93	92.6±62.19
Total weight, g	min-max	5.89-58.4	0.22-17.69	0.97-196.13
	M±s	32.89±19.405	4.68±4.880	40.6±67.88
Weight without interns, g	min-max	4.68-43.35		0.64-153.11
	M±s	24.92±14.416		34.9±55.48
Condition factor by Fulton	min-max	1.96-2.26	1.85-2.75	1.72-4.03
	M±s	2.10±0.091	2.27±0.303	2.30±0.730
Condition factor by Clark	min-max	1.56-1.67		1.24-2.88
	M±s	1.60±0.045		1.70±0.574
Age groups		3	4	4
Maximum age		4	3	6
Minimum size(l)/age of sexual maturity approach		67 mm/ 1 year	No date	67 mm/1 year

Reduction in the size and life span of sattar snowtrout may be caused by changes in the hydrological regime and / or catch and pollution of water resources. Morfopathological analysis (Table 3) revealed no serious violations in the external structure and internal organs of sattar snowtrout. The obtained values of the IUS to zone match the relative environmental wellbeing according to the proposed ratings system of Y.S.Reshetnikov et al. (1999).

Table 3. Morfopathological analysis of the sattar snowtrout selections

Selection	Appearance	Gill	Liver	Kidneys	IUS
The Arystandy River, 2012	Curvatures of beams in fins, lane violation in scales	Slightly anemic	Normal	Normal	1.5±0.53
The Arystandy River, 2013	Curvatures of beams in fins, lane violation in scales	Slightly anemic	Grey, mosaic	White, speckled	3.7±1.07
The Tastak River, 2013	Normal	Slightly anemic	Normal	Normal	0.7±0.42
The Keles River, 2012	Curvatures of beams in fins, lane violation in scales	Dual stamens	Normal	Normal	0.4±0.52
The Arys River, 2012	Exophthalmus	Anemic	Unevenly painted	Normal	2.0±0.93

The entire region is characterized by a high level of anthropogenic load, which did not allow to choose a “clean” sattar snowtrout as a quality control. Therefore, the most far away from each other rivers were selected for the histological study - the headwaters of Arys and Arystandy. Histological examination of the bodies found the following in the river Arys:

In the gills of sattar snowtrout, all examined fish experienced a slight swelling in the multilayered epithelium of the primary gill lamellae at the base. There is a large increase in the number and mucous cells in the outer layer. Figure 3 shows the foci of necrosis in the lamellae. The majority of fish have presence of parasitic infestation, accompanied by proliferation of the primary gill epithelium. There is an expansion of the intercellular space in the lamellae and delamination of the lamellae from the basal layer. Also, there is a desquamation of the primary gill’s epithelium (Fig. 4). Individual respiratory cells are in the condition of necrobiosis. There is a swelling the primary and secondary gill’s epithelium.

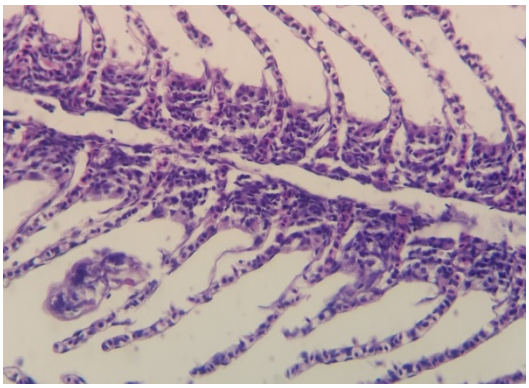


Fig. 3. Parasitic infestation in the gill epithelium
Coloring by hematoxylin and eosin
Increasing X 200

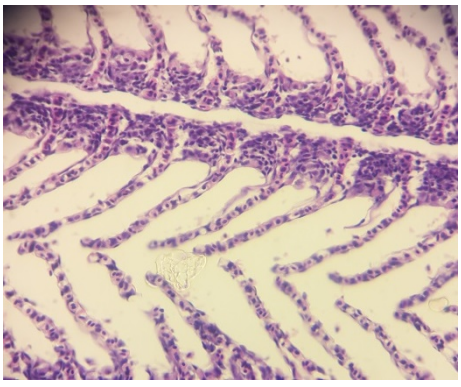


Fig. 4. Desquamation of the primary gill’s epithelium
Coloring by hematoxylin and eosin
Increasing X 200

In the liver, the studied fish sattar snowtrout had foci of necrosis accompanied by karyopyknosis phenomena and the advent of nuclear-free hepatocytes, foci of necrosis of the parenchyma and bile duct epithelium (Fig. 5).

