Feeding characteristics and assessment of histopathological changes in the organs of the Sandpiper goby (*Neogobius fluviatilis*)

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Abstract. The paper presents data from ichthyological, trophological and histological studies of the Sandpiper goby (Neogobius fluviatilis) endemic to the Caspian Sea, which has an important role in trophic chains, as food object for sturgeon, semi-migratory fish species, marine migratory Clupeidae family and Caspian seal. Place of sampling for the study were stations in the Northeastern part of the Caspian Sea, which is inferior in trophic content to its other parts, which had confirmed by data from trophic studies. The basis of the studied fish nutrition was Polychaeta, Bivalvia, Crustacea and in some cases juvenile fish, which indicates their nutritional plasticity, and the fact that they are not competitors to each other in nutrition. Depending on results of fish's visceral organs histological studies, structural changes were determined, which were more adaptive in nature and were a response to the impact of aquatic environment factors. In gills was found protozoan invasion causing hyperplasia of secondary lamellae epithelium and sloughing of respiratory epithelium. There were growths of the epithelial layer of villi in the intestine. In the liver and pancreas were detected fatty degeneration and necrotic changes. In all organs were found microcirculatory disorders, including the presence of erythrocytes with hyperchromic cytoplasm.

1 Introduction

The Caspian Sea ecosystem is currently facing instability as a result of prolonged human activity. If this anthropogenic pressure continues to increase, there is a possibility of irreversible negative changes in the ecological state of the Caspian Sea. That is why it is crucial to prioritize the implementation of marine environmental monitoring. This monitoring should encompass the evaluation of pollutant effects on both the marine environment and the biota, including non-commercial fish species that play a vital role in the ecosystem.The presence of Gobiidae species in the Caspian Sea has a significant impact on the food chains

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within the ecosystem. The ichthyofauna in the eastern part of the Northern Caspian Sea interacts with these gobies in two main ways. Firstly, they serve as prey for other fish species, establishing a "predator-prey" relationship [1]. Secondly, they compete with other fish species for food resources. The Gobiidae family displays a high level of adaptability, allowing them to thrive even in the face of fluctuating environmental conditions [2,3].

The nutritional composition of the organisms consumed by gobies is similar to that of other benthophage fishes, including crustaceans, mollusks, worms, and insect larvae [4]. According to the majority of scientists, histopathological changes can serve as a valuable bioindicator of the environment effects on the fish's body. In fish, when exposed to toxic substances at concentrations that do not cause visible signs of poisoning, certain morphological changes occur in the internal organs. The lack of clinical symptoms of poisoning can be attributed to the phenomena of adaptation and compensation in the body's functions [4]. The aim of the work was to assess the nutrition and histopathological condition of Sandpiper gobies (*Neogobius fluviatilis*) in the Kazakh waters of the Caspian Sea.

2 Material and methods

Material for studying the gobies nutrition was taken from catches of 4.5-meter trawl at 30 stations in the north-eastern part of the Caspian Sea, according to the state order program of the “Fisheries Committee of the Republic of Kazakhstan: Biological basis. Conducting comprehensive marine research to assess the biological resources state of the Kazakh part of the Caspian Sea"[3].

Fig. 1. Caspian Sandpiper gobies (*Neogobius fluviatilis*)

Sandpiper goby (*Neogobius fluviatilis*) 60 samples selected from catches for trophological and histological analysis had no appereance of the pathological process and were fixed in 10% neutral formaldehyde for 24 hours (Fig.1). After washing from formalin, a biological analysis was carried out to determine the age using spine, length, weight, gender [5] and then the digestive tract of 15 different size samples was selected for trophological analysis, the studies were carried out in accordance with the standard quantitative-weight and quantitative method [6]. The identification of invertebrates was carried out using the Atlas of Invertebrate Animals of the Caspian Sea [7,8]. An EP613C torsion scale was used to check the nutrient node weight. An MBS-9 stereo microscope and a MicroOptix light microscope were used to identify nutrient node. The diagrams were made in Microsoft Excel. Gills, digestive tract, liver, heart of 15 samples were processed in accordance with the standards of histological studies to obtain serial sections 5 μm thick, coloured with hematoxylin–eosin [9,10]. Analysis and microphotography of histological preparations coloured with

hematoxylin and eosin were carried out using a Leica DM LB2 light optical microscope with a Leica DFC320 digital camera at magnifications of 10x, 20x, 40x and 100x.

