Helminth community structure and diet of three Afrotropical anuran species: a test of the interactive-versus-isolationist parasite communities hypothesis

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Abstract. The interactive-versus-isolationist hypothesis predicts that parasite communities should be depauperated and weakly structured by interspecific competition in amphibians. A parasitological survey was carried out to test this hypothesis using three anuran species from Nigeria, tropical Africa (one Bufonidae; two Ranidae). High values of parasite infection parameters were found in all three species, which were infected by nematodes, cestodes and trematodes. Nonetheless, the parasite communities of the three anurans were very depauperated in terms of number of species (4 to 6). Interspecific competition was irrelevant in all species, as revealed by null models and Monte Carlo permutations. Cluster analyses revealed that, in terms of parasite community composition, the two Ranidae were similar, whereas the Bufonidae was more different. However, when prevalence, intensity, and abundance of parasites are combined into a multivariate analysis, each anuran species was clearly spaced apart from the others, thus revealing considerable species-specific differences in terms of their parasite communities. All anurans were generalists and probably opportunistic in terms of dietary habits, and showed no evidence of interspecific competition for food. Overall, our data are widely consistent with expectations driven from the interactive-versus-isolationist parasite communities hypothesis.

1 Introduction

The most successful theoretical framework for parasite community ecology research has been the interactive versus isolationist classification of parasite communities (Holmes and Price, 1986; Esch et al., 1990; Sousa, 1994), now viewed as extremes of a continuum rather than a dichotomy given the huge variability observed in natural assemblages of parasites (Poulin, 2001; Poulin and Luque, 2003). According to this theoretical framework, interactive parasite communities consist of many species, most with high host colonization rates (Holmes and Price, 1986). These features result in infracommunities (Bush et al., 1997) with many co-occurring para-

site species, several having large infrapopulation sizes, and thus niche overlap and interspecific interactions are potentially extensive (Poulin, 2001; Poulin and Luque, 2003). In contrast, isolationist communities consist of fewer species, most with limited colonisation abilities; their infrapopulations are generally small and only rarely co-occur with those of other species in the same host at intensities high enough to result in significant interactions (Holmes and Price, 1986; Poulin, 2001; Poulin and Luque, 2003). This dichotomy is certainly useful but still difficult to apply because (i) most parasite communities fall somewhere in-between these extremes, and (ii) when applied to real data, the classification of parasite communities along the continuum has been the matter of subjective judgment as there is no single measure of the general degree of interactivity in parasite communities (but see Poulin and Luque (2003) for the development of an index).

Helminthes are important parasites of free-ranging amphibians (Baker, 1987; Bursey et al., 2001), with an ecological role in regulating amphibian population demography being still little studied (Baker, 1987; Aho, 1990; Anderson, 2000; McAllister et al., 2010). Typically, helminth communities of amphibians are species-poor assemblages where interactions are unlikely (Aho, 1990; Barton, 1999). Several factors have been advocated to explain the fact that amphibian helminth communities are depauperated (Aho, 1990) and belong to the isolationist extremity of the continuum, including a generalized opportunistic diet, simple intestinal system, low vagility and ectothermic metabolism of their hosts (Barton, 1999).

Most of the studies analyzing community structure of helminthes parasitizing amphibians were conducted in temperate regions (e.g., Bolek and Coggins, 2001; Muzzall et al., 2001; Brooks et al., 2006), whereas studies on tropical helminthes and their amphibian hosts are uncommon in the international literature, and mainly confined to Central and South American study systems (e.g., Bursey et al., 2001; Hamann et al., 2006). Studies on African tropical systems are even rarer, and concerning the helminth fauna of amphibians in Nigeria, there are just a few studies available to date (Anyacho, 1997; Anosike and Keke, 2002; Aisien et al., 2003). All these studies were simply descriptive. No study, however, analyzed the frog helminthes in a community ecology perspective (Friggens and Brown, 2005).

The lack of community ecology research on helminthes parasitizing anurans in African tropical regions is particularly unfortunate, not only in the light of the theoretical framework above-mentioned but also because many helminth species exploit food-web relationships, and are valuable as means of reflecting the biodiversity of their habitat (Marcogliese, 2001). Host feeding and diet are important in helminth acquisition, and the habitat from which the bulk of food is obtained is as important as the diet in parasite fauna acquisition (Guidelli et al., 2003).

