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*MORPHOFUNCTIONAL FEATURES OF COREGONIDAE IN THE MOUTH OF THE OB RIVER AS INTEGRAL ESTIMATION OF THEIR HABITAT CONDITIONS**

SUMMARY. Water ecosystems of the Ob-Irtysh basin are under intensive anthropogenic load. The most important bioindicator of prolonged water pollution is the fish as the most mobile, fecund, and high-organized water biocenosis component. Water intoxication causes fish lesions, mostly Coregonidae are affected. To avoid annual winterkilling in the middle Ob and in the lower Ob they go to the Gulf of Ob, where they hibernate until the ice melts and the water refreshes. To estimate the water quality in the ecosystem of the lower Ob and the Gulf of Ob during winter season and on the whole, over a number of years, the condition of gills, liver, gonads, and other morphological features of peled, muksun, and Siberian whitefish during their anadromous migration to the Ob River are studied. It is established that the plankton-eating peled has minor abnormalities of gonads, liver, and branchial epithelium. Despite the fact that the Gulf of Ob has a low level of pollution, the plankton-benthos-eating muksun is proved to have liver deviations and considerable pathomorphological changes of branchial epithelium, though gonad abnormalities are infrequent. The benthos-eating Siberian whitefish has minor abnormalities of the internals. Registered species abnormalities can be caused by the high level of pollution in the middle Ob, where muksun, and sometimes peled, return to breed. On the contrary, the bulk of Siberian whitefish and some of peled spawn in the pure waters of the Urals.

KEY WORDS. The Gulf of Ob, peled, muksun, Siberian whitefish, migration, habitat conditions, gonads, liver, gills, development disorders.

The existence of a species is caused by high reliability and stable rhythmical functioning of the reproductive system and other body systems in various environmental conditions [1-6], considerably transformed under the influence of anthropogenic load. The latter affects, to a great extent, water ecosystems [7-9] due to their natural accumulative characteristics. The final trophic link is the fish as the most mobile, fecund, and high-organized water biocenosis component. Numerous published works [10-20] indicate the effective use of fish as bioindicators of prolonged water pollution. Among the fishes of the Ob-Irtysh basin, *Coregonidae* are under the greatest anthropogenic load. Depleted by intensive capture fishery,

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they are less resistant to intoxication and considerable season fluctuation of water conditions. Most of *Coregonidae* migrate to the Gulf of Ob, avoiding winterkill, but some inhabit it permanently (whitefish) or spend there most of their lives (muksun). There they fatten and mature, and migrate to this area after the spawning season [9]. The Ob River discharges its waters with pollutants into the Gulf of Ob, accumulating the polluted river flows from the Urals, Kazakhstan and other territories of Western Siberia. The level of petroleum hydrocarbons pollution of water and bottom sediments of this estuary is characterized as low. The level of heavy metal pollution is high, with excessive concentrations of zinc, nickel, chrome, and copper in bottom sediment [21]. This factor should be considered the determinant in the assessment of gills, liver, and gonads primarily of benthos-eating *Coregonidae*. The condition of these internals of *Coregonidae* that hibernate in the Gulf of Ob and that migrate to the Ob River after wintering was studied earlier [9], [22], [23]. However, the continuous changing of climate with the lowering water content of the Ob basin, accompanied by increasing pollution, explains the relevance of this research.

The purpose of this research is to identify the condition of gonads, liver, and gill apparatus of *Coregonidae* with various types of feeding during the anadromous migration period in the mouth of the Ob River after wintering in the Gulf of Ob in the low water conditions.

The data and methods of the research. The ichthyological data were collected in June, 2-15, 2012 in the estuarial part of the Ob River (near Yamburg settlement). *Coregonidae* with various feeding preferences were investigated: the plankton-eating peled, the plankton-benthos-eating muksun, and the benthos-eating Siberian whitefish. The fish were measured for the fork length and weighed on the ScoutPRO-600 (*Ohaus*) scales. The age was determined by scale reading. Hemoglobin content (Hgb) in the blood from the hemal canal was determined with the *MiniGem 523* hemoglobinometer. Gonads, liver, and gills were fixed in the Bouin's solution, preserved in 4% neutral formalin. The histology test of the gonads was carried out in the Center for Environmental Research and Reconstruction of Biosystems, the faculty of Biology, Tyumen State University, using standard methods [24]. After the dehydration of objects in high-proof alcohol and paraffin embedding in the EC-350 (*Microm*) embedding center, the sections 5 μm thick were prepared by means of the *HM 355S* (*Microm*) rotary microtome, stained with *Heidenhain*'s iron hematoxylin, and then placed in the *Bio Mount* (*Bio Optica*) synthetic medium. The specimens were analyzed with 40x, 100x, 200x, 400x, and 1000x magnifications using the *AxioImager A1* (*Zeiss*) microscope, and recorded with the *AxioCam MRc5* video camera, using the licensed *AxioVision 4.7.1.* software.

