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Influence of *Triaenophorus nodulosus* invasion on morphobiological parameters of European perch (*Perca fluviatilis L.*) in Shatsk National Nature Park reservoirs

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It was conducted comprehensive ichthyological and ichthyopatological research of European perch *(Perca fluviatilis L.)* populations in lakes of Shatsk National Nature Park and the impact of *Triaenophorus nodulosus* invasion on its size, weight and reproductive ability. Morphometric analysis, absolute and relative fecundity of fish were determined by the methods generally accepted in ichthyology. Clinical examination of fish (2,225 specimens, including European perch -1650 specimens) was performed during control catches. Simultaneously, the weight, size, and age of the fish were determined. Morphometric analysis was performed on 120 specimens of perch with the length of 104.4-194.8 mm and the weight of 16.4-177.6 g. Pathoanatomic autopsy of the fish was performed in the field and laboratory conditions. Parasitological studies were performed by the method of incomplete parasitological analysis. The extent and intensity of the invasion were determined. When conducting research on the parasitofauna of native fish species in the lakes of the Shatsk National Nature Park, it was found their infestation with helminths of various systematic positions (*Triaenophorus nodulosus, Khawia sinensis, Raphidascaris. acus,* and *Philometroides lusiana*). Perch was the most infected with the helminths. The invasion caused by the plerocercoid of Cestoda *T. Nodulosus* was the most intensive (18.2-56.3%, depending on the reservoir). There was a significant difference in the measurements of non-infested and infested with *T. nodulosus* fish individuals of the same age, we also registered significant lag in growth and a decrease in body weight among the infested specimens. A steady trend towards a decrease in fecundity parameters was also observed in infested fish. Thus, in non-infested individuals of 4-year perch, the highest individual absolute fecundity (IAF) was 6.7 thousand eggs against 3.1 thousand eggs in the infested perch (Lucimer lake). The lowest IAF – 3.9 thousand eggs against 2.0 thousand eggs in the infested perch specimesn was found in Chorne Velyke lake. In non-infested individuals of 5-year perch, the highest IAF index was 9.4 thousand eggs when compared to 6.8 thousand in the infested perch (Pulemetske lake). The lowest IAF was 6.3 thousand eggs (Chorne Velyke lake). The highest IAF was recorded in perch from Pulemetske lake (34.6 thousand eggs in non-infested versus 10.3 thousand eggs in infested specimens). The lowest IAF was observed in perch from Chorne Velyke lake (9.1 and 6.6 thousand eggs, respectively). There was decrease in fish size, weight and fecundity in perch infested with *Tr. nodulosus*, but it did not affect the reproductive fish ability.
Key words: ichthyofauna; perch; parasitic fauna; helminths; plerocercoids; infestation; *Triaenophorus*; fecundity.

Introduction

Shatsk National Nature Park (ShNNP) was established in 1984 with the aim of preserving, recreating and effective use of natural resources. It has a high recreational potential, but its main task in modern conditions is to preserve and restore the lake ecosystems of Ukraine. The total area of the Park is 32,830 ha, a significant part of its territory (6,348. 8 ha) is occupied by 23 lakes, located between Western Bug and Pripyat Rivers. (Yurchuk et al., 2009). Shatsk lakes are characterized by different origins, low flow, weak external water exchange, which determines their sensitivity to anthropogenic impact and causes variability in the chemical composition of water. Because of water saturation with mineral and biogenic substances, the water quality in Shatsk lakes has undergone negative changes during the past 40-50 years (Yevtushenko et al., 2009). The largest by area and economic value are Svityaz, Pulemetske, Luky-Peremut and Lucimer lakes. Most lakes are connected by channels for reclamation of swamp areas and regulation of flow during flooding (Yurchuk et al., 2011).

