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Coexistence of helminth species in *Lysapsus limellus* (Anura: Pseudidae) from an Argentinean subtropical area: influence of biotic and abiotic factors

Received: 8 February 2000 / Accepted: 27 June 2000 / Published online: 27 July 2000
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Abstract The need for studies on helminth communities of South American amphibians was addressed by examining changes in composition and population dynamics of the helminth component and infracommunities in the frog *Lysapsus limellus* Cope, 1862, from 1994 to 1996. Two pond types were considered, one permanent and one semipermanent. The main goals of this study were (1) to investigate the relationships between pond type, season, study time, frog body size, and frog sex and the presence-absence and counts of helminth parasite species in the frog host, *L. limellus*, and (2) to examine the co-occurrence of the different parasite species in the frog host in terms of the structure, assembly, and dynamics of the helminth infracommunity. Parasite presence and absence were analyzed using logistic regression and parasite counts were analyzed using log-linear modeling. To examine the association between parasite species, a principal components analysis was carried out on the correlation matrix for the counts. The pattern of co-occurrences was also examined through a randomization test. The main results were as follows: (1) five parasite species were found in the infracommunity; (2) the pond type and the host size class were the principal factors related to the presence and absence of parasites for the three dominant species, while the year of study and the host sex were only important for one of the species; (3) for the parasite counts, many more factors were significant, with body size class and pond type always important factors for the three core species of the infracommunity, with season, year of study, and sex also sometimes important, and (4) strong associations were observed between some helminth species of the host from the permanent pond,

but the same species did not co-occur in frogs in the semipermanent pond. The life histories of the five species can be considered as opportunistic or “*r* strategists.”

Keywords Helminth infracommunity · Helminths co-occurrences · Semipermanent and permanent pond · Host ecology · Subtropical habitat

Introduction

The increasing attention being paid to parasite community ecology has generated an active discussion about the nature of parasite assemblages, with special emphasis on the controversy concerning parasite species interaction. The study of factors regulating the arrangement of natural communities has always been at the core of ecology (Strong et al. 1984; Diamond and Case 1986; Ricklefs and Schluter 1993; Kehr and Hamann 1995; Poulin 1995; Hamann and Kehr 1998). Parasite assemblages can be studied at different scales and, recently, much effort has gone into resolving and determining the number of parasite species recorded for several host species over their whole geographical range, for example with fish, amphibian, reptilian, and bird hosts (Price and Clancy 1983; Aho 1990; Gregory 1990; Bell and Burt 1991; Gregory et al. 1991; Kennedy 1997; Hamann and Kehr 1998).

The richness (number of species) of parasite infracommunities varies among host individuals in a population, among host populations, and among host species. The rate at which the host can be infected by parasites and the persistence of infection are considered to be key factors determining the infracommunity richness and co-occurrences among the various parasite species (Holmes and Price 1986). Hence, variation in habitat, host size, and diet are considered to be important in explaining differences in infracommunity richness among host populations or species.

One approach to examining the question of interactive versus non-interactive parasite assemblages would be to

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study the dynamics of an assemblage across a broad range of abiotic (e.g., pond type and time) and biotic (e.g., host body size) conditions. Janovy et al. (1990) suggested that parasite assemblage attributes such as diversity, species density, and co-occurrence may be determined by biotic factors, but characteristics that respond essentially to variations in the host's abiotic habitat may not always be of ecological significance.

The distribution of species richness among infracommunities within a component community constituted of n parasite species may be exclusively determined by the prevalence of the parasite species and by how they co-occur (Janovy et al. 1995). The number of parasite species found in infracommunities is only one feature of their structure. Infracommunities may be random assemblages of species from the component community, or may represent structured, repetitive subsets of species whose composition is extremely predictable (Bush 1990; Thoney 1993; Guégan and Huguény 1994).

Parasites generally spend most or all of their life cycle in association with one or more hosts. Numerous morphological and physiological adaptations to these hosts are exhibited, and parasite life histories also display synchrony with host biology. Selective pressure from many other sources also acts on parasite assemblages (Poulin 1995).

Lysapsus limellus is a member of the frog family Pseudidae. It is distributed exclusively in South America, throughout tropical and subtropical lowlands east of the Andes and in the Magdalena Valley, Colombia (Duellman and Trueb 1986). In Argentina, *L. limellus* is restricted to the areas adjacent to the Paraguay and Paraná Rivers. It may be included among amphibians with a great preference for aquatic habitats, and occurs in semipermanent and permanent ponds (Hamann and Kehr 1997).

The main objectives of this study were: (1) to investigate the influence of pond type, season, study time, frog body size, and frog sex on the presence and abundance of helminth parasite species in the frog host, *L. limellus* and (2) to examine the co-occurrence between parasite species in the frog host, illustrating the structure, assembly, and dynamics of the helminth infracommunity.

Materials and methods

Study sites

Two lentic environments in the north of the Riachuelo basin in the province of Corrientes, Argentina (27°25' S, 58°40' W) were selected. One of these sites was semipermanent and the other a permanent pond.

Pond 1: a triangular-shaped semipermanent pond covering approximately 1 ha and with a maximum depth of approximately 0.8 m. During the study period, the dominant vegetation was *Cyperus* spp., *Salvinia* sp., *Ludwigia peploide*, *Nymphoides* sp. and *Paspalum* sp. The water level in this pond differed in the 2 study years. In 1994, the weather was characterized by low rainfall, lowering the pond water level toward December. In

1995, rainfall was high only during the first 4 months, with the pond drying between September and December.

