Nematode Infestation in *Rana arvalis* Nilsson, 1842: The Role of Age and Geographic Location

E. A. Malkova[a], *, A. V. Burakova[a], I. A. Kshnysev[a], and V. L. Vershinin[a, b]

[a] *Institute of Plant and Animal Ecology, Ural Branch, Russian Academy of Sciences, Yekaterinburg, 620144 Russia*
[b] *Ural Federal University, Yekaterinburg, 620002 Russia*
*e-mail: bay@ipae.uran.ru
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Abstract—Ten parasite species have been identified in populations of *Rana arvalis* Nilsson, 1842 (from the western macroslope of the Middle Urals to the Ishim Plain), with the nematodes *Oswaldocruzia filiformis* (Goeze, 1782) and *Rhabdias bufonis* (Schrank, 1788) being dominant. A two-component (hurdle) model combining binomial regression (infection probability) and gamma regression (infection intensity) provides the best biological and statistical fit for nematode infestation patterns. Both infection probability and intensity increase with host age, peaking at 3–4 years: each unit increase in $\log_2$-transformed age ($\log_2(\text{Age} + 1)$) raises the odds of infestation 6.2-fold (95% CI: 4.9–7.8) and intensity 1.4-fold (1.2–1.5). Intensity also increases 2.1-fold (1.8–2.5) per additional nematode species. Geographic trends reveal latitudinal and longitudinal variability: infestation odds decrease 2.1-fold (1.5–2.7) per degree northward, while infection intensity increases 1.14-fold (1.1–1.2) per degree eastward. Neither host morph (striata/maculata) nor sex significantly influence nematode infestation parameters.

INTRODUCTION

Amphibians are an important link of the food chain and indicators of the state of the environment (Hocking and Babbitt, 2014). However, animals are subject to various threats, including infections by endoparasites (Garner et al., 2016; Hallinger al., 2020; Svinin et al., 2020). Understanding the mechanisms of infection and the factors affecting its intensity is of great importance for conserving amphibian biodiversity. Global trends, such as climate change and the decline in amphibian populations (Gardner, 2001), exacerbate these problems.

Abiotic conditions, such as temperature and humidity, influence the spread and intensity of parasitic infestation. An increase in ambient temperature increases the efficiency of helminth transmission (Mordecai et al., 2013). This often leads to hyperinfection or to the dominance of one species, which negatively affects the condition of intermediate and definitive hosts, up to the death of the latter (Vershinin et al., 2017; Mordecai et al., 2013). The humidity of the environment and the presence of abundant vegetation are important for maintaining the viability of eggs and larvae of endoparasites with a direct life cycle, and they are directly related to the risk of infection of amphibians (Barton, 1998; Wacker, 2018; Silva et al., 2018). However, high temperatures can also negatively affect the viability of free-living stages of nematodes, and lack of oxygen, due to excessive moisture, can cause the death of their larvae (Spieler and Schierenberg, 1995).

In this case, the individual characteristics of the animals should also be taken into account. Ecological and physiological features of the organism, such as age, sex (Gustafson et al., 2015), or genetic polymorphism in the population (Vershinin, 2008), can affect susceptibility to parasites throughout the life cycle. It has been shown that the taxonomic spectrum of helminths expands in sexually mature amphibians (Chikhlyaev, 2004; Rezvantseva et al., 2010; Zhigileva and Kirina, 2014; Burakova et al., 2022).

The endoparasite infection of amphibians of different sexes depends on the behavioral characteristics of the animals (Assemian et al., 2016). According to a number of researchers, the infection of males and females with helminths is either at a similar level (Saglam and Arikan, 2006) or males are more infected (Vasnetko and Siddikov, 1999). Under certain conditions, the species diversity of helminths in males can also be higher (Rezvantseva et al., 2010).