The ichthyofauna of these lakes includes 29 fish species along with introduced species like Chudsk whitefish, white omul, Amur carp, mottled silver carp, river eel, and zander (Sidorenko, Sinchuk, 2008). The most numerous species in the lakes of ShNNP are the cyprinid fishes and the perciforms used for industrial catches. The maximum fish productivity in these lakes was observed in 1977-1983, while the native fish species had a significant advantage in catches. The highest fish productivity was in Lucimer lake - 68.4 kg/ha, the lowest fish productivity was in Svityaz lake (12.4 kg/ha). The minimum industrial fish productivity was observed in 1993-1996 and now it registered at the same level, whereas in some lakes the industrial fishing has been

stopped (Sidorenko, 2009). In recent decades, the number of valuable commercial species (zander, pike) has decreased in the reservoirs of the Shatsk National Nature Park, and in Luky-Peremut and Svityaz lakes they disappeared.

The emergence of a qualitatively new invasion, including infestation with Triaenophorus, can lead to the penetration and subsequent naturalization of the parasites (Mineeva, Mineev, 2019). When conducting initial ichthyological and ichthyopathological studies in Shatsk reservoirs, we found a significant infestation of several fish species with parasites, among which the helminths were predominated. This, the aim of our research was to evaluate the European perch (*Perca fluviatilis* L.) population in the lakes of the Shatsk National Nature Park and impact of Triaenophorus nodulosus invasion on fish morpho-biological parameters.

Materials and Methods

We performed field studies in the lakes of the Shatsk National Natural Park (Lucimer, Chorne Velyke, Svityaz, and Pulemetske lakes) during spring and autumn month of 2016-2019. The largest catch of perch was observed in nets with a mesh size of 18-40 mm.

In total, they were subjected to ichthyological studies 5,786 fish specimens of different age groups: cyprinid fishes (roach – *Rutilus rutilus L.*, bream – *Abramis brama L.*, rudd – *Scardinius erythrophtalmus L.*, Prussian carp – *Carassius gibelio Bloch*, white bream – *Blicca bjoerkna L.*, gobies – *Gobio gobio L.*, common bleak – *Alburnus alburnus L.*, tench – *Tinca tinca L.*); perches (European perch – *Perca fluviatilis L.*, ruffe – *Gymnocephalus cernua L.*, zander – *Lucioperca lucioperca L.*); ictaluridae (brown bullhead – *Amiurus nebulosus Le Sueur*); pikes (pike – *Esox lucius L.*).

Control catches were carried out using industrial set nets with a mesh size of 18, 22, 25, 27, 30, 35, 40, 45, 50, 60, and 80 mm, and a minnow seine. The material was collected and processed according to Kostousov (2005). *Morphometric analysis* was performed on 120 perch specimens with length of 104.4-194.8 mm and weight of 16.4-177.6 g. To determine the age of fish, we used standard ichthyological methods for calculating age rings on the scale plate. To determine the fecundity of perch, 1 g of eggs was taken from the middle of the ovary at the IV stage of maturity of producers. Individual absolute and relative fecundity was determined according to Pravdin (1966).

Clinical examination of fish (2,225 specimens, including 1,650 perch specimens) was performed during control catches. Simultaneously, the weight, size, and age of the fish were determined. Pathoanatomic autopsy of fish was performed in field and laboratory conditions, using live or just dead fish (Musselius et al., 1983). In parasitological studies, the method of incomplete parasitological analysis was used. Parasites were collected, fixed, and processed in the laboratory using methods of Bykhovskaya-Pavlovskaya (1985). The extensivity (percentage of infested individuals in the total number of examined fish) and the intensity of invasion (minimum and maximum number of parasites in one infested fish) were determined.

The obtained data was processed by MS Excel software.

