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Communities of trematodes in Ponto-Azov gravel snail *Lithoglyphus naticoides* (C. Pfeiffer, 1828) Gastropoda: Hydrobiidae) and their potential impact on the development of marginal host populations in the Volga River basin

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ABSTRACT: Recently the prosobranch freshwater mollusk *Lithoglyphus naticoides* successfully established in the Rybinsk and Uglich reservoirs and became a permanent element of the Upper Volga fauna. The present paper sets to study the characteristics of trematode communities in the colonies of *L. naticoides*, formed under relatively unfavorable habitat conditions for the host at the northeastern border of the range, and to characterize the host-parasite relations. We revealed the parthenitae of *Apophallus muehlingi*, *Apophallus* (=*Rossicotrema*) *donicus*, *Parasymphylodora markewitschi*, *Nicolla skrjabini*, *Sanguinicola volgensis* in the marginal populations of this invasive mollusk. Besides, we found *Xiphidiocercaria* sp., representing a new host record for *L. naticoides* in the Uglich Reservoir, the species identity of which has not yet been clarified. The possibility of using indicators of the occurrence of trematodes (the prevalence, P±SE, %) to assess the degree of pathogenic influence on this host and competitive relations between trematodes is studied. The results of statistical analysis of the data on the prevalence of parthenitae are confirmed by studies of the hepatopancreas of adult specimens of *L. naticoides* performed using histological methods. In particular, it is noticeable that even fully developed daughter rediae of *Apophallus* sp. exhibit weakest effect on the digestive gland of *L. naticoides.* The body of these very mobile rediae is relatively thin, and they easily move between the lobes of the hepatopancreas, practically without squeezing them. It is shown that the rediae of *A. muehlingi*, *A. donicus*, and *P. markewitschi* usually dominate at the initial stage of *L. naticoides* colony development. At the stage of full establishing of *L. naticoides*, the prevalence of *N. skrjabini* or *S. volgensis* sporocysts can increase quickly. Based on analysis of the data on the prevalence of parthenitae and histological examinations we assume that the small size of the digestive gland of *L. naticoides* prevents the full development of a mixed infection with parthenitae of two or more species. The maximum proportions of mixed infections were 6.87–8.82% of the total number of individuals studied. Of the six variants of double infection in the Upper Volga basin, the most common cases are *Nicolla+Sanguinicola*, *Nicolla+Parasymphylodora*, *Sanguinicola + Parasymphylodora*. Mixed infection with *Apophallus+Parasymphylodora*, *Sanguinicola + Xiphidiocercaria*, *Parasymphylodora+Xiphidiocercaria* is less common. For the populations of *L. naticoides* we studied, due to the short period of joint coevolution, during which the

helminth adapted to this host, the trematode *S. volgensis* is considered to be the most pathogenic parasite, both at the organismic and at the population levels. The negative effect of the communities of trematodes sharply increases with an increase in the population density of *S. volgensis*.
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KEY WORDS: alien species, Gastropoda, *Lithoglyphus naticoides*, marginal populations, parasites, Trematoda, mixed infections.

Сообщества трематод у понто-азовского моллюска *Lithoglyphus naticoides* (C. Pfeiffer, 1828) (Gastropoda: Hydrobiidae) и их потенциальное влияние на развитие краевых популяций хозяина в бассейне реки Волга

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РЕЗЮМЕ: Переднежаберный пресноводный моллюск *Lithoglyphus naticoides* недавно стал постоянным элементом фауны Верхней Волги – после успешной натурализации в Рыбинском и Угличском водохранилищах. Основные задачи исследования — изучение особенностей сообществ трематод в поселениях *L. naticoides*, сформировавшихся в относительно неблагоприятных для хозяина условиях обитания на северо-восточной границе ареала, и описание паразито-хозяинных отношений. В краевых популяциях этого моллюска-вселенца мы выявили партениты *Apophallus muehlingi*, *Apophallus* (=*Rossicotrema*) *donicus*, *Parasymphylodora markewitschi*, *Nicolla skrjabini*, *Sanguinicola volgensis*. Кроме того, в Угличском водохранилище мы впервые обнаружили *Xiphidiocercaria* sp., видовая принадлежность которой пока не выяснена. Изучена возможность использования показателей встречаемости трематод (the prevalence, P±SE, %) для оценки степени патогенного влияния на этого хозяина и конкурентных отношений между трематодами. Результаты статистического анализа данных по встречаемости партенит подтверждаются исследованиями пищеварительной железы у взрослых особей *L. naticoides* выполненными с использованием гистологических методов. В частности, отмечено, что даже полностью развитые дочерние редии *Apophallus* sp. оказывают слабое воздействие на пищеварительную железу *L. naticoides.* Тело этих очень подвижных редий относительно тонкое, они легко перемещаются между долями гепатопанкреаса, практически их не сдавливая. Показано, что на начальном этапе развития поселения *L. naticoides* обычно доминируют редии *A. muehlingi*, *A. donicus* и *P. markewitschi.* На этапе полноценной натурализации *L. naticoides* может быстро повышаться встречаемость спороцист *N. skrjabini* или *S. volgensis*. По результатам анализа данных о встречаемости партенит и гистологического исследования мы пришли к выводу, что небольшой размер пищеварительной железы *L. naticoides* препятствуют полноценному развитию смешанного заражения партенитами двух или более видов. Максимальные значения доли смешанных заражений не превысили 6.87–8.82% от общего числа исследованных особей. Из шести вариантов двойного заражения в бассейне Верхней Волги наиболее распространены случаи *Nicolla*+*Sanguinicola*, *Nicolla* + *Parasymphylodora*, *Sanguinicola*+*Parasymphylodora*. Менее распространено смешанное заражение *Apophallus*+*Parasymphylodora*, *Sanguinicola*+*Xiphidiocercaria*, *Parasymphylodora*+*Xiphidiocercaria*. Для исследованных нами популяций *L. naticoides*, из-за небольшого периода коэволюции, в течение которого гельминт адаптировался к данному хозяину, трематода *S. volgensis* рассматривается в качестве наиболее патогенного паразита, как на организменном, так и на популяционном уровнях. Негативное влияние сообществ трематод резко возрастает при росте численности популяций *S. volgensis*.

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КЛЮЧЕВЫЕ СЛОВА: чужеродные виды, Gastropoda, *Lithoglyphus naticoides*, краевые популяции, паразиты, Trematoda, смешанные заражения.

Introduction

Parasites can be important factors that determine the success of an invasion and its impact on the recipient community (Dunn, 2009; Poulin *et al.*, 2011; Tyutin *et al.*, 2013; Zhokhov *et al.*, 2019). Freshwater systems, when compared to terrestrial habitats, may offer certain advantages in the study of disease emergence caused by the introductions of non-indigenous species (Poulin *et al.*, 2011). Recently, the freshwater prosobranch mollusk *Lithoglyphus naticoides* (C. Pfeiffer, 1828) has a relatively large range within Europe. Some of the trematodes associated with *L. naticoides* are pathogens for fish, fish-eating birds and mammals; therefore, this mollusk is included in the lists of European invasive species, the dispersal of which is dangerous and can lead to negative economic and environmental consequences (Panov *et al.*, 2009; The most dangerous..., 2018). The penetration of this mollusk into the lower reaches of the Volga River through the Volga-Don Canal occurred no later than 1971 (Pirogov, 1972). This was accompanied by the development of large foci of helminthoses caused by some trematodes associated with the invasive mollusk. By 1997, parthenitae of at least 13 species of trematodes were identified in *L. naticoides* in the Volga River delta, at least three of which are considered invaders (Biserova, 2005, 2010; Ivanov, 2008).

