

# Parasites in a man-made landscape: contrasting patterns of trematode flow in a fishpond area in Central Europe

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## SUMMARY

We have explored a large body of novel data focusing on small-scale temporal and spatial patterns in the composition and structure of larval trematode communities in *Lymnaea stagnalis* (L.) from a typical Central European agricultural landscape. The 5 eutrophic fishponds studied provide excellent environments for the development of species-rich and abundant trematode communities. Nine prevalent species were consistently present in component communities, but had differential contribution to the parasite flow in the 5 ponds resulting in significant contrasting patterns of community similarity and the prevalence of the 3 major transmission guilds driving this similarity. Component communities split into 2 groups: (i) those from the large pond dominated by anacid and larid generalists with active miracidial transmission; and (ii) those from the smaller ponds dominated by 2 plagiariochideans infecting snails *via* egg ingestion. We put forward 3 hypotheses for the remarkable differences in larval trematode flow in the similar and closely located eutrophic ponds: (i) species-specific differences in parasite colonization potential displayed by an 'active-passive' dichotomy in miracidial transmission strategies of the species; (ii) top-down effects of pond context on transmission pathways of the trematodes; and (iii) competition as an important mechanism in eutrophic environments with a bottom-up effect on component community structure.

Key words: *Lymnaea stagnalis*, trematode community structure, spatial variation, parasite flow, eutrophic fishponds, Central Europe.

## INTRODUCTION

Eutrophication is one important anthropogenic ecosystem stressor frequently suggested as being associated with increased parasitism in fishes and invertebrates (e.g. Lafferty, 1997; Lafferty and Kuris, 1999; Marcogliese, 2005) and a recent modelling study has shown a greater frequency of positive associations of eutrophication with the impact of infectious disease (Lafferty and Holt, 2003). High eutrophication of water bodies, associated with colonization by snails and nesting ducks, is suggested as an important factor leading to re-emergence of cercarial dermatitis (caused by *Trichobilharzia* spp., see Allgöwer and Effelsberg, 1991; Beer and German, 1993; Kolářová *et al.* 2010 and references therein). However, empirical evidence linking infection levels in invertebrate hosts with productivity, other than the frequently used examples (i.e. Beer and German, 1993 and Marcogliese *et al.* 1990; see e.g. Lafferty, 1997; Lafferty and Kuris, 1999; Marcogliese, 2005) has been gathered only recently. Thus, both field data from large-scale surveys in North America (Johnson

*et al.* 2002, 2004; Johnson and Chase, 2004) and mesocosm manipulative experiments (Johnson *et al.* 2007) have provided strong evidence that eutrophication enhances infection with *Ribeiroia ondatrae* in the second intermediate hosts, frog larvae, *via* nutrient-mediated increases in the density of the first intermediate host and cercarial production per-snail. A similar mechanism relating nutrient levels and snail host density has been suggested for echinostome infections (species of *Echinostoma* and/or *Echinoparyphium*) in amphibians (Skelly *et al.* 2006; see Johnson and McKenzie, 2009 for a review).

However, all of the above studies concern single species dynamics. Given that eutrophication boosts both the abundance of snail and bird final hosts (Wiśniewski, 1958; Esch, 1971; Johnson *et al.* 2007) and the dynamics of individual trematode species, leading to cascading effects up the trophic chains, how would these changes translate into the composition and structure of larval trematode communities? Unravelling determinants of community structure in parasites in eutrophic environments can largely benefit from a comparative approach in which several populations of a single snail host species are investigated simultaneously. Here we explore a large body of novel data on the composition and structure of communities of larval trematodes in 5 populations of the great pond snail, *Lymnaea stagnalis* (L.), in a

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model system (eutrophic fishponds in South Bohemia, Czech Republic), typical of a central European agricultural landscape.

Fishponds, a specific type of man-made wetlands, represent environmentally important landscape features, characteristic of historical agriculture-dominated landscapes in Central Europe that support significant animal and plant diversity (Pokorný and Hauser, 2002). Originating as early as the 11th century fishponds are common in many countries in central Europe (e.g. Austria, Czech Republic, France, Germany, Hungary and Poland). Fishpond systems are particularly dense in the Czech Republic (Pouličková *et al.* 2009) and represent the only type of wetland providing the most important habitats for water birds. Although having lost their artificial appearance (i.e. they “look like natural lakes”, Pokorný and Hauser, 2002) fishponds represent managed aquatic ecosystems in which water level, fish stocks, and the nutrient input are under human control (Pechar, 2000). These ecosystems have suffered from over-exploitation during the second half of the 20th century (e.g. fertilizer run-off, liming and fertilization, high fish stock densities, added fish feed) that resulted in increasing trophic status, ultimately reaching a state of hypertrophy (Pechar, 2000).

The host-parasite system selected, the freshwater pulmonate snail *L. stagnalis* (Gastropoda: Lymnaeidae) and its trematode parasites, is ideally suited to assessment of levels of parasitism in European wetlands and in ponds in particular. *L. stagnalis* is common and abundant in slow moving or stagnant water bodies and widely distributed throughout Europe (Glöer, 2002). This snail serves as first (and in many cases also as second) intermediate host for a wide variety of trematodes whose larval morphology, life cycles and geographical distribution have been subjected to intense studies originating in Europe as early as the 19th century (reviewed by Faltýnková and Haas, 2006; Faltýnková *et al.* 2007; Żbikowska and Nowak, 2009). A current cumulative estimate of the trematode richness in *L. stagnalis* in Central Europe results in 24 species representing multiple evolutionarily independent lineages (12 families) (Brown *et al.* 2011; see also Faltýnková *et al.* 2007 and references therein). Of these, 19 species require birds as definitive hosts; 3 and 2 species develop in mammals and amphibians, and 1 in fish. Thus, the bulk of parasites in *L. stagnalis* utilize birds as definitive hosts that become infected mostly *via* trophic webs. A quantitative assessment of infection levels in snail populations, therefore, provides an opportunity to infer habitat-related occupational and trophic linkages for this important wetland component in Europe. However, although isolated data exist from eutrophic lakes in northern Europe (Väyrynen *et al.* 2000; Żbikowska *et al.* 2006) quantitative studies on larval

parasite communities in molluscs from European wetlands are lacking.

We focused on quantification of small-scale temporal and spatial patterns of the composition and structure of larval trematode communities using prevalence data for individual parasite species and functional/taxonomic groupings in 83 distinct snail population samples. Based on evidence on snail-trematode systems from coastal wetlands similarly dominated by parasites maturing in birds (e.g. Mouritsen and Poulin, 2002; Thieltges *et al.* 2009) in which a linkage between the bird and parasite abundance and diversity has often been shown on small to medium spatial scales (e.g. Smith, 2001; Hechinger and Lafferty, 2005; Fredensborg *et al.* 2006) we hypothesized that larval parasite richness, prevalence and community structure would show substantial homogeneity due to the small geographical extent of the study. This expectation was further enforced by the linkage across scales of the distribution-abundance relationship for the larval trematodes in *L. stagnalis* recently indicated by Brown *et al.* (2011) who found that the most widespread species in the large-scale inventories in Europe (i.e. *Diplostomum pseudospathaceum*, *Opisthioglypseranae*, *Plagiorchis elegans*, *Echinostoma revolutum* and *Echinoparyphium aconiatum*) are also locally abundant in the area of our study (i.e. attained high prevalence in component communities). Conversely, pond context, i.e. size, function and management of the ponds, could induce differential changes of the dynamics of parasite populations and this would lead to differentiation in community structure. We therefore, evaluated whether the different roles of the ponds in the carp breeding management cycle and associated timing of high fish stock densities and periodical drawdown would result in noticeable variations in larval community composition and structure among ponds.

Pond occupancy by birds also varies seasonally especially during spring and autumn migrations when large numbers accumulate in the area of study. We hypothesized that, although some parasites are recruited to the snail hosts in pulses (e.g. during spring and autumn bird migrations), the continuous presence of nesting birds and the low specificity to the definitive hosts typical for the trematodes parasitizing *L. stagnalis* (see e.g. Sitko *et al.* 2006; Kavetska *et al.* 2008 and references therein) would tend to reduce the effects of the seasonal heterogeneity of recruitment. We, therefore, did not expect significant seasonal change in the levels of parasitism and community structure in our system.

Results of this first relatively long-term assessment of larval communities in a snail species from Europe providing evidence for increased transmission rates and contrasting pattern of parasite flow in the closely located model eutrophic water bodies, may lead to a



Fig. 1. Map of the study area in the Czech Republic showing all (outlined) and study ponds (filled). Key to ponds: V, pond Vlkovský; HS, pond Hluboký Sax; HH, pond Hluboký u Hamru; VD, pond Velký Dvorecký; Z, pond Zavadil.

better understanding of parasitism in eutrophic environments.

#### MATERIALS AND METHODS

##### *Study area and fishponds*

Our study was carried out in South Bohemia which is emblematic with its network of lentic habitats (fishponds) (Fig. 1). The fishponds selected were representative of 3 typical size categories in the landscape: large (>40 ha); medium-sized (10–20 ha) and small (<5 ha) (see Table 1 for details). Three fishponds (Vlkovský, Hluboký Sax and Hluboký u Hamru, further referred to as ponds V, HS and HH) are located in the Třeboň Basin Biosphere Reserve and Protected Landscape Area (BR/PLA) which contains 465 fishponds and encompasses 2 wetlands of international importance protected by the Ramsar Convention (Třeboň Fishponds, Important Bird Area code CZ008). Třeboň Basin BR/PLA is especially important for birds (*ca* 200 species recorded) providing suitable habitats for nesting (*ca* 150 species) and stop-over sites for waterfowl during migration (up to 15 000–20 000 individuals). Since 2004 most of the area has belonged to the European network Natura 2000. Two small ponds (Velký Dvorecký and Zavadil, further referred to as ponds VD and Z) are located at the border of Třeboň Basin

BR/PLA in the area of the neighbouring Jindřichův Hradec. The 2 groups of ponds are 16 km apart so that regional differences in abiotic characteristics are minimized and the condition that snails would be exposed to infection by parasites from a common regional pool is satisfied. The mean monthly temperatures in the 2 areas were very similar during the entire sampling period (range in temperature difference 0–0.8 °C) as were the mean annual temperatures (8.5, 9.6 and 9.3 °C *vs* 8.2, 9.2 and 8.9 °C for 2006, 2007 and 2008 in Třeboň and Jindřichův Hradec area, respectively). On average, the ponds are ice-covered from November to March.

