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**THE INFLUENCE OF TREMATODE METACERCARIAE
ON THE INDIVIDUAL FECUNDITY OF BITHYNIA TROSCHELI
(GASTROPODA: BITHYNIIDAE)**

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Fifteen trematode species belonging to seven families parasitizing the females of *Bithynia troscheli* (Paasch, 1842) at metacercarial stage, were recorded in Kargat River (Lake Chany, South of West Siberia, Russia). The breeding mollusk females have less metacercarial diversity (9 species vs 15) and lower mean parasite abundance, comparing to non-ovigerous mollusks. The hypothesis of the metacercarial influence on individual fecundity of aquatic gastropods was tested. The individual fecundity parameters of the *B. troscheli* females, uninfected and infected with the trematode metacercariae belonging to fam. Echinostomatidae, Cyathocotilidae, Strigeidae, Cyclocoelidae, were compared. The percentage of the breeding *B. troscheli* females was less and their fecundity parameters were lower for the hosts infected (with the Strigeidae or Cyclocoelidae metacercariae) comparing to non-infected ones. Trematode metacercariae found in the mantle or somatic musculature had no significant effects on the fecundity of the host. However, our results showed that trematode metacercariae affect the reproduction of *B. troscheli* in dual ways. All fecundity parameters of the mollusk females with the high infection rate were significantly lower than those for uninfected females: the percentage of fecund females ($\chi^2 = 6.73$, $p < 0.01$), number of clutches per female, number of egg capsules per clutch and number of normal egg capsules per female (Tukey HSD, $p < 0.001$). Although the females with low intensity of metacercarial infection lay egg clutches significantly more frequently than uninfected ones ($\chi^2 = 4.18$, $P = 0.04$); the fecundity parameters were approximately equal for both groups of the mollusk females. These pioneer results prove the reality of influence of metacercariae on the individual fecundity of aquatic gastropods. The regulatory population mechanisms that may compensate the host reproduction loss caused by metacercaria, are discussed.

Key words: host-parasite system, Gastropoda, trematode, metacercariae, Strigeidae, Cyclocoelidae, Echinostomatidae, Cyathocotilidae, Western Siberia.

ВЛИЯНИЕ МЕТАЦЕРКАРИЙ ТРЕМАТОД
НА ИНДИВИДУАЛЬНУЮ ПЛОДОВИТОСТЬ *BITHYNIA TROSCHELI*
(GASTROPODA: BITHYNIIDAE)

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У самок *Bithynia troscheli* (Paasch, 1842) из р. Каргат (оз. Чаны, юг Западной Сибири, Россия) обнаружены метацеркарии трематод 15 видов 7 семейств. У размножающихся самок обнаружено меньше видов метацеркарий (9 из 15) и более низкие значения индекса обилия этих паразитов, чем у самок, не отложивших кладки. Проверена гипотеза о влиянии паразитирования метацеркарий трематод на индивидуальную плодовитость *B. troscheli*. Проведено сравнение индивидуальной плодовитости самок, зараженных метацеркариями трематод (сем. Echinostomatidae, Cyathocotilidae, Strigeidae, Cyclocoelidae) и незараженных. Доля плодовитых самок *B. troscheli*, зараженных метацеркариями трематод сем. Strigeidae или Cyclocoelidae, была значительно меньше и показатели их плодовитости достоверно ниже, чем у незараженных. Метацеркарии трематод в тканях мантии или мышцах не оказывали значимого воздействия на плодовитость хозяина. Показано, что метацеркарии трематод оказывают двойственное влияние на плодовитость *B. troscheli*. Все показатели плодовитости самок с высокой интенсивностью инвазии были значимо ниже, чем у незараженных: доля плодовитых самок ($\chi^2 = 6.73$, $p < 0.01$); число кладок на самку, число яйцевых капсул кладке и число нормальных яйцевых капсул на самку (Tukey HSD тест, $p < 0.001$). Однако самки с низкой интенсивностью инвазии значимо чаще продуцировали кладки, чем незараженные ($\chi^2 = 4.18$, $p = 0.04$), хотя показатели плодовитости этих двух групп были примерно равными. Эти результаты впервые доказывают влияние метацеркарий на индивидуальную плодовитость водных брюхоногих моллюсков. Обсуждаются возможные внутрипопуляционные механизмы, компенсирующие репродуктивные потери хозяев.

Ключевые слова: система паразит—хозяин, Gastropoda, метацеркарии трематод, Strigeidae, Cyclocoelidae, Echinostomatidae, Cyathocotilidae, Западная Сибирь.

The metacercariae of trematodes may affect the growth (Shigin, 1980; Sessions, Ruth, 1990; Thieltges, 2006; Shaw et al., 2010), biomass (Pampoulie et al., 1999; Wegeberg, Jensen, 2003; Shaw et al., 2010), survival (Shigin, 1980; Jensen et al., 1998; Ferreira et al., 2005; Orlofske et al., 2009), mortality (Meissner, Bick, 1999; Pampoulie et al., 1999; Kuris et al., 2004; Overstreet, Curran, 2004; Bates et al., 2010), and behavior (Shigin, 1980; Babirat et al., 2004; Sukhdeo, Sukhdeo, 2004; Hansen, Poulin, 2005) of the second intermediate hosts. Mollusks are common hosts of metacercariae in marine and freshwater ecosystems. Metacercariae were detected in bivalves (Calvo-Ugarteburu, McQuaid, 1998; Thieltges, 2006; Nikolaev et al., 2006; Lassalle et al., 2007), as well as in gastropods (Pulmonata and Prosobranchia) (Filimonova, Shalyapina, 1979; Serbina, 2002, 2011; Morley et al., 2004; Yurlova, Serbina, 2004; Yurlova et al., 2006). However, the trematode-induced influence on the second molluscan hosts, is not well studied so far. The pathogenic effects induced by metacercariae in bivalves include: the reduced ability to burrow, reduced byssal thread production, reduced tolerance to anoxia, impaired growth and enhanced mortality (Calvo-Ugarteburu, McQuaid, 1998; Wegeberg, Jensen, 2003; Thieltges, 2006).