The northward expansion of *L. naticoides* in the cascade of the Volga reservoirs began against the background of a significant increase in mean annual temperatures in the 1990s. As a result, the mollusk massively established in the sandy biotopes of the shallow-water zone of the reservoirs (Yakovlev *et al.*, 2010; Perova *et al.*, 2018; Kurina, Seleznev, 2019; Kurina *et al.*, 2021) including the cascade of reservoirs on the largest Volga tributary, the Kama River. In this region it even dominated by biomass in the Volga-Kamsky and Kamsky reaches of the Kuibyshev Reservoir. In the shallow Nizhnekamsk Reservoir, the species also occurs *en masse*, but its wide northward spread is probably limited by a number of factors: a smaller area of warmed-up areas of the coastal zone, low water mineralization and oxygen deficiency at the end of winter (Sharapova, 2007; Yakovlev *et al.*, 2010). The influence of another possible limiting factor, the water pollution, has not been assessed yet. However, a suggestion has been made that in some regions of the Central Europe the species either disappeared or is at the brink of extinction due to water pollution (Animal Base, 2013: http://www.animalbase.uni-goettingen.de/zooweb/servlet/AnimalBase/home/species?id=2317). It was also noted that threats to this species include water pollution and habitat degradation throughout its range; however, the species appears to be tolerant of poor environmental conditions and siltation (László *et al*., 2000).

The expansion of this invasive mollusk into the Volga reservoirs was accompanied by the range extension of trematodes specific to this host (Zhokhov *et al*., 2006, 2019, 2021; Tyutin, Slynko, 2010; Tyutin *et al*., 2013; Tyutin, Izvekova, 2013). On the northeastern border of the range, the colonies of *L. naticoides* were formed under relatively unfavorable habitat conditions. In the Rybinsk Reservoir the appearance of *L. naticoides* and, as a consequence, trematodes of the genus *Apophallus* Lühe, 1909 was recorded in 2005–2006 (Tyutin, Slynko, 2010). According to our observations, the process of establishing of *L. naticoides* in this reservoir took at least five to seven years (Tyutin *et al*., 2013, 2020, 2022; Structure and functioning..., 2018). At the initial stage of establishing in 2005–2009 there was an increase in the area of the first permanent colony of the mollusk in the Volga Reach of the reservoir. By that time the parasite composition, including five species of trematodes associated with this host, was determined. Judging by the increase in fish infestation and the rapid dispersal of *L. naticoides* throughout the Volga Reach, by 2010–2011 the establishing of the mollusk and the associated formation of foci of some helminthoses were completed. The invader did not spread over the entire area of the Rybinsk Reservoir. However, the existence of a fairly large population of *L. naticoides* with a relatively stable abundance in the area of the Volga Reach created the prerequisites for an increase in the number of associated helminths in the water bodies of the Upper Volga basin. In 2013–2015 the distribution of *L. naticoides* and some associated trematodes in the Uglich Reservoir located upstream of the cascade was noted, which makes it possible to consider this mollusk as a permanent element of the fauna of the Upper Volga basin (Perova *et al*., 2018).

Already at the initial stage of the development of the marginal populations of *L. naticoides*, the total infection of mature individuals with trematodes may exceed 90% (Biserova, 2010; Tyutin *et al*., 2020, 2022). However, the degree of pathogenicity of sporocysts and rediae is not practically studied even in the common species of trematodes parasitizing *L. naticoides*. Short life cycle (13–15 months), rheophilicity and confinement of most aggregations to biotopes with weakly silted sandy or stony bottom sediments, usually occupying a relatively small area of the water body are among the main features of this invasive mollusk (Mouthon, 2007; Yakovlev *et al*., 2010). There is a widespread opinion that the decrease, instead of expected rise, in the prevalence of parthenitae in mollusks of older size-age groups or in individuals of the same age group by the beginning of autumn may be considered as a consequence of the selective death of infected host specimens (Biserova, 2010). In the Rybinsk Reservoir in 2009–2010 there were the cases of a decrease in the infection of adult *L. naticoides* with rediae of the genus *Apophallus* from ~64% to ~49% during the summer and to ~19% by August of the next year (Tyutin, Izvekova, 2013). Other species of trematodes associated with *L. naticoides* are less studied in this regard. It was shown in *L. naticoides* from the downstream Volga populations, that parthenitae of unidentified trematodes species may negatively affect the respiratory function of the host (Arakelova, 1998). There are no other published data on the influence of trematodes on the physiology of this mollusk at the organismic level and the studies of the hepatopancreas of *L. naticoides* performed using histological methods. It should be noted that many trematodes can be important factors for some native mollusks in the upstream Volga reservoirs. Thus, in the Volga Reach of the Rybinsk Reservoir the prevalence of parthenitae of the genus *Bunodera* was >30% in small (1+) and >60% in large (2+ and 3+) individuals of the clams *Pisidium amnicum* (O.F. Müller, 1774) (Tyutin, 1996).

The goal of the present paper is to analyze, on the example of large colonies of *L. naticoides* located in the Rybinsk and Uglich reservoirs, the relations in several parasite-host systems at the organism and population levels.

Material and methods

The two water bodies, where the materials were sampled, differ in their hydrological characteristics

(The river Volga…, 1979). The Uglich Reservoir is the valley-type reservoir with a poorly developed coastline. The Rybinsk Reservoir (lacustrine-type) is a larger man-made lake. Sandy deposits occupy a significant part of the bottom of this reservoir. The configuration of the water surface is very complex: with a lacustrine Main Part, separated by the Mologa and Sheksna reaches, several widened estuaries of other large tributaries, and an elongated section between the Uglich dam and the Main Part (the Volga Reach). In the present study we analyzed group samples of *L. naticoides* collected in these reservoirs in the summer months of 2009–2019.

The mollusks were sampled mainly by manual collection (using a hydrobiological scraper). In some cases, a modified DAK-100 grab sampler with a sampling area of 0.01 m^2 or a DAK-250 grab sampler (0.025 m^2) were used according to the standard methods previously used by the authors (Tyutin *et al*., 2013; Perova *et al*., 2018). The main sampling of *L. naticoides* was carried out in the colonies formed on a river-type site within the Volga Reach of the Rybinsk Reservoir (distance of about 80 km from 57°47′N, 38°28′E to 58°06′N, 38°41′E). In total, more than 1000 specimens of gravel snail from this reservoir were examined. Also five specimens of native *Bithynia tentaculata* (Linnaeus, 1758) and ten specimens of native *Viviparus viviparus* (Linnaeus, 1758) were examined in August 2019.

In order to study the features of the structure of the trematode community in a reservoir with a relatively high water turnover rate, 131 specimens of *L. naticoides* from a younger population were examined in August 2019. This population was formed in the upper section of the Uglich Reservoir (the mollusks were sampled at a depth of 4 m on the bottom covered with silted sand); approximate coordinates of the geographical center of the sampled colony: 56°53′N, 37°25′E. The choice of this sampling point was also determined by the relatively high (for the Upper Volga basin) density of this colony: to 520 ind./m^2 (Perova *et al*., 2018).