For the statistical analysis, STATISTICA Statsoft, Inc. (v.6) and MS Excel (2007) were used.

Various methods of analysis were employed, and 51 specimens of *Coregonus peled*, 50 specimens of *C. muksun*, and 33 specimens of *C. lavaretus pidschian* were investigated.

The water samples from the Gulf of Ob and the estuary of the Ob were taken at the end of winter and in the early summer, respectively. They were analyzed in the Ecological Research Laboratory of Tyumen State University, with the *I-130* ionomer, the *ANION 410* ionomer-conductometer, the *ICS-2100* ion chromatograph, the *ICS-1100* (*Dionex*), and the *UNICO-2100 UV* spectrophotometer.

Results. After the ice clearance in the mouth of the Ob River, fish migrated from the Gulf of Ob where they had entered because of suffocating waters. It is the part of the river that provides the most reliable analysis of functional fish condition for the time being. During these two weeks of migration, the water temperature changed from 6-7°C in the southern part of the Gulf of Ob to 16-17°C in the estuary of the Ob River (Yamburg settlement), the hydrochemical research is presented in Table 1. The water is supposed to be pure for all the components under study, except for the iron and copper concentrations. In comparison with the middle Ob, there are no large plants or urban agglomerations that could cause water pollution in the lower Ob and in the Gulf of Ob. However, toxicants accumulate considerably in soil [21].

Table 1

Chemical water composition in the Gulf of Ob and in the estuary of the Ob River in spring-summer period, 2012

Index	Sampling Location		Maximum allowable concentration of fishery waters
	The Gulf of Ob, near Novy Port settlement (April, 21)	The estuary of the Ob River, near Yamburg settlement (June, 15)	
pH	7.1	6.9	6.5-8.5
Specific electrical conductivity, μS/cm	235.0	55.5	not rated
HCO_3^-, mg/l	113.166	21.607	not rated
Total alkali, mmol/l	1.8552	0.3542	not rated
Li^+, μg /l	0.004	0.001	80
Na^+, mg /l	9.6	2.3	120
NH_4^+, mg /l	0.08	0.025	0.5
K^+, mg /l	1.4	0.6	10
Mg^{2+}, mg /l	0.3	2.1	40
Ca^{2+}, mg /l	27.5	6.5	180
F^-, mg /l	0.123	0.044	0.05*
Cl^-, mg /l	7.3	1.5	300
NO_2^-, mg /l	0.007	0.02	0.08
Br^-, mg /l	0.019	not detected	1.35
NO_3^-, mg /l	2.7	0.11	40
SO_4^{2-}, mg /l	8.9	3.0	100
PO_4^{3-}, mg /l	0.05	not detected	0.15
Si, mg /l	7.3	1.27	not rated
Fe (total), mg /l	3.169	3.236	0.1
Mn^{2+}, μg /l	4.81	0.901	10
Cu^{2+}, μg /l	0.765	1.404	1
Cr^{3+}, μg /l	<0.5	<0.5	70
Ni^{2+}, μg /l	0.765	<0.5	10
Pb^{2+}, μg /l	<0.5	<0.5	6
Cd^{2+}, μg /l	<0.1	<0.1	1

* In addition to the background fluoride content, but not higher than their total content of 0.75 mg/l.

Peled. The catches were exemplified by immature and fertile specimens, some of them were missing out the regular spawning season, the other were ready for the upcoming spawning. The age of the fish varied from 4 to 7 years. The length and weight characteristics of fish are given in Table 2. Almost all of them (96%) had internal parasites: plerocercoids of gull-tapeworm *Diphyllobothrium dendriticum*. In some fish, protozoan cysts were found in the liver and helminthes in the gastrointestinal tract. Most of fish had poor intestine filling and low fat content. The average hemoglobin content was 98 g/l in the blood of female fish and 90 g/l in the blood of male ones (Table 2). The gonadosomatic index of immature females did not exceed 0.6%.