Thieltges (2006) showed that a trematode infection could be an important determinant of the bivalve growth, with potential economic implications for the mussels' cultivation. The growth of *Mytilus edulis* Linnaeus, 1758 was negati-

vely correlated with the number of metacercariae. It is known that the fecundity increases with size of bivalves (Zhokhov, Pugacheva 1995; Calvo-Ugarteburu, McQuaid, 1998; Sytnik et al., 2010). Thus a reduction in growth can be regarded as an indirect negative effect of metacercariae on mollusks' fecundity. However, quantitative data on the effects of trematodes on their second intermediate molluscan hosts are scarce and fragmentary. There is an evidence that metacercariae affect the fecundity of gastropods. Foster (1958) demonstrated, that slugs (*Milax sowerbii* Férussac and *Agriolimax reticulatus* Müller) infested with non-encysted brachylaimid metacercariae in the kidney had an extensive necrosis of the organ. In *A. reticulatus*, the rate of food ingestion and the rate of egg production were reduced by infection.

The Bithyniidae are dioecious snails that are common in the Ob and Irtysh Rivers and their tributaries and lakes in West Siberia (Karpenko et al., 2008; Serbina, 2010a; Serbina, Bonina, 2011). They make up to 16 % of gastropod biomass in water areas of the south of West Siberia (Serbina, 2013). During cold periods, bithyniid snails live completely buried in a sediment. The life span of snails varies from 4 up to 6 years. It is also possible to estimate the individual age (Serbina, 2002, 2008; Kozminsky, 2003), and even conditions in particular years, based on winter growth rings on the shell (Serbina, 2010a). The reproductive biology of bithyniid snails in the south of West Siberia (in natural reservoir conditions and in laboratory aquaria) has been investigated by Serbina (2005). The breeding age of females was from two to six years (Serbina, 2002). In previous studies, Filimonova and Shalyapina (1979), and Serbina (2002, 2010b, 2011) showed that the bithyniid snails from West Siberia played a role of the second intermediate hosts for the twenty trematode species.

Five objectives were set here to assess the influence of metacercariae on the fecundity of *Bithynia trosscheli* (Paasch, 1842): a) to determine the individual fecundity of *B. trosscheli* females; b) to make up a list of species of metacercariae and estimate their occurrence and prevalence in the host females; c) to evaluate the infection rate difference between breeding and non-breeding females in different years; d) to compare a fecundity of the *B. trosscheli* females with different intensities of metacercariae infection; e) to evaluate differences in the proportion of fecund females and their fecundity characteristics between the infected and uninfected mollusks.

MATERIAL AND METHODS

Material was sampled in 1994—2007 in the estuary of Kargat River (54°37.76' N; 78°13.07' E). This study was carried out at the Chany Field Station of the Institute of Systematics and Ecology of Animals, Russian Academy of Sciences. The sampling area has been previously characterized by Yurlova et al. (2006). The Bithyniidae snails were collected from May to September (twice in 10-day period) by hand from 4—6 plots of 0.25 m² at a depth of 0.1—0.7 m. In the laboratory, each snail was placed into a separate Petri dish with filtered river water. The number of clutches, egg capsules, and embryos were calculated every day under a stereomicroscope at 16x magnification. The ovigerous *B. trosscheli* females were named as the «fecund» or «breeding females». The total number of egg capsules laid by a single female during reproductive period

Table 1
Number the trematode species (parthenites and metacercariae)
in samples of *Bithynia troscheli* snails from Kargat River
(1996, 1999, 2000, 2002—2003)

Years	Studied snails		Number of trematode species in the snail females	
	All	Females from them	Parthenites	Metacercariae
1996	546	317	11	11
1999	540	308	10	4
2000	340	224	12	10
2002	1075	646	11	11
2003	1346	646	10	7
A total	3847	2141	20	15

(June—July) was defined as the «individual fecundity». After 15—20 days the non-ovigerous *B. troscheli* were dissected to determine mollusks' sex and parameters of the trematode parthenitae and metacercariae infection. The sex of the snails was determined based on genital system structures. Females of breeding age that did not lay egg capsules were defined as the «non-breeding females».

The individual fecundity of *B. troscheli* females was studied in 1996, 1999 and 2000, 2002, and 2003. The 524 females produced 1386 clutches containing 13, 218 egg capsules and 13, 169 embryos. The individual fecundity parameters are: number of clutches per female (NC); size of clutches — number of egg capsules per clutch (SC); number of normal egg capsules per female (containing an embryo within each egg capsule) (nEC); number of egg capsules containing no embryos per female; number of capsules with 2—3 embryos, per female.

In total, 3, 847 *B. troscheli* specimens were analyzed (table 1); females were classified into four groups: infected with parthenitae (1) infected with metacercariae (2) infected with parthenitae and metacercariae (3) uninfected (4). To evaluate the metacercariae influence on female fecundity, the snails infected with parthenitae were excluded from the analysis (groups 1 and 3). Young females of *B. troscheli* (shell size <5.5 mm) and females sampled in August and September did not lay egg capsules and were therefore also excluded. The data on uninfected females (n = 878) and metacercariae-infected females (n = 780) were compared. The infected females were classified into three groups according to the number of metacercariae: 1—2 metacercariae (low intensity infection); 3—10 metacercariae (moderate intensity infection); >10 metacercariae (high intensity infection). The species of metacercariae were identified using keys of Filimonova and Shalyapina (1979) and Sudarikov et al. (2002). The meanings of prevalence and mean abundance were used according to Beklemishev (1970) and Bush et al. (1997).