3 Results and discussion

All studied fish had stomachs filled with fully digested food. The fullness index was equal to - 195.76%00. In the nutrient node, from all the examined samples were observed Polychaeta bristles, Bivalvia mollusks, fragments of Crustacea and fish (Fig. 2).

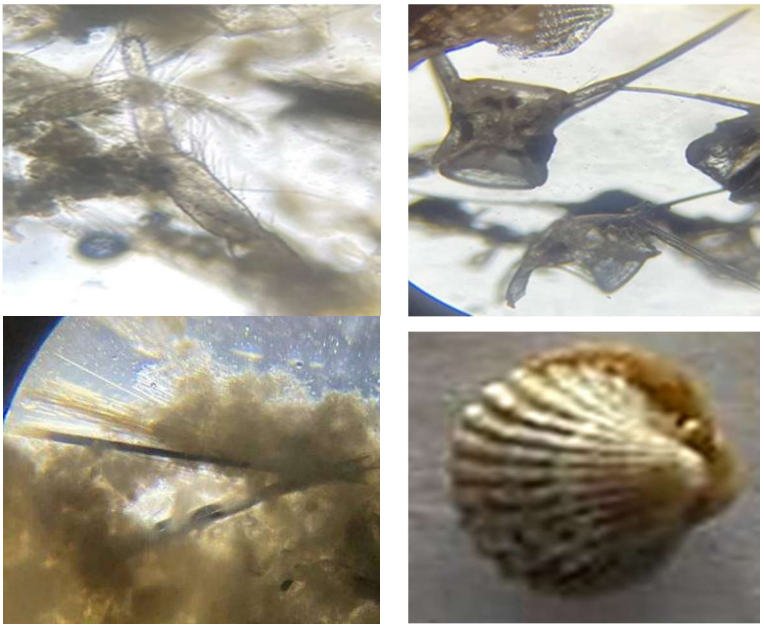


Fig. 2. A –Polychaeta bristles. B - fish vertebrae. C - limb of a crustacean. D - Bivalvia

The age of the studied Caspian sand gobies was within 2-3 years, length ranged from 7.7 to 10.0 cm, weight - from 7.84 to 19.8 g. Fatness was high and averaged according to Fulton - 1.91, according to Clark 1.76 (Table 1).

Table 1. Biological parameters of the Caspian sand goby

Length, cm			Weight, g			Fatness, %		
lim	lim x± S x	CV, %	lim	lim x± S x	CV, %	Fulton	Clark	n
7.7-10.0	8.95±0.18	7.67	7.84-19.8	14.02±1.06	29.41	1.91	1.76	15

The repetition frequency of components: 60% were Polychaeta, 20% were Bivalvia, 10% were fragments of fish and Crustacea (Fig.3).

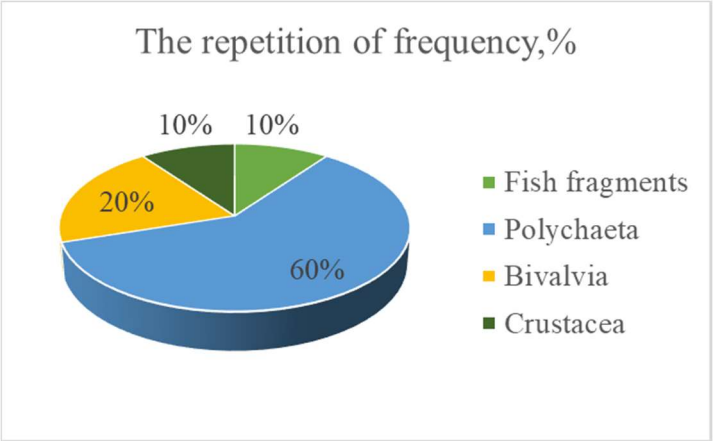


Fig. 3. The repetition frequency of components in the digestive tract of the sand goby