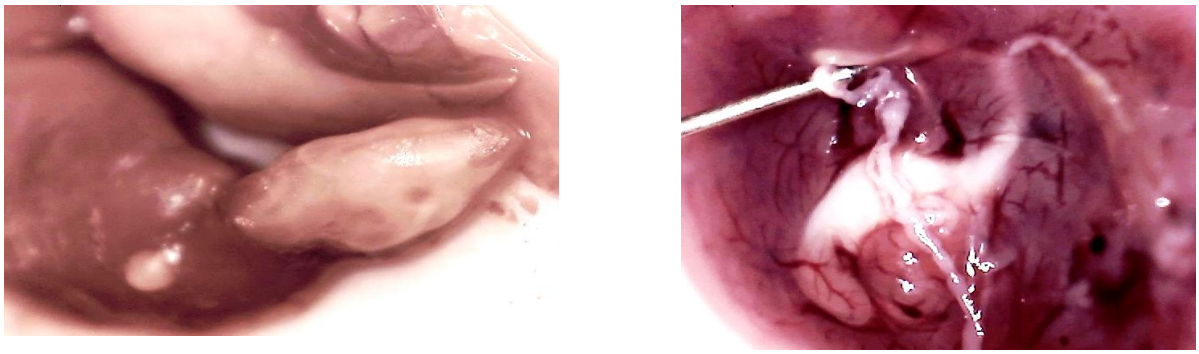
Results and discussions

We determined that core of fish community in the studied lakes consisted of cyprinid fishes (60-75 % from total catch), perciforms (15-35%), ictaluridae (5-10 %), amd pikes – about 3%. We registered sticklebacks only in Lucimer lake.

Perch ration in the lakes was 25-35%. As a benthophage, it affected the other commercial fish species and their juveniles. We also revealed the infestation of individual species by helminths form various systematic groups (*Triaenophorus nodulosus, Khawia sinensis, Raphidascaris acus,* and *Philometroides lusiana*) and determined the presence of microsporidia. We determined that ectoparasites were absent in smears from the gills and surface covers of the examined fish, which could be explained by unfavorable ecological state of reservoirs and specific features of fish parasitofauna. Helminths were most often found in perch.

The invasion caused by the *T. nodulosus* cestode was the most intensive. Plerocercoids of the pathogen (150.0–300.0 mm long, 2.0–4.0 mm wide) were localized in the liver in single or multiple capsules with a diameter of 3-6 mm, which formed a bumpy surface of the organ (Figs. 1, 2).

Fig. 1. Perch and its liver with the capsule



a b

Fig. 2. Capsules with plerocercoids in the liver of perch; **a** – capsules with plerocercoids; **b** – extraction of *T. nodulosus* plerocercoid from the capsule

During four years of research, the extent of invasion by T. nodulosus plerocercoids of the examined perch specimens varied: Lucimer lake – from 32.0 to 56.3 %, Chorne Velyke lake - from 28.6 to 52.4%, Svityaz lake – from 18.2 to 35.7%, and Pulemetske lake - from 30.0, for II – 2 -7 specimen/fish.

We found that perch of non-infested and infested groups had significant differences in size and weight indicators. According to the specified invasion, there is a significant lag in the growth of the infested individuals (Fig. 3).