Pond 2: a permanent pond covering approximately 2 ha. It is oval-shaped with well-defined margins and a maximum depth of 2 m. It is wider and less vegetated than pond 1. The predominant vegetation was *Salvinia* sp., *L. peploide*, and *Nymphoides* sp.

Analytical procedure

Frogs were collected monthly from March 1994 to September 1995 (pond 1, $n=106$) and November 1995 to September 1996 (pond 2, $n=436$). Animals were captured with a dip net (width 65 cm, height 45 cm, depth 45 cm) 2–4 m from the pond shore where the vegetation was more abundant. The sampling time was 1 h and the samples were always taken between 0900 and 1200 hours. All possible microhabitats were included in each sample. Frogs were transported to the laboratory where snout-vent length and sex were recorded.

During each sampling session, water temperature (°C), conductivity, dissolved oxygen, pH, and pond water level were recorded. The Centro de Ecología Aplicada del Litoral meteorological station provided rainfall and air temperature data.

Other variables recorded were: (1) POND, coded 1 for the semipermanent pond and 2 for the permanent pond; (2) SEAS (season), coded 1 for fall (21 March–20 June), 2 for winter (21 June–20 September), 3 for spring (21 September–20 December), and 4 for summer (21 December–20 March); (3) YEAR, coded 1–3 for 1994–1996, respectively; (4) SIZE, the body length of the frog (mm); (5) SIZC, the body size class of the frog coded 1 for under 12 mm, 2 for 12 to under 15 mm, 3 for 15 to under 17 mm, and 4 for 17 mm or more; (6) SEX, coded 1 for female and 2 for male; (7) S1–S5, counts for the trematode parasite species *Glythelminis vitellinophilum*, *Catadiscus propinquus*, *Lophosicyadiplostomum* sp., Echinostomatidae gen. sp. 1, and *Cylindrotaenia* sp., respectively. Three other parasite species were registered (the nematodes *Camallanus* sp., *Cosmocerca* sp., and a trematode, Diplostomidae gen. sp. 1) but are not analyzed here because they were very rare (only present in a few hosts in very low numbers).

Helminths detached from the excretory bladder, alimentary canal (divided into stomach and oesophagus, duodenum, rectum), lungs, liver and gall bladder, kidneys, heart, and muscle were counted and fixed in Railliet-Henry solution, stained with carmine hydrochloride, cleared in creosote and mounted in Canada balsam.

Statistical analysis of parasite presence-absence

Logistic regression was carried out to determine how the presence of each parasite species depended on the factors POND, SEAS, YEAR, SIZC, and SEX. With this method, an equation of the form:

$$p = \exp(\Sigma\theta_i) / [1 + \exp(\Sigma\theta_i)]$$

(called the logistic function) was fitted to the data, where $\Sigma\theta_i$ represents a sum of parameters that allows for the effects of the factors included in the model. This is a standard method for analysing presence-and-absence data (Manly 1992). The goodness of fit of models is measured by a quantity called the "deviance," which is analogous to the residual sum of squares with ordinary analysis of variance. The effects for the factors POND, SEAS, YEAR, SIZC, and SEX were fitted successively, followed by all of the two-factor associations, using the computer program GLIM 4 (Francis et al. 1993).

Following this initial analysis, reduced models were fitted for the presence and absence of the different parasite types. These models always included the factors POND, YEAR, SIZC, and SEX because these were all found to have significant effects with one or more of the parasite types. In addition, for each of the parasite species, the reduced model included any association that was statistically significant at the 5% level.

For each parasite species, the fitted logistic function gives an estimated probability of the presence of the species in a frog. To assess the goodness of fit of the model, the observed proportions with different parasite types were compared with these estimated probabilities. Standardized residuals were calculated as $R=(p-\theta)/\sqrt{[\theta(1-\theta)/n]}$, where p is the sample proportion, θ is the estimated probability of a parasite type being present, and n is the number of frogs for the particular pond-year-size-sex combination being considered. Most of these standardized residuals should be within the range -2 to $+2$ for a model that fits well, with almost all between -3 and $+3$.

Statistical analysis of parasite counts

Log-linear models were used to analyze the abundance distribution of individual parasite species. Here, the expected abundance X of a parasite is assumed to be given by an equation of the form:

$$E(X)=\exp(\Sigma\theta_i)$$

where $\Sigma\theta_i$ represents a sum of parameters that allow for the effects of various factors. This is a standard method of analysis for count data (Manly 1992). For each species, a model was fitted including the factors POND, SEAS, YEAR, SIZC, and SEX, together with all of the two-factor associations. As with logistic regression, the goodness of fit of models is measured by the deviance, which is analogous to the residual sum of squares in ordinary analysis of variance.

Because of the sparse nature of the data, with many zero counts, and some evidence of more variation in some counts than can be expected from the standard log-linear model, the deviance for each effect and association was tested as recommended by McCullagh and Nelder (1989). A scale factor H was estimated by dividing the Pearson χ^2 statistic for the full model by its degrees of freedom (df_s). The deviance D for an effect with df_i degrees of freedom was then tested by forming an F -statistic from the equation $F=(D/df_i)/H$. This is compared with critical values from the F -distribution with degrees of freedom df_i and df_s .

