NOTE

Larval digenean communities in *Biomphalaria* species in two contrasting wetlands from South America

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ABSTRACT: A key factor in the colonization probabilities of parasites is the host's characteristic habitat. Considering that rice fields are simple habitats compared with natural wetlands, we comparatively analyzed the composition of the cercariae communities in *Biomphalaria* snails in an agricultural and a nonagricultural habitat of Corrientes Province, Argentina. We collected 2998 *Biomphalaria* snails from November 2011 to May 2012: *B. tenagophila*, *B. occidentalis*, *B. peregrina*, and *B. orbignyi* in the nonagricultural habitat and *B. straminea* in the agricultural habitat. Nine cercaria species were found in the nonagricultural habitat and 12 in the agricultural habitat, with overall parasite prevalence of 9.27 and 7.69%, respectively. The overall prevalence and mean species richness of cercariae showed no significant differences between habitats. The cercariae communities of the 2 habitats showed low similarity (38%), sharing 4 species. The mean species diversity of cercariae was higher in the nonagricultural habitat. Our results suggest that rice fields provide conditions for the hosts and cercariae that are somewhat similar to those of nonagricultural wetlands, although the contrasting characteristics of habitats can influence the presence of different intermediate and definitive hosts and consequently impact parasite diversity.

KEY WORDS: Digenea · Cercariae · Rice fields · Natural wetlands · Biodiversity

INTRODUCTION

The component community of cercariae in snails and their spatial and temporal heterogeneity is related to the abundance, distribution (temporal and spatial), and richness of the definitive hosts in the area (Smith 2001, Hechinger & Lafferty 2005). In this sense, the host's characteristic habitat is a key factor in the colonization probabilities of parasites, because a disturbance in the environment that may affect the host or transmission at any point of their life cycle reduces the abundance of cercariae measured in populations of the first intermediate host (Huspeni et al. 2005).

In recent times, human activities, including agricultural expansion, have increased the loss of natural wetlands. Flooded rice fields, which are presently one of the world's dominant forms of agriculture, act as temporary artificial wetlands (Ramsar Convention of 2011; www.ramsar.org). Different studies have shown that rice fields provide habitats for many species of invertebrates and vertebrates (Elphick et al. 2010, Blanco & de la Balze 2011, Maltchik et al. 2011), that is to say, a wide range of possible hosts in the life cycle of digeneans.

We analyzed the community of cercariae in the first intermediate host, snails of the genus *Biomphalaria* Preston, 1910, by considering 2 contrasting environ-

ments: a rice field (representing agricultural habitat) and a natural wetland (i.e. a nonagricultural habitat) in Corrientes Province, Argentina. Rice fields are habitats with very low heterogeneity, dominated by a single plant species, with uniform water depths that fluctuate on similar schedules, predictable disturbance patterns, and relative uniformity with many very similar units (Elphick 2010). In contrast, natural wetlands are more stable, i.e. with smaller fluctuations in water levels, and heterogeneous, i.e. with more available microhabitats.

The species of *Biomphalaria*, highly studied due to their medical importance in the transmission of schistosome parasites, inhabit a wide variety of environments, including shallow, temporary, and standing or slow-flowing freshwater bodies, and are widely distributed in Argentina, mainly in the northeast (Rumi & Núñez 2013). To test whether cercaria infections differed between the agricultural and nonagricultural habitat, the main goals of the present study were (1) to determine the cercariae community in *Biomphalaria* species in both habitats; (2) to compare the prevalence of larval infection between habitats; (3) to describe the degree of similarity in cercariae communities; and (4) to determine the mean species richness and diversity of parasite communities.

