

Helminth infracommunities of the maculated toad *Amietophrynus regularis* (Anura: Bufonidae) from Ismailia, Egypt

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ABSTRACT: The objective of the present work was to study the helminth infracommunities of *Amietophrynus (Bufo) regularis* and the possible effects of intrinsic and extrinsic factors on infracommunity structure and on the infection parameters of each parasite species involved. A total of 129 *A. regularis* were collected from Ismailia, Egypt, over 3 seasons. Helminth infracommunities consisted of 8 helminth taxa (1 monogenean, 1 digenean, 1 cestode, 3 nematodes, and 2 acanthocephalans [1 adult and 1 cystacanth]). *Aplectana macintoshii* had the highest prevalence (82.94%), mean abundance (73.74), and mean intensity (88.91) and can be considered a core species. *A. macintoshii* dominated in 68.99% of the infracommunities, with a high Berger-Parker index value (0.9). Only 9 toads were uninfected; the remainder harbored between 1 and 7 helminth species and 1 to 632 ind. Mean species richness and abundance were 2.13 ± 0.13 and 81.34 ± 13.60 , respectively, while evenness and diversity were 0.3 and 0.44, respectively. The results revealed that season, host sex, and age played significant roles in determining infracommunity species richness. The patterns of helminth infracommunity richness and diversity were similar to those previously observed in other amphibian hosts. This study indicated that the helminth community of *A. regularis* was depauperate.

KEY WORDS: *Amietophrynus regularis* · Helminth infracommunity · Body size · Season · Sex · Parasite load

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INTRODUCTION

Studies on the helminth communities of amphibians have increased in recent years (Aho 1990, Burse & Goldberg 1998, Bolek & Coggins 2000, 2001, 2003, Goldberg et al. 2002, Khidr et al. 2002, Paredes-Calderon et al. 2004, Brooks et al. 2006, Hamann et al. 2006). The recent interest in amphibian parasites probably stems from the hypothesis that some parasitic species may be responsible for some amphibian declines. Johnson et al. (1999, 2001) offer compelling experimental evidence that a trematode parasite, *Ribeiroia* sp., is responsible for the dramatic limb deformities observed in certain amphibian populations in several areas of North America.

Most studies on the helminth community structure of amphibian hosts have been conducted in species distributed in temperate latitudes (Aho 1990, Muzzall 1991, Yoder & Coggins 1996, McAlpine 1997, Bolek &

Coggins 2001, 2003, Muzzall et al. 2001); therefore, it is necessary to examine more species from different localities in tropical latitudes to determine if there is general correspondence with the patterns described by Aho (1990) for amphibian helminth communities (Paredes-Calderón et al. 2004).

Amietophrynus (Bufo) regularis is widespread in savanna regions south of the Sahara and in a region stretching from Senegal through West Africa to Central Africa, and through North Africa to Egypt. This species feeds on a wide variety of vertebrates and invertebrates. However, the main components of its diet are invertebrates, including beetles, odonates, and spiders (Rödel 2000). Although there are reports of helminths of *A. regularis* (Khidr et al. 2002) from Egypt, no studies exist on the factors that structure the helminth infracommunities of this toad. The objective of the present work was to study the helminth infracommunities of *A. regularis* and the possible effects of intrinsic fac-

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tors (host sex, weight and age) and an extrinsic factor (season) on the structure of these infracommunities, notably on the prevalence, mean abundance and mean intensity of each parasite species involved.

MATERIALS AND METHODS

A total of 129 specimens of *Amietophrynus regularis* (5.1 to 7.9 cm in snout–vent length) were collected from Nefesha, Ismailia (32° N, 30° 30' E), in 3 seasons: winter (February 2005), spring (April 2005) and summer (July 2005). Toads were collected by hand during spring and summer. During the hibernation season (winter), specimens were collected by digging muddy-sand areas. The toads collected from this area can be described as semi-terrestrial. Parasitological analysis was carried out within 72 h of capture. Toads were killed using chloroform, and then dissected and examined for helminth parasites. The stomach, intestine, lungs, kidneys and urinary bladder were dissected and placed separately in Petri dishes containing 0.9% saline solution. The body cavity was also searched for helminths. Contents were cleaned in saline solution and examined under a dissecting microscope.