Following the initial analysis to determine significant effect, reduced models were fitted for the parasite counts. The factors POND, SEAS, YEAR, SIZC, and SEX were included for all models, but only the significant associations were included for each parasite type.

Statistical models for associations between parasites

The association between parasite species was further examined using a principal components analysis on the correlation matrix for the counts. Two other somewhat non-standard analyses were also conducted. One examined whether within sample months there was any evidence that the total parasite load in individual frogs is more or less variable than expected if the parasite types occur independently. The other examined whether the co-occurrences of parasites are what would be expected if the different types occur independently.

The first non-standard analysis begins by converting the counts to Z -scores, separately for each parasite species, i.e., the counts for each species were scaled to have a mean of zero and a variance of 1 over all frogs. The purpose of this scaling was to remove any effects of abundance differences between the different parasite types. Next, the variances of the Z -scores were calculated for each month in which there were at least two frogs. The variance of the sum $S=Z_1+Z_2+Z_3+Z_4+Z_5$ was also calculated for each month. The expectation was then that $\text{Var}(S)=\text{Var}(Z_1)+\text{Var}(Z_2)+\text{Var}(Z_3)+\text{Var}(Z_4)+\text{Var}(Z_5)$ if the distributions of the parasite types are independent. If $\text{Var}(S)$ is larger than this expected value then it indicates some tendency for the high counts to occur together and the low counts to occur together for the different parasite types. On the other hand, if $\text{Var}(S)$ is lower than expected, some tendency for the total parasite load to be constrained for individual frogs is indicated. To assess whether

$\text{Var}(S)$ was significantly different from expectation, the observed values for each month, and the total of $\text{Var}(S)$ for all months can be compared with the distributions obtained when the Z -scores are independently allocated to the frogs for each of the parasite species, within each of the months. This is a randomization test for parasite associations (Manly 1997).

The second extra analysis used the generalized Monte Carlo test described by Manly (1995) to examine whether there is any tendency for pairs of parasite species to occur together, or not to occur together, given the number of parasite types possessed by each frog and the total number of occurrences of each parasite species. For this purpose, the data were examined in two parts. First, the frogs from pond 1 with at least one parasite type were considered. Next, the frogs from pond 2 with at least one parasite type were considered.

Results

Habitat abiotic variables

Pond 1

During the study period, water temperatures ranged between 13 and 36°C. Conductivity was low, ranging between 9 and 100 $\mu\text{S cm}^{-1}$; pH varied between 5.3 and 8.9. Dissolved oxygen ranged from 1.2 to 4.0 ppm. Monthly data of these variables are presented in Hamann and Kehr (in press).

Pond 2

During the study period, the water temperatures ranged between 9 and 28°C. Conductivity was low, oscillating between 10 and 70 $\mu\text{S cm}^{-1}$; pH varied between 5 and 7. Dissolved oxygen ranged from 2.1 to 14.2 ppm.

Presence-absence of parasites

Table 1 shows the results of the logistic regression. The factor SEAS was never significant and was therefore not considered. The results for each species were as follows: (1) the probability of *G. vitellinophilum* being present depended on the pond, the size and sex of the frogs, with the pond effect varying for males and females; (2) the probability of *C. propinquus* being present depended on the pond and the size of the frogs, with the size effect varying with the pond; (3) the probability of *Lophosicyadiplostomun* sp. being present depended on the pond, the year, the size and the sex of the frogs, with the size effect varying for different years; (4) the probability of Echinostomatidae gen. sp. 1 being present depended on the year and on the size of the frogs; (5) the probability of *Cylindrotaenia* sp. being present depended on the year and on the sex of the frogs, with the sex effect varying from year to year.

The probabilities of the parasite species being present according to the fitted models are shown in Table 2. It appears that: (1) the probability of *G. vitellinophilum* being present increased with the size of frogs, and was

higher for males in pond 1 but higher for females in pond 2; (2) the probability of *C. propinquus* being present decreased with frog size in pond 1 but increased with frog size in pond 2; (3) the probability of *Lophosicyadiplostomun* sp. being present was high only in

pond 1 and year 1, where it was absent in the size class 1 males, but highest in size class 2, and generally higher in males than in females; (4) the probability of Echinostomatidae gen. sp. 1 being present was always quite low, but it was zero in pond 1 in year 1, and otherwise generally decreased with frog size; (5) the probability of *Cylindrotaenia* sp. parasites being present was always low, being zero in pond 1 in year 1, and slightly higher for males than for females in year 2 only.

Table 1 Analysis of deviance for logistic regression models. The "deviances" can be tested against the χ^2 distribution. A significantly large value indicates that including the corresponding effect in the model gives a significant improvement in fit over a model that includes the effects listed above this effect. For example, a significant deviance for YEAR indicates that a model including this factor is better than a model that includes POND and SEASON only. The interaction POND×YEAR cannot be estimated. Parasites species: S1 (*Glypthelminis vitellinophilum*), S2 (*Catadiscus propinquus*), S3 *Lophosicyadiplostomun* sp.), S4 (Echinostomatidae gen. sp. 1), and S5 (*Cylindrotaenia* sp.). Host species: *Lysapsus limellus*

