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The role of non-commercial cyprinids in maintenance and spread of the opisthorchiasis focus in the middle Ob River basin (Tomsk region, Russia)

Anastasia V. Simakova [a,*], Irina B. Babkina [a], Nakul Chitnis [b,c], Alexey V. Katokhin [d], Alexandr M. Babkin [a], Olga S. Fedorova [e]

[a] *Biological Institute, Tomsk State University, Tomsk, Russian Federation*
[b] *Department of Epidemiology and Public Health, Swiss Tropical and Public Health Institute, Basel, Switzerland*
[c] *University of Basel, Basel, Switzerland*
[d] *Institute of Cytology and Genetics, Siberian Branch of the Russian Academy of Sciences, Novosibirsk, Russian Federation*
[e] *Department of Faculty Pediatrics, Federal State Budget Educational Institution of Higher Education, Siberian State Medical University, Ministry of Healthcare of Russian Federation, Tomsk, Russian Federation*

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ABSTRACT

The study assessed the role of non-commercial cyprinid species in maintaining the opisthorchiasis focus in the middle Ob River basin, Tomsk region, Russia. The source of *O. felineus* infection for humans and carnivores is fish of the family Cyprinidae. This is the most numerous family, 14 species live in the middle Ob River basin, which includes 6 commercial species and 8 non-commercial species.

This study aimed to investigate the current situation on infestation of non-commercial cyprinids with *O. felineus* metacercariae and their role in maintaining and spreading the natural focus of opisthorchiasis in the middle Ob River basin.

We investigated 4 non-commercial species (tench, sunbleak, common bleak, gudgeon), which are highly abundant in water bodies. Tench, common bleak and gudgeon are objects of amateur fishing. These species are traditionally included in the diet of the local population.

Opisthorchis felineus metacercariae were recorded in muscles of all the examined fish species. The identification of metacercariae was confirmed by morphological methods and PCR diagnostics.

Tench and sunbleak are the main sources of opisthorchiasis infection in the floodplain lakes of the Ob River basin (the prevalence of tench infection is 89.3% and mean intensity of infection is 11.2 metacercariae per fish, the prevalence of sunbleak infection is 50.9% and the intensity of infection is 4.25 metacercariae per fish).

The prevalence of infection in the introduced common bleak from the rivers of the middle Ob River basin is rapidly increasing from 2.4 (2016–2018) to 37.5% (2020–2021), and mean intensity of infection increased from 1 to 4.15.

The epizootic state of water bodies in the middle Ob River basin remains unfavorable in relation to opisthorchiasis. Tench, common bleak and sunbleak, along with ide and dace, are the main source of infection for humans and animals, which is evidenced by high infection with *Opisthorchis felineus* metacercariae in these numerous fish species. They pose the greatest danger

* Corresponding author.
E-mail address: anastasiasimakova@yahoo.com (A.V. Simakova).

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of infection of people and animals with opisthorchiasis. These species should be included in the campaign to avoid raw and poorly cooked fish in the diet. In addition, such species as roach, bream and sunbleak also pose the danger of infection with opisthorchiasis, but to a lesser extent.

1. Introduction

Opisthorchis felineus is a parasite predominantly of carnivores throughout much of its range, only occasionally causing human disease (Pozio et al., 2013) except in eastern Russia where prevalence in humans can be high and poor sanitary conditions make the spread of eggs via human faeces possible (Mordvinov et al., 2012). It was estimated that 80 million people were at risk of food-borne opisthorchiasis, approximately 1.2 million people are infected with *O. felineus* infection (Keiser and Utzinger, 2009). In Russia, opisthorchiasis infection accounting for 78.5% of all recorded helminthiases in this country (Federal Service on Surveillance in the Sphere of Consumer Rights Protection and Human Welfare, 2018).

The issue of the incidence of opisthorchiasis in the population is still poses a significant risk, not only in Russia, but throughout the world (Marcos et al., 2008; Ogorodova et al., 2015; Petney et al., 2013).

Humans become infected by consuming cyprinid fish infected with metacercariae. In the Ob River basin, the family Cyprinidae is represented by 14 species, of which 6 are commercial species, and the rest are non-commercial species.

