HISTOPATHOLOGIES IN FISH FROM NORTH CASPIAN SEA [1].

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Histopathological changes of fishes are possible to use as a biomarkers of pollution impact in different water bodies. Histological changes and lesions were analyzed in gobies and other species in the Caspian Sea for several years.

The most numerous investigations are fulfilled with monkey goby (*N. fluviatilis*) one of the most abundant and wide distributed species in the Caspian Sea. Histological changes finding for this species cover almost the whole specter of changes in Caspian fish. Also round goby (*N. melanostomus*), syrman goby (*N. syrman*), gorlap goby (*N. gorlap*), Caspian sprat (*Clupeonella delicatula*), Caspian sand-smelt (*Atherina boyeri*), Caspian roach (*Rutilus rutilus*) and other species were investigated.

Also three sturgeon species were under investigation. Great sturgeon, Russian sturgeon and Starred sturgeon have annual long-distance migrations from Volga and Ural deltas to the South Caspian Sea and back. All three sturgeon species feeding on fish, shellfish and benthic invertebrates, but Great sturgeon prefer fish (up to 90%), Russian sturgeon – shellfish and polychaete, and Starred sturgeon – other benthic impetrates including small crabs.

MATERIALS AND METHODS

Six Great sturgeons (*Huso huso*), two Starred sturgeons (*Acipenser stellatus*) and fourteen Russian sturgeons (*Acipenser guldenstaedti*) were investigated. Organs of sampling were liver, spleen, kidney, and gill. All sturgeons were taken from the commercial fishing in the Ural maritime area (Kazakhstan). Time from taken fishes from the water to dissection was no more two hours. Majority of fishes were still alive, with rare heart activity. After dissecting organs were placed in formalin immediately. Sampling organs for all species except sturgeons were gills, heart, liver, spleen, pancreas, intestine, and kidney. For histology analyses organs were fixed in the field in 10% buffered formalin

Pesticide analysis in fish liver. Pesticides and arochlors widely distributed in the Caspian region were determined in the liver of all investigated fishes. In the field 1 g of liver (exact weights) were ground up, placed in different glass vials and 10 ml n-hexane added. Samples were held at ambient temperature for return to the laboratory. Samples were extracted with *n*-hexane. Hexane was removed and replaced by a new portion of 10-ml *n*-hexane and stirred for 10 minutes. This was repeated three times. All hexane fractions (one from fixing and three from extraction) were pooled in one sample. 2-ml sulfuric acid (concentrated) was added to the extract. The mix was intensively stirred for 15 minutes. The sample was then allowed to stratify and the sulfuric acid removed. This was repeated several times until the extract was clear (usually 2-3 times). For removal of traces of sulfuric acid, the extract was washed three times with 15-ml of distilled water. For removal of the traces of water 2 g of Na_2SO_4 (anhydrous) was added to the extract. The extract was decanted and evaporated to 2 ml: If necessary, volume was adjusted by a new portion of hexane. Measurement of the concentration and type of pesticide was carried out on the gas-liquid chromatograph CHROM-5, using standard solutions of known substances for comparison.

Histopathology. Tissue sections from the organs listed above were stained routinely with hematoxylin and eosin (Luna, 1968) and examined for histopathological abnormalities. The severity of abnormalities were scored on a scale of 0-3: Liver: macrophage aggregation – 0, fat degradation and regeneration – 1; Cloudy swelling of cytoplasm – 2; necrosis – 3. Kidney: macrophage

[1] - These data were presented on the poster session of 10th European Congress of Ichthyology (ECI X) in Prague, September 3-7, 2001 and 26th Session of the International Seminar on Planetary emergences in Erice (Italy) August 24-25, 2001

aggregation, tubula vacuolation, and parasites – 1; glomerulla vacuolation – 2. Spleen: macrophage aggregation – 0; fat degradation and regeneration – 1; vacuolation –2. Gill: aneurysm and parasites – 1; macrophage aggregation and epithelia proliferation within gill – 2. Neoplasms, fibrosis, granulomas, spongiosis hepatis and parasites were noted as being present or absent for each organ.

