Helminth communities in amphibians from Latvia, with an emphasis on their connection to host ecology

A. Čeirāns[1] [iD], E. Gravele[1], I. Gavarane[1], M. Pupins[1], L. Mezaraupe[1], I. Rubenina[1], Y. Kvach[2], A. Skute[1], O. Oskyrko[3], O. Nekrasova[4], O. Marushchak[4] and M. Kirjushina[1]

[1]Institute of Life Sciences and Technologies, Daugavpils University, Parades iela 1A, LV-5401, Daugavpils, Latvia; [2]Institute of Marine Biology, NAS of Ukraine, Pushkinska Street, 37, 65048 Odessa, Ukraine; [3]Faculty of Science, Charles University, Viničná 7, 12844 Prague, Czech Republic and [4]I.I Schmalhausen Institute of Zoology, NAS of Ukraine, B. Khmelnytskogo Street, 15, 01030, Kyiv, Ukraine

Abstract

Helminth infracommunities were studied at 174 sites of Latvia in seven hosts from six amphibian taxa of different taxonomical, ontogenic and ecological groups. They were described using a standard set of parasitological parameters, compared by ecological indices and linear discriminant analysis. Their species associations were identified by Kendall's rank correlation, but relationships with host size and waterbody area were analysed by zero-inflated Poisson and zero-inflated negative binomial regressions. The richest communities (25 species) were found in post-metamorphic semi-aquatic *Pelophylax* spp. frogs, which were dominated by trematode species of both adult and larval stages. Both larval and terrestrial hosts yielded depauperate trematode communities with accession of aquatic and soil-transmitted nematode species, respectively. Nematode loads peaked in terrestrial *Bufo bufo*. Helminth infracommunities suggested some differences in host microhabitat or food object selection not detected by their ecology studies. Associations were present in 96% of helminth species (on average, 7.3 associations per species) and dominated positive ones. Species richness and abundances, in most cases, were positively correlated with host size, which could be explained by increasing parasite intake rates over host ontogeny (trematode adult stages) or parasite accumulation (larval *Alaria alata*). Two larval diplostomid species (*Strigea strigis*, *Tylodelphys excavata*) had a negative relationship with host size, which could be caused by parasite-induced host mortality. The adult trematode abundances were higher in larger waterbodies, most likely due to their ecosystem richness, while higher larval abundances in smaller waterbodies could be caused by elevated infection rates under high host densities.

Introduction

Helminths are an important component of natural ecosystems, where they exert effects on their hosts' growth, physical performance, fecundity and population sizes, which affect connections throughout the ecological network (Thomas *et al.*, 1999). Helminth infracommunities in amphibians are typically less diverse than those in birds or mammals, but they contain relatively high numbers of helminths that are core species in several hosts (Aho, 1990). Amphibians are placed in the middle of food webs, being important prey items for many vertebrates and major predators of invertebrates (Duellman & Trueb, 1994), which determines their role as intermediate, definitive or paratenic hosts for many parasites with complex life cycles (Smyth & Smyth, 1980; Galaktionov & Dobrovolskij, 2003; Toledo & Fried, 2019).

There are many studies on helminth community compositions in amphibian hosts across Europe. Relatively recent works have been published concerning post-metamorphic ranid frogs from Germany (Andreas, 2006) and Poland (Popiolek *et al.*, 2011; Okulewicz *et al.*, 2014), bufonid toads from Belarus (Shimalov & Shimalov, 2001) and *Pelophylax* spp. frogs from Serbia (Bjelić-Čabrilo *et al.*, 2009) and Ukraine (Kuzmin *et al.*, 2020). Annotated helminth check lists are available for the main post-metamorphic anuran hosts (*Rana* spp., *Bufo bufo*, *Pelophylax* spp.) from the Volga River basin of Russia (Chikhlyaev & Ruchin, 2014, 2021; Chikhlyaev *et al.*, 2018a, b, 2019a, b, 2020) and the Ural Region of Russia (Burakova & Vershinin, 2016; Burakova & Baytimirova, 2017; Vershinin *et al.*, 2017). Brief reports on helminthological investigations in post-metamorphic newts have been made for Greece (Sattaman, 1990) and Belarus (Shimalov *et al.*, 2001), and recently the *Lissotriton vulgaris* helminth community composition has also been studied by combining morphological identification and DNA sequencing (Sinsch *et al.*, 2019). Older studies, often published as brief notes in local sources in national languages, have also been summarized (Ryzhikov *et al.*, 1980;

Sudarikov *et al.*, 2002). However, helminth community studies in European amphibian larval stages are very few (Avery, 1971), in contrast with many studies in North America (e.g. Bursey & Dewolf, 1998; Koprivnikar *et al.*, 2006; Rhoden & Bolek, 2015).

A number of local or intrinsic factors affect helminth aggregations in amphibians, such as spatial heterogeneity, host body size, parasite dimensions and virulence, and parasite and intermediate-host productivities (Campião *et al.*, 2015; Johnson & Wilber, 2017; Toledo *et al.*, 2017; Mihaljevic *et al.*, 2018). In addition, fish studies suggest a high importance of individual host susceptibility (Tinsley *et al.*, 2020) and show more complicated mechanisms determining helminth infection rates, such as ontogenic dietary shifts that change parasite intake pathways (Saad-Fares & Combes, 1992), or the presence of certain parasite infection levels that are optimal for the host body condition (Maceda-Veiga *et al.*, 2016). Common invasion pathways or biological interactions (such as mutual facilitation or competition) may cause parasite species associations in hosts (Dallas *et al.*, 2019) that, so far, have been noted in very few amphibian helminth community studies (Hamann *et al.*, 2006a, 2010, 2013a, 2014). Many studies have shown the effect of anthropogenic habitats, such as soybean agriculture (Koprivnikar & Redfern, 2012), pasture and rice agriculture (Hamann *et al.*, 2006b, 2020; Campião *et al.*, 2017), crop vs. livestock land uses (Draghi *et al.*, 2020; Portela *et al.*, 2020), and urban and pesticide polluted areas (King *et al.*, 2007), on their composition, species richness and abundances. Helminths may provide clues to freshwater trophic state and water quality (Zargar *et al.*, 2011), but, in general, the effect of freshwater habitat qualities on helminth communities in vertebrate hosts remains understudied. Thus, to our knowledge, there are no studies on the effect of waterbody size, which clearly affects habitat spatial diversity and waterbody buffering capacities against external pressures (Biggs *et al.*, 2016) that could be important for complex trematode life cycles (Galaktionov & Dobrovolskij, 2003).

Surprisingly few studies in Western Palearctic amphibians have paid attention to species associations, relationship with host size or the habitat effect other than simple terrestrial vs. aquatic division. Some of these surveys confirm species richness or parasite total abundances as being higher in larger hosts (Andreas, 2006; Kuzmin *et al.*, 2020), while others indicate the prevalence and intensity being positively related to the host size in separate trematode species (Abdel-Gaber *et al.*, 2017; Ozoliņa *et al.*, 2021) or describe changes in the post-metamorphic ranid frog infracommunities along the urbanization gradient (Vershinin *et al.*, 2017). Antagonism between some nematode and trematode species has been noted in communities in the Danube basin (Bjelić-Čabrilo *et al.*, 2009).

Amphibian helminth communities in Latvia and the east Baltic region were virtually unstudied before the present survey, with the single exception being a study on *Alaria alata* mesocercariae infections in ranid and bufonid amphibian hosts (Ozoliņa *et al.*, 2021). Since 2016, amphibians have been targeted by several state-wide monitoring surveys in Latvia, which allowed us to visit many sites and perform extensive sampling for parasitological investigations. For the present study, we sampled amphibians from different taxonomical (anurans from two families and newts), ontogenic (larval and post-metamorphic) and ecological (terrestrial, semi-aquatic, aquatic) groups. The aims of this study were: (1) to provide basic information on helminth infracommunities in Latvian amphibians (such as species lists and standard parasitological parameters); (2) to compare helminth communities between hosts from various taxonomical, ecological and ontogenic groups; (3) to identify species associations; and (4) to find out helminth infection relationships with host ontogeny (stage, size) and waterbody size, which could be related to ecological traits or life cycles of both hosts and parasites.

Materials and methods

Study sites and sampling

Amphibians were collected in June–August of 2017–2020 at 174 sites covering the whole territory of Latvia (fig. 1). We sampled seven hosts from six amphibian taxa: 370 post-metamorphic water frogs (a species complex, *Pelophylax* spp.) from 107 sites (range 1–26; median 2 per site); 90 water frog tadpoles from ten sites (1–70; 2.5); three moor frogs (*Rana arvalis*) from two sites; 26 common frogs (*Rana temporaria*) from seven sites (1–16; 1); 53 common toads (*B. bufo*) from 22 sites (1–18; 1); 249 larval smooth newts (*L. vulgaris*) from 53 sites (1–22; 2.5); and 18 larval great crested newts (*Triturus cristatus*) from 13 sites (1–2; 1). The water frog (*Pelophylax*) species complex was identified only to a generic level, because the species separation in a typical for Latvia mixed *lessonae-esculentus* populations produce many errors when based solely on morphological data (Mayer *et al.*, 2013), but some other study showed a lack of substantial effect of water frog genetics on helminth communities (Popiolek *et al.*, 2011).

Water frogs, their tadpoles and newt larvae were collected by hand net in waterbodies, while terrestrial amphibians (*Rana*, *Bufo* post-metamorphs) were collected by hand. A sampler spent ∼ 20 min at each site, typically collecting all available amphibians, except *T. cristatus*, which is a rare and protected species in Latvia and, therefore, its samples were limited to 1–2 specimens per site, depending on observed abundance. Amphibians were placed into separate plastic boxes with water and aeration holes and transported to the laboratory. Post-metamorphic amphibians were measured and weighed. Their size ranges covered the variation in the whole population: *Pelophylax* spp. 2.1–11.1 (median 5.4); *R. temporaria* 1.8–9.2 (5.2); *R. arvalis* 2.9–6.4 (5.3); and *B. bufo* 2.0–10.1 (6.8) cm.