According to the analysis of nutrition spectrum: 43.48% were Polychaeta, 26.06% were fish fragments, 17.39% were Crustacea and 13.04% were Bivalvia (Figure 4).

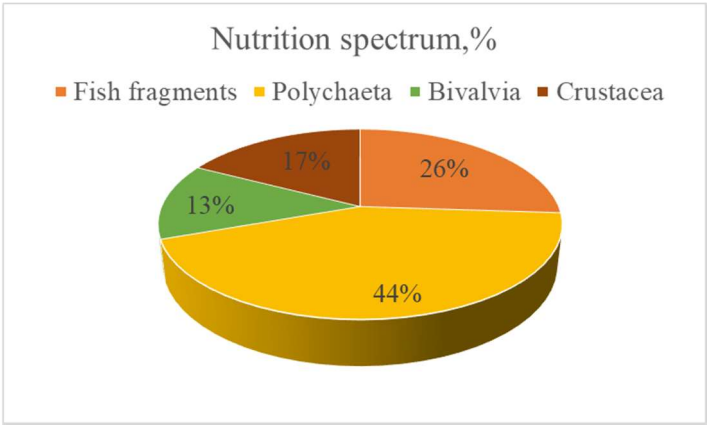
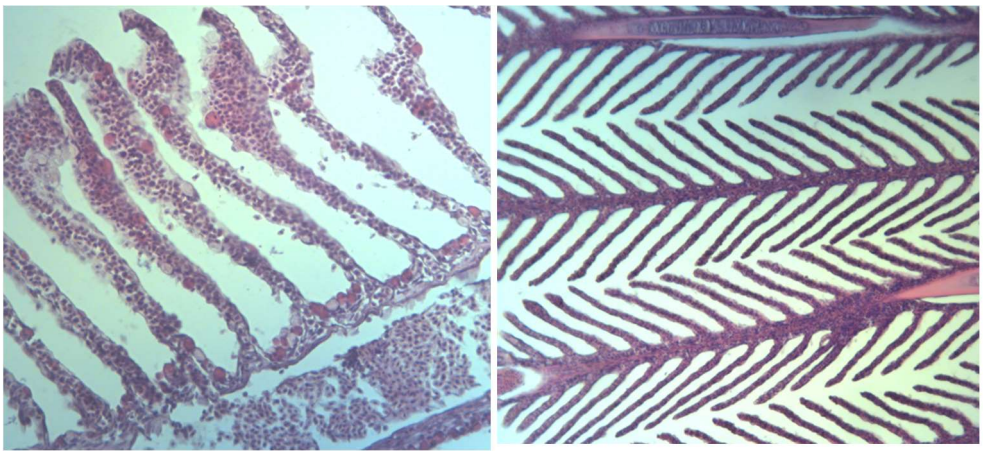