Hepatocytes look exhausted with a centrally located large nucleus (Fig. 5, 6). At a high magnification of microscope vacuolization of cells can be seen in the cytoplasm.

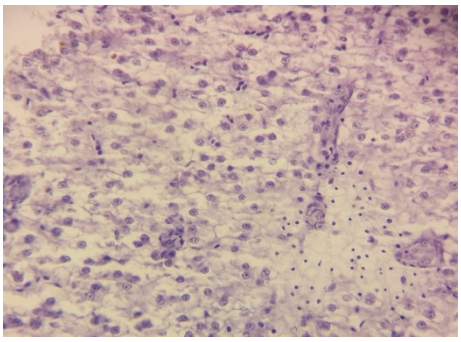


Fig. 5. Karyopyknosis, nuclear-free hepatocytes
Coloring by hematoxylin and eosin
Increasing X 200

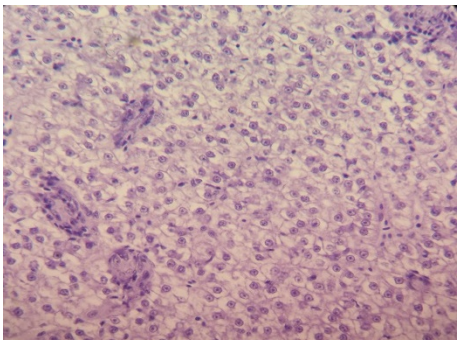


Fig.6. Proliferation of the bile ducts
Coloring by hematoxylin and eosin
Increasing X 200

Inflammatory infiltrates were detected in all tested fish in terms of cell proliferation as lymphocytic series, which could be spotted both in the field of portal tract and in the thickness of the parenchyma (Figure 5, 6). There is a proliferation of the bile ducts, and a violation of the integrity of the deformation of the bile duct epithelium. Depending on the intensity of the process, we were able to observe and focal emergence, sporadically occurring on different parts of the parenchyma.

Histological examination of the organs found in the river Arystandy showed:

On microscopic examination of gills of sattar snowtrout there were areas with normal and pathologically changed structure of the gill apparatus.

Respiratory cells located at the outer layer are larger. Mature mucous cells are too large and located in the outer layer of the primary gill epithelium and between lamellaes space; they are characterized by the displacement of the nucleus to the periphery. There are areas in the lysed between lamellas space.

Also, there is a faint swelling primary epithelium and strong swelling secondary gill epithelium presented in Figures 7, 8.

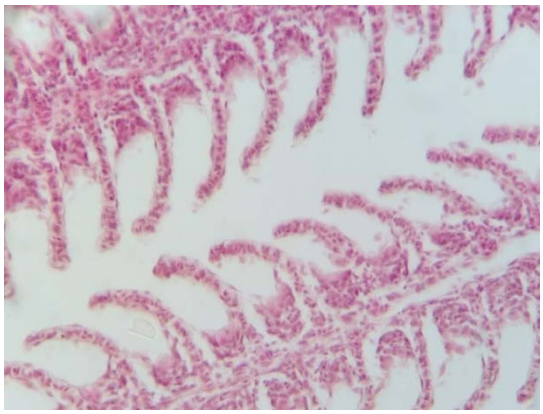


Fig.7. The intercellular space between the layers of the secondary gill epithelium
Coloring by hematoxylin and eosin