It has been shown that amphibians of different phenotypes are not equally susceptible to certain types of helminths (Lebedinsky, 1994; Mineeva, 2006). For example, maculata morphs are more heavily infected
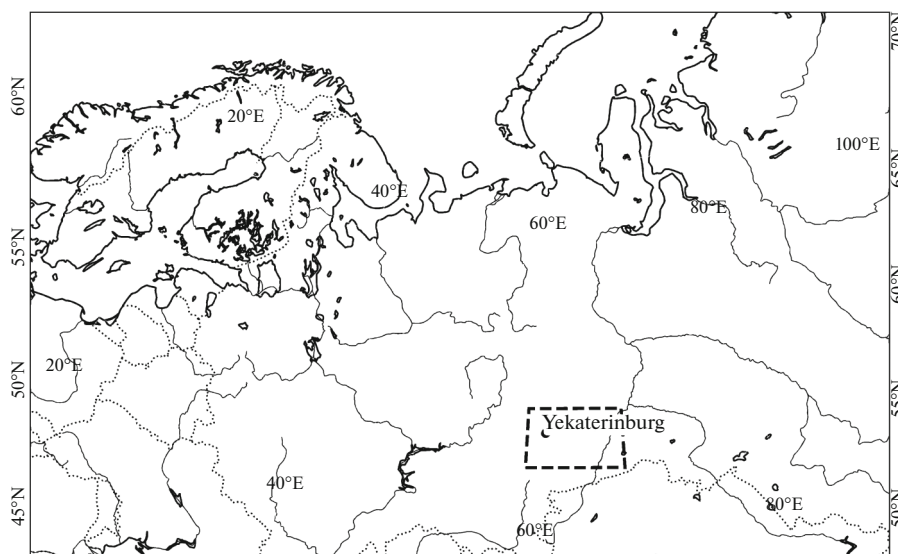
Fig. 1. Geographical location of the study region (dashed line).

with the nematode *Cosmocerca ornata* (Dujardin, 1845), which is associated with a longer period of their activity, in comparison with the striata morphs (Kirillova and Kirillov, 2015).

It has been shown for most studied regions of the Russian Federation that nematodes with a direct life cycle predominate in the helminth fauna of the moor frog (Ruchin and Chikhlyaev, 2013; Zhigileva and Kirina, 2015; Vershinin et al., 2017). Therefore, in this paper we investigate the influence of individual characteristics of specimens and the geographical location of the habitat on nematode infestation of moor frogs. The proposed approach makes it possible to quantify the contribution of individual factors to the variability of infestation of amphibians with nematodes.

MATERIALS AND METHODS

Study area. Frogs were captured by hand from May to August 2010–2014. We collected a total of 815 individuals of the moor frog were caught: the western slope of the Middle Urals, 100 ind.; the eastern slope of the Middle Urals, 502 ind.; the Turinskaya Plain (Western Siberia), 24 ind.; and the Ishim Plain (Western Plain), 189 ind. (Fig. 1). The contrast between the northernmost point and the southernmost point is ≈3° N, and between the westernmost point and the easternmost point it is ≈8° E.

Analysis of climatic conditions. The database of actual observations on temperature and humidity for the period from 2010 to 2014 was compiled on the basis of open information available at the rp5 website (*Raspisanie pogody*, 2025). The nearest weather station was selected for each trapping location. Data on atmospheric precipitation were used as an indicator of the environmental humidity. When analyzing the heat

supply of habitats, the days with an average daily temperature of 10°C and above were selected. In the analysis of the thermal provision of habitats, days were selected where the average daily temperature was 10°C and above. The choice of this threshold value is due to the activity of food items (Mellanby, 1939; Abdullah, 1961) and *R. arvalis* (Ledentsov, 1989). The selected period is consistent with the literature data on the activity of tailless amphibians in similar conditions (April to September/October) (Vershinin, 2007). Further, the use of the terms "active days" and "active period" implies the specified threshold value of the average daily temperature.

Parasitological analysis. In the laboratory, the animals were euthanized and subjected to a complete helminthological autopsy (Ivashkin et al., 1971). The helminth species were identified using the keys of K.M. Ryzhikov et al. (1980) and V.E. Sudarikov et al. (2002). The parasitic infection was assessed by the main parameters: the prevalence (P) and the infestation intensity (I), as well as the parasite abundance index (A) (Breev, 1976).

Absolute age determination. The age of animals was determined by the method of skeletochronology modified by A.V. Ledentsov (1990).

For this purpose, the cross sections stained with Ehrlich's hematoxylin were made from the middle of the third phalange of the fourth toe of the hindlimb. In addition to counting the lines of arrested growth, we also measured the diameters to confirm the accuracy of determining the lines corresponding to the first and second hibernation. The diameters of the annual layers and the areas of the toe phalanges of the amphibians were measured using the Levenhuk ToupView program.