Infestation of commercial and introduced species (bream, ide, goldfish, silver carp, dace, and roach) was studied earlier It was found that ide and dace are the main carriers of *O. felineus* metacercariae (Simakova et al., 2019, 2021). Non-commercial species (sun bleak, common bleak, gudgeon, tench, three species of minnows, carp), which may not only be abundant, but also have good taste (for example, tench, common bleak, gudgeon), have not been properly studied. Non-commercial species can also be the source of infection for humans and animals and play an important role in maintaining and expanding the focus of opisthorchiasis. The study of these fish species for larval of *O. felineus* infestation was sparse prior to our study.

Of the studied non-commercial fish species, tench and gudgeon are the objects of human nutrition. According to survey data (Simakova et al., 2020), the share of these species in the diet of people in the Tomsk region is up to 1.3%. Bleak is also an object of amateur fishing, and in recent years it has begun to be considered by fishery statistics in the Tomsk region.

The issue of the incidence of opisthorchiasis is still one of the urgent and socially significant in Tomsk region, despite the preventive measures taken; therefore, the study of the biology, ecology of not only commercial, but also non-commercial cyprinids, and their infection with *O. felineus* metacercariae, are crucial for the control and prevention of opisthorchiasis.

The aim of the study is to assess the current situation on the infection of non-commercial cyprinids with *O. felineus* metacercariae and their role in maintaining and spreading the natural focus of opisthorchiasis in the middle Ob River basin.

We have studied the most numerous non-commercial cyprinid fish species that are confined to the floodplain of the largest river in Siberia and floodplain lakes, where there are all links for opisthorchiasis circulation. These are tench, sunbleak, common bleak and gudgeon.

2. Materials and methods

2.1. Study area

The study was conducted in the Tomsk region situated in the southeast of Russia (Western Siberian Plain), which the middle Ob River basin. Tomsk city is the capital of the Tomsk region.

2.2. Fish harvesting and contamination assessment

Infection of non-commercial fish was estimated in 2020–2021 on fish caught in the Ob River basin (Tomsk region): in the Basandaika River (second-order tributary; 56°24′41.00" N, 84°58′50.70″ E), Tom River (first-order tributary; 56°27′45.4"N, 84°55′48.1″E), Ob River in the Shegarsky (56°32′50,92" N, 84°09′36.83″ E) districts, and in several lakes (56°25′59.131"N, 85°3′43.949″E; 56°17′56"N, 84°53′29″E) of the Tomsky district (Tomsk region).

The fish were selected from commercial catches and crayfish trap. These fish were accidentally caught during commercial fishing and selected from commercial catches. In total, 206 individuals were assayed in 2020–2021, including 32 common bleak; 135 sunbleak, 10 gudgeon and 28 tench.

The fish were identified to a species level and gender to measure the standard length (SL, cm) and body weight (W, g). Otoliths were used to determine the age. The back of the fish's head was transected to extract otoliths. In small fish, otoliths were examined as a whole, and in large fish they were separated. Examination was performed using a stereo microscope, up to 90×, MSP-1, LOMO. One transparent and one opaque zone together were taken as an indicator of annual growth.

To examine the presence of trematode metacercariae in fish, the compression method (Sayasone et al., 2007; Vichasri et al., 1982), In this study, we employed the compression method only, because the sizes of common bleak, sunbleak, gudgeon were small enough (mostly <10 cm, only few over 10 cm but still <15 cm) to examine by the compression method (Touch et al., 2009; Rim et al., 2008). Tench was examined by this method in order to correctly compare the results. In addition, this method has traditionally been and is used by Russian scientists (Beer, 2005). Therefore, we used this method to correctly compare our results with previous studies in

Siberia.

Opisthorchis felineus metacercariae were identified using a standard patented method (Voronin et al., 2019).