For the analysis of the occurrence of parasites, large group samples of mollusks were collected, including at least 100 adult specimens of *L. naticoides*. To study age differences in infection, we used the previously applied (Tyutin, Izvekova, 2013) division of each sample into two size-age groups: 4.0–6.9 mm and 7.0–9.0 mm. The first size group of *L. naticoides* that we distinguished consists of young underyearlings (0+). The second one consists mainly of two-year-old specimens (1+). The level of infestation in mollusks was assessed by the emission of cercariae or by the presence of parthenitae at dissection. At that, light microscopes MBS-9, MBS-10, and MBI-3 were used. To study the degree of influence of trematodes of different species on the host using histological methods, 39 specimens of mollusks aged 1+ were selected from the sample collected in the last ten days of June 2019 in the Volga Reach of the Rybinsk Reservoir. Totally, we examined seven specimens of mollusks with monoinfection with rediae of *Apophallus muehlingi* (Jägerskiöld, 1899) or *Apophallus* (=*Rossicotrema*) *donicus* (Skrjabin et Lindtrop, 1919), five specimens with rediae *Parasymphylodora markewitschi* Kulakowskaja, 1947, five specimens with sporocysts *Nicolla skrjabini* (Iwanitzky, 1928), and ten specimens infected with sporocysts of *Sanguinicola volgensis* (Razin, 1929). In addition, we examined the tissues of the digestive gland in 12 control individuals of *L. naticoides*, free from infection with trematode parthenitae. The body of the mollusk was preserved with 10% formaldehyde solution. Using a microtome MNTUK-64-1281, a series of 7 μm thick paraffin sections of hepatopancreas tissues were prepared. The mounts were stained following the standard technique with iron hematoxylin (according to Heidenhain). The digital photographs of the sections illustrating the study results were obtained using a Keyence VHX-1000 light microscope with a Z500R lens (a constant magnification was ×500).

The prevalence of each parasite in the sample of mollusks (the proportion of infected individuals with the calculation of the standard statistical error P±SE, %) was used as the main indicator (Sokal, Rohlf, 1995; Bush *et al*., 1997). A total prevalence (the proportion of all infected individuals with one and two trematode species in the sample) was used also. The analysis was performed applying the Pearson's χ^2-test (Chi-square) with Yates correction by method of contingency tables at different levels of significance. The calculations were performed using the STATISTICA 6.0 and Microsoft Excel software packages. Some methodological aspects and data on the levels of *L. naticoides* infestation, mainly related to the seasonal and age dynamics of *Apophallus* spp. in the Rybinsk Reservoir in 2005–2010, are partially given in previously published articles (Tyutin, Izvekova, 2013; Tyutin *et al*., 2013; Perova *et al*., 2018).

Results

According to our observations, in the Upper Volga reservoirs, *L. naticoides* spawns in mass at the beginning of summer, and young mollusks quickly reach a size of >4 mm. At such a shell height, the volume of the host's hepatopancreas already ensures the full development of microhemipopulations of parthenitae of all the identified species of trematodes. Six large samples of *L. naticoides* collected at the end of summer (July–August) in which mollusks of different

Table 1. Prevalence (P±SE, %) of trematode parthenitae in the different size groups of *Lithoglyphus naticoides*.

Таблица 1. Встречаемость (P±SE, %) партенит трематод в разных размерных группах *Lithoglyphus naticoides*.

Trematode species	Size group of mollusks		Statistical significance of differences
	4.0–6.9 mm	7.0–9.0 mm	
Group sample no.1. Initial stage of establishing of *Lithoglyphus naticoides* in the Rybinsk Reservoir (July 2009, n=118)			
Apophallus spp.	64.29±5.73	77.08±6.07	χ^2=1.6375, p>0.20067
Parasymphylodora markewitschi	8.57±3.35	10.42±4.41	χ^2=0.0003, p>0.98692
Nicolla skrjabini	0	0	–
Sanguinicola volgensis	0	0	–
Group sample no. 2. Initial stage of establishing of *Lithoglyphus naticoides* in the Rybinsk Reservoir (August 2009, n=105)			
Apophallus spp.	49.12±6.62	66.67±6.80	χ^2=2.5977, p>0.10702
Parasymphylodora markewitschi	14.04±4.60	16.67±5.38	χ^2=0.0102, p>0.91936
Nicolla skrjabini	1.75±1.74	0	–
Sanguinicola volgensis	0	0	–
Group sample no. 3. Final stage of establishing of *Lithoglyphus naticoides* in the Rybinsk Reservoir (August 2010, n=163)			
Apophallus spp.	3.30±1.87	19.44±4.66	χ^2=9.5578, p<0.00199*
Parasymphylodora markewitschi	6.59±2.60	9.72±3.49	χ^2=0.1946, p>0.659126
Nicolla skrjabini	0	1.39±1.38	–
Sanguinicola volgensis	3.30±1.87	0	–
Group sample no. 4. Final stage of establishing of *Lithoglyphus naticoides* in the Rybinsk Reservoir (July 2011, n=102)			
Apophallus spp.	5.00±2.81	11.90±5.00	χ^2=0.8143, p>0.36684
Parasymphylodora markewitschi	18.33±5.00	16.67±5.75	χ^2=0.0022, p>0.96285
Nicolla skrjabini	71.67±5.82	66.67±7.27	χ^2=0.1034, p>0.74773
Sanguinicola volgensis	8.33±3.57	2.38±2.35	χ^2=0.6887, p>0.406597
Group sample no. 5. The stage of stable permanent population of *Lithoglyphus naticoides* in the Rybinsk Reservoir (August 2019, n=175)			
Apophallus spp.	16.83±3.72	52.70±5.80	χ^2=23.6332, p<0.0001*
Parasymphylodora markewitschi	9.90±2.97	8.11±3.17	χ^2=0.0199, p>0.887814
Nicolla skrjabini	9.90±2.97	25.68±5.08	χ^2=6.5884, p<0.010264*
Sanguinicola volgensis	28.71±4.50	6.76±2.92	χ^2=11.7872, p<0.000596*
Group sample no. 6. The stage of stable permanent population of *Lithoglyphus naticoides* in the Uglich Reservoir (August 2019, n=131)			
Apophallus spp.	4.21±2.26	30.56±7.68	χ^2=15.3670, p<0.00009*
Parasymphylodora markewitschi	6.32±2.50	11.11±5.24	χ^2=0.3071, p>0.579442
Nicolla skrjabini	4.21±2.06	16.67±6.21	χ^2=4.1141, p<0.042528*
Sanguinicola volgensis	73.68±4.52	36.11±8.01	χ^2=14.2985, p<0.000156*

Notes. n — number of mollusks examined, ind. * — P±SE differences are statistically significant by Pearson's χ^2-test at a high level of significance (p<0.01). "Dash" — insufficient amount of data for statistical processing.

