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THE ROLE OF SPATIAL AND TEMPORAL HETEROGENEITY AND COMPETITION IN STRUCTURING TREMATODE COMMUNITIES IN THE GREAT POND SNAIL, *LYMNAEA STAGNALIS* (L.)

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ABSTRACT: We assessed how spatial and temporal heterogeneity and competition structure larval trematode communities in the pulmonate snail *Lymnaea stagnalis*. To postulate a dominance hierarchy, mark-release-recapture was used to monitor replacements of trematode species within snails over time. In addition, we sampled the trematode community in snails in different ponds in 3 consecutive years. A total of 7,623 snails (10,382 capture events) was sampled in 7 fishponds in the Jindřichův Hradec and Třeboň areas in South Bohemia (Czech Republic) from August 2006 to October 2008. Overall, 39% of snails were infected by a community of 14 trematode species; 7% of snails were infected with more than 1 trematode species (constituting 16 double- and 4 triple-species combinations). Results of the null-model analyses suggested that spatial heterogeneity in recruitment among ponds isolated trematode species from each other, whereas seasonal pulses in recruitment increased species interactions in some ponds. Competitive exclusion among trematodes led to a rarity of multiple infections compared to null-model expectations. Competitive relationships among trematode species were hypothesized as a dominance hierarchy based on direct evidence of replacement and invasion and on indirect evidence. Seven top dominant species with putatively similar competitive abilities (6 rediae and 1 sporocyst species) reduced the prevalence of the other trematode species developing in sporocysts only.

In ecology, there is considerable disagreement about the extents to which communities of similar species are structured, i.e., whether species have statistical associations or are random assemblages. At the heart of the structure debate is the role of interspecific competition. While most authors agree that interspecific competition can occur, the extent to which it affects the distribution and abundance of competitors is controversial (Gotelli and McCabe, 2002). Trematodes in snails make good model systems for investigating community structure because each snail host is a potential community of trematodes, permitting many communities to be analyzed (Esch and Fernández, 1993). Many trematode species can infect a snail species, but it is uncommon to find more than 1 species of trematode parthenitae infecting the same individual snail. A lack of multiple infections is one form of evidence that indicates a parasite community is structured. The reason for this structure has been the subject of substantial research (reviewed by Esch and Fernández, 1993, 1994; Kuris and Lafferty, 1994; Esch et al., 2001, 2002; Curtis, 2002). Molluscs are almost exclusively the first intermediate hosts for digenetic trematodes. They are model systems for addressing questions about community structure in ecology because each host is a patch of habitat with limited nutrient and spatial resources for which trematodes potentially compete (e.g., Kuris, 1990; Sousa, 1990, 1992, 1994; Lafferty et al., 1994).

An extensive series of experiments by Lie and co-workers (see reviews by Lie et al., 1968; Lim and Heyneman, 1972) found that, within the individual host, trematode communities are structured via antagonistic interactions (competition) that are hierarchical in nature so that dominant species fend off, or replace, subordinate species. Competition occurs because asexual reproduction within the snail host results in dense populations of rediae, or sporocysts, or both, in the same target organs (usually hepatopancreas and gonads). Kuris (1990) examined trematode communities in the salt marsh prosobranch snail, *Cerithidea californica*, at the infracommunity (within a host) level. He concluded that competition was the major structuring force in this system and suggested criteria for interactions to strongly affect infracommunity structure, i.e., a high overall prevalence, a dominance hierarchy between potential competitors, and predominance of interference competition. An independent study on the same snail–trematode system provided support for the deterministic assembly rules at the level of individual hosts (Sousa, 1993).

However, for trematodes to compete they must first infect the same snail. An alternative hypothesis for the lack of multiple infections in trematode communities was proposed by Cort et al. (1937), who emphasized the importance of spatial and temporal factors that affect the transmission of trematodes from the definitive vertebrate hosts to snails. They proposed that there is variability in recruitment in space and time, isolating trematode species, making multiple infections improbable and, therefore, competition rare. A study of *C. californica* (see Sousa, 1990), but especially of the freshwater pulmonate snails *Helisoma anceps* and *Physa gyrina*, found heterogeneity in recruitment generated by the behavior of the definitive hosts, snail population dynamics (mortality, recruitment, size structure), habitat characteristics, and the nature of infective agents (eggs or miracidia) (Fernandez and Esch, 1991a, 1991b; Williams and Esch, 1991; Snyder and Esch, 1993). These authors assumed, though did not explicitly test, that such spatial and temporal heterogeneity isolated trematode species from each other, explaining the relative infrequency of double infections.

Lafferty et al. (1994) developed a method of sequential analysis that allows assessment of the effects of spatial and temporal heterogeneity and competition using samples replicated in time and space. From this work, it became apparent that heterogeneity could intensify or reduce species interactions. Therefore, demonstration of heterogeneity, per se, did not necessarily explain a lack of multiple infections in trematode communities. Across many studies, heterogeneity, formerly thought to be isolated species, actually leads to the intensification of interactions; subsequently,

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competition significantly reduces the prevalence of subordinate species (Kuris and Lafferty, 1994).

We now know that at least 3 mechanisms (spatial heterogeneity, temporal heterogeneity, and competition) can structure trematode communities so that they depart from a random assemblage of the available species (Kuris and Lafferty, 1994). One way to measure community structure is to compare the observed frequency of multiple infections with expected frequencies determined by a null model of independent assortment. Trematode communities might be structured if final hosts are not evenly distributed in space. As proposed by Cort et al. (1937), spatial heterogeneity could isolate species if niche partitioning by final hosts leads trematode species to recruit in different locations, reducing the potential for multiple infections in a snail. Alternatively, aggregated distributions of final hosts would lead most trematodes in the community to infect the same subpopulation of snails, resulting in a higher than expected frequency of double infections. For instance, sites with high abundances and diversity of final host birds have snails with a high prevalence of infection with diverse communities of trematodes (Hechinger and Lafferty, 2005). Finally, genetic or physiological differences in hosts could make some populations more susceptible to infection than others. Temporal variation in trematode recruitment can lead to similar increases or decreases in trematode interactions, as can spatial heterogeneity, particularly if trematode recruitment has strong seasonal patterns. Furthermore, species interactions might structure trematode communities. Infection by 1 species might impair the immune defense of a snail, increasing the ability of other species to invade, leading to a higher than expected frequency of double infections (e.g., Lim and Heyneman, 1972; Adema and Loker, 1997; see also Lafferty, 2002). In a single trematode community, each mechanism above may operate to varying degrees, leading to a net frequency of multiple infections that may, or may not, vary from random assortment.

In the present study, we investigated the structure of larval trematode communities in a lymnaeid snail from Europe. The freshwater pulmonate *Lymnaea stagnalis* (L.) (Gastropoda: Lymnaeidae) is one of the most widespread snails in Europe and Asia (invasive in North America, Australia, and New Zealand). In Europe, *L. stagnalis* is the first intermediate host for at least 24 trematode species (Brown et al., 2011; see also Faltýnková et al., 2007 and references therein) whose larval morphology and life cycles have been subjected to extensive studies originating in Europe as early as the 19th Century. Previously, we examined the composition and structure of communities of larval trematodes in 5 populations of *L. stagnalis* in eutrophic fishponds in South Bohemia (Czech Republic) that represented typical wetland habitats in central Europe. This first, relatively long-term assessment of larval communities in a snail species from Europe used a mark-release-recapture method and revealed high levels of parasitism as well as species-rich and abundant trematode component communities (within a host population) in *L. stagnalis* (Soldánová et al., 2011). This study also depicted a significant spatial differentiation in community structure that, in association with high recruitment rates (Soldánová and Kostadinova, 2011), indicated that the *Lymnaea*–trematode system offers an excellent opportunity to address questions about the role of spatial and temporal heterogeneity and subsequent competition in structuring trematode communities.