Helminth investigations

Parasitological investigations of collected animals were carried out during the 24 h after samples were delivered to the laboratory (Daugavpils University Ethical committee decision no. 26/2). The animals were euthanized in accordance with Directive 2010/63/EU of the European Parliament on the protection of animals used for scientific purposes and according to the guidelines of the Federation of European Laboratory Animal Science Associations (FELASA) (Guillen, 2012), by a percussive blow to the head under the supervision of a FELASA Category C certified specialist. A full standard parasitological investigation of the euthanized animals was carried out (Skryabin, 1928; Justine *et al.*, 2012), including examination of skin that was peeled off and rinsed in distilled water and all internal organs, body cavity, visceral membranes and limb musculature that were dissected, compressed between two slides and examined with the aid of microscopy. Encapsulated larval stages were released from surrounding tissues and analysed at ×100–400 magnification. A total of 841 host specimens were analysed. Helminth identification was based solely on their morphology. They were identified,
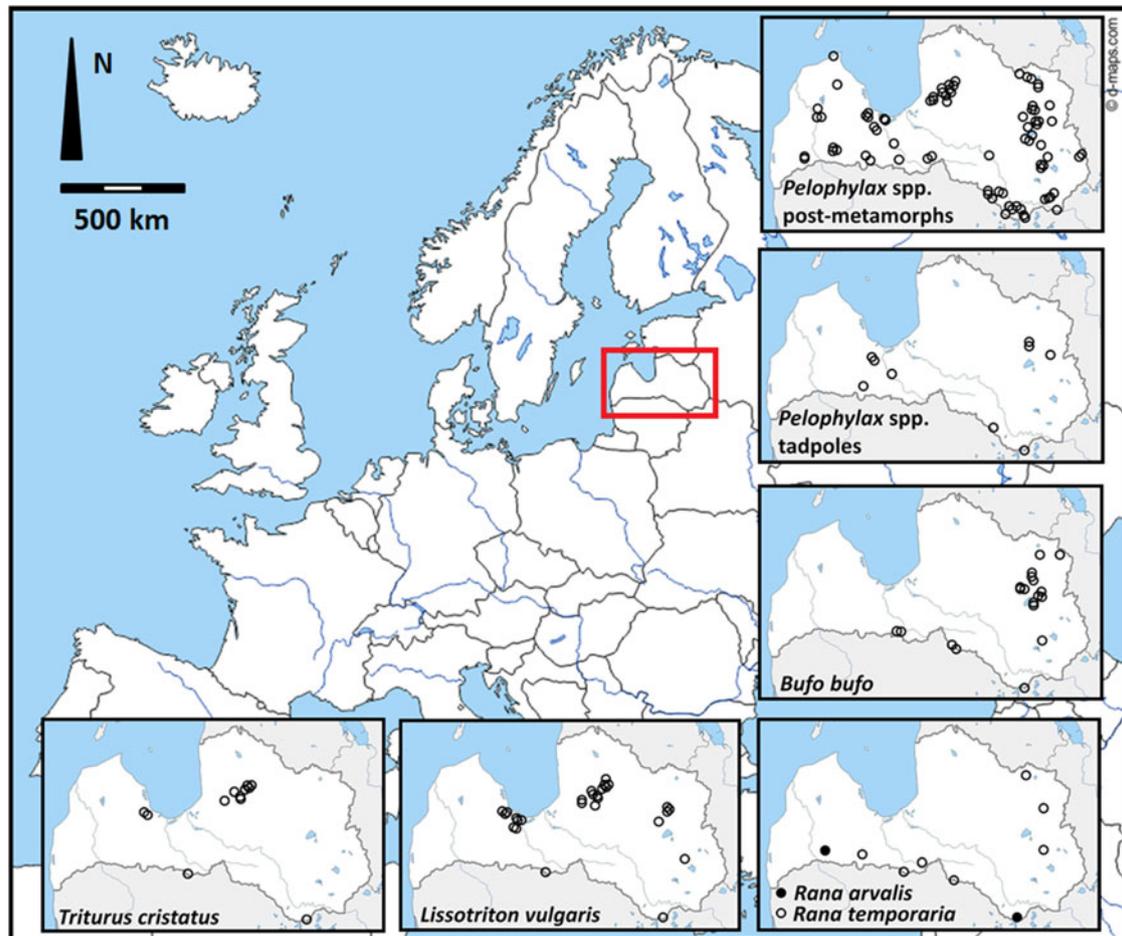
Fig. 1. Location of sampled sites.

mainly to species level, with the aid of essential references containing taxonomic keys and species descriptions (e.g. Ryzhikov et al., 1980; Sudarikov et al., 2002; Jones et al., 2005).

Data analyses

The role of each helminth taxon in the community was described by a standard set of parameters used in parasitology (Bush et al., 1997), such as abundance (A), prevalence (P) and infection intensity (I). For host-specific infracommunity descriptions, we used species richness (S), natural logarithm Shannon–Wiener diversity index (H′) and Pielou evenness (J′) (Shannon & Weaver, 1949; Pielou, 1966). We chose the Sorensen index (Magurran, 2004) for species composition (or qualitative) comparison due to the small relative biases caused by undersampling (Chao et al., 2006), and the Morisita–Horn index (Horn, 1966) for comparisons of helminth proportions in infracommunities (or quantitative comparison), due to its suitability for surveys with unequal sampling (Chao et al., 2006). We performed Kendall's rank correlation (KR) to identify helminth associations in hosts. In each helminth species, we also estimated the host-specific dominance index, which was calculated as the abundance ratio to the abundance of the most numerous species (Poulin et al., 2008). We performed linear discriminant analysis (LDA) to dimensionally reduce infracommunity data and visualize their placement along canonical axes. In the LDA, we used a data set with samples

containing at least two parasite species (sample sizes: Pelophylax spp. post-metamorphs 203; Pelophylax spp. tadpoles 32; B. bufo 44; R. temporaria 15; R. arvalis 2; L. vulgaris 32; T. cristatus 2).

We performed zero-inflated Poisson regression (ZIP) and zero-inflated negative binomial regression (ZINB) with a constant inflation option to detect statistically significant helminth infection relationships with host size or waterbody area. While both these regression types are used to deal with data sets with excessive zeroes, ZINB is generally a recommended option for overdispersed data distributions (Yang et al., 2017), which was typical for our helminth counts. However, ZIP models may actually perform better on empirical overdispersed count data (Zell et al., 2019) and, therefore, we used both ZIP and ZINB in our analyses. The independent unit in our parasite–host size analyses was a sample, but in the parasite–waterbody size analyses it was a site. We pooled data from both hosts' sexes because their separate analyses produced basically the same results but strongly reduced data pool, and several studies have confirmed the absence of effect of sex on helminth prevalence and intensities in given host taxa (Andreas, 2006; Abdel-Gaber et al., 2017; Ozoliņa et al., 2021).

In our parasite response to host size analysis, we created a series of host and parasite taxon-specific data sets, where we placed only the samples from sites where a given helminth taxon was detected in a given host. For statistically significant models, we referred to McFadden's pseudo R^2 as a measure of goodness of fit for non-nested models (Menard, 2000) and the z-score as a

measure of data dispersion in the correlations (Sprinthall, 2011) to evaluate which host size parameter – length (cm) or weight (g) – better fitted the data. Populations of the same amphibian species can vary across sites in length–weight response curves. This could be caused by site-specific food resource availability, habitat thermal properties, sex, population genetics, etc. (Duellman & Trueb, 1994) that may blur a parasite–host size relationship when the pooled data from several sites are being used. However, it may cause only Type II errors (showing a poorer relationship than actually occurs), which cannot invalidate the detected overall (or typical) trend in a given parasite–host system.

In the parasite response to waterbody size analysis, we created host-specific data sets where helminth average abundances per site, site average and total species richness were dependent variables and the waterbody area (ha) was a predictor. This analysis was limited to two hosts (*Pelophylax* spp. post-metamorphs and *L. vulgaris* larvae) and to sites with at least three samples per given host present. Here, we also omitted sites where samples were collected in unclosed linear habitats (channels, ditches or riverbanks) that did not allow meaningful area measurements. Waterbody areas were measured on orthophoto maps using the Google Earth Pro software (Google LLC, Mountain View, California, USA) and ranged from 0.01 to 16.92 (median 0.09) ha.

The possible host size relationship with the waterbody size was tested by the Poisson regression (PR). The correlation was absent (dependent variable: host length (cm); predictor: waterbody area (ha); log likelihood ratio chi-square = 1.25, McFadden's pseudo $R^2 = 0.001$, likelihood ratio chi-square test $P = 0.264$).

LDA was performed on Past 4 (developed by Ø. Hammer, D.A.T. Harper and P.D. Ryan from the Natural History Museum, University of Oslo, Norway). KR, PR, ZIP and ZINB were performed on STATA 14.2 (StataCorp LLC, College Station, Texas, USA) with the Stata Technical Bulletin insertion 'Scalar measures of fit for regression models' (developed by J. Scott Long, Indian University and Jeremy Freese, University of Wisconsin-Madison, USA).

Results

The full results of the parasitological investigation are given in supplementary tables S1–S5. We identified a total of 17 trematode, one monogenean, seven nematode and one acanthocephalan species in our samples (table 1). The richest were the infracommunities in post-metamorphic water frogs (*Pelophylax* spp.), which contained 25 species. The poorest were larval *T. cristatus* infracommunities, with only four species, and the most skewed were also communities in amphibian larval stages (*Pelophylax* spp., *L. vulgaris*). Dominant or sub-dominant helminth species in Latvian amphibians were trematodes – *A. alata* (in *R. temporaria*, *Pelophylax* spp.), *Diplodiscus subclavatus* (in *R. arvalis*), *Opisthioglyphe ranae* (in *Pelophylax* spp., *L. vulgaris*) and *Tylodelphys excavata* (in *Pelophylax* spp.), and nematodes – *Hedruris androphora* (in *T. cristatus*), *Neoraillietnema praeputiale*, *Oswaldocruzia filiformis* and *Rhabdias bufonis* (all in *B. bufo*, *R. temporaria*) (table 2).

Helminth infracommunity similarities depended on both host taxonomic closeness and common habitat (table 3). A terrestrial Ranidae frog (*R. temporaria*) yielded infracommunities that quantitatively (in species proportions) were more similar to those of a terrestrial Bufonidae toad (*B. bufo*), while qualitatively (in species composition alone) they had about equal similarities to both a Bufonidae toad from the same habitat and a Ranidae frog (*Pelophylax* spp.) from a different habitat. In semi-aquatic

Ranidae frog (*Pelophylax* spp.) post-metamorphs, the helminth species composition was more similar to that in the terrestrial Ranidae post-metamorphs, while quantitatively their communities were more similar to those in their own larval stages. In amphibian larval stages, infracommunities in the first newt species (*L. vulgaris*) were more similar to those in frog tadpoles than those in the other newt species in both qualitative and quantitative indices, while the opposite was true in the second newt species (*T. cristatus*).

In the LDA, which we used to discriminate infracommunities from various hosts, the first two canonical axes explained 92.4% of the total variation (fig. 2). The first axis (eigenvalue 1.82, 78.6% of variation) alone was the main discriminant function, which arranged hosts along the aquatic–terrestrial habitat gradient, with the highest positive loadings being from dominant terrestrial nematodes, *R. bufonis* (0.243) and *N. praeputiale* (0.159), but negative from a rare trematode (*Diplostomum spathacerum* −0.332), an acantocephalan (−0.180), a monogenean (−0.374) and aquatic nematodes (*H. androphora* −0.287; *Gyrinicola tba* −0.235; *Heligmosomoides polygyrus* −0.156; *Cosmocera ornata* −0.146). All trematode species with a dominance index >0.1 and the dominant nematode, *O. filiformis*, had loadings within the interval between −0.05 and +0.05. The second axis was far less important (eigenvalue 0.32, 13.8% of variation), and separated hosts within the aquatic ecosystem, with the highest positive loading coming from the nematode *H. androphora* (4.373) but negative loadings from some other aquatic nematodes (*G. tba* −0.294; *C. ornata* −0.267), a monogenean (−0.373) and some trematodes (*Haematoloechus variegatus* −0.171; *Skrjabinoeces similis* −0.138). Hence, the LDA analysis separated larval great crested newt (*T. cristatus*) infra-communities from those in other aquatic and semi-aquatic hosts, mainly by the dominance of the aquatic nematode, *H. androphora*, but the dominance of terrestrial nematodes (*R. bufonis*, *N. praeputiale*) distinguished terrestrial amphibian (*B. bufo*, *R. temporaria*) infracommunities.

The Kendall rank correlation matrix for helminth species associations is given in supplementary table S6. All but one (nematode *H. androphora*) helminth species were correlated to the other species (on average, 7.3 associations per species), where their positive associations (present in 96% of species) were far more typical than negative ones (present in 36% of species), and, with the single exception of *Paralepoderma cloacicola*, positive associations were also statistically stronger (fig. 3). The most involved were adult plagiorchiid trematodes, which always had associations with other species from their own guild, diplostomid larval trematodes and an acanthocephalan. Gastrointestinal nematodes showed notable difference in aquatic vs. terrestrial infection route species since aquatic *C. ornata* possessed an association pattern similar to that of the plagiorchiid trematodes, while terrestrial *N. praeputiale* and *O. filiformis* were associated mainly with other terrestrial nematodes. A markedly negative association was found between the lung nematode, *R. bufonis*, and the plagiorchiid larvae guild.