Table 2

Coregonidae morphofological parameters during the anadromous migration from the Gulf of Ob

Parameter	Species					
	Peled		Muksun		Siberian Whitefish	
	females (38 specimens)	males (13 specimens)	females (26 specimens)	males (24 specimens)	females (24 specimens)	males (9 specimens)
Fork length, mm	318.1±3.6 288-402	295.9±4.5 266-335	507.6±6.4 447-560	506.3±6.5 442-557	313.7±7.2 202-386	296.1±10.9 237-340
Weight, g	340.9±15.1 233-771	263.9±14.5 220-407	1,846.8±85.2 1,245-2,580	1,750.3±71.2 1,160-2,273	336.7±21 77-558	275.4±30.8 117-401
Gonadosomatic index, %	0.84±0.04 0.4-1.3	0.25±0.01 0.17-0.34	1.30±0.2 0.25-3.3	0.25±0.02 0.06-0.56	1.5±0.1 0.37-3.6	0.20±0.05 0.04-0.40
Hemoglobin concentration in blood, g/l	98.8±4.6 62-162	90.3±3.5 74-115	100.1±3.7 70-136	102.9±3.05 74-131	90.4±4.7 52-130	99.3±7.3 60-121
Fat content, points	1.8±0.1 1-4	1.3±0.2 1-3	3.2±0.1 1-5	3.3±0.1 2-5	2.1±0.2 1-4	1.3±0.1 1-2
Intestine filling, points	2.1±0.1 1-3	2.0±0.2 1-3	2.9±0.03 2-3	2.9±0.04 2-3	1.9±0.1 0-3	1.6±0.2 1-3

Note: the numerator is the average arithmetic and standard error, and the denominator represents the variation limits.

Previtellogenic oocytes were present in gonads at the phase of physiological maturation of germinal cells (stage II). There were oogonial nests and early meiotic prophase oocytes (EMPO) between the previtellogenic oocytes. The mature fish GSI (gonadosomatic index) increased to 1.2% (Fig. 1). Degenerating follicles from the previous spawning were present in gonads at the phase of functional maturation and physiological maturity (stage IV and IIIa). Oogonial nests and early meiotic prophase oocytes (EMPO) were formed in contact with the degenerating follicles. The maturation of germinal cells was exemplified by oocytes at the phase of cytoplasmic vacuolization (Fig. 2a). Some gonads of immature males had poorly developed generative tissue, extruded by connective tissue bands (Fig. 2b), the germinal cells were exemplified by Type A spermatogonia germ cells, and less frequently by Type B germ cells (Fig. 2c).

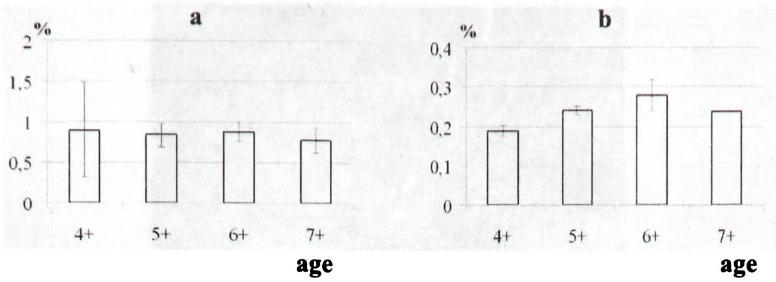

Fig. 1. Gonadosomatic index of Peled females (a) and males (b) of different age groups during the anadromous migration to the estuary of the Ob River

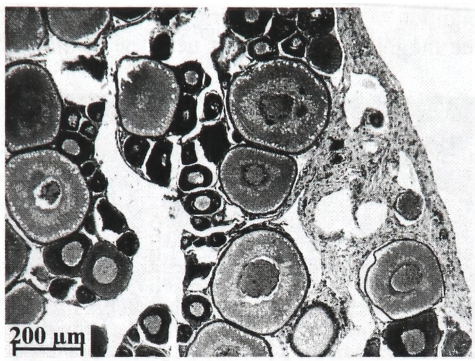

Fig. 2a. Condition of internals during the anadromous migration
a —numerous previtellogenic oocytes among oocytes at the phase of vacuolization; empty follicles from the previous spawning under the ovaries surface;

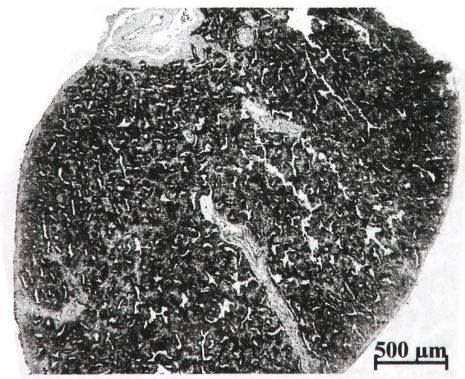

Fig. 2b. Condition of internals during the anadromous migration
b — a male gonad of immature fish;