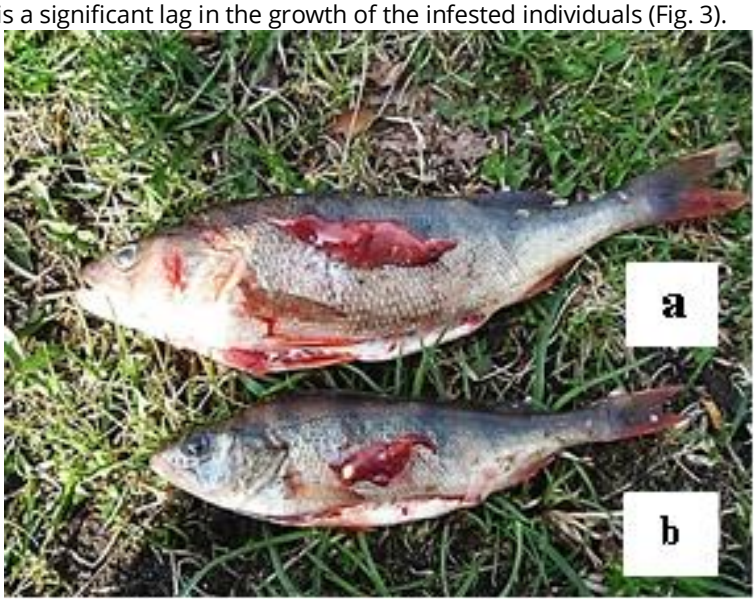
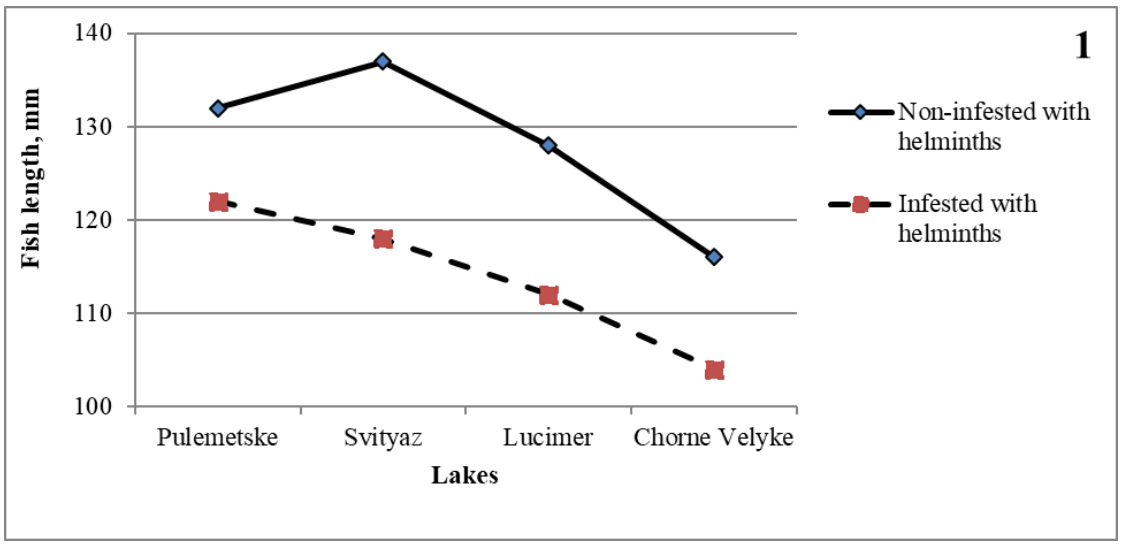


Fig. 3. Size of three-year-old perch and their liver in *T. nodulosus* infested and non-infested specimens; **a** - non-infested perch, **b** - infested perch (liver with incysted plerocercoids)

Differences in the body length of the infested and non-infested 5-year old perch are shown in Fig. 4.