Statistical analyses were performed using STATISTICA 6.0 and Excel 2003. Data were compared using the one-way ANOVA, Pearson's correlation, Chi-square test, Student's t-test and Tukey tests with a 95 % confidence interval ($P < 0.05$). If no significant differences were found, the replicates were pooled for further analysis.

RESULTS

The individual fecundity of *Bithynia troscheli*

During the whole research period of 1995—2007, snails sampled before May 29 and after July 20 did not lay clutches of egg capsules. The earliest clutches were obtained using a separated alimentation on June 2 from females sampled on May 31. The latest clutches were obtained on July 21 from a female sampled on July 15. After collection, females of *B. troscheli* laid egg capsules from the first day of observation up to 11th day. For the following period (from the 12th to 50th day), there was no egg production at all. Females of breeding age (from 2 to 6 years) had shell heights ranging from 5.6 to 11.2 mm and snail weights between 61 and 197 mg. Females of *B. troscheli* laid egg capsules in one to nine clutches during a single reproductive period (fig. 1). The number of clutches laid by fecund females in one reproductive season varied from 1.45 ± 0.11 to 3.35 ± 0.27 in different years. Usually the number of egg capsules in the first clutch exceeded the number of egg capsules in all subsequent clutches (fig. 2).

However, a positive correlation between number of egg capsules and number of clutches was found (Pearson's coefficient $r = 0.38$, $P < 0.001$). Because of the multiple laying of clutches, the mean number of egg capsules laid in a single reproductive period varied from 18.31 ± 1.47 or 18.86 ± 1.17 (in 1999 or 2002, respectively) to 27.11 ± 1.22 (in 2003) per breeding female. The maximum individual fecundity was 91 egg capsules. As a rule, female fecundity increases with age: from 16 egg capsules per breeding female in one-year-old (1+) to 23 egg capsules per breeding female in five-year-old (5+) mollusks. There was positive correlation between number of clutches and age (Pearson's coefficient $r = 0.12$; $p < 0.001$) or number of clutches and size of a shell ($r = 0.21$;

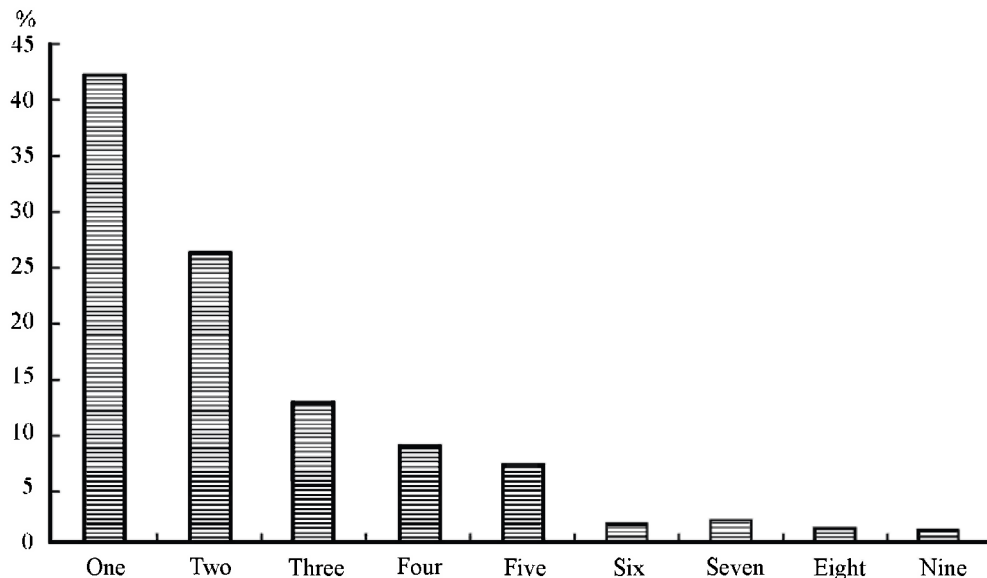


Fig. 1. Females of *Bithynia troscheli* laid different numbers of clutches.

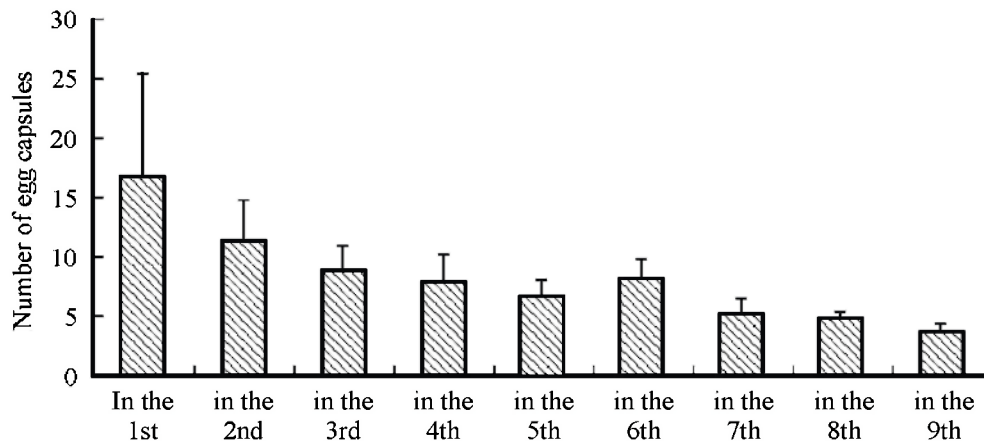


Fig. 2. Number of egg capsules, from the 1st up to the 9th clutch.