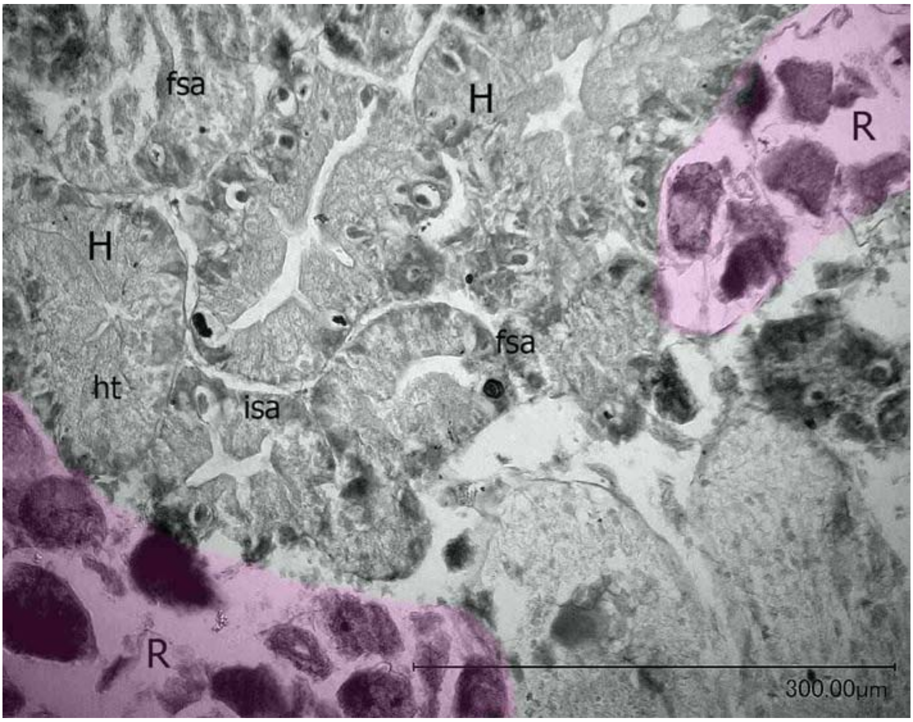

Fig. 1. The daughter rediae with cercariae of *Apophallus* sp. in the inner part of digestive gland of *Lithoglyphus naticoides*. Abbreviations: H — tissues of hepatopancreas, R — rediae with cercariae (violet color), isa — initial stages of the starvation autolysis of the digestive cells, fsa — final stages of the starvation autolysis of the digestive cells, ht — healthy tissue of hepatopancreas.
Рис. 1. Дочерние редии с церкариями *Apophallus* sp. во внутреннем участке пищеварительной железы *Lithoglyphus naticoides*. Обозначения: H — ткани гепатопанкреаса, R — редии с церкариями (фиолетовый цвет), isa — начальные стадии автолизиса пищеварительных клеток, fsa — финальные стадии автолизиса пищеварительных клеток, ht — здоровая ткань гепатопанкреаса.

ages were presented, were analyzed for the degree of pathogenicity of trematodes (Table 1). In the Uglich Reservoir, with a higher density of the host population, the structure of trematode communities was more complex. In addition to the five species of trematodes common for the Upper Volga basin, sporocysts with *Xiphidiocercaria* sp. have been revealed here (previously they assigned to members of the family Echinostomatidae, but the species identity has not yet been precisely identified). Probably, the distribution of *Xiphidiocercaria* sp. in the Uglich Reservoir took place already at the initial stages of establishing of *L. naticoides* in 2013–2015. At the stage of stable permanent population, the prevalence of *Xiphidiocercaria* sp. was 10.53±3.15% in small and 5.56±3.82% in large individuals of *L. naticoides* (August 2019). This difference is statistically insignificant (χ^2=0.2929, p>0.58835). We did not record the expected increase in the total prevalence of trematodes to the level of 100% in *L. naticoides* samples.

The population of *L. naticoides* from the Volga Reach of the Rybinsk Reservoir showed, that at the initial stage of establishing of this mollusk (the group samples no. 1 and no. 2), only trematodes of two species from the genus *Apophallus*, which are difficult to differentiate at the parthenita stages, reached the high prevalence. Despite the absence of serious competition from other trematode species, we did not record the expected increase in the prevalence of *Apophallus* spp. in large individuals (7.0–9.0 mm), in comparison with smaller individuals (4.0–6.9 mm). Statistically significant differ-

Fig. 2. The daughter sporocysts with short-tail cercariae of *Nicolla skrjabini* in the peripheral part of digestive gland of *Lithoglyphus naticoides*. Abbreviations: H — tissues of hepatopancreas, S — sporocysts with cercariae (violet color), fsa — final stages of the starvation autolysis of the digestive cells.
Рис. 2. Дочерние спороцисты с короткохвостыми церкариями *Nicolla skrjabini* в периферическом участке пищеварительной железы *Lithoglyphus naticoides*. Обозначения: H — ткани гепатопанкреаса, S — спороцисты с церкариями (фиолетовый цвет), fsa — финальные стадии автолизиса пищеварительных клеток.

ences between the two size-age groups of *L. naticoides* appeared only at the final stage of establishing of gravel snail (the group samples no. 3 and no. 4) and persisted after the formation of a more complex trematode community at the stage of a stable constant host population (the group sample no. 5). Significant year-to-year fluctuations in the prevalence of *Apophallus* spp. took place only in 2009–2010, when in the Upper Volga region a long winter was followed by an abnormally hot summer. High adaptation of *Apophallus* spp. at the stage of stable permanent population is confirmed by statistically significant differences in the age dynamics of infestation in *L. naticoides* from the Uglich Reservoir (the group sample no. 6).

In all cases, the low prevalence of *P. markewitschi* rediae was recorded in mollusks of both age groups. There was not a single case of a statistically significant increase in the prevalence of parthenitae of this species in mollusks of the older size-age group. Relations in the sporocyst system *N. skrjabini*–*L. naticoides* at the stage of a stable permanent host population are clearly closer to those existing in the *Apophallus*–*L. naticoides* system. An opposite picture is characteristic of the trematode *S. volgensis*. In both the Rybinsk and Uglich reservoirs, even at the stage of the stable permanent populations of the host, a statistically significant decrease in the incidence of sporocysts of this species in mollusks of the older size-age group was recorded.

Based on the results of statistical analysis of the data on the prevalence of parthenitae we believe that the degree of pathogenicity of trematodes increases in the following order: *Apophallus* spp., *N. skrjabini*, *P. markewitschi*, *S.*