Of the 22 helminth infection–host length models, ZIP performed better in 18 cases, as indicated by the Bayesian information criterion (BIC), but in two cases the models were equal (table 4). In two more cases, ZINB had slightly higher BIC values, but ZINB and ZIP had equal, or the latter had slightly higher, pseudo R^2 values. Hence, ZIP was clearly preferable over ZINB. Both helminth abundances and species richness had positive relationships with the host size. In adult trematode abundances, there was a better relationship with host weight; while in adult trematode species richness, larval trematode and nematode abundances

Table 1. Helminth infracommunities in amphibians from Latvia: abundance (A; average for all samples ± standard deviation), prevalence (P, %), species richness (S; total and average for infected samples ± standard deviation), diversity (H′) and evenness (J′) in major taxonomic groups and life stages.

Host	Bufo bufo	Rana temporaria	Rana arvalis	Pelophylax spp.	Pelophylax spp.	Lissotriton vulgaris	Triturus cristatus
Sample size	53	26	3	370	90	249	18
Taxonomy	Anura – Bufonidae	Anura – Ranidae	Anura – Ranidae	Anura – Ranidae	Anura – Ranidae	Caudata – Salamandridae	Caudata – Salamandridae
Life stage	Post-metamorphic	Post-metamorphic	Post-metamorphic	Post-metamorphic	Larval	Larval	Larval
Habitat	Terrestrial	Terrestrial	Terrestrial	Semiaquatic	Aquatic	Aquatic	Aquatic
Monogenea							
A	0	0	0	0.00 ± 0.05	0	0	0
P, %	0	0	0	<1	0	0	0
S	0	0	0	1	0	0	0
Trematoda, larv							
A	0.04 ± 0.27	2.77 ± 8.04	0.67 ± 1.15	12.98 ± 33.38	6.25 ± 12.47	3.02 ± 6.96	0.67 ± 1.57
P, %	2	19	33	57	71	35	22
S	1	2; 1.0 ± 0.0	1	10; 1.6 ± 0.9	6; 1.5 ± 0.5	4; 1.3 ± 0.5	3; 1.5 ± 0.6
H′	n.e.	0.45	n.e.	1.78	0.79	0.81	0.98
J′	n.e.	0.65	n.e.	0.77	0.44	0.59	0.81
Trematoda, ad							
A	0.15 ± 0.97	0.54 ± 1.68	4.00 ± 6.93	7.87 ± 24.39	0.05 ± 0.31	0.57 ± 3.44	0
P, %	6	15	33	55	3	5	0
S	2; 1.0 ± 0.0	5; 1.5 ± 0.6	1	8; 1.7 ± 0.9	1	3; 1.1 ± 0.3	0
H′	0.35	1.13	n.e.	1.67	n.e.	0.51	n.e.
J′	0.50	0.70	n.e.	0.80	n.e.	0.46	n.e.
Trematoda, total							
A	0.25 ± 1.04	3.46 ± 8.33	4.67 ± 5.25	20.91 ± 45.15	6.36 ± 12.37	3.59 ± 8.03	0.67 ± 1.57
P, %	9	27	67	77	72	37	22
S	3; 1.2 ± 0.4	7; 1.6 ± 0.5	2; 1.0 ± 0.0	17; 2.3 ± 1.4	7; 1.5 ± 0.5	6; 1.3 ± 0.5	3; 1.5 ± 0.6
H′	0.76	1.01	0.41	2.27	0.83	0.86	0.98
J′	0.69	0.52	0.59	0.80	0.43	0.48	0.81
Cestoda[a]							
A	0	0	0	0.02 ± 0.42	0.02 ± 0.21	0	0
P, %	0	0	0	<1	2	0	0

(Continued)

Table 1. (*Continued.*)

Host	Bufo bufo	Rana temporaria	Rana arvalis	Pelophylax spp.	Pelophylax spp.	Lissotriton vulgaris	Triturus cristatus
Nematoda							
A	27.00 ± 29.04	8.00 ± 9.13	3.33 ± 4.16	0.99 ± 3.94	0.09 ± 0.55	0.03 ± 0.20	1.11 ± 2.25
P, %	87	88	67	23	4	2	28
S	5; 2.8 ± 0.6	3; 1.9 ± 0.8	3; 2.0 ± 0.0	5; 1.3 ± 0.5	1	1	1
H′	1.14	1.08	0.94	1.12	n.e.	n.e.	n.e.
J′	0.71	0.98	0.86	0.70	n.e.	n.e.	n.e.
Acantocephala							
A	0.62 ± 3.99	0.08 ± 0.39	0	0.17 ± 0.67	0	0	0
P, %	8	4	0	9	0	0	0
S	1	1	0	1	0	0	0
Total							
A	27.81 ± 31.38	11.53 ± 18.01	8.00 ± 7.21	22.01 ± 45.87	6.41 ± 12.41	3.62 ± 8.05	1.78 ± 2.51
P, %	87	92	67	78	73	39	44
S	9; 3.0 ± 0.8	11; 2.4 ± 1.3	5; 3.0 ± 0.0	25; 2.4 ± 1.8	9; 1.5 ± 0.6	7; 1.3 ± 0.5	4; 1.4 ± 0.7
H′	1.27	1.68	1.31	2.43	0.91	0.90	0.99
J′	0.58	0.73	0.81	0.75	0.42	0.46	0.72
Samples	53	26	3	370	92	249	18
Sites	22	7	2	107	10	53	13

[a]Unidentified taxa. larv, larvae; ad, adults; n.e., not estimable.

Table 2. Dominance index for helminth taxa in post-metamorphic (PM) and larval (L) amphibian hosts.

Taxa	Stage[a]	*B. bufo* PM	*R. temporaria* PM	*R. arvalis* PM	*Pelophylax* spp. PM	*Pelophylax* spp. L	*L. vulgaris* L	*T. cristatus* L
Monogenea								
Polystoma integerrimum	ad	0	0	0	0.001	0	0	0
Trematoda								
Alaria alata	msc	0	**0.652**	0.167	**1.000**	**1.000**	0	0
Diplodiscus subclavatus	ad	0.014	0.022	**1.000**	0.231	0.012	0.006	0
Diplostomum spathacerum	mtc	0	0	0	0.002	0.019	0	0
Echinoparyphium recurvatum	mtc	0	0	0	0.235	0.005	0.041	0.200
Encyclometra colubrimurorum	mtc	0	0	0	0.041	0.012	0	0
Gorgodera varsoviensis	ad	0	0	0	0.022	0	0	0
Haematoloechus variegatus	ad	0	0	0	0.043	0	0	0
Opisthioglyphe ranae	ad	0	0.011	0	0.308	0	0.232	0
	mtc	0	0	0	**0.607**	0.328	**1.000**	0.350
Paralepoderma cloacicola	mtc	0	0	0	0.117	0.034	0.382	0
Pleurogenes claviger	ad	0.002	0.011	0	0.107	0	0	0
Pleurogenoides medians	ad	0	0	0	0.463	0	0.039	0
Prosotocus confusus	ad	0	0.011	0	0.059	0	0	0
Skrjabinoeces similis	ad	0	0.098	0	0.201	0	0	0
Strigea falconis	mtc	0	0	0	0.043	0	0	0
Strigea sphaerula	mtc	0.004	0.130	0	0.072	0	0.043	0.050
Strigea strigis	mtc	0	0	0	0.044	0	0	0
Tylodelphys excavata	mtc	0	0	0	**0.614**	0	0	0
Cestoda								
Cestoda indet.	ad	0	0	0	0.005	0	0	0
	larv	0	0	0	0	0.005	0	0
Nematoda								
Cosmocera ornata	ad	0.004	0	0	0.034	0	0	0
Gyrinicola tba	ad	0	0	0	0	0.019	0	0
Hedruris androphora	ad	0	0	0	0	0	0.016	**1.000**
Heligmosomoides polygyrus	ad	0.039	0	0	0.010	0	0	0
Neoraillietnema praeputiale	ad	**0.946**	**0.620**	0.417	0.067	0	0	0
Oswaldocruzia filiformis	ad	**1.000**	**1.000**	0.333	0.088	0	0	0
Rhabdias bufonis	ad	**0.568**	**0.717**	0.083	0.001	0	0	0
Acantocephala								
Acantocephalus ranae	ad	0.059	0.022	0	0.038	0	0	0

[a]msc – mesocercariae, mtc – metacercariae, larv – larvae, ad – adults. Index values >0.5 in bold.

Table 3. Infracommunity comparisons between hosts by Sorensen index for qualitative (upper-right section) and Morisita–Horn index for quantitative (lower-left section) similarities (*R. arvalis* skipped due to small sample size).

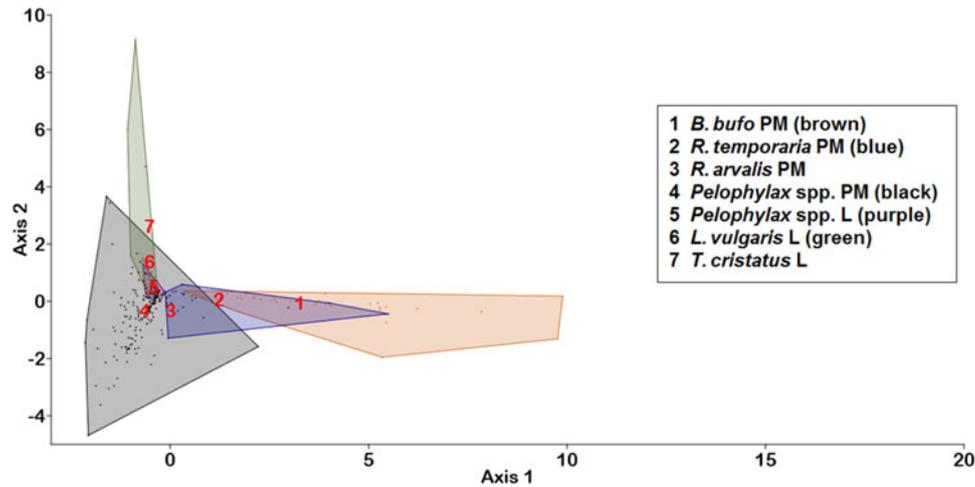|	*B. bufo* PM	*R. temporaria* PM	*Pelophylax* spp. PM	*Pelophylax* spp. L	*L. vulgaris* L	*T. cristatus* L
B. bufo PM	x	0.667	0.500	0.111	0.250	0.154
R. temporaria PM	0.856	x	0.623	0.300	0.333	0.267
Pelophylax spp. PM	0.059	0.323	x	0.483	0.444	0.250
Pelophylax spp. L	<0.001	0.365	0.496	x	0.500	0.308
L. vulgaris L	<0.001	0.005	0.249	0.292	x	0.727
T. cristatus L	<0.001	0.004	0.073	0.102	0.316	x