For a large original dataset on infracommunities in *L. stagnalis*, we assessed the relative importance of spatial and temporal heterogeneity and competition among larval trematodes in structuring trematode communities, focusing on the extent to which competition at the infracommunity level affects component community structure.

MATERIALS AND METHODS

Sampling system and procedure

From August 2006 to October 2008, individual *L. stagnalis* were collected every 3 wk along predefined sampling transects at 7 fishponds in 2 areas of South Bohemia in the Czech Republic. Three ponds (Vlkovský, Hluboký Sax, and Hluboký u Hamru; referred to as ponds V, HS, and HH) are located in the Třeboň Basin Biosphere Reserve and Protected Landscape Area and 4 (Zavadil, Velký Dvorecký, Bartoňovský, and Špitálský; referred to as ponds Z, VD, B, and S) are located c. 20 km away near Jindřichův Hradec. Data for the ponds are summarized in Table I. In all ponds, sampling transects (approximately 30–50 m wide, 1.5–2 m long, and 0.5–1 m deep) were along shores dominated by stands of *Phragmites australis* and *Typha* spp. Snails were collected haphazardly with a strainer or hand-picked from the emergent vegetation in the littoral zone. These samples provided an opportunity to investigate how variation in the trematode population intensified interactions or isolated species from one another.

A mark-release-recapture procedure was used to investigate trematode infections over time in individual snails so that multiple infections and species switches could be monitored (see Soldánová et al., 2011 for more details on methodology). The purpose of the mark-recapture portion of the study was to postulate dominance relationships among the potentially competing species. A total of 7,623 *L. stagnalis* was collected, checked for trematode infection, marked, and released. Each recaptured snail was considered as an independent observation, resulting in 10,382 capture events comprising 124 distinct samples.

Sampling was uninterrupted during the first winter (2006–2007) due to the unusually mild weather as compared to the second sampling winter (2007–2008), when ponds were covered by ice or drained for 4 to 5 mo (see Soldánová et al., 2011 for details). Fewer samples were collected from 2 of the small ponds, pond B and pond S (9 and 8 samples, respectively), which remained half-drained for most of the sampling period (November 2006 to March 2008); this affected littoral macrophytes in the snail habitat. Because a higher prevalence of infection was observed in larger, mature snails (Żbikowska et al., 2006), and because we sought to obtain large quantities of infected snails, sampling was focused on mature individuals with shell heights ca. 40–50 mm. Sampling from a narrow size range helped reduce the effect of snail age–size on trematode community structure, allowing us to focus on spatial and temporal factors. Snails were examined for cercariae emission only; however, our surveys of the same host–parasite system demonstrated no significant differences between the levels of infection recorded on the basis of cercarial emission and after snail dissection (Brown et al., 2011; Soldánová et al., 2011). Identification of cercariae to species was carried out using a compound microscope following Faltýnková et al. (2007) and Našincová (1992).

Data analysis

Snail populations in both regions were infected by a common pool of parasites (Soldánová et al., 2011). The total dataset comprised 10,382 capture events and 124 distinct samples (Table I). These data were used to detect non-random structure, analyze species associations, and construct a putative dominance hierarchy. A more restricted dataset comprised 10,147 capture events and 98 distinct samples and excluded small samples for which prevalence could not be estimated (Table I). The restricted dataset was used to partition the effect of potential structuring factors on trematode communities and to assess the effect of interactions on the species composition and abundance in component communities. Prevalences were computed by dividing the number of snails infected with a given trematode by the number of snails examined in the relevant sample. When multiple infections were found, the data were included in the prevalence estimates for each species.

First, we sought evidence for any form of non-random structure in the trematode community, e.g., the combined effect of spatial and temporal heterogeneity and interactions among species. Our measure of structure was the difference in the observed and expected frequencies of double infections in the total sample. The null model for estimating the overall expected frequencies of double infections was generated by first pooling all samples (assuming no spatial or temporal component of the sampling) and then estimating how many double infections would occur if the observed trematodes had recruited with equal probability to each snail and then coexisted if multiple species recruited to the same snail. The expected frequency of double infections for each species pair *i*, *j* was calculated by multiplying the sample size, N, by the prevalence (p) of trematode species *i* (p_i) and the prevalence of trematode species *j* (p_j), or Np_ip_j-null model (Kuris, 1990). This model is a simple and conservative way to assess structure (Lafferty et al., 1994) and provided our initial estimate for structure in this community. Because the null model only predicts double infections, each observed triple infection was treated as 3 observed double infections (Kuris, 1990; Kuris and Lafferty, 1994). Observed and expected values were compared by chi square at the significance level of 0.05. The Np_ip_j-null model may underestimate the frequency of double infections if competitive exclusion reduces the prevalence of subordinate species (Lafferty et al., 1994). The formula derived by Lafferty et al. (1994) to estimate pre-interactive species prevalences, i.e., prevalences free of the competition effect, is an alternative null model, Ne_ie_j, where e_i is an estimate of the proportion of snails that species *i* recruited in the absence of any effects of competition or facilitation (see Lafferty et al., 1994 for detailed description). Calculating e_i for a species requires a postulated dominance hierarchy, though dominance need not be absolute or fully resolved.

We postulated a dominance hierarchy of the competitive relationships among trematodes infecting *L. stagnalis* using direct and indirect evidence (Kuris, 1990); the direct evidence was based on observations from our mark-release-recapture study, e.g., if species A replaced species B, A was assumed to be dominant to B. In many cases, replacements were inconsistent, i.e., species B also replaced species A, leading us to extend this method with an assumption that dominance was probable. When direct evidence was not available, we used indirect evidence for dominance based on logic and studies in other systems. These lines of indirect evidence were (1) that related species had similar dominance characteristics, and (2) that rediae species dominated sporocyst species (rediae larval forms have a muscular pharynx used to consume host tissue and also to consume heterospecifics; see e.g., Lie, 1967). In the text and tables, the dominant member of a pair is followed by "$>$", e.g., DP $>$ EA signifies the proposed dominance of *Diplostomum pseudospathaceum* (DP) over *Echinoparyphium aconiatum* (EA).