All ponds are surrounded by agricultural fields with forested patches nearby and are used for fish farming in the traditional manner (i.e. stocking and fishing every year, with winter storage in hibernation ponds), common carp (*Cyprinus carpio* L.) being the main cultured species. However, size, pond use and associated management differ between the ponds (see Table 1 for details). The largest pond V serves as the rearing (or main) fishpond (i.e. for accommodation of 2-year-old fish stocks during the third growing season) whereas the 4 much smaller ponds are used as hibernation ponds (i.e. for storage of yearlings or 2-year-old fish in the winter period). The main pond V is drained for fish harvesting at the beginning of November; filling starts after 1 month and the pond is filled to capacity at the end of March – beginning

Table 1. Summary data for the ponds studied, snail samples and overall rates of larval trematode infections (Key to ponds: V, pond Vlkovský; HS, pond Hluboký Sax; HH, pond Hluboký u Hamru; VD, pond Velký Dvorecký; Z, pond Zavádil.)

Pond	V	HS	HH	VD	Z
Ponds					
Geographical coordinates	49°08'56"N 14°43'51"E	49°09'42"N 14°45'55"E	49°09'38"N 14°46'19"E	49°04'43"N 14°56'20"E	49°04'37"N 14°55'56"E
Size (ha)	47.09	12.0	11.9	4.75	1.86
Function	Main pond	Hibernation pond	Hibernation pond	Hibernation pond	Hibernation pond
Draining for fish harvest	Autumn	Spring	Spring	Autumn	Autumn
Time drained	One month	One week	One week	One month	One month
Fill to capacity	End of March – beginning of April	in 4–14 days	in 4–14 days	Beginning of March	December
Rehabilitation during study	No	Sept. – Dec. 2007	No	No	No
Snails					
Total no. of snails marked and released	1120	843	1394	1720	1457
Total no. of capture events	1554	1265	2085	2171	1833
No. of samples	19	16	17	16	15
No. of snails per sample, range (mean ± s.d.)	20–184 (82 ± 46)	20–144 (79 ± 40)	36–278 (123 ± 61)	21–352 (136 ± 84)	20–256 (122 ± 85)
Mean snail height (range, mm)	41–52	46–51	40–53	35–44	36–50
Mean snail width (range, mm)	21–28	23–28	20–32	17–23	18–31
Overall prevalence (range, %)	6.6–63.5	27.6–89.0	5.6–100	0.9–71.4	1.7–66.7
Percentage of samples with overall prevalence >50%	26.3	75.0	29.4	25.0	20.0

of April of the following year. The medium-sized hibernation ponds HS and HH are drained in spring (beginning of March – beginning of April) for fishing the overwintered stocks (end of March – beginning of April); filling starts after a week and is completed within 4 days (fast filling) to up to 2 weeks (slow filling). The smallest hibernation ponds VD and Z are drained/fished in autumn (mid-September to mid-November); filling starts within 1 week after fishing and the fill to capacity is reached within 1 (pond Z) to 3 (pond VD) months. All ponds supported viable persistent populations of *L. stagnalis*.

#### Sampling of the model host-parasite system

We surveyed each pond tri-weekly from August 2006 to October 2008 with the exception of January and February 2008 when the ponds were covered by ice. Not all planned samples (28 per pond) were collected and/or used in statistical tests due to the low density of adult snails in the early spring (all ponds) and during/after summer droughts (pond HH, summer of 2007). Sampling was interrupted by the regular annual draining of the ponds (all ponds) and restoration of pond HS (sediment removal from the bottom of the pond was carried out between September and December 2007). Nevertheless, the 83 distinct samples gathered reflect the actual state of trematode transmission in the ponds studied.

Snails were sampled randomly with hand-nets or hand picked from the surface and/or aquatic

vegetation along selected transect near the shore of each pond (typically 40–45 m long by 1.5 m wide and extending to a depth of 0.5 m). Only snails of the sexually mature cohorts (shell height *ca* 40–50 mm) were sampled since the output of infective larval stages from this part of the snail population largely contributes to trematode transmission in the habitats of *L. stagnalis* (see e.g. Shigin, 1980; Źbikowska *et al.* 2006). To standardize the sampling effort, sampling was carried out by 3 persons for 30 min at each site. Sampling times were extended at low densities of mature snails but target sample size (i.e. at least 50 snails) in most of these cases was not achieved. Thus, although the size of the snail samples reflected population density at the time of sampling, samples of <20 snails were excluded from analyses.

In the laboratory snails were measured with electronic digital callipers (accuracy 0.5 mm), labelled (individual numbers) with permanent paint, and screened for the presence of patent trematode infections (i.e. cercarial emission only). Snails were placed into individual beakers with dechlorinated tap water under a light source for 24 h to stimulate the emergence of cercariae. Live cercariae were examined and identified to the species level using the keys of Faltýnková *et al.* (2007). Representative samples of each trematode species were fixed in hot 4% formaldehyde solution. Identification was carried out within 2 days of collection; during this time non-emitting snails were repeatedly checked. A snail was recorded as uninfected if no cercarial emission was



detected during the 2-day period. Snails were then released back into their habitats.

We used a mark-release-recapture technique in order to simultaneously investigate long-term trematode infracommunity dynamics (i.e. screening infections of individual snails). This non-destructive technique ensured observation of an undisturbed system (i.e. without introducing changes in snail population dynamics due to frequent removal/dissection of large fractions of the host populations). Under this protocol we recorded snail infection status based on the presence/absence of the patent stage of infection during which cercariae (free-living dispersal stages in the trematode life cycle) are produced and released. While cercarial emergence is generally considered to reflect accurately the prevalence of patent infections (Esch *et al.* 2001), there is a possibility that the presence of false negatives (i.e. infected snails showing no cercarial emission during the time of screening) can result in an underestimation of the overall prevalence, a problem especially pronounced in the long-lived marine snail-trematode systems (Curtis and Hubbard, 1990).

Since no data exist on the host-parasite system studied, and as a quality assurance measure, a control group of 781 snails comprising 5 spring and 6 autumn samples (the seasons when the probability for false negatives is assumed to be high) was studied. The infection status of these snails was determined both by cercarial emergence within 2 days of collection and by snail dissection. Seven infections (0.89%) were not detected by screening for cercarial emergence. The differences between the actual overall prevalence (i.e. including false negatives) and the prevalence based on cercarial emergence ranged between 0–1.15% and 0–5.26% in spring and autumn samples, respectively. There was no significant effect of season on these differences (Mann-Whitney test,  $U=8$ ,  $n=5$ , 6;  $P=0.19$ ). No statistically significant differences were found between the prevalences detected from cercarial emergence and dissection (Fisher's exact test, all  $P=1$ ). We, therefore, concluded that detection of infection status by cercarial emergence adequately reflects the actual levels of infection of the most epidemiologically relevant cohorts of *L. stagnalis* in Central Europe.

### Terminology

Ecological terms used follow those of Bush *et al.* (1997). Prevalence (expressed as a percentage) is defined as the proportion of snails in a sample infected with a given trematode species; snails with multiple infections were counted as infected for each parasite species in the infection. Overall prevalence denotes infection status irrespective of the parasite species. Data were gathered at the component community level (all parasite species in a distinct snail population sample i.e. taken at one point in time

in a given pond). Due to the overall high infection levels the following prevalence scheme was adopted using the terminology of Holmes and Price (1986) for simplicity and with no implication to the 'core-satellite' hypothesis (Hanski, 1982): (i) species with a prevalence of >20% in a sample were designated as 'core'; (ii) species with prevalences between 5 and 20% were designated as 'secondary' and (iii) species with a prevalence of <5% were designated as 'satellite'.

Focusing on the mode of infection of the first intermediate host and the transmission pathways to definitive hosts we recognize 3 trematode groupings further referred to as 'transmission guilds' or 'guilds' for simplicity. The first represents *D. pseudopathaceum*, a generalist parasite of fish-eating (predominantly larid) birds, characterized by infection of *L. stagnalis* by active miracidial penetration and the use of fish second intermediate hosts. The second comprises a group of anatid generalist species infecting *L. stagnalis* by active miracidial penetration (the only exception being *Notocotylus attenuatus* dispersing *via* eggs) and using mollusc second intermediate hosts or directly transmitted to birds. The third includes 2 plagiorchioideans, *P. elegans* and *O. ranae*, which infect *L. stagnalis* passively *via* egg ingestion and use non-fish/mollusc intermediate hosts in their life cycles. Two of the species found during our study are not assigned to a guild: the specific parasite of rallid birds *Moliniella anceps* and the specific parasite of cormorants *Paryphostomum radiatum*.

### Data analysis

Ponds were considered individual wetland patches within the landscape that were subsequently (e.g. richness estimates; contrasts in community composition) assigned to 3 size groups as described above. Prevalence was calculated for each snail population sample. In order to meet assumptions for parametric tests prevalence data (expressed as proportions) were arcsin square-root-transformed; data on snail size and sample size natural-log-transformed; and species richness data natural-log ( $x+1$ )-transformed. Spearman rank correlations ( $r_s$ ) and Kruskal-Wallis (K-W) non-parametric ANOVA were applied when assumptions for parametric tests were not fully met. Statistical analyses were carried out with Statistica 7.0 (StatSoft, Inc., Tulsa, OK, USA).

Sample sizes did not differ significantly between ponds (ANOVA;  $F_{4,78}=1.95$ ,  $P=0.111$ ) but snails were generally larger in the large and medium-sized ponds (ANOVA on mean snail length;  $F_{4,78}=21.07$ ,  $P<0.0001$ ). Snail density estimated by sample size exhibited a weak negative correlation with overall prevalence and a positive correlation with component community richness ( $r_s=-0.277$  and  $0.353$ , respectively,  $P<0.05$ ) whereas mean snail length

was associated with overall prevalence ( $r_s=0.483$ ,  $P<0.05$ ). Therefore, we examined the variations in component community parameters with respect to 'pond' and 'season' as factors in two-way analysis of covariance designs (separate ANCOVAs; general linear model procedure in Statistica) after the first-order effects of snail size and, as a precaution, snail density, were accounted for (entered as covariates). We examined 2 sets of models: (i) using data on component community richness, overall prevalence (and infracommunity richness) and prevalence of the major transmission guilds from 3 seasons (spring, summer and autumn) from all ponds (Analysis 1); and (ii) using data from all 4 seasons from 3 ponds (V, HS and HH) for which more winter replicates (December to February) were available (Analysis 2).