$P < 0.001$). Mean number of egg capsules per female of *B. troscheli* varied seasonally being highest in first ten-days of June and the lowest in the third ten-days of July (28.59 ± 0.18 and 6.5 ± 0.48 , respectively; Tukey test, $P < 0.01$).

Infection of the *Bithynia troscheli* females with trematode metacercariae

In females of *B. troscheli*, we found metacercariae of 7 trematode families: *Asymphylogadora* sp. Szidat, 1943 [Monorchidae Odhner, 1911]; *Moliniella anceps* Molin, 1859; *Echinoparyphium aconiatum* Dietz, 1909; *Echinoparyphium recurvatum* Linstow, 1873; *Echinostoma revolutum* Frohlich, 1808; *Echinostoma uralensis* Skrjabin, 1915; *Echinostoma grandis* Baschkirova, 1946; *Hypoderaeum conoideum* Bloch, 1782; *Hypoderaeum cubanicum* (Artyukh, 1958); [Echinostomatidae (Looss, 1899) Dietz, 1909]; *Cyathocotyle bushiensis* Khan, 1962; *Cyathocotyle bithyniae* Sudarikov, 1974; [Cyathocotilidae (Mühling, 1898) Poche, 1925]; *Cotylurus cornutus* Rudolphi, 1808; [Strigeidae, Railliet, 1919]; *Lecithodoliffusia arenula* Creplin, 1825 [Lecithodendriidae Odhner, 1911]; Cyclocoelidae gen. sp. [Cyclocoelidae Kossack, 1911], and *Atriophallophorus minutus* (Becker, 1900) Price, 1934 [Microphallidae (Ward, 1901) Travassos, 1920]. In total, 15 species of metacercariae were found during this study (table 2). The total number of species of metacercariae recorded each year varied from 4 (1999) to 11 (1996 and 2002).

Infections parameters in breeding and non-breeding females for different years were compared. The prevalence of metacercariae in breeding females was higher than in the non-breeding females in all years (fig. 3, *a*). The number of trematode species, and mean abundance in breeding females, was lower than in non-breeding females (fig. 3, *b*, *c*). Similar results were obtained for each dominating species of trematode metacercariae. E.g., the highest mean abundance of *C. bithyniae* in non-breeding females was 15.8; 11.8 and 13.9 in 2000; 2002 and 2003, respectively. The parasite mean abundance in fecund females was significantly lower; 2.4; 1.9 and 3.6, respectively (Student's *t*-test: $t = 2.8$, $DF = 190$, $p < 0.01$; $t = 3.3$, $DF = 628$, $p < 0.001$; $t = 1.97$, $DF = 507$, $p < 0.05$). Also, the higher abundance of metacercariae in non-breeding females than in breeding fe-

Table 2

Characteristics of trematode metacercariae parasitizing the *Bithynia troscheli* females from Kargat River (1996, 1999, 2000, 2002—2003)

Species	Locali- zation*	Prevalence, %	Abundance		Intensity of infection
		Min—max**	Min—max**	Mean ± SE	Max
<i>Asymphylodora</i> sp.	SM, gill	0.63—1.95	0.009—0.05	2.248 ± 0.156	15
<i>Moliniella ansceps</i>	M	0.41—9.32	0.009—0.31	0.003 ± 0.001	4
<i>Echinoparyphium aconiatum</i>	M	1.16—10.74	0.163—0.55	0.477 ± 0.054	56
<i>E. recurvatum</i>	M	0.02—0.45	0.005—0.02	0.057 ± 0.015	5
<i>Echinostoma revolutum</i>	M	0.01—0.45	0.005—0.01	0.015 ± 0.006	8
<i>E. uralensis</i>	M	0.45—0.79	0.005—0.01	0.004 ± 0.002	2
<i>E. grandis</i>	M	0.16—0.98	0.002—0.06	0.015 ± 0.009	15
<i>Hypoderaeum conoideum</i>	M	0.20—2.05	0.006—0.06	0.004 ± 0.002	8
<i>H. cubanicum</i>	M	0.12***	0.006	0.002	4
<i>Cyathocotyle bushiensis</i>	M, SM	0.16—8.93	0.005—0.23	0.132 ± 0.010	23
<i>C. bithyniae</i>	T, F, head	2.35—34.3	0.268—4.41	0.053 ± 0.017	800
<i>Cotylurus cornutus</i>	H, G	0.44—3.23	0.022—0.44	0.042 ± 0.009	8
<i>Lecithodoliffusia arenaria</i>	H, G	0.45—0.63	0.08—31.92	0.026 ± 0.013	153
<i>Atriophallophorus minutus</i>	H, G	0.45*	31.3	3.269	7000
<i>Cyclocoliidae</i> gen. sp.	M, SM, H	0.45—1.95	0.027—0.07	0.126 ± 0.039	42

Note. * — Abbreviations: M — mantle, H — hepatopancreas, G — gonad, T — tentacle, F — foot, SM — somatic musculature. ** — Minimum and maximum parameters, in different years. Mean abundance for all years. *** — detect only in one sample (one year).

males, was recorded for *C. bushiensis* (1.7 and 0.4; $t = 4.4$, $DF = 507$, $p < 0.001$) and *E. aconiatum* (3.6 and 2.4; $t = 2.2$, $DF = 507$, $p = 0.03$).

To evaluate the effect of metacercariae on fecundity of *B. troscheli* females, the individuals infected with parthenitae were excluded from analysis (fig. 4).