Fig. 2. LDA chart for helminth infracommunities in post-metamorphic (PM) and larval (L) hosts. Numbers denoting the host taxa are placed on group means; hosts with less than three samples have no convex hulls in the chart. Axis 1 can be interpreted as an aquatic–terrestrial habitat gradient, while axis 2 separates hosts in the aquatic habitat.
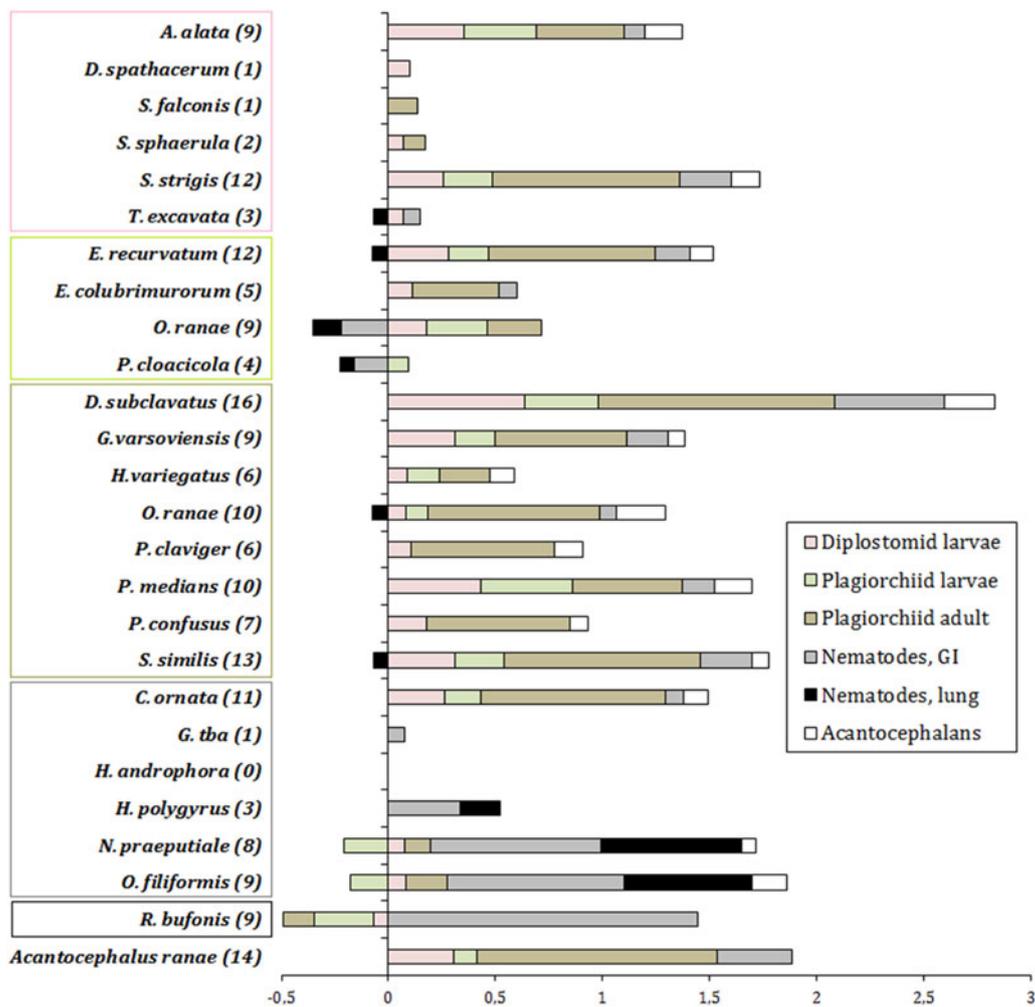

Fig. 3. The strengths of species associations within helminth infra-communities given as the summations of the statistically significant ($P < 0.05$) tau-b statistics in the Kendall's rank correlation matrix (number of associations in parentheses).

and species richness relationships were better with host length (table 5). Relationships with host size were also present in six trematode and three nematode species. Larval trematode, *A.* *alata*, and gastrointestinal nematodes *N. praeputiale* and *O. filiformis* infections were better correlated to host length, while adult trematode – *Pleurogenes claviger* – to the host weight. Two

Table 4. Summary statistics for zero-inflated Poisson regression (ZIP) and zero-inflated negative binomial regression (ZINB) models for helminth abundance (A) or species richness (S) relationships with host length and in post-metamorphic amphibian hosts.

Dependent variable	ZIP				ZINB			
	logLIK	McPsR^2	BIC	P	logLIK	McPsR^2	BIC	P
***Pelophylax spp.* host**								
A *A. alata*, msc	93.4	0.024	3096.3	0.0000	5.6	0.008	−60.6	0.0178
A *E. recurvatum*, mtc	21.8	0.033	36.7	0.0000	2.8	0.006	−135.1	0.0970
A *P. claviger*, ad	121.9	0.411	−120.3	0.0000	4.2	0.029	−148.7	0.0390
A *P. medians*, ad	16.3	0.010	1224.1	0.0001	0.7	0.002	−36.8	0.3921
A *S. strigis*, mtc	83.4	0.044	1144.8	0.0000	0.1	0.000	−213.2	0.7640
A *T. excavata*, mtc	335.0	0.129	1513.0	0.0000	6.9	0.012	−192.1	0.0088
A Trematoda, larv	100.4	0.010	8113.7	0.0000	4.2	0.002	−23.2	0.0402
A Trematoda, ad	79.3	0.012	4286.7	0.0000	16.9	0.010	−317.8	0.0000
S Trematoda, larvae	13.3	0.015	−1172.0	0.0003	12.4	0.014	−1166.2	0.0000
S Trematoda, ad	30.2	0.033	−1186.6	0.0000	30.8	0.033	−1186.6	0.0000
S Trematoda, total	38.0	0.030	−853.6	0.0000	36.2	0.029	−873.6	0.0000
A *N. praeputiale*, ad	43.1	0.149	−123.0	0.0000	9.7	0.034	−129.0	0.0045
A *O. filiformis*, ad	39.0	0.115	−134.7	0.0000	4.4	0.018	−194.6	0.0367
A Gi Nematoda[a]	159.6	0.165	−46.2	0.0000	19.0	0.032	−269.6	0.0000
S Nematoda	16.0	0.033	−1594.2	0.0001	16.0	0.033	−1583.3	0.0001
***Bufo bufo* host**								
A *N. praeputiale*, ad	22.2	0.043	325.8	0.0000	4.0	0.013	152.8	0.0446
A *O. filiformis*, ad	101.0	0.117	594.6	0.0000	11.7	0.036	151.7	0.0006
A *R. bufonis*, ad	12.6	0.032	188.4	0.0004	9.9	0.033	96.0	0.0016
A Gi Nematoda[a]	119.1	0.106	806.9	0.0000	15.0	0.035	212.8	0.0001
S Nematoda	10.5	0.059	−32.5	0.0012	5.0	0.059	−32.5	0.0012
***Rana temporaria* host**								
A *O. filiformis*, ad	50.8	0.279	60.8	0.0000	8.8	0.076	39.5	0.0030
A Gi Nematoda*	69.6	0.311	81.2	0.0000	16.8	0.119	56.2	0.0000

[a]Gastrointestinal nematodes, all nematode species of our study except *R. bufonis*. logLIK, log likelihood ratio chi-square; McPsR^2, McFadden's pseudo R^2; BIC, Bayesian information criterion; P, likelihood ratio chi-square test.

other trematode species (*Echinoparyphium recurvatum*, *P. medians*) and the lung nematode, *R. bufonis*, had a similar goodness of fit in both length and weight models. Two larval trematode species (*Strigea strigis*, *T. excavata*) had a negative relationship with host size.

Of eight helminth infection–waterbody size models, ZIP was better in seven cases, but in one case BIC was slightly higher in ZINB (table 6). Species richness was not related to waterbody area, but a positive relationship was found in pooled adult trematode abundance and in two separate adult trematode species (*P. claviger*, *P. medians*). A negative correlation with waterbody size was found in pooled larval trematode abundance in two hosts, in two separate larval trematode species (*A. alata*, *O. ranae*) and in the nematode, *O. filiformis*.

Discussion

The results of our study fit general patterns with semi-aquatic amphibians yielding the richest helminth communities

(Aho, 1990), nematodes dominating in terrestrial amphibian taxa and trematodes in semi-aquatic taxa (Hamann *et al.*, 2013b; Okulewicz *et al.*, 2014; Burakova & Vershinin, 2016). The water frogs (*Pelophylax* spp.) at our study sites were the core hosts, with rich trematode communities of both larval and adult stages with diverse life cycles and ecological connections (fig. 4). Nematode species richness was similar in several hosts, but their loads peaked in terrestrial *B. bufo*. Amphibian larval stages basically yielded a depauperated community of semi-aquatic frogs, supplemented by some rare nematode species (*G. tba*, *H. androphora*). The admission of *H. androphora* separated larval newt, especially *T. cristatus*, communities from other aquatic and semi-aquatic hosts. It is noteworthy that the post-metamorphic newts from the neighbouring Belarus had infracommunities very similar to those of *Pelophylax* spp. frogs in our study (Shimalov *et al.*, 2001), while they were very different in more distant Germany (Sinsch *et al.*, 2019). There were two dominant trematode species in Latvia – *A. alata* and *O. ranae* – that infected both larval and post-metamorphic amphibian stages. Unlike *O. ranae*,

Table 5. Comparison of goodness of fit and dispersion in zero-inflated Poisson regression (ZIP) models for helminth abundances (A) or species richness (S) relationships with host length vs. host weight.

Dependent variable	Host	Samples (non-zero observations)	Length		Weight	
			McPsR^2	z [pr]	McPsR^2	z [pr]
Trematoda						
A *A. alata*, msc	Pkl	153 (65)	0.024	9.51	0.004	3.92
A *E. recurvatum*, mtc	Pkl	125 (47)	0.033	4.62	0.034	4.75
A *P. claviger*, ad	Pkl	77 (15)	0.411	9.99	0.043	13.13
A *P. medians*, ad	Pkl	87 (32)	0.010	4.12	0.010	4.26
A *S. strigis*, mtc	Pkl	138 (40)	0.044	−8.86	0.005	−3.14
A *T. excavata*, mtc	Pkl	154 (54)	0.129	−16.84	0.131	−15.22
A, larvae only	Pkl	360 (203)	0.010	10.02	0.003	6.07
A, ad only	Pkl	360 (197)	0.012	8.97	0.024	13.50
S, larvae only	Pkl	360 (203)	0.015	3.62	0.008	2.65
S, ad only	Pkl	360 (197)	0.033	5.78	0.022	5.02
S, total	Pkl	360 (275)	0.030	6.38	0.017	4.82
Nematoda						
A *N. praeputiale*, ad	Pkl	85 (31)	0.149	6.89	0.074	5.31
	BB	53 (43)	0.043	4.62	0.030	3.99
A *O. filiformis*, ad	Pkl	98 (31)	0.115	6.21	0.032	3.57
	RT	26 (16)	0.279	6.49	0.222	6.37
	BB	53 (43)	0.117	9.64	0.069	7.96
A *R. bufonis*, ad	BB	53 (41)	0.032	3.28	0.031	3.54
A, gastrointestinal[a]	Pkl	169 (81)	0.165	13.27	0.118	12.54
	RT	26 (21)	0.311	7.60	0.237	7.37
	BB	53 (46)	0.106	10.56	0.078	9.63
S, total	Pkl	360 (81)	0.033	4.05	0.014	2.80
	BB	53 (46)	0.059	3.34	0.028	2.66

Only statistically significant relationships given. Hosts: Pkl, *Pelophylax* species complex; RT, *Rana temporaria*; BB, *Bufo bufo*; McPsR^2, McFadden's pseudo R^2; z [pr], *z*-score for the predictor.
[a]Pooled for all the species except *R. bufonis*; likelihood ratio chi-square test $P < 0.01$ in all the models.

A. alata had a statistically significant positive relationship with host size that may look like a steady accumulation over host ontogeny. However, plotting of prevalence and infection loads against ontogenetic stage and host size classes showed a sharp decline in prevalence in the first post-metamorphic size (fig. 5), which could be caused by the elevated mortality of infected tadpoles around the metamorphosis stage. In larval *O. ranae* infections, such a decline was absent.