In this paper, we analyze the helminth fauna and the diet parameters of three contrasted species of African tropical anurans: a terrestrial toad (*Amietophrynus* (= *Bufo*) *maculatus*; Bufonidae), a large-sized aquatic frog (*Hoplobatrachus occipitalis*; Ranidae), and a smaller sized aquatic frog (*Ptychadena aequiplicata*, Ranidae) from Port Harcourt, Nigeria. More specifically, we address the following key questions: (i) do helminth communities of the three anuran species mentioned above belong to the interactive versus isolationist side of the continuum? Our hypothesis is that they should belong to the isolationist side, as predicted from the majority of studies on amphibian parasites from elsewhere (Aho, 1990; Barton, 1999); (ii) are the helminth communities structured by interspecific competition? We hypothesize that interspecific competition should be irrelevant for these helminth communities, as suggested by previous amphibian studies (Aho, 1990; Barton, 1999); (iii) are the studied anuran species generalists in diets? We predict that they should be general-

ist, because dietary generalism is one of the factors producing depauperated communities of parasites in these ectothermic vertebrates, at least for trematodes and cestodes (Barton, 1999). We explored these issues by using null models (Gotelli and Graves, 1996), with emphasis on determining whether the helminth communities are non-randomly structured within their anuran hosts, i.e. whether the location of the various helminth species within the host's gastrointestinal system may be determined by resource partitioning patterns (for the case of cestodes within elasmobranchs, see Friggens and Brown, 2005).

2   Materials and methods

2.1   Study area

We randomly collected amphibians from ponds, wet grasses and refuse dumps around residential areas of Port Harcourt (co-ordinates 04°45′ N; 07°01′ E; Rivers State), in the Niger Delta region of southern Nigeria. The study sites were precisely: Rumuokwurusi (N 04°51.643, E 007003.576), Woji (N 04°49.385, E 007003.710′), Azubie (N 04°48.682′ E 007003.112′), and Rumunduru (N 04°52.239′ E 007001.806′). All collection sites were geographically close, so the data was pooled. We did not sample intensively a single area for conservation reasons, i.e. for avoiding removal of an excessive number of anuran individuals from a same population. The study area has relatively slight monthly fluctuations in maximum and minimum temperatures (27–33 °C), and high mean relative humidity of over 65 % in most months of the year. The dry season extends from November to April and the wet season extends May to October, with peaks in July and September.

2.2   Sampling protocol

Overall, 116 specimens of *Amietophrynus maculatus* (69 males and 47 females), 9 specimens of *Hoplobatrachus occipitalis* (6 males and 3 females) and 15 specimens of *Ptychadena aequiplicata* (9 females and 6 males) were collected within short period (May to November 2008) in order to minimize the seasonal variations on enteric helminth infracommunity structure occurring in the hosts (Verma and Singh, 2000; Bolek and Coggins, 2001). We decided not to sacrify more individuals of the two latter species for conservation purposes, because they are widespread but usually not abundant in the studied ponds.

The specimens were transported alive to the laboratory where they were humanely sacrificed with MS 222, commercially available as Tricaine Methane Sulfonate (TMS). Tricaine is a benzoic acid derivative and, in water of low alkalinity (<50 mg L^{-1} as CaCO$_3$), the solution was buffered with sodium bicarbonate. A 10 g L^{-1} stock solution was made, and sodium bicarbonate added to saturation, resulting in a pH between 7.0 and 7.5 for the solution (Anonymous, 2006).

The body cavity was opened by longitudinal incision from throat to vent and the gut was detached by cutting across the esophagus and rectum (Goldberg and Bursey, 2007). Thereafter, it was severed into four regions: esophagus, stomach, intestine, and colon. Each segment was opened longitudinally and placed separately in a watch glass containing normal saline to extract the helminthes. They were preserved in vials containing 70 % ethanol until later examination. Nematodes were identified after clearing in a drop of undiluted glycerol on a glass slide; cestodes and trematodes were regressively stained in haematoxylin, dehydrated in graded ethanol and mounted in balsam for examination (Goldberg and Bursey, 2007). The helminthes were subsequently identified to possible taxonomic level, using a high resolution compound microscope (Model: National Motic A07270). After counting, (i) prevalence was calculated as the percentage of infected individuals of each species; (ii) mean intensity as the number of helminthes per infected frog; and (iii) species-richness was the number of helminth species per frog (Verma and Singh, 2000). Voucher helminthes and frogs were deposited in the Museum, Department of Applied and Environmental Biology, Rivers State University of Science and Technology, Port Harcourt.