Once we determined that the trematode community was not a random assemblage, we sought to partition this structure into the effects of space, time, and species interactions following the method of Lafferty et al. (1994). We first calculated the expected number of double infections for each sample, e.g., with the Np_ip_j-null model. We then combined the data into a pooled, well-mixed sample, and calculated the expected number of double infections again. This gave a measure of community structure that assumes no spatial or temporal heterogeneity. Comparing the estimate of the pooled data with the sum of the separate estimates gave a measure of heterogeneity in the trematode community. For example, if, in a sample of trematodes from 100 snails there were 20 species A and 30 species B at site 1 (6 expected A and B), and 60 species A and 10 species B at site 2 (also 6 expected A and B), the sum of the expected double infections would be 12 while the expected double infections from the pooled sample would be $80 \times 40/200 = 16$, the difference being due to a slight isolation between A and B from spatial heterogeneity among ponds. Comparison of the expected number of double infections if recruitment was assumed homogeneous (pooled), with the expected number of double infection summed across the individual samples, indicates whether spatial or temporal heterogeneity in recruitment isolates (pooled $>$ summed) or intensifies (summed $>$ pooled) interactions among species. Specifically, the structuring effect of spatial heterogeneity, independent of time and competition, was determined by summing the expected frequency of double infections ("sum of expected") calculated separately for each pond (pooled across dates) and comparing this to the expected frequency of double infections calculated from the observations summed over all 7 ponds ("expected of pooled"). The structuring effect of temporal heterogeneity independent of space and competition was similarly determined by summing the expected frequency of double infections ("sum of expected") calculated separately for each date at a given pond and comparing this to the expected frequency of double infections calculated from the pooled sampling dates ("expected of pooled"). To evaluate any effect of species interactions after the structuring effects of spatial and temporal heterogeneity, we compared the frequency of observed double infections with the sum of the expected double infections calculated separately for each sample.

Finally, we assessed the importance of competition on the trematode component community by estimating the relative abundance of the trematode species lost to competition or gained due to facilitation (by comparing the observed and expected frequencies).

RESULTS

Of 7,623 snails collected, marked, and released over the 26-mo sampling period, 1,976 (25.9%) individuals were recaptured 21–364 days after release. Of these, 1,397 (70.7%) were recaptured once, 420 (21.2%) twice, 123 (6.2%) 3 times, 27 (1.4%) 4 times, and 9 (0.5%) 5 times. Altogether, this amounted to 10,382 observations for patent infections. Table I summarizes these data. Parthenitae of 14 species belonging to 7 families were found to infect 4,028 (38.8%) *L. stagnalis*; 7 were rediae species (cercariae develop in rediae) and 7 were sporocyst species (cercariae develop in sporocysts only). Table II lists the trematode species and their prevalence in the total and restricted datasets by pond and for pooled data across all 7 ponds. Prevalence was highest in late summer and early autumn (Soldánová et al., 2011). Overall prevalence was generally high (exceeding 40% in 4 ponds); there were only slight differences in prevalence between the total and restricted datasets. Seven species were found in all ponds and 2 species were found in a single pond; the remaining 5 occurred in 2–6 ponds. Two species, *Plagiorchis elegans* (PE) and *Opisthioglyphe ranae* (OR), had the highest prevalences (Table II). Due to the high prevalence, we observed many multiple infections, and changes in trematode species within a snail were detected over time.

Multiple infections and species replacements

A total of 280 (7%) of all infected snails harbored more than 1 parasite species, comprising 16 types of double (275) and 4 types of triple infections (5). No combinations of rediae species were detected. Of the 5 triple infections observed, 2 comprised a combination of 2 sporocyst and 1 redia species, i.e., *O. ranae*, *Australapatemon burti* (AB), and *Echinostoma revolutum*, and *O. ranae*, *P. elegans*, and *Paryphostomum radiatum* (PR), respectively. The remaining 3 comprised 4 sporocyst species, i.e., *O. ranae*, *P. elegans*, and *Trichobilharzia szidati* (TS) (1 case), and *O. ranae*, *P. elegans*, and *D. pseudospathaceum* (2 cases). Observed numbers of snails infected by a given combination of 2 trematode species, including conversions from triple infections, are presented in Table III. *Opisthioglyphe ranae* and *T. szidati* were the most frequently represented in the double infection types. The former species and *P. elegans* were the most frequent of the double infections (78.9%) followed by *O. ranae* and *D. pseudospathaceum* (7.6%).

A change in the trematode species within individual hosts was detected in 123 recaptured snails. Single species infections changed to a different species 32 times (Table IV). The most frequently recorded replacements were of 1 sporocyst species replacing another sporocyst species (17 cases), whereas a redia species replacing a redia species was observed in just 3 snails. Replacement of sporocyst species by redia species was observed 8

TABLE I. Summary data for the fishponds studied in South Bohemia (Czech Republic) and samples of *Lymnaea stagnalis* for the total (T) and restricted (R) datasets. Abbreviations for ponds: V, Vlkovský; HS, Hluboký Sax; HH, Hluboký u Hamru; Z, Zavadil; VD, Velký Dvorecký; B, Bartoňovský; S, Špitálský.

Parameter	Třeboň area						Jindřichův Hradec area											
	V		HS		HH		Z		VD		B		S		Pooled data	
	T	R	T	R	T	R	T	R	T	R	T	R	T	R	T	R
Geographic 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′37″N 14°55′56″E		49°04′43″N 14°56′20″E		49°07′18″N 14°54′53″E		49°07′15″N 14°55′08″E			
Size (ha)	47.09		12.00		11.90		1.86		4.75		1.62		3.88			
No. of snails marked and released	1,141	1,120	895	843	1,418	1,394	1,474	1,457	1,729	1,720	568	568	398	381	7,623	7,483
No. of capture events	1,611	1,554	1,333	1,265	2,123	2,085	1,873	1,833	2,185	2,171	824	824	433	415	10,382	10,147
No. of samples	25	19	21	16	20	17	23	15	18	16	9	9	8	6	124	98

times but, surprisingly, there were also 4 opposite cases when sporocyst species replaced redia species. We observed 47 cases where a double infection changed to a single infection. These concerned either double infections in which 1 of the species was eliminated or single infections invaded by another species that subsequently eliminated the first infecting species (31 and 16 cases, respectively). Based on the dissections of other snail samples, all trematode species parasitizing *L. stagnalis* occupied the same microhabitat, the hepatopancreas, expanding to the gonad where the larval infrapopulations grow and eventually castrate the snail.

There were 19 negative ($P < 0.05$) and 3 positive ($P < 0.05$) associations among species pairs (Table V). We used these associations to help inform the dominance hierarchy, not for statistical inference about structure; note that the critical alpha for judging a specific species-pair interaction should be adjusted for multiple comparisons. A statistical analysis of the total dataset (10,382 capture events) revealed fewer observed double infections than would be expected by chance (290 vs. 520; $\chi^2_{(3)} = 107.09$, $P < 0.001$) for the observed 19 species combinations, indicating that trematode communities in *L. stagnalis* exhibit non-random structure driven by competitive or isolating mechanisms (or both).

Dominance hierarchy

A putative dominance hierarchy was constructed for all 14 trematode species of *L. stagnalis* observed in our study (Fig. 1), using direct evidence of replacement or invasion (inferring the latter species to be dominant) (Table VI), or indirect evidence based on past studies (Kuris, 1990). *Opisthioglyphe ranae* and *P. elegans* were the most frequently observed species in infection changes (93 of 123 cases, i.e., 75.6%). Of these, *P. elegans* was evaluated as dominant over *O. ranae* 50 times vs. 30 in which *O. ranae* dominated over *P. elegans*. *Diplostomum pseudospathaceum* was found to dominate over some of the species in 13 cases. When direct evidence was not available or showed partial dominance, i.e., inconsistent observations, e.g., NA (*Notocotylus attenuatus*) > OR; or HC (*Hypoderaeum conoideum*) > PE; PE > TS; see Table VI, indirect evidence that sporocyst species are subordinate to rediae species and that related species likely interact in similar manner was used. Decisions regarding putative dominance were conservative. In some cases, there was no significant interaction among species pairs but there was limited evidence of replacement in the recapture data. In these cases, dominance was not postulated because, if it occurred, its effects on species abundance were not detectable in this study. When direct evidence contrasted with indirect evidence, the direct evidence was applied (DP > EA; PE > MA [*Moliniella anceps*]; see Table VI).

