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(Received 3 October 2023; received in revised form 12 December 2023; accepted 28 December 2023)

Study of Charyn river naked osman (*Diptychus dybowskii*) nutrition and ichthyopathological analysis

Abstract. The paper discusses the study of nutrition and ichthyopathological analysis of naked osman from the Charyn River, which is located on the territory of the Charyn State National Natural Park in the Almaty region. The naked osman belongs to the carp family. The fish does not have any scales on other part of the body except the lateral line. The naked osman currently dwells in Kazakhstan, China, Kyrgyzstan, Uzbekistan, India, Nepal and other Asian countries. The purpose of the work is to determine the Charyn River's food base and water condition by studying the food spectrum, fish pathology. To carry out the given study the fish were caught in the autumn in 2021. Only 15 samples of the caught fish were taken for the analysis. According to the ichthyological analysis results, the absolute length of 15 samples of the fish ranged from 8.3 cm to 12.7 cm, and their mass was from 4.81 g to 17.60 g. The standard quantitative – weight method was used for ichthyotrophological research. The special histological method was employed for ichthyopathological study. According to the ichthyotrophological study, the fullness index was about 10.75%00, and insect larvae were an essential food. Histological studies did not reveal an obvious pathology. As a result, the nutrient reserves in the reservoir were found to be at a good level and the external environment was found not to produce a negative effect on the fish.

Key words: Charyn River, naked osman, *Diptychus dybowskii*, ichthyotrophology, ichthyopathology, nutrition study.

Introduction

The territory of the Republic of Kazakhstan has 85,000 rivers and temporary water bodies. The distribution of water resources in Kazakhstan is uneven. One-third of water resources is for the eastern regions, and 1/4 is for the southeastern and southern regions [1]. One of the largest rivers, the Ili, provides Lake Balkhash with water. 30% of the Ili River water resources is on Kazakhstan territory. Besides Sharyn and Shelek, there are several mountain rivers such as Turgen, Esik, Talgar, Kaskelen which flow into the Ili River on the left [2].

The source of the Sharyn River originates from the southern ranges of the Ketmen Mountains. Kegen, the middle part of the river, that is also called Sharyn flows from the exit to Zhalanash hill [3,4]. The acclimatization works of the last century led to

the migration of such fish as carp, pikeperch, bream, asp, catfish, roach, grass carp, white and spotted silver carp to the Balkhash-Ili basin. Over the past 14 years, alien species from China have appeared, but they were included in the Red Book as native species (rudd fish, Balkhash perch). Other aborigines (catfish, naked osman) migrated to mountain rivers. In addition, the habitat of rudd fish is the Sharyn River and Kaskelen Bay [5,6].

The naked osman is one of the main species inhabiting in the Sharyn River. Naked osman is found in mountainous regions of Central Asia. The short length of the head, a small indicator of the maximum height of the body, a high caudal stem and shortened pectoral fins are the characteristics of the Sharyn Osman. These morphological features determine the “long and slender” body shape of the naked osman compared to the body

shape of the remaining geographical races of this species [7-9].

Naked osman belongs to bentophages by the nutrition type. Ichthyotrophological studies were carried out by the method of benthos fish nutrition study. The studies of the nutrition of bentophages differ in the stomach presence or absence as well as in the sections of the digestive fish canal. In fish with a stomach, food is better preserved and more easily digested. When investigating fish with no stomach, food is digested throughout the digestive tract. The nutrition of benthos fish with no stomach was carried out by combined method, which includes methods for benthos, planktonophage and predatory fish [10]. Ichthyopathological studies of the fish were made by the special histological methods. As numerous studies show histopathological changes can be an excellent bioindicator of the biological effects of environmental influences on the organism. They can range from the presence of minor parasitic infestation to severe necrotic processes and indicate tumor formations. Histological changes can rarely be caused by the effects of a particular substance. These changes are a general response to the impact of the entire complex of environmental toxicants. Histological processing of the materials obtained in the the study included the following operations: dehydration, paraffin freezing, cutting with microtome and studying under the microscope [11-13].

The given research is considered to be relevant due to the fact that the study of the Charyn River ichthyopathological and ichthyotrophological characteristics has conducted for the first time. So, the investigation of its ichthyofauna, nutritious resources and water conditions through fish and their pathology is an important part of the given research. The ichthyotrophological and ichthyopathological studies of Charyn River naked osman have been made for the first time which makes the given study more valuable and novel in the field of ichthyology.