Food items of anuran individuals were also removed, identified and counted. With some exceptions, order level was used as the operational taxonomic unit (OTU, Sneath and Sokal, 1973) for identification. The use of descriptive ecological terms is according to Bush et al. (1997).

2.3 Statistical analyses

The following parameters were calculated: abundance (number of helminthes or prey items), richness (number of parasite species), diversity and evenness. Parametric tests were used after having verified data normality and homoscedasticity. Non-normal data were log-transformed to achieve normality, and then parametric tests were used. ANOVA was used to calculate differences between means in multiple population groups. Frequency differences in helminthes among the various segments of the gastrointestinal system of anurans were analyzed by observed versus expected χ^2 test. UP-GMA dendrogram (with Euclidean distances) were used to cluster dissimilarities between anurans in terms of their parasite species, and between parasites in terms of their occurrence among the three anuran species.

Helminth communities within each anuran species were analyzed for random/nonrandom organization patterns by null models (Gotelli and Graves, 1996), with each sector of the anuran gastrointestinal tract being considered a different microhabitat resource. The same approach was previously used by Friggens and Brown (2005) for cestode communities of elasmobranchs. To evaluate whether each helminth community was structured randomly or not, we contrasted the actual data matrix with 30 000 random "pseudo-communities" generated by Monte Carlo simulations (Gotelli and Graves,

1996). Pianka's (1986) overlap formula was calculated for all communities, and the original species utilization matrices from which Pianka's overlap was calculated were randomized by shuffling the original values among the resource states. We used two randomization algorithms (RA2 and RA3) of Lawlor (1980), as they are particularly robust for niche overlap studies (Gotelli and Graves, 1996). RA2 tests for structure in the generalist-specialist nature of the resource utilization matrix by conserving guild structure, but destroying observed niche breadth (Gotelli and Graves, 1996; Luiselli, 2006, 2008). RA3 tests for guild structure by conserving niche breadth for each species, but destroying guild structure manifested by the zero structure of the resource utilization matrix (Gotelli and Graves, 1996; Luiselli, 2006, 2008). Niche overlap values were calculated for each of these randomly generated matrices, and species-pair and community-summary statistics were computed (Friggens and Brown, 2005). Actual overlap values were then compared to the distributions of the expected values. Structure was assumed when $P_{\mathrm{obs} \leq \mathrm{exp}} < 0.05$ (Gotelli and Graves, 1996). In all cases, equiprobable resource use was assumed. The same algorithm procedures and the same number of random Monte Carlo permutations were also performed to test niche partitioning patterns in food habits of the three sympatric anurans. We used the EcoSim software (Acquired Intelligence Corp., Kesey-Bear) to calculate overlap indices and to generate Monte Carlo simulations.

Apart from null models, all the other statistical tests were done according to Zar (1984) and Fowler and Cohen (1994), and performed by a Statistica for Windows (version 6.0) software.

3 Results

3.1 General patterns of helminth distribution across anuran species and gastrointestinal tracts

A total of 2903 helminthes were extracted from the anurans examined; 2495 (85.9 %) from *Amietophrynus maculatus*, 240 (8.3 %) from *Hoplobatrachus occipitalis*, while 168 (5.8 %) were from *Ptychadena aequiplicata*. In all, there were nine helminth species infecting the anurans; 3 nematodes, 3 trematodes, and 3 cestodes (Table 1), with the highest helminth burden record being due to the nematodes, *Rhabdias bufonis* and *Oxyuris* sp.

Six helminth species were recovered from *Amietophrynus maculatus* specimens, including nematodes, cestodes, and trematodes (two species each). *Hoplobatrachus occipitalis* harbored four helminth species, including 2 nematodes, 1 cestode, and 1 trematode. Five helminth species were recovered from *Ptychadena aequiplicata*, including 2 nematodes, 1 cestode, 1 trematode and 1 monogenean. Two helminthes (*Oxyuris* sp. and *Eurytrema* sp.) were found in all anurans, two in two anurans, and the rest were found in only an anuran species.

Table 1. Summary of the numbers of helminthes extracted from different segments of the anuran guts in three species from Port Harcourt, Nigeria.

