Helminth’s assemblage of a small frog in the Brazilian semiarid: parasite-host-environment relationships

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ABSTRACT. Parasite infections may contingency different aspects of the hosts’ lives, and are usually expected to directly or indirectly compromise host reproduction. However, although amphibians have historically been study models in parasite ecology, the effects of parasitism on amphibians’ reproduction remain unknown. Thus, we investigated how the parasite load varies as a function of climatic seasonality (rainy and dry season) and intrinsic host characteristics (size and sex), as well as the relationship between parasite load and reproductive investment in males and females of a small frog (Pseudopaludicola pocoto Magalhães, Loebmann, Kokubum, Haddad & Garda, 2014) in the Brazilian semiarid region. The parasitological parameters of the studied population were not influenced by the season of the year or by the hosts’ body size, but females of P. pocoto had a higher prevalence and intensity of infection than males. The number of oocytes and the volume of the testes were not related to the parasite load, revealing that the parasitism did not negatively impact the gonadal investment in P. pocoto. Our findings suggest that short-lived species, such as the tiny Pseudopaludicola species, have a high reproductive investment independent of their parasitic interactions. In addition, this should be true mainly in those species that live in seasonally dry environments, such as P. pocoto, in which reproduction is even more constrained by the shortened and unpredictable rainy period.

KEYWORDS. Amphibia, Pseudopaludicola, seasonality, reproductive allocation, Nematoda.

RESUMO. Assembleia de helmintos em uma pequena rã no semiárido brasileiro: relação parasito-hospedeiro-ambiente. Como infecções parasitárias podem contingenciar diversos aspectos da vida dos hospedeiros, de modo geral, espera-se que o parasitismo comprometa diretamente ou indiretamente a reprodução dos hospedeiros. Contudo, apesar dos anfíbios historicamente terem sido modelos de estudos em ecologia parasitária, os efeitos do parasitismo na reprodução de anfíbios permanecem incompreendidos. Neste trabalho investigamos como a carga parasitária varia em função da sazonalidade climática (período chuvoso e seco) e de características intrínsecas dos hospedeiros (tamanho e sexo), bem como a relação entre a intensidade de infecção e o investimento reprodutivo em machos e fêmeas de um pequeno anfíbio (Pseudopaludicola pocoto Magalhães, Loebmann, Kokubum, Haddad & Garda, 2014) no semiárido brasileiro. Os parâmetros parasitológicos da população estudada não foram influenciados pela estação do ano nem pelo tamanho corpóreo dos hospedeiros, mas as fêmeas de P. pocoto apresentaram maior prevalência e intensidade de infecção que os machos. A carga parasitária não foi relacionada com o número de ovócitos nem com o volume dos testículos, revelando que o parasitismo não impactou negativamente o investimento gonadal em P. pocoto. Nossos achados sugerem que espécies com baixa longevidade, como as diminutas espécies de Pseudopaludicola, apresentam um alto investimento reprodutivo independente de suas interações parasitárias. Complementarmente, isso deve ocorrer sobretudo naquelas espécies que vivem em ambientes sazonalmente secos, como P. pocoto, nos quais a reprodução é ainda mais contingenciada pela brevidade e imprevisibilidade do período chuvoso.

PALAVRAS-CHAVE. Amphibia, Pseudopaludicola, sazonalidade, esforço reprodutivo, Nematoda.

Macroparasites, such as helminths, can interact with their hosts in many different ways, which can cause severe damage, subtle injuries, or even not generate apparent disadvantages for the hosts (POULIN, 2007). These different consequences may be related to factors such as environmental conditions where interactions are established (MINCHELLA, 1985), as well as intrinsic host and parasite features (MINCHELLA, 1985; GALVANI, 2003; DYBDahl & STORFER, 2003; POULIN, 2007).

Parasites that live in environments with high climatic seasonality might have contingencies in terms of survival, transmissibility and reproduction (ALTIZER et al., 2006; CHANDRA & GUPTA, 2007; POULIN, 2007). For example, in semiarid regions where rainfall is scarce, unpredictable and has high variability between years (Ab’Sáber, 2003), helminths can be affected by the high risk of desiccation of eggs and larvae, as well as the unavailability of active hosts most of the time (Perry, 1999; Madelaine et al., 2020). Thus, in these environments, rainy periods tend to be more favorable for the infection, development and reproduction of helminths (POULIN & MORAND, 2000; CHANDRA & GUPTA, 2007).
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Aspects inherent to hosts such as size, sex and vagility can substantially influence helminth infection rates (AHO, 1990; PRICE, 1990; HAMANN et al., 2006; TOLEDO et al., 2015, 2018). For example, hosts with larger body sizes tend to harbor a greater number of parasites (POULIN, 2007; CAMPÃO et al., 2015). The impairment or inhibition of reproduction in hosts is one of the ways the parasites maintain the host in good energetic conditions (HURD, 2001; HEINS et al., 2004). Thus, it is plausible to expect that parasitism can compromise the reproductive investment of hosts (HURD, 2001). However, if on the one hand a reduction in fecundity can be an adverse effect of energy limitation provoked by infection. On the other hand, it can constitute a host defensive strategy, resulted of greater investment in survival at the expense of reproduction (HURD, 2001).