The aim of the study is to determine the nutritional base of the Sharyn River and the state of the fish by conducting the naked osman ichthyotrophological and ichthyopathological studies.

Materials and methods

To determine the food spectrum and conduct histological studies the species of naked osman (*Diptychus dybowski*) were caught in the autumn of 2021 in the Charyn River using the following coordinates: inspection 1, part 5, allotment 8 ($43^{\circ}40'14.84''\text{N}$, $79^{\circ}23'24.17''\text{E}$; Figure 1, A), inspection 5, part 49, allotment 7 ($43^{\circ}30'31.87''\text{N}$, $79^{\circ}13'20.20''\text{E}$; Figure 1, B), inspection 19, part 120, allotment 7 ($43^{\circ}21'40.81''\text{N}$, $79^{\circ}9'52.81''\text{E}$; Figure 1, C).



Figure 1 – The coordinates used for fish catch in the Charyn River.

Note: A – $43^{\circ}40'14.84''\text{N}$, $79^{\circ}23'24.17''\text{E}$; B – $43^{\circ}30'31.87''\text{N}$, $79^{\circ}13'20.20''\text{E}$; C – $43^{\circ}21'40.81''\text{N}$, $79^{\circ}9'52.81''\text{E}$

The caught fish were preliminary placed in 10% formalin, then they were delivered to the laboratory where 15 samples of the fish were selected for analysis (Figure 2).



Figure 2 – Naked osman used for study

In the laboratory of the Department of Biodiversity and Bioresources, Al-Farabi Kazakh National University, ichthyological, ichthyotrophological and ichthyopathological studies of 15 samples of the fish were made. Ichthyological studies and measurements were made using Pravdin method. During the ichthyological studies, absolute and short length of the samples were measured using a caliper, large and small weights were determined using the MW-Micro Digital Computing Scale (Korea). The indicator of fish fatness was calculated according to Fulton and Clark formula [14,15].

The components of food in the digestive tract of naked osman were investigated by a standard weight-quantity method. Processing was performed quantitatively, i.e., by counting and measuring the gastrointestinal tract. During the ichthyotrophological analysis, the gastrointestinal tract of fish was taken, the intestine was divided into 3 parts, and the nutrient node of each section was placed onto filter paper and dried. The dried nutrient node was weighed on an EP613C torzion scale (Switzerland). The nutrient node composition of food supply was determined by a binocular magnifying glass (MBS-9 stereomicroscope (SCOPICA, Russian)) and a microscope (MicroOptix light microscope (MicroOptx, Inc., Austria)) [10,16-18].

To identify the physiological state of the fish organs, a histological study was conducted using special histological methods. The fragments of different organs were placed in cassettes and prepared for dehydration using the research method. Dehydration process is carried out in a battery of

butyl and ethyl alcohol starting with 70° alcohol and then increasing its concentration by 10°. The materials were transferred from one solution to another. They were dried on the filter paper. The time of each alcohol exposure was 35 minutes. After the material was immersed in alcohol, it was dipped in alcohol – butanol mixture, and then it was placed in pure butanol. After the immersion in butanol-II, the material was put in molten paraffin. Then it was placed in a thermostat overnight at 56° C to impregnate the sample with paraffin. When the material solidified in paraffin, a microtome was used to obtain 5-micron histological sections. To remove paraffin from the surface of the sections before their coloring, they were passed through the solutions of xylene-I, xylene-II, then 96 percent ethanol-I, 96 percent ethanol-II, 70 percent ethanol. The colored sections were investigated using optical microscope (Motic BA-400 microscope (Motic Asia, Hong Kong, China)) [11,12,19]. Statistical data processing was done by standard methods and statistical programs in Microsoft Excel.

Results and discussion

Ichthyological analysis allowed determining the value of the fish length, large and small weight. The absolute length of the studied fish ranged from 8.3 cm to 12.7 cm, the average length was 10.7 cm, the large weight was from 4.81 to 17.60 g, the average weight was 10.3 g (Table 1).

Table 1 – Length and weight of the studied naked osman

No.	L, cm	l, cm	Q, g	q, g
1	12.7	10.5	17.60	14.06
2	11.2	9.1	13.81	10.35
3	11.8	9.6	13.62	10.81
4	11.1	8.9	10.63	8.81
5	11	9	10.70	9.23
6	11.5	9.4	12.01	10.06
7	10.7	8.9	9.74	8.42
8	11.1	9	10.95	9.22
9	10.7	8.6	10.23	8.39
10	10.7	8.6	10.91	8.74
11	10.8	8.5	9.82	7.69
12	10.1	8.4	8.34	7.21
13	9.6	7.8	6.21	5.40
14	8.8	7.1	5.23	4.43
15	8.3	6.7	4.81	3.91

Table 1 shows the data measured in the ichthyological study. The table gives the values of the absolute length (L), short length (l), large (Q) and small (q) weight, which show notable changes in the parameters.