Anura (Host) species	Helminth species	Phylum	Esophagus	Stomach	Intestine	Colon	Total
Amietophrynus maculates							
	Rhabdias bufonis	Nematoda	12	357	782	28	1179
	Oxyuris sp.	Nematoda	0	17	78	929	1024
	Eurytrema sp.	Trematoda	0	119	108	32	259
	Mehraorchis sp.	Trematoda	0	0	5	0	5
	Davainea sp.	Cestoda	0	1	12	0	13
	Diphyllobothrium sp.	Cestoda	0	0	15	0	15
Hoplobatrachus occipitalis							
	Aplectana acuminata	Nematoda	0	5	16	0	21
	Oxyuris sp.	Nematoda	0	0	3	106	109
	Eurytrema sp.	Trematoda	0	35	62	7	104
	Diphyllobothrium sp.	Cestoda	0	0	6	0	6
Ptychadena aequiplicata							
	Aplectana acuminata	Nematoda	0	8	20	0	28
	Oxyuris sp.	Nematoda	0	0	2	62	64
	Eurytrema sp.	Trematoda	0	21	46	3	70
	Polystoma sp.	Monogenea	0	0	1	0	1
	Raillietina sp.	Cestoda	0	0	5	0	5
TOTAL			12	563	1161	1167	2903
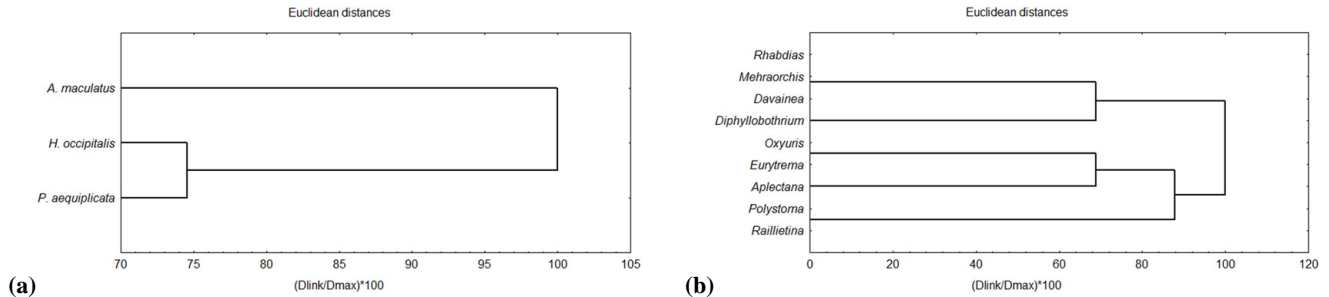
Figure 1. UPGMA dendrograms (standardized to 100 %) showing dissimilarity between host-anuran species in terms of their helminth communities **(a)**, and among helminth species in terms of their associations with the three studied anuran species **(b)**.

A UPGMA dendrogram showed that the two Ranidae clustered together in terms of helminth species parasitizing them, with *Amietophrynus maculatus* being substantially different (Fig. 1a). A same type of cluster analysis revealed three helminth clusters in terms of their association with anurans (Fig. 1b): (i) a cluster formed by *Rhabdias, Mehraorchis*, and *Davainea*, (ii) *Oxyuris* and *Eurytrema*; (iii) *Polystoma* and *Raillietina*.

The frequency of occurrence of helminthes was strongly uneven among tracts of the gastrointestinal system, either pooling the three anuran species ($\chi^2 = 1268.63$, $df = 3$, $P < 0.0001$) or considering them separately (*A. maculatus*: $\chi^2 = 1067.81$, $df = 3$, $P < 0.0001$; *H. occipitalis*:

$\chi^2 = 125.63$, $df = 3$, $P < 0.0001$; *P. aequiplicata*: $\chi^2 = 83.00$, $df = 3$, $P < 0.0001$). In all the anurans, the esophagus harbored the least number of helminthes with only one species (*Rhabdias bufonis*) observed, and intestine was the favored sector of the anuran gastrointestinal system by all helminth species apart from *Oxyuris* sp. (Table 2). *Rhabdias bufonis* also occurred in other segments. The total number of helminthes extracted in the various segment indicate that their segment preference followed the order: (colon = intestine) > stomach > esophagus.