After accounting for spatial and temporal heterogeneity, the observed number of double infections between sporocyst and rediae species was substantially lower than expected (27 vs. 244; $\chi^2_{(3)} = 197.63$; $P < 0.0001$) (Table V), consistent with the expectation that rediae species and sporocyst species compete (Lafferty et al., 1994). All sporocyst species except *Australapatemon minor* (AM) and *Cotylurus cornutus* were negatively associated (Table V). No double infections among rediae species were observed in our study, suggesting that interactions among rediaee species led rapidly to competitive exclusion. The difference in the observed and expected number of co-occurrences of sporocyst species was lower than for rediae species (263 vs. 381;

TABLE II. Trematode species in *Lymnaea stagnalis* from the fishponds in South Bohemia (Czech Republic) and their abbreviations used in the text and tables. Prevalence (%) is provided for each pond studied for the total (T) and restricted (R) datasets. Multiple infections were treated as infections of each involved species. Abbreviations for ponds: V, Vlkovský; HS, Hluboký Sax; HH, Hluboký u Hamru; Z, Zavadil; VD, Velký Dvorecký; B, Bartoňovský; S, Špitálský.

			Třeboň area						Jindřichův Hradec area									
			V		HS		HH		Z		VD		B		S		Pooled data	
Intramolluscan stage/Family	Species code	Species	T	R	T	R	T	R	T	R	T	R	T	R	T	R	T	R
Rediae species																		
Echinostomatidae	ER	*Echinostoma revolutum*	2.86	2.96	1.73	1.42	0.66	0.67	0.96	0.87	1.46	1.47	0.85	0.85	7.62	7.95	1.67	1.64
	HC	*Hypoderaeum conoideum*	4.41	4.25	0.53	0.47	0.52	0.53	0.69	0.55	1.97	1.93	1.70	1.70	4.39	4.58	1.71	1.66
	PR	*Paryphostomum radiatum*	0.37	0.39	0.98	1.03	0.57	0.58	2.46	2.45	3.39	3.41	5.46	5.46	8.78	9.16	2.25	2.30
	EA	*Echinoparyphium aconiatum*	2.36	2.32	0.53	0.47	1.13	1.15	0.27	0.27	0.14	0.14	–	–	–	–	0.74	0.73
	MA	*Moliniella anceps*	0.37	0.39	0.38	0.40	–	–	–	–	–	–	–	–	–	–	0.11	0.11
	ERE	*Echinoparyphium recurvatum*	0.06	0.06	–	–	–	–	–	–	–	–	–	–	–	–	0.01	0.01
Notocotylidae	NA	*Notocotylus attenuatus*	1.30	1.35	0.15	0.16	0.52	0.53	0.05	–	0.05	0.05	0.12	0.12	–	–	0.36	0.35
Sporocyst species																		
Plagiorchiidae	PE	*Plagiorchis elegans*	2.11	2.12	50.34	50.28	13.24	12.76	25.41	25.10	14.42	14.37	11.04	11.04	0.69	0.72	18.02	17.75
Telorchiidae	OR	*Opisthioglyphe ranae*	0.43	0.45	14.10	14.23	25.72	26.00	1.66	1.47	6.82	6.86	21.48	21.48	10.85	10.84	11.03	11.11
Diplostomidae	DP	*Diplostomum pseudospathaceum*	18.13	18.08	1.13	1.11	3.06	3.07	0.16	0.05	1.05	1.06	0.36	0.36	4.39	4.58	4.05	3.99
Schistosomatidae	TS	*Trichobilharzia szidati*	3.66	3.60	0.98	0.95	0.09	0.10	0.11	0.11	0.37	0.37	3.52	3.52	0.92	0.72	1.13	1.10
Strigeidae	AM	*Australapatemon minor*	–	–	–	–	0.05	0.05	0.53	0.55	0.14	0.14	–	–	0.46	0.48	0.15	0.16
	AB	*Australapatemon burti*	0.19	0.19	–	–	–	–	–	–	–	–	0.24	0.24	6.24	6.51	0.31	0.32
	CC	*Cotylurus cornutus*	0.06	0.06	–	–	–	–	–	–	–	–	–	–	–	–	0.01	0.01
Overall prevalence			35.3	35.1	63.5	63.2	41.1	41.0	31.6	30.9	27.9	27.9	42.6	42.6	43.4	44.6	38.8	38.5

TABLE III. Double infections observed (O) in *Lymnaea stagnalis* in South Bohemia (Czech Republic). Data from total (T) and restricted (R) datasets include conversions from triple infections indicated by an "*". Triple infections were counted as 3 double infections. Abbreviations for ponds: V, Vlkovský; HS, Hluboký Sax; HH, Hluboký u Hamru; Z, Zavadil; VD, Velký Dvorecký; B, Bartoňovský; S, Špitálský.

	Třeboň area									Jindřichův Hradec area														
	V			HS			HH			Z			VD			B			S			Pooled data		
Trematode species combination	O	T	R	O	T	R	O	T	R	O	T	R	O	T	R	O	T	R	O	T	R	O	T	R
Sporocyst–sporocyst species																								
Opisthioglyphe ranae + Plagiorchis elegans	2	2	2	84	85	79	72	75	74	10	10	8	37	37	37	12	12	12	–	–	–	217	221	212
O. ranae + Australapatemon burti	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1	2	2	1	2	2
O. ranae + Diplostomum pseudospathaceum	1	1	1	2	2	2	14	16	16	1	1	–	2	2	2	1	1	1	–	–	–	21	23	22
O. ranae + Trichobilharzia szidati	–	–	–	–	1	1	–	–	–	–	–	–	–	–	–	3	3	3	–	–	–	3	4	4
T. szidati + P. elegans	1	1	1	4	5	5	–	–	–	1	1	1	1	1	1	1	1	1	–	–	–	8	9	9
T. szidati + D. pseudospathaceum	1	1	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1	1	1
P. elegans + D. pseudospathaceum	–	–	–	1	1	1	–	2	2	–	–	–	–	–	–	–	–	–	–	–	–	1	3	3
Sporocyst–redia species																								
O. ranae + Hypoderaeum conoideum	–	–	–	–	–	–	–	–	–	–	–	–	1	1	1	–	–	–	–	–	–	1	1	1
O. ranae + Echinostoma revolutum	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1	2	2	1	2	2
O. ranae + Notocotylus attenuatus	–	–	–	–	–	–	2	2	2	–	–	–	–	–	–	1	1	1	–	–	–	3	3	3
*O. ranae + Paryphostomum radiatum**	–	–	–	–	–	–	–	1	1	–	–	–	–	–	–	–	–	–	–	–	–	–	1	1
T. szidati + N. attenuatus	2	2	2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	2	2	2
T. szidati + H. conoideum	2	2	2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	2	2	2
T. szidati + E. revolutum	2	2	2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	2	2	2
T. szidati + Moliniella anceps	6	6	6	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	6	6	6
P. elegans + E. revolutum	–	–	–	–	–	–	–	–	–	1	1	–	–	–	–	–	–	–	–	–		1	1	–
P. elegans + M. anceps	–	–	–	5	5	5	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	5	5	5
*P. elegans + P. radiatum**	–	–	–	–	–	–	–	1	1	–	–	–	–	–	–	–	–	–	–	–	–	–	1	1
*A. burti + E. revolutum**	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1	1	–	1	1
Total no. of double infections	17	17	17	96	99	93	88	97	96	13	13	9	41	41	41	18	18	18	2	5	5	275	290	279

TABLE IV. Changes of infection with larval trematodes observed in *Lymnaea stagnalis* in South Bohemia (Czech Republic). Three categories of change were distinguished. Abbreviations for ponds: V, Vlkovský; HS, Hluboký Sax; HH, Hluboký u Hamru; Z, Zavadil; VD, Velký Dvorecký; B, Bartoňovský; S, Špitálský.