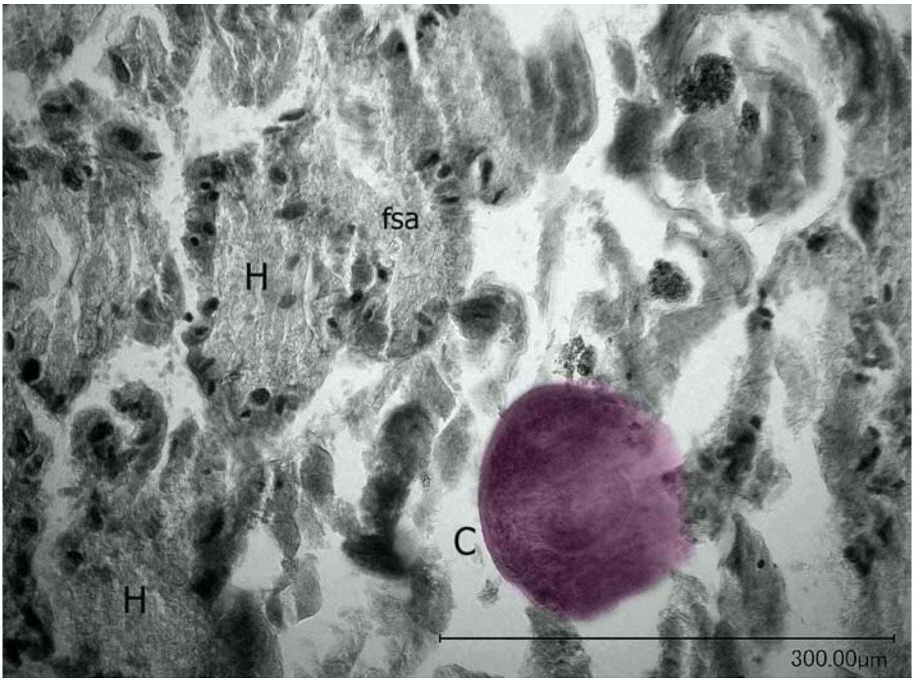

Fig. 3. The cercarieum of *Parasymphylodora markewitschi* in the inner part of digestive gland of *Lithoglyphus naticoides*. Abbreviations: H — tissues of hepatopancreas, C — cercarieum (violet color), fsa — final stages of the starvation autolysis of the digestive cells.
Рис. 3. Церкариеум *Parasymphylodora markewitschi* во внутреннем участке пищеварительной железы *Lithoglyphus naticoides*. Обозначения: H — ткани гепатопанкреаса, C — церкариеум (фиолетовый цвет), fsa — финальные стадии автолизиса пищеварительных клеток.

volgensis. In general, the results given above are confirmed by studies of the hepatopancreas of *L. naticoides* performed using histological methods. No cases of serious damage to other organs were found in the studied individuals of gravel snail. Minimal tissue abnormalities of the hepatopancreas were found at the parasitizing with parthenitae of the genus *Apophallus* (Fig. 1). On the series of the paraffin sections of hepatopancreas, it is noticeable that even fully developed daughter rediae of *Apophallus* sp. exhibit weakest effect on the inner parts of the digestive gland of *L. naticoides*. The body of these very mobile rediae, with a length 0.9–1.1 mm, is relatively thin (~0.2 mm). They easily move between the lobes of the hepatopancreas, practically without squeezing them. No large aggregations of parasite excretion products were found. The presence of isolated bacteria near the lumens of the ducts of the digestive gland, which is probably a consequence of the migration of cercariae, is noticeable. The body of cercariae of the genus *Apophallus* are relatively small (0.15–0.20 mm); therefore, in spite of the large volumes of their emission (up to several hundred specimens per day), no noticeable injury to the inner parts of the hepatopancreas was revealed. In the peripheral areas of the hepatopancreas, the density of *Apophallus* sp. is much higher, but the mechanical compression of the host tissues is relatively weak as well.

Thicker and less mobile daughter sporocysts of *N. skrjabini*, with a length 0.6–0.8 mm and localization similar to that of the *Apophallus* rediae, have a greater effect on the host tissues. Some parts of the *L. naticoides* hepatopancreas, both in depth and in the periphery, may be half-filled with sporocysts of this fluke. At the sites of their contact with the host tissues, pronounced edema of the walls of the hepatopancreas ducts, their deformation and large dark granules are noted, which is probably associated with the accumulation of excretion products or the effect of toxins secreted by parasites on

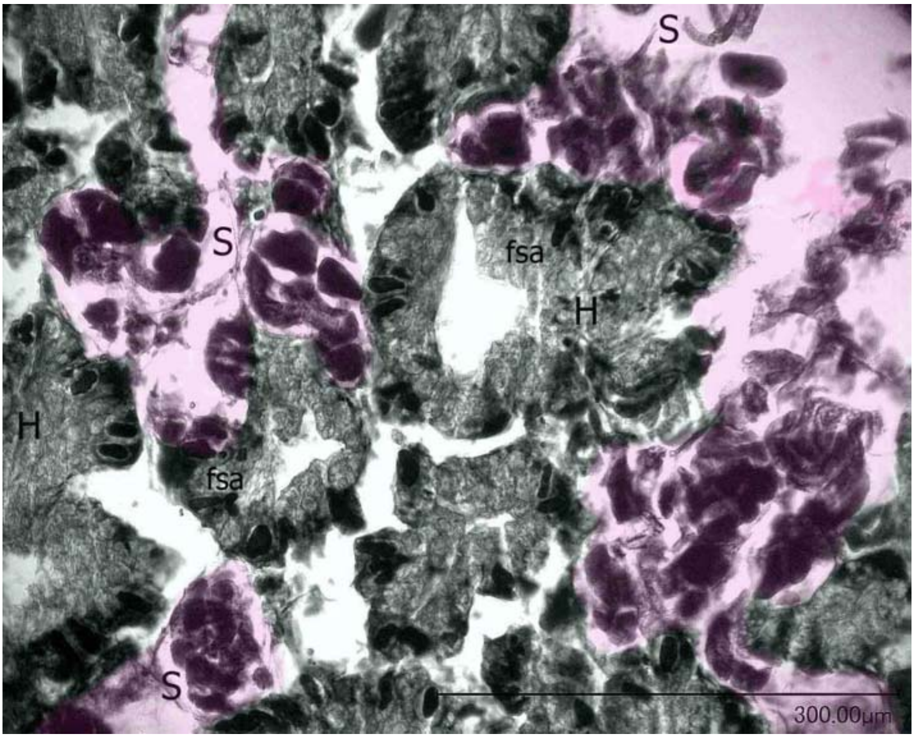

Fig. 4. The daughter sporocysts with cercariae of *Sanguinicola volgensis* in the inner part of digestive gland of *Lithoglyphus naticoides*. Abbreviations: H — tissues of hepatopancreas, S — sporocysts with cercariae (violet color), fsa — final stages of the starvation autolysis of the digestive cells.

Рис. 4. Дочерние спороцисты с церкариями *Sanguinicola volgensis* во внутреннем участке пищеварительной железы *Lithoglyphus naticoides*. Обозначения: H — ткани гепатопанкреаса, S — спороцисты с церкариями (фиолетовый цвет), fsa — финальные стадии автолизиса пищеварительных клеток.

the cells of the digestive gland (Fig. 2). The number of bacterial cells near aggregations of *N. skrjabini* sporocysts is less than expected, which may be explained by a small number of small short-tailed cercariae emerging from mollusks (usual daily emission ~10–15 ind.). The consequences of parasitizing with *P. markewitschi* in the hepatopancreas of gravel snail are even more pronounced (Fig. 3). The tissues of the digestive gland often look depleted and deformed, and the voids between them present frequently. Such damage is associated not only with the migration of big rediae, but also with the active movement of large cercariae of this trematode when they leave the mollusk.