MATERIALS AND METHODS

Study sites

Biomphalaria snails were captured in 2 different types of habitats spaced by 20 km, in the northwest of Corrientes Province, Argentina. The agricultural habitat was a rice field situated at 27° 40′ S, 58° 48′ W. The sampled site had a surface area of 51 076 m². During the sampling period, the depth of the irrigated channels ranged between 5.0 and 50.0 cm. The nonagricultural habitat corresponded to 2 semipermanent water bodies that are very close together in the basin of the Riachuelo River, situated at 27° 29′ S, 58° 45′ W and 27° 29′ S, 58° 46′ W. These ponds have surface areas of 24 172 and 25 071 m² and depths of approximately 1.5 and 0.9 m, respectively. During the sampling period, the depth of the littoral zone ranged between 10.0 and 46.0 and between 10.0 and 45.0 cm.

Collection and examination of snails and cercariae

The snails were collected from the littoral zone of the ponds and from the edge of the cultivated parcels and irrigated channels (2 m in width and 260 m in length, approximately) for 1.5 h by 2 people using simple mesh nets (25 cm in frame diameter). The methodology used in the study of cercariae was described by Fernández et al. (2016), and measurement data are presented in previous publications (Fernández et al. 2013, 2014, 2016, Fernández 2014, Fernández & Hamann 2017). Identification of cercariae was carried out following Yamaguti (1975), Ostrowski de Núñez (1992), and Ostrowski de Núñez et al. (1990, 1991, 1997). Specimens were deposited in the Helminthological Collection of the Centro de Ecología Aplicada del Litoral (CECOAL), Corrientes, Argentina.

Statistical analysis

Prevalence of cercariae was calculated according to Bush et al. (1997), and abundance of cercariae was considered as the number of snails parasitized by a particular species of cercariae. To characterize the cercariae community from each habitat, we used species richness (S), the Shannon index (H'), and dominance (D). The diversity index was used with decimal logarithms ($\log_{10}$). Since the sample sizes in the nonagricultural and agricultural habitats were different, we used rarefaction methods (implemented in Eco-Sim 7.7 software; Gotelli & Entsminger 2004) to compare the mean diversity and the mean richness of cercariae. Wilcoxon′s V-test was used to compare differences in species diversity and richness between habitats. The Sorensen similarity index (S) was used to compare the degree of similarity in cercariae communities between habitats. The Z-test statistic was used to compare 2 proportions (prevalence) of cercariae infection between samples and habitats. A t-test was used to test for significant differences in cercariae diversity. The software used for analyses were Xlstat 7.5 and Bio-Dap.

RESULTS

In total, we collected 2998 *Biomphalaria* snails on 5 sampling dates between November 2011 and May 2012, from both the nonagricultural habitat (*B. tenagophila* n = 440, *B. occidentalis* n = 511, *B. peregrina* n = 18, *B. orbignyi* n = 13) and the agricultural habitat (*B. straminea* n = 2016). The sample sizes differed between habitats, due to the progressive drying of the nonagricultural habitat, where the number of snails sampled in March and April 2012 was lower (Table 1).

Table 1. Collection date, monthly temperature (mean ± SD, min.–max.), sample size of *Biomphalaria* (n), and overall prevalence (Prev.) of cercariae for each sample obtained from nonagricultural and agricultural habitats in Corrientes, Argentina. Statistical differences between 2 habitats in overall prevalence of cercariae (*Z*) marked in **bold**

Collection date	Temperature (°C)	Nonagricultural		Agricultural		*Z*	p
		n	Prev. (%)	n	Prev. (%)		
Dec 2011	25.3 ± 3.3 (12.2–40.7)	395	11.64	137	5.11	2.44	**0.015**
Jan 2012	27.3 ± 2.3 (16.2–38.9)	143	6.29	215	20.93	−4.09	**0.0001**
Feb 2012	28.4 ± 2.6 (15.0–38.7)	337	9.49	512	8.07	0.59	0.558
Mar 2012	25.0 ± 4.6 (7.9–39.4)	102	2.94	751	6.79	−1.73	0.084
Apr 2012	20.9 ± 4.1 (6.6–33.8)	5	20.00	401	2.49	0.41	0.680
Total		982	9.27	2016	7.69	−1.51	0.132

We found a total of 17 cercariae species in the 2 habitats: 12 species in the agricultural habitat and 9 species in the nonagricultural habitat (Fig. 1). The most abundant species (*D*) was the group of Echinostamatidae (Table 2).