We used species richness accumulation curves produced by the analytical formulae of Uglund *et al.* (2003) (UGE curves) in PRIMER v6 software (Clarke and Gorley, 2006) to compare local richness estimates among ponds using component community data (999 randomizations; samples entered in random order).

Similarity in species composition and structure between pairs of communities was investigated by calculating the Bray-Curtis similarity index using square-root transformed prevalence data. We analysed variations in community structure using 2 different similarity approaches, a permutational regression approach (Legendre *et al.* 1994) and non-parametric analysis of similarities (ANOSIM, Clarke and Gorley, 2006). First, we tested whether the distance among ponds had any effect on compositional similarity by regressing Bray-Curtis similarity matrix on the distance matrix (linear distances in km among all pairs of localities obtained from maps). The significance of the best regression model was based on 9999 random permutations of the dependent variable matrix (Manly, 1997) using RT 2.1 program (Western EcoSystems Technology, Inc., Cheyenne, Wyoming, USA). Secondly, we visualized similarities between component communities in the 5 ponds through a non-metric multi-dimensional scaling ordination (MDS procedure in PRIMER v6) performed on a Bray-Curtis similarity matrix derived from square-root transformed prevalence data. We then carried out a non-parametric analysis of compositional similarity (ANOSIM procedure in PRIMER v6) between *a priori* defined groups by factors: (i) 'pond' (5 contexts, i.e. each pond considered a defined individual wetland patch); (ii) 'pond size' (3 contexts, i.e. small, medium-sized and large); (iii) 'pond function' (2 contexts associated with both fish density and permanence of occupancy, i.e. rearing and hibernation ponds), and (iv) 'season' (4 contexts). We performed randomization tests (R-statistic evaluated for 9999 permutations) on similarity matrices to test the null hypothesis of no differences in parasite community structure between community groupings

defined by factors (i)–(iii) allowing for the fact that there may be seasonal differences (2-way crossed layout ANOSIM with factors 'pond' and 'season'; 'pond size' and 'season'; and 'pond function' and 'season', respectively). To avoid the confounding effect of pond size, we examined the influence of the timing of the annual drawdown on the subset of communities from medium-sized and small ponds [2-way crossed layout ANOSIM with factors 'season' and 'drawdown' (2 contexts, i.e. autumn-winter and spring drawdown)]. The R-statistic provides a general measure of the degree of separation of *a priori* defined groups in the high-dimensional space represented by the resemblance matrix for the communities and reaches its maximum value of 1 when all dissimilarities between the groups exceed any dissimilarity within either group (Clarke *et al.* 2008).

## RESULTS

### *General characteristics of larval trematode communities*

A total of 6534 snails was captured, marked and released in the 5 ponds during the 26-month period of study. Patent trematode infections were detected in 3374 of the 8908 (37.9%) capture events. Although the overall prevalence of larval trematodes varied widely among samples (0.9–100%) infection levels were generally high, reaching in excess of 50% in 35% of the samples (range per pond 20–75% of the samples; the upper limit reflects distinctly higher prevalences at pond HS; see Table 1). Of the 6534 marked and released snails, 1703 were recaptured (26.1%). Most of these were recaptured once ( $n=1210$ ; 71.1% of recaptured snails) or twice ( $n=357$ ; 21.0%), and 136 snails (8.0%) were recaptured 3–5 times ( $n=109$ ;  $n=21$ ; and  $n=6$ , respectively). There were no differences in recapture rates between ponds across our complete dataset ( $F_{4,75}=0.136$ ,  $P=0.968$ ) but these were significantly higher in winter ( $F_{3,75}=24.29$ ,  $P<0.0001$ ) (main effects ANOVA,  $R^2=0.504$ ,  $F_{7,75}=10.90$ ,  $P<0.0001$ ). Snails retained marks up to 364 days.

The 5 populations of *L. stagnalis* were parasitized by a total of 14 species belonging to 7 trematode families (see Table 2 for full species/family names and second intermediate/definitive host groups). With the exception of the amphibian parasite *O. ranae*, all species mature in aquatic birds (1 of these, *P. elegans*, also completes its life cycle in mammals) and utilize 6 transmission pathways involving different second intermediate host groups (indicated by numbers in Table 2 and Fig. 3) to reach the definitive hosts: (i) no second intermediate host (*T. szidati* and *N. attenuatus*); (ii) mollusc-bird (*E. aconiatum*; *E. recurvatum*; *E. revolutum*; *H. conoideum*; *M. anceps* and *C. cornutus*); (iii) fish-bird (*D. pseudospathaceum* and *P. radiatum*); (iv) amphibian-amphibian (*O. ranae*); (v) leech-bird (*Australapatemon burti* and *A. minor*); and (vi) larval insect-bird/mammal (*P. elegans*).

Table 2. Trematodes recorded in *Lymnaea stagnalis* in the ponds studied, their second intermediate and definitive host groups and transmission pathway number (as indicated in Fig. 4)

(Data on definitive bird and amphibian hosts are based on surveys from the Czech and Slovak Republics only: Sitko *et al.* (2006) (birds); Vojtková (1974 *a, b*) (amphibians); Rudolfová *et al.* (2007) (bird hosts for *T. szidati* as confirmed by a sequencing study). Mammalian and reptilian host numbers are from Sharpilo and Iskova (1989); no data are currently available for *A. burti* but we assume this species has a life cycle similar to that of the closely related *A. minor* and also infects multiple species of waterfowl. See Fig. 4 for a graphical illustration of transmission pathways.)

Species	Abbreviation	Second intermediate host groups	Definitive host groups	Transmission pathway no.
Family Diplostomidae Poirier, 1886				
<i>Diplostomum pseudospathaceum</i> Niewiadomska, 1984	DP	Fish	Fish-eating birds (5 spp.)	3
Family Echinostomatidae Looss, 1899				
<i>Echinoparyphium aconiatum</i> Dietz, 1909	EA	Molluscs	Ducks (4 spp.)	2
<i>Echinoparyphium recurvatum</i> (Linstow, 1873)	ERE	Molluscs	Waterfowl (25 spp.)	2
<i>Echinostoma revolutum</i> (Frölich, 1802)	ER	Molluscs	Waterfowl (38 spp.)	2
<i>Hypoderaeum conoideum</i> (Bloch, 1782)	HC	Molluscs	Waterfowl (12 spp.)	2
<i>Moliniella anceps</i> (Molin, 1858)	MA	Molluscs	Coots (4 spp.)	2
<i>Paryphostomum radiatum</i> (Dujardin, 1845)	PR	Fish	Cormorants (2 spp.)	3
Family Notocotyliidae Lühe, 1909				
<i>Notocotylus attenuatus</i> (Rudolphi, 1809)	NA	– <sup>a</sup>	Waterfowl (19 spp.)	1
Family Plagiorchiidae Lühe, 1901				
<i>Plagiorchis elegans</i> (Rudolphi, 1802)	PE	Larval insects	Birds (28 spp.); mammals (6 spp.); reptiles (4 spp.)	6
Family Schistosomatidae Stiles & Hassall, 1898				
<i>Trichobilharzia szidati</i> Neuhaus, 1952	TS	– <sup>b</sup>	Ducks (2 spp.)	1
Family Strigeidae Railliet, 1919				
<i>Australapatemon burti</i> (Miller, 1923)	AB	Leeches	Waterfowl	5
<i>Australapatemon minor</i> (Yamaguti, 1933)	AM	Leeches	Waterfowl (15 spp.)	5
<i>Cotylurus cornutus</i> (Rudolphi, 1808)	CC	Molluscs	Waterfowl (17 spp.)	2
Family Telorchhiidae Looss, 1899				
<i>Opisthioglyphe ranae</i> (Frölich, 1791)	OR	Amphibians	Amphibians (13 spp.)	4

<sup>a</sup>Cercariae encyst on vegetation; <sup>b</sup>cercariae infect birds directly.

Thirteen of the 14 species in the total regional list comprised the trematode species pool of the largest pond V, whereas those of the medium-sized and small ponds consisted of 10 (ponds HS, HH and VD) and 9 species (pond Z). The species not recorded in most of the smaller ponds were *E. recurvatum*, *A. burti*, *C. cornutus* (all 4 ponds); *M. anceps* (ponds HH, VD and Z), *A. minor* (pond HS) and *N. attenuatus* (pond Z). A comparison of the species accumulation UGE curves illustrates the higher local species diversity at the large pond V (i.e. non-asymptotic UGE curve in contrast to those at the medium-sized and small ponds, see Fig. 2). Component communities at pond V also had on average more species (mean 5.73 *vs* 3.87–4.71) but differences were not significant (ANOVA,  $R^2 = 0.108$ ,  $F_{4,78} = 2.37$ ,  $P = 0.06$ ).

However, infracommunity richness was typically low (means <1 with 2 exceptions) with somewhat higher values in communities from pond HS (ANOVA,  $R^2 = 0.220$ ,  $F_{4,78} = 5.50$ ,  $P = 0.0005$ ; mean 0.66 *vs* 0.32–0.46). These values reflected the overall low number of multiple infections (248 out of 6534

marked and released snails and of 3374 capture events when infections were detected, i.e. 3.8% and 7.4%, respectively). Of these, 244 (98.4%) were double and represented by 12 species combinations: 6 involving *T. szidati* as one of the species in the pair ( $n = 20$ ); 4 involving *O. ranae* ( $n = 218$ ; in 196 of these in combination with *P. elegans*); and a further 2 involving *P. elegans* ( $n = 6$ ). The 4 triple infections (1.6%) comprised combinations of *O. ranae* and *P. elegans* with 3 other species: *D. pseudospathaceum* ( $n = 2$ ); and *P. radiatum* and *T. szidati* ( $n = 1$  each).