The fish fatness was calculated using the Fulton and Clark formula, Fulton results were from 1.31 to 1.83, Clark results were from 1.19 to 1.37 (Table 2).

Table 2 shows the results of fatness calculated by Fulton and Clark formula. According to Fulton formula, the maximum value was 1.27, and the minimum was 0.7. According to Clark formula the maximum value was 0.74, and the minimum was 0.61. These indicators demonstrate a good level of fish fatness.

During ichthyotrophic studies, fullness index of 15 samples of naked osman was calculated. The overall value of the fullness index was 161.22%00 and the average value was 10.75% (Figure 3).

Table 2 – Fatness results of the studied fish by Fulton and Clark

No.	Fulton	Clark
1	0.86	0.67
2	1.27	0.74
3	0.83	0.66
4	0.78	0.64
5	0.80	0.69
6	0.79	0.66
7	0.80	0.69
8	0.80	0.67
9	0.84	0.68
10	0.89	0.71
11	0.79	0.61
12	0.81	0.7
13	0.70	0.61
14	0.82	0.69
15	0.84	0.68
medium	0.84	0.68

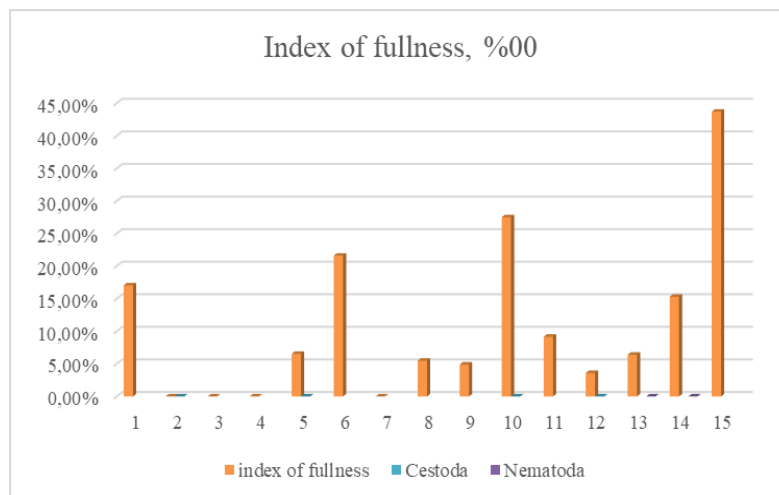


Figure 3 – Fullness index of the studied naked osman

Figure 3 shows the fullness index variability. Of the 15 fish species studied, 4 were completely free and cestodes were shown to be found in 4 samples and nematodes were present in 2 samples.

The results of ichthyotrophic studies mainly indicates the presence of insect fragments and unidentified pieces of digested food in the digestive tract of the naked osman (Figure 4).

Figure 4 illustrates the chitinous legs and claws of the insect found in the nutrient node, where completely digested food was also discovered but it could not be identified.

Fragments and nests of caddisfly larvae were discovered in the intestine (Figure 5).

The images given above show the parts of the caddisfly larvae which consist of a nest and fragments of a wing mixed with digested food. Nests are usually made of stones and plants, wings have a chitinous structure, so they cannot be digested.

In addition, the fragments of water beetle larvae were present in the intestine part (Figure 6).

The image above illustrates the part of the water beetle chitinous head and the digested food elements. Since the parts of the insects are chitinous, they cannot be completely digested.

Nematodes were in 4 species of the studied fish, and cestodes were in 2 species (Figure 7).