The ecological terminology follows that described by Bush et al. (1997). Analyses of helminth infracommunity structure, irrespective of site of infection, included measurements of mean number of helminths (abundance), species richness, mean diversity, and evenness (Brillouin's index). Numeric dominance was determined using the Berger-Parker dominance index. Age class was divided into 2 groups: immature refers to individuals with both forelimbs and hind limbs, which lack tails or tail stubs, and are not of breeding size; adult refers to individuals of breeding size. Sex was determined by the external appearance of mature toads (e.g. the presence of a vocal sac in males), and by dissection in the case of immature individuals. The effects of both individual and interacting factors (host sex, host age and season) on infection was statistically analyzed using the General Linear Interactive Model (GLIM) after normalization of the data by $\log_{10}(x + 1)$ transformation (Crawley 1993, Wilson & Grenfell 1997). Chi-square (χ^2) test was used to test for differences in prevalence, and Mann-Whitney test (U) for differences in parasite abundance between host sex and age. Abundance among seasons was analyzed using the Kruskal-Wallis test. Correlations of helminth abundance with host size and weight were tested using Spearman rank correlation coefficient (r_s). The degree of aggregation of the different parasite species was calculated by the Index of Dispersion I (variance to mean ratio, where $I > 1$ indicates overdispersed data) and the Index of Discrepancy D as described by Poulin

(1993) ($D = 0$ indicates an even distribution across all hosts, and $D = 1$ indicates that all parasites are aggregated in a single host). All statistical tests were performed using the software package SPSS 12.00.

RESULTS

The structure of the sampled host population by season of capture and host sex is shown in Table 1. Eight species of helminth parasites were found (Table 2) and 120 toads (93.02%) carried at least one of these helminth species. *Aplectana macintoshii* was the most common (82.94%) and *Polystoma integerrimum* (6.98%) was the rarest. Two of the 8 species were rare, with low prevalence (<11%) and low mean abundance (<0.5 helminths toad⁻¹). *A. macintoshii* dominated in 68.99% of the infracommunities, with a high Berger-Parker index value (0.9). *A. macintoshii* had the highest prevalence (82.94%), mean abundance (73.74) and mean intensity (88.91) and can be considered a core species. Only 9 toads were uninfected, while the remainder harbored between 1 to 7 helminth species and 1 to 632 ind. The overall mean species richness and abundance were 2.13 ± 0.13 and 81.34 ± 13.60 , respectively. Cumulative species richness curves indicated that 85.34% of the helminth species found were recovered from only 38 toads. Evenness and diversity were 0.3 and 0.44, respectively.

The distribution of infracommunity species richness (Fig. 1) showed a good fit with positive binomial ($\chi^2 = 7.2$; $df = 5$, $p = 0.204$) but not with normal distribution ($\chi^2 = 22.88$; $df = 5$, $p < 0.0001$). Three-way ANOVA in GLIM with normal errors revealed that season, host sex and host age played significant roles in determining infracommunity species richness (Table 3, Fig. 2). There were strong significant interactions between these factors (Table 3). Species richness was significantly higher in spring and summer and lower in winter ($F_{2,126} = 5.85$, $p = 0.004$). Species richness also differed significantly between sexes ($t = 4.22$; $p < 0.0001$), with male toads having higher mean species richness

Table 1. *Amietophrynus regularis*. Structure of the sampled host population by season of capture and host sex

Season	No. of collected toads				Total
	Male		Female		
	Mature	Immature	Mature	Immature	
Winter	18	0	17	0	35
Spring	16	10	11	3	40
Summer	9	13	14	18	54
Total (mature and immature)	66		63		129

Table 2. Overall prevalence, abundance, and indices of aggregation of helminths infecting *Amietophrynus regularis* collected from Ismailia, Egypt. *I*: index of dispersion, *D*: index of discrepancy