It is worth noting that the revealed disorders of the digestive gland of *L. naticoides*, caused by parasitizing with the parthenitae of *Apophallus* sp., *N. skrjabini*, and *P. markewitschi*, were usually local in nature. Significantly more serious changes in the structure of the hepatopancreas of gravel snail were caused by the sporocysts of *S. volgensis*. In a specific case, small, but very numerous daughter sporocysts of a round or irregular oval shape may form large and dense clusters practically throughout the entire volume of the hepatopancreas (Fig. 4). In the internal regions of the digestive gland, significant deformation of both the duct walls and the sporocysts themselves is observed. In the places of direct contact of sporocysts with host tissues, numerous large dark granules are noted (probably these are the products of parasite excretion or products of impaired metabolism of the mollusk itself). All ducts of the hepatopancreas, even relatively slightly deformed, show

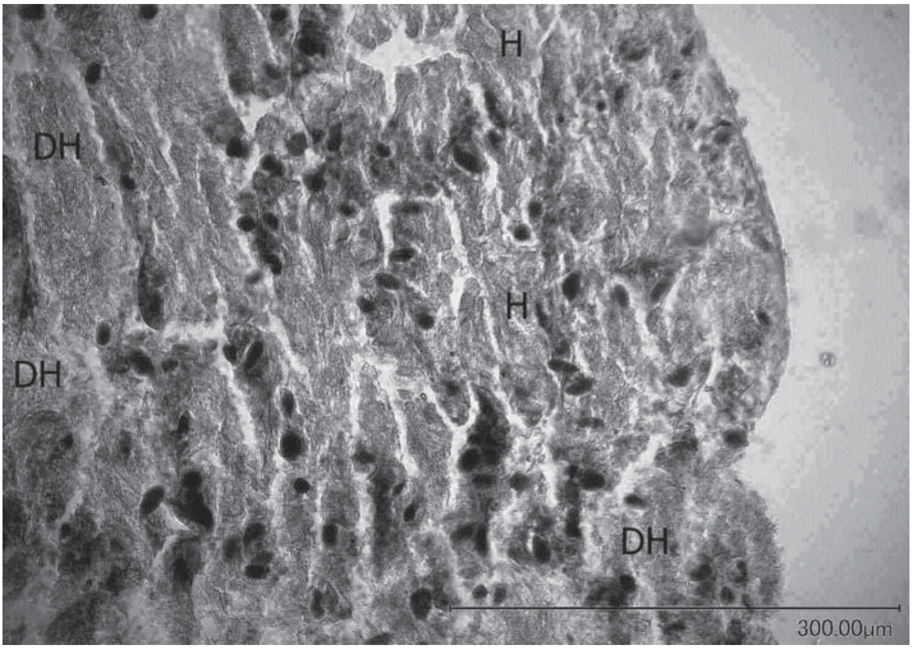

Fig. 5. The peripheral part of digestive gland of a non-infected specimen of *Lithoglyphus naticoides* in the spawning period. Abbreviations: H — zones with relatively intact structure of hepatopancreas tissues, DH — zones with pronounced dystrophy or degeneration of hepatopancreas tissues.
Рис. 5. Краевой участок пищеварительной железы незараженной особи *Lithoglyphus naticoides* в период нереста. Обозначения: H — зоны с относительно сохранной структурой тканей гепатопанкреаса, DH — зоны с выраженной дистрофией или дегенерацией тканей гепатопанкреаса.

signs of edema. In some ducts, the internal boundaries are not clearly visible, which may be considered as the beginning of a degenerative process. The same pattern is observed in the marginal areas of the digestive gland. Apparently, with the massive development of daughter sporocysts of *S. volgensis*, a significant part of the ducts is completely pinched and does not function properly.

The materials for the preparation of histological mounts were preserved in June, i. e. in the period of intensive growth of adult specimens of *L. naticoides*. As a result, in most of the mollusks free of parasites the hepatopancreas tissues appear weakened (Fig. 5). In the internal regions of the digestive gland, rather large areas with partial or almost complete degeneration of hepatopancreas tissues are observed. At some points, tissue destruction is visible to the state of an amorphous cell mass. Even in the areas with a relatively intact structure of hepatopancreas tissues, signs of their depletion are noticeable in the form of a decrease in the size of cells, as well as the appearance of deposits of metabolic products. In the marginal areas of the digestive gland in spawning *L. naticoides*, cases of complete tissue disintegration were not observed, but all signs of tissue dystrophy and disruption of the normal functioning of the duct system were present.

By data on the dynamics of the prevalence of trematodes in the summer months, the lowest balance of host-parasite relations takes place in the first years after gravel snail was introduced into the new water body (Table 2). In approximately equal in number samples of *L. naticoides* (4.0–9.0 mm), collected at the beginning and at the end of summer at the initial stage of establishing in the Rybinsk Reservoir, the differences in the prevalence of trematodes are not statistically significant. At the stage of a stable permanent population of *L. naticoides*, a statistically significant increase in the prevalence of *Apophallus* spp. and *P. markewitschi* from June to

Table 2. Seasonal variations in the prevalence (P±SE, %) of trematode parthenitae in *Lithoglyphus naticoides* in the Volga Reach of the Rybinsk Reservoir.
Таблица 2. Сезонные изменения встречаемости (P±SE, %) партенит трематод у *Lithoglyphus naticoides* в Волжском плесе Рыбинского водохранилища.

Trematode species	Months of sampling		Significance of differences
	June–July	August	
Initial stage of establishing of *Lithoglyphus naticoides* (2009, n=223)			
Apophallus spp.	69.49±4.24	57.14±4.83	χ^2=3.1486, p>0.07599
Parasymphylodora markewitschi	9.32±2.68	15.24±3.51	χ^2=1.3137, p>0.25172
Nicolla skrjabini	0	0.95±0.95	–
Sanguinicola volgensis	0	0	–
Total	78.81±3.76	73.33±4.32	χ^2=0.6433, p>0.42249
The stage of stable permanent population of *Lithoglyphus naticoides* (2019, n=343)			
Apophallus spp.	14.88±2.75	32.00±3.53	χ^2=12.9927, p<0.00031*
Parasymphylodora markewitschi	1.79±1.02	9.14±2.18	χ^2=7.5164, p<0.00611*
Nicolla skrjabini	20.24±3.10	16.57±2.81	χ^2=0.5435, p>0.46099
Sanguinicola volgensis	51.79±3.86	19.43±2.99	χ^2=37.9002, p<0.00001*
Total	88.69±2.44	77.14±3.17	χ^2=8.1517, p<0.00432*

Note. Designations as in Table 1.

Table 3. Number of cases of mixed infections by trematode parthenitae in samples of *Lithoglyphus naticoides*.
Таблица 3. Количество случаев смешанного заражения партенитами трематод в выборках *Lithoglyphus naticoides*.

Type of mixed infection	Water body		Total
	The Rybinsk Reservoir	The Uglich Reservoir	
Nicolla+Sanguinicola	6	3	9
Nicolla+Parasymphylodora	4	0	4
Sanguinicola+Parasymphylodora	0	3	3
Apophallus+Parasymphylodora	2	0	2
Sanguinicola+Xiphidiocercaria	0	2	2
Parasymphylodora+Xiphidiocercaria	0	1	1
Total	12	9	21

August at a significant decline in the prevalence of *S. volgensis* sporocysts is observed. At the same time, the infestation with *N. skrjabini* sporocysts changes to the least extent. In a specific case, this is determined by the death of a significant part of the individuals that received a double infection with *Nicolla+Sanguinicola* closer to autumn. After the complication of structure of the trematode community by 2019, a decrease in the total infection of *L. naticoides* during the summer also became more noticeable. All specimens of native gasropods (*B. tentaculata* and *V. viviparus*) were free from infection with parthenitae in this point.