The overall frequency of multiple infections differed significantly between ponds (K-W  $H_{4,83} = 19.87$ ,  $P = 0.0005$ ) being higher in communities from medium-sized ponds HS and HH (on average 10.4% and 8.9% of all infections, respectively, *vs* 2.9% from the large pond V and 1.9% and 4.5% from the small ponds Z and VD, respectively). The rates of multiple infections were distinctly higher and reached maxima in autumn (K-W  $H_{3,83} = 17.78$ ,  $P = 0.0005$ ). Notably, double infections with *O. ranae* prevailed numerically ( $n = 218$ , i.e. 88.0%) and were registered mostly in medium-sized ponds ( $n = 80$  and

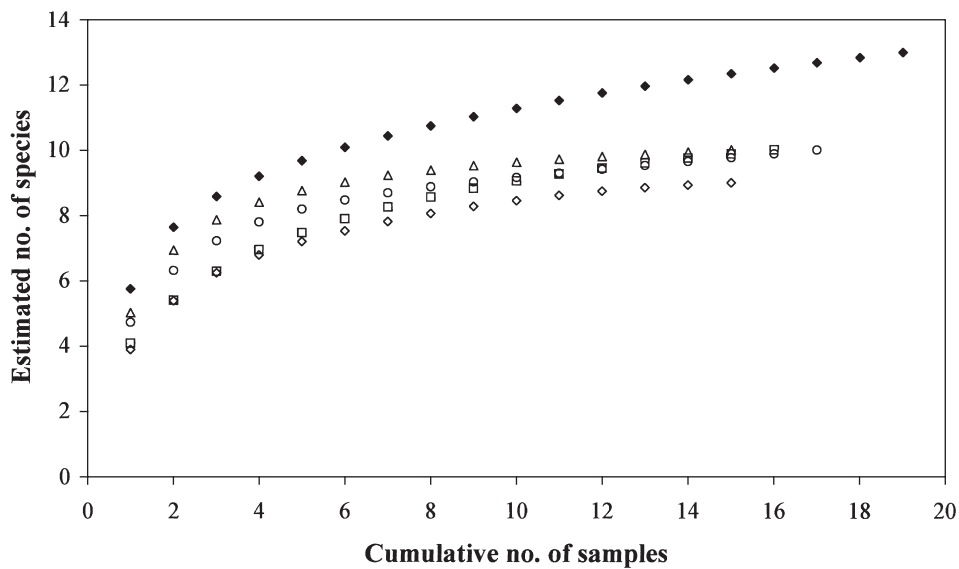


Fig. 2. Comparison of the richness of local species pools at the study ponds estimated from component community richness data (UGE curves, 999 randomizations, samples entered in random order). Key to symbols: pond V (filled diamonds); pond HS (open squares); pond HH (open circles); pond VD (open triangles); pond Z (open diamonds).

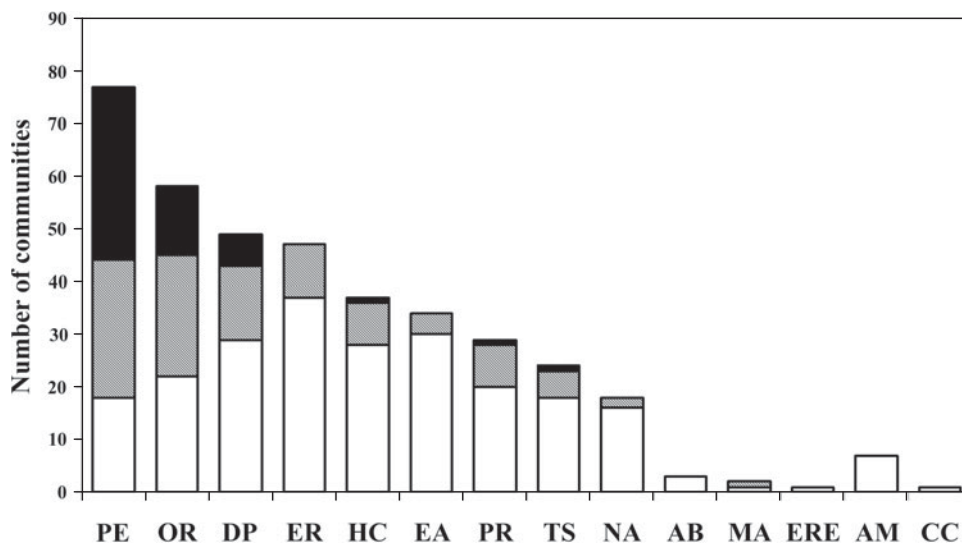


Fig. 3. Occurrence-prevalence patterns of the trematodes ranked by their overall occurrence in larval trematode communities in *Lymnaea stagnalis* in the study area. The breakdown by area for each species indicates the proportion of communities where a species was core (filled area), secondary (checkerboard area) and satellite (open area). Abbreviations as in Table 2.

$n = 87$  in pond HS and HH, respectively) and in the small pond VD ( $n = 40$ ). This species was most frequently found (and again in these smaller ponds) in combination with *P. elegans* (in 90% of double infections involving *O. ranae*). Collectively the 2 species of the plagiurchioidean guild were involved in 94.8% of all multiple infections.

*Patterns of species occurrence and parasite flow*

The breakdown by prevalence status (i.e. core, secondary and satellite) for the species in component communities across our complete dataset provided

in Fig. 3 illustrates their differing abundance-distribution patterns. Nine species occurred in at least 20% and up to 93% of the communities studied; of these 3, *P. elegans*, *O. ranae* and *D. pseudospathaceum*, were most frequent and prevalent (i.e. attained core and secondary status in at least 40% and up to 77% of communities where present). The remaining 5 species exhibited low erratic occurrence (in less than 10% of communities) with typically low prevalence (<5%; with a single exception all satellite). Eight of the 9 common (i.e. most frequent and prevalent) trematodes occurred in all 5 ponds (*N. attenuatus* was not registered at pond Z) and 7 occurred in all seasons



(*T. szidati* was absent in communities sampled in winter and *P. radiatum* was present in communities sampled in summer and autumn only). On the other hand, the 5 rare species (at the right side of the plot) occurred typically in a single community per pond (up to 3; *A. minor* in ponds VD and Z and *A. burti* in pond V) and in a single season (*M. anceps* in spring and *Australapatemon* spp., *C. cornutus* and *E. recurvatum* in autumn). It is worth noting that cercarial emergence was readily detected during winter screening (i.e. December, January and February) for 7 of the 9 common species in our study. These were (in order of mean winter prevalence and frequency): *P. elegans*, *O. ranae*, *D. pseudospathaceum*, *E. revolutum*, *N. attenuatus*, *H. conoideum* and *E. aconiatum* (see Table 3). Furthermore, the daily cercarial production of the 3 most frequent and prevalent species was within the range observed during the warm seasons (9302–56 642 cercariae for *P. elegans*; 204–1890 cercariae for *O. ranae*; and 633–4032 cercariae for *D. pseudospathaceum*).

Mapping the summed overall occurrence of the core and secondary species onto the pathway scheme in Fig. 4 indicates a contrasting pattern of parasite flow in the ponds studied. The large pond V was characterized by the predominance of trematodes utilizing pathways 1–3 (i.e. utilizing mollusc and fish intermediate hosts or lacking second intermediate host) whereas the species utilizing pathways 4 and 6 (i.e. using amphibians and larval insects as second intermediate hosts) were consistently most prevalent in the smaller ponds. None of the *Australapatemon* spp. utilizing pathway 5 was found to attain core or secondary status in any of the 83 component communities studied. The temporal sequence of the prevalence of the 9 common species summarized in Table 4 indicates that this contrasting pattern of parasite flow is repeatable at least for the time-scale of the study (see Table 3 for ranges and means for prevalence of the individual species). In spite of the variations, the larid generalist parasite *D. pseudospathaceum* (utilizing pathway 3) and the guild of anacid generalists (pathways 1 and 2) were most prevalent in the seasonal community samples from pond V whereas the 2 species comprising the plagiorchioidean guild (*O. ranae* and *P. elegans*; pathways 4 and 6, respectively) consistently exhibited high prevalence in the medium-sized and small ponds.

#### Patterns of spatial and temporal variation of component communities

Semi-logarithmic function (ln-transformed similarity *vs* non-transformed distance) provided the best fit for the relationship between component community similarity and distance between ponds. However, with only 2% of the variance in similarity accounted for by inter-pond distance, there was no significant distance effect (Mantel test,  $P=0.168$ ).

An exploratory non-metric MDS ordination of component communities (Fig. 5A) revealed 2 cohesive groups with similar community structure: (i) the smaller group on the left consisting of communities from the large pond V; and (ii) the larger group comprising those from the medium-sized ponds (lower right) which exhibited a significant degree of overlap with communities from the small ponds. The latter, however, showed the greatest variation (i.e. the loose points to the upper right and a single point at the lower left of the plot, see Fig. 5A) in spring (April and May 2007) and early summer (June 2007, 2008). A two-way crossed ANOSIM confirmed a significant effect of both 'pond' and 'season' to the differences identified from MDS with greater differentiation of communities in relation to pond of origin (global  $R=0.521$ ,  $P=0.001$  *vs*  $R=0.178$ ,  $P=0.02$ ). Larval trematode communities from pond V exhibited the greatest differentiation (pairwise test  $R$  statistics ranging from 0.676 to 0.949; all  $P=0.001$ ; average dissimilarity range 82.1–89.6%) due to the overall higher prevalences of *D. pseudospathaceum* and the anacid generalist guild and the absence or very low prevalence of the plagiorchioidean guild (SIMPER procedure; see prevalences of the 3 transmission guilds superimposed on the MDS plot in Fig. 5B–D). The greater within-pond variability observed in the small ponds Z and VD (Fig. 5A) probably contributed to the higher differentiation of communities sampled in spring from those sampled in autumn (pairwise test  $R=0.531$ ,  $P=0.001$ ) and winter (pairwise test  $R=0.489$ ,  $P=0.044$ ). The former (points deviating from the main cluster at the lower right in Fig. 5A) in fact represent the first post-drawdown communities since no overwintered mature snails were found (or sample sizes were too small to be included in the analysis) in March and the beginning of April from the small ponds. Larval communities also exhibited significant differentiation (global  $R=0.568$ ,  $P=0.001$ ) in relation to pond size context (i.e. small, medium-sized and large) and an overall homogenization with respect to season (global  $R=0.160$ ,  $P=0.003$ ). Pairwise tests revealed significant differentiation of communities sampled at the 3 pond size-types (all  $P=0.001$ ) but with most pronounced differences in community structure in those sampled from the large pond (pairwise test  $R=0.840$  and 0.777 for contrasts with medium-sized and small ponds, respectively, *vs*  $R=0.293$  for contrasts between medium-sized and small ponds).