Helminth species	Microhabitat	Sex of host	Prevalence (%)	Abundance		Indices of aggregation	
				Mean \pm SE	Range	<i>I</i>	<i>D</i>
<i>Aplectana macintoshii</i>	Small and large intestine	Male	92.42	120.03 \pm 21.64	0–600	50.89	0.36
		Female	73.01	25.25 \pm 12.70	0–576		
		Combined	82.94	73.74 \pm 13.32	0–600		
<i>Oxysomatium ranae</i>	Large intestine	Male	28.78	4.32 \pm 1.20	0–47	22.63	0.85
		Female	11.11	0.79 \pm 0.49	0–30		
		Combined	20.15	2.60 \pm 0.67	0–47		
<i>Rhabdias bufonis</i>	Lung	Male	36.36	0.71 \pm 0.14	0–6	1.82	0.78
		Female	17.46	0.24 \pm 0.07	0–2		
		Combined	37.13	0.48 \pm 0.08	0–6		
<i>Polystoma integerrimum</i>	Urinary bladder	Male	12.12	0.18 \pm 0.06	0–2	1.53	0.92
		Female	1.59	0.02 \pm 0.02	0–1		
		Combined	6.98	0.10 \pm 0.03	0–2		
<i>Pleurogenoides sitapurii</i>	Small intestine	Male	12.12	3.11 \pm 1.26	0–42	32.91	0.93
		Female	6.35	0.19 \pm 0.09	0–3		
		Combined	9.30	1.68 \pm 0.66	0–42		
<i>Nematotaenia dispar</i>	Small intestine	Male	42.42	2.33 \pm 0.39	0–10	4.34	0.72
		Female	25.39	0.98 \pm 0.24	0–6		
		Combined	26.36	1.67 \pm 0.24	0–10		
<i>Acanthocephalus bufonis</i>	Small intestine	Male	34.84	0.86 \pm 0.15	0–4	3.01	0.77
		Female	20.63	0.86 \pm 0.24	0–6		
		Combined	27.91	0.86 \pm 0.14	0–6		
Cystacanth	Body cavity, mesenteries and attached to kidney	Male	13.63	0.30 \pm 0.11	0–4	2.36	0.92
		Female	6.35	0.10 \pm 0.05	0–2		
		Combined	10.08	0.20 \pm 0.06	0–4		

(2.65) than females (1.59). Species richness did not differ between mature and immature hosts (Table 3; $t = 0.33$, $p = 0.7$).

Infection prevalence was higher in immature toads than in adults for *Aplectana macintoshii*, *Oxysomatium ranae*, *Nematotaenia dispar*, *Acanthocephalus bufonis* and the cystacanth. Conversely, infection prevalence was higher in adult toads for *Rhabdias bufonis*, *Polystoma integerrimum* and *Pleurogenoides sitapurii* (Fig. 3). Significant differences in mean abundance by host age were found only in *Aplectana macintoshii*, *Oxysomatium ranae* and *Pleurogenoides sitapurii* (Table 4). Immature toads had higher mean abundance

of *Aplectana macintoshii* and the cystacanth when compared to adults. On the other hand, adult toads had higher mean abundance than immature individuals for *Oxysomatium ranae*, *Rhabdias bufonis*, *Polystoma integerrimum*, *Nematotaenia dispar* and *Acanthocephalus bufonis*. Overall helminth prevalence and mean abundance were higher in immature than in adult toads (Fig. 3).

Infection prevalence differed between sexes (Table 2), with % prevalence in males being significantly higher than in females (Table 3). Mean abundance also differed significantly between sexes for all helminth species (Tables 3 & 4). Male toads had signif-

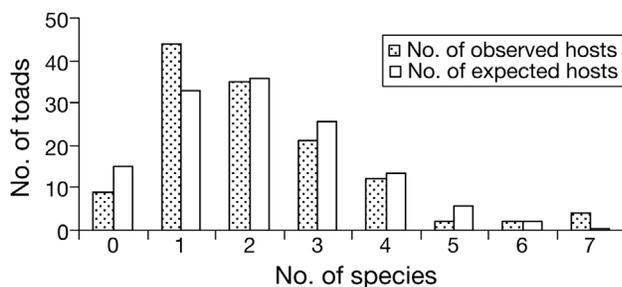


Fig. 1. *Amietophrynus regularis*. Frequency distribution of helminth infracommunity species richness

Table 3. Main factors affecting species richness of helminth infracommunity of maculated toad *Amietophrynus regularis*

Factors	SS	df	χ^2	<i>F</i>	<i>p</i>
Sex	31.10	1	31.10	29.57	<0.0001
Age	17.44	1	17.44	16.58	<0.0001
Season	68.43	2	34.22	32.53	<0.00001
Sex \times Age	18.07	1	18.08	17.19	<0.00001
Sex \times Season	67.44	2	33.73	32.06	<0.00001
Age \times Season	6.16	1	6.16	5.85	0.01
Sex \times Age \times Season	13.55	1	13.54	12.88	0.0004
Error	125.16	119	1.05		
Total	883	129			