Cases of mixed infection are quite rare and are usually observed in medium-sized individuals of the host. No cases of triple infection have been identified (Table 3). It should be noted that, despite the relatively high values of the prevalence of rediae of the genus *Apophallus*, the degree of their participation in double infections is small. Double infestation usually occurs after preliminary weakening of the mollusk by the sporocysts of *S. volgensis*. Thus, at the stage of stable permanent population in 2019 in the Rybinsk Reservoir, only a single case of double contamination with parthenitae (*Nicolla* + *Sanguinicola*) was recorded. In the conditions of the Uglich Reservoir, where *Xiphidiocercaria* sp. is registered, and the prevalence of *S. volgensis* is very high, mixed parasite infection is more common. In general, at the examination of more than 1200 mollusk individuals, 21 cases of mixed infection were identified, in two thirds (66.67±10.29%) of which *S. volgensis* was involved. The most common pairs are *Nicolla* + *Sanguinicola, Nicolla* + *Parasymphylodora*, *Sanguinicola*+*Parasymphylodora*. Less common variants are *Apophallus* + *Parasymphylodora*, *Sanguinicola*+*Xiphidiocercaria*, *Parasymphylodora*+*Xiphidiocercaria*. The maximum proportion of double infections in the total number of studied mollusks was noted in 2011 in the Rybinsk Reservoir (8.82±2.28%). The persistence of a high level of mixed infections in the Uglich Reservoir (6.87±2.21% in 2019) even at the stage of a stable permanent population may be partly explained by the higher density of gravel snail colonies in this reservoir.

Discussion

It is known that the degree of host-parasite antagonism in very diverse parthenitae–mollusk systems may vary from an extremely aggressive parasite–“extremist” strategy in trematodes with high pathogenicity to an alternative parasite strategy (parasite–“flexible politic”) in trematodes with low pathogenicity (Gorbushin, 2000; Galaktionov *et al*., 2002). The pathogenic effect of trematode parthenitae on the host organism is minimized when they are localized in the gonads, which is usually observed in long-lived species of mollusks (Taskinen *et al*., 1997; Gorbushin, 2000). In long-lived mollusks the cases of degenerations of sporocysts microhemipopulations were registered (Ginetzinskaya, Dobrovolsky, 1983). For mollusks of the subclass Prosobranchia a localization of trematode parthenitae in the hepatopancreas is more characteristic. At the same time, both the physiological (associated with the release of toxic substances) and the mechanical (due to the migration of the parthenitae themselves and of emerging cercariae) pathogenic effects, increases significantly. The most noticeable pathogenicity of trematodes is manifested under the action of unfavorable environmental factors in cases of insufficient adaptation of the parasite-host systems to them (Galaktionov *et al*., 2002). In the natural conditions, seasonal and age-related mortality is evident, e. g. in marine prosobranch mollusks in the Subarctic, where seasonal fluctuations in water temperature are very pronounced (Gorbushin, 2000; Nikolaev *et al*., 2020). The sporocysts of *Cercaria parvicaudata* Stunkard and Shaw, 1931 (Renicolidae) are capable of causing the death of mollusks of the genus *Littorina* within one year (Nikolaev *et al*., 2020). In the same colonies of *Littorina* spp. microhemipopulations of mobile rediae of *Himasthla littorinae* Stunkard, 1966 (Himasthlidae) may produce cercariae for more than one warm season. The level of functional disorders of the hepatopancreas in these cases depends not only on the intensity of infection (the density of microhemipopulations of parthenitae), but also on their localization in the digestive gland. In general, in mollusks of the genus *Littorina*, the degree of pathogenicity increases markedly in the following order: rediae (Cryptocotyle) localized on the periphery of the digestive gland, small mobile rediae (Himasthla), and sporocysts (Renicola) (Gorbushin, 2000). On the other hand, there is evidence that with well-balanced host-parasite relations in the rediae–mollusk system, the effect of trematodes on the survival of the host can be minimal even under the action of an additional stress factor (for example, temperature). Thus, in the conditions of a chronic laboratory experiment, it was shown that microhemipopulations of parthenitae of the trematode *Philophthalmus rhionica* (Olenev, Tichomirov, 1976) during the development in the hepatopancreas of freshwater prosobranch mollusk *Melanopsis praemorsa* (Linnaeus, 1758) statistically significantly affected the survival of the host only at of the tolerable temperature range (34–36 °C) (Ataev, 1991). As for another potential stress factor that could be effective in this respect — the influence of pollutants — we didn’t find any relevant data in the available publications.

The cases of parasitism of parthenitae of trematodes in *L. naticoides* examined in the present study do not contradict the above-mentioned pattern. As a rule, in the natural conditions, well-adapted parasites do not cause complete exhaustion and death of an already mastered host during the summer months without additional exposure to negative environmental factors. We believe that the use of seasonal data for the analysis of the mortality of *L. naticoides* is complicated due to the presence of two differently directed processes in the summer months: the death of a part of infected two-year-old specimens (age 1+) and a simultaneous increase in body size and degree of infection in individuals of the first year of life. Nevertheless, the decrease in the total infection by all species of trematodes in the Rybinsk Reservoir from June to August 2009 (at the initial stage of establishing) may indicate that the death of mollusks is associated with the consequences of infection with some parasites. At the stage of stable permanent population of *L. naticoides*, this seasonal mortality rate increased. The seasonal decline in the prevalence of *S. volgensis* from 51.79% to 19.43% may be interpreted as a result of the death of mollusks, associated precisely with infection with sporocysts of this species. The data of our observations in the wild are supported by the results of histological examination. Based on our histological studies only *S. volgensis* sporocysts demonstrate great pathological effects at the organismic level.

It is worth noting that many digestive gland cells, in snails of the genus *Littorina* heavily parasitized with parthenitae, undergo exactly the same stages of autolysis as described in mollusks subjected to starvation (James, 1965). In these cells, no food particles were found. In heavily infected with some rediae or sporocysts freshwater snail *B. tentaculata* (Gastropoda, Bithyniidae), the digestive gland may be reduced to about two-thirds of its normal size (Reader, 1971). The breakdown of the digestive gland may be the reason for the high death rate observed in infected *B. tentaculata* during the winter months. Probably, in many cases, the negative impact of trematodes on the population of the first intermediate host can be limited by a simple decrease in reproductive potential. In these cases, there may be no direct mechanical effect of parthenitae on the gonads. On the example of the prosobranch mollusk *Bithynia troschelii* Paasch, 1842, which is close to *L. naticoides* in size, the negative effect of redioid and sporocystoid trematodes on the development of females and the number of fully formed embryos produced by them is described (Serbina, 2015).