Category of change	Třeboň area			Jindřichův Hradec area				Pooled data
	V	HS	HH	Z	VD	B	S	
Single infection to a different single infection	8	3	7	2	6	6	–	32
Single–double infection to a double–triple infection	2	14	17	1	6	4	–	44
Double infection to a single infection	3	20	21	1	2	–	–	47
Total	13	37	45	4	14	10	0	123

$\chi^2_{(3)} = 37.94$, $P < 0.0001$), suggesting relatively weak competitive interactions among sporocyst species. Three species pairs combining 2 sporocyst species (*P. elegans* and *T. szidati*) and 2 rediae species (*M. anceps* and *N. attenuatus*) were significantly more frequent than would be expected by chance, suggesting facilitation. Another combination of sporocyst species occurred at random, whereas for the 2 remaining sporocyst species, the expected number of double infections was zero (Table V). However, there were contradicting direct observations, i.e., *P. elegans* was found 3 times to eliminate *M. anceps* and *N. attenuatus* replaced *T. szidati* in 1 infection. A conservative approach (co-dominance) and indirect evidence were applied for these observations. Six combinations of double infections occurred as frequently as could be expected by chance ($P \geq 0.05$), suggesting neither competition nor facilitation. There was insufficient statistical power to test relationships between species in the remaining 19 combinations of the relatively rare species; only 1 of these combinations was observed in our study (*A. burti* and *E. revolutum*).

Figure 1 displays the hypothesized dominance hierarchy. Seven species were putatively co-dominant. Table V shows that most of the co-dominant species were highly interactive, but the order of dominance was not clear. In 4 snails, a replacement was detected among the dominants (Table VI), but we opted not to assign dominance on such limited evidence. We suspect dominance in these top species may be based on priority of occupancy. *Echinoparyphium recurvatum* (ERE) was placed in this group, despite its rarity, based on indirect evidence (its relatedness to the other echinostomes in this group). Although 5 of these species are echinostomatids and were found to be dominant in other studies (Lie, 1973; Kuris, 1990; Sousa, 1990; Lafferty et al., 1994), 1 was a notocotylid and 1 was a diplostomid, with the latter species having only sporocysts in the life cycle. Six species were presumed dominant over *P. elegans*, and all of them but the notocotylid were dominant over *O. ranae*. As a second tier in the hierarchy, *O. ranae* and *P. elegans* were abundant trematodes that co-occurred as per random expectations (Table V). Recapture data also showed that they frequently interacted with other species, i.e., in 80 cases with *P. elegans* supplanting *O. ranae* 50 times (Table VI). The echinostome *M. anceps* seems to be subordinate to *O. ranae*, *P. elegans*, and *T. szidati* based on the limited evidence of replacement and on weak interactions (Tables V, VI). There was insufficient power to detect any significant interactions between this rare echinostomatid and other species (Table V). *Plagiorchis*

TABLE V. Observed/expected numbers of pairwise associations among larval trematodes in *Lymnaea stagnalis* in South Bohemia (Czech Republic). Observed double infections were pooled over all ponds and expected double infections were calculated using species prevalence from total dataset. Statistically significant associations indicated in bold, values for which statistical test could not be performed are in italic, and empty cells indicate double zeros. Abbreviations for species names: AB, *Australapatemon burti*; AM, *Australapatemon minor*; CC, *Cotylurus cornutus*; DP, *Diplostomum pseudospathaceum*; EA, *Echinoparyphium aconiatum*; ER, *Echinostoma revolutum*; ERE, *Echinoparyphium recurvatum*; HC, *Hypoderaeum conoideum*; MA, *Moliniella anceps*; NA, *Notocotylus attenuatus*; OR, *Opisthioglyphe ranae*; PE, *Plagiorchis elegans*; PR, *Paryphostomum radiatum*; TS, *Trichobilharzia szidati*.*

Intramolluscan stage	Species code	Rediae species							Sporocyst species					
		ER	HC	PR	EA	MA	ERE	NA	PE	OR	DP	TS	AM	AB	CC
Rediae species	ER														
	HC	*0/3*													
	PR	**0/4^{n}**	**0/4^{n}**												
	EA	*0/1*	*0/1*	*0/2*											
	MA														
	ERE														
	NA	*0/1*	*0/1*	*0/1*											
Sporocyst species	PE	**1/31^{n}**	**0/32^{n}**	**1/42^{n}**	**0/14^{n}**	**5/2^{p}**		**0/7^{n}**							
	OR	**2/19^{n}**	**1/20^{n}**	**1/26^{n}**	**0/8^{n}**	*0/1*		3/4^{non}	221/206^{non}						
	DP	**0/7^{n}**	**0/7^{n}**	**0/9^{n}**	*0/3*			*0/1*	**3/76^{n}**	**23/46^{n}**					
	TS	2/2^{non}	2/2^{non}	*0/3*	*0/1*	**6/0^{p}**		**2/0^{p}**	**9/21^{n}**	**4/13^{n}**	1/5^{non}				
	AM								*0/3*	*0/2*	*0/1*				
	AB	*1/1*	*0/1*	*0/1*					**0/6^{n}**	2/4^{non}	*0/1*				
	CC														

* ^{n} = negative interactions ($P < 0.05$); ^{p} = positive interactions ($P < 0.05$); ^{non} = non-significant interactions ($P \geq 0.05$).