Effect	df	Deviance				
		S1	S2	S3	S4	S5
POND	1	39.7***	171.2***	72.0***	0.0	0.1
SEAS	3	6.6	5.2	1.1	4.4	2.2
YEAR	2	4.2	3.3	13.8**	8.5*	5.3
SIZC	3	47.3***	26.1***	2.7	9.4*	5.9
SEX	1	0.7	3.7	5.2*	0.8	0.2
POND×SEAS	3	5.9	3.4	3.5	2.3	2.8
POND×YEAR	0	—	—	—	—	—
POND×SIZC	3	0.5	23.2***	5.4	6.0	2.7
POND×SEX	1	5.7*	0.0	0.2	1.0	0.1
SEAS×YEAR	1	2.2	0.6	1.2	0.0	0.0
SEAS×SIZC	9	11.9	10.4	7.5	8.7	8.9
SEAS×SEX	3	3.6	1.6	2.4	0.3	2.3
YEAR×SIZC	5	7.3	5.6	15.8**	0.0	1.5
YEAR×SEX	2	0.3	3.0	2.7	0.0	6.7*
SIZC×SEX	3	1.6	1.4	5.9	1.3	3.0
Residual	501	555.8	327.0	130.8	101.6	116.5

*P<0.05; **P<0.01%; ***P≤0.001

Table 2 also shows the numbers of frogs sampled in the different size classes in the different ponds and years. The frogs in the smallest size class were found at low frequencies in pond 1 in years 1 and 2, and in pond 2 in year 3. However, in pond 2 in year 2, the small frogs were the most common.

Analysis of parasite counts

Figure 1 shows the counts of the different parasite species plotted against the pond, the season when the frog was sampled, the year, the size class and the sex of the frog.

G. vitellinophilum specimens had their highest counts in pond 1, autumn, year 1, size class 4, and females. Counts were never very high in spring, and increased steadily with the size class. The *C. propinquus* parasites had the highest counts in pond 1, autumn, year 1, and size class 2. Counts were never very high in winter and summer, year 3, and size class 1. The *Lophosicyadiplostomun* sp. parasites had the highest counts in pond 1, autumn, years 1 and 2, size classes 2–4, and females. The counts were always very low in spring, and appeared to increase steadily with the size class. The Echinostomatidae gen. sp. 1 parasites had the highest counts in pond 2, autumn, year 3, and size classes 2 and 3. The counts were always very low in spring and summer, year 1, and size classes 1

Table 2 Estimated probabilities of the presence of parasites of different species in the host frog *L. limellus*. Dashes indicate that no frogs of a particular size were sampled at that time and loca-

tion. The numbers of female and male frogs sampled is also shown. Parasite species codes as in Table 1

Pond	Year	Size	Female frogs					Male frogs							
			n	S1	S2	S3	S4	S5	n	S1	S2	S3	S4	S5	
1	1	1	0	—	—	—	—	—	2	0.06	1.00	0.00	0.00	0.00	0.00
		2	2	0.31	0.80	0.82	0.00	0.00	2	0.18	0.86	0.68	0.00	0.00	
		3	10	0.48	0.60	0.92	0.00	0.00	1	0.31	0.68	0.83	0.00	0.00	
		4	13	0.60	0.59	0.32	0.00	0.00	5	0.42	0.67	0.17	0.00	0.00	
	2	1	0	—	—	—	—	—	0	—	—	—	—	—	
		2	1	0.51	0.90	0.00	0.12	0.05	3	0.34	0.93	0.00	0.09	0.11	
		3	3	0.68	0.76	0.20	0.12	0.02	11	0.52	0.82	0.10	0.09	0.04	
		4	35	0.78	0.75	0.23	0.02	0.04	18	0.64	0.82	0.12	0.02	0.09	
	2	2	1	32	0.08	0.00	0.02	0.05	0.01	30	0.11	0.00	0.01	0.04	0.02
			2	22	0.23	0.07	0.00	0.03	0.07	22	0.30	0.10	0.00	0.02	0.13
			3	4	0.38	0.21	0.05	0.03	0.03	3	0.47	0.28	0.02	0.02	0.06
			4	8	0.50	0.42	0.06	0.01	0.06	8	0.59	0.51	0.03	0.00	0.11
3		1	12	0.05	0.00	0.00	0.07	0.01	8	0.07	0.00	0.00	0.06	0.00	
		2	52	0.16	0.03	0.01	0.04	0.04	48	0.22	0.04	0.01	0.03	0.02	
		3	45	0.28	0.08	0.00	0.04	0.02	48	0.37	0.12	0.00	0.03	0.01	
		4	63	0.39	0.20	0.05	0.01	0.03	31	0.49	0.26	0.02	0.01	0.02	
			302	240											

Fig. 1 Counts of helminth parasite species in the host frog *Lysapsus limellus* by pond number (POND), season of sampling (SEAS), year of sampling (YEAR), host size class (SIZC), and sex of frog (SEX). Parasite species: S1 (*Glypthelmins vitellinophilum*), S2 (*Catadiscus propinquus*), S3 (*Lophosicyadiplostomun* sp.), S4 (Echinostomatidae gen. sp. 1), and S5 (*Cylindrotaenia* sp.)