There was a strong differentiation of communities in relation to pond function context (i.e. rearing and hibernation ponds) allowing for the fact that there may be seasonal effects (global  $R=0.769$ ,  $P=0.001$  and  $R=0.122$ ,  $P=0.001$ , respectively). Three species contributed collectively to 56% of the dissimilarity (average 71.4%, SIMPER procedure) between the 2 community groupings: *P. elegans* (21.3%); *D. pseudospathaceum* (19.1%); and *O. ranae* (15.4%).

Table 3. Summary of seasonal data (range (mean)) for parasite community richness, overall prevalence (P%) of larval trematode infections and the prevalence (species name abbreviation (%)) of the 9 most frequent and abundant trematodes in *Lymnaea stagnalis* in the studied fishponds

(Abbreviations as in Tables 1 and 2.)

Pond	Season	Mean snail height (mm)	Total no. of species	P (%)	OR (%)	PE (%)	DP (%)	EA (%)	ER (%)	HC (%)	PR (%)	TS (%)	NA (%)
V	Winter	43–47 (45)	4–5 (4·3)	17–33 (27)	–	0–3 (1·4)	3–18 (10·8)	0–2 (0·6)	0–8 (3·8)	0–7 (3·5)	–	–	3–12 (6·6)
	Spring	49	7	56	2·4	2·4	7·3	2·4	7·3	–	–	41·5	–
	Summer	41–52 (48)	3–7 (4·8)	7–64 (34)	0–6 (0·9)	0–4 (2·0)	3–45 (19·4)	0–15 (4·6)	0–4 (1·6)	0–15 (3·2)	0–2 (0·2)	0–7 (1·4)	–
	Autumn	42–52 (45)	4–9 (7·3)	29–60 (44)	0–2 (0·5)	0–10 (2·9)	5–39 (18·0)	1–10 (3·3)	0–6 (3·3)	1–40 (10·6)	0–2 (0·3)	0–11 (4·1)	0–4 (1·4)
HS	Winter	50 (50)	2–3 (2·5)	69–71 (70)	4–6 (4·6)	65–69 (66·8)	0–1 (0·6)	–	–	–	–	–	–
	Spring	46–51 (48)	2–7 (3·7)	50–89 (75)	3–18 (11·3)	49–87 (71·5)	0–1 (0·3)	0–2 (0·6)	0–2 (0·6)	0–4 (1·4)	–	–	–
	Summer	46–49 (48)	4–5 (4·2)	28–57 (44)	2–24 (11·9)	13–52 (24·8)	0–3 (1·1)	0–3 (1·3)	0–6 (3·1)	–	0–1 (0·2)	0–7 (1·4)	–
	Autumn	47–50 (49)	3–5 (4·5)	35–82 (64)	10–37 (20·7)	25–64 (46·4)	0–3 (1·0)	0–3 (0·6)	0–5 (2·2)	0–3 (0·5)	0–3 (1·3)	0–1 (0·3)	0–1 (0·2)
HH	Winter	48–53 (50)	4–6 (5·0)	30–49 (39)	18–31 (23·1)	10–12 (10·9)	2–4 (3·2)	1–2 (1·4)	0–1 (0·3)	–	–	–	0–2 (0·9)
	Spring	47	5	46	17	30	–	–	3	2·9	–	–	1·4
	Summer	40–49 (44)	2–6 (4·1)	6–61 (28)	0–50 (18·4)	3–16 (7·7)	0–3 (0·9)	0–1 (0·1)	0–2 (0·7)	0–3 (0·9)	0–1 (0·2)	0–1 (0·1)	0–1 (0·2)
	Autumn	46–50 (48)	3–6 (5·2)	28–100 (59)	16–85 (40·6)	10–32 (18·0)	0–11 (5·9)	0–5 (2·4)	0–1 (0·2)	–	0–3 (1·3)	–	0–4 (1·1)
VD	Winter	41	3	50	5	38	–	–	5	–	–	–	–
	Spring	35–40 (38)	0–3 (1·7)	0–23 (9)	–	0–19 (6·7)	–	–	–	0–4 (2·0)	–	–	0·3
	Summer	39–44 (42)	1–8 (5·4)	1–63 (30)	0–18 (7·6)	0–29 (14·3)	0–4 (1·5)	0–1 (0·2)	0–9 (1·9)	0–5 (1·1)	0–16 (5·0)	0–1 (0·6)	–
	Autumn	37–43 (40)	3–8 (5·3)	14–71 (44)	2–17 (9·9)	6–57 (25·6)	0–1 (0·5)	0–1 (0·2)	0–10 (2·8)	0–8 (3·4)	0–22 (4·4)	0–1 (0·2)	–
Z	Spring	36–38 (37)	1–2 (1·7)	2–48 (20)	–	2–44 (17·6)	–	–	–	0–4 (2·3)	–	–	–
	Summer	40–50 (44)	2–7 (4·4)	9–67 (36)	0–2 (0·9)	8–64 (29·9)	0–1 (0·1)	0–2 (0·3)	0–5 (1·2)	0–2 (0·4)	0–9 (2·9)	0–1 (0·1)	–
	Autumn	39–43 (41)	4–6 (4·8)	41–67 (49)	3–17 (6·9)	32–38 (35·2)	–	–	0–4 (2·3)	0–1 (0·5)	1–13 (4·3)	–	–

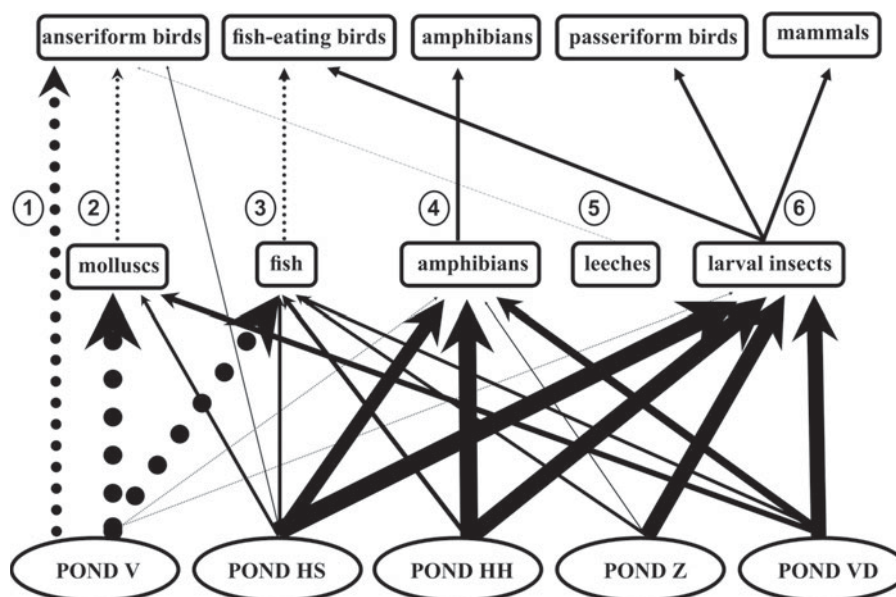


Fig. 4. Diagrammatic representation of the trematode transmission pathways (numbered), with respect to the definitive (top level) and second intermediate (middle level) host groups, of the species parasitizing *Lymnaea stagnalis* in the study ponds (bottom level). Thickness of the arrows to second intermediate host groups is proportional to the frequency of attaining core and secondary status in communities at each pond by the trematodes utilizing a given pathway. Dotted arrows: large pond (V); filled arrows: medium-sized (HS and HH) and small ponds (Z and VD).

Draining in autumn-winter induced a higher compositional variation in the small ponds that resulted in a significant effect of the timing of the annual drawdown on community structure in the medium-sized and small ponds (global  $R=0.293$ ,  $P=0.001$ ).

The above patterns were consistent with the results from separate two-way ANCOVAs on community parameters and the prevalence of the major transmission guilds after the effects of snail mean density and size were partialled out. The first analysis carried out on the data from spring, summer and autumn samples from all ponds (Analysis 1 in Table 5) revealed a significant both temporal and spatial differentiation in component community richness with communities from the large pond V harbouring more species than those from the remaining ponds (Table 5). The mean numbers of species in communities attained higher levels in autumn and those sampled in spring exhibited a higher variation. However, there was a significant effect on overall prevalence (and infracommunity richness; data not shown) by factor ‘season’ only; both parameters exhibited similar increased variability in spring and higher levels in autumn (Table 5; Fig. 6A).

The prevalences of *D. pseudopathaceum* and the species of the guild of anatid generalists did not differ significantly among seasons but were distinctly higher at the large pond V (Fig. 6B, C; Table 5). The average prevalence of *D. pseudopathaceum* at pond V was 16.9% (vs 0.04–3.0% in the smaller ponds) and the average prevalence of the anatid guild was 18.2% (vs 2.9–4.6% in the smaller ponds). Notably, the prevalence of the latter guild exhibited a low in summer communities at pond V which

resulted in a significant interaction between factors ‘pond’ and ‘season’ (Table 5). On the other hand, there was a significant effect of both ‘pond’ and ‘season’ on the prevalence of the plagiogorchioidean guild which exhibited distinctly lower levels at pond V (mean across seasons 2.9%) in comparison with the smaller ponds (means 40.2% and 61.1% for medium-sized ponds HH and HS and 24.1% and 32.0% for small ponds VD and Z) and overall higher levels in autumn and increased variation in spring (Fig. 6D; Table 5). A second analysis was performed on data from 4 instead of 3 seasons, however, only for the 3 ponds (V, HS and HH) for which more winter samples (December to February) were available. The separate two-way ANCOVAs revealed similar patterns of spatial and temporal variability of community parameters the only difference being the significant variation among seasons of the prevalence of the anatid generalists guild (Analysis 2 in Table 5).