Opisthioglyphe ranae was also a dominant or common species in studies in Germany (Andreas, 2006), Poland (Okulewicz *et al.*, 2014), Ukraine (Kuzmin *et al.*, 2020), Serbia (Bjelić-Čabrilo *et al.*, 2009), Russia's Volga River (Chikhlyaev *et al.*, 2018a) and the Ural region (Vershinin *et al.*, 2017), where *A. alata* was absent or rare. The lack of *A. alata* at many of these sites could be explained by habitat type since our survey showed its preference for smaller waterbodies, but waterbodies were large, or their type was not specified in the studies above. One other study showed *A. alata* domination in frogs in some smaller pools behind beaver dams (Chikhlyaev & Ruchin, 2020). *Alaria alata* preferences toward particular amphibian host species may also

vary across sites. While its abundance and prevalence were higher in *Pelophylax* frogs than in *Rana* (represented mainly by *R. temporaria*) frogs in our study, the opposite was true for France (Patrelle *et al.*, 2015) and Germany (Andreas, 2006). In Russia, the highest loads have been registered in *R. arvalis*, while *Pelophylax* spp. and *R. temporaria* were much less infected (Chikhlyaev & Ruchin, 2020). *Alaria alata* may be present at low loads in the common toad (*B. bufo*), while the natterjack toad (*Epidalea calamita*) that breeds exclusively in small, very shallow pools (Drobenkov, 2015), can be extremely heavily infected, reaching 50% prevalence and loaded with up to 1600 larvae per single host (Shimalov & Shimalov, 2001).

Trematode infracommunities in post-metamorphic amphibians from Latvia were more variable than those from sub-tropical Argentina (Hamann *et al.*, 2013b): while semi-aquatic *Pelophylax* spp. yielded more species in total (but not on average per host specimen) and had more even (as indicated by Pielou's J′) trematode communities, terrestrial *B. bufo* and *R. temporaria* yielded poorer and typically more skewed communities compared with the Argentinian ones. Nematode-dominated communities in

Table 6. Summary statistics for zero-inflated Poisson regression (ZIP) and zero-inflated negative binomial regression (ZINB) models for helminth abundance relationships with waterbody area in semi-aquatic and aquatic amphibian hosts.

	A. alata, msc	*O. ranae*, mtc	*P. claviger*, ad	*P. medians*, ad	Trematoda, ad	Trematoda, larv	Trematoda, larv	*O. filiformis*, ad
Host	Pkl	Lvulg	Pkl	Pkl	Pkl	Pkl	Lvulg	Pkl
Sites	40	26	40	40	40	40	26	40
Non-zero sites	15	8	7	6	33	34	14	9
ZIP								
logLIK	92.3	34.8	57.9	134.6	250.2	40.5	22.1	23.6
McPsR^2	0.225	0.327	0.582	0.578	0.410	0.054	0.137	0.232
BIC	181.9	−3.2	−95.0	−38.3	222.9	567.3	64.5	−58.4
z [pr]	−7.47	−2.50	9.95	10.39	19.64	−3.28	−3.75	−3.27
P	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
ZINB								
logLIK	5.4	2.5	14.9	4.3	10.7	2.7	0.5	6.8
McPsR^2	0.043	0.042	0.263	0.068	0.047	0.010	0.006	0.122
BIC	−8.2	−13.9	−91.3	−74.4	85.4	133.2	15.7	−84.1
z [pr]	−2.02	−1.62	9.95	1.30	2.34	−1.86	−0.78	−1.85
P	0.0204	0.1115	0.0001	0.0382	0.0011	0.1034	0.4637	0.0092

Pkl, post-metamorphic *Pelophylax* spp.; Lvulg, *Lissotriton vulgaris* larvae; logLIK, log likelihood ratio chi-square; McPsR^2, McFadden's pseudo R^2; BIC, Bayesian information criterion; P, likelihood ratio chi-square test; z [pr], z-score for the predictor.

terrestrial anurans from Brazilian rainforest had lower species richness and were more variable in diversity and evenness than those from Latvia (Toledo *et al.*, 2017). Most of the helminth species of our study were involved in associations with other helminths. Dominant species typically had many associations, with the only exceptions being *T. excavata* and *H. androphora*. Latvian infracommunities yielded much more associations per parasite species (7.3 on average) than those in Argentina (0.1–0.3 on average), where negative associations were equal to or more frequent than positive ones (Hamann *et al.*, 2006b, 2010, 2013a). In Latvia, positive associations dominated, which is consistent with the data from small mammals (Dallas *et al.*, 2019), suggesting that this could be a more typical pattern in small tetrapod communities. Negative associations between soil-transmitted nematodes *O. filiformis*, *N. praeputiale*, *R. bufonis* and many trematodes (especially larval *O. ranae* and *P. cloacicola*) of our study could be explained by terrestrial vs. aquatic infection routes. However, in trematodes, we did not find evidence for the intraguild association dominance over inter-guild ones and causes for species associations in most cases are unclear and require further study.

Helminth infracommunity structure could be a good indicator of its host's habitat and feeding habit preferences, showing differences in a microhabitat or food object selection not detected by their ecology studies. In our study, a terrestrial frog, *R. temporaria*, yielded a transitional community between that of a semi-aquatic frog (*Pelophylax* spp.) and a terrestrial toad (*B. bufo*), as indicated by its depauperate trematode infracommunities and intermediate nematode loads (fig. 4). *Rana temporaria* is an explosive breeder, present in waterbodies only in early spring, to a lesser extent than *B. bufo* (Čeirāns *et al.*, 2020), but it has more water-permeable skin (Bentley & Yorio, 1976) and may move to shoreline habitats

during the summertime (Vences *et al.*, 2000), where trematodes may ascend with occasional aquatic prey. The other *Rana* species in our study – *R. arvalis* – had only three samples but they were in line with other studies (Andreas, 2006; Okulewicz *et al.*, 2014; Vershinin *et al.*, 2017; Chikhlyaev & Ruchin, 2020) that showed trematode infection rates in *R. arvalis* being higher than those of *R. temporaria*. Together, this suggests an increase of terrestriality in the following order: *Pelophylax* spp. – *R. arvalis* – *R. temporaria* – *B. bufo*, whereas the last three hosts are usually regarded as equally terrestrial in their ecology accounts (e.g. Arnold & Ovenden, 2002; Speybroeck *et al.*, 2016).

The nematode *R. bufonis* was virtually absent in *Pelophylax* spp. frogs, but it was frequent in *R. temporaria* frogs, and especially in *B. bufo* toads. *Rhabdias bufonis* differs from other nematodes of our study in being a lung parasite, which invades the amphibian host through skin penetration by the larvae produced by free-living generation in soil, where they arise from hosts' faeces (Spieler & Schierenberg, 1995). Its free-living form has an extremely short life-span (Gems, 2002), and it is highly unlikely that terrestrial amphibians could attain the 50% prevalence observed in this nematode (Okulewicz *et al.*, 2014) from contact with random soil. Since amphibians prefer shelters with moist soil (Cohen & Alford, 1996), which facilitate nematode survival (Coleman & Wall, 2015), we suggest that the host will be infested mainly in regularly used terrestrial shelters where they defecate, but host-specific *R. bufonis* infection rates could be caused by differences in host fidelities in the use of such shelters.

The higher overall infestation of larger hosts in our study was in line with other studies (Andreas, 2006; Abdel-Gaber *et al.*, 2017; Toledo *et al.*, 2017; Kuzmin *et al.*, 2020) and could be caused either by higher parasite intake rates by larger hosts or by the accumulation of parasites with host age (Poulin, 2007).
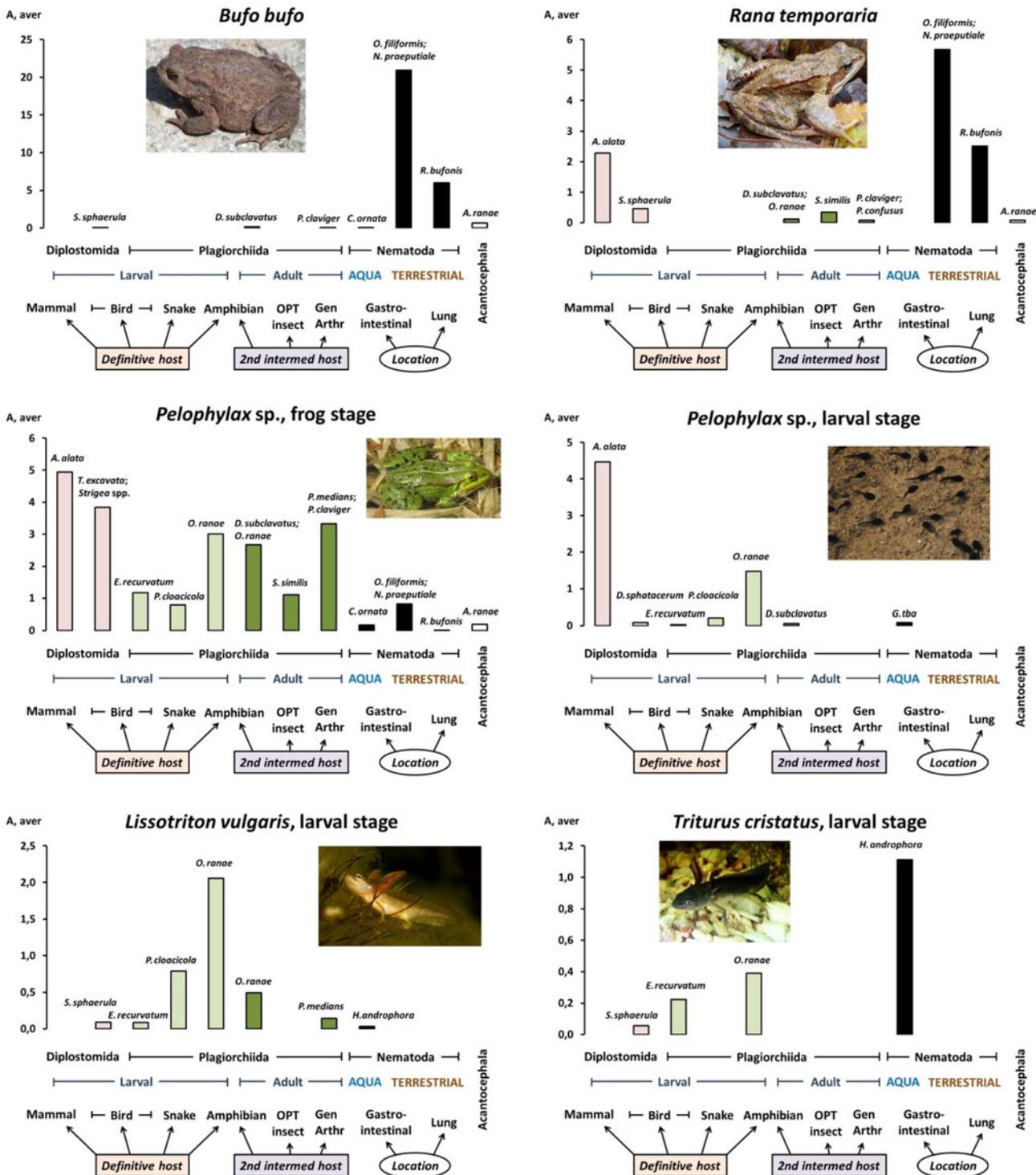
Fig. 4. Structure of helminth infracommunities in amphibian hosts from Latvia (*R. arvalis* omitted due to small sample size). Typical helminth species from each unit given on top of their columns. x-axis: first row = taxonomic group; second row = life stage in amphibians (trematodes; from aquatic habitat) or parasite larval habitat (nematodes); third row = other hosts (trematodes) or location in hosts (nematodes). Abbreviations: AQUA, aquatic; OPT, Odonata, Plecoptera, Ephemeroptera, Trichoptera; Gen arthr, wide range of arthropod hosts.