2.3. Molecular genetic methods

Morphological identification of O. *felineus* was confirmed by molecular genetic methods, i.e. PCR diagnostics based on the genetic marker ITS2 (Internal Transcribed Spacer 2 of ribosomal RNAs gene). Species specific nucleotide divergence in the marker has been shown to be sufficient to distinguish from O. *felineus* other opisthorchid species by PCR (Müller et al., 2007; Brusentsov et al., 2010; Kiyan et al., 2018). DNA from 6 metacercariae were individually isolated using STAB method (Rusch et al., 2018). Metacercaria specimens were: 2 from sunbleak, 2 from tench (Medvezhye Lake), and 2 from dace (the Tom River). For PCR reaction, standard PCR kits were used. The universal for Opisthochiidae reverse primer ITS2exR (5′- GGAACGACCTGAACACCA −3′) in combination with the O. *felineus* species-specific forward primer OfF (5'-ATGATTTCCCCACGCAT-3′) generated a PCR product with a length of 408 nt. The PCR products were analyzed by electrophoresis in 1% agarose gel. The product of PCR using DNA isolated from *O. felineus* adult worm was taken as positive control (Fig. 1).

The prevalence of infection, PI (the percentage of infested individuals relative to the total number of the examined ones), mean infection intensity (average number of parasites in infected hosts), mean infection intensity per 1 kg of fish weight (the average number of parasites in terms of 1 kg of weight in all examined fish). The index of mean abundance (the average number of larvae per examined individual) was calculated by dividing the total number of the observed larvae by the number of examined individuals.

The fish species are ordered in the Results section according to the intensity of their infection. Statistical analyses were performed using R (3.6.1), a free software environment for statistical computing and graphics. Continuous data were presented in mean and standard deviation. To assess the correlation between the biological parameters of fish and the number of metacercariae in the muscles, the Pearson correlation coefficient (r) was used. Linear regression analysis was used to assess the relationship between the number of *O. felineus* metacercariae and body weight of fish. The nonparametric Kruskell – Wallis test was used to compare the level of infection in groups of different sex and age (Zar, 2010). A P-value of 0.05 or less was considered significant.

3. Results

3.1. Fish contamination assessment

The examined non-commercial cyprinid species included tench *Tinca tinca* Linnaeus, 1758, sunbleak *Leucaspius delineatus* Heckel, 1843, common bleak *Alburnus alburnus* Linnaeus, 1758, and gudgeon *Gobio gobio* (Linnaeus, 1758) (Fig. 2).

3.2. Tench

Tench species were collected in the floodplain lakes of the Ob River basin. The age cohort of the sample was represented by individuals aged 4+ to 7+ years, with a body length of 17.6–25.5 cm (average of 19.9) and weight of 150–588 g (average of 223 g) (Fig. 3A). The female to male ratio was 1:2. The prevalence of tench infection amounted to 89.3%; the infection prevalence was observed to increase with age: 75% of fish aged 4+, 92% of fish aged 5+, and 100% of fish aged 6+ and older. The mean infection intensity was 11.2 and varied from 1 to 36; however, one individual aged 4+ exhibited a higher infestation (63) (Table 1). The infection intensity was observed to increase with age: 3.8 for fish aged 4+, 6.2 for fish aged 5+, 15.4 for fish aged 6+, 21.5 metacercariae for fish aged 7+ (Fig. 3B). There were no significant differences in the infection of males and females.

3.3. Sunbleak

Sunbleak species were collected in the floodplain lakes of the Tom River. The age cohort of the sample was represented by individuals aged 1+ to 3+ years, with a body length of 2.0–5.3 cm (average of 3.9) and weight of 0.13–2.55 g (average of 1.04 g) (Fig. 4A). The sample included immature and sexually mature individuals, and the female to male ratio was 1:2. The prevalence of infection was more than 50%, and the prevalence of immature individuals was significantly lower than that of sexually mature

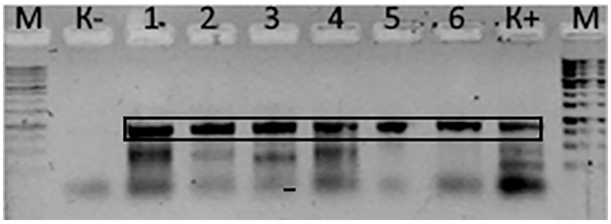

Fig. 1. Gel electrophoresis of PCR products generated with DNA preparations from *O. felineus* metacercariae. Lanes 1, 2 – from dace (Tom River, Tomsk city district); 3, 4 – from tench (Medvezhye Lake); 5, 6 – from sunbleak (Medvezhye Lake); K+ − positive control; K- – negative control; M – DNA Ladders (by 250 bp) up to 10 kb.