The overall prevalence of cercariae showed no significant differences between habitats (p > 0.05; Table 1). In December 2011, the prevalence was significantly higher in the nonagricultural habitat, whereas in January 2012, the prevalence was significantly higher in the agricultural habitat (Table 1). Echinocercariae was the most prevalent group in both habitats, and its prevalence showed no significant differences between habitats (Fig. 2). The prevalence of furcocercariae and xiphidiocercariae

showed significant differences between habitats (p < 0.05; Fig. 2). The infection prevalence showed significant differences between furcocercariae and echinocercariae in January and February 2012 for agricultural habitat, and in December 2011 and February 2012 for nonagricultural habitat (Fig. 3).

Between habitats, the cercariae community showed a low similarity (S = 0.38). Using a rarefaction method, the number of individuals was combined, and the values of mean richness and mean diversity for each of the habitats were obtained (Table 3). The mean species richness of the cercariae did not differ between the 2 habitats (Wilcoxon *V*-test = 7, p = 0.074, n_1 = 9, n_2 = 9), whereas mean species diversity of cercariae
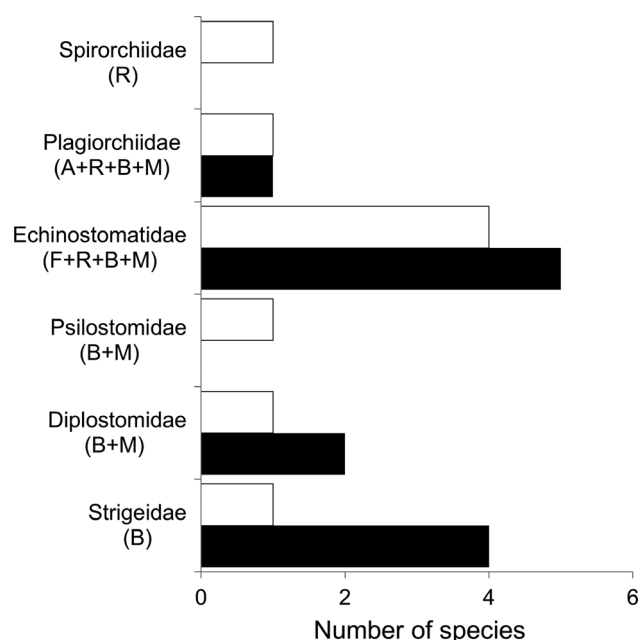