Statistical analysis. The analysis of the average daily temperature and the number of days of the active
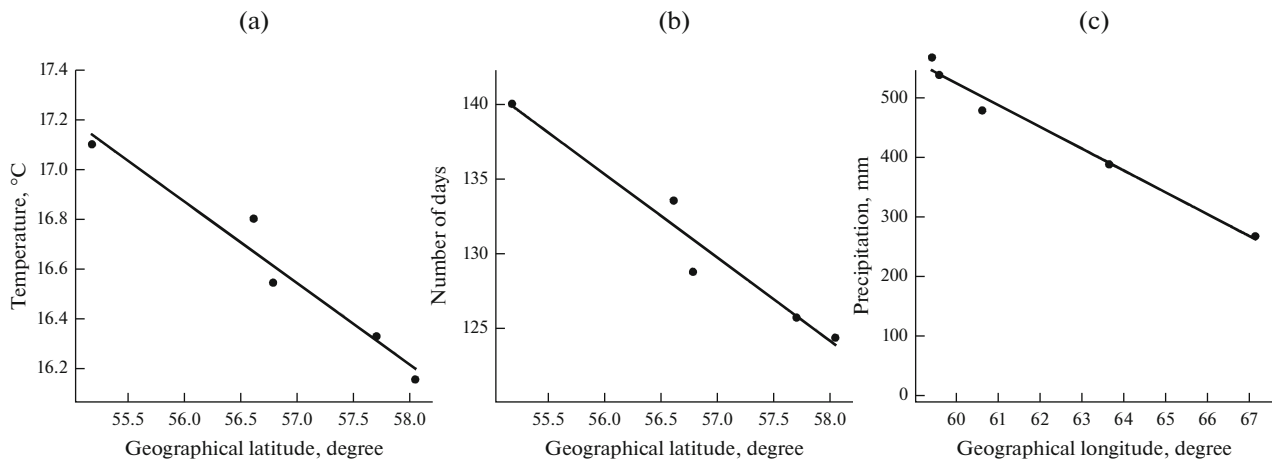
Fig. 2. Dependence of climate characteristics as a linear function of latitude and longitude: (a) average daily temperature of the active period, (b) duration of the active period, and (c) average annual precipitation. The points are the average values for weather stations, the straight line is regression.

period, as well as the average annual precipitation, was carried out in the orthogonal directions north to south and west to east.

For statistical modeling of the variability of nematode infection rates in the moor frog, the hurdle models were used, which assume a combination of two components of data generation (Mullahy, 1986; Cameron, and Trivedi, 1998; McDowell, 2003; Orlova et al., 2015). The risk of infection was modeled by binary regression (1), and the nonzero average abundance of the parasite in infected individuals ($y > 0$) was modeled by multiplicative regression (2):

(1) $\eta = b_0 + \Sigma X_i b_i$, $\eta = \log[\mu/(1 - \mu)]$ ($y_i = 0$ and $y_i \geq 1$, then $y_i = 1$),

(2) $\eta = \mathrm{Exp}(b_0 + \Sigma X_i b_i)$, if $y_i \geq 1$.

The estimates of parameters were obtained by the maximum likelihood method, and the Akaike information criterion was used to select the optimal models (Anderson et al., 1994): $\mathrm{CAIC} = -2LL + K[\log(n) + 1]$. Statistical inference was based on the best model if its weight significantly exceeded the others. The effects of the following predictors (X_i) were evaluated: host age (discrete age classes or the logarithm of age in years $+1$), latitude and longitude of habitat (degrees), morph (striata/maculata), host sex, and number of nematode species in an individual. The parameters and their 95% CI (confidence interval) are given after potentiation, $\exp(b_i)$ or $1/\exp(b_i)$, which are interpreted as an n-fold change in response with a single change in the predictor.

The data was analyzed using Statistica v. 10.0 (Stat-Soft, Inc) and Python (NumPy, seaborn, matplotlib, and scipy.stats).

RESULTS

Weather data analysis. It is found that the contrast in the average daily temperatures of the active period between the extreme southern and northern points of the study region was less than 1°C. At the same time, a statistically significant decrease in temperature was recorded in the northern direction ($r = -0.98$, $p < 0.01$) (Fig. 2a). The relationship between the average daily temperatures of the active period and the geographical location was not statistically significant ($p = 0.29$) in the longitudinal direction, despite the relatively high value of the correlation coefficient ($r = 0.6$). The duration of the active period of tailless amphibians within the study region increases by several weeks at a latitude of 58° compared with a latitude of 55° (Fig. 2b), and the amount of precipitation decreases from west to east ($r = -0.99$, $p < 0.001$) (Fig. 2c).