#### DISCUSSION

The 14 species found in our geographically restricted study comprised ca 60% of the larval trematode parasites of *L. stagnalis* reported throughout its distributional range in Europe (24 species; see Faltýnková *et al.* 2007 and references therein; Brown *et al.* 2011). The regional trematode richness in *L. stagnalis* in the fishponds of South Bohemia is much higher than that observed in the classical 3-year study of the large eutrophic lake Družno (a total of 9 and 5 species in the qualitative and quantitative samples, respectively; see Wiśniewski, 1958). The faunal richness reported here is also higher than that

Table 4. Temporal sequence of prevalence of the 9 common trematode species in larval trematode communities in *Lymnaea stagnalis*

(C and S indicate species that attained core and secondary status, respectively, in at least one component community within a season. Satellite status is indicated by a "+". The period of rehabilitation at pond HH when sampling was interrupted is indicated by na. Abbreviations as in Tables 1 and 2.)

Pond	Season	PE <sup>a</sup>	OR <sup>a</sup>	DP <sup>b</sup>	ER <sup>c</sup>	HC <sup>c</sup>	EA <sup>c</sup>	TS <sup>c</sup>	NA <sup>c</sup>	PR <sup>d</sup>
V	Summer 2006			S	+	+	+			
	Autumn 2006	+		S	S	S	+	S	+	+
	Winter 2006	+		S	S	S	+		S	
	Spring 2007	+	+	S	S		+	C		
	Summer 2007	+	S	C	+	S	S	S		+
	Autumn 2007			S		C	S	S		
	Summer 2008	+	+	C	+	+	S	+		
	Autumn 2008	S	+	C	+	+	+	+	+	
HS	Summer 2006	S	S	+	+					
	Autumn 2006	C	C	+	+		+	+	+	
	Winter 2006	C	S	+						
	Spring 2007	C	S	+	+		+			
	Summer 2007	C	+			+	+	S		
	Rehabilitation	na	na	na	na	na	na	na	na	na
	Summer 2008	C	C	+	S		+			S
	Autumn 2008	C	C		S	+	+			S
HH	Summer 2006	+	S	+	+					+
	Autumn 2006	C	C	S	+		+		+	+
	Winter 2006	S	C	+	+		+		+	
	Spring 2007	C	S		+	+			+	
	Summer 2007	S	C	+	+	+		+	+	+
	Summer 2008	S	C	+		+	+		+	
	Autumn 2008	S	C	+			+		+	
VD	Summer 2006	C	+	+	+	+				+
	Autumn 2006	C	S	+	+	S				+
	Spring 2007	S				+			+	
	Summer 2007	C	S	+	S	S	+	+		+
	Autumn 2007	C	S		S	+	+	+		+
	Summer 2008	S	S	+	+	+		+		S
	Autumn 2008	S	S		+					C
Z	Summer 2006	C	+		+					+
	Autumn 2006	C	+		+	+				+
	Winter 2006	C	+		+					
	Spring 2007	C				+				
	Summer 2007	C	+	+	+	+	+	+		S
	Autumn 2007	C	S		+					S
	Spring 2008	S				+				
	Summer 2008	C	+		+	+				+

<sup>a</sup>Plagiorchioidean guild; <sup>b</sup>*Diplostomum pseudospathaceum*; <sup>c</sup>Anatid generalists guild; <sup>d</sup>*Phalacrocorax* spp. specialist.

detected in the few recent studies on larval trematodes in *L. stagnalis* in productive water bodies in Finland, Czech Republic, Poland and Germany (range 6–12 species, with an almost complete overlap with the common species in our dataset; see Väyrynen *et al.* 2000; Faltýnková, 2005; Żbikowska *et al.* 2006; Soldánová *et al.* 2010; Brown *et al.* 2011). Surprisingly, we have detected more species than the 2 recent extensive faunistic studies which report pooled data from a wide range of ponds in Germany and Poland (10 and 13 species, respectively; Faltýnková and Haas, 2006; Żbikowska, 2007). Our relatively long-term dataset thus appears to have captured exhaustively the diversity of the trematode species parasitizing *L. stagnalis* in Europe's wetlands.

The diversity of our system, however, does not appear to be associated with accumulation with time since a similar regional pool (12 species) has been identified in 2009 at 2 of the ponds of this study (pond V and HH, see Brown *et al.* 2011).

Not all trematodes of the regional species pool were consistently present in component communities. The 9 species identified by us as common are also among the most frequently recorded in previous inventories of larval trematodes of *L. stagnalis* in Europe (Väyrynen *et al.* 2000; Faltýnková, 2005; Faltýnková and Haas, 2006; Żbikowska *et al.* 2006; Żbikowska, 2007; Faltýnková *et al.* 2007). Further, the most frequent and prevalent trematodes which contributed substantially to the structure of



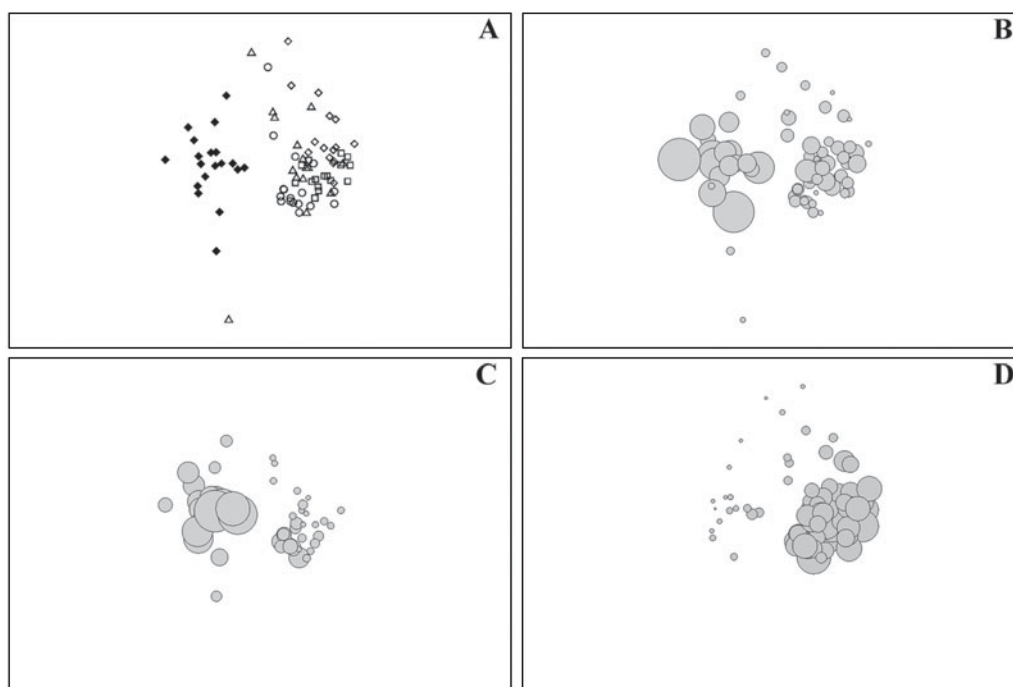


Fig. 5. (A) Two-dimensional MDS configuration plot of the 83 trematode component communities based on the similarity in community structure (Bray-Curtis index). (B) The same MDS plot but with the prevalence of the guild of anatiid generalists superimposed. (C) The same MDS plot but with the prevalence of the larid generalist *Diplostomum pseudospathaceum* superimposed. (D) The same MDS plot but with the prevalence of the plagiorchioidean guild superimposed. The size of the circles is directly proportional to the prevalence of the respective guild in each component community. Key to symbols: pond V (filled diamonds); pond HS (open squares); pond HH (open circles); pond VD (open triangles); pond Z (open diamonds).

component communities and patterns of parasite flow within ponds, *P. elegans*, *O. ranae* and *D. pseudospathaceum*, were recognized in these faunistic studies as either “dominant” (Faltýnková and Haas, 2006; Faltýnková *et al.* 2007) or “most common” (Žbikowska *et al.* 2006) and “most frequent” (Žbikowska, 2007). In spite of the different spatial configuration, these 3 most widespread species in the large-scale inventories in Europe exhibited consistently high prevalences (i.e. local abundance) in their respective habitats during the entire course of sampling. Our study, therefore, provides evidence for a linkage across scales of the distribution-abundance relationship for the larval trematodes in *L. stagnalis* recently indicated by Brown *et al.* (2011).

Although overwintering of larval trematode infections by *P. elegans* and *D. pseudospathaceum* has been inferred from the presence of infections in *L. stagnalis* sampled in April (Žbikowska *et al.* 2006), our study is the first to show that the larval stages of almost all common species of *L. stagnalis* overwinter in the snails. Further, not only the snail populations sampled in winter carried infections with 3 to 6 species but cercarial emergence occurred readily during screening in the laboratory. The emergence of large numbers of cercariae indicates that the functional activity in the germinal masses has not been arrested in both wintering daughter sporocysts and rediae. To date, the only seasonal data on

reproduction of trematode parthenogenetic generations are based on observations of larval parasites in populations of *Hydrobia* spp. and *Littorina saxatilis* from the Barents and White seas (Galaktionov and Dobrovolskij, 2003). Our data thus provide an exceptional example shedding light on winter development of larval infrapopulations from freshwater and less harsh environments, especially with respect to representatives of families for which no data exist (Diplostomidae, Plagiorchioidae and Telorchioidae) or arrested functional activity of germinal masses has been demonstrated (Echinostomatidae).

Overwintering of infections in snails may have ramifications far beyond the knowledge on arrested development in larval trematodes. First, species whose cercarial embryonic development is not blocked by low winter temperatures may have the competitive advantage over the rest due to the fast restart of cercarial emergence as the water warms up. This would affect the development and structure of local trematode communities and consequently the distribution-abundance relationship in trematodes parasitizing *L. stagnalis*. This is supported by the fact that all 7 species overwintering in snail populations in the area of our study attained core status in larval trematode communities and that the 3 species with the highest prevalence and frequency in winter samples, *P. elegans*, *O. ranae* and *D. pseudospathaceum*, are identified as the most widespread and

Table 5. ANCOVA statistics for the variation in trematode component community richness, overall prevalence and the prevalence of infection of the populations of *Lymnaea stagnalis* with the 3 major transmission guilds as a function of season and pond of origin and their interactions

(Differences between the 2 analyses are indicated in bold.)