Fig. 1. Number of different cercariae species per family in nonagricultural (white bars) and agricultural (black bars) areas, and groups of definitive hosts (F: fish; A: amphibians; R: reptiles; B: birds; M: mammals)
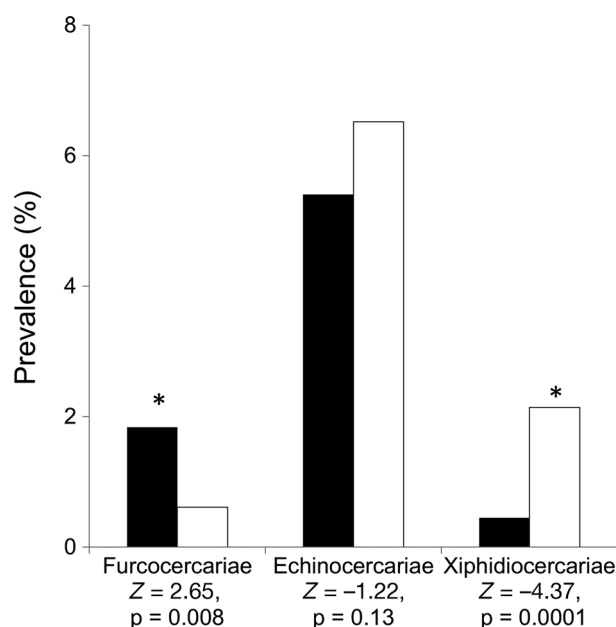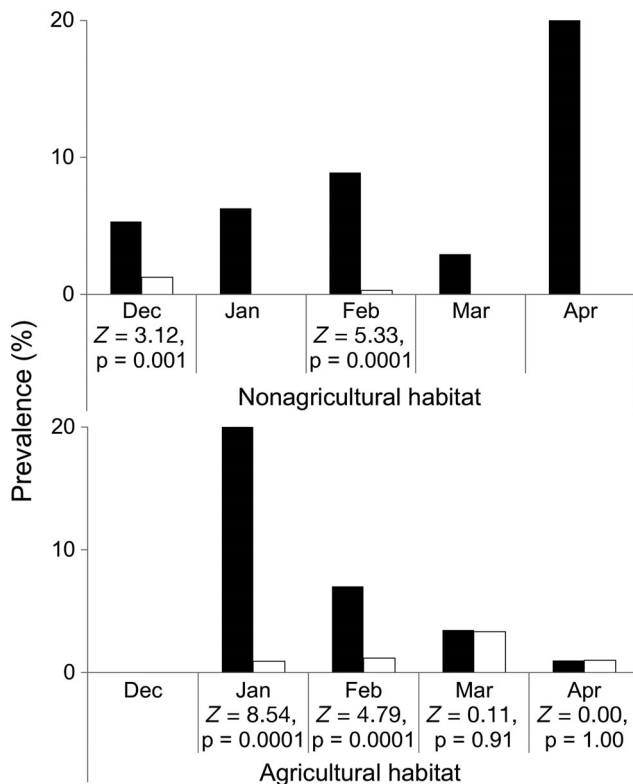

Fig. 2. Overall prevalence of trematode groups in *Biomphalaria* snails in nonagricultural (white bars) and agricultural (black bars) habitats in Corrientes, Argentina. *Significant difference (p < 0.05) between the 2 habitats in overall prevalence of furcocercariae and xiphidiocercariae

Table 2. Cercariae records in the genus *Biomphalaria* from nonagricultural and agricultural habitats in Corrientes, Argentina. A: abundance; *D*: dominance; BT: *B. tenagophila*; BO: *B. occidentalis*; BOR: *B. orbignyi*; BP: *B. peregrina*; BS: *B. straminea*

Larval digenean	Family	Nonagricultural Host	A	*D*	Agricultural Host	A	*D*	Reference(s)
Furcocercaria V	Strigeidae	–	–		BS	21	0.13	Fernández & Hamann (2017),
Cercaria *Spirorchiidae*	Spirorchiidae	BT	1	0.01	–			Fernández et al. (2016)
		BO	1	0.01				
Furcocercaria sp. XV	Strigeidae	–	–		BS	1	0.006	Fernández et al. (2013)
Furcocercaria sp. XVI	Strigeidae	–	–		BS	2	0.01	Fernández et al. (2013)
Furcocercaria sp. XVII	Diplostomidae	BT	1	0.01	BS	9	0.06	Fernández et al. (2013)
		BO	1	0.01				
Furcocercaria sp. XVIII	Schistosomatidae	–	–		BS	2	0.01	Fernández et al. (2013)
Furcocercaria sp. XX	Strigeidae	–	–		BS	2	0.01	Fernández & Hamann (2017),
Furcocercaria sp. III	Strigeidae	BO	2	0.02	–			Fernández et al. (2016)
Cercaria *Ribeiroia* sp.	Psilostomidae	BO	5	0.05	–			Ostrowski de Núñez et al. (1991), Fernández et al. (2016)
Echinocercaria sp. IV	Echinostomatidae	BT	1	0.01	–			Fernández et al. (2016)
		BO	9	0.10				
		BP	1	0.01				
Echinocercaria sp. V	Echinostomatidae	–			BS	75	0.48	Ostrowski de Núñez et al. (1997), Fernández (2014)
Echinocercaria sp. XIII	Echinostomatidae	–			BS	3	0.02	Fernández et al. (2014)
Echinocercaria sp. XIV	Echinostomatidae	BO	2	0.02	BS	2	0.01	Fernández et al. (2014)
Echinocercaria sp. XV	Echinostomatidae	–			BS	1	0.006	
Echinocercaria sp. XVI	Echinostomatidae	BT	2	0.02	BS	28	0.18	Fernández et al. (2014)
		BO	4	0.04				
Echinocercaria sp. 1	Echinostomatidae	BT	12	0.13	–			Fernández et al. (2016)
		BO	28	0.31				
Xiphidiocercaria sp. I	Plagiorchiidae	BT	8	0.09	BS	9	0.06	Ostrowski de Núñez et al. (1990), Fernández (2014)
		BO	7	0.08				
		BP	4	0.04				
		BOR	2	0.02				