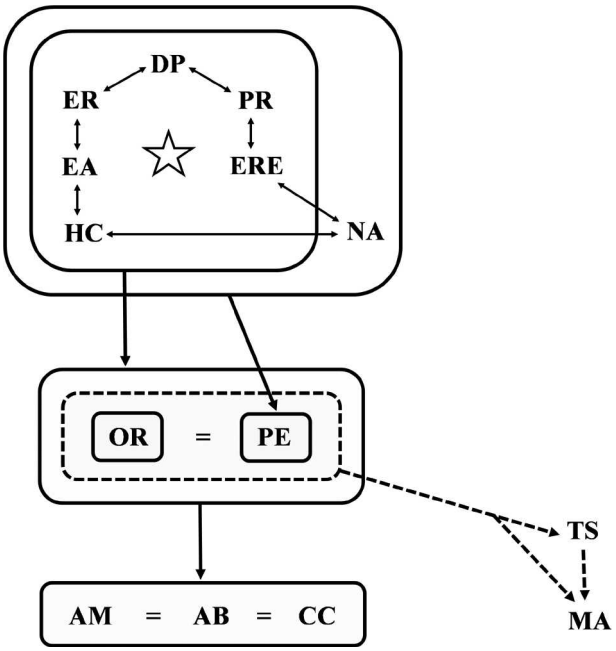

FIGURE 1. Putative competitive dominance hierarchy for the larval trematodes in *Lymnaea stagnalis*. A star indicates that all dominant species interact with each other and double-headed arrows indicate that 1 species is dominant over the other, but the direction of the interaction cannot yet be determined (prior occupancy may be the determinant). Solid lines and arrows represent transitive dominance relationships (all species subordinate to species X are also subordinate to the species that dominate species X; see Kuris, 1990), whereas interrupted lines and arrows indicate non-transitive dominance relationships. Key to species: AB, *Australapatemon burti*; AM, *Australapatemon minor*; CC, *Cotylurus cornutus*; DP, *Diplostomum pseudospathaceum*; EA, *Echinoparyphium aconiatum*; ER, *Echinostoma revolutum*; ERE, *Echinoparyphium recurvatum*; HC, *Hypoderaeum conoideum*; MA, *Moliniella anceps*; NA, *Notocotylus attenuatus*; OR, *Opisthioglyphe ranae*; PE, *Plagiorchis elegans*; PR, *Paryphostomum radiatum*; TS, *Trichobilharzia szidati*.

elegans and *O. ranae* were the only species found in a negative association with the avian schistosome, *T. szidati*. The lack of dominance over *T. szidati* by the echinostomatids indicates that this hierarchy is not fully transitive (Table V). The 3 strigeids were uncommon in this system and appeared to be subordinate to other species based on limited observations on negative association between *P. elegans* and *A. burti* (Table V) and indirect evidence. One of them, *A. burti*, did co-occur with *O. ranae* or *E. revolutum* in multiple infections, so it may not be readily suppressed by otherwise dominant species.

The hypothesized dominance hierarchy made it possible to develop a null model that accounted for individuals lost to competitive exclusion. It is important to emphasize that this model does not assume that competition occurs, only that if it occurs, it is possible to predict the outcome. In the following section, both the basic (Np_ip_j) and dominance (Ne_ie_j) null models were used to assess the effects of heterogeneity and competition on community structure, and results are given for both.

Effects of spatial and temporal heterogeneity and competition on community structure

Spatial heterogeneity had the net effect of isolating species; Np_ip_j-null model: 531 summed expected double infections vs. 628 expected double infections from the pooled data; $\chi^2_{(3)} = 15.97$, $P < 10^{-4}$; Ne_ie_j-null model: 626 summed expected double infections vs. 755 expected double infections from the pooled data; $\chi^2_{(3)} = 23.81$, $P < 10^{-4}$) (Fig. 2). In other words, had final hosts been homogeneously distributed among the sampling sites, we would have observed more double infections among trematode recruits. However, spatial heterogeneity did not explain all of the structure in the trematode community. After the isolating effects of spatial heterogeneity were controlled for, there was a significant reduction in the number of observed double infections that could be attributed to competitive exclusion (Np_ip_j-null model: 531 expected double infections vs. 279 observed double infections; $\chi^2_{(3)} = 126.20$, $P < 10^{-4}$; Ne_ie_j-null model: 626 expected double infections vs. 279 observed double infections; $\chi^2_{(3)} = 205.00$, $P < 10^{-4}$) (Fig. 2).

Temporal heterogeneity did not appear to isolate, or concentrate, species in time in 4 ponds. Using the Np_ip_j-null model, no significant differences in the expected number of double infections

TABLE VI. Direct evidence for dominance relationships among larval trematodes in *Lymnaea stagnalis* in South Bohemia (Czech Republic). (1) Dominant trematode species observed replacing subordinate species, e.g., ER → EA = EA > ER. (2) Dominant trematode species observed infecting a snail having a previous trematode infection, e.g., OR → OR+NA = NA > OR. (3) Dominant trematode species observed eliminating subordinate species in double infections, e.g., ER + TS → ER = ER > TS. Numbers represent direct observations; inconsistent direct observations indicated by an "*". Abbreviations for species names: AB, *Australapatemon burti*; AM, *Australapatemon minor*; CC, *Cotylurus cornutus*; DP, *Diplostomum pseudospathaceum*; EA, *Echinoparyphium aconiatum*; ER, *Echinostoma revolutum*; ERE, *Echinoparyphium recurvatum*; HC, *Hypoderaeum conoideum*; MA, *Moliniella anceps*; NA, *Notocotylus attenuatus*; OR, *Opisthioglyphe ranae*; PE, *Plagiorchis elegans*; PR, *Paryphostomum radiatum*; TS, *Trichobilharzia szidati*.

	Direct evidence		
Dominance relationships	1	2	3
EA > ER	1	–	–
NA > HC	1	–	–
NA > OR	–	1	–
NA < OR*	1*	–	–
NA > TS	1	–	–
PR > ER	1	–	–
PR > PE	3	–	–
PR > OR	3	–	–
PR < OR*	1*	–	–
ER > TS	–	–	1
HC > PE	1	–	–
HC < PE*	1*	–	–
HC > TS	–	–	1
DP > EA	1	–	–
DP > PE	1	–	1
DP < PE*	1*	–	–
DP > OR	–	–	8
DP < OR*	–	2*	–
DP > TS	2	–	–
PE > OR	5	18	27
PE < OR*	8*	16*	6*
PE > TS	–	2	–
PE < TS *	–	2*	–
OR > TS	–	2	–
OR < TS*	–	1*	–
PE > MA	–	–	3

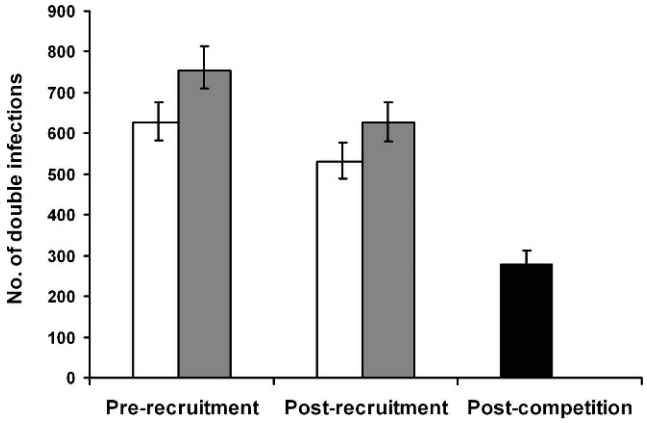

FIGURE 2. Comparative evaluation of the effects of spatial heterogeneity in recruitment and competition on trematode community structure among the ponds studied. The expected number of double infections was calculated using the Np_ip_j-null model (white bars) and Ne_ie_j-null model (grey bars). The black bar represents the observed number of double infections. The first pair of bars indicates the number of expected double infections in the absence of competition if recruitment were spatially homogeneous among ponds (expected of pooled or pre-recruitment). The second pair of bars indicates the number of expected double infections in the absence of competition with the observed spatial variation in recruitment among ponds (sum of expected or post-recruitment). Error bars represent 95% confidence limits. Both null models indicate that spatial heterogeneity significantly reduced interactions and that competition significantly reduced the number of double infections that persisted after recruitment.

between the summed and pooled data were found in ponds V, HS, B, and S; the same result was obtained using the Ne_ie_j-null model (Table VII). Figure 3A illustrates an example of the non-significant effect of temporal heterogeneity in recruitment on the potential number of co-occurrences (pond V). In contrast, intensification of interactions by temporal heterogeneity was apparent in the other 3 ponds tested (Table VII). At 1 of these ponds (pond Z), the effect was only significant for the Ne_ie_j-null model, though both null models were in the same direction (Fig. 3C). Both null models for the remaining ponds (VD and HH; see Fig. 3B for the latter) revealed the same pronounced effect, i.e., towards intensification of interactions due to seasonal peaks in trematode recruitment. Competition appeared to substantially reduce double infections after controlling for temporal variation (ponds V, HH, and Z; Table VII, Fig. 3).