Analysis of the parasite fauna. A total of ten species of helminths of two phyla were found in *R. arvalis*: Platyhelminthes, Nematoda, and two species of endosymbiotic protozoa of the Phylum Bigyra Cavalier-Smith, 1998. The species composition of helminthes is as follows:

Phylum Nematoda Cobb, 1932: *Oswaldocruzia filiformis* (Goeze, 1782), *Cosmocerca ornata* (Dujardin, 1845), *Neoraillietnema praeputiale* (Skrjabin, 1916), *Aplectana acuminata* (Schrank, 1788), and *Rhabdias bufonis* (Schrank, 1788).

Phylum Platyhelminthes: *Dolichosaccus rastellus* (Olsson, 1876); *Opisthioglyphe ranae* (Froelich, 1791); *Haplometra cylindracea* (Zeder, 1800); *Echinoparyphium recurvatum* (Linstow, 1873), mtc.; and *Holostephanus volgensis* (Sudarikov, 1962), mtc.

Phylum Bigyra Cavalier-Smith, 1998: *Opalina ranarum* Purkinje et Valentin, 1835, and *Cepedea dimidiata* (Metcalf, 1923).

Trematodes were found at the stage of marita (sexually mature) and metacercariae. Sexually mature forms are found for nematodes. The helminthofauna of *R. arvalis* is dominated by nematodes with a direct life
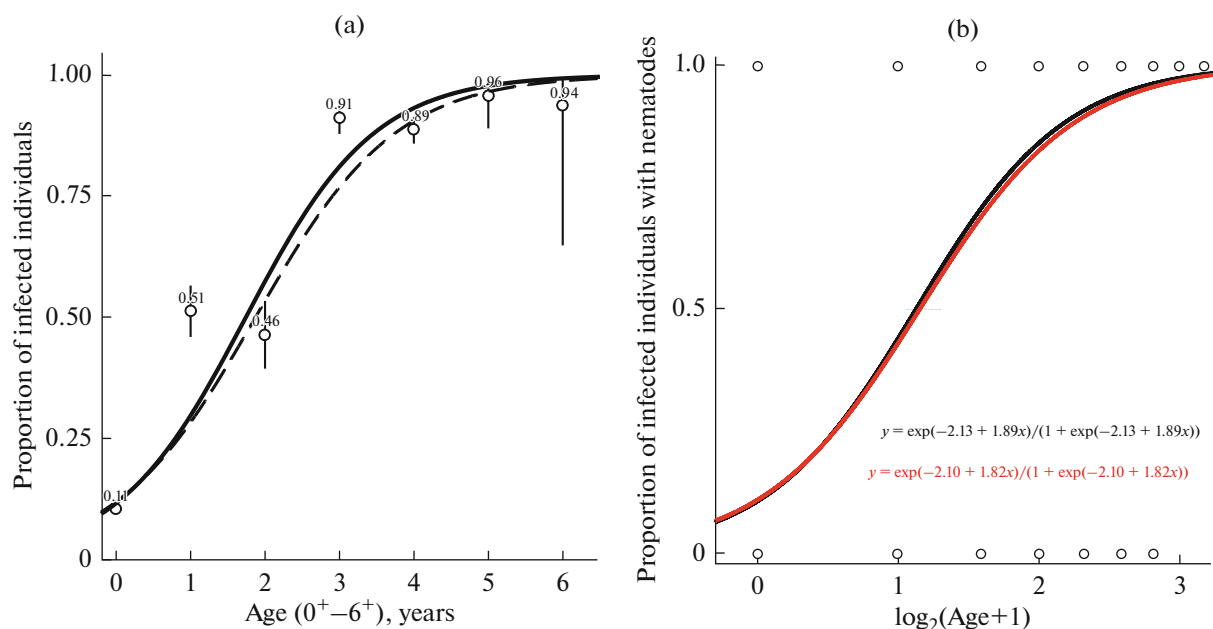
Fig. 3. (a) Risk of infection with nematodes as a function of the age of *R. arvalis*. Points are the observed proportion of infected individuals (segments, 95%CI), single-factor logit model with discrete age ($df = k - 1 = 6$). Smooth curves are the same, but with continuous age ($df = 1$). The bold line indicates a model without other covariates, and the long stroke is a model with covariates; (b) is the same for the log-transformed age. The black line is for the one-factor model, and the gray line is for the covariate model, longitude.

cycle such as *O. filiformis* ($P = 0.30$; $A = 1.94$ ind./specimen) and *R. bufonis* ($P = 0.14$; $A = 1.22$ ind./specimen), for which the moor frog is the definitive host. The contribution of other nematode species (*C. ornata*, *N. praeputiale*, and *A. acuminata*) is insignificant. The probability and intensity of infestation for them is significantly lower ($P \leq 0.04$, $A \leq 0.03$ ind./specimen) than for the dominant species.