Fig. 3. Monthly prevalence of echinocercariae (black bars) and furcocercariae (white bars) infecting snails of the genus *Biomphalaria* in nonagricultural and agricultural habitats in Corrientes, Argentina

was significantly higher in the nonagricultural habitat (Wilcoxon *V*-test = 45, p = 0.004, $n_1 = 9$, $n_2 = 9$).

Four species of cercariae were common to both habitats: *Furcocercaria* sp. XVII, *Echinocercaria* sp. XIV, *Echinocercaria* sp. XVI, and *Xiphidiocercaria* sp. I. The diversity of these cercariae showed no significant differences between habitats (*t*-test = −0.01, *V* = 19.31, p > 0.05). The inequality of species abundance of the nonagricultural habitat (*H'* = 0.46) was similar to that of the agricultural habitat (*H'* = 0.47).

DISCUSSION

In this study, the cercariae fauna in *Biomphalaria* snails exhibited a high species richness (17), ranging between 12 in the agricultural habitat and 9 in the nonagricultural habitat. The parasitic infection of this last habitat was similar to that previously described

Table 3. Summary of main results (±SD) of the rarefaction method. n: number of parasitized snails; Nonag: nonagricultural habitat; Ag: agricultural habitat

n	Mean richness		Mean diversity	
	Nonag	Ag	Nonag	Ag
10	4.44 ± 1.01	4.41 ± 1.06	1.28 ± 0.27	1.22 ± 0.29
20	6.04 ± 1.11	5.91 ± 1.20	1.44 ± 0.20	1.40 ± 0.22
30	7.02 ± 1.03	6.97 ± 1.26	1.51 ± 0.16	1.47 ± 0.18
40	7.71 ± 0.92	7.81 ± 1.25	1.56 ± 0.12	1.51 ± 0.16
50	8.17 ± 0.79	8.44 ± 1.26	1.58 ± 0.11	1.54 ± 0.13
60	8.53 ± 0.62	8.97 ± 1.22	1.60 ± 0.08	1.56 ± 0.12
70	8.78 ± 0.43	9.56 ± 1.20	1.61 ± 0.06	1.57 ± 0.10
80	8.94 ± 0.23	9.95 ± 1.11	1.62 ± 0.04	1.59 ± 0.10
91	9.00 ± 0.00	10.43 ± 0.09	1.63 ± 0.00	1.60 ± 0.08

in *Biomphalaria* spp. from other natural wetlands in northeastern Argentina, with maximum richness of 9 larval species (Ostrowski de Núñez et al. 1990, 1991, 1997). In addition, the low similarity (38%) in cercariae communities between habitats could be explained by the contrasting characteristics of the habitats that can influence the presence of different definitive hosts, e.g. birds and fish (see Blanco & de la Balze 2011) and availability of intermediate gastropod hosts. In this sense, the presence of different species of *Biomphalaria* in the 2 habitats could have partially influenced the parasite diversity, considering the low specificity of the cercariae for the *Biomphalaria* species from similar environments (Fernández 2014). In turn, among the 4 shared species in both habitats, the dominant species was different in each habitat, which also indicates inequality in the abundance of definitive hosts. Finally, the higher mean diversity observed in the nonagricultural vs. agricultural habitats is in agreement with several studies that demonstrated that, in impacted environments, cercariae are less abundant and less diverse in first intermediate hosts (Huspeni et al. 2005).