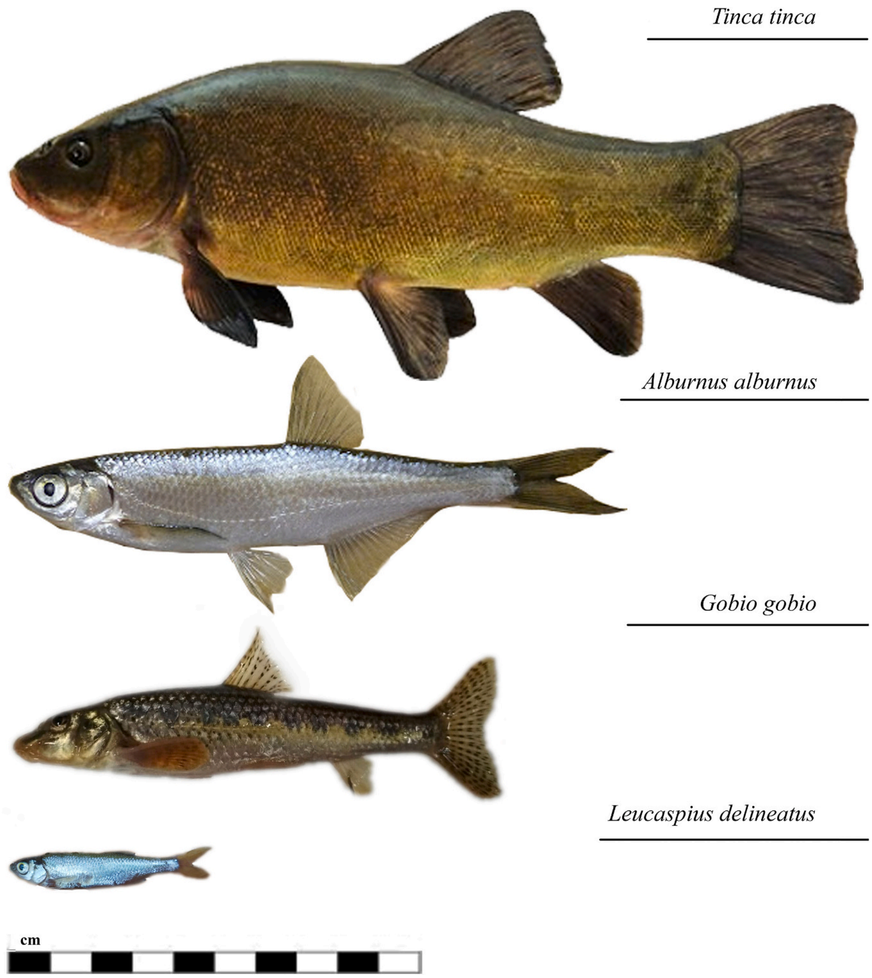

Fig. 2. The researched fish species from middle Ob River basin.

individuals (38.2 and 56.6%, respectively). The mean infection intensity amounted to 4.25, and the abundance index was 2.6 (Table 1). The mean intensity of immature fish was significantly lower than that of sexually mature fish (1.84 and 4.97; wilcox.test, $p < 0.05$). The infection intensity was similar for males and females. No correlation was found between the sunbleak infestation and its size and age (Fig. 4).

3.4. *Common bleak*

The examined common bleak individuals were collected in different water bodies of the middle Ob River basin – the Ob and Tom rivers. The sample contained individuals aged 3+ to 5+ years with a body length of 9.6–12.7 cm (average of 11.2) and weight of 10.8–23.4 g (average of 15.8 g) (Fig. 5). The female to male ratio was 1:1.5.

The prevalence of infection was 37.5%; there were no significant differences in the infection of males (36.8%) and females (38.5%). The mean infection intensity was 11.1 and varied from 1 to 13; however, one individual exhibited a high infection intensity (95) (Fig. 5; Table 1). No significant difference was found in the infection intensity depending on the sex and age and size of the fish.