Our data suggest the presence of both mechanisms in the studied infracommunities: in intermediate-host generalist plagiorchiid trematodes, *P. claviger* and *P. medians*, which have a short-living adult stage in frogs (Sudarikov *et al.*, 2002), higher infection rates in larger frogs could be attained by an increase of prey intake volumes over the ontogeny, while in *A. alata* mesocercaria, a similar trend could be caused by a larger frog's ability to consume infected prey – for example, tadpoles (Pearson, 1956) – and subsequent accumulation of a long-living parasite stage (Möhl *et al.*, 2009).
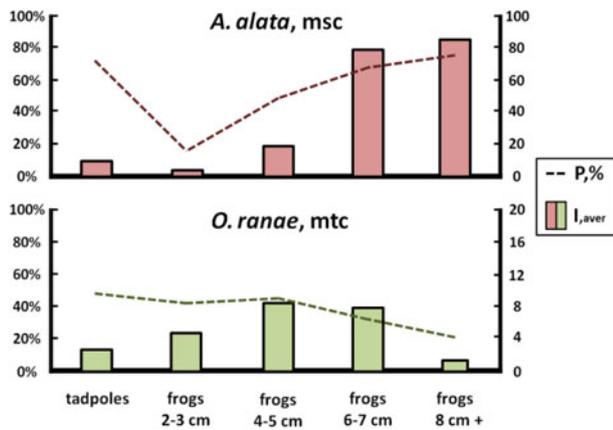
Fig. 5. Prevalence (P, %; dashed line) and infection intensities (I, aver; columns) in two dominant trematode species larval stages over water frog (*Pelophylax* spp.) host ontogeny. x-axis = ontogenic stage and post-metamorphs' size class; left y-axis = prevalence; right y-axis = infection intensity (data from two *A. alata*-infected and five *O. ranae*-infected sites). Abbreviations: msc, mesocercariae; mtc, metacercariae; aver, average.

In amphibians, disease or infection is usually accompanied by emaciation and weight loss (Bancila *et al.*, 2010). Diplostomid trematode (*A. alata*, *S. strigis*) larvae and gastrointestinal nematode infections were better correlated with host length than with weight, probably due to their adverse effect on host body condition (Hendrikx & Van Moppes, 1983; Koprivnikar *et al.*, 2012; Svinin *et al.*, 2020) and accompanying weight loss that increased data dispersion. A similar pattern was also observed in trematode species richness. Adult trematode infections, however, were better correlated with host weight. This could be explained by higher parasite intake rates with food by active and healthy hosts, and lower pathogenicity of the adult stages (Koprivnikar *et al.*, 2012) that did not significantly affect host body conditions.

Post-metamorphic amphibians are considered to have a generally low susceptibility to trematode cercaria infections compared to tadpoles (Koprivnikar *et al.*, 2012). However, with few exceptions (*A. alata*, *D. spathacerum*, *O. ranae*), larval trematodes in our study were less abundant in tadpoles, several diplostomid species were missing and larval trematodes, as a group, increased in larger hosts in both species richness and sheer abundances, indicating a substantial intake by post-metamorphic frogs. Their intake pathways were not always clear. Thus, we found a trend towards infection increase over host ontogeny in the echinostome, *E. recurvatum* metacercaria. Although echinostome infections are well studied in larval amphibians (e.g. Holland, 2009; Orlofske *et al.*, 2013; Goren *et al.*, 2014), this particular species was extremely rare in our tadpole samples and we did not find a description of its infection routes into post-metamorphic frogs. The typical second intermediate host for this species are regarded to be snails and not amphibians (Huffman & Fried, 2012). Larvae predilection sites in frogs in our study (fig. 6) may give some clues – for example, *E. recurvatum* metacercaria were located mainly around the eyes, which suggests that the main penetration route for the cercaria could be through the eye mucosa, while a significant proportion of metacercaria of some other taxa (*Encyclometra colubrimurorum*, *P. cloacicola*, *Strigea* spp.) were located in the walls of the mouth cavity and gastrointestinal tract. The absence of substantial infections of internal organs and better infection rate correlation with host weight in *E. recurvatum* suggests that this echinostome may have little effect on its host's health.

Opposite trends with lower infection rates in larger hosts were found in several diplostomid (*S. strigis*, *T. exavata*) metacercaria infections. There is no good evidence for self-clearance from well-established diplostomid infections in post-metamorphic amphibians (e.g. Rohr *et al.*, 2009; Raffel *et al.*, 2011; Poulin & Lagrue, 2015), yet latter larval stages may eliminate plagiorchiid metacercaria due to some unknown mechanism (Holland, 2009). Strigeid infections may cause amphibian population decline (Sinsch *et al.*, 2018), and lower infection rates in both diplostomids in larger frogs could be explained by a parasite-induced host mortality (Loot *et al.*, 2001). In our study, most *T. excavata* metacercaria were found in the brain and spinal cord (supplementary table S2), which may inhibit the host's physical reaction to threats and facilitate *T. excavata* transmission to its definitive host – the white stork (*Ciconia ciconia*) (Sitko *et al.*, 2006; Girisgin *et al.*, 2017) – in that way, fitting the optimal virulence strategy (Cressler *et al.*, 2016).

Waterbody size did affect helminth abundances but not their species richness in our study. The nematode *O. filiformis* is also common in sand lizards (*Lacerta agilis*) from dry terrestrial habitats (Kirillova *et al.*, 2020). It infects hosts through ingestion of its larvae with terrestrial food (Chikhlyaev *et al.*, 2019b) and its higher infection rates in frogs from smaller waterbodies could be caused by higher proportions of terrestrial prey items in such habitats (relevant studies on waterbody-size-specificity of their diets are absent). Trematode ontogenetic stages had opposite trends to waterbody size. Overall adult trematode infections and also infections of adults of two plagiorchiid species (*P. claviger*, *P. medians*) were higher in larger waterbodies, which could be caused by richer arthropod food resources (Heino, 2009) and possibly higher intake rates of occasional aquatic prey by mainly onshore-feeding *Pelophylax* spp. frogs. Both these plagiorchiids are intermediate-host generalists, having metacercariae in an extremely wide range of aquatic arthropods, with frogs being definitive hosts (Sudarikov *et al.*, 2002). There are hemimetabolous insects (Plecoptera, Ephemeroptera, Odonata), which pass metacercariae into the imago stage (Mariluan *et al.*, 2012) and are potentially better vectors for trematode transfer to onshore-feeding hosts, but their imago stages are rare in *Pelophylax* spp. diets (Tyler, 1958; Balint *et al.*, 2008; Paunovic *et al.*, 2010). Interestingly, infections of several plagiorchiids (*Gorgodera varsoviensis*, *S. similis*), having a second intermediate stage mainly in hemimetabolous Odonata (Sudarikov *et al.*, 2002), had no correlation with the waterbody area in our study.

First intermediate hosts for studied trematode communities are always gastropod snails, from which emerge free-swimming stages aiming to invade the next host (Galaktionov & Dobrovolskij, 2003). In water they may reach a biomass of more than 150 mg per m^3 (Preston *et al.*, 2013). The smallest waterbodies of our study had an area of 0.01 ha, which is the pond size with the highest larval amphibian densities (Semlitsch *et al.*, 2015). Elevated host densities result in increased cercariae and metacercariae prevalence in snails (Zimmermann *et al.*, 2016), and high tadpole and frog densities are known to be positive for trematodes (Hartson *et al.*, 2011). Hence, higher levels of larval trematode infections in amphibians from the smaller waterbodies of our study could be caused by higher host (snail, tadpole or both) densities that increase chances for short-living, free-moving miracidium and cercaria stages to meet their hosts. Trematode, especially diplostomid, larval infections may increase amphibian mortality (Sinsch *et al.*, 2018) and the use of small waterbodies by amphibians could be a trade-off between benefits
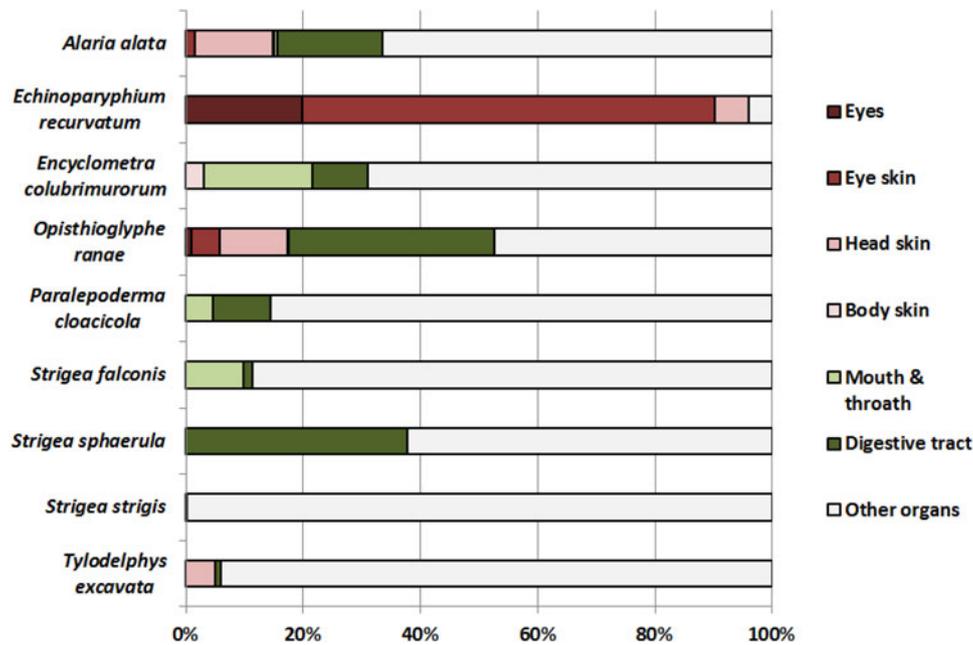
Fig. 6. Percentages of trematode larvae located in subcutaneous tissues and visual organs (shades of red), and in walls of gastrointestinal organs (shades of green) of *Pelophylax* spp. post-metamorphs.

from getting a breeding site free from fish predators that eradicate their larval stages (Hartel *et al.*, 2007; Kloskowski, 2011) and elevated risks of diseases caused by parasite infections.

References

Abdel-Gaber R, Maher S, Kamel R and El Deeb N (2017) Supplementary studies of *Pleurogenoides medians* (Digenea: Lecithodendriidae) infecting the marsh frog *Rana ridibunda* (Amphibia: Ranidae) in Egypt. *Beni-Suef University Journal of Basic and Applied Sciences* **6**, 154–159.

Aho JM (1990) Helminth communities of amphibians and reptiles: comparative approaches to understanding patterns and processes. pp. 157–195 *in* Esch GW, Bush AO and Aho JM (*Eds*) *Parasite communities: patterns and processes.* Dordrecht, Springer.

Andreas K (2006) Helminthen einheimischer Froschlurche. 123 pp. Dissertation, The Free University of Berlin, Berlin, Germany.

Arnold EN and Ovenden DW (2002) *Reptiles and amphibians of Europe.* 288 pp. Princeton and Oxford, Princeton University Press.

Avery RA (1971) Helminth parasite populations in newts and their tadpoles. *Freshwater Biology* **1**, 113–119.

Balint N, Citrea L, Memetea A, Jurij N and Condure N (2008) Feeding ecology of the *Pelophylax ridibundus* (Anura, ranidae) in Dobromir. *Romania. Biharean Biologist* **2**, 27–37.