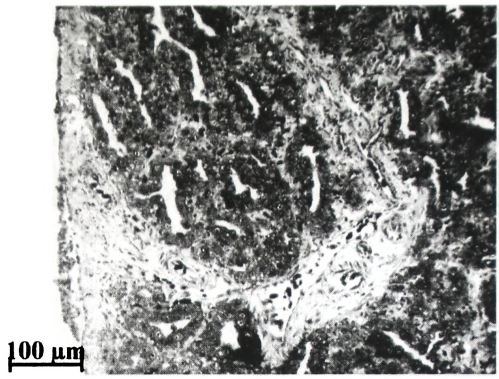

Fig. 2c. Condition of internals during the anadromous migration
c —the same magnified; a vast scar near the seminiferous tubule

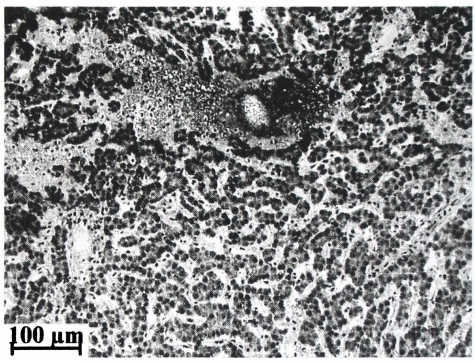

Fig. 2d. Condition of internals during the anadromous migration
d — hepatocytes near cirrhotic enlargement area

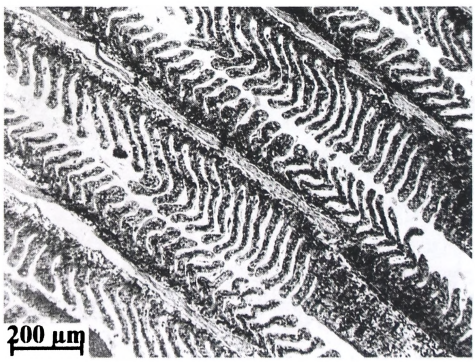

Fig. 2e. Condition of internals during the anadromous migration
e — respiratory lamellar thickening of gill filaments

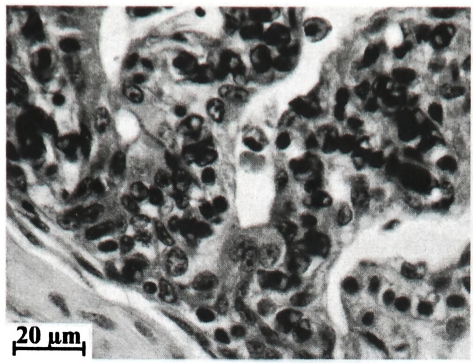

Fig. 2f. Condition of internals during the anadromous migration
f — the same magnified; infiltration of erythrocytes and cell hypertrophy of associative epithelium and respiratory lamella

The vascularized liver in male and female fish didn't have any obvious changes. However, the microscopic examination frequently revealed cells with lipophanerosis among basophilic hepatocytes, and the appearance of caverns as a result of their degeneration. Cirrhotic enlargement of vascular walls was quite frequently observed (Fig. 2d).

There were no abnormalities detected in gills of peled, they were moderately hyperemic. The epithelium of afferent filaments mostly consisted of 3-4 layers of cells; the respiratory lamella in the whole had no abnormalities, though thickened and square-headed forms were quite often observed (Fig. 2 e, f), and some fish had coalescence in some parts of gills surface (up to 20%) followed by accompanied cytolysis. Gill ectoparasites (*Ergasilus sp.*) were often detected in *Coregonus peled,* extensive invasion of fish tested was 82.4%.

Thus, at the end of the wintering period immature males of plankton-eating peled were revealed to have some abnormalities of reproductive system, some male and female fish had minor liver and gill epithelium abnormalities. The high level of luminal infestation and ectoparasitic invasion could be caused by decreased defense mechanisms during over-wintering in the Gulf of Ob.

Muksun. The age of the examined fish varied between 7 and 12 years. The characteristics are given in Table 2. All specimens had high fat content and intestine filling. There were much less fish with luminal infestation (26%) and with gill ectoparasites (16%) in comparison with peled. Muksun matures quite late, females mature between 8-9 years of age; males do a year earlier. The gonadosomatic index increased respectively. The reduction with significant range of values can be observed, reflecting missing out the spawning season after the previous one at the level of 9+...10+ for males and 10+...11+ for females (Fig. 3). No abnormalities were detected in muksun gonads. Female fish were exemplified as missing out the spawning season, preparing for the upcoming one. At the phase of physiological maturity of sexual cells (stage IIIa) oocytes at the phase of cytoplasmic vacuolization were present in female gonads, and corpus luteum was formed. The old interstitial gland cells and the remains of incompletely degenerated follicles were detected. Apparently, these fish spawned two years before, they missed out the spawning a year before,

and the next spawning will be no earlier than the next year. This fact proves that muksun fish have three years' genesial cycle, its detailed description is given in the works [9], [23].