Optimization of the representation of amphibian age. The determination of the absolute age of amphibians is based on the calculation of the number of hibernations they have survived. The number of hibernations corresponds to discrete age classes and is determined by the lines of arrested growth on the bone sections. The representation of particular age classes in the samples may be low, which dictates the need to consider age not as a discrete feature, but as a rank one. It is found that the dependence of the logarithm of the odds of infection and the intensity of infestation is quite adequately approximated by a monotonic dependence on the logarithm of age in years, transformed by the formula $X = \log_2(\text{Age} + 1)$, rather than by classification into six discrete age groups (0+ to ≥5+) (ΔCAIC > 3) (Fig. 3). The most intense infection of animals with nematodes occurs in the first years of life, while the proportion of individuals infected with nematodes increases with age, reaching a maximum by the age of 3 years (Fig. 3a).

Risk of infection. The prevalence of infection was 33.9% (276 individuals out of 815). To determine the importance of the factors determining the probability

of nematode infection in amphibians, 32 statistical models were constructed, including the null hypothesis. Among the models considered, the model with only two of the considered predictors showed the greatest statistical support: $\log_2$-transformed age and the north–south gradient (Table 1).

It is found that the odds of infection increase by 6.2 times (4.9–7.8) with a change in the value of $\log_2(\text{Age} + 1)$ by one (Table 2), which corresponds to a comparable increase in risk during the transition: from underyearlings (0+) to individuals aged 1+, from 1+ to 3+, and from 3+ to 7+ years (Fig. 3b). Thus, the odds of infection increase approximately 12.4 times during the first 3 years of the animal's life. The odds of infection show an inverse relationship with latitude, decreasing 2.1-fold (2.7–1.5) by a degree north (Table 2) within the study region.

Infection intensity. An analysis of the average infection intensity based on a naive approach (standard one-parameter Poisson distribution, ignoring other predictors) showed that nematode infection in underyearlings of *R. arvalis* ($\lambda_0 = 3.4$) is similar to that in yearlings ($\lambda_1 = 2.7$) (Fig. 4b). However, starting from the age of two ($\lambda_2 = 7.4$), there was a significant increase in the average abundance of nematodes, reaching a peak by the age of four ($\lambda_4 = 14.4$). The observed infection intensity becomes more variable in the older age groups (5+, 6+), demonstrating both an apparent decrease ($\lambda_5 = 10.3$) and an increase ($\lambda_6 = 15.7$) (Fig. 4b). The observed distribution of infection inten-
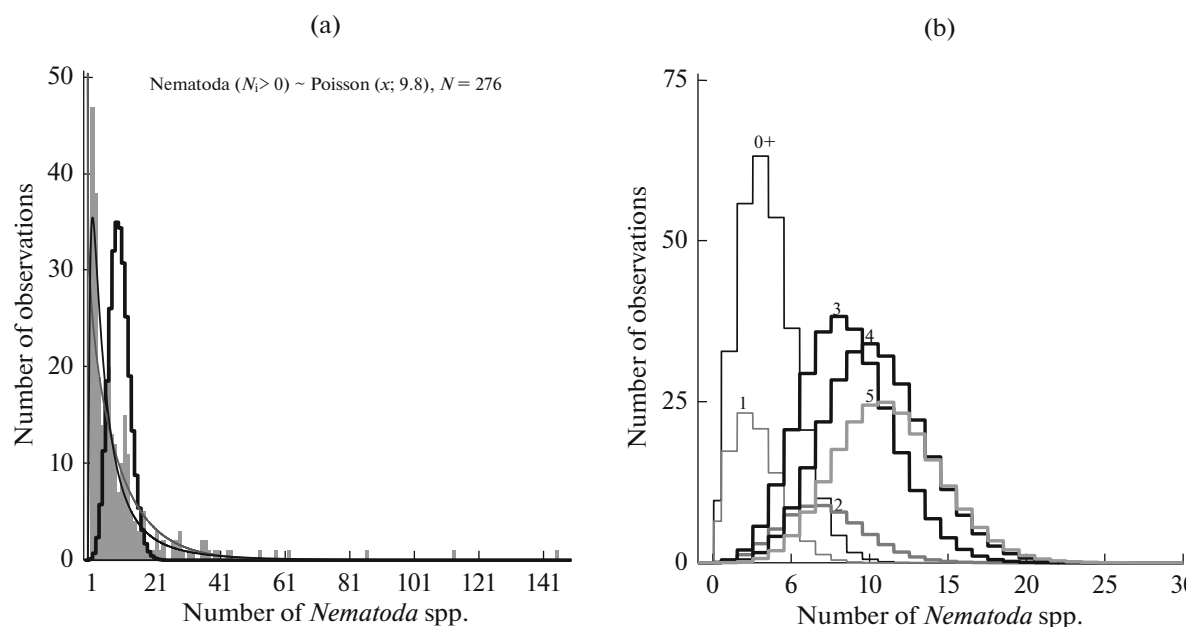
(a)   (b)