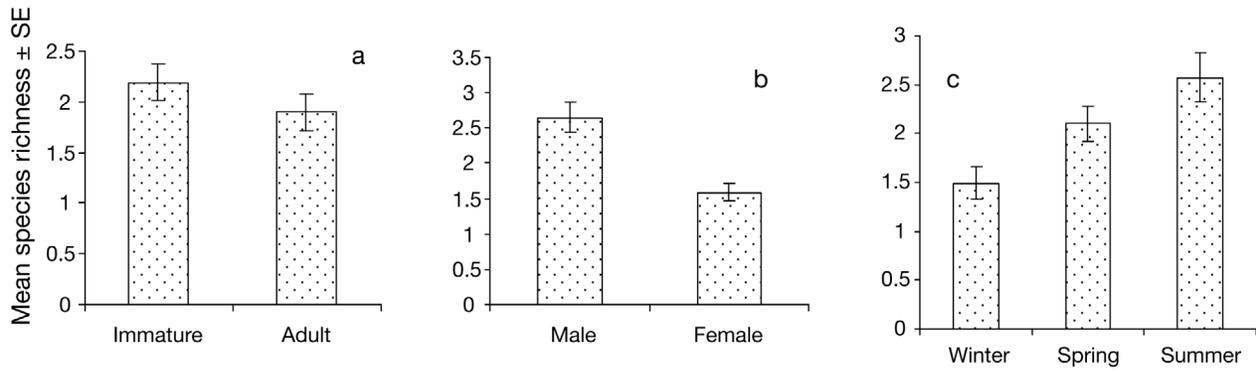


Fig. 2. *Amietophrynus regularis* infected by helminths. Variation of helminth infracommunity species richness in relation to (a) host age (host maturity), (b) host sex, and (c) season