3.5. *Gudgeon*

Gudgeon species were collected in the Basandaika River (a second-order tributary of the Ob River). The age cohort of the sample was represented by individuals aged 1+ to 4+ years, with a body length of 5.6–12.5 cm (average of 9.8) and weight of 2.4–32.0 g (average of 16.6 g) (Fig. 6). The male to female ratio was 1:2. The prevalence of infection was 10% (Table 1). One *O. felineus* larva was found in the individual aged 3+ years (Fig. 6).

Thus, all the examined non-commercial cyprinid species were infected with *O. felineus* larvae. Tench and sunbleak were the most infected species in floodplain lakes, and bleak was the most infected species in rivers.

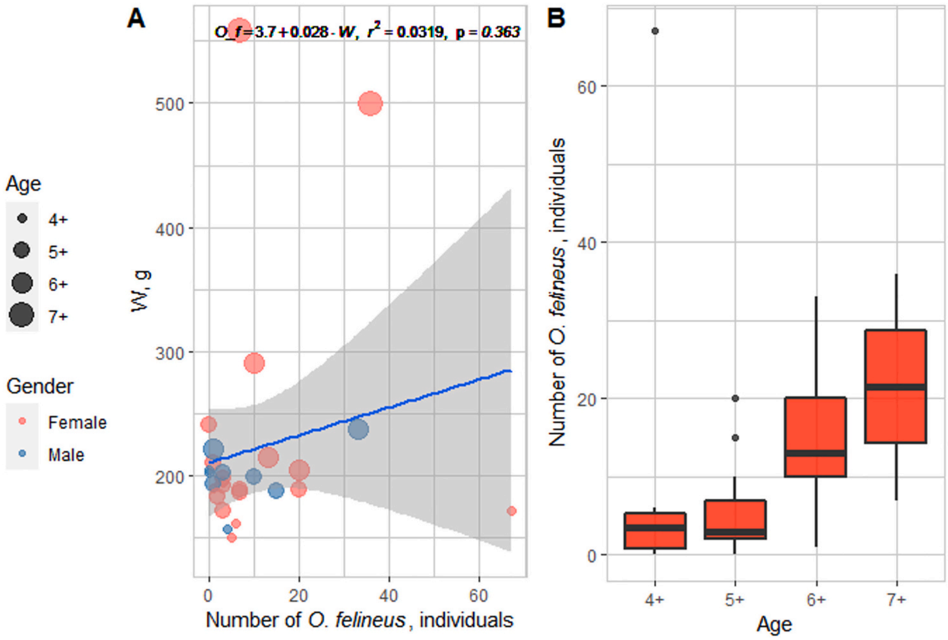

Fig. 3. Size, age, and infection characteristics of tench *Tinca tinca* of different age in the middle Ob River basin (2020–2021). A: Number of *O. felineus* (*O_f*) plotted against the body weight (*W*, weight). B: Number of *O. felineus* on fish of different age. Each box includes three horizontal lines which denote 25, 50 (median) and 75% of the data (from 25 to 75% of the data are enclosed into a rectangle); the upper whisker extends from the first quartile to the maximum value no further than 1.5 × IQR (where IQR is the interquartile range); lower from the third quartile to the smallest value, no further than 1.5 × IQR of data; (point), values beyond ±1.5 × IQR.

Table 1
Characteristics of the infection of cyprinid fish by *O. felineus* larvae (middle Ob River basin, 2020–2021).

Species	Examined in total	Number of infected	PI, %	Mean intensity per fish	Mean abundance, individuals	Mean intensity per kg
				M ± s.e. (min–max)		
Tench *Tinca tinca*	27	24	89.3	11.2 ± 2.99 (1–67)	10.03	45.3
Sunbleak *Leucaspius delineatus*	110	56	50.9	4.25 ± 1.14 (1–61)	2.16	2239.8
Common bleak *Alburnus alburnus*	32	12	37.5	11.08 ± 7.69 (1–95)	4.15	202.2
Gudgeon *Gobio gobio*	10	1	10	1	0.1	4.7

4. Discussion

We provide the first report of metacercariae of *O. felineus* in tench, sunbleak, common bleak, and gudgeon in the middle Ob River basin. Previous studies reported no *O. felineus* in these hosts in this system (Bocharova, 2007). The morphological identification of metacercariae of *O. felineus* was supported by PCR amplification of species-specific markers. According to our study, all the examined non-commercial cyprinid species are infected with *O. felineus* larvae. The highest prevalence indices were found for tench and sunbleak. The infection for tench is comparable to that for dace (Simakova et al., 2021). The most consumed cyprinids fish are dace, ide and bream, as well as non-commercial species tench and gudgeon (Simakova et al., 2021).