Fig. 4. The nutrition spectrum in the digestive tract of sand goby

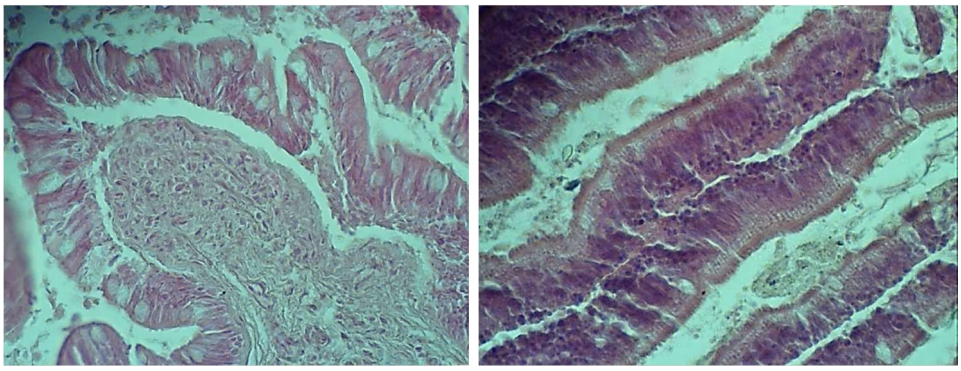
Histological examination of fish gills shows the presence of protozoa invasion in the lamellae, causing hyperplasia of the secondary gill epithelium and an increase in mucous cells at their locations. The affected parts of the lamellae are deformed and expanded due to an increase in macrophages and lymphocytes (Figure 5A). In healthy petals, the primary and secondary respiratory epithelium had normal thickness (Figure 5B).



A B

Fig. 5. Images of histological section of *Neogobius fluviatilis* gills. A- Hyperplasia in lamellas, 40x magnifying; B - normal gills, 10x magnifying. Hematoxylin and Eosin stain

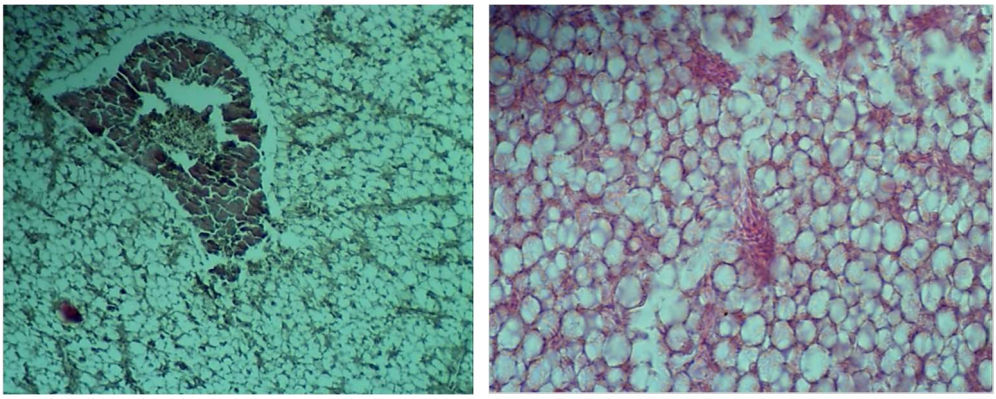
The basic organization of the intestinal structure is formed by four layers: mucous, submucosal, muscular, and serous membranes. Layer of the mucous membrane of the anterior intestine with elongated and branched villi with many goblet cells. The midgut has flatter villi with a small number of goblet cells scattered among the enterocytes. In general, necrotic enterocytes are noted in the anterior and middle sections (Figure 6).



A B

Fig 6. Representative photographs of the *Neogobius fluviatilis* digestive tract histostructure. A- Anterior section, 20x magnifying; B- middle section, 40x magnifying. Hematoxylin and Eosin stain