The vitellogenesis occurs in the terminal oocytes of females, entering the spawning population of the current year (Fig. 4a), with previtellogenic oocytes settled in between them. In such female gonads, the reserve fund of the sexual cells (oogonia and early meiotic prophase oocytes) is absent (Fig. 4b). Male gonads are at the final gonad maturation stage (stage II), but the period of spermatogenesis has not come yet, the walls of seminiferous tubules contain Type A spermatogonial cysts, less frequently they contain Type B small spermatogonia (Fig. 4c). The cysts of primary spermatocytes rarely occur on the periphery of the male gonad.

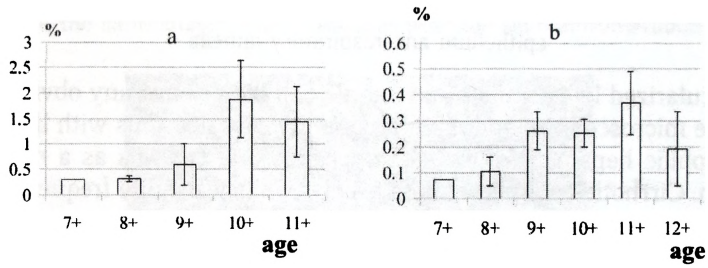

Fig. 3. Gonadosomatic index of muksun females (a) and males (b) during the anadromous migration to the estuary of the Ob River

A high fat content was found in liver of all examined males of muksun with no obvious other abnormalities. Some fish had areas (the number varies) of degeneration of hepatocytes and erythrocyte hemolysis (Fig. 4d).

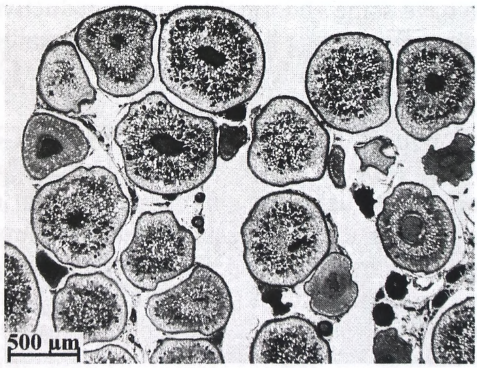

Fig. 4a. Condition of gonads and liver during the anadromous migration from the Gulf of Ob. a —female gonad of mature adult (maturation stage IIIb)

Fig. 4b. Condition of gonads and liver during the anadromous migration from the Gulf of Ob. b — the same magnified; active vitellogenosis starts in the ooplasm of oocyte

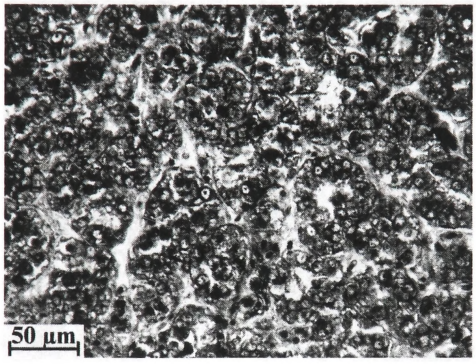

Fig. 4c. Condition of gonads and liver during the anadromous migration from the Gulf of Ob. c — in the walls of seminiferous tubules Type A spermatogonia, less frequently Type B; interstitial tissue is well-developed

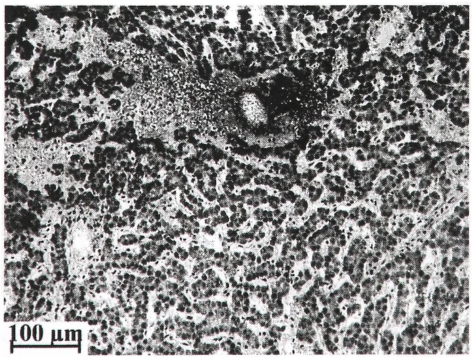

Fig. 4d. Condition of gonads and liver during the anadromous migration from the Gulf of Ob. d — in the liver, a vast area with degeneration of hepatocytes is well-observed