Tench and sunbleak are predominantly lacustrine species. It was established that these species are the main carriers of metacercariae in the floodplain lakes of the middle Ob River basin, since other lacustrine cyprinid species exhibit either low prevalence and intensity infection, for example, roach (1.8%, 0.06), or are noninfected (*Carassius gibelio* and *Carassius carassius*) (Simakova et al., 2021).

The prevalence of tench infection, both in the European part of Russia and in Siberia, is not high (0–33%), the infection intensity is low (Romashova, 2015; Shibitov, 2019; Sous and Bocharova, 2001; Strokin, 2007). According to our data, the prevalence of tench infection in the floodplain lakes of the middle Ob River basin is 3-fold higher than that in other regions.

Different levels of infection in cyprinids can be associated with both the type of water body (river, floodplain lake, continental lake) and the localization of fish in the water body (Babkina et al., 2021). For fish with weak migratory activity, confinement to living in a

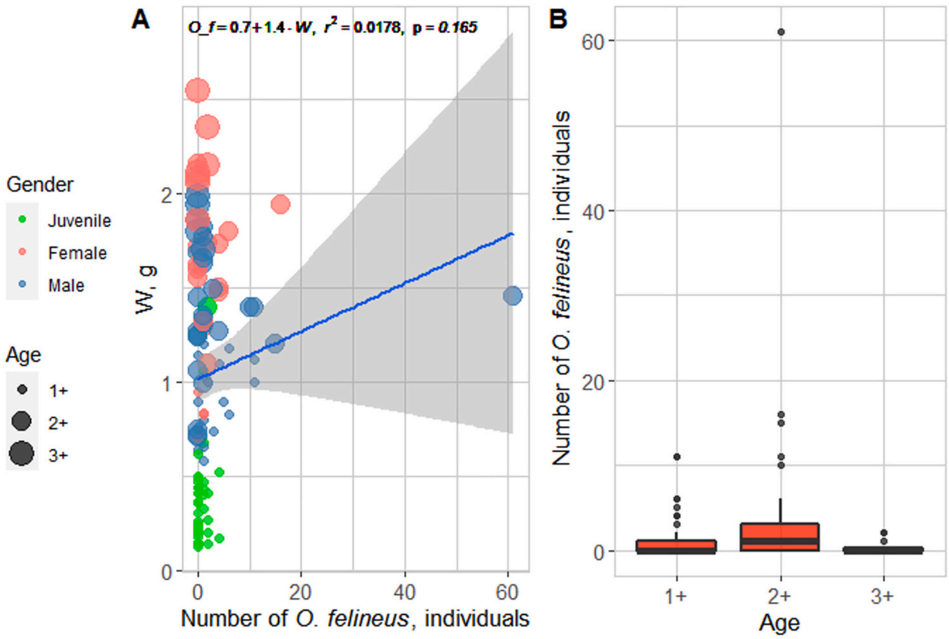

Fig. 4. Size, age, and infection characteristics of sunbleak *Leucaspius delineatus* of different age in the middle Ob River basin (2020–2021). A: Number of *O. felineus* (*O_f*) plotted against the body weight (*W*, weight). B: Number of *O. felineus* on fish of different age. Each box includes three horizontal lines which denote 25, 50 (median) and 75% of the data (from 25 to 75% of the data are enclosed into a rectangle); the upper whisker extends from the first quartile to the maximum value no further than 1.5 × IQR (where IQR is the interquartile range); lower from the third quartile to the smallest value, no further than 1.5 × IQR of data; (point), values beyond ±1.5 × IQR.