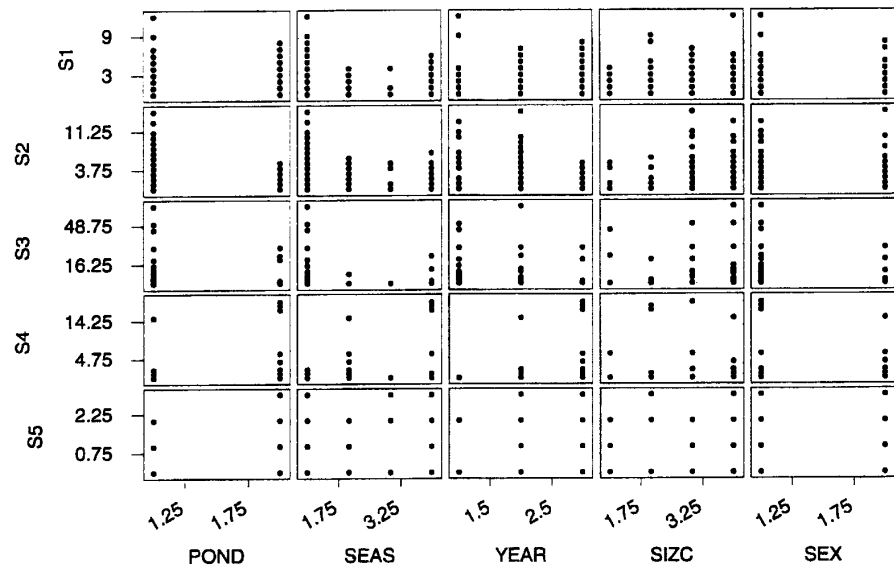


Table 3 Analysis of deviance for log-linear models for parasite counts in the host frog *L. limellus*. The deviances are tested by dividing them by the estimated scale factor to form an *F*-statistic, as explained in the text. A significantly large *F*-value indicates that including the corresponding effect in the model gives a significant improvement in fit over a model that includes the effects listed above it. For example, a significant deviance for YEAR indicates that a model including this factor is better than a model that includes POND and SEASON only. The association POND×YEAR cannot be estimated. Parasite species codes as in Table 1

Effect	df	Deviance				
		S1	S2	S3	S4	S5
POND	1	95.4***	715.6***	1202.5***	0.0	0.2
SEAS	3	14.1	14.1*	42.5	94.4***	5.1
YEAR	2	1.1	0.1	508.9***	20.3**	12.0***
SIZC	3	57.3***	33.5***	120.1***	149.1***	14.0***
SEX	1	0.3	4.0	73.6***	30.6***	0.2
POND×SEAS	3	3.6	8.7	31.7	71.2***	3.2
POND×YEAR	0	—	—	—	—	—
POND×SIZC	3	2.3	75.3***	98.3**	15.7*	2.1
POND×SEX	1	7.0	0.6	57.2**	3.8	0.0
SEAS×YEAR	1	2.9	0.8	0.1	0.0	0.0
SEAS×SIZC	9	11.9	18.4	84.8	23.2	18.6*
SEAS×SEX	3	0.6	0.8	35.5	0.2	6.5*
YEAR×SIZC	5	14.8	26.1**	104.1**	0.0	2.2
YEAR×SEX	2	3.9	5.1	17.3	0.0	7.4**
SIZC×SEX	3	5.5	0.8	85.0**	2.2	4.3
Residual	501	725.3	522.8	1241.1	374.7	140.8
Pearson χ^2	501	979.0	772.0	3201.4	936.9	350.2

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

and 4. The *Cylindrotaenia* sp. parasites never had a count of more than 3. The counts were all zero for year 1, but this is the only noticeable pattern.

Table 3 summarizes the results obtained from fitting log-linear models to the counts. The observed mean counts were compared with the expected counts from the reduced models. Generally, the agreement was good. The standardized residuals appeared, as required, to approximately follow a standard normal distribution, with most values in the range from -2 to $+2$ and almost all values in the range from -3 to $+3$. This analysis shows that the counts of the parasites varied in a complicated way, with all of the factors being important for one species or another. Given this situation, the log-linear models appear to describe the counts in a satisfactory manner.

Association between parasites

Parasite numbers appeared to be related. This is shown in Fig. 2, where each of the parasite counts is plotted against the others. In general, high numbers for one parasite seemed to occur when the other parasites were either absent or in low numbers. The only exception to this rule in the data was for one frog where *C. propinquus* and *Lophosicyadiplostomun* sp. were both quite high at the same time.

The principal components and the percentages of variation for which they account are shown in Table 4. The first component mainly reflects the sum of the counts of *G. vitellinophilum*, *C. propinquus*, and *Lophosicyadiplostomun* sp. The second component is mainly

Fig. 2 Helminth parasite numbers in the host frog *L. limellus* plotted against each other. Parasite species codes as in Fig. 1