Fig. 4. (a) Distribution of the number of nematodes of infected individuals of *R. arvalis*, approximation by distributions: Poisson (bold stepwise), log-normal, and gamma (smooth). (b) Same, but for particular age classes, approximation by the Poisson distribution; the numbers near the distribution curves are age classes.

sity (excess of zeros, a heavy right tail; Fig. 4a) does not correspond to the Poisson distribution for both the entire group of infected individuals (Kolmogorov–Smirnov test, $p < 0.01$) and for particular age classes (Fig. 4b).

Since the estimate of the variance ratio ($\varphi = X^2/dfRes = 12.2 \gg 1$) for the count regression (Pois-

son) does not satisfy the assumptions of homoscedasticity (overdispersion), gamma regression with logarithmic transformation of the response was used to describe the dynamics of the average number of nematodes per individual (Tables 3, 4).

Sixty-four models were studied to determine the importance of predictors that determine the infection

Table 1. Selection of logit regression models to describe the risk of infection by the nematode *R. arvalis* s

No.	Predictors	k	$-2LL$	$\Delta CAIC$	w
1	**Latitude, $\log_2(Age + 1)$**	**3**	**561.1**	**0**	**0.889**
2	Latitude, $\log_2(Age + 1)$, morph	4	559.3	5.95	0.045
4	Latitude, longitude, $\log_2(Age + 1)$	4	560.7	7.33	0.023
12	Latitude, longitude, $\log_2(Age + 1)$, morph, sex	6	558.4	20.37	≈ 0
31	*H_0*	*1*	*1043.4*	*466.93*	*≈ 0*
32	Morph	2	1037.3	468.53	≈ 0

k, number of parameters; $-2LL$, maximum of the logarithm of the likelihood function; $\Delta CAIC$, difference between CAIC of a particular model and the minimum CAIC among all models; w, relative likelihood of the model; H_0, null hypothesis. The model number corresponds to its position from best to worst in the ranking. Min CAIC = 584.20.

Table 2. Estimates of the parameters of the best logit regression model for describing the average risk of infection by the nematode *R. arvalis*

Parameters	b	se	Wald-X^2	95%CI		exp	95%CI	
Interc.	38.34	8.38	20.94	21.89	54.78			
Latitude	−0.72	0.15	23.22	−1.01	−0.43	2.1^{-1}	2.7^{-1}	1.5^{-1}
$\log_2(Age + 1)$	1.82	0.12	236.85	1.59	2.06	6.2	4.9	7.8

b, regression coefficient; se, standard error; Wald-X^2(1), Wald statistics:$=[b_i/se(b_i)]^2$; Exp, odds ratio.

Table 3. Selection of gamma regression models for the average number of nematodes in infected individuals of *R. arvalis*

No.	Predictors	k	ΔCAIC	w
1	**Longitude, $\log_2(\text{Age} + 1)$, NspNem**	**4**	**0**	**0.860**
2	Latitude, Longitude, $\log_2(\text{Age} + 1)$, NspNem	5	5.15	0.065
8	Longitude, $\log_2(\text{Age} + 1)$, Latitude, sex, morph, NspNem	7	18.16	0.000
61	H_0	1	195.99	≈ 0

See the note to Table 2; NspNem, the ~~average geometric~~ number of nematode species per host. Min CAIC = 1622.75.

Table 4. Estimates of the parameters of the best gamma regression model for the expected number of nematodes in infected individuals of *R. arvalis*

Parameters	b	se	Wald-X^2	95%CI		exp	95%CI	
Interc	−8.04	0.799	101.2	−9.60	−6.47			
Longitude	0.13	0.013	108.1	0.11	0.16	1.14	1.11	1.17
$\log_2(\text{Age} + 1)$	0.30	0.051	35.3	0.20	0.40	1.35	1.23	1.50
NspNema	0.75	0.092	66.0	0.57	0.93	2.11	1.76	2.53

See the note to Table 3.

intensity in amphibians by nematodes. The model with three predictors received the maximum statistical support: logarithmically transformed age, west−east gradient, and number of nematode species per individual (Table 3). The estimated increase in the infection intensity with an increase in $\log_2(\text{Age} + 1)$ per unit was 1.4 times (1.2−1.5) (Table 4). This indicates that the maximum increase in the infection intensity was observed in the first years of life. The geometric average of the number of nematodes per individual increases by 1.14 (1.1−1.2) times per degree from west to east in the study region (Table 4). Each new nematode species increases the infection intensity by 2.1 (1.8−2.5) times.