RESULTS

α, β, and γ HCH isomers were founded. α-HCH are the most common among HCH. It was determined in 5 Great Sturgeons and 8 Russian Sturgeons. β-HCH was determined in one Great sturgeon and 4 Russian sturgeons. Only once β-HCH was founded in absence of α-HCH. This is almost true for γ-HCH. Usually concentration of all isomers HCH is not very high and varied from 0.09 μg/g to 0.74 μg/g. Only once concentration of γ-HCH was 1.03 μg/g in the liver of Russian sturgeon. No α-HCH or β-HCH was founded in this fish.

DDT and/or its isomers were founded in all investigated fishes. DDT and DDE are more common then DDD. Usually concentration of DDT is several times higher, then DDD or DDE.
Policholbiphenile A50 was determined in all six Great sturgeons, one Starred sturgeon, and 10 Russian sturgeon (13 Russian sturgeon were investigated). It concentration varied from 2.01 μg/g to 12.95 μg/g. It seems, that concentration of all pollutants are higher in Great sturgeon comparative to Russian sturgeon.

Table 1. Concentration of organochlorides in the liver of fishes (μg/g, wet weight)
Caspian sea 2000, (Kazakhstan part, near the Ural Delta)

species	A50	DDT	DDD	DDE	γ-HCH	β-HCH	α-HCH
Great sturgeon	4,53	67,92	16,46	19,67	-	-	0,18
Great sturgeon	12,95	11,58	-	3,94	-	0,4	0,26
Great sturgeon	5,56	4,42	3,12	16,18	-	-	0,25
Great sturgeon	5,18	3,47	-	5,12	-	-	0,32
Great sturgeon	6,83	8,69	-	4,23	-	-	-
Great sturgeon	4,13	7,53	5,16	26,49	0,35	-	0,74
Russian sturgeon	4,14	3,17	-	2,16	1,03	-	-
Russian sturgeon	2,54	4,54	-	1,16	0,12	-	0,1
Russian sturgeon	3,89	10,42	2,45	1,18	-	0,4	0,65
Russian sturgeon	5,55	-	0,65	0,79	-	0,5	0,35
Russian sturgeon	-	-	-	-	-	-	0,31
Russian sturgeon	5,54	-	2,89	1,39	-	-	0,79
Russian sturgeon	2,13	2,01	-	2,01	0,11	-	0,09
Russian sturgeon	2,21	15,2	-	0,52	-	0,19	0,1
Russian sturgeon	-	-	-	-	-	-	-
Russian sturgeon	-	6,08	-	-	-	-	-
Russian sturgeon	2,01	11,15	-	0,63	-	-	0,18
Russian sturgeon	2,07	-	-	0,81	-	-	-
Russian sturgeon	4,34	9,1	-	1,71	-	0,36	-
Russian sturgeon	-	7,13	-	-	-	-	-
Starred sturgeon	2,17	4,86	-	0,85	-	-	-
Starred sturgeon	-	8,1	1,29	1,28	0,28	-	-

Goby species

Monkey goby – *Neogobius fluviatilis pallasi* (Berg, 1916) – 77 specimens
Round goby – *Neogobius melanostomus affinis* (Eichwald, 1831) – 14 specimens
Syrman goby – *Neogobius syrman eurystomus* (Kessler, 1877) – 5 specimens
Gorlap goby – *Neogobius gorlap* Iljin, 1927 = N. kessleri gorlap – 8 specimens