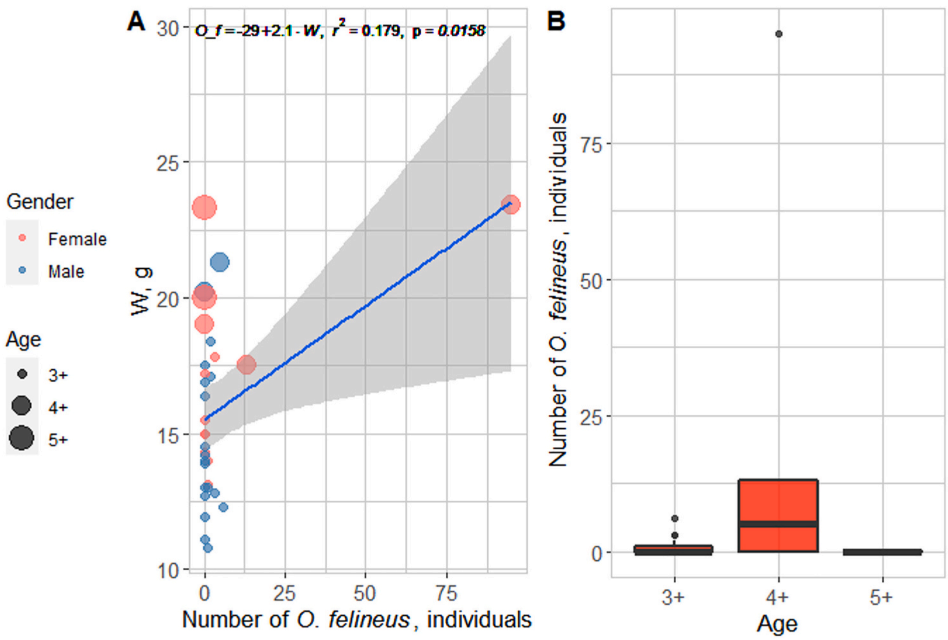

Fig. 5. Size, age, and infection characteristics of common bleak *Alburnus alburnus* of different age in the middle Ob River basin (2020–2021). A: Number of *O. felineus* (*O_f*) plotted against the body weight (*W*, weight). B: Number of *O. felineus* on fish of different age. Each box includes three horizontal lines which denote 25, 50 (median) and 75% of the data (from 25 to 75% of the data are enclosed into a rectangle); the upper whisker extends from the first quartile to the maximum value no further than 1.5 × IQR (where IQR is the interquartile range); lower from the third quartile to the smallest value, no further than 1.5 × IQR of data; (point), values beyond ±1.5 × IQR.

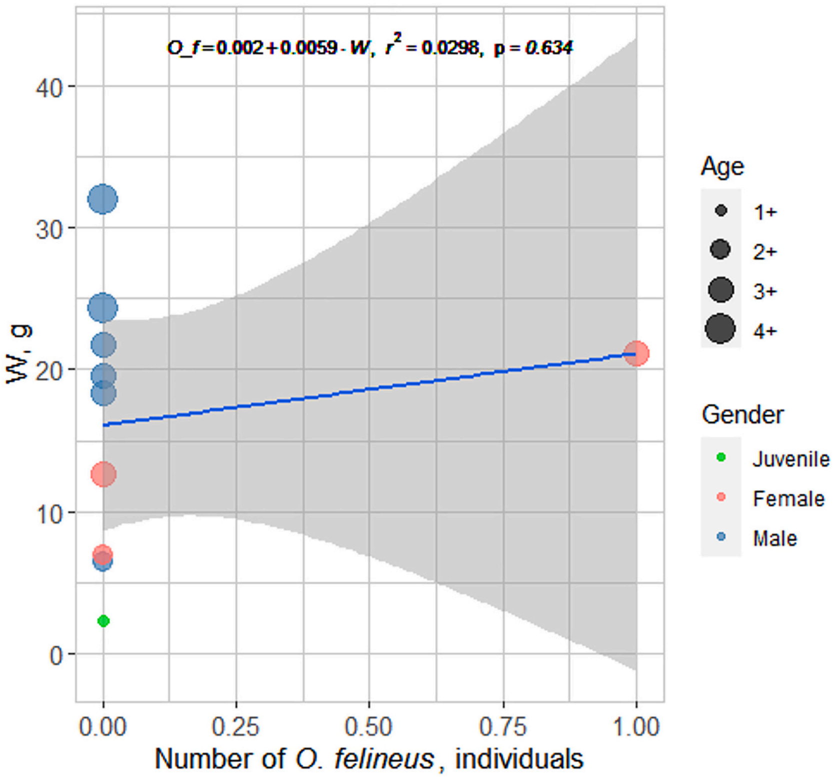

Fig. 6. Number of *O. felineus* (*O_f*) plotted against the body weight (*W*, weight) of gudgeon *Gobio gobio* in the middle Ob River basin (2020–2021).