Dependence of the *Bithynia troscheli* fecundity on the intensity of infection with metacercariae

The molluskan females were subdivided into three groups based on intensity of infection with metacercariae. The percentages of fecund females were compared among these three groups. The fecundity parameters of infected snails were compared with those of uninfected ones, used as a control. A percentage of fecund females (41 %) in the first group (low infection intensity) was even significantly higher than the percentage of fecund uninfected females (33.8 %; $\chi^2 = 4.18$, $P = 0.04$). Moderate intensity of infection with metacercariae did not cause much damage to *B. troscheli*, as the proportion of fecund uninfected females and females infected in average with 3—10 metacercariae did not show

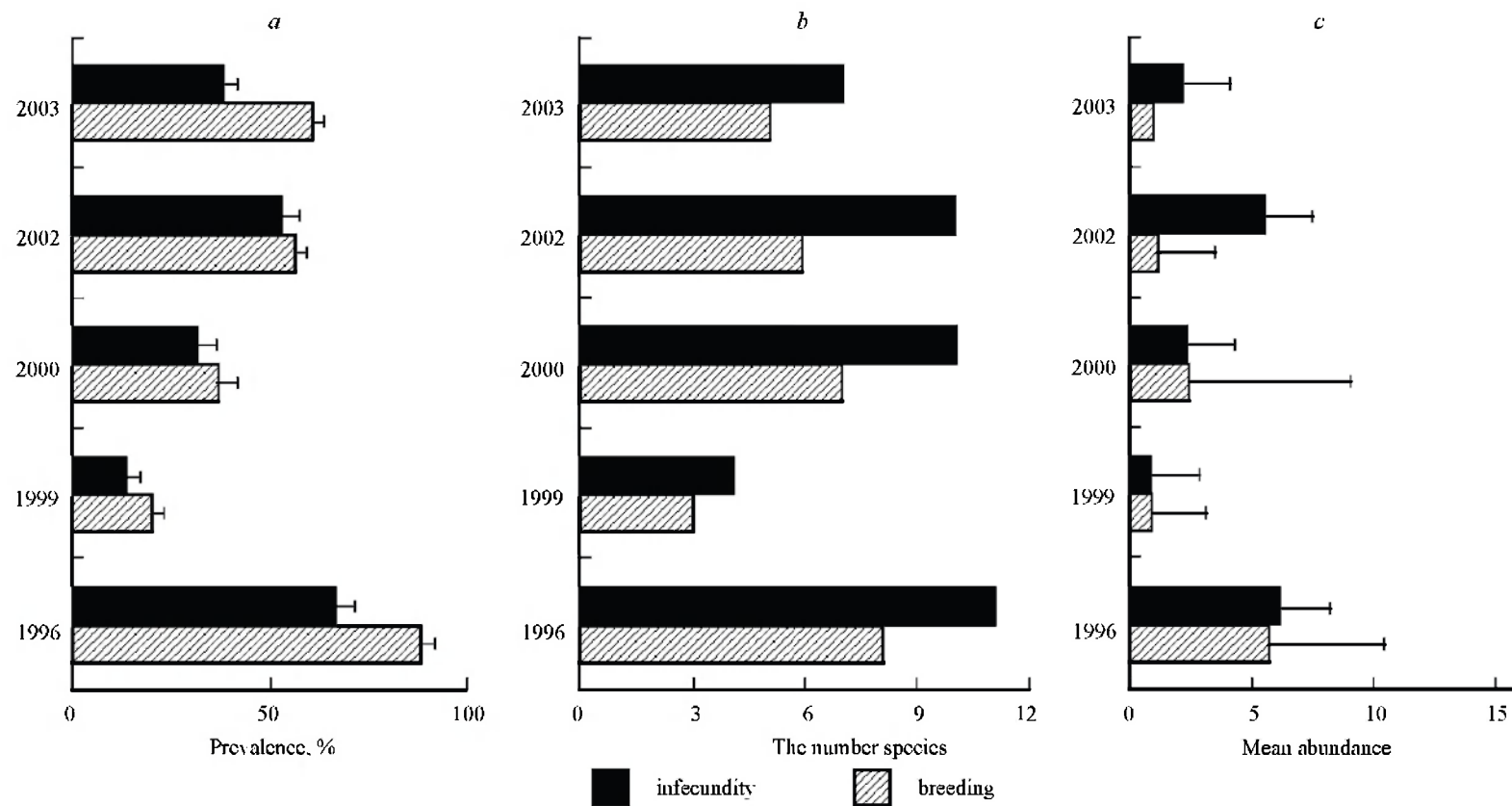


Fig. 3. Dynamics of infection rate parameters for the females of *Bithynia troscheli* infected with trematode metacercariae, from Kargat River (1996, 1999, 2000, 2002—2003).

a — prevalence (% \pm SE); *b* — number of species, Student's *t*-test 1996 — 1.07 and 1.41; $t = 2.6$ $p < 0.01$; 2000 — 0.35 and 0.77; $t = 4.1$ $p < 0.001$; *c* — mean abundance, Student's *t*-test 1996 — 5.50 and 8.22; $t = 1.81$ $p = 0.007$; 2002 — 4.26 and 1.01; $t = 3.5$ $p < 0.001$.

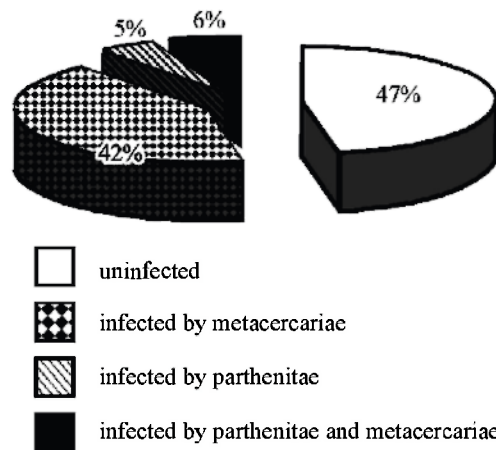


Fig. 4. Groups of the *Bithynia troscheli* females, uninfected and infected with trematodes (parthenites and metacercariae) from Kargat River (1996, 1999, 2000, 2002—2003). All studied females considered as 100 %.