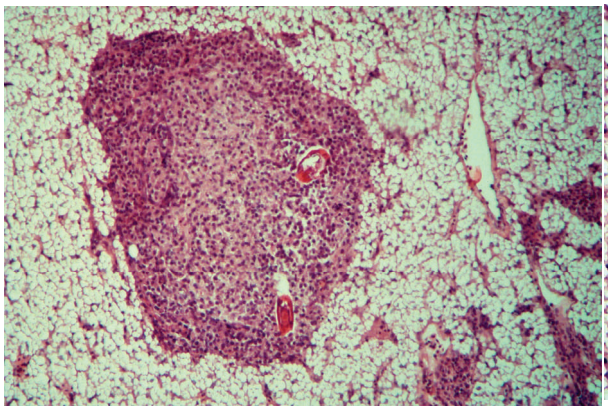

Fig. 1. Tumor in the liver of round goby

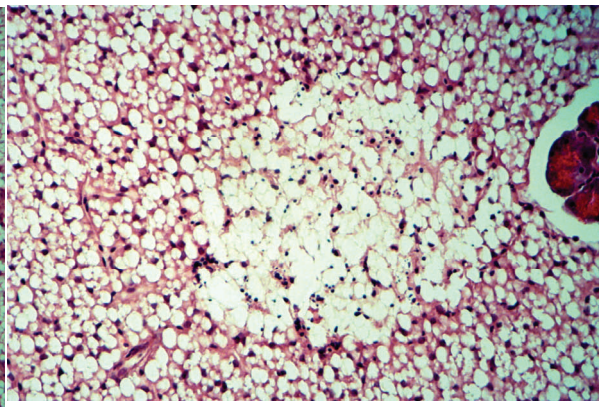

Fig. 2. Necrosis in the liver of monkey goby

Caspian goby – *Neogobius caspicus* (Eichwald, 1831) – 2 specimens
Deepwater goby – *Neogobius bathybius* (Kessler, 1877) – 9 specimens
Blackspot goby – *Mesogobius nigronotatus* (Kessler, 1877) – 3 specimens
Gobies of all species were investigated from several locations in the eastern part of North Caspian Sea (95 specimens) and from three locations in the Middle Caspian Sea around western coast (28 specimens)

Liver

Fatty degeneration was found out for all goby species. The big lipid vacuole appeared in the hepatocytes and driven cytoplasm and nucleus to the walls. Very often it lead to atrophy of the nucleus.

Collangioma (cirrhosis**)** - proliferation of connective tissue inside the liver.

Necrosis**.** Cytoplasm become colorless, nuclei decrease in size and destroyed. Destroying of cell's wall begin. (Fig. 2)

Basophilic foci**.** Cytoplasm of hepatocytes become dark blue. Nuclei increase in size, and have intensive coloration. No vacuoles at all.

Tumor. Once we found something looking as a tumor in the liver of round goby. (Fig. 1)

Spleen

Macrophage aggregations were found out almost in all investigated fishes.

Atrophy. Erythrocytes disappear. Cytoplasm of some cells become colorless, nuclei have pale coloration.

Proliferation of connective tissue**.** External tunica of the spleen becomes thicker. Additional connective cords appear inside the organ.

Necrosis was found out in one monkey goby, simultaneously with parasite.

Cell vacuolation and tumors were found out several times.

Pancreas

Degradation of acinar cells around intestine**.** Acinar cells lose granules of zymogen, become pale, cytoplasm become homogeneous, nuclei don't see well. It is found out in many goby specimens.

Vacuolation of acinar cells detected out only in parts of pancreas inside the liver. It was found out in *N. fluviatilis* only.

Intestine

Degradation of intestine walls

Vacuolation of epithelial cells very often accompany inflammation of intestine walls.

Macrophages were found out in *N. fluviatilis* and *N. melanostomus*.

Kidney

Destruction of canaliculus were one of the most often changes. Cells of canaliculus become round and lose connections with each other.

Vacuolation of canaliculus**.** Hydropic vacuole appears in single cells. Cytoplasm and nucleus

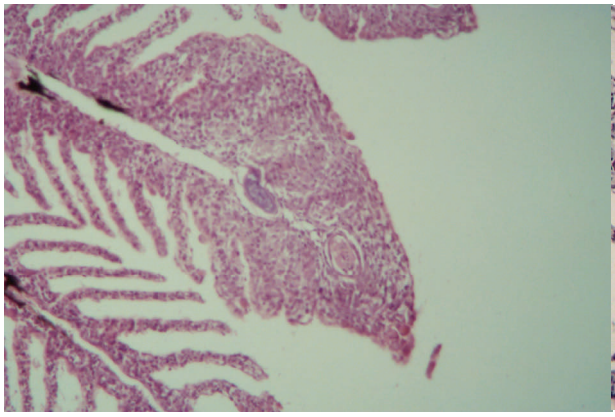

Fig. 3. Hyperplasia of gill's epithelial cells of monkey goby

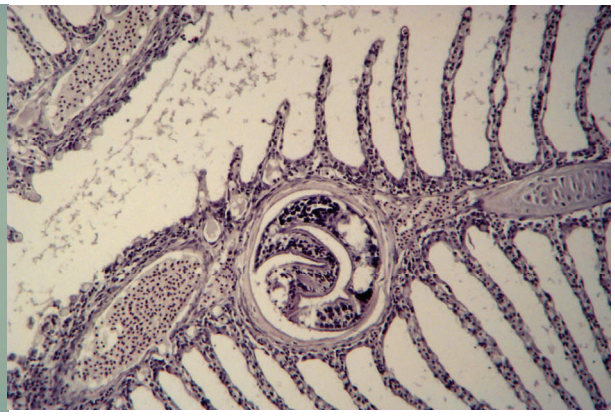

Fig. 4. Parasite in the gill of monkey goby

are driven to the wall.