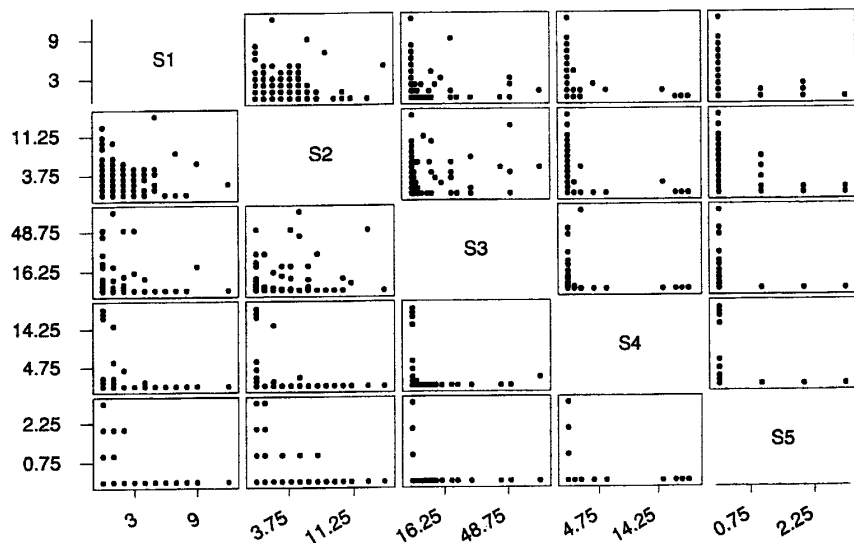


Fig. 3 Principal components (PCs) of helminth parasite species in the host frog *L. limellus* plotted against the pond number (POND), the season (SEAS), the year (YEAR), the host size class (SIZC), and the sex of the frog (SEX). Parasite species codes as in Fig. 1

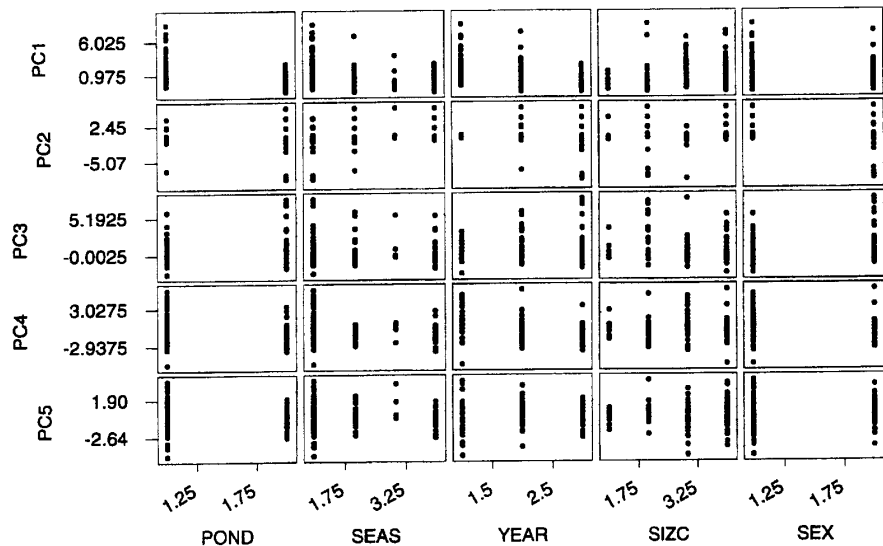


Table 4 Results of a principal component analysis: coefficients of standardized counts, and the percentage of variation explained. Parasite species codes as in Table 1. Host species: *L. limellus*

	Coefficients				
	PC1	PC2	PC3	PC4	PC5
S1	0.507	-0.024	-0.281	-0.677	-0.452
S2	0.669	0.089	0.104	-0.033	0.730
S3	0.531	0.000	0.311	0.606	-0.504
S4	-0.039	-0.711	0.643	-0.282	0.018
S5	-0.107	0.697	0.633	-0.306	-0.091
Percent variation explained	29.2	20.4	19.8	18.3	12.3
Cumulative	29.2	49.6	69.4	87.7	100.0

a contrast between the counts of Echinostomatidae gen. sp. 1 and *Cylindrotaenia* sp. The third component mainly reflects the sum of the counts of Echinostomatidae gen. sp. 1 and *Cylindrotaenia* sp. The fourth component is mainly a contrast between the counts of *Lophosicyadiplostomun* sp. and *G. vitellinophilum*. The fifth component is mainly a contrast between *C. propinquus* and the sum of *G. vitellinophilum* and *Lophosicyadiplostomun* sp.

Figure 3 shows the scores from the principal components plotted against the pond, the season, the size class, and the sex of the frog. The results can be summarized as follows. (1) PC1: the sum of the counts of *G. vitellinophilum*, *C. propinquus*, and *Lophosicyadiplostomun* sp. is

Table 5 Comparison of the observed number of co-occurrences of parasites species in the host frog *L. limellus* from permanent pond 2 with the means and SDs that were obtained from randomized data. The *italicized* observed values are those that seem un-

usual in comparison with the randomized distribution, and thus contribute most to the significant results obtained for the Manly (1995) test for species interactions. Parasite species codes as in Table 1

	Observed					Randomization mean					Randomization SD				
	S1	S2	S3	S4	S5	S1	S2	S3	S4	S5	S1	S2	S3	S4	S5
S1	119	26	2	5	2	119.0	20.4	3.0	5.6	5.9	0.0	0.3	0.2	0.3	0.2
S2		46	3	0	3		46.0	1.1	1.8	2.1		0.0	0.1	0.1	0.2
S3			7	0	0			7.0	0.3	0.3			0.0	0.1	0.1
S4				13	0				13.0	0.5				0.0	0.1
S5					14					14.0					0.0

higher in pond 1 than in pond 2, tends to decrease with the season number and the year number, is lower for size class 1 than the other size classes, and tends to be higher for females than for males; (2) PC2: the difference between the counts for Echinostomatidae gen. sp. 1 and *Cylindrotaenia* sp. tends to decline with the season number, and may be lower in size classes 1 and 4 than in size classes 2 and 3; (3) PC3: the sum of the counts for Echinostomatidae gen. sp. 1 and *Cylindrotaenia* sp. tends to increase with the pond and the year; (4) PC4: the difference between the counts for *Lophosicyadiplostomun* sp. and *G. vitellinophilum* is more variable for pond 1 than for pond 2, more variable for season 1 than for the other seasons, and more variable for females than for males, and the level of variation also increases with the season number, (5) PC5: the contrast between the count for *C. propinquus* and those for *G. vitellinophilum* and *Lophosicyadiplostomun* sp. follows a similar pattern to that for PC4 in terms of variation.