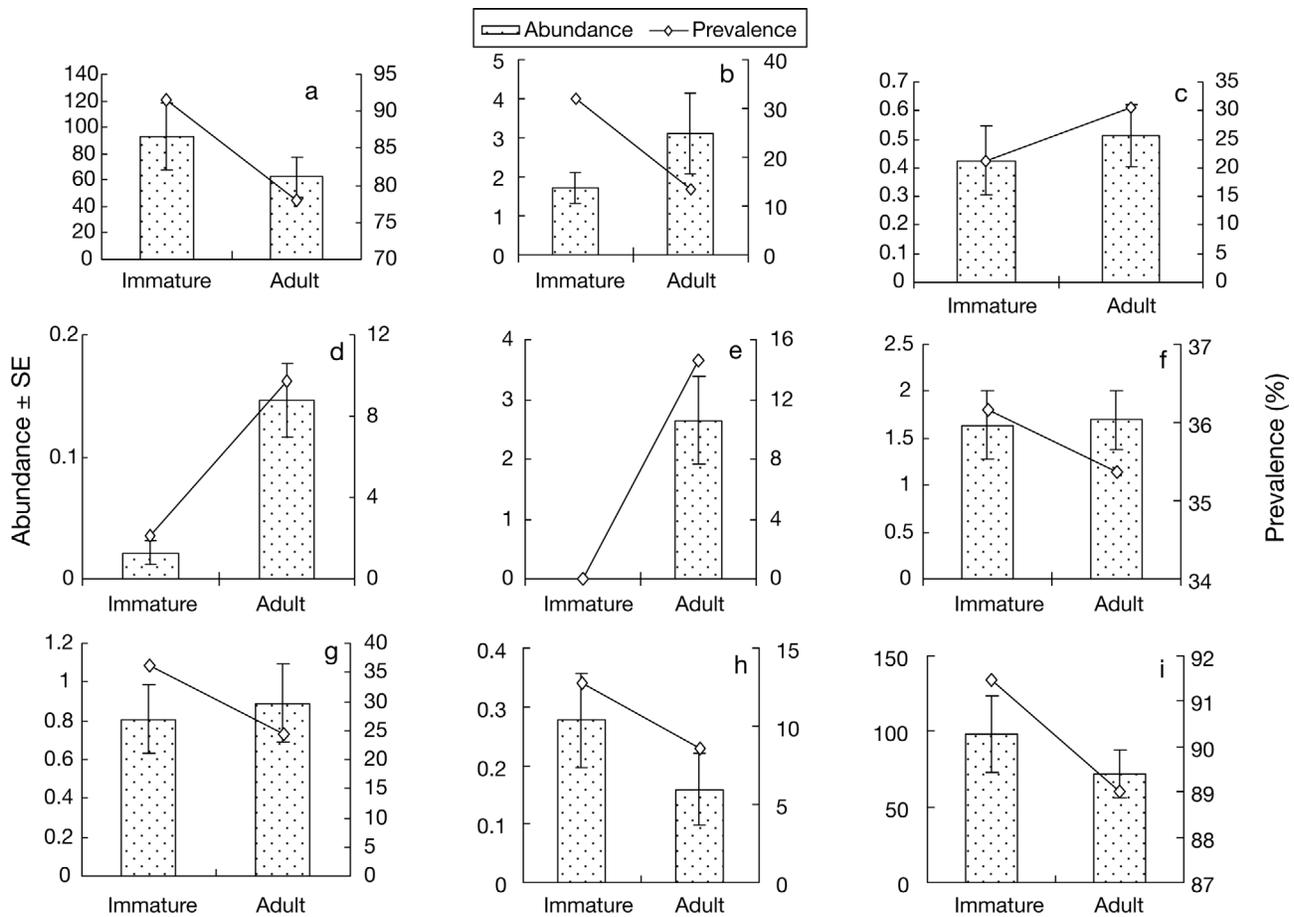


Fig. 3. *Amietophrynus regularis* infected by helminths. Prevalence (lines), and mean abundance (bars) ± SE of helminths in relation to host age (maturity): (a) *Aplectana macintoshii*, (b) *Oxysomatium ranae*, (c) *Rhabdias bufonis*, (d) *Polystoma integerrimum*, (e) *Pleurogenoides sitapurii*, (f) *Nematotaenia dispar*, (g) *Acanthocephalus bufonis*, (h) cystacanth, and (i) total helminths combined

icantly higher mean abundance than females for all helminth species except *Acanthocephalus bufonis*. The overall mean abundance of helminths combined differed significantly between sexes (Table 4; $U = 852$, $p < 0.0001$).

Season had significant effect on infection prevalence of *Aplectana macintoshii*, *Oxysomatium ranae* and *Acanthocephalus bufonis* (Fig. 4). Overall mean abundance also differed significantly among different seasons (Table 4; $\chi^2 = 6.65$, $p = 0.04$), increasing from

Table 4. *Amietophrynus regularis* infected by helminths. Relationship between mean abundance and host age (host maturity), host sex, season, host size (snout–vent length), and weight. *U*: Mann-Whitney statistic; χ^2 : chi-square; r_s : Spearman rank correlation coefficient

Helminth species	Host age		Sex		Season		Host size		Host weight	
	<i>U</i>	<i>p</i>	<i>U</i>	<i>p</i>	χ^2	<i>p</i>	r_s	<i>p</i>	r_s	<i>p</i>
<i>Aplectana macintoshii</i>	1359	0.005	1026	<0.0001	10.95	0.004	0.09	0.27	-0.23	0.008
<i>Oxysomatium ranae</i>	1641	0.04	1663	0.005	11.33	0.003	0.02	0.76	-0.24	0.007
<i>Rhabdias bufonis</i>	1804	0.44	1638	0.008	0.44	0.80	0.26	0.003	0.12	0.19
<i>Polystoma integerrimum</i>	1778	0.09	1858	0.01	5.06	0.08	-0.07	0.45	-0.13	0.15
<i>Pleurogenoides sitapuri</i>	1645	0.006	1943	0.02	2.19	0.33	0.15	0.08	0.09	0.33
<i>Nematotaenia dispar</i>	1915	0.94	1626	0.013	3.65	0.16	0.09	0.23	0.069	0.43
<i>Acanthocephalus bufonis</i>	1763	0.31	1812	0.11	8.95	0.01	-0.08	0.36	0.116	0.19
Cystacanth	1845	0.44	1821	0.015	1.27	0.52	-0.12	0.15	0.07	0.39
Total helminths	1605	0.11	852	<0.0001	6.65	0.04	0.006	0.95	-0.124	0.16