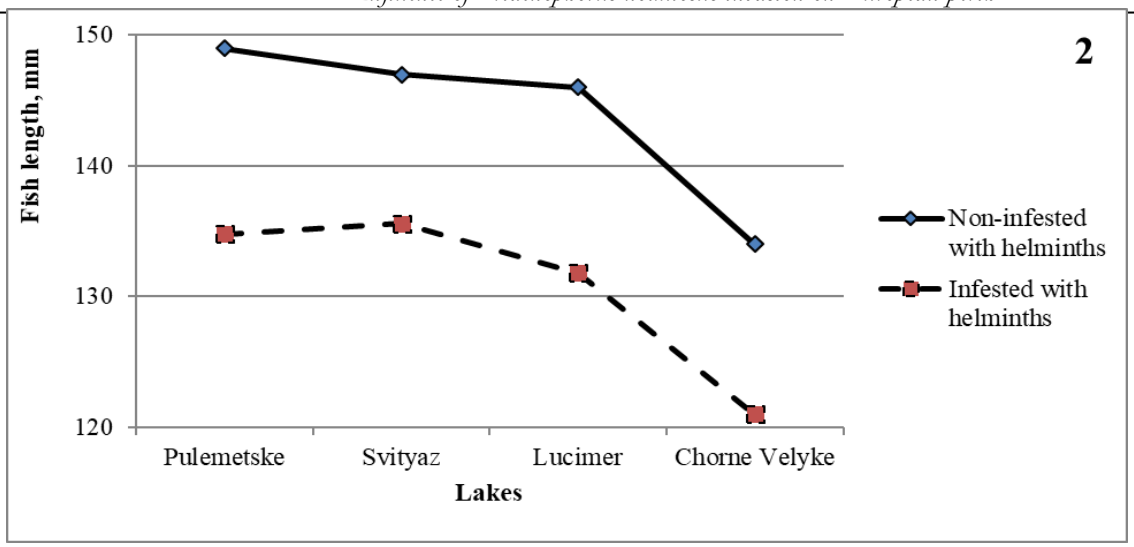


Fig. 4. The body length of triaenophorus infested and and non-infested 4-year (**1**) and 5-year perch (**2**) in various reservoirs of the Shatsk National Nature Park.

There is a clear significant difference in the measurements of non-infested and infested with *T. nodulosus* fish specimens of the same age, there was a significant lag in growth and body weight among the infested specimens (Table 1).

Table 1. Morpho-biological parameters of perch, non-infested and infested with plerocercoids of *T. nodulosus* (n = 120)

Age, years	Fish length, mm M±m	CV%	Fish weight, g M±m	CV%	IAF, thousand eggs M±m	CV,%	RF, eggs/g M±m	CV%
			Pulemetske Lake					
4	132.4±2.3**	3.9	35.6±1.0**	6.3	6.3±0.2*	8.4	179±2.4	3.0
	122.0±1.4	2.5	28.4±0.8	6.7	5.4±0.3	12.7	192±6.1	7.1
5	149.2±2.2**	3.2	55.9±1.6	6.3	9.4±0.5**	11.4	168±4.2	5.5
	134.8±2.2	3.5	36.2±1.4	8.7	6.8±0.2	9.6	188±2.6	3.0
6	194.8±3.6****	4.1	177.6±10.7	13.4	34.6±2.4	15.4	194±5.9	6.7
	158.0±2.3	3.2	61.6±2.3	8.2	10.3±0.8	18.0	167±7.5	10.0
			Svityaz Lake					
4	137.2±5.4***	8.7	39.2±3.0**	17.2	5.8±0.4***	16.8	152±4.8	7.2
	118.0±1.4	2.6	26.1±1.1	9.5	4.3±0.2	13.2	168±3.0	4.0
5	147.0±2.***	4.1	55.9±1.7	6.8	7.9±0.4***	12.8	143±4.3	6.7
	135.6±2.3	3.8	37.7±1.2	6.9	6.1±0.3	11.9	162±4.0	5.6
6	166.0±1.9**	2.6	86.2±2.0	5.2	16.9±0.9	12.5	195±7.1	8.1
	148.8±1.8	2.7	59.2±1.0	3.7	8.2±0.3	9.0	138±3.3	5.4
			Lucimer Lake					
4	128.8±2.1**	3.6	33.2±1.2****	8.3	6.7±0.3****	9.7	201±5.9	6.6
	112±3.2	6.3	17.0±0.8	10.2	3.1±0.3	18.0	182±7.2	8.8
5	146±1.4**	2.1	52.1±1.1	4.7	8.2±0.6*	15.0	156±7.9	11.4
	131.8±1.9	3.3	31.7±1.1	7.4	6.7±0.3	8.6	210±3.7	3.9
6	167.2±2.9**	3.8	83.7±2.2	5.7	14.7±0.9**	13.4	175±6.3	8.0
	150.8±1.8	2.6	54.2±1.3	5.6	9.7±0.6	14.6	179±7.7	9.6
			Chorne Velyke Lake					
4	116±1.4**	2.7	26.2±1.3**	11.0	3.9±0.3**	16.1	150±3.5	5.1
	104.2±1.3	2.9	16.4±0.5	6.9	2.0±0.1	12.7	123±4.1	7.4
5	134.2±1.8**	3.0	35.8±0.9**	5.9	6.3±0.3**	11.5	175±5.1	6.5
	121.2±1.3	2.3	22.3±1.4	13.7	4.1±0.3	16.5	185±3.2	3.9
6	148.6±1.5**	2.2	48.7±1.1**	5.0	9.1±0.4**	10.9	184±5.0	6.1
	137.4±1.2	1.9	37.3±1.4	8.2	6.6±0.3	9.2	177±4.2	5.2