Table 5 summarizes the results of the randomization test on variances of standardized species counts to examine whether within sample months there is evidence that the parasite species load per frog occurs independently between the frogs. Overall, there is little evidence against the null hypothesis of independence ($P=48.3\%$ on a two-tailed test). There is a significant result only in month 4, where the expected value of $\text{Var}(S)$ is 8.5 and the observed value is 18.8 ($P=4.7\%$ on a two-tailed test). Given the number of tests carried out, this can be attributed to chance. These results suggests that within months, the total parasite load in individual frogs is similar to what would be expected if parasite species occur independently.

The other test for association between species that was applied is the generalized Monte Carlo test described by Manly (1995). This examines the co-occurrences between parasite species given the number of parasite species for each frog and the total number of occurrences of each parasite species. First, the 96 frogs from pond 1 with at least one parasite species were considered. The estimated significance level for the overall test statistic for randomness was found to be 19.54%, with a standard error of 0.42%, so no evidence of associations was found. The serial algorithm used was repeated 100 times with a series length of 5,000. Second, the 160 frogs from pond 2 with

at least one parasite type were considered. In this case, evidence for associations was found. Overall, for all parasites considered together, the significance level was estimated to be 1.05%, with a standard error of 0.08%. The significance levels (SE) for the individual parasite species were: *G. vitellinophilum*, 1.30% (0.09%); *C. propinquus*, 1.11% (0.07%); *Lophosicyadiplostomun* sp., 19.18% (0.5%); Echinostomatidae gen. sp. 1, 43.32% (0.8%); and *Cylindrotaenia* sp., 3.65% (0.17%). Thus it seems that the distribution was not random for any of the species except *Lophosicyadiplostomun* sp. (Table 5).

Table 5 shows the number of co-occurrences observed for the parasite species together with their mean and standard deviation. The italicized values are unusual in comparison with the randomized distribution, and are mainly responsible for the significant test result. It seems, therefore, that *G. vitellinophilum* and *C. propinquus* occur together more often than expected by chance. The same is true for *C. propinquus* and *Lophosicyadiplostomun* sp. On the other hand, *G. vitellinophilum* and *Cylindrotaenia* sp. do not occur together as often as expected, and the same is true for *C. propinquus* and Echinostomatidae gen. sp. 1.

Discussion

The helminth infracommunities of *L. limellus* were similar for hosts from the semipermanent and permanent ponds in terms of species composition and dominance. Three dominant or "core" species of helminths were observed in the host for both ponds: *G. vitellinophilum*, *C. propinquus*, and *Lophosicyadiplostomun* sp. The effect of pond type on the number of parasites for the host was more important for *Lophosicyadiplostomun* sp. than for *C. propinquus* and *G. vitellinophilum*, decreasing in that order. The probability of the presence of the three species was greater in the semipermanent than in the permanent pond.

The probability of the presence of the species *G. vitellinophilum* increased with the size of the frogs. In pond 1, the probability of presence was greater for males than females, but this was reversed in pond 2. In addition, the proportion of females and males in pond 1 differed significantly, with more females present, probably

due to differential dispersion behavior by the two sexes. In contrast, in pond 2, the sex ratio was close to 1:1. *G. vitellinophilum* was located in the anterior part of the small intestine. The life cycle of this trematode resembles that of *G. quieta* as described by Leigh (1946). The metacercariae can infect the frog by penetrating its skin, before moving to the small intestine (Smyth and Smyth 1980).

For individuals of *C. propinquus*, the probability of presence was low for larger frog size in pond 1. However, the probability increased for the larger frogs in pond 2. This parasite species is located in the large intestine. Frogs become infected by eating molted skin containing metacercariae. For both *G. vitellinophilum* and *C. propinquus*, *L. limellus* is the definitive host.

The probability of the presence of *Lophosicyadiplostomun* sp. was high in pond 1 during the first year, and highest in male frogs with body size class 2. In general, this probability was higher in males than in females. This species is represented by its metacercariae located in the kidney. Frogs become infected when cercariae pass through the body and encyst in the kidney. The frog may be a good intermediate host for *Lophosicyadiplostomun* sp. because it is eaten by birds (Combes et al. 1994). Birds are the definitive host for this species.

The parasites Echinostomatidae gen. sp. 1 and *Cylindrotaenia* sp. occurred at low frequency. These species could be considered satellite species in this infracommunity. The probability was zero in pond 1 in year 1. Echinostomatidae gen. sp. 1 occurs in the metacercariae stages in the kidney, while those of *Cylindrotaenia* sp. are present in the adult stage in the middle region of the small intestine.