In contrast with the internals examined, the gill epithelium of muksun had great cytomorphological abnormalities. Some fish (no more than 25%) had minor

abnormalities, the associative epithelium was no more than 3-4 layers of cells, attached to the cartilage matrix of the gill filament (Fig. 5a). Some respiratory lamellae had hyperplasia, certain filaments had lamellar bulging, though these lamellar merges are quite rare (Fig. 5b). However, most of the muksun males were revealed to have some significant gill epithelium abnormalities, concerning certain filaments, as well as groups of filaments, leading to gill apparatus disorders. Thus, the thickness of associative epithelium along the whole length varied from 2 to 4-5 cells, the associative epithelium of some filaments was detached from the cartilage matrix, and the space was filled with the aplastic mass of cell debris (Fig. 5 c, d). Thereby, the terminal sequences of respiratory lamellae coalesce, the centre of the filament and its initial part coalesce with the lamellae of adjoining filaments, causing gaseous exchange decrease in this area. Generally, from a third to a half of the lamellae have various abnormalities.

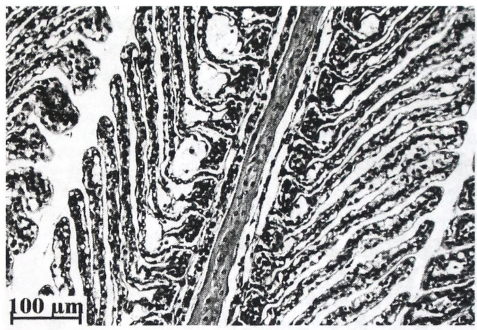

Fig. 5a. Gill apparatus of muksun in the estuary of the Ob River
a — common respiratory lamellae of common species or with an aneurysm on terminals

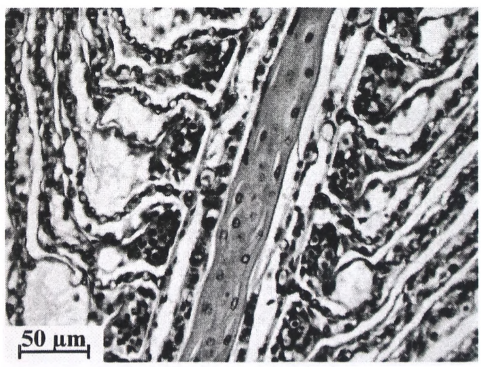

Fig. 5b. Gill apparatus of muksun in the estuary of the Ob River
b — the same magnified; the associate epithelium area, consisting of 4-5 layers of cells, there are extended cavities at the base of some lamellae

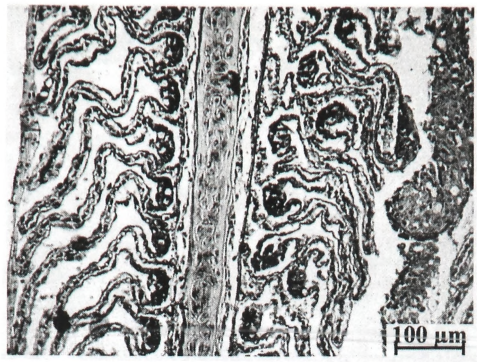

Fig. 5c. Gill apparatus of muksun in the estuary of the Ob River
c — some parts of the associative epithelium are detached from the filament, some terminal sequences of respiratory lamellae coalesce

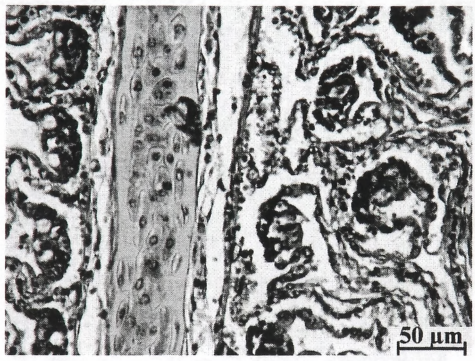

Fig. 5d. Gill apparatus of muksun in the estuary of the Ob River
d — the space of detached epithelium is filled with some elements of cell debris

Thus, after wintering in the Gulf of Ob, the migrating muksun has a high level of feed intake, a significant nutrient reserve and is able to provide the sufficient development of its reproductive system, though there are some abnormalities in liver. Gill epithelium abnormalities are detected in most cases, probably affected by the negative hydrochemical process during the wintering period. It seems to be a result of respiratory depression, which will have a negative effect on spawners' condition in future.

Siberian whitefish. Immature and fertile specimens were detected among migratory fish, the age of all fish varied between 3 and 8 years. There were some fish with luminal infestation (9%), more fish with gill ectoparasites (18%), which is equatable to muksun. The average hemoglobin content in females' blood was 90 g/l, in males' blood it was 99 g/l (Table 2). GSI of immature females was 0.3%, GSI of mature females was 1.4% or higher, GSI of males was 0.2% (Fig. 6).