Numerator - non-infested, denominator – infested specimens. IAF – individual absolute fecundity, RF – relative fecundity; GSI – gonadosomatic index. * p< 0.05, ** p <0.01, *** p <0.02, **** p< 0.001

As it can be seen from the table, perch of non-infected and infected groups had differences (sometimes significant) in morpho-biological indicators. We found definite difference ($p<0.05$-0.001) in the length, weight, and individual absolute fecundity between infested and non-infested fish specimens. However, we noted delay in non-infested fish growth compared to precious reports related to 4-6 year perch. According to these repots, the length of perch varied from 220.0 to 264.0 mm, and weight – from 140.0 to 300.0 g (Drozzhina, Fedorova,1982), which was higher than our perch measurements from Shatsk lakes.

Among the indicators that can indirectly characterize the species state and living conditions is fecundity. The amplitude of changes in relative fecundity among specimens of one generation indicates the degree of sexual maturation of females in terms of the intensity of generative exchange, which ultimately determines the life expectancy of most individuals and, accordingly, the dynamics of population decline (Spanovska, 1976). Individual absolute fecundity (IAF) and RF are indicators not only of the number of eggs laid by a female, but also of the state of producers and the quality of sexual products, which together affect the descendants of this species of fish, aggravating the situation with reproduction (Nikolsky, 1974).

We established that in non-infested specimens of 4-year perch, the highest IAF was 6.7 thousand eggs against 3.1 thousand eggs (lake Lucimer), and the lowest - 3.9 thousand eggs against 2.0 thousand eggs in infested ones (lake Chorne Velyke). In non-infested specimens of 5-year old perch, the highest rate of IAF was 9.4 thousand eggs against 6.8 thousand in the infested ones (lake Pulemetske), the lowest - 6.3 thousand eggs (Chorne Velyke lake). In 6-year perch, the highest IAF was recorded in Pulemetske lake (34.6 thousand eggs in non-infested against 10.3 thousand eggs in infested fish). The smallest IAF was observed in perch from Chorne Velyke lake (9.1 and 6.6 thousand eggs, respectively) with a slight difference (Fig. 5).

We determined a steady trend of decreasing fecundity in infested fish. Comparing the obtained results with the data of previous years, the productive properties of perch in Shatsky lakes, in particular in Svityaz lake have changed significantly. Thus, with a body length of 24.0-36.0 cm and a mass of 320.0-1000.0 g, the IAF was 32.2-130.9 thousand eggs, which significantly exceeds the IAF in these studies.

We found that male perch in the reservoirs of Shatsk lakes first spawn at the age of 2 and 3 years with a body length of about 90 mm and a weight of 10.0 g, females - at the age of 3 to 4 years with a body length of 84 mm and a weight of 8.0 g. In some males, the puberty occurs at the age of one year. Fecundity significantly increases with the age. In young specimens, the function of the sex glands increases with each successive cycle, which was also registered (see Table 1). We detected that 4-year perch had 2.0-6.7 thousand eggs, 5-year – 4.1-9.4 thousand eggs, and 6-year –6.6-34.5 thousand eggs.