Figure 4 – A, B – Images of insect fragments and pieces of naked osman digested food

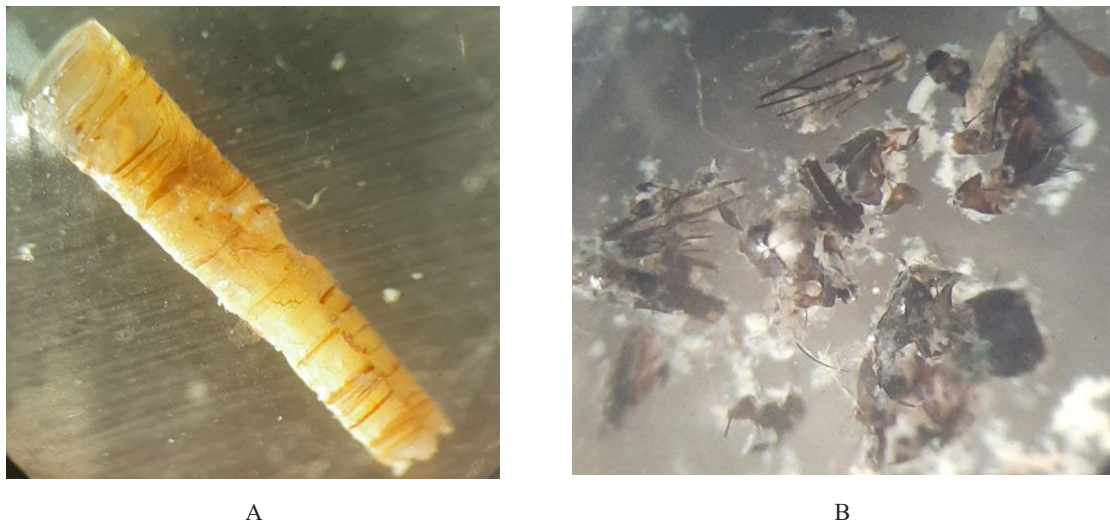


Figure 5 – A – Image of caddisfly larvae nest. B – Image of caddisfly larvae fragments



Figure 6 – Images of water beetle larvae fragment

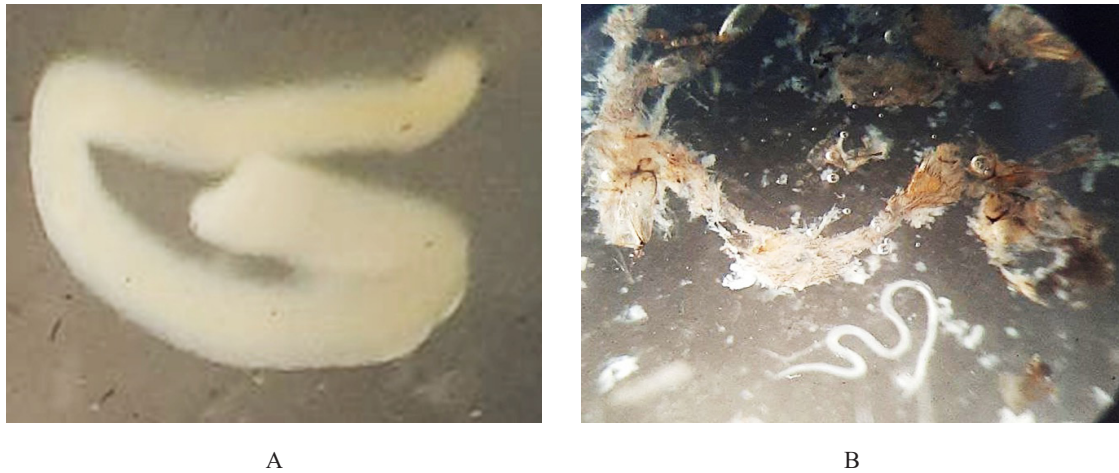


Figure 7 – A- Image of cestoda found in intestine part.
B – Image of nematoda found in intestine part

Figure 7 shows the cestodas and nematodas found inside the intestinal tract of the samples. In total, 4 nematodes and about 17 cestosdes were found in 15 fish.

The repetition frequency of the identified fragments and the number of fragments were determined using Microsoft Excel (Table 3).

Table 3 – The repetition frequency and the number of components in the digestive tract of the naked osman

Components	The repetition frequency	The number of components
Insect	90%	83.30%
Larvae of water beetle	20%	4.50%
Caddisfly larvae	30%	6.10%
Plant	20%	6.10%

From the results we can see that the food spectrum of the studied fish consists mainly of insects and their larvae. The naked osman eats plants very rarely, however the intestinal sections contain plants because the caddisfly larvae can build their nests near plants.

The results of histological studies demonstrate that the condition of the gills of 15 samples of naked osman was normal. Hyperplasia was one of the most frequent changes in histological preparation analysis (Figure 8).