Models/Analyses	Source of variation	Analysis 1 All ponds: 3 seasons			Analysis 2 Large and medium-sized ponds: 4 seasons		
		D.F.	F	P	D.F.	F	P
Component community richness (R <sup>2</sup> =0.516/0.602) <sup>a</sup>	Global model	16, 57	3.74	0.0001	13, 38	4.42	0.0001
	Pond	4	3.01	0.026	2	12.00	<0.0001
	Season	2	5.45	0.007	3	3.19	0.034
	Pond × Season	8	1.55	ns	6	2.25	ns
Overall prevalence (R <sup>2</sup> =0.610/0.673)	Global model	16, 57	4.95	<0.0001	13, 38	4.05	0.0004
	Pond	4	0.973	ns	2	2.77	ns
	Season	2	7.76	0.001	3	4.01	0.014
	Pond × Season	8	1.86	ns	6	0.71	ns
<i>D. pseudospathaceum</i> prevalence (R <sup>2</sup> =0.823/0.804)	Global model	16, 57	16.60	<0.0001	13, 38	11.98	<0.0001
	Pond	4	18.80	<0.0001	2	37.97	<0.0001
	Season	2	2.29	ns	3	2.32	ns
	Pond × Season	8	1.78	ns	6	0.53	ns
Anatid generalists guild prevalence (R <sup>2</sup> =0.643/0.722)	Global model	16, 57	6.42	<0.0001	13, 38	7.60	<0.0001
	Pond	4	14.47	<0.0001	2	23.70	<0.001
	Season	2	2.64	<b>ns</b>	3	4.67	<b>0.008</b>
	Pond × Season	8	2.96	0.008	6	2.90	0.020
Plagiorchioidean guild prevalence (R <sup>2</sup> =0.739/0.831)	Global model	16, 57	10.09	<0.0001	13, 38	14.43	<0.0001
	Pond	4	15.29	<0.0001	2	37.50	<0.001
	Season	2	7.67	0.001	3	3.98	0.015
	Pond × Season	8	1.77	ns	6	1.36	ns

<sup>a</sup>(R<sup>2</sup> Analysis 1/R<sup>2</sup> Analysis 2); P-values >0.05 are indicated by ns.

prevalent in the large-scale inventories carried out in Europe (see above). Secondly, the overwintering of infected snails may compound the effects of eutrophication among years by ensuring higher densities of infected snails for the following year (Johnson *et al.* 2007). This effect may have resulted in the relative stability of the temporal sequence of the prevalence of the common species and the weak seasonal effects on prevalence and community structure in our system.

In fact, the significant differentiation in community structure with respect to the size of the ponds (i.e. small, medium-sized and large) was largely due to the higher variation and differentiation observed in first post-drawdown communities sampled at the small ponds. The overall higher variability in prevalence in spring communities, and of the plagiorchioidean guild in particular, substantially contributed to the significant seasonal effect on overall prevalence. Winter snail mortality, as well as natural spring mortality of the oldest cohort, may have partly contributed to this effect in all ponds. However, the higher variation in both snail populations and trematode community structure in overwintered snails in early summer at the ponds VD and Z, suggests that communities in small ponds are less resilient to winter dewatering; this was supported by

the significant differentiation in relation to the timing of the annual drawdown. These 2 ponds are subjected to prolonged late autumn-winter drawdown in contrast to short spring drawdown in the medium-sized ponds HS and HH. Snails are weaker in dry environments at low temperatures (Hong *et al.* 2002). Therefore, winter drawdown-induced snail mortality appears a plausible explanation for the changes in larval trematode communities in the season following drawdown. However, the disturbance effects did not persist in communities later in the year.

Although the large pond V supported a higher proportion of total diversity (93% *vs* 64–71% for smaller ponds) and this was reflected in the significant differences in component community richness among ponds, we detected no effect of pond context on overall prevalence and infracommunity richness and this was in accord with our initial hypothesis of substantial homogeneity of larval trematode infections in *L. stagnalis* based on the small geographical extent of the study and the vagility of birds acting as the final hosts for most of the species in this bird-parasite dominated snail-trematode system.

However, the spatial heterogeneity in recruitment of larval trematodes to the populations of their

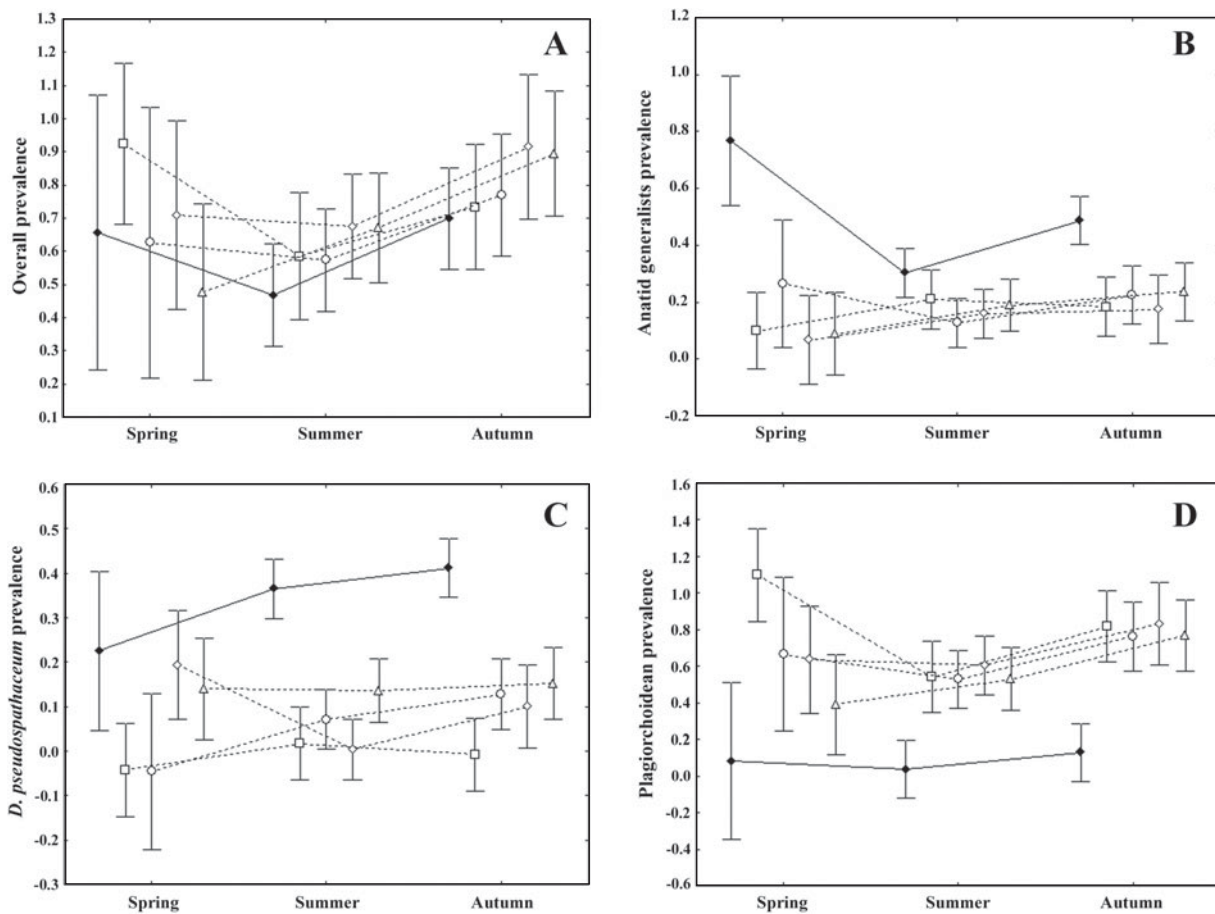


Fig. 6. ANCOVA interaction plots showing aggregate differences (least squares means) in trematode prevalence (arcsin square-root-transformed) among seasons and between ponds. (A) Overall prevalence. (B) Prevalence of the guild of anatic generalists. (C) Prevalence of the larid generalist *Diplostomum pseudospathaceum*. (D) Prevalence of the plagiorchioidean guild. Vertical bars denote 0.95 confidence intervals. Key to symbols: pond V (filled diamonds); pond HS (open squares); pond HH (open circles); pond VD (open triangles); pond Z (open diamonds).

first intermediate snail hosts was unexpected. This was indicated semi-quantitatively by the differential contribution to the parasite flow in the 5 ponds of the 9 common species and supported by the significant contrasting patterns of component community similarity in relation to pond context (size and function) and the prevalence of the 3 major transmission guilds driving this similarity. Notably, the distance between ponds was not associated significantly with the observed variations in similarity in the dataset studied. The 5 seemingly similar ecosystems generally split into 2 groups with respect to the composition and structure of component communities of larval trematodes in *L. stagnalis*. Those sampled at the large pond V, which also supported more species, were dominated by anatic and larid generalists with active miracidial transmission that infect birds directly or *via* consumption of the mollusc and fish intermediate hosts. On the other hand, communities sampled in the medium-sized and small ponds were dominated by the 2 species of the plagiorchioidean guild infecting *L. stagnalis* *via* egg ingestion and using larval insects or amphibians as intermediate hosts in their life cycles.

What is driving this spatial heterogeneity of larval trematode community composition and structure in an area where a large number of species have generally had the opportunity to colonize and persist in the snail populations? We put forward 3 hypotheses for the remarkable differences in larval trematode flow in the similar and closely located eutrophic ponds in South Bohemia. The first plausible prediction is that larval parasite communities in the studied populations of *L. stagnalis* reflect species-specific differences in parasite colonization potential. Species may also vary in their capacities to colonize snails in smaller ponds that represent more unstable habitats less resilient to disturbance such as a prolonged annual drawdown. We suggest that the 'active-passive' dichotomy in miracidial transmission strategies of the available species in the regional pool in interaction with the pond context (i.e. variability related to size) may have resulted in the observed dichotomy in the representation of the major transmission guilds in component communities. This is supported by the fact that the large, and presumably less variable pond V, sustained dispersal-dependent or 'pulsed transmission' communities which were

dominated by species with active miracidial dispersal strategies which generally possess narrower transmission windows due to the discontinuous presence of the final bird hosts. On the other hand, the smaller and less resilient to disturbance ponds supported 'press transmission' (or continual presence) communities dominated by 2 species dispersing *via* environmentally resistant infective stages.