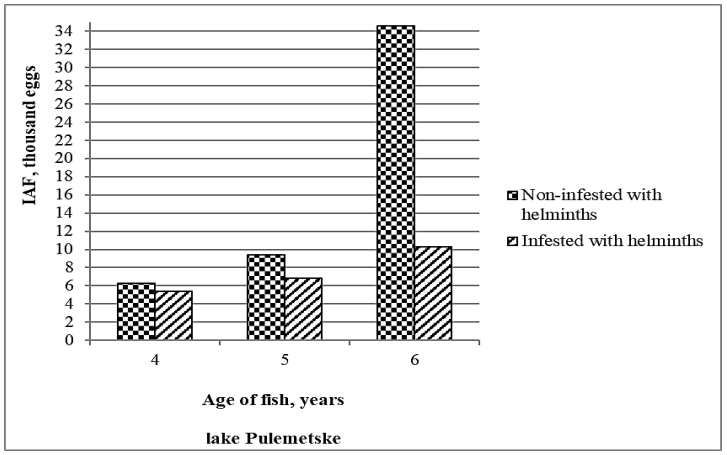
We detected tha significant differences in absolute fecundity between non-infested and infested perch specimens, which indicates a deterioration in its productive characteristics in terms of infestation with triaenophorus.

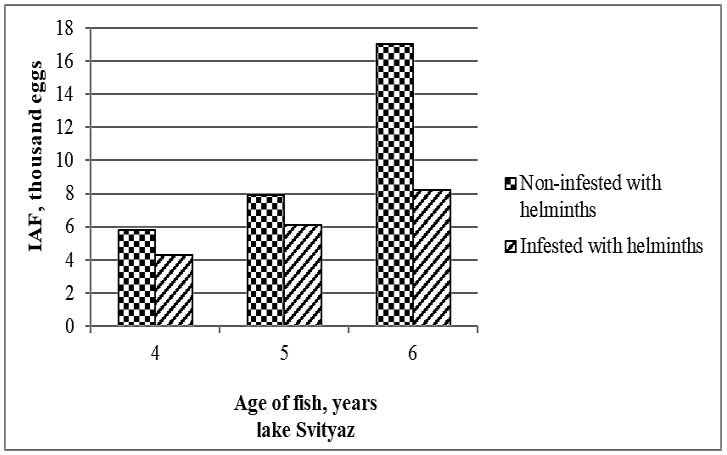
The absolute fecundity was highest in specimens of middle-aged groups. So, individual absolute fecundity in 6-year perch in Pulemetske lake was the highest and amounted up to 34.6 thousand eggs. Individual absolute fecundity of various age groups is shown in Fig. 5. We also registered a decrease in the productive characteristics of perch in the studied reservoires, mainly caused by T. Nodulosus infestation.

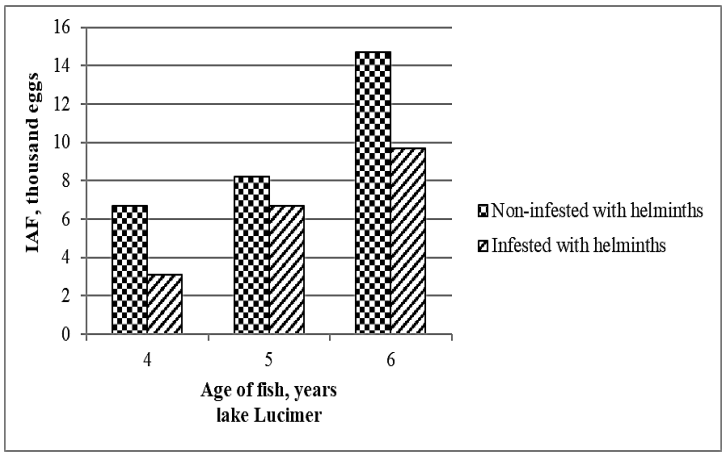
The growth rate of the fish of one population and the timing of puberty can vary significantly under the influence of abiotic and biotic factors. This results in decline of the industrial size and weight, caused by increased anthropogenic impact on aquatic ecosystems, reduced forage, interspecific and intraspecific competition for food resources, disturbances in the fishery. This, the gradual decrease in the growth rate, which leads to the formation and dominance of slow-growing individuals was observed previously (Kryzhanovsky, Dyachuk, 1985; Zaitsev, Rostovtsev, 2006; Yevtushenko et al., 2009). However, our research shows that a significant infestation of perch with T. nodulosus also leads to a decrease in size and weight and negatively affects the reproductive capacity.