The study of histological samples of fish muscles showed no pathological changes. The largest and

smallest cross-sectional sizes of the muscles were taken for study and compared. The smallest muscles were 35.03 nm, 33.83 nm, 42.51 nm, and the largest muscles were 82.71 nm, 86.22 nm, 93.35 nm (Figure 9).

There is no pathology in the histological samples of the naked osman stomach parts taken for the study. The walls are not damaged and straight (Figure 10).

Histological tests of the studied fish the liver showed no pathology. Stem cells lined with blood capillaries are clearly visible. Hepatocyte cells were basophilic. No fat content was observed in these fish species (Figure 11).

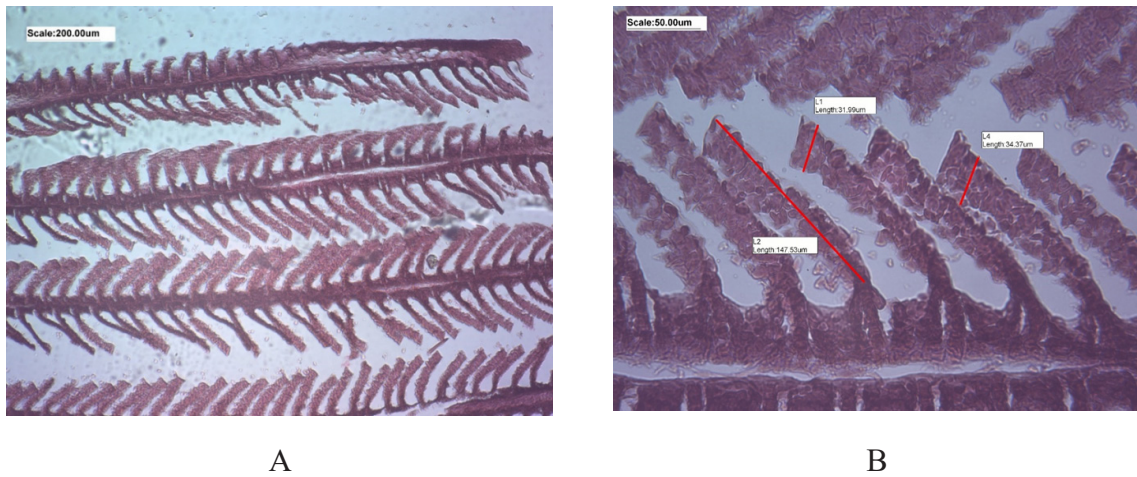


Figure 8 – Images of histological section of naked osman gills coloured by haematoxylin-eosin A-10x magnifying, B-40x magnifying

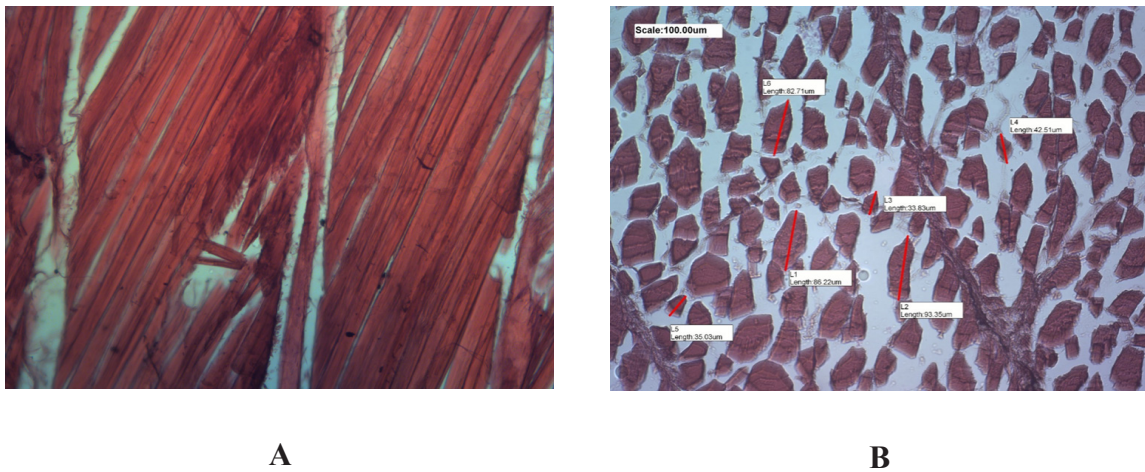


Figure 9 – A, B- 20 x image of histological section of the naked osman muscles colored by haematoxylin-eosin