Table 2. Results of statistical analysis showing different species-specific preferences of helminth species for particular sectors of the gastrointestinal system of anuran species from Port Harcourt, Nigeria. When a same helminth species occurred in more than one anuran species, statistical analyses were performed on pooled numbers.

| Helminth species	χ^2 test ($df = 3$), P-value	Sector Favored
Rhabdias bufonis	1331.27, <0.0001	Intestine
Oxyuris sp.	2848.40, <0.0001	Colon
Eurytrema sp.	198.54, <0.0001	Intestine
Mehraorchis sp.	15, <0.0019	Intestine
Davainea sp.	31.61, <0.0001	Intestine
Diphyllobothrium sp.	63.00, <0.0001	Intestine
Aplectana acuminata	70.59, <0.0001	Intestine
Polystoma sp.	Too small sample size for analysis	
Raillietina sp.	15, <0.0019	Intestine
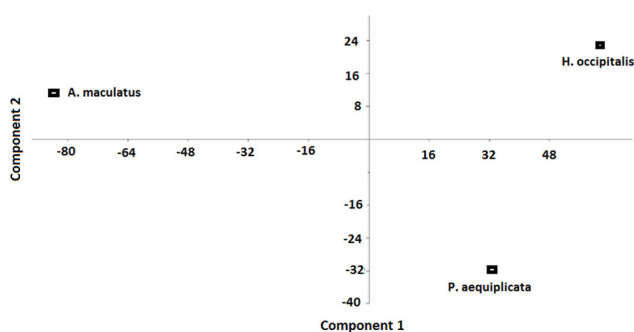
Figure 2. Scatterplot of a Principal Component Analysis classifying the three anuran species on the basis of the prevalence, intensity, and abundance of their gastrointestinal parasites.

3.2 Helminth community structure

Prevalence, intensity of infection, abundance and diversity for each helminth species in each of the three hosts are shown in Table 3. *Amietophrynus maculatus* showed more richness and diversity of helminth communities, having six helminth species, than *H. occipitalis* and *P. aequiplicata*. Combining prevalence, intensity, and abundance of parasites into a PCA (eigenvalues: component 1 = 6175.09 with 88.14 % of variance explained; component 2 = 830.63 with 11.86 % of variance explained), it resulted that each of the three anuran species was very different and spaced apart into the multivariate space (Fig. 2).

Niche overlaps between helminth species in the use of four gastrointestinal system sectors ranged 0.083–1 in *A. maculatus* ($x = 0.622$; variance = 0.145), 0.027–0.973 in *H. occipitalis* ($x = 0.495$; variance = 0.231), and 0.032–0.997 in *P. aequiplicata* ($x = 0.585$; variance = 0.217). Helminth communities were randomly organized in *A. maculatus* according to RA3 (mean of simulated indices = 0.350; variance of simulated indices = 0.0089; $P_{obs \leq exp} = 0.984$) and RA2 (mean of simulated indices = 0.621; variance of sim-

ulated indices = 0.0087; $P_{obs \leq exp} = 0.506$). Also in *H. occipitalis* there was no evidence of structured community of helminthes according to both randomization algorithms (RA3: mean of simulated indices = 0.348; variance of simulated indices = 0.023; $P_{obs \leq exp} = 0.849$; RA2: mean of simulated indices = 0.595; variance of simulated indices = 0.020; $P_{obs \leq exp} = 0.259$). No community structure was also found in *P. aequiplicata* with both RA3 (mean of simulated indices = 0.326; variance of simulated indices = 0.015; $P_{obs \leq exp} = 0.954$) and RA2 (mean of simulated indices = 0.639; variance of simulated indices = 0.017; $P_{obs \leq exp} = 0.354$).

3.3 Anuran dietary habits

All anuran species were insectophagous (Coleoptera were particularly frequently eaten) with Gastropoda being also commonly consumed (Table 4). Few plant parts were also found in anuran guts, but these were certainly ingested secondarily by them as all amphibians are strictly carnivorous. All species appeared clearly generalists in habits: excluding plant remains, a total of 10 different prey type categories were found in *A. maculatus*, and 9 prey types in each of the two Ranidae.

In terms of percentage of occurrence of food items, there were high niche overlaps values in all pairwise combinations (*A. maculatus* versus *H. occipitalis* = 0.796; *A. maculatus* versus *P. aequiplicata* = 0.871; *P. aequiplicata* versus *H. occipitalis* = 0.906; $x = 0.858$; variance = 0.003), with no evidence of food niche partitioning using both RA3 (mean of simulated indices = 0.467; variance of simulated indices = 0.008; $P_{obs \leq exp} = 0.999$) and RA2 (mean of simulated indices = 0.710; variance of simulated indices = 0.005; $P_{obs \leq exp} = 0.992$).