Connection between interstitial cells destroyed**.**

Necrosis of canaliculus and destruction of glomerulus. External tunica of glomerulus destroyed, cells lose connection with each other and distance between them become more than a half of its diameter.

Parasites were detected twice inside kidney.

Heart

Single changes were found out only in monkey goby. All types of changes were observed only once. It is cytoplasm granulation, fibrosis of muscle cells, atrophy.

Gills

Hyperplasia of epithelial cells is one of the most common lesions. Lamellar and interlamellar epithelium proliferates and packs the space between secondary lamellas.

Aneurysm of secondary lamellas. Dilatation of lamellas and stagnation of blood in it.

Parasites. Many specimens have different type of parasites encapsulated in secondary and/ or primary lamellas. Identification of parasites weren't done.

Caspian Roach *Rutilus rutilus caspicus* (Jakowlew, 1870)

Only 38 specimens not far from the Ural delta were investigated.

Liver: Macrophage aggregations were found in all investigated fish and once hydropic vacuolation of hepatocytes was find out.

Spleen: Macrophage aggregations were found out in all investigated fishes.

Kidney: Vacuolation of canaliculus were found out in 8 fishes and macrophages were found out in 7 fishes.

Intestine: Only once macrophages inside intestine submucosa were found out.

Pancreas: In all fishes acinar cells around intestine were displaced by adipose tissue. No changes in pancreas inside liver were observed.

Heart: Only once macrophages were found out. In the same fish macrophages were found in the intestine.

Gills: Once proliferation of interlamellar epithelia and twice aneurysm of secondary lamellas were found out.

Caspian sprat *Clupeonella delicatula caspia* (Nordmann, 1840)

Only 11 specimens from North and Middle Caspian Sea were investigated.

Liver: There were no changes in the liver of fishes from the North Caspian Sea. In fishes from Middle Caspian Sea several lesions were observed. Macrophage aggregations, blood stagnation, necrosis of hepatocytes, granulation of cytoplasm, and parasite were found out.

Spleen: In all investigated fishes macrophage aggregations were found out.

Pancreas: In the fishes from the Middle Caspian Sea macrophage aggregation and replacement of acinar cells by adipose tissue were found out.

Heart: No changes were found.

Gills: Only once aneurysm and parasite were detected in the fish from the Middle Caspian Sea

Gonads: Proliferation of connective tissue was found out in one male.

Caspian sand-smelt *Atherina boyeri caspia* (Eichwald, 1831) = Atherina mochon pontica natio caspia

Only six specimens from two locations in the North Caspian Sea and seven specimens from one location in the Middle Caspian Sea were investigated.

Liver: Fatty degradation, macrophage aggregation, and granulation of cytoplasm were found out both in North and Middle Caspian locations.

Spleen: Only two fishes were examined. Both have macrophage aggregations and one has collangioma.

Kidney: One fish from North Caspian Sea has parasites and degradation of canaliculus. One fish from Middle Caspian Sea has vacuolation of canalicullus

Intestine: No changes were found.

Pancreas: No changes were found in fishes from North Caspian Sea. All fishes from Middle Caspian Sea have replacement of acinar cells by adipose tissue. One fish has macrophages between acinar cells.

Heart: No changes were found.

Gills: No changes were found.

Gonads: No changes were found.

Sturgeon species (*Huso huso*), (*Acipenser stellatus*), (*Acipenser guldenstaedti)*

Liver:

Fat degradation. Vacuole with glycogen and lipids are volume the whole cell. Nuclei are not evident, cytoplasm are only near cell's wall and become colorless. Cell's walls are regular. It is found out in two Great sturgeons and three Russian sturgeons, and don't found out in Starred sturgeon

Macrophage aggregation. Macrophages usually founded near big blood vessels, hemopoetic cell, and among hepatocytes. It is founded in all fishes.