Infestation with Triaenophorus is a common disease of predatory fish (perch, whitefish, grayling, dace, tench, catfish, trout, smelt, gangfish), mainly in rivers, lakes and reservoirs. At the plerocercoid stage, the parasite in the fish body can live for several years and is a striking example of species specificity – sexually mature helminths parasitize in the intestines of pike, while the larval stage (in the form of cysts) – in the liver of perch. The development cycle of T. nodulosus occurs with the participation of cyclops of Cyclops strenuus, Paracyclops fimbriatus, Mesocyclops oithonoides, Eucyclops serrulatus, Acanthocyclops bicuspidatus and others (intermediate hosts) and an additional host – fish. Definitive host of the helminth is pike. The helminthiasis of fish is widely distributed in various types of reservoirs. It is rarely recorded in pond farms and only in trout (Hayevskaya, 2006). With a high intensity and extensiveness of infestation, they cause significant damage to both the ichthyofauna of natural reservoirs and aquaculture facilities. There is a risk of infection with triaenophorus when using Cyclops spas as a live feed for growing juveniles (Lahnsteiner et al., 2009). According to special literature data, the ratio of digestive enzymes changes in perch of different age groups infested with triaenophorus plerocercoids, their activity decreases, especially of those that provide the initial stages of assimilation of protein components of food, which cannot but affect the effectiveness of fish nutrition. (Frolova et al., 2018). There is also information about the negative impact of T. nodulosus invasion on the body and some biochemical parameters of the liver (Morley, Lewis, 2017; Borvinskaya et al., 2019).

We believed that under optimal conditions, the parasites have little effect on the physiological characteristics of hosts, but it is an important selective factor that in specific environmental conditions or under the stress, significantly affect the stability of host body.







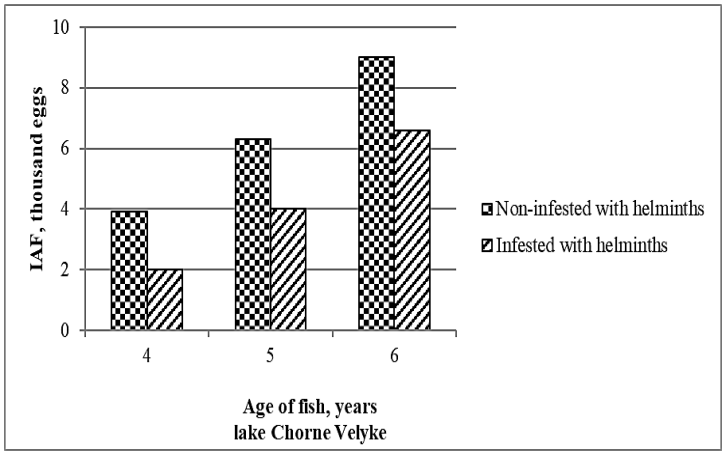


Fig. 5. Individual absolute fecundity of perch different age groups (the lakes of Shatsk National Nature Park)

Conclusions

We registered a significant infestation of perch with plerocercoidscaused by *Triaenophorus nodulosus*. The extent of infestation fluctuated in the range of 18.2–56.3% with the intensity of invasion 2-7 specimens per fish depending on the reservoir. We also found significant difference in morpho-biological parameters between non-infested and infested by *T. nodulosus* fish specimens of the same age, moreover, we detected a significant lag in growth, weight loss and fertility among the infested specimens, which, however, did not affect the fish reproductive ability.

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