floodplain with a large number of mollusks can lead to a significant increase in infestation with metacercariae. These will be discussed in a separate article in the future. The snail prevalence of infection was 3–4% during the period 1971–1975 (Beer, 1982). The tench, as well as the sunbleak for our study, were collected from floodplain lakes (in the mainland lake, the sunbleak was free from invasion), the gudgeon from the river Basandaika, common bleak from the Ob and Tom rivers. However, in the same collection site, different species show significant differences in the infection. Possibly, the indicators of fish infection also depend on the biochemical parameters of integument and muscles (Beer, 2005).

In Siberia, the infection prevalence in natural populations of sunbleak varies from 2% to 100%, while the infection intensity is mostly low (Bonina and Serbina, 2011; Fedorov et al., 1996; Sous and Rostovtsev, 2006). Our data confirm the results of studies into the infection of sunbleak from other regions of Siberia. Common bleak was accidentally introduced into the Ob River basin. It was first recorded in 1990 (Babkina et al., 2013). In the native habitat, the infection of common bleak are mostly high (Fattakhov et al., 2011; Romashova, 2015). According to the previous studies, in the 1990s, bleak inhabiting the Ob River basin was not infected with opisthorchiasis (Bocharova, 2007). Our studies have shown that common bleak can be infected with *O. felineus* metacercariae (Simakova et al., 2019). At present, the prevalence of infection in this species from the middle Ob River basin was found to significantly increase from 2.4% (2016–2018) to 37.5% (2020–2021), and the mean infection intensity increased from 1 to 4.15. Previously, the infection intensity was 1 metacercria per individual, and to date the maximum value of the infection intensity attains 95. The load index per ton increased 200 fold. Thus, the infection of common bleak inhabiting the rivers of the middle Ob River basin is comparable with the infection of ide and dace (Simakova et al., 2021). The increase of common bleak infection may be associated with the active dispersal of fish along the watercourses of the Ob basin in recent years. Consequently, common bleak is actively involved in the circulation of opisthorchiasis in Siberia.

In addition to the commercial species, ide and dace (Babkina et al., 2021; Simakova et al., 2021), non-commercial species such as tench, common bleak and sunbleak significantly contribute to maintenance and spread of the natural focus of opisthorchiasis in the middle Ob River basin. The role of gudgeon is negligible. Tench and sunbleak are the main carriers of *O. felineus* metacercariae in floodplain lakes; in the rivers of the Ob River basin, these are ide, dace and common bleak.

5. Conclusions

Our studies have proven that in addition to commercial cyprinid species, non-commercial species are actively involved in the circulation of opisthorchiasis in water bodies of the middle Ob River basin, Siberia, Russia. A number of these species significantly contribute to maintenance and spread of the focus of opisthorchiasis. In rivers, dace, ide (commercial species) and common bleak (non-commercial species) play a crucial role in maintaining and spreading the focus of opisthorchiasis caused by *Opisthorchis felineus*; in floodplain lakes, tench and sunbleak (non-commercial species) are most important spreaders of opisthorchiasis. The role of gudgeon (non-commercial species), as well as bream and roach (commercial), is insignificant, but these species are involved in the opisthorchiasis circulation in the study area.

Opisthorchiasis burden in Siberia could be more efficiently reduced by targeting control interventions at of such fish species as ide, dace, tench, common bleak, including campaigns promoting the cooking of these fish before consumption. Control measures should target these fish species. This study is part of the planned integrated interdisciplinary project on opisthorchiasis in the middle Ob River basin in Tomsk region, which focuses not only on the fish infection, but also on the infection of the intermediate host, *Bithyniidae* snails, and the definitive host, domestic and wild animals.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fawpar.2022.e00146.

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