Bancila RI, Hartel T, Plaiasu R, Smets J and Cogalniceanu D (2010) Comparing three body condition indices in amphibians: a case study of yellowbellied toad Bombina variegata. *Amphibia-Reptilia* **31**, 558–562.

Bentley PJ and Yorio T (1976) The passive permeability of the skin of anuran amphibia: a comparison of frogs (*Rana pipiens*) and toads (*Bufo marinus*). *The Journal of Physiology* **261**, 603–615.

Biggs J, von Fumetti S and Kelly-Quinn M (2016) The importance of small waterbodies for biodiversity and ecosystem services: implications for policy makers. *Hydrobiologia* **793**, 3–39.

Bjelić-Čabrilo O, Popović E and Paunovic A (2009) Helminthofauna of *Pelophylax* kl. *esculentus* (Linne, 1758) from Petrovaradinski Rit marsh (Serbia). *Helminthologia* **46**, 107–111.

Burakova AV and Baytimirova EA (2017) The species composition of parasites of *Bufo bufo* (Amphibia: Bufonidae) in the surrounding area of Visimsky State Nature Biosphere Reserve. *Russian Journal of Parasitology* **42**, 320–324 (in Russian).

Burakova AV and Vershinin VL (2016) Analysis of parasitofauna in syntopically cohabitating representatives of Anura. *Вестник СПбГУ* **3**, 31–36 (in Russian).

Bursey CR and Dewolf WF (1998) Helminths of the frogs, *Rana catesbeiana*, *Rana clamitans*, and *Rana palustris*, from Coshocton county, Ohio. *Ohio Journal of Science* **98**(2), 28–29.

Bush A, Lafferty KD, Lotz JM and Shostak AW (1997) Parasitology meets ecology on its own terms: Margolis *et al.* Revisited. *Journal of Parasitology* **83**, 575–583.

Campião KM, Ribas A, Morais DH, Silva R and Tavares LER (2015) How many parasites species a frog might have? Determinants of parasite diversity in South American anurans. *PLoS One* **10**, e0140577.

Campião KM, Ribas ACA, Silva ICO, Dalazen GT and Tavares LER (2017) Anuran helminth communities from contrasting nature reserve and pasture sites in the Pantanal wetland, Brazil. *Journal of Helminthology* **91**, 91–96.

Čeirāns A, Pupina A and Pupins M (2020) A new method for the estimation of minimum adult frog density from a large-scale audial survey. *Scientific Reports* **10**, 8627.

Chao A, Chazdon RL, Colwell RK and Shen TJ (2006) Abundance-based similarity indices and their estimation when there are unseen species in samples. *Biometrics* **62**, 361–371.

Chikhlyaev IV and Ruchin AB (2014) The helminth fauna study of European common brown frog (*Rana temporaria* Linnaeus, 1758) in the Volga basin. *Acta Parasitologica* **59**, 459–471.

Chikhlyaev IV and Ruchin AB (2020) Helminths of amphibians (Amphibia) in beaver ponds in the central Russia. *Aquaculture, Aquarium, Conservation & Legislation – International Journal of the Bioflux Society* **13**, 3810–3821.

Chikhlyaev IV and Ruchin AB (2021) An overview of the helminths of moor frog *Rana arvalis* Nilsson, 1842 (Amphibia: Anura) in the Volga basin. *Diversity* **13**, 61.

Chikhlyaev IV, Kirillova NY and Kirillov AA (2018a) Ecological analysis of trematodes (Trematoda) of marsh frog *Pelophylax ridibundus* (Ranidae, Anura) from various habitats of the national park "Samarskaya Luka" (Russia). *Nature Conservation Research. Заповедная наука* **3**, 36–50 (in Russian).

Chikhlyaev IV, Ruchin AB and Fayzulin AI (2018b) An overview of the trematodes fauna of pool frog *Pelophylax lessonae* (Camerano, 1882) in the Volga basin, Russia: 1. Adult stages. *Nusantara Bioscience* **10**, 256–262.

Chikhlyaev IV, Ruchin AB and Fayzulin AI (2019a) An overview of the trematodes fauna of the pool frog *Pelophylax lessonae* (Camerano, 1882) in the Volga basin, Russia: 2. Larval stages. *Nusantara Bioscience* **11**, 106–111.

Chikhlyaev IV, Ruchin AB and Fayzulin AI (2019b) Parasitic nematodes of pool frog (*Pelophylax lessonae*) in the Volga basin. *Revista MVZ Cordoba* **24**, 7314–7321.

Chikhlyaev IV, Ruchin AB and Kirillov AA (2020) Ecological analysis of the helminth fauna in *Bufo bufo* (Amphibia: Anura) from various habitats. *Nature Conservation Research. Заповедная наука* **5**, 1–10.

Cohen MP and Alford RA (1996) Factors affecting diurnal shelter use by the cane toad, *Bufo marinus*. *Herpetologica* **52**, 172–181.

Coleman DC and Wall DH (2015) Chapter 5 – soil fauna: occurrence, biodiversity, and roles in ecosystem function. pp. 111–149 in Paul EA (*Ed.*) *Soil microbiology, ecology and biochemistry*. 4th edn. Amsterdam, Elsevier, Academic Press.

Cressler CE, McLeod DV, Rozins C, Van Den Hoogen J and Day T (2016) The adaptive evolution of virulence: a review of theoretical predictions and empirical tests. *Parasitology* **143**, 915–930.

Dallas TA, Laine A-L and Ovaskainen O (2019) Detecting parasite associations within multi-species host and parasite communities. *Proceedings of the Royal Society B, Biological Sciences* **286**, 20191109.

Draghi R, Drago FB, Saibene PE and Agostini MG (2020) Helminth communities from amphibians inhabiting agroecosystems in the Pampean region (Argentina). *Revue Suisse de Zoologie* **127**, 261–274.

Drobenkov S (2015) Reproductive ecology and population dynamics of the natterjack toad, *Epidalea calamita*, in agricultural landscape of Belarus. *Herpetological Facts Journal* **2**, 15–24.

Duellman WE and Trueb L (1994) *Biology of amphibians*. 670 pp. Baltimore, Johns Hopkins University Press.

Galaktionov KV and Dobrovolskij AA (2003) *The biology and evolution of trematodes: an essay on the biology, morphology, life cycles, transmissions, and evolution of digenetic trematodes*. 592 pp. Dordrecht, Springer.

Gems D (2002) Ageing. pp. 801–880 in Lee DL (*Ed.*) *The biology of nematodes*. London, New York, Taylor & Francis.

Girisgin AO, Birlik S, Senlik B and Yildirimhan HS (2017) Intestinal helminths of the white stork (*Ciconia ciconia* Linnaeus 1758) from an inter-route site in Turkey. *Acta Veterinaria Hungarica* **65**, 221–233.

Goren L, Routtu J and Ben-Ami F (2014) Trematode-associated morbidity and mortality of tadpoles in Israel. *Parasitology Research* **113**, 3833–3841.

Guillen J (2012) FELASA guidelines and recommendations. *Journal of the American Association for Laboratory Animal Science* **51**, 311–321.

Hamann MI, González CE and Kehr AI (2006a) Helminth community structure of the oven frog *Leptodactylus latinasus* (Anura, Leptodactylidae) from Corrientes, Argentina. *Acta Parasitologica* **51**, 294–299.

Hamann MI, González CE and Kehr AI (2006b) Species affinity and infracommunity ordination of helminths of *Leptodactylus chaquensis* (Anura: Leptodactylidae) in two contrasting environments from northeastern Argentina. *Journal of Parasitology* **92**, 1171–1179.

Hamann MI, Kehr AI and González CE (2010) Helminth community structure of *Scinax nasicus* (Anura: Hylidae) from a south American subtropical area. *Diseases of Aquatic Organisms* **93**, 71–82.

Hamann MI, Kehr AI and González CE (2013a) Helminth communities in the burrowing toad, *Rhinella fernandezae*, from northeastern Argentina. *Biologia* **68**, 1155–1162.

Hamann MI, Kehr AI and González CE (2013b) Biodiversity of trematodes associated with amphibians from a variety of habitats in Corrientes province, Argentina. *Journal of Helminthology* **87**, 286–300.

Hamann MI, Kehr AI and González CE (2014) Helminth community structure in the Argentinean bufonid *Melanophryniscus klappenbachi*: importance of habitat use and season. *Parasitology Research* **113**, 3639–3649.

Hamann MI, González CE and Fernández MV (2020) Trematode parasites associated with amphibians from a rice field in the northeastern Argentina. *Food Webs* **22**, e00139.

Hartel T, Nemes S, Cogălniceanu D, Öllerer K, Schweiger O, Moga CI and Demeter L (2007) The effect of fish and aquatic habitat complexity on amphibians. *Hydrobiologia* **583**, 173–182.

Hartson RB, Orlofske SA, Melin VE, Dillon RT and Johnson PTJ (2011) Land use and wetland spatial position jointly determine amphibian parasite communities. *EcoHealth* **8**, 485–500.

Heino L (2009) Biodiversity of aquatic insects: spatial gradients and environmental correlates of assemblage-level measures at large scales. *Freshwater Reviews* **2**, 1–29.

Hendrikx WML and Van Moppes MC (1983) *Oswaldocruzia filiformis* (Nematoda: Trichostrongylidae): morphology of developmental stages, parasitic development and some pathological aspects of the infection in amphibians. *Parasitology Research* **69**, 523–537.

Holland MP (2009) Echinostome metacercariae cyst elimination in *Rana clamitans* (green frog) tadpoles is age-dependent. *Journal of Parasitology* **95**, 281–285.

Horn HS (1966) Measurement of "overlap" in comparative ecological studies. *The American Naturalist* **100**, 419–424.

Huffman J and Fried B (2012) The biology of *Echinoparyphium* (Trematoda, Echinostomatidae). *Acta Parasitologica* **57**, 199–210.

Johnson PTJ and Wilber MQ (2017) Biological and statistical processes jointly drive population aggregation: using host–parasite interactions to understand Taylor's power law. *Proceedings of the Royal Society B, Biological Sciences* **284**, 20171388.

Jones A, Bray RA and Gibson DI (Eds) (2005) *Keys to the Trematoda*. **Volume II**. 768 pp. London, The Natural History Museum, Commonwealth Agricultural Bureau International (CABI Publishing).

Justine J-L, Briand MJ and Bray RA (2012) A quick and simple method, usable in the field, for collecting parasites in suitable condition for both morphological and molecular studies. *Parasitology Research* **111**, 341–351.

King KC, McLaughlin JD, Gendron AD, Pauli BD, Giroux I, Rondeau B, Boily M, Juneau P and Marcogliese DJ (2007) Impacts of agriculture on the parasite communities of northern leopard frogs (*Rana pipiens*) in southern Quebec, Canada. *Parasitology* **134**, 2063–2080.

Kirillova NY, Kirillov AA, Shchenkov SV and Chikhlyaev IV (2020) *Oswaldocruzia filiformis* sensu lato (Nematoda: Molineidae) from amphibians and reptiles in European Russia: morphological and molecular data. *Nature Conservation Research. Заповедная наука* **5**, 41–56.

Kloskowski J (2011) Consequences of the size structure of fish populations for their effects on a generalist avian predator. *Oecologia* **166**, 517–530.

Koprivnikar J and Redfern JC (2012) Agricultural effects on amphibian parasitism: importance of general habitat preturbations and parasite life cycles. *Journal of Wildlife Diseases* **48**, 925–936.

Koprivnikar J, Baker RL and Forbes MR (2006) Environmental factors influencing trematode prevalence in grey tree frog (*Hyla versicolor*) tadpoles in southern Ontario. *Journal of Parasitology* **92**, 997–1001.