The comparison of the prevalence of each species before and after competition revealed a total of 11.2% of trematode infections lost to competition (Table VIII). In line with the competitive dominance hierarchy, rediae species suffered fewer losses than did sporocyst species in both null models.

DISCUSSION

Spatial and temporal heterogeneity

The trematode community in *L. stagnalis* is species-rich and overall prevalence is high, thus offering conditions for potentially intense competitive interactions among trematode species. Spatial heterogeneity in recruitment among species appeared to reduce the frequency of interactions. In contrast, for some of the ponds seasonality in recruitment seems to have intensified interactions among trematode species. Most interactions among species led to competitive exclusion, with dominant species replacing subordinate species or by prior residents excluding new recruits. For this reason, multiple infections were substantially less abundant than expected. Many of the observed double infections were likely transient.

Our observation of spatial heterogeneity derives from spatial differences in the distribution of 2 groups of trematodes (Soldánová et al., 2011; Soldánová and Kostadinova, 2011). The first group includes those species using waterfowl as definitive hosts (the echinostomatids, the notocotylid, and the schistosomatid), and larid birds (*D. pseudospathaceum*). All but *N. attenuatus* are transmitted by active miracidia. The second group contains *P. elegans* (a parasite of birds and small mammals) and

TABLE VII. Summary data for the double infections in restricted dataset and results from the χ^2 tests for temporal heterogeneity (first line) vs. competition (second line). Expected double infections are provided for both null models, Np_ip_j-null model and Ne_ie_j-null model, and calculated separately for each sampling date at a given pond (sum of expected) and from the pooled data across all sampling dates (expected of pooled). Observed double infections also shown. Abbreviations for ponds: V, Vlkovský; HS, Hluboký Sax; HH, Hluboký u Hamru; Z, Zavadil; VD, Velký Dvorecký; B, Bartoňovský; S, Špitálský.

		Sum of expected		Expected of pooled		χ^2		*P*-value	
Pond	Observed	Np_ip_j	Ne_ie_j	Np_ip_j	Ne_ie_j	Np_ip_j	Ne_ie_j	Np_ip_j	Ne_ie_j
V	17	76	105	73	94	0.13	1.37	ns*	ns
						48.16	79.10	<0.0001	<0.0001
HS	93	135	153	141	162	0.29	0.57	ns	ns
						14.62	26.77	0.0001	<0.0001
HH	96	161	379	126	137	10.35	457.62	0.001	<0.0001
						28.44	258.40	<0.0001	<0.0001
Z	9	40	50	32	35	2.04	6.55	ns	0.01
						24.56	34.56	<0.0001	<0.0001
VD	41	97	124	67	72	13.86	38.84	0.0002	<0.0001
						33.84	58.92	<0.0001	<0.0001
B	18	53	69	57	72	0.30	0.14	ns	ns
						24.70	41.14	<0.0001	<0.0001
S	5	36	67	35	54	0.03	3.60	ns	ns
						29.23	68.42	<0.0001	<0.0001

* ns = $P \geq 0.05$.

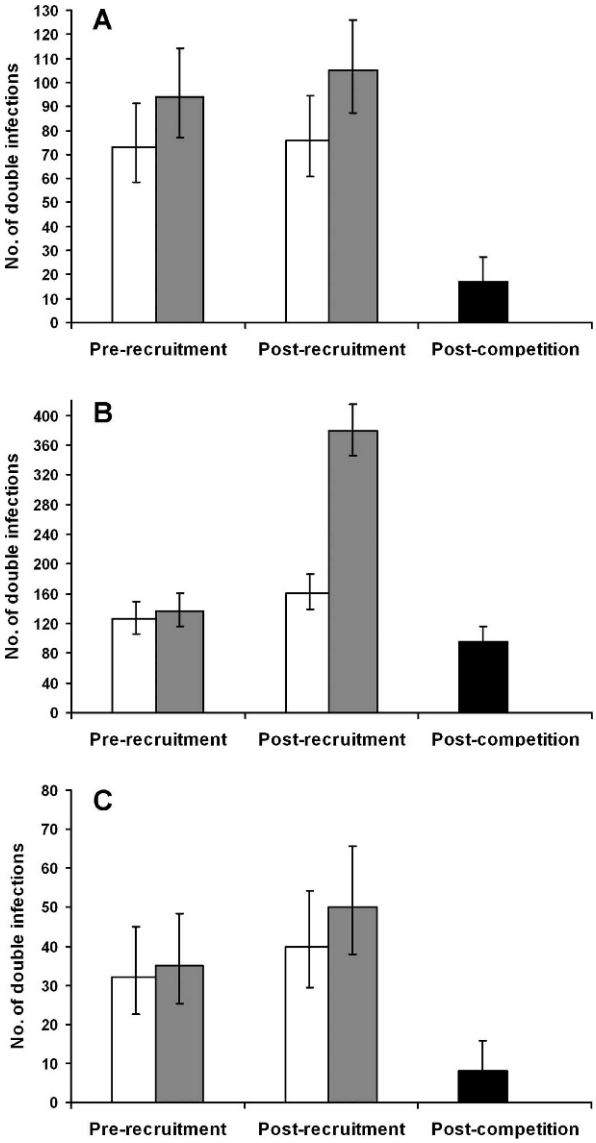

FIGURE 3. Comparative evaluation of the effects of temporal heterogeneity in recruitment and competition on trematode community structure for 3 representative cases showing: (**A**) no significant effect of temporal heterogeneity (both null models; communities at pond V); (**B**) significant effect of temporal heterogeneity (both null models; communities at pond HH); and (**C**) significant effect of temporal heterogeneity (Ne_ie_j-model only; communities at pond Z). The expected number of double infections was calculated using the Np_ip_j-null model (white bars) and Ne_ie_j-null model (grey bars). In each case, the tendency was for seasonality to intensify interactions (second pair of bars; post-recruitment) compared to a null model for homogenous recruitment in time (first pair of bars; pre-recruitment). The effects were stronger for the Ne_ie_j-null model (grey bars). The observed number of double infections (black bar) was significantly lower, indicating a strong structuring effect of competition. Error bars represent 95% confidence limits.