DISCUSSION

The intestinal nematodes *O. filiformis* and *R. bufonis* dominate among the endoparasites in the studied populations of *R. arvalis*. The predominance of these species, mainly *O. filiformis*, was traced in parasitic complexes and other species of tailless amphibians that lead a terrestrial lifestyle (Burakova, 2011; Zhigileva and Kirina, 2015; Burakova and Malkova, 2021; Burakova and Malkova, 2023). This is due to the wide distribution of these species in the Palearctic, parasitization on many species of amphibians and reptiles (Ryzhikov et al., 1980; Chikhlyaev et al., 2016), the terrestrial lifestyle of the host, and synchronization of active phases of parasite reproduction with the features of frog ontogenesis (Okulewicz et al., 2013; Ruchin and Chikhlyaev, 2013; Zhigileva and Kirina, 2015).

The use of a multimodel approach allowed us to establish that each additional nematode species increases the infection intensity of the host by 2.1 (1.76−

2.53) times. Parasites are characterized by the spatial distribution within the host organism, occupying specific areas of the host biotope (Nasurdinova and Zhigileva, 2007). The dominant species of nematodes occupy different trophic niches: *O. filiformis* is localized mainly in the anterior parts of the small intestine and *R. bufonis* in the lungs. The accompanying species (*C. ornata*, *N. praeputiale*, and *A. acuminata*) inhabit the rectum, where *C. ornata* is more common in the distal sections, and *A. acuminata* is more common in the proximal sections (Bjelić-Čabrilo et al., 2009; Chikhlyaev and Ruchin, 2014). The abundance/number of species using a single host biotope can be regulated by competitive interactions. It is known that *O. filiformis* limits the spread of *C. ornata* in the intestine. However, the heterogeneity of the intestinal environment (differences in pH, content composition, and surface area) allows several species to coexist, each of which occupies its own niche (Bjelić-Čabrilo et al., 2009). Thus, an increase in the number of nematode species leads to an increase in the infection intensity due to spatial separation (different parts of the intestine or organs), trophic specialization (use of different resources), and a decrease in direct competition between species. These mechanisms allow parasites to use host resources more efficiently, increasing the total abundance of helminths in the body (Nasurdinova and Zhigileva, 2007).

The age of *R. arvalis* has confirmed its prognostic value for assessing both the risk of infection and the infection intensity by nematodes. The increase in the infection intensity in the moor frog (Wacker, 2020) and other amphibians with age has been reported in a number of works (Odnokurtsev and Sedalishchev, 2008). Researchers usually use qualitative age catego-

ries: underyearlings, immature, and sexually mature individuals (Rezvantseva et al., 2010). Sometimes the size and age groups are distinguished based on the body length (Odnokurtsev and Sedalishchev, 2008; Burakova, 2011; Wacker, 2020). In this study, using the example of *R. arvalis*, changes in the risk and intensity of infection were calculated using the absolute age of animals in years. It is found that the increase in the risk of infection is the highest in *R. arvalis* from underyearlings to 3 years of age. During this period, the active growth of the entire body was observed in moor frogs, which determines the maximum annual increase in the bone tissue and high energy requirements. It is known that the feeding rate of amphibians decreases with age and is related to body size. For example, in juvenile *R. arvalis*, it is 4.2% of the body weight per day, decreasing to 1.3% in adults (Loman, 1979). This is due to differences in the rate of digestion of food items, as well as in hunting strategy. Juvenile individuals consume mainly small invertebrates, while adults use an energy-efficient strategy of ambushing, focusing on large prey, which reduces the number of specimens caught per day. In addition, the energy consumption for growth decreases with age (Loman, 1979; Ishchenko and Skurykhina, 1981). Since the infection with *O. filiformis* occurs by accidental ingestion with food items and the infection with *R. bufonis* occurs through the skin and nasal passages, fewer consumed items and a sedentary hunting strategy in adults reduce the probability of infection with nematodes. In conditions of the continental climate, individuals of *O. filiformis* and *R. bufonis* are able to survive wintering in the host body (Vakker, 2020). However, the lifespan of adult parasitic nematodes is only several months (Dubinina, 1950; Goater, 1992). This probably affects mainly seasonal differences in the infection intensity, rather than the accumulation of nematodes with age, since the generation of these parasites is renewed annually. It is the increase in the linear body size of amphibians and the associated features of feeding behavior that increases the risk of infection with parasites in the first years of life. After 3 years, the rate of increase in the risk of infection decreases. This is explained by several reasons for this. First, the growth slows down in sexually mature individuals (Baitimirova and Vershinin, 2017; Lyapkov, 2024). Second, they switch to a less active way of hunting, which leads to a decrease in the number of ingested items. At the same time, the balance is established between the entry of new invasive larvae of *O. filiformis* and *R. bufonis* into the host organism (orally and percutaneously, respectively) and the death of helminths of the previous year's generation (Vakker, 2020).