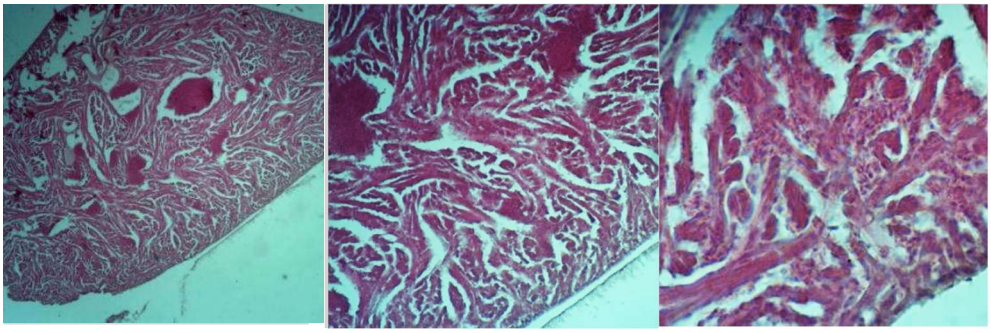
Large hepatocytes with protruding nuclei, vacuolated cytoplasm without granules, clear boundaries with layers of fibrous connective tissue. Overall, no obvious vacuolar degeneration, inflammatory cell infiltration or steatosis. The biliary system consists of ducts and bile canaliculi (Figure 7).



A B

Fig. 7. Representative photographs of the *Neogobius fluviatilis* liver histostructure. A – 10x magnifying; B - 40x magnifying. Hematoxylin and Eosin stain

There is no enlargement of the atria and ventricles. Compact and spongy myocardium are differentiated, there are no signs of fibrosis in the places of their contact. In the heart not found reciprocal increase in the spongy myocardium, which occurs due to a decrease in the compact one. In the spongy tissue, trabecular plates and lacunae filled with blood are differentiated (Figure 8).



A B C

Fig 8. Representative photographs of the *Neogobius fluviatilis* heart histiostructure. A – 10x magnifying; B – 20x magnifying; C – 40x magnifying. Hematoxylin and Eosin stain

4 Conclusion

The fauna of gobies in the Caspian Sea basin currently includes 43 species, of which 35 species are endemic, 7 are native and one species is exotic [11]. The sub-basins of the Northern Caspian Sea are home to a diverse range of species, totaling 21 in number. However, these species have been largely overlooked due to their limited economic value, small size, and preference for deep-sea habitats that are difficult to access. Consequently, there is a significant lack of knowledge regarding their biological characteristics and ecological dynamics. The Ponto-Caspian basin, which includes the Caspian Sea, as well as the Black and Azov Seas, is inhabited by the Gobiidae family. Within this family, there are two distinct lineages: the Ponto-Caspian bentophylline gobies of the Gobius lineage and the

benthophiline gobies of the Pomatoschistus lineage. The Ponto-Caspian bentophylline gobies consist of (genera Neogobius Iljin, 1927, Ponticola Iljin, 1927, Mesogobius Bleeker, 1874, Proterorhinus Smitt, 1899, Babka Iljin, 1927). On the other hand, the benthophiline gobies (Anatirostrum Iljin, 1930, Benthophilus Eichwald, 1831, Benthophiloides Beling & Iljin, 1927, Caspiosoma Iljin, 1927). Additionally, there are the Pomatoschistus line (Knipowitschia Iljin, 1927 and the monotypic genus Hyrcanogobius Iljin, 1928) [12]. The genus Neogobius includes five valid species: *N. caspius*, *N. pallasi*, *N. bathybius*, *N. fluviatilis* is endemic to the Caspian Sea, *N. melanostomus* is in the Ponto-Caspian basin. The present studies found several significant biological characteristics in Sandpiper goby (*Neogobius fluviatilis*) with food resources, area, season, and year. As is known, the sand goby is a low-migratory and highly flexible species. Regardless of the area and location of sampling, features were distributed in significant proportions. The results of histological studies on fish's visceral organs indicated that structural changes were more adaptive and a result of the impact of different aquatic environment factors. Thus, in the spring, as the water warms up, males of Sandpiper goby (*Neogobius fluviatilis*) migrate to shallow water; females begin to enter shallow water at the end of April, increasing their numbers in May. Sandpiper goby (*Neogobius fluviatilis*) actively feed on small macroinvertebrates, especially crustaceans. They themselves act as objects of consumption by vertebrates, including sturgeon (*Acipenseridae*) and the Caspian seal (*Pursa caspian*).

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