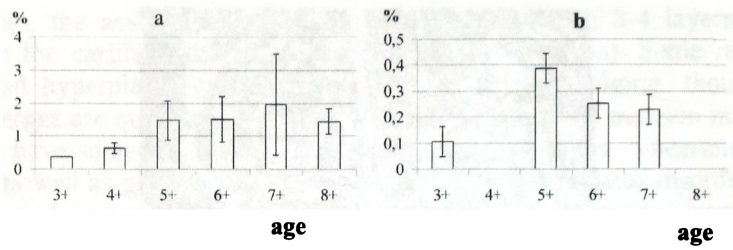

Fig. 6. Gonadosomatic index of females (a) and males (b) of Siberian whitefish during the anadromous migration to the estuary of the Ob River

Previtellogenic oocytes were exemplified by gonads at the phase of physiological maturation of germinal cells (stage II), with the presence of oogonial nests and early meiotic prophase oocytes among them. Vitellogenic oocytes were performed in gonads at the phase of physiological maturity of germinal cells (stage III) (Fig. 7a), with the presence of previtellogenic oocytes and bands of resorbing follicles between them (Fig. b). Male gonads were mostly performed by Type A spermatogonia germ cells (Fig. 7c). There were no obvious gonad abnormalities of specimens detected.

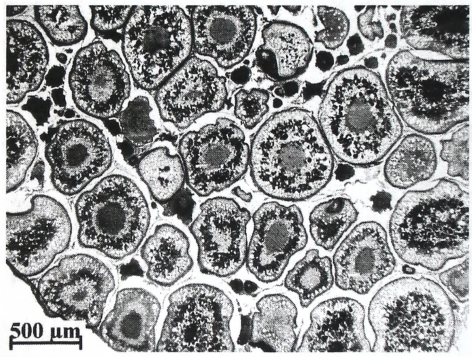

Fig. 7a. Internals of Siberian whitefish in the estuary of the Ob River
a, b — female gonads at the phase of physiological maturity of sexual cells (stage IIIb), among vitellogenic oocytes, empty resorbing follicles are observed

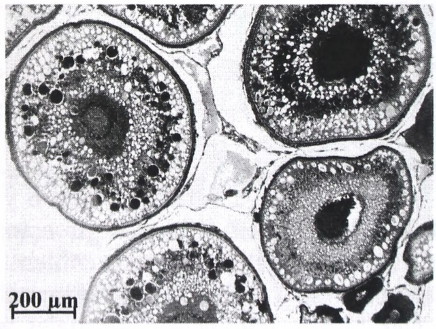

Fig. 7b. Internals of Siberian whitefish in the estuary of the Ob River
a, b — female gonads at the phase of physiological maturity of sexual cells (stage IIIb), among vitellogenic oocytes, empty resorbing follicles are observed

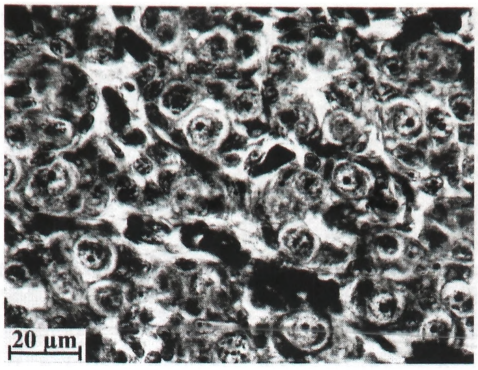

Fig. 7c. Internals of Siberian whitefish in the estuary of the Ob River
c — in male gonads at the phase of physiological maturation (stage II) Type A spermatogonia germ cells are performed

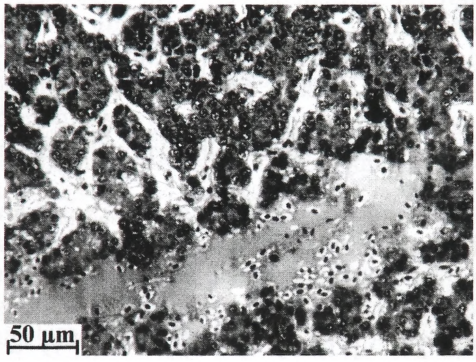

Fig. 7d. Internals of Siberian whitefish in the estuary of the Ob River
d — hyperemic area of the liver