Climate change can modify the vulnerability of species (Pacifici et al., 2017). Since preindustrial times, the average global temperature has increased by 1.0°C (*Climate Change…*, 2021). The role of climate change in reducing the populations of amphibians remains controversial. Outbreaks of parasitic infec-

tions are considered an indirect mechanism of this process (Luedtke et al., 2023). In the present work we studied the habitats of *R. arvalis* located within the temperate zone and characterized by a slight difference in the main climatic characteristics due to the geographical distance. The difference in the average annual temperatures between the extreme points does not exceed 1°C, which is comparable to the RCP2.6 scenario, which assumes minimal global warming by 2100 (Duan et al., 2016). Nevertheless, the differences seen in the thermal regime have a measurable effect on the duration of the active period of amphibians. Its increase averages 15−20 days at 55° N compared to 58° N. Based on estimates of the odds ratio, it is found that a longer period of activity increases the chances of infection with parasites by 6.3 (4.5−8.1) times in southern populations compared with northern populations within the study region.

The difference in the average annual amount of precipitation between the studied western and eastern regions is about 300 mm, which is reflected in the change of plant communities. We should note that, in this study, the southern biotopes coincide with the eastern ones. They are located on the territory of the Ishim Plain, a significant part of which is occupied by the forest−steppe landscapes (Kotlyakov, 2003). Under conditions of moisture deficiency, amphibians are forced to concentrate in relief depressions near freshwater water bodies (Misyura and Marchenkovskaya, 2011), which contrasts with the more moistened forests of other studied areas, where animals are protected from direct sunlight under the canopy of the forest. The littoral zones with dense vegetation cover provide optimal conditions for the development of nematode eggs and subsequent host infection (Vakker, 2018). The high local density of hosts and favorable conditions for the realization of the life cycles of parasites ensure a high density of invasive larvae in the environment, which, of course, increases the risk of infection.

At the same time, the infection intensity increases significantly only from west to east. Perhaps this is due to the fact that the high degree of aggregation of amphibians near water bodies in the steppe biotopes of the Ishim Plain, when the probability of infection has already reached its maximum, has a major impact on the increase in the intensity of infection.

Further studies will make it possible to clarify the understanding of the mechanisms of climate influence and its dynamics on the spread and intensity of parasitic infections in amphibians.

CONCLUSIONS

The nematodes *O. filiformis* and *R. bufonis* dominate in the study habitats of *R. arvalis*, which is due to the ecological characteristics of the host species. The age of amphibians and the number of nematode species in individuals are important individual character-

istics that determine the infection intensity. The use of a two-component model of generalized regression made it possible to quantify the effect of age and geographical location of the habitat on both the risk of infection and the infection intensity in *R. arvalis* within the study region. It is shown that the probability of infection decreases in the northern direction, and the infection intensity increases to the east. These results can be used to develop strategies for monitoring and preserving amphibian populations in the face of global environmental changes.

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AUTHOR CONTRIBUTION

E.A. Malkova collected the material, determined the age of animals, performed the statistical analysis, discussed the results, and wrote and edited the text. A.V. Burakova collected the material, carried out the parasitological analysis, discussed the results, and wrote and edited the text. I.A. Kshnyasev performed the statistical analysis, discussed the results, and wrote and edited the text. V.L. Vershinin collected the material and edited the text.

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ETHICS APPROVAL
AND CONSENT TO PARTICIPATE

The animals were collected, processed, and euthanized in accordance with the protocol of November 3, 2023, approved by the Commission on Bioethics of the Institute of Plant and Animal Ecology, Ural Branch, Russian Academy of Sciences.

CONFLICT OF INTEREST

The authors of this work declare that they have no conflicts of interest.

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