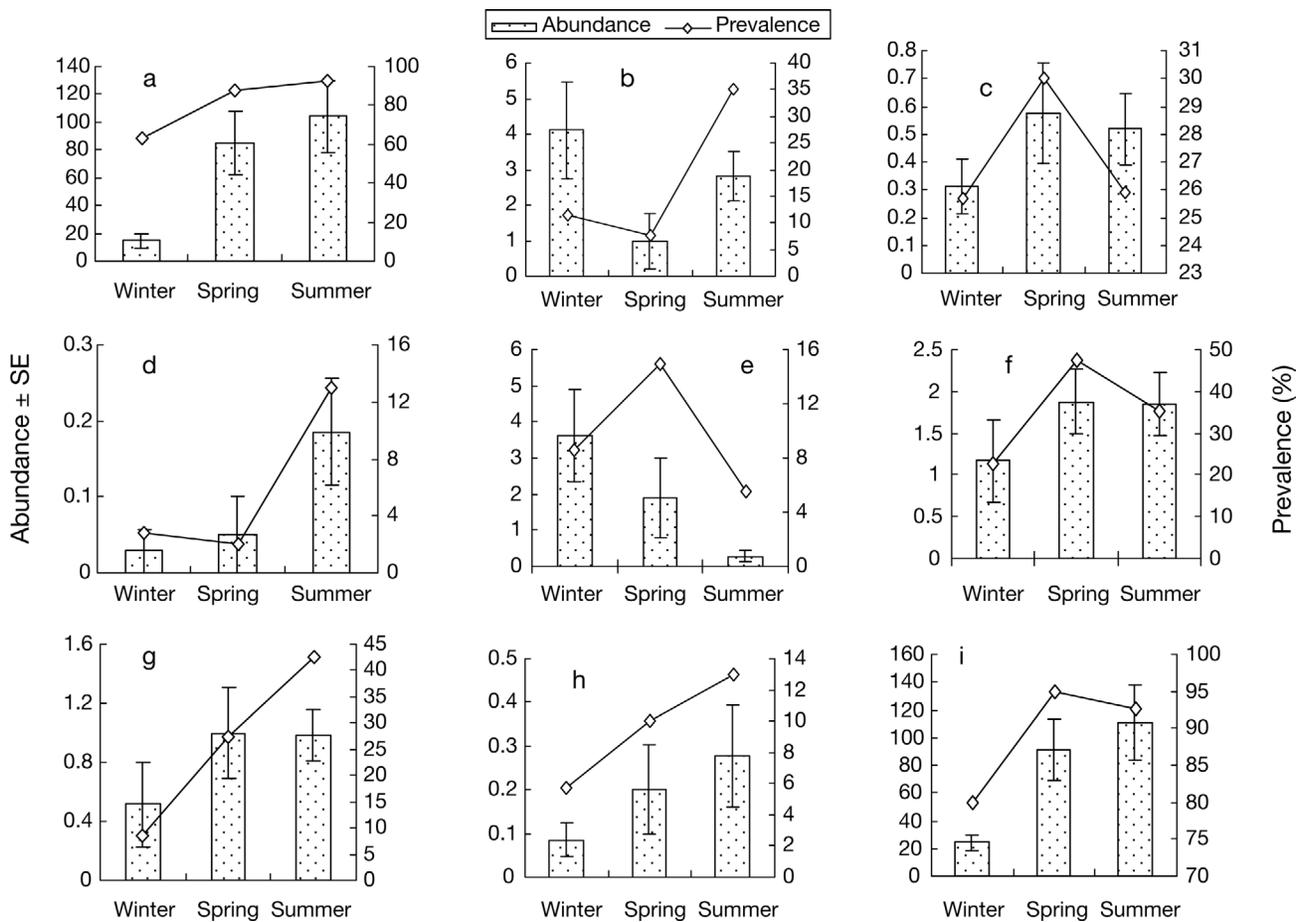


Fig. 4. *Amietophrynus regularis* infected by helminths. Seasonal variation in prevalence (lines), and mean abundance (bars) \pm SE of helminths: (a) *Aplectana macintoshii*, (b) *Oxysomatium ranae*, (c) *Rhabdias bufonis*, (d) *Polystoma integerrimum*, (e) *Pleurogenoides sitapuri*, (f) *Nematotaenia dispar*, (g) *Acanthocephalus bufonis*, (h) cystacanth, and (i) total helminths combine

24.22 ind. in winter to 110.87 ind. in summer (Fig. 4). Of the 8 parasites, *Aplectana macintoshii*, *Oxysomatium ranae* and *Acanthocephalus bufonis* showed significant difference in mean abundance among seasons (Table 4, Fig. 4). The frequency distribution of helminth abundance showed that they were over-

dispersed, with $I > 1$ (Table 2). The mean intensity differed significantly among different seasons in *Oxysomatium ranae*, *Rhabdias bufonis*, *Pleurogenoides sitapuri* and *Acanthocephalus bufonis*.