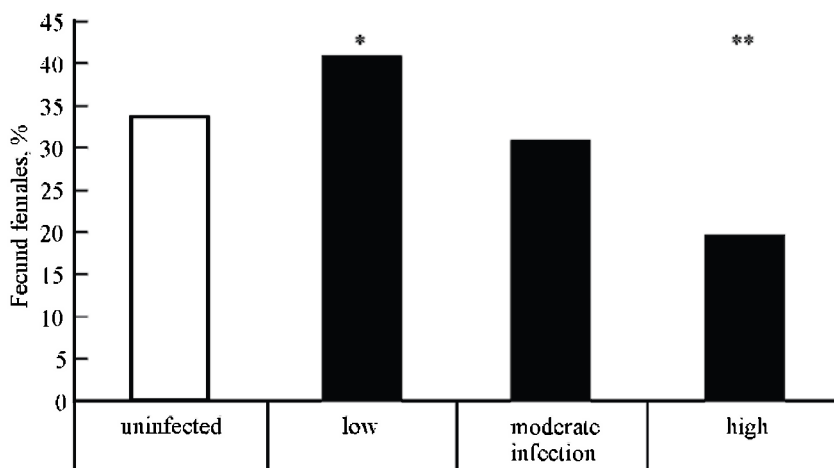


Fig. 5. The percentage of fecund females of *Bithynia troscheli*, uninfected and infected with trematode metacercariae.

Chi-square, * $p < 0.05$; ** $p < 0.01$. Group uninfected females (control). Groups of snail females infected with metacercariae: 1 — low intensity; 2 — moderate intensity; 3 — high intensity of infection.

significant differences. However the percentage of fecund females (19.4 %) in the third group (high intensity of infection) was significantly less ($\chi^2 = 6.73$, $P < 0.01$) than in the control group (fig. 5).

Fecundity of the *B. troscheli* females was depended on intensity of infection with metacercariae, as it was shown by the one-way ANOVA. Mean number of clutches and normal egg capsules per female show different values for groups with different intensity of infection with metacercariae (ANOVA $F = 9.36$ and $F = 6.74$, $DF = 3$, $P < 0.001$) (table 3). It was lowest in the high-intensity group (Tukey test, $P < 0.001$). Mean number of egg capsules per a clutch

Table 3

Fecundity parameters (mean \pm SE) of the *Bithynia troscheli* females, uninfected and infected with trematode metacercariae

Fecundity indicators	Uninfected	Intensity of infection			ANOVA
		low	moderate	high	
NC	0.815 \pm 0.036	0.817 \pm 0.081	0.846 \pm 0.102	0.379 \pm 0.081***	F = 9.36 df = 3 p < 0.001
SC	4.664 \pm 0.208	4.082 \pm 0.394	2.980 \pm 0.426**	2.062 \pm 0.497***	F = 3.52 df = 3 p < 0.01
n EC	8.167 \pm 0.335	7.797 \pm 0.736	6.181 \pm 0.769*	3.384 \pm 0.727***	F = 6.74 df = 3 p < 0.001
n	861	349	254	177	

Note. NC — Number clutches per female; SC — number of egg capsules in per clutch; n EC — normal egg capsules per female (one embryo in one egg capsule). Marks: Tukey HSD test * — p < 0.05; ** — p < 0.01; ***p < 0.001.

Table 4

Fecundity parameters of the *Bithynia troscheli* females, uninfected and infected with common trematode metacercariae, from Kargat River

Species	NC	SC	n EC	0 embryos	2—3 embryos
	Mean \pm SE				
<i>Cyathocotyle bithyniae</i>	0.682 \pm 0.066	3.082 \pm 0.324	6.004 \pm 0.579	0.122 \pm 0.037	0.083 \pm 0.029
<i>C. bushiensis</i>	0.963 \pm 0.135	3.436 \pm 0.434	7.346 \pm 0.951	0.112 \pm 0.064	0.080 \pm 0.041
<i>Echinoparyphium aconiatum</i>	0.815 \pm 0.091	3.845 \pm 0.505	6.673 \pm 0.769	0.096 \pm 0.038	0.280 \pm 0.111
<i>E. recurvatum</i>	0.714 \pm 0.362	2.380 \pm 0.773	7.343 \pm 2.389	0.029 \pm 0.029	0
<i>Cotylurus cornutus</i>	0.219 \pm 0.219**	0.165 \pm 0.165***	1.125 \pm 1.125**	0	0
<i>Cyclocolliidae</i> gen. sp.	0.192 \pm 0.111**	1.250 \pm 0.772**	1.885 \pm 1.145**	0	0
Uninfected	0.815 \pm 0.036	4.664 \pm 0.208	8.167 \pm 0.335	0.085 \pm 0.026	0.101 \pm 0.023

Note. NC — number of clutches per female; SC—number of egg capsules per clutch; n EC — number of normal egg capsules per female (containing one embryo in one egg capsule); 0 embryos — number of egg capsules without embryos, per female; 2—3 embryos — number of egg capsules with 2—3 embryos in one egg capsule, per female; uninfected females were controls. Marks: Tukey HSD test * — p < 0.05; ** — p < 0.01; *** — p < 0.001. Females of *Bithynia troscheli* co-infected with the trematode metacercariae of 2, 3 and 4 species were excluded from the table 4.

varied between groups of females different in their infection intensity with metacercariae (ANOVA $F = 3.52$, $DF = 3$, $p < 0.01$). Values of these parameters were the highest in the uninfected group or in the low infection intensity group (Tukey test, $P > 0.05$) and the lowest in the heavily infected group (Tukey test, $P < 0.01$).