Amphibians has been historically good study models for parasitic ecology, as they harbor a high diversity of parasites (CAMPÃO et al., 2014) and many species are locally abundant and easy to sample (DUELLMAN & TRUEB, 1994). It is known that parasitic infections can affect anuran population dynamics (JOHNSON & MACKENZIE, 2009; VREDENBURG et al., 2010), with parasitological parameters that can vary ontogenetically (CAMPÃO et al., 2009; TODELO et al., 2015) and differ between the sexes (Van SLUYS et al., 2006). As reproduction in amphibians is an activity with high energy demand (WELLS, 2007), it is expected that parasitic infections may also compromise their reproductive efficiency. However, there is a relative lack of information on the general effects of parasitism on the reproductive investment of hosts (HURD, 2001; GALDINO et al., 2014; KISS et al., 2019), particularly for amphibians (PROHIL et al., 2013).

Parasitic interactions in amphibians have been more studied in wet tropical environments (SANTOS & AMATO, 2010; AGUIAR et al., 2015; TOLEDO et al., 2015, 2017, 2018) than in dry tropical environments (MORETTI et al., 2017; SILVA et al., 2018a; MADELAIRE et al., 2020). The Caatinga corresponds to the largest area of Tropical Dry Forest in South America (DA SILVA et al., 2017) and harbor one of the richest amphibian fauna among semiarid regions in the world (GARDA et al., 2017). However, knowledge about parasitism in Caatingan anurans is basically constituted by punctual infection records (TELES et al., 2014, 2017, 2018) and lists of helminth fauna for some host species (TELES et al., 2015; ALCANTARA et al., 2018; OLIVEIRA et al., 2019). Since these semiarid environments have a marked seasonality in relation to water availability, which is a limiting factor both for amphibians (CARVALHO et al., 2010; MADELAIRE & GOMES, 2016) and for helminths (PERRY, 1999; MADELAIRE et al., 2020), it reinforces the need to provide information on host-parasite interactions between helminths and anurans in the Caatinga domain.

In the Brazilian semiarid region, most frogs concentrate their reproductive activities in the short rainy period of the year (ARZABE et al., 2005; JARED et al., 2019). This is also the case of the tiny frog Pseudopaludicola pocoto Magalhães, Loembmann, Kokubun, Haddad & Garda, 2014, a species described in the last decade, which despite being common and widely distributed in the Brazilian semiarid region (PEREIRA et al., 2015; LANTYER-SILVA et al., 2016), has little ecological information available (SILVA et al., 2018a, b). The community of helminths that parasitize P. pocoto is known for only one location in the Caatinga (SILVA et al., 2018a) and the relationships between its parasite load and its reproductive investment are completely unknown. In this study, we evaluated the ecological interactions between P. pocoto and its associated helminth fauna, specifically investigating: 1) how marked rainfall seasonality affects the parasitological parameters; 2) in what extension host body size and sex influence the parasite load and; 3) what are the relationships between the parasite load with the reproductive investment of females and males hosts.

MATERIAL AND METHODS

Fieldwork was carried out in Assú municipality (5°34′38″S, 36°54′30″W), a semiarid area located in the western region of the state of Rio Grande do Norte, Brazil. This macroregion is classified according to Köppen-Geiger as presenting a Warm Semiarid Climate (BSH), which may present a variation in annual precipitation from 400 to 1000 mm depending on the location, and annual average temperatures ranging between 22°C and 35°C (DA SILVA et al., 2017; INMET, 2018). The Brazilian semiarid region is characterized by high temperatures and seasonal rains, with short rainy periods of three to four months, with unpredictable rainfall, and the dry season lasting from 7 to 10 months (DA SILVA et al., 2017). Assú municipality has historically an average annual precipitation of 587.4 mm, with a concentration of rainfall between February and May, whilst September use to be the driest month in the region (MEDEIROS et al., 2005).

We sampled specimens of Pseudopaludicola pocoto through active searches along breeding sites (SCOTT JR & WOODWARD, 1994). The samplings took place in three temporary ponds, in the rainy months (March and May 2017, February, April and May 2018) and in the dry months (September and October 2017). We collect 94 individuals of Pseudopaludicola pocoto, 41 females and 53 males, including 23 collected in rainy and 71 in dry season. Individuals were manually captured and collected under licenses from Instituto Chico Mendes de Conservação da Biodiversidade (n°. 58431-1 and n°. 35650-1). The sampled individuals were transferred to the Laboratório de Ecologia e Comportamento Animal – LECA of the Universidade Federal Rural do Semi-Árido – UFERSA, in the municipality of Mossoró, Rio Grande do Norte state, where their body size was measured in terms of snout-vent length (SVL) with a caliper (resolution 0.01 mm). After that, the individuals were euthanized following the recommended ethical precepts (RESOLUÇÃO DO CONSELHO FEDERAL DE BIOLOGIA – CFBO nº. 301/2012) and had their internal organs (gonads, lungs, stomach, small and large intestines) removed for parasite inspection. For proper fixation of