Three-way ANOVA in GLIM after data normalization (by $\log_{10}(x+1)$ transformation) showed that sex

Table 5. Test of interaction (general linear model) between factors affecting mean abundance of helminth infracommunity of the maculated toad *Amietophrynus regularis*. Values of $p < 0.05$ are significant

Helminth species Factor considered	SS	df	χ^2	F	p
<i>Aplectana macintoshii</i>					
Sex	6.88	1	6.88	17.82	<0.0001
Age	0.97	1	0.97	2.51	0.11
Season	8.67	2	4.33	11.23	<0.0001
Sex × Age	6.07	1	6.07	15.73	0.0001
Sex × Season	7.11	2	3.55	9.21	0.0001
Age × Season	12.20	1	12.20	31.61	<0.0001
<i>Oxysomatium ranae</i>					
Sex	1.40	1	1.40	10.98	0.001
Season	1.93	2	0.96	7.57	0.001
Sex × Age	0.82	1	0.82	6.45	0.01
Sex × Season	3.16	2	1.58	12.37	<0.0001
Age × Season	0.50	1	0.50	3.92	0.05
Sex × Age × Season	0.64	1	0.63	4.97	0.02
<i>Rhabdias bufonis</i>					
Sex	0.32	1	0.32	10.55	0.001
Age	0.20	1	0.20	6.74	0.01
Season	0.26	2	0.13	4.37	0.01
Sex × Season	0.72	2	0.36	11.92	<0.0001
Sex × Age × Season	0.12	1	0.12	3.94	0.04
<i>Polystoma integerrimum</i>					
Sex	0.08	1	0.08	22.64	<0.0001
Age	0.17	1	0.17	47.35	<0.0001
Season	0.29	2	0.15	40.98	<0.0001
Sex × Age	0.20	1	0.20	55.55	<0.0001
Sex × Season	0.27	2	0.14	37.81	<0.0001
Age × Season	0.12	1	0.12	34.34	<0.0001
Sex × Age × Season	0.15	1	0.15	41.38	<0.0001
<i>Pleurogenoides sitapurii</i>					
Age	0.67	1	0.67	6.41	0.01
<i>Nematotaenia dispar</i>					
Season	0.94	2	0.47	4.30	0.01
Sex × Age	1.09	1	1.09	9.98	0.002
Age × Season	2.05	1	2.05	18.74	<0.0001
<i>Acanthocephalus bufonis</i>					
Age	0.35	1	0.35	6.53	0.01
Season	0.92	2	0.46	8.59	0.001
Sex × Season	1.36	2	0.68	12.71	<0.0001
Age × Season	0.28	1	0.28	5.20	0.02
Sex × Age × Season	0.22	1	0.22	4.08	0.04
Total helminths					
Sex	6.61	1	6.61	20.64	0.0001
Season	5.99	2	2.99	9.37	0.0001
Sex × Age	4.40	1	4.40	13.76	0.0003
Sex × Season	7.69	2	3.85	12.02	0.0001
Age × Season	7.88	1	7.88	24.64	0.0001

and season had significant main effects on total parasite abundance (Table 5). The main effect of age was also significant except in *Oxysomatium ranae* and *Nematotaenia dispar*. There were also interactions between host age, sex and season in some of the helminth fauna. A positive correlation was found between

weight and mean intensity of *Acanthocephalus bufonis* ($r_s = 0.48$, $p = 0.003$). On the other hand, a negative correlation was found between weight and mean abundance of *Aplectana macintoshii* ($r_s = -0.23$, $p = 0.008$) and *Oxysomatium ranae* ($r_s = -0.24$, $p = 0.007$) and mean intensity of *Rhabdias bufonis* ($r_s = -0.33$, $p = 0.04$).