Also in terms of percentage of abundance of food items, the results were not substantially different. Indeed, there were high niche overlaps values in all pairwise combinations (*A. maculatus* versus *H. occipitalis* = 0.967; *A. maculatus*

Table 3. Infection parameters of the helminthes parasitizing three anuran species from Port Harcourt, Nigeria. Symbols: n = number of infected hosts. Exam = number of examined hosts. N = overall number of helminthes.

Anuran species	Helminth species	Prevalence n/Exam %	Intensity Range mean	Abundance N mean
Amietophrynus maculatus				
	Rhabdias bufonis	98/116	1–28	1134
		84.5	11.6	11.7
	Oxyuris sp.	102/116	1–24	1024
		87.9	10.0	11.0
	Davainea sp.	6/116	1–4	13
		5.2	2.2	2.2
	Diphyllobothrium sp.	5/116	1–5	15
		4.3	3.0	3.0
	Eurytrema sp.	20/116	2–30	262
		17.2	13.1	13.1
	Mehraorchis sp.	2/116	2–3	05
		1.7	2.5	2.5
Hoplobatrachus occipitalis				
	Aplectana acuminata	8/9	1–5	21
		88.9	2.6	2.6
	Oxyuris sp.	9/9	9–20	109
		100.0	12.1	12.1
	Diphyllobothrium sp.	3/9	1–3	06
		33.3	2.0	2.0
	Eurytrema sp.	9/9	5–22	104
		100.0	11.6	11.6
Ptychadena aequiplicata				
	Aplectana acuminata	11/15	1–5	28
		73.3	2.6	2.6
	Oxyuris sp.	13/15	2–10	64
		86.7	4.9	4.9
	Raillietina sp.	2/15	2–3	05
		13.3	2.5	2.5
	Eurytrema sp.	7/15	3–21	71
		46.7	10.1	10.1
	Polystoma sp.	1/15	0–1	01
		6.7	1.0	1.0

versus *P. aequiplicata* = 0.953; *P. aequiplicata* versus *H. occipitalis* = 0.990; x = 0.970; variance = 0.251), with again no evidence of food niche partitioning using both RA3 (mean of simulated indices = 0.251; variance of simulated indices = 0.018; $P_{obs \leq exp}$ = 1.00) and RA2 (mean of simulated indices = 0.709; variance of simulated indices = 0.005; $P_{obs \leq exp}$ = 1.00). Hence, we conclude that the three anuran species were substantially similar in terms of food composition, with no evidence of any interspecific competition for food among them.

4 Discussion

To begin with, we should stress that our samples were small for two of the three species (*H. occipitalis* and *P. aequiplicata*), and this fact may have somewhat influenced the results. This potential shortcoming aside, our study has nonetheless revealed some clear patterns concerning the communities of gastrointestinal parasites of the three studied anurans. Firstly, our study clearly showed that the helminth communities were quite depauperate in all the three species, with 4 to 6 species being detected in each host species. In this regard, our data perfectly agree with earlier studies

Table 4. Diet descriptors of the three anurans from Port Harcourt, Nigeria. Symbols: T = total number of prey items; $\%\,P$ = percentage of occurrence; $\%\,N$ = percentage of abundance.

Prey type	A. maculatus			H. occipitalis			P. aequiplicata		
	T	$\%\,P$	$\%\,N$	T	$\%\,P$	$\%\,N$	T	$\%\,P$	$\%\,N$
Coleoptera	210	86.2	76.4	53	77.8	52.5	39	73.3	46.4
Lepidoptera	03	1.7	1.1	03	22.2	3.0	02	6.7	2.4
Diptera	04	2.6	1.5	02	22.2	2.0	03	6.7	3.6
Orthoptera	04	3.5	1.5	03	33.3	3.0	04	26.7	4.8
Hymenoptera	03	0.9	1.1	03	33.3	3.0	04	26.7	4.8
Isoptera	01	0.9	0.4	02	11.1	2.0	04	20.0	4.8
Unidentified insects	20	94.8	7.3	17	100.0	16.8	13	66.7	15.5
Acarina	02	0.9	0.7	–	–	–	03	20.0	3.6
Miriapoda	05	2.9	1.8	03	22.2	3.0	–	–	–
Gastropoda	16	13.8	5.8	11	100.0	10.9	07	33.3	8.3
Plant parts	07	6.0	2.6	04	33.3	3.8	05	33.3	6.0

indicating that amphibian parasite communities are typically in the proximity to the isolationist extremity of the continuum (Aho, 1990). Also, null model analyses revealed that the helminth communities were not structured by niche resource partitioning in any of the anuran hosts. Hence, our data fit well with the idea that parasite communities form unstructured assemblages with little resource limitation and competitive influence (Rohde, 1991; Gotelli and Rohde, 2002). This pattern is also consistent with the theoretical predictions of the isolationist model for parasite communities. Overall, Afrotropical anurans conform with both temperate and Neotropical amphibians in terms of the general characteristics of their parasite communities.