Regeneration. Regeneration hepatocytes very often displace in narrow stripes with width in two-three cells. Usually it is small area. Nuclei are increased in size and have intensive color. Cytoplasm also has intensive coloration and contains no vacuoles. Usually it is more basophilic comparative to surrounding hepatocytes. It is found out in two Great sturgeons.

Necrosis. Hepatocytes are distracted, all cell's structures destroyed, cytoplasm is almost colorless. Cell's walls are partly destroyed. Two loci of necrosis are found in one Great sturgeon. One of the loci is big enough.

Cloudy swelling. Hepatocytes lose all vacuoles, it become more eosinophilic and granular. Inside cytoplasm more condense parts appear. It is found only once in Great sturgeon.

Spleen

Macrophage aggregation. Macrophages in spleen contain lot of hemosiderin and their appearances confirm misbalance in hemopoetic process. It is found in one Great sturgeon and two Russian sturgeons.

Vacuolation. Nuclei are of normal size and shape and displace to cell walls. It is found in one Great Sturgeon.

Regeneration. Cells have increased nuclei, cytoplasm are paler. It is found in one Great Sturgeon.

Fat degradation. Big lipid vacuoles appear in hemopoetic cells. Nuclei are driven to the walls. One or several vacuoles take more than a half of cell's volume.

Kidney

Macrophage aggregation. It is found in three Great sturgeons and one Russian sturgeon.

Renal tubular vacuolation (RTV). Some cells of canalicules are vacuolated. Nuclei and cytoplasm are driven to the walls. Usually it is only one vacuole and not very big. It is found in one Great sturgeon, one starred sturgeon and five Russian sturgeons.

Glomerulus's destruction. External tunica of glomerulus is destroyed, cells lose connection with each other, intercell's distances increase to half cell's diameter. All cell's structures are normal. Some cells can be vacuolated. Vacuole is not big situated near the wall. It is found in one Russian sturgeon, but without tunica destruction and with vacuoles.

Gills

Aneurysm of secondary lamellas. Dilatation of lamellas and stagnation of blood in it. It is found in three Great sturgeons, one Starred sturgeon, and two Russian sturgeon.

Hyperplasia of interlamellar epithelia. Interlamellar epithelial cells proliferate and volume all the space between lamellas. It is found in two Russian sturgeons.

Proliferation of epithelia. Lamellar and interlamellar epithelial cells proliferate simultaneously. It is found in one Great sturgeon.

DISCUSSION

Comparing to goby species roach has fewer lesions and changes of inner organs. Nevertheless, there are some abnormalities detected only for roach and never observed in other species. There were hydropic vacuolation of hepatocytes, macrophages between muscle cells in the heart, focal tumors and necrosis in testes. We never observe in the roach such lesions as necrosis, degeneration, and atrophy of pancreas. In general there were more macrophage aggregation in roach, than in other species. So, roach has your own specter of pathologies not identical to goby specter even habitat on the same loci. There was more pathology in all organs, except kidney, in goby

Table 2. Histopathological changes in sturgeons (per cent of investigated specimens)

organ	pathology	Great sturgeon		Russian sturgeon		Starred sturgeon	
		1999	2000	1999	2000	1999	2000
liver	M	9.4	9.4	9.4	9.4	9.4	9.4
	Cl	9.9	16.67	-	-	-	-
	Fd	-	33.33	21.43	21.43	1.6	-
	R	-	33.33	-	-	-	-
	Nc	-	16.67	-	-	-	-
kidney	M	28.57	50.00	64.71	1.7	58.33	-
	RTV	28.57	16.67	58.82	35.71	26.67	50.00
	V	-	-	-	-	1.6	-
	Pr	-	-	-	-	20.00	-
	G	-	-	-	1.7	-	-
spleen	M	9.4	50.00	9.4	1.7	87.5	-
	V	-	16.67	9.9	-	1.12	-
	R	-	16.67	-	-	-	-
	Fd	-	-	-	21.43	-	-
gill	A	36.36	50.00	1.12	14.28	30.77	50.00
	Pl	-	16.67	-	14.28	30.77	-
	M	-	-	-	-	25.00	-
	Pr	9.9	-	-	-	-	-

M – macrophages RTV – tubula vacuolation G – glomerulla destruction
Nc – necrosis Fd – fat degeneration Cl – cloudy swelling of cytoplasm
Pr – parasites R – regeneration Pl – proliferation of gill (lamellar and/or interlamelar) epithelia
A – aneurysm

species comparative to roach. On the contrary in kidney roach has much more pathology than the most sensitive goby species – syrman goby.