Both plagiorchioidean species that determined the structure of 'press transmission' communities possess a number of characteristics that indicate they could sustain both continual presence and high infection levels in the populations of their intermediate hosts. First, both species exhibit low host specificity to the second intermediate and final hosts. Although *L. stagnalis* serves as the main intermediate host of *O. ranae*, this species also infects successfully *R. auricularia*, *Radix ovata* (Draparnaud) and *Stagnicola palustris* (Müller); this is viewed as a mechanism compensating the lack of actively infecting miracidium in the life cycle (Galaktionov and Dobrovolskij, 2003). In addition to a wide specificity for the second intermediate hosts (benthic larvae of aquatic insects of 6 orders, including 35 species of Odonata; see Sharpilo and Iskova, 1989), infective cercariae of *P. elegans* exhibit behavioural adaptations reducing the probability of superinfection of the insect hosts (Lowenberger and Rau, 1994) and ensuring completion of the life cycle by avoiding parasite-induced mortality of the second intermediate hosts. Secondly, plagiorchioideans disperse *via* environmentally resistant eggs in which the miracidia can remain infective for 5–6 months (Galaktionov and Dobrovolskij, 2003). Accumulation in both space and time of the eggs in combination with massive daily cercarial production (1000–3000 cercariae per snail on average; Lowenberger and Rau, 1994; Galaktionov and Dobrovolskij, 2003) ensures high infection probabilities *via* saturation of the environment with trematode dispersal stages. These plagiorchioidean adaptations to transmission, therefore, represent an advantage in colonizing snail populations in unstable habitats such as the small fishponds studied by us resulting in characteristic 'press' communities which sustain drawdown disturbance. However, the 'active-passive' dispersal dichotomy cannot explain the remarkably low occurrence and prevalence of both species of the plagiorchioidean guild in communities from the large pond V.

The second hypothesis we put forth is that the size and function (and associated management) of the ponds are major determinants of the structure of larval communities in *L. stagnalis* in the eutrophic ponds studied. Thus increased transmission rates of trematodes maturing in anatid and larid birds at the large pond V may reflect higher abundance and/or more permanent occupancy patterns by birds of these waterfowl guilds. Whereas anseriform birds may be

dependent on a larger availability of feeding and nesting habitats existing in large ponds, fish-eaters (gulls, terns and cormorants in particular) can be readily attracted by nearly permanently maintained high fish densities at the large pond V (e.g. Suter, 1994; Musil *et al.* 1995). We suggest that this latter aspect of pond management, i.e. the differential use of the ponds with respect to the duration of high fish standing crops (reaching up to 1000 kg ha<sup>-1</sup>; Kořínek *et al.* 1987), has a top-down effect on transmission pathways of the trematodes in the studied ponds, which in turn, leads to marked differentiation of community composition and structure in spite of the high colonization potential of the plagiorchioidean trematodes.

Three lines of evidence support our suggestion. Fish presence in both farm dams and ponds and natural lakes is the main factor for dramatic decreases in richness and abundance of anuran tadpoles (see Gillespie and Hero, 1999 for a review). The large pond V is used as a rearing fishpond for accommodation of carp fish stocks during the third growing season and is also regularly stocked with predatory fish, *Esox lucius* L., *Sander lucioperca* (L.) and *Silurus glanis* L., whereas the presence of the carp stock in the 4 smaller hibernation ponds is restricted to winter when fish cluster together and cease movements and feeding. Therefore, the continuous fish stocking and especially of the large predators at pond V, may have reduced amphibian reproduction and abundance as evidenced by the contrasted low prevalence and temporary unstable occurrence of *O. ranae*. However, even if amphibian populations at pond V have been impaired due to the continuous presence of fish stocks, resulting in a drastic reduction of probabilities of infection of *L. stagnalis* with *O. ranae*, there must be another factor acting in a similar manner on the populations of *P. elegans*, a species that utilizes a different transmission pathway.

Increased fish predation on benthic invertebrates can be an additional mechanism leading to a top-down response of plagiorchioidean abundance in the area of study. Although fish feed is supplied, the predator pressure on invertebrates might still be very high, especially in spring when, according to the pond management cycle, carp feed on natural food. Consequently, differential predator pressure on benthic invertebrate intermediate hosts may account for disparate infection levels of *P. elegans* between ponds. Finally, although carp is omnivorous, the bulk of its diet consists of detritus (Chapman and Fernando, 1994; Michel and Oberdorff, 1995; García-Berthou, 2001). Such feeding results in the uprooting of macrophytes and an increase of water turbidity in the shallow microhabitats where plagiorchioidean transmission occurs, thus reducing the probability of egg encounter by snails. Therefore, small-scale differences in trematode transmission



habitats between the main and hibernation ponds associated with carp feeding may have also contributed to the differential distribution and abundance of the passively dispersing species of the plagiorchioidean guild. The above considerations collectively support the idea that the differential use of the ponds by definitive bird and amphibian hosts combined with the nature of infective stages colonizing snail populations and the variation in the abundances of second intermediate hosts contribute to the contrasting patterns of infection with the 3 major transmission guilds in our system.

However, it is possible that the high productivity of the ponds associated with increased density of both intermediate and final hosts, may have driven the infection rates above the threshold when interspecific interactions can take place as indicated by the observed rates of species co-occurrence in infracommunities. Although we consider the number of multiple infections as low with respect to producing sizeable effects on the distributions of infracommunity richness among ponds, the overall rates are, to the best of our knowledge, among the highest observed in snail-trematode systems. Compared with the data on European populations of *L. stagnalis*, the overall rate of multiple larval trematode infections in the area of our study is more than 10 times higher than those of a dataset collected over 20 years by Loy and Haas (2001; calculated from their Table 1) and of less-inclusive short-term studies of Faltýnková (2005) and Faltýnková and Haas (2006): 3.8% (out of 6534 examined snails) vs 0.30% (out of total sample of 43 441 snails); 0.21% (out of 473 snails); and 0.33% (out of 1219 snails), respectively. Compared with the available data from freshwater pulmonates studied in North America, the proportion of multiple infections in *L. stagnalis* is much higher than that in *Helisoma anceps* (Menke) (in 3.8% vs 0–0.18% of all snails examined; see Fernandez and Esch, 1991; Williams and Esch, 1991; Esch *et al.* 1997) and appears closer to the values observed in *Stagnicola emarginata angulata* (Sowerby) (7.2%; see Cort *et al.* 1937) and *Physa gyrina* (Say) (7.2%; see Snyder and Esch, 1993). A notable similarity with the latter 2 host-parasite systems is that the bulk of multiple infections in *L. stagnalis* were associated with egg-transmitted species.

We, however, observed heterogeneity in the distributions of multiple infections that was associated with the heterogeneity in species composition among ponds. Whereas the low rates of trematode co-occurrence in the small ponds is probably due to the higher variability of these habitats affecting the relative abundance of the trematode infective stages, and thus worsening the probability of interspecific encounter, the distinctly higher rates of multiple infections in the medium-sized ponds HS and HH as opposed to the large pond V deserve a comment. The high infection rates in our system indicate

increased opportunities for trematode recruitment so that the main assumption of a competition model (Kuris, 1990; Sousa, 1990; Esch *et al.* 2002) may appear fulfilled in the larger ponds. A possible scenario for the development of infracommunities in *L. stagnalis* would include an initial colonization by subordinate species *sensu* Kuris (1990) followed by competitive exclusion by dominant species *sensu* Kuris (1990).

The rich and abundant trematode species pool in the eutrophic environments studied appears well suited to this scenario. The 3 transmission guilds recognized by us differ not only in their use of the second intermediate hosts but also in their intramolluscan development which might be associated with their competitive abilities at the infracommunity level: (i) sporocyst–redia i.e. dominant species (the anatid guild except *T. szidati*) and (ii) sporocyst–sporocyst i.e. subordinate species (the plagiorchioidean guild and *D. pseudospathaceum*) (see Kuris, 1990; Fernandez and Esch, 1991 and references therein). The 2 species of the plagiorchioidean guild with their wide distribution and host specificity, egg dispersal and long life span of the miracidia, coupled with snail susceptibility (e.g. within day of hatching, see Zakikhani and Rau, 1999) are perhaps the best candidates for initial colonizers of the snail populations. An overlaid ‘pulsed’ massive infection with species of the anatid guild (? and *D. pseudospathaceum*) in pond V may have caused subsequent exclusion through competition of the inferior plagiorchioideans which otherwise (i.e. at the conditions of sporadic presence and low prevalences of the dominant guilds in smaller ponds) would co-exist in infracommunities as evidenced by the distinctly higher infracommunity richness and levels of double infections with the 2 plagiorchioidean species in the medium-sized ponds. Therefore, in spite of the variable strength of trematode transmission, eutrophic fishponds appear to provide conditions in which interspecific interactions may be an important mechanism with a bottom-up effect on community structure, maintaining the observed parasite flow dichotomy in our system.

Summarizing, although pond context emerges as a major determinant for the contrasting patterns of trematode flow observed in the eutrophic fishponds in South Bohemia, it in no way negates the alternative hypothesis for a bottom-up structuring of larval communities in the model snail-trematode system studied. We believe that our system offers excellent opportunities to test the hypothesis of competition as an important process in eutrophic environments in Central Europe and elsewhere especially because it meets 2 of its 3 assumptions (Kuris, 1990): (i) increased transmission rates as evidenced by the high overall prevalence; and (ii) co-occurrence of potential competitors (species of the 3 transmission guilds identified in our study). We have established

a dominance hierarchy for the trematodes in *L. stagnalis* and are currently addressing the rates of recruitment in this system and the influence of heterogeneity induced by pond context on the patterns and rates of interspecific interaction at the infracommunity level. Our study indicates that whatever the driving force, heterogeneity in recruitment or interspecific interactions, or both, they can define predominant pathways of trematode transmission in eutrophic environments.

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