The influence of the metacercariae of common species on the fecundity of *Bithynia troscheli*

Among 15 trematode species found in the *B. troscheli* females sampled in Kargat River (table 2), the six species of 25 % occurrence, were regarded as common; and the rest nine species of 20 % occurrence in primary samples, were defined as rare (fig. 6). The breeding females were infected with 9 of 15 recorded trematode species. Six of nine species were common, whereas other three species were rare (*Asymphylodora* sp.; *Echinostoma revolutum* and *E. grandis*).

The snail females infected with metacercariae of common species (belonging to the fam. Echinostomatidae or Cyathocotilidae) were compared with uninfected females in a percentage of the fecund females, and no significant differences were detected (Chi-square test, $p > 0.05$) (fig. 6). However, the percentages of fecund females infected with Cyclocoelidae gen. sp. metacercariae (11.54 %; $\chi^2 = 6.86$, $p < 0.01$) or *Cotylurus cornutus* metacercariae (3.13 % $\chi^2 = 15.61$, $p < 0.001$) were significantly less than values of these parameters

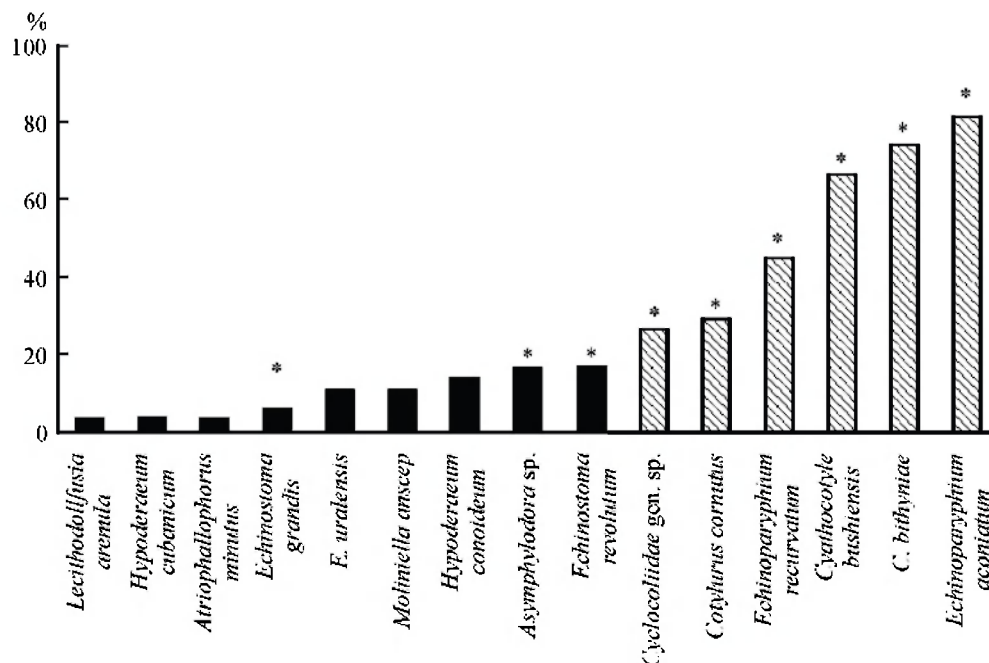


Fig. 6. Frequency of metacercariae occurrence.

* — trematode species in breeding females of *B. troscheli*. Six species > 25 % of primary samples were common species. Nine species 20 % of primary samples were rare species.

for uninfected females. It was concluded that the fecundity parameters of females infected with the trematode metacercariae were significantly less than those for uninfected female snails (Tukey HSD test, $P < 0.01$; table 4).

DISCUSSION

Metacercariae of 15 trematode species were found in the *B. troscheli* females from Kargat River are common in West Siberian freshwater gastropods; 12 of them have been previously recorded in the same ecosystem parasitizing the pulmonates (Yurlova et al., 2006). Metacercaria of *Lecithodoliffusia arenula* and *Atriophallophorus minutus* are developed in the first intermediate host. These species are specific for bithyniid snails (Serbina, 2010b). Though the trematode species of the fam. Monorchidae were rare in Chany Lake (Serbina, 2004), the percentage of the monorchid parthenitae in infected bithyniidae snails from Ob River varied from 10 % up to 28 % in different years (Serbina, Bonina, 2011). The number of trematode species at metacercarial phase found in the West Siberian bithyniid snails are as follows: 17 for Kargat River, 7 for Ob River, 8 for Ob River affluents, 6 for Karasuk River, and 12 for Krotovo Lake (Serbina, 2002, 2011, 2013; Yurlova, Serbina, 2004). The species *Echinoparyphium aconiatum*, *Cyathocotyle bithyniae*, and *C. bushiensis* parasitizing bithyniid snails were recorded in all the surveyed rivers and lakes, occurring in more than 65 % of samples (fig. 6). They also exhibited the highest values of prevalence and abundance (table 2). However, the number of trematode species and their mean abundance were lower in breeding females than in non-breeding ones (fig. 3, b, c). Similar results were obtained for *Palaemonetes pugio* Holthuis which non-ovigerous females were more heavily infected than ovigerous ones (Pung et al., 2002). As in *B. troscheli*, the reduction of individual fecundity was already