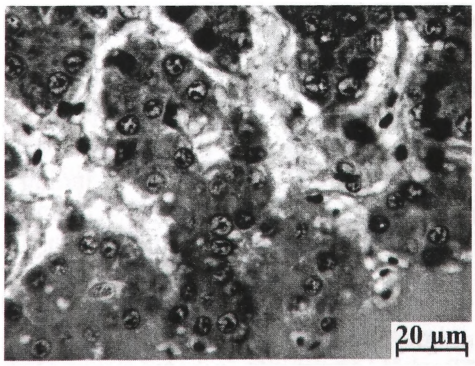

Fig. 7e. Internals of Siberian whitefish in the estuary of the Ob River
e — the same magnified; cytoplasm of most of the hepatocytes is basophilic; intercellular spaces are filled with fat

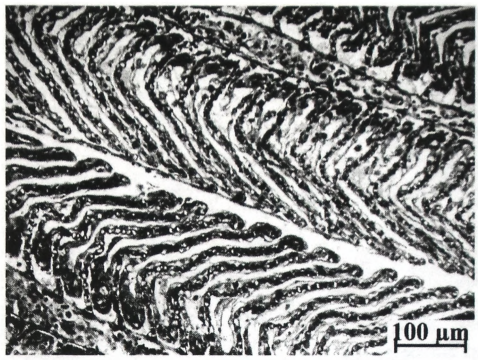

Fig. 7f. Internals of Siberian whitefish in the estuary of the Ob River
f — respiratory lamellae in some parts of filament are thin, and desquamation occurs.

There were no significant abnormalities detected in the hyperemic liver of Siberian whitefish, high fat content is observed, hepatocytes degeneration with cavitation was detected in some parts of the liver (Fig. 7 d, e). There were also no considerable pathological changes in the gills of this species (Fig. 7 f). Only certain fish had abnormalities up to 20-25% at the base of filaments in condition of respiratory lamellae, evident as desquamation of coating surface and cytolysis of respiratory cells, often coalescing with their terminal sequences. The associative epithelium had 2-3 layers of cells.

Thus, though the benthos-eating Siberian whitefish had low level of feed intake and low fat content during the anadromous migration period in the estuary of the Ob River, no pathomorphological abnormalities of male and female germinal cells were detected. No considerable abnormalities in liver and gills apparatus were detected, with comparatively low level of parasitic invasion. Observed abnormalities were mostly fragmentary.

Discussion. The research of morphological functions, as well as the histological evaluation of the condition of gonads, liver, and gills apparatus in peled, muksun, and Siberian whitefish during the migration period from the Gulf of Ob to the Ob River demonstrated distinct species-specific features. If plankton-eating peled had minor abnormalities of reproductive system, liver and gills epithelium, but 100% of them were infested by helmints, then in low-infested muksun with large luminal fat reserve the gonad abnormalities were not usually registered. However, muksun had considerable abnormalities of the liver and particularly of the gills apparatus. It could be caused by a wide range of food items, including plankton and benthos (the main part of petrochemicals and heavy metals accumulate in soils), as well as by periodic spawning migration to the polluted middle Ob. The low infestation was registered for typical benthos-eating Siberian whitefish, their internals had minor abnormalities. Since the number of spawning population in the middle Ob is not large, the main stock migrates for spawning to the Ural tributaries of the lower Ob which are still pure. The wide variability in hemoglobin concentration, detected in female peled and Siberian whitefish, is obviously caused by different requirements of oxygeneous repair processes after post-spawning resorption of empty follicles and by preparing for the upcoming spawning season. The repair processes in males and females of muksun are less active because of longer two- and three-year intervals

between spawning periods [23], during which the physiological processes normalize. The sexual cycle rhythmicity and the reproduction potential of *Coregonidae* are mostly determined by the effective functioning of gills apparatus and liver [4], [16]. Moreover, the morphological functions of the condition of gonads, liver, and gills in *Coregonidae* are supposed to be the test data for health evaluation of certain populations in the Ob River ecosystems. This is also the indicator of adaptative plasticity of these species and the basis for ecological forecasting.

In conclusion, it should be noted that the richest resources of valuable fish species are formed and preserved in the Gulf of Ob. Here young specimens of sturgeon, nelma, muksun, peled, Siberian whitefish, broad whitefish, and other fish fatten, most of populations escape from winterkill. As we mentioned earlier [9], the Gulf of Ob is one of the most important water supplies in the ecosystem of the Ob-Irtysh basin, and *Coregonidae* come to different areas of it to fatten, survive, restore their reproduction potential, and rehabilitate in different seasons. This estuary condition should be considered as the indicator of life proper for *Coregonidae* in the Ob River during their biocycle.

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