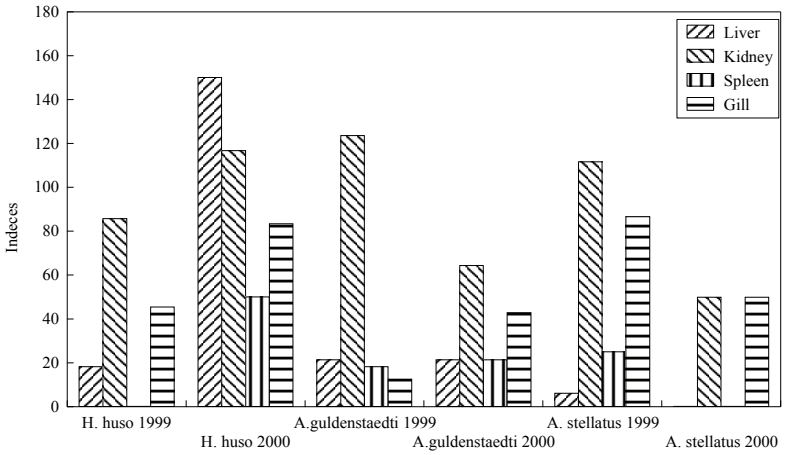

Fig.5. Histopathological changes in sturgeon from the Ural delta

Sprat and sand-smelt are the pelagic fishes with feeding different from gobies and roach. Fat accumulation in sprat occurs around intestine as in roach. This two species never have fatty degeneration of hepatocytes. In sand-smelt fat accumulates in the liver as in goby species and all those species have fatty degeneration of hepatocytes. Sand-smelt and sprat have granulation of cytoplasm in hepatocytes, which never observed in goby species and roach. Both pelagic species were investigated simultaneously on several locations. In all cases there were more pathologies in all organs in sprat, than in sand-smelt. It means that sprat is more sensitive to inconvenient environment, than sand-smelt. There were more pathologies in sprat than in goby species habitat on the same location. Number of pathologies in inner organ of sand-smelt is almost equal to deepwater goby from the same location.

According to the number of different lesions sprat is more sensitive than all other investigated species. Goby species were divided into two groups. For the first group (*N. melanostomus; N. bathybius*) more pathological changes were found in the liver and spleen, and for the second group (*N. fluviatilis, N. caspicus, N. syrman*) more changes were found in the kidney and gills. Gorlap goby is more resistant, than other gobies. (Fig. 6, 7).

Comparative to 1999 there are more pathological changes in Great sturgeon liver (Table 2, Fig. 5). Fat degradation, regeneration, and necrosis were observed in the liver of Great sturgeon only in 2000. In kidney of Great sturgeon we found the same changes as in 1999, but macrophage aggregation become more common, and renal tubule vacuolation on the contrary become rarer. In the spleen macrophage aggregation were founded only in 50% of fishes (100% in 1999), and vacuolation and regeneration were founded only in 2000. Aneurysms of secondary lamellas (gill) become more common in 2000, also proliferation of gill epithelia, but there were no parasites founded in the gill and other organs. Indices of pathological changes become higher for Great sturgeon in 2000 for all organs. This means, that environment become worth for Great sturgeon.

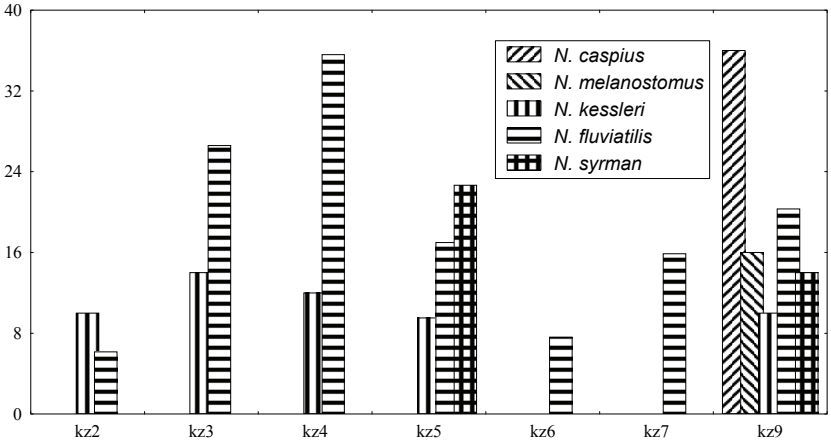

Fig. 6. Estimation of all pathologies in different goby species in the North Caspian Sea