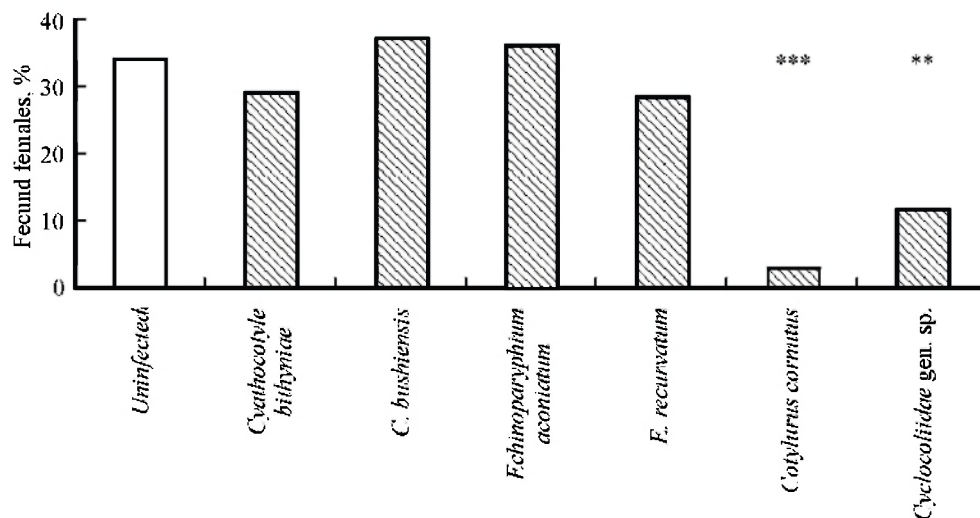


Fig. 7. The percentage of fecund females of *Bithynia troscheli* uninfected and infected with the metacercariae of the common trematode species.

Chi-square: *** $p < 0.001$; ** $p < 0.01$.

revealed for amphipod or isopod hosts infected with trematode metacercariae (Thomas et al., 1995; Ferreira et al., 2005).

In this study it was shown that the trematode metacercariae in the host's hepatopancreas and/or gonad may reduce significantly the fecundity of the *B. troscheli* females. Female snails infected with metacercariae of *Cotylurus cornutus* and Cyclocoliidae gen. sp. bred significantly rarer (fig. 7; Chi-square test, $p < 0.01$) and their fecundity parameters are significantly lower (table 4). Metacercariae localized in the mollusk's mantle or somatic musculature had no significant effects on the parameters of the host. Here is shown that metacercariae effect the fertility of *B. troscheli* resulting in the dual impact. They can significantly reduce the individual fecundity of snails, or increase the percentage of reproductively active females.

The proportion of fecund females with high intensity of infection was significantly lower, than the percentage of fecund uninfected females (fig. 5; Chi-square test, $p < 0.01$). All of the fecundity parameters of females with high intensity of infection were significantly lower, than in uninfected females (Tukey HSD test $P < 0.001$; table 3). The negative impact of the trematode metacercariae at high infection intensity on the survival of second intermediate hosts has been demonstrated for amphipods, tadpoles, juvenile fishes (Fredensborg et al., 2004; Orlofske et al., 2009; Kelly et al., 2010). Low intensity of infection with metacercariae does not lead to population loss of *B. troscheli*.

The fecundity parameters of *B. troscheli* with low intensity of infection with metacercariae did not differ from the fecundity parameters of uninfected females (table 3). However, the former bred significantly more frequently than uninfected ones (fig. 5; Chi-square test, $p < 0.05$). Similar data were obtained in study on the *Schistosoma*—*Biomphalaria* system (Minchella, Loverde, 1981; Thornhill et al., 1986). Snails exposed to *Schistosoma* miracidia, whether they became infected or remained uninfected, laid more eggs following exposure than unexposed controls. This phenomenon is known as the «fecundity compensation». By present time, two variants of the «fecundity compensation» have been studied: adult females allocated more resources to reproduction (Minchella, Loverde, 1981) and the juvenile females matured earlier (Thornhill et al., 1986). It should be noted that the «fecundity compensation» was not revealed in the system of *Potamopyrgus*—*Microphallus* (Krist, Lively, 1998). The infection of intermediate hosts with *Schistosoma* involves the penetration of snail by an actively swimming miracidium, while the host of *Microphallus* becomes infected by ingestion of an immobile embryonated egg (the passive transmission). Hence, snails exposed to the *Schistosoma* miracidia may get an obvious cue of the threat of infection whereas the *Potamopyrgus* snails do not get such cue from *Microphallus*. In the life cycles, the trematodes can be transmitted from the first intermediate host to the next molluscan host in two ways: either through swimming larvae that leave the first intermediate host or as metacercariae that are formed inside the first intermediate host (the passive transmission, e. g. some species of fam. Microphallidae and Lecithodendriidae) (Filimonova, Shalyapina, 1979; Krist, Lively, 1998). The latter case corresponds to the secondary dixenic life cycle type (Galaktionov, Dobrovolskij, 2003). The species *L. arenula* and *A. minutus* caused castration of bithyniidae snails even at the phase of parthenitae (Serbina, Kozminsky, 2010). All the 9 species found in fecund females of *B. troscheli* infected them actively (as the *Schistosoma* miracidium). It

can be assumed that adult females perceive the infection with 1—2 metacercariae as a threat, starting to breed. As an interpretation of results here, it is reasonable to suppose the presence of population mechanisms to compensate the hosts' reproductive losses. Irrespective of the mechanisms involved, the present work shows that the trematode metacercariae may have a significant impact on aquatic snails. Our results were obtained from naturally infected snails, and not via experimental infection; thus, it remains possible that host's fecundity is associated with infection but is not caused by it. The present study shows that metacercariae do not sterilize bithyniids, but they may affect the fecundity of snails depending on the infection intensity. Further studies are needed to understand the mechanisms which trematodes use to interfere with the fecundity processes of *B. troscheli* and other hosts.

In conclusion, infection with trematode metacercariae does not cause parasitic castration but it can significantly reduce the individual fecundity of the *B. troscheli* females at high intensity of infection with metacercariae. The revealed increase of the breeding host females' percentage at low level of infection may be considered as the host fecundity regulatory compensation, which is significantly reduced with increasing of the trematode infection intensity.

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