Koprivnikar J, Marcogliese DJ, Rohr JR, Orlofske SA, Raffel TR and Johnson PTJ (2012) Macroparasite infections of amphibians: what can they tell us? *EcoHealth* **9**, 342–360.

Kuzmin Y, Dmytrieva I, Marushchak O, Morozov-Leonov S, Oskyrko O and Nekrasova O (2020) Helminth species and infracommunities in frogs *Pelophylax ridibundus* and *P. esculentus* (Amphibia: Ranidae) in northern Ukraine. *Acta Parasitologica* **65**, 341–353.

Loot G, Lek S, Dejean J and Guegan JF (2001) Parasite-induced mortality in three host populations of the roach *Rutilus rutilus* (L.) by the tapeworm *Ligula intestinalis* (L.). *Annales de Limnologie* **37**, 151–159.

Maceda-Veiga A, Green AJ, Poulin R and Lagrue C (2016) Body condition peaks at intermediate parasite loads in the common bully *Gobiomorphus cotidianus*. *PLoS One* **11**, e0168992.

Magurran A (2004) *Measuring biological diversity*. 264 pp. Oxford, Blackwell Publishing.

Mariluan GD, Viozzi GP and Albarino RJ (2012) Trematodes and nematodes parasitizing the benthic insect community of an Andean Patagonian stream, with emphasis on plagiorchiid metacercariae. *Invertebrate Biology* **131**, 285–293.

Mayer M, Hawlitschek O, Zahn A and Glaw F (2013) Composition of twenty Green Frog populations (Pelophylax) across Bavaria, Germany. *Salamandra* **49**, 31–44.

Menard S (2000) Coefficients of determination for multiple logistic regression analysis. *The American Statistician* **54**, 17–24.

Mihaljevic JR, Hoye BJ and Johnson PTJ (2018) Parasite metacommunities: evaluating the roles of host community composition and environmental gradients in structuring symbiont communities within amphibians. *Journal of Animal Ecology* **87**, 354–368.

Möhl K, Grosse K, Hamedy A, Wüste T, Kabelitz P and Lücker E (2009) Biology of *Alaria* spp. and human exposition risk to *Alaria* mesocercariae – a review. *Parasitology Research* **105**, 1–15.

Okulewicz A, Hildebrand J, Łysowski R, Bunkovska K and Perec-Matysiak A (2014) Helminth communities of green and brown frogs from Poland (lower Silesia region). *Journal of Herpetology* **48**, 34–37.

Orlofske SA, Belden LK and Hopkins WA (2013) Larval wood frog (*Rana [= Lithobates] sylvatica*) development and physiology following infection with the trematode parasite, *Echinostoma trivolvis*. *Comparative Biochemistry and Physiology - Part A: Molecular & Integrative Physiology* **164**, 529–536.

Ozoliņa Z, Deksne G, Pupins M, Gravele E, Gavarane I and Kirjušina M (2021) *Alaria alata* mesocercariae prevalence and predilection sites in amphibians in Latvia. *Parasitology Research* **120**, 145–152.

Patrelle C, Portier J, Jouet D, Delorme D and Ferte H (2015) Prevalence and intensity of *Alaria alata* (Goeze, 1792) in water frogs and brown frogs in natural conditions. *Parasitology Research* **114**, 4405–4412.

Paunovic A, Bjelic-Cabrilo O and Simic S (2010) The diet of water frogs (*Pelophylax esculentus* "complex") from the Petrovaradinski Rit marsh (Serbia). *Archives of Biological Sciences* **62**, 797–806.

Pearson JC (1956) Studies on the life cycles and morphology of the larval stages of *Alaria arisaemoides* Augustine and Uribe, 1927 and *Alaria canis* LaRue and Fallis, 1936 (Trematoda: Diplostomidae). *Canadian Journal of Zoology* **34**, 295–387.

Pielou EC (1966) The measurement of diversity in different types of biological collections. *Journal of Theoretical Biology* **13**, 131–144.

Popiolek M, Rozenblut-Koscisty B, Kot M, Nosal W and Ogielska M (2011) Endoparasitic helminths of water frog complex in Poland: do differences exist between the parental species *Pelophylax ridibundus* and *Pelophylax lessonae*, and their natural hybrid *Pelophylax esculentus*? *Helminthologia* **48**, 108–115.

Portela A, Dos Santos T and Dos Anjos L (2020) Changes in land use affect anuran helminths in the South Brazilian grasslands. *Journal of Helminthology* **94**, e206.

Poulin R (2007) Are there general laws in parasite ecology? *Parasitology* **134**, 763–777.

Poulin R and Lagrue C (2015) The ups and downs of life: population expansion and bottlenecks of helminth parasites through their complex life cycle. *Parasitology* **142**, 1–9.

Poulin R, Luque JL, Guilhaumon F and Mouillot D (2008) Species abundance distributions and numerical dominance in gastrointestinal helminth communities of fish hosts. *Journal of Helminthology* **82**, 193–202.

Preston DL, Orlofske SA, Lambden JP and Johnson PTJ (2013) Biomass and productivity of trematode parasites in pond ecosystems. *Journal of Animal Ecology* **82**, 509–517.

Raffel TR, Lloyd-Smith JO, Sessions SK, Hudson PJ and Rohr JR (2011) Does the early frog catch the worm? Disentangling potential drivers of a parasite age-intensity relationship in tadpoles. *Oecologia* **165**, 1031–1042.

Rhoden HR and Bolek MG (2015) Helminth community structure in tadpoles of northern leopard frogs (*Rana pipiens*) and Woodhouse's toads (*Bufo woodhousii*) from Nebraska. *Parasitology Research* **114**, 4685–4692.

Rohr JR, Raffel TR and Sessions SK (2009) Digenetic trematodes and their relationship to amphibian declines and deformities. pp. 3067–3088 *in* Heatwole H and Wilkinson JW (*Eds*) *Amphibian biology. Vol. 8. Amphibian decline: diseases, parasites, maladies, and pollution*. Chipping Norton, Surrey Beatty & Sons.

Ryzhikov KM, Sharpilo VP and Shevechenko NN (1980) *Helminths of amphibians of the fauna of the USSR*. 278 pp. Moscow, Izdatelstovo Nauka (Publishing Science) (in Russian).

Saad-Fares A and Combes C (1992) Abundance/host size relationship in a fish trematode community. *Journal of Helminthology* **66**, 187–192.

Sattaman H (1990) Endohelminths of some amphibians from northern Greece (Trematoda, Acanthocephala, Nematoda; Amphibia: Triturus, Rana, Bombina). *Herpetozoa* **3**, 67–71.

Semlitsch RD, Peterman WE, Anderson TL, Drake DL and Ousterhout BH (2015) Intermediate pond sizes contain the highest density, richness, and diversity of pond-breeding amphibians. *PLoS One* **10**, e0123055.

Shannon CE and Weaver W (1949) *The mathematical theory of communication*. 35 pp. Urbana, University of Illinois Press.

Shimalov VV and Shimalov VT (2001) Helminth fauna of toads in Belorussian Polesie. *Parasitology Research* **87**, 84–84.

Shimalov VV, Shimalov VT and Shimalov AV (2001) Helminth fauna of newts in Belorussian Polesie. *Parasitology Research* **87**, 356–356.

Sinsch U, Kaschek J and Wiebe J (2018) Heavy metacercariae infestation (*Parastrigea robusta*) promotes the decline of a smooth newt population (*Lissotriton vulgaris*). *Salamandra* **54**, 210–221.

Sinsch U, Heneberg P, Těšínský M, Balczun C and Scheid P (2019) Helminth endoparasites of the smooth newt *Lissotriton vulgaris*: linking morphological identification and molecular data. *Journal of Helminthology* **93**, 332–341.

Sitko J, Faltýnková A and Scholz T (2006) *Checklist of the trematodes (Digenea) of birds of the Czech and Slovak Republics*. 111 pp. Praha, Academia.

Skryabin KI (1928) *The method of complete helminthological dissections of vertebrates including humans*. 45 pp. Moscow, Moscow State Univ. Press (in Russian).

Smyth JD and Smyth MM (1980) *Frogs as host-parasite systems I: an introduction to parasitology through the parasites of Rana temporaria, R. esculenta and R. Pipiens*. 112 pp. London, Macmillan Publishers Limited.

Speybroeck J, Beukema W, Bok B, Van VJ and Velikov I (2016) *Field guide to the amphibians and reptiles of Britain and Europe*. 432 pp. London, New York, Bloomsbury Publishing Plc.

Spieler M and Schierenberg E (1995) On the development of the alternating free-living and parasitic generations of the nematode *Rhabdias bufonis*. *Invertebrate Reproduction & Development* **28**, 193–203.

Sprinthall RC (2011) *Basic statistical analysis*. 9th edn. 659 pp. Harlow, Pearson Education.

Sudarikov V, Shigin A, Kurochkin Y, Lomakin V, Stenko R and Yurlova N (2002) *Metacercariae of trematodes-parasites of freshwater aquatic organisms in central Russia*. 298 pp. Moscow, Nauka (in Russian).

Svinin AO, Bashinskiy IV, Litvinchuk SN, et al. (2020) *Strigea robusta* causes polydactyly and severe forms of Rostand's anomaly P in water frogs. *Parasites & Vectors* **13**, 381.

Thomas F, Poulin R, de Meeüs T, Guégan J-F and Renaud F (1999) Parasites and ecosystem engineering: what roles could they play? *Oikos* **84**, 167–171.

Tinsley R, Rose Vineer H, Grainger-Wood R and Morgan E (2020) Heterogeneity in helminth infections: factors influencing aggregation in a simple host–parasite system. *Parasitology* **147**, 65–77.

Toledo R and Fried B (Eds) (2019) *Digenic trematodes*. 2nd edn. 487 pp. Cham, Springer International Publishing.

Toledo GM, Schwartz HO, Nomura HAQ, Aguiar A, Velota RAMV, da Silva RJ and Anjos LA (2017) Helminth community structure of 13 species of anurans from Atlantic rainforest remnants, Brazil. *Journal of Helminthology* **92**, 438–444.

Tyler MJ (1958) On the diet and feeding habits of the edible frog (*Rana esculenta* Linnaeus). *Proceedings of the Zoological Society of London* **131**, 583–595.

Vences M, Galan P, Palanca A, Vieites DR, Ni Eto S and Rey J (2000) Summer microhabitat use and diel activity cycles in a high altitude Pyrenean population of *Rana temporaria*. *Herpetological Journal* **10**, 49–56.

Vershinin VL, Burakova AV and Vershinina SD (2017) Comparative analysis of the parasitocenoses of amphibians from the family Ranidae (Anura) in the urbanization gradient. *Russian Journal of Ecology* **48**, 466–475.

Yang S, Harlow LI, Puggioni G and Redding CA (2017) A comparison of different methods of zero-inflated data analysis and an application in health surveys. *Journal of Modern Applied Statistical Methods* **16**, 518–543.

Zargar UR, Yousuf AR, Chishti MZ, Ahmed F, Bashir H and Ahmed F (2011) Effects of water quality and trophic status on helminth infections in the cyprinid fish, *Schizothorax Niger* Heckel, 1838 from three lakes in the Kashmir Himalayas. *Journal of Helminthology* **86**, 70–76.

Zell J, Rohner B, Thürig E and Stadelmann G (2019) Modeling ingrowth for empirical forest prediction systems. *Forest Ecology and Management* **433**, 771–779.

Zimmermann MR, Luth KE and Esch GW (2016) Transmission pattern differences of miracidia and cercariae larval stages of digenetic trematode parasites. *Acta Parasitologica* **61**, 680–688.