On the contrary to Great sturgeon, in Russian sturgeon there are less pathologies comparative to 1999. We found the same pathologies as in 1999, but it become rarer. The only exception are the gills. Percent of fishes with aneurysm of secondary lamellas are similar in 1999 and 2000, but proliferation

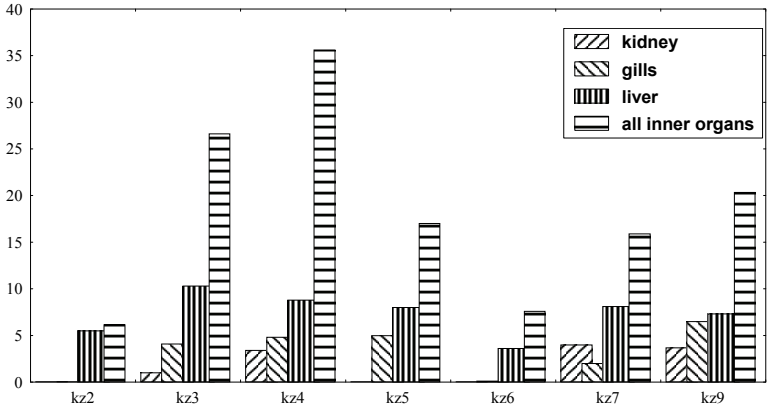

Fig.7. Pathological changes in monkey goby from several locations in the North Caspian Sea

activity of epithelium cells becomes significantly higher. This means, that environment for Russian sturgeon become a little bite better or the same as in 1999.

It is difficult to compare results of 1999 and 2000 as only two specimens of Starred sturgeon were investigated in 2000. But it seems, that situation is equal to Russian sturgeon, either becomes a little bite better.

Maximum of pathological changes in the North Caspian Sea were found in fishes from the mouth of Ural delta. Its evidence, that environment for fishes here is worth. The better environment was in the open sea not far from the Ural delta. Ecology conditions in the mouth of Ural delta are not stable during the year and depend from the amount of pollutants incoming with river influx. (Fig. 6) .

Only two specimens of gobies were investigated from the south of Middle Caspian Sea near the Azerbaijan cost. Both specimens have significant lesions in liver, kidney, and pancreas. Hepatocytes contain small amount of lipid vacuoles. Biological condition of both fishes is critical. On location near to Baku city and oil drilling field many goby specimens have necrosis, tumors, and blood stagnation in the liver, proliferation of connective tissue in the spleen. All fishes had heavy lesions of gills. Biological condition of all fishes is critical. On the north of Middle Caspian Sea near to the boarder with North Caspian Sea gobies, sprat, and sand-smelt were investigated. In the liver of goby species accumulation of glycogen in the liver was from small to medium. Some gobies had blood stagnation in the liver. Some fishes had necrosis and proliferation of connective tissue in different organs. Many fish had lesions in the gills. Biological condition of sprat is dangerous. There were not so much pathologies in the sand-smelt from the same location. Biological condition of gobies and sand-smelt is good enough.

Conclusion

- For the future monitoring sprat and complex of gobies species (*N. fluviatilis – N. melanostomus – N. gorlap*) are the most suitable.
- Biological condition of fishes in locations remote from deltas is good. Biological condition of fishes in the mouth of the Ural Delta is not stable and changes from good to critical during the year. According to the number of pathological changes zone around mouth of Ural delta is one of the most polluted site in the eastern part of North Caspian Sea.
- Biological condition of all fish species is worse in the Middle Caspian Sea (Western coast line) comparing to the North Caspian Sea. It is especially true for polluted sites around oil drilling fields.

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***Жимбей Е. Н.*, Митрофанов И. В.**.* Случаи гистопатологии у рыб Северного Каспия**

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