We assume that for the species associated with *L. naticoides*, the period of joint coevolution, during which the parasite adapted to this host, is clearly important. Species of the genus *Apophallus*, which at the stage of metacercaria cause known varieties of “black-spotted disease” in a wide range of fish (apophallesis and rossicotremosis), and at the marita stage, parasitizing many fish-eating birds and mammals, are highly specific parasites of *L. naticoides* and often give outbreaks of abundance at different points of its habitat (Biserova, 2005; Ivanov, 2008; Tyutin, Slynko, 2010; Tyutin *et al*., 2013, 2020; The most dangerous..., 2018). Another parasite species highly specific to the gravel snail, *N. skrjabini*, reaches a large number only in some water bodies with a high population density of the second intermediate (mainly crustaceans of the order Amphipoda) and definitive (usually fish of the family Gobiidae) hosts (Stanevičiūtė *et al*., 2008; Biserova, 2010; Tyutin *et al*., 2013, 2020, 2022; Zhokhov *et al*., 2006, 2019). According to the published data, many species of trematodes belonging to the genera *Parasymphylodora* and *Sanguinicola* can change the first intermediate hosts within the range or represent mixed groups of species that are difficult to differentiate at the parthenita stages (Biserova, 2005, 2010; Stanevičiūtė *et al*., 2008; Zhokhov *et al*., 2021).

Based on our histological results and field data, the trematode *S. volgensis* demonstrates the greatest pathogenicity, both at the organismic and at the population levels. Presumably, in the autumn and winter months, the high degree of antagonism of host-parasite relations takes place. We assume that trematodes *A. muehlingi*, *A. donicus*, and *N. skrjabini* demonstrate the highest parasitic specificity to gravel snail and, as a result, their pathogenicity in natural conditions is usually relatively weak. It is likely that the degree of antagonism was quite high at the initial stage of establishing these parasite-host systems. At the population level, this is supported by our data on the seasonal dynamics of the prevalence of trematodes. The formation of stable parasitic systems of *A. muehlingi* and *A.*

donicus took about 20 years after the introduction of the mollusk into the Volga River delta (Ivanov, 2008). In the conditions of the Upper Volga reservoirs, it happened much faster. Probably, the successful adaptation of *L. naticoides* and its parasites to new conditions was facilitated by the fact that the water temperature was significantly higher than the climatic norm (Structure and functioning..., 2018) in 2000–2012 in the Rybinsk Reservoir during the entire growing season (from late April to early November). In the reservoirs of the Upper Volga the formation of a complex multispecies community of trematodes in *L. naticoides* populations also occurred fairly fast. At the same time, the dominance of trematodes of the genus *Apophallus*, which is so characteristic of the Lower Volga (Biserova, 2010), is not always observed. We assume that the currently observed spread of *S. volgensis* clearly leads to a sharp increase in competition in the communities of trematodes, and can increase their overall negative impact on the host population. In our opinion, in addition to the ability to maintain its abundance at a high level, the negative impact of a trematode at the population level depends on its ability to participate in mixed infections.

The zones with optimal conditions for the life cycle of a trematode usually confine to biotopes occupying a relatively small area of the water body (Ginetzinskaya, Dobrovolsky, 1983; Galaktionov, Dobrovolskij, 2003). Laboratory modeling of periodical drainage of littoral (exposure to extremely high temperatures, desalination, and prolonged drying) in some marine prosobranch mollusks revealed a significantly higher mortality rate of infected individuals due to dysfunction of the mechanism of shell opening with a cap (Galaktionov *et al*., 2002). In cases of mixed infection with participation of *S. volgensis* sporocysts, the sporocysts of *N. skrjabini* are usually the second component. The low prevalence of *P. markewitschi* rediae indicates a more pronounced antagonism of the parasite-host relationship. There was not a single case of a statistically significant increase in the prevalence of parthenitae of this species in mollusks of the older size-age group. It is possible that stimulation of the mollusk immunity at the initial stage of development of the microhemipopulation of *S. volgensis* parthenitae prevents reinfection with redioid trematodes. Our data showed no cases of double infection with *Sanguinicola*+*Apophallus*, although such mixed infection was detected at other points of the *L. naticoides* range (Stanevičiūtė *et al*., 2008; Biserova, 2010). A more detailed analysis of these relationships is hindered by a rather low level of knowledge on the European species of the genus *Sanguinicola*. According to one of the latest review articles on this topic (Zhokhov *et al*., 2021), at least four species of trematodes of this genus parasitize fish and mollusks in the Upper Volga basin. One more endemic species is known in Western Europe. Thus, the list of the first intermediate hosts for the genus *Sanguinicola* includes more than 20 species of mollusks from seven families: Lymnaeidae, Planorbidae, Valvatidae, Neritidae, Lithoglyphidae, Bithyniidae, and Melanopsidae. The formation of well-adapted parasite-host pairs is not observed. For example, within Eastern Europe in the prosobranch mollusk *Bithynia leachii* (Sheppard, 1823), which is close to *L. naticoides* in size and biology, the following species were recorded: *Sanguinicola armata* Plehn, 1905, *S. intermedia* Ejsmont, 1926 and cercariae of this genus not identified as species ("Cercaria cristata"). In this regard, many authors prefer to indicate the species found in *L. naticoides* as *Sanguinicola* sp. or *Sanguinicola* spp. (Stanevičiūtė *et al*., 2008; Biserova, 2010; Zhokhov *et al*., 2021). Judging by the presence of sporocysts with different body shapes in this mollusk, in the Volga River basin, gravel snail may be parasitized by both introduced and native species from the genus *Sanguinicola.*

Conclusions

In the reservoirs of the Upper Volga basin the parthenitae of all trematode species associated with colonies of the mollusk *L. naticoides* develop rapidly and massively affect the host's hepatopancreas, i.e. fall into the category of parasites not intended to increase the lifespan of the host. However, at the stage of a stable permanent population the degree of antagonism of host-parasite relations is relatively low for most species. According to our data we suggest that the influence of trematodes increases not only on the host population, but also on the ecosystem of the reservoir as a whole. For example, the negative effect of *N. skrjabini* on the host population sharply increases with an increase in the population density of *S. volgensis.*

The study of the tissues of the digestive gland of adult mollusks of the second year of life (1+) collected in June 2019 in the Rybinsk Reservoir revealed a relatively weak influence of the parthenitae of all the considered species of trematodes on the host during this period. We assume that the pathogenicity of all the considered species of trematodes is manifested to a much greater extent in the autumn period, when the trematodes respond to a decrease in temperature, and switch from the emission of cercariae to the emergence of additional individuals of the daughter generation.

Analysis of the data on the prevalence of parthenitae along with histological examinations of the tissues of the digestive gland of *L. naticoides* suggests an increase in pathogenicity in the following order: rediae of *A. muehlingi* and *A. donicus*, sporocysts of *N. skrjabini*, rediae of *P. markewitschi*, sporocysts of *S. volgensis*. Trematoda *S. volgensis*, which does not show its specificity to *L. naticoides*, stands out. We suggest that the small size of the digestive gland of *L. naticoides* is a serious obstacle to the full development of hemipopulations of parthenitae of two or more species; therefore, the most common cases of double contamination recorded were *Nicolla+Sanguinicola*, *Nicolla + Parasymphylodora*, *Sanguinicola + Parasymphylodora.* It is likely that mixed infection is the most typical for the final stage of *L. naticoides* establishing, when the number of species included in it is already quite large, but the relative equilibrium in the number of their populations in the ecosystem of the recipient reservoir has not yet been achieved. Taking into account the continuing trend towards an increase in mean annual temperature values in the Upper Volga region (Structure and functioning..., 2018), we can expect a further complication of the structure of the communities of trematodes associated with *L. naticoides* on the northeastern border of its range.

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Compliance with ethical standards

CONFLICTS OF INTEREST: The authors declare that there is no conflict of interest regarding the publication of this article.

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