DISCUSSION

Helminth communities of amphibians are generally regarded as depauperate and isolationist (Aho 1990, Muzzall 1991, Yoder & Coggins 1996, Bolek & Coggins 2001, 2003). However, Hamann et al. (2006) found no fixed pattern, i.e. helminth communities were at an intermediate point between isolationist and interactive. In this study, helminth infracommunities of *Amietophrynus regularis* show low diversity and species richness, and can thus be considered depauperate. The characteristics of the infracommunities studied herein correspond with the patterns described for helminth communities of amphibians by Aho (1990), particularly in the sense that they are depauperate, and are highly variable. Mean species richness in this study was 2.13 as compared with 0.8 in temperate regions (Aho 1990). Mean species richness recorded in other amphibian species in tropical regions were 3.49 (Paredes-Calderón et al. 2004) and 2.40 (Hamann et al. 2006). Aho (1990) identified 2 interacting factors responsible for the observed patterns in amphibians: host vagility and energetic demands of poikilothermy. He suggested that reduced host vagility would restrict exposure to many helminth species, and that the low energetic demands of poikilothermy would reduce consumption of potential intermediate hosts.

Our results are in agreement with Aho (1990), who noted the number of nematode species and individuals to vary between host species, but to frequently dominate the helminth community composition. *Amietophrynus regularis* infracommunities were also dominated by nematodes in both number and species

composition (3 species of nematode and 9910 ind.). The majority of parasite communities of bufonid amphibians show a higher number of nematode species (Goldberg & Bursley 1991, 1992, Goldberg et al. 1995, Bolek & Coggins 2000, 2003, Luque et al. 2005) than trematodes, as observed for several ranid hosts (McAlpine 1997, Bolek & Coggins 2000, Muzzall et al. 2001, Paredes-Calderón et al. 2004). Terrestrial toads predominantly feed on ants, beetles and other terrestrial invertebrates, thereby preventing them from becoming infected with several species of trematodes which commonly infect aquatic amphibians such as ranids (Luque et al. 2005).

Results showing that male toads had higher mean species richness (2.65) than females (1.59) are in agreement with those of Vashetko & Siddikov (1999), who reported richer helminth fauna in male *Amietophrynus viridis* than in females.

The overall helminth prevalence and mean abundance were higher in immature than in adult toads—contrary to the findings of Muzzall (1991) and McAlpine (1997) on various ranid species. These differences could be explained by ontogenetic changes in the host diet and by behavior; both characteristics have important roles in parasite recruitment and may be correlated with the host's body size.

The present study showed that male toads had higher prevalence and mean abundance of parasites than females for all helminth species except *Acanthocephalus bufonis*. This result is in agreement with Khidr et al. (2002) who found that male *Bufo regularis* have higher infection rate than females. Testosterone has been shown to have a negative impact on immune response in some vertebrates (Casto et al. 2001, Hughes & Randolph 2001). There are at least 2 potential pathways in which increased steroid levels can influence parasite load in free-ranging animals. First, testosterone may increase movement, display rates and aggression, which can lead to higher exposure to parasites (Klein 2000). Second, testosterone may increase susceptibility to infection or infestation by directly lowering the immunocompetence of the individual via suppression of the immune system (Hillgarth & Wingfield 1997).

The higher overall prevalence of parasites in spring, and the higher mean combined abundance in summer agree with the previous findings of Wetzel & Esch (1996), Khidr et al. (2002), and Hamann et al. (2006). The ectothermic condition of amphibians limits helminth recruitment potential and community development in winter by affecting both feeding rates and foraging behavior (Aho 1990, Bolek & Coggins 2000, Paredes-Calderón et al. 2004). Seasonal variation in the prevalence and abundance of helminths may be due to a combination of factors, including variation in

ingestion rates of infective stages, breeding period, age, sex and foraging activity of hosts.

This study focused on the contribution of one extrinsic (season) and 2 intrinsic factors (age and sex) in attempting to explain variations in prevalence and mean abundance of helminth infracommunities of *Amietophrynus regularis*. These factors interact in various ways to shape the infracommunity structure in a given habitat at a specific time. Apparently, the main forces determining infracommunity structure are ectothermy (as a regulator of the ingestion rate), and the feeding habits of the hosts, because most of the helminth taxa enter the host by ingestion of intermediate hosts.

In conclusion, more extensive studies of amphibian helminth communities and incorporating them into a phylogenetic context are required to make more robust predictions about patterns and processes in these host-parasite systems.

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