For parasite counts, significant effects were similar for *G. vitellinophilum* and *C. propinquus*, but were not the same as those found when only presence and absence were analyzed. For *Lophosicyadiplostomun* sp., Echinostomatidae gen. sp. 1, and *Cylindrotaenia* sp., there were large differences. In particular, there were more significant factors with the analysis on counts than for the analyses of presence and absence. Body size class was always an important factor, the pond was important for *G. vitellinophilum*, *C. propinquus*, and *Lophosicyadiplostomun* sp., with higher counts in the semipermanent pond, season was important for *C. propinquus* and Echinostomatidae gen. sp. 1, year was important for *C. propinquus*, Echinostomatidae gen. sp. 1, and *Cylindrotaenia* sp., and sex was important for *Lophosicyadiplostomun* sp. and Echinostomatidae gen. sp. 1 (with higher counts in females). Host body size has long been recognized as an important determinant of parasite richness (Dogiel 1964; Price 1980; Bell and Burt 1991; Hamann and Kehr 1998). Counts of *Lophosicyadiplostomun* sp. were related to host sex (Lees 1962; Plasota 1969; Hollis 1972), with females being most heavily infected.

For the *G. vitellinophilum*, the probability that a frog had at least one parasite increased with frog body size at pond 1. This probability was higher in males than in fe-

males. Considering the counts of parasites per host, the female frogs at pond 1 belonging to size class 4 had the most *G. vitellinophilum* parasites. The female frogs had a greater body length than males and were more abundant. The higher probability of male infection with *G. vitellinophilum* may be due to their earlier arrival, and therefore longer presence, at the pond after filling by rainwater. Males spent more time than females in pond 1.

The higher counts of *C. propinquus*, in pond 1 and in size class 2, may be due to their appearance after *G. vitellinophilum*, when the frogs had already had sufficient time to reach metamorphosis. The other dominant species in this infracommunity was *Lophosicyadiplostomun* sp. The highest counts were observed in frog size classes 2 and 4, with greater numbers in females than in males.

The species *G. vitellinophilum*, *C. propinquus*, and *Cylindrotaenia* sp. are considered to be autogenic because they complete their life cycles completely within the pond and use definitive hosts which are almost permanently restricted to the pond (e.g., frogs, turtles, snakes). *Lophosicyadiplostomun* sp. and Echinostomatidae gen. sp. 1 are considered to be allogenic species because they occur in definitive hosts that are not permanent residents of the pond. These hosts are usually migratory birds (Esch et al. 1988).

Co-occurrence of parasites was only seen in the hosts of pond 2. Positive associations were recorded only for those species whose individuals were located mainly in different organs of the body, or in different parts of the same organ. *G. vitellinophilum* and *C. propinquus* co-occurred more often than expected by chance. While *G. vitellinophilum* was located in the anterior part of the small intestine, *C. propinquus* was found in the large intestine. For both species, *L. limellus* was the definitive host. This association between individuals of *G. vitellinophilum* and *C. propinquus* is likely due to the separation of their ecological niches. Perhaps their fundamental niches overlap while their realized niches are different spaces in the intestine, allowing them to coexist (Kennedy 1975; Price 1980; Poulin 1998). Here, coexistence may depend on the pond type. In the permanent pond 2, the parasites encountered an *L. limellus* age structure reflecting the stability of the habitat. Periodic drying of semipermanent pond 1 will influence the host age structure, the mobility of its members or its metapopulation dynamics being important for pond recolonization. It is very possible that not all age classes are well represented after a dry period.

Lophosicyadiplostomun sp. and Echinostomatidae gen. sp. 1 showed no co-occurrence. They develop inside the same organ, the kidney. For both species, *L. limellus* is an intermediate host and must be eaten by birds, snakes, or other vertebrates for them to complete their life cycles. We find it impossible to determine whether the lack of coexistence of these species in the same organ is due to functional responses to competition for one or both species.

The three dominant parasite species found in *L. limellus* follow an opportunistic or "r" strategy, colonizing

the host size class that is most numerous in the frog population. Dominant parasite species were most abundant in the unpredictable pond. Desiccation is an important perturbation factor, playing a fundamental role for these host and parasite populations. If disturbance occurs frequently, the diversity succession will always be composed of pioneer or earlier species, and their population age structures will be very different from those in a stable pond. In our study, the difference in the helminth infracommunities between ponds was restricted to the abundance of the parasites. Parasite species in *L. limellus* were similar at both sites.

In summary, the helminth communities in *L. limellus* were depauperate, having low diversity in the two ponds considered. No fixed pattern in terms of infracommunity type, that is, isolationist or interactive, was observed. In interactive communities, competition (or biotic factors) is the main force structuring the infracommunities. In this situation, the species component has a high probability of colonizing the host. It predicts saturated, equilibrium communities structured by biotic interactions. Helminth infracommunities infesting in pond 2 (the stable habitat) had the characteristics of interactive infracommunities. Isolationist communities have a low probability of colonization, predicting unsaturated and non-equilibrium communities composed of species dispersed individualistically. We think that isolationist communities were observed in the host of the unstable pond 1.

Bush et al. (1990) suggest that contemporary ecological processes are the most significant factors affecting helminth community richness. If the presence of a species of parasite in a specific host has an evolutionary component within a determined area, the presence of a parasite in a host at a particular habitat still appears to be mainly a function of ecology. McAlpine (1997) concluded that contemporary ecology, including host and parasite life histories and abiotic features of particular wetlands, was the most important factor structuring helminth communities in three species of ranid frog in Canada. In agreement with this author, in our study, pond type was the most important factor shaping the helminth infracommunity structure in the frog host, *L. limellus*.

Acknowledgements We are extremely grateful to Donald F. McAlpine, Robert Poulin, and Frank R. Hensley for helpful comments on an earlier version of this manuscript which greatly improved it.

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