The helminth communities found in the three anurans examined here differed slightly: three nematodes were recovered from *A. maculatus*, while two were recovered from both *H. occipitalis* and *P. aequiplicata*. *Oxyuris* sp. was common to all three anurans. Although this latter is primarily a mammal parasite, it has already been recorded as a parasite of amphibians elsewhere (e.g., Lehmann, 1960). *Aplectana acuminata* occurred in *H. occipitalis* and *P. aequiplicata*, while *Rhabdias bufonis* occurred only in *A. maculatus*. Of the cestodes, *Diphyllobothrium* sp. was common to *A. maculatus* and *H. occipitalis*. The trematode, *E. pancreaticum* occurred in all three hosts. The only monogenean recovered was from a *P. aequiplicata* specimen. No single community included all species locally available.

Our data revealed that, in terms of helminth community composition (presence/absence of the various parasites), there was an higher similarity between the two Ranidae than between each of the Ranidae and the only Bufonidae studied here. It is likely that this higher similarity may depend on the more similar general ecological habits of the two Ranidae (semi-aquatic) whereas the Bufonidae is terrestrial. An habitat effect on the abundance and richness of helminth parasites

was also reported in earlier studies (e.g., McKenzie, 2007). However, when prevalence, intensity, and abundance of parasites are combined into a multivariate analysis, each anuran species was clearly spaced apart from the others, thus revealing considerable species-specific differences in terms of their parasite communities. We tentatively attribute these differences to microhabitat differences between the anuran species. For instance, *P. aequiplicata* tends to be more forest-linked than *H. occipitalis*, which is found also in open grassland ponds (Rödel, 2000; Akani et al., 2005).

Our diet data of the three anurans showed a generalist trophic spectrum for all species, with insects (especially coleopterans) and gastropods being important food items. Dietary generalism of the host has been predicted to be an ecological correlate of the isolationist structure of parasite communities (Poulin and Luque, 2003), and also in this regard our study fits well with theoretical expectations. It is likely that prey availability around the habitat of anurans was very important in regulating the food items, and that anurans ate all moving objects in their sight range that they can ingest (e.g., Toshiaki and Masafumi, 2001). However, it is noteworthy in our study that the overall dietary spectrum was similar among anuran species (as testified by the high niche overlap values observed), despite we included in the analyses anuran species with very different habitat and life-style characteristics. For instance, *A. maculatus* is strictly terrestrial, whereas the other two species are aquatic (Rödel, 2000), and they also differ locally in terms of microhabitat characteristics at most sites (e.g., Akani et al., 2005). In addition, our anuran species paralleled their helminth communities in that they did not compete interspecifically for food, as clearly shown by our null model analyses with both RA2 and RA3 algorithms. Also in this case, lack of interspecific competition for food is expectable among sympatric predators with generalist and opportunistic dietary habits (Pianka, 1986).

Price (1990) stated that predation was an integral part of transmission in many animal parasite systems. In our study case, the three anurans were clearly indiscriminate, unspecialized predators of invertebrate organisms. This is widely in agreement with previous studies on anurans, and, because of this indiscriminated predatory activity, the degree of specificity of helminth parasites to particular species of hosts should be fairly low (Prudhoe and Bray, 1982). However, the nematodes found in this study are monoxenous and do not require an intermediate host, as infection is by skin penetration or egg ingestion, thus diet being unimportant in their case. Diet is however important to trematodes and cestodes (Anderson, 2000). In the case of nematodes, habitat should be of course important in the transmission. Considering that (i) primarily mammalian (and also human) parasites are common in the studied anurans, and that (ii) in part of the study areas there was an intense human activity (including the use of these ponds also as latrines), it is possible that this might have influenced the probability of transmission of these parasites to anuran hosts. Further researches are needed in order to confirm this hypothesis.

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