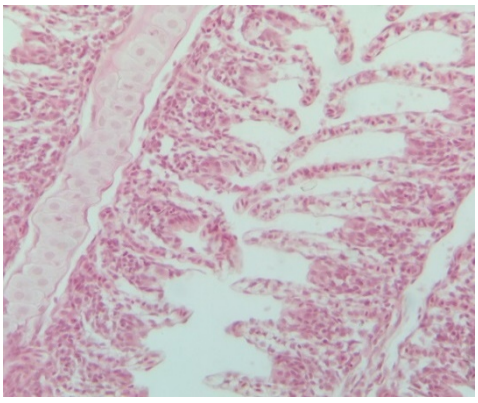


Fig.8. Swelling, destruction of the vascular lammelae layer
Coloring by hematoxylin and eosin

Increasing X 200 Increasing X 200

At the base of the lamellae an expansion of the intercellular space between the layers of the secondary gill epithelium was marked, and in some places on the lamellae too (Fig. 7). Patchy fusion of lamellae was as destruction of the apical part. Vascular disorders were observed in the form of extensions of capillaries in lamellae (Fig. 8).

In the liver, all the tested specimens of sattar snowtrout hepatocytes with well-defined boundary cells, a few desolate (light colored or completely colorless) with a centrally located round nucleus, which contains one, rarely two nucleuses.

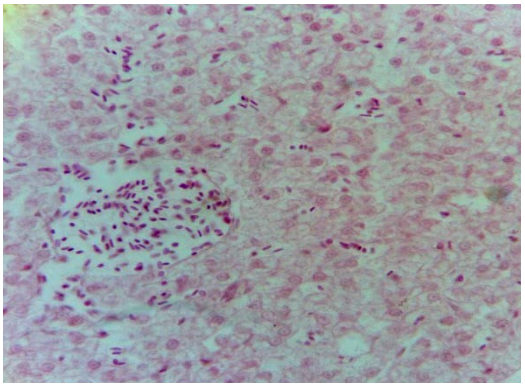


Fig.9. Extensive foci necrosis of parenchyma

Coloring by hematoxylin and eosin

Increasing X 200

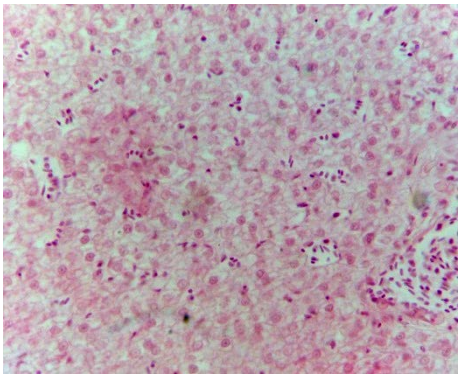


Fig. 10. Hepatocytes with two-nuclear and with absent nucleus

Coloring by hematoxylin and eosin

Increasing X 200

Hepatocytes with absent nucleus are noted absently (Fig. 9, 10). Epithelial integrity of the vessel is broken, torn at the top (Fig. 9). Micronecrosis and foci necrosis, accompanied karyopyknosis. There is a plethora of vessels and expansion of sinusoids. (Fig. 9, 10).

Marked signs of swelling in the gills of fish are an adaptive response to the action of water pollutants. Increasing the number of mucous cells was compensatory - adaptive reaction that increases the protective mucus layer on the surface of the gills in contact with water pollution. In gills sattar snowtrout observed compensatory-adaptive processes and destructive nature.

Processes compensatory-adaptive character manifested in the mucous cells hyperplasia, edema and inflammation phenomena, as well as the appearance of a fairly numerous mucous cells in the secondary gill epithelium. Destructive changes were detected in the composition in the form of a lamella necrosis leading to degradation of entire lamellae. The changes of a destructive nature prevailed over reactive compensatory, indicating that high levels of xenobiotics in the studied reservoirs.

It should be noted in the liver sattar snowtrout pathological changes are mainly compensatory-adaptive character and reduce the viability of populations of this species.

4. CONCLUSIONS

1. Modern variety of fish population consists of 23 native and 11 alien fish species.
2. Sattar snowtrout is one of the most common species of fish.
3. Under the current anthropogenic load decreased maximum size and longevity sattar snowtrout.
4. Histological study of the gills and liver of sattar snowtrout revealed significant pathological changes in these organs.
5. In general, the results indicate poor condition of habitat, but at the same time the high adaptability of sattar snowtrout to contamination. That allows to recommend this kind of as a biological indicator.

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