O. ranae (a parasite of amphibians) that are recruited to snails through ingestion of eggs. Past work in this system indicates that the distance among ponds did not drive differences in trematode communities (Soldánová et al., 2011). Instead, pond size and management associated with behavioral patterns of birds and amphibians, and abundance of second intermediate hosts, contributed to the diverse distribution and abundance of passively and actively transmitted species (Soldánová et al., 2011). In particular, pond V supported higher species diversity and higher infection levels of generalist parasites of waterfowl (especially ducks and larids). Because most of the dominant competitors involving rediae species and *D. pseudospathaceum* were in pond V, there was an opportunity for some subordinate species in other ponds to avoid competition.

TABLE VIII. Effect of competition on the relative abundance of trematode species estimated from restricted dataset across all sites. First two columns represent occurrences (single and double infections with each species combined) calculated from expected (before competition) and observed (after competition) prevalences. Third column (Change %) represents percentage losses due to competition for each species. Abbreviations for species names: AB, *Australapatemon burti*; AM, *Australapatemon minor*; CC, *Cotylurus cornutus*; DP, *Diplostomum pseudospathaceum*; EA, *Echinoparyphium aconiatum*; ER, *Echinostoma revolutum*; ERE, *Echinoparyphium recurvatum*; HC, *Hypoderaeum conoideum*; MA, *Moliniella anceps*; NA, *Notocotylus attenuatus*; OR, *Opisthioglyphe ranae*; PE, *Plagiorchis elegans*; PR, *Paryphostomum radiatum*; TS, *Trichobilharzia szidati*.

Trematode species	Before competition	After competition	Change (%)
HC	176.8	168	−5.0
ERE	1.1	1	−5.7
ER	179.6	167	−7.0
NA	39.1	36	−7.8
EA	80.4	74	−7.9
PR	253.5	233	−8.1
DP	454.0	406	−10.6
PE	2,019.4	1,804	−10.7
OR	1,285	1,130	−12.1
TS	136.6	112	−18.0
CC	1.5	1	−33.8
AB	59.0	32	−45.7
AM	34.3	16	−53.4
MA	0	11	no estimate
Total	4,720.5	4,191	−11.2

The structure of larval trematode communities was also significantly affected by temporal heterogeneity. In 3 ponds (HH, VD, and Z), seasonal pulses of autumn infections intensified trematode interactions. These double infections were driven mostly by a high prevalence of *P. elegans* and *O. ranae*. However, this pattern did not occur in pond HS (sample sizes were too small to assess seasonality in ponds B and S). Because trematode species do not necessarily have to recruit at the same time to interact, temporal heterogeneity may not strongly affect the frequency of interactions (Kuris and Lafferty, 1994).

Competition

Larval trematode communities in *L. stagnalis* were strongly influenced by competitive exclusion. Very few double infections were seen relative to those expected after accounting for temporal and spatial heterogeneity (Fig. 2). The vast majority of multiple infections were between the prevalent sporocyst species *P. elegans* and *O. ranae* due to their high recruitment rates (Soldánová and Kostadinova, 2011). These 2 species are probably able to co-exist for some time and the outcome may vary.

Our hierarchy, postulated for the larval trematodes of *L. stagnalis*, is similar to that of *Lymnaea rubiginosa* provided by

Kuris (1990). The suite of trematodes in the latter includes echinostomatids, schistosomatids, and strigeids in shared genera and an unidentified plagiorchiid. The *L. rubiginosa* system is uniquely informative because interactions among larval trematodes were demonstrated using experimental double infections (reviewed by Lie et al., 1968; Lim and Heyneman, 1972). The postulated interspecific competitive relationships among trematodes in our system corresponds, to a degree, to the common pattern in the published hierarchies in that species developing in sporocysts only are generally subordinate to rediae species that are capable of active predation, i.e., direct antagonism of Lie (1967; Lie et al., 1968; Lim and Heyneman, 1972; Kuris, 1990; Fernandez and Esch, 1991a; Sousa, 1992, 1993). The exclusion of sporocyst species by rediae species occurred within 21–28 days (Soldánová and Kostadinova, 2011). However, the sporocyst species *D. pseudospathaceum* appeared to outcompete the other sporocyst species and prevent co-infection by rediae species. An advantage of prior arrival is also seen for the closely related *Diplostomum scheuringi* (Fernandez and Esch, 1991a). Perhaps as an adaptation for prior arrival, *D. pseudospathaceum* primarily infects juvenile snails. This is in line with the suggestion of Riley and Chappell (1992; reviewed in Adema and Loker, 1997) for the ability of *Diplostomum spathaceum* (probably *D. pseudospathaceum*, see Niewiadomska, 1986) to induce changes in *L. stagnalis'* immature defense system. However, we found that *D. pseudospathaceum* also frequently infected mature snail cohorts, perhaps due to its high colonization rates in the area (Soldánová and Kostadinova, 2011). The subordinate position of the strigeids in our hierarchy is similar to that of the unidentified strigeids and *Cotylurus lutzi* in the postulated hierarchies for the trematodes in *C. californica* and *L. rubiginosa* (Kuris, 1990). Among the rediae species, *E. aconiatum*, *P. radiatum*, and *N. attenuatus* were observed to replace *E. revolutum* and *H. conoideum* (HC), which possess smaller rediae (Našincová, 1992). One possible explanation could be that large rediae tend to dominate small rediae (Kuris, 1990). Otherwise, prior occupancy may determine the winner in interactions between the 7 dominant species, a pattern demonstrated for some pairs of echinostomatids involving *Echinostoma audyi*, *Echinostoma hystricosum*, *Hypoderaeum dingeri*, and *Echinoparyphium dunni* in a related snail species, *L. rubiginosa* (Lie et al., 1966).

In *L. stagnalis*, the interactions were sometimes complex and not fully transitive, e.g., *T. szidati* and *M. anceps*. This is not without precedent. For instance, Lie (1973) experimentally demonstrated a circular competitive hierarchy by combining 2 rediae species, *H. dingeri* and *E. audyi*, with a sporocyst species, *Trichobilharzia brevis*, in the same snail host; the presence of degenerating rediae of *H. dingeri* due to suppression by *T. brevis* inhibited the development of the highly predatory *E. audyi* and, therefore, *T. brevis* was not excluded. Prior infection may impair a snail's immune system, making the infected host more susceptible to another infection (Lie et al., 1973; Loker, 1994). As with other avian schistosomes of the genera *Trichobilharzia* and *Austrobilharzia*, *T. szidati* may be an obligate secondary invader that only infects snails with compromised immunity or may be able to persist for a very long time in double infections with otherwise dominant species (Lie et al., 1966; Lie, 1973; Walker, 1979; Kuris, 1990). This is consistent with our observations, as *T. szidati* was 1 of the species most frequently involved in multiple infections. In contrast, direct evidence of species replacement indicates *T. szidati* was otherwise subordinate in our system. Another secondary invader was *M. anceps*, which occurred exclusively in double infections with *T. szidati* and *P. elegans*, always being found to invade the latter. However, *M. anceps* may be rapidly excluded from these co-infections through strong, non-predatory antagonism by a sporocyst species, a mechanism similar to that demonstrated for a redia–sporocyst-only species combination (Ow-Yang and Lie, 1972).

Overall, our analysis demonstrates that competition in individual hosts structures the component trematode community at the snail population level. As a result, we estimate that competition eliminated 11.2% of the trematode infections from the system. The model system studied thus provides general insight into the extent to which species interactions can structure communities in the midst of spatial and temporal variation in habitat use.

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