The infection prevalence during the study period showed no significant differences between environments, which suggests that both environments are generally suitable for the definitive hosts, miracidia and *Biomphalaria* species. However, we found no *Biomphalaria* species overlap between the 2 environments, which may be related to the different available microhabitats (Rumi et al. 2002); moreover, pesticide use could affect the community of intermediate hosts by reducing the availability of food to benthic macroinvertebrates (Gagneten 2002), although the longevity and infectivity of digenean larval stages appear not to be affected (Raffel et al. 2009). The smaller prevalence observed in the agricultural vs.

nonagricultural habitat in December 2011 coincided with the initial stage of the flooding of the field, when suitable conditions for the establishment of the hosts are not yet available, i.e. not all of the flora in the irrigated channels that serve as substrate for snails are established. Nevertheless, the higher prevalence observed in the rice field in January 2012 can indicate more abundance of definitive hosts and higher concentration of snails for the transmission of free larval stages.

The echinocercariae was the most prevalent group in both habitats, which coincides with the observations of Ostrowski de Núñez et al. (1990, 1997) in different freshwater habitats of Corrientes, Argentina. Furcocercariae were more prevalent in the rice field than in the non-agricultural wetland and represented the richest group with respect to the number of species in the families Strigeidae and Diplostomidae, which use mainly birds as definitive hosts (Fig. 1). Birds are highly mobile and therefore may access rice fields more easily than say, e.g. fishes or reptiles, which may have a smaller home range and relatively slow dispersal. In this sense, the rice fields from northeastern Argentina are alternative habitats for many waterbird species that forage on a variety of prey, nest in the crop and in fringing vegetation, and use the rice fields for staging during migration (see Lesterhuis 2011). Finally, the low occurrence of *Xiphidiocercaria* sp. I in the agricultural wetland would indicate that some factor (e.g. reduced microhabitat, use of pesticides) either limited or reduced the presence of their definitive host, considering that this cercaria was present in several *Biomphalaria* species (Table 2).

In both habitats, when echinocercariae reached their maximum prevalence, furcocercariae exhibited an opposite trend. These trends were previously observed in Planorbidae from Corrientes Province (Ostrowski de Núñez et al. 1990, 1991) and Argentine Patagonia (Flores et al. 2010). Moreover, the peak infection of furcocercariae and echinocercariae (from January to April 2012) observed at different stages of rice cultivation (echinocercariae: January, eared/mature rice and deeply flooded; furcocercariae: March, rice stubble with water) could be linked to the temporary presence of definitive hosts in the environment. In this sense, Blanco et al. (2006) demonstrated that the succession of microhabitats provided by the rice fields throughout the growing cycle favors the differential use of certain groups of aquatic birds that, according to their particular requirements, are temporarily present in the environment.

This study is the first to emphasize the role of habitat type in the transmission dynamics of cercariae in the first intermediate host from agricultural and nonagricultural wetlands in Argentina. It should be pointed out that in this study we analyzed the family-level diversity, due to the complexity of arriving at species, therefore the species-level diversity story may be more complicated. The species diversity of cercariae found in the snails from the rice field shows that this environment offers an alternative to natural wetlands for definitive hosts. There is a need to further study cercariae in rice fields (from morphological, molecular, and life-cycle perspectives) to elucidate interactions between the habitat quality, hosts, and digenean parasites.

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