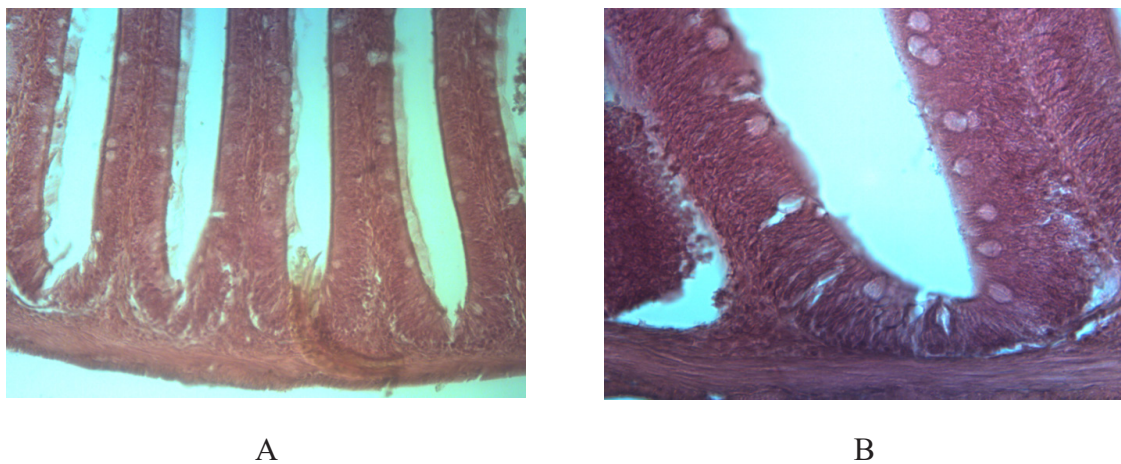


Figure 10 – Image of histological section of the naked osman stomach parts colored by haematoxylin- eosin 20x magnifying, B- 40x magnifying

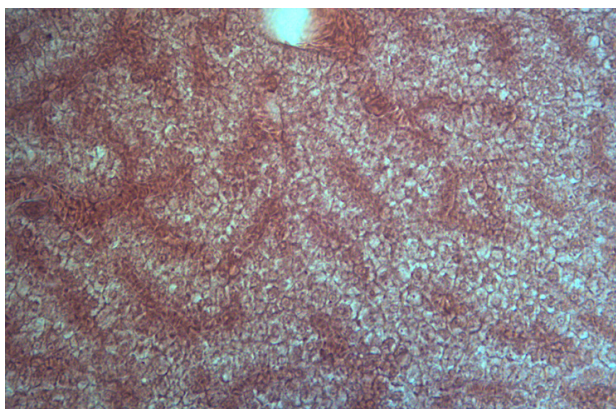


Figure 12 – 20x image of histological section of the naked osman liver colored by haematoxylin-eosin

The research gives a detailed analysis of the naked osman food spectrum and its ichthyopathological state. It reveals the Charyn River nutritious resources and water conditions. The given study is considered to be a pioneering one since such investigations have never been conducted before and the objects used for the study have also been never researched.

Conclusion

The results of ichthyotrophological studies indicate 90% of insect fragments, 30% of caddisfly larvae, 20% of water beetle larvae and plants. The composition of these samples included 83.30% of the fragments of insects, 4.5% of the larvae of water

beetles, 6.10% of the larvae of caddisflies and plants. The studied fish are benthophagous, according to the frequency of food fragments and their number of components, insect larvae fragments was their main food. Out of 15 fish, 4 had completely empty digestive tract. 11 fish samples had digested food in their tract, the total fullness index equaled 161.22%00. The studies also showed the sufficient supply of nutrients to the reservoir.

Histological analysis revealed that protozoal infestation caused such changes as hyperplasia and lamella shortening in gills in addition to the normal state of the structure of the gill lamella of the fish. There are no pathologies very dangerous for the fish in the digestive system of the fish. Epithelium desquamation was observed on some villi of the fish intestine. The liver was in a normal state. Hepatocyte cells were arranged in a right order and were clearly visible. In most cases, the fibers of the fish skeleton muscles were normal, the sarcolemma was not damaged, the horizontal lines were also clearly visible, and the nuclei were located on the periphery. According to the study results the external environment produced no negative effect on the body of the naked osman. As the nutrition results of the Sharyn River fish show, nutrition base is good which prevents pathologies in organisms. Results can be useful for further studies on naked osman and on the Charyn River. In addition, ichthyotrophological and histological methods were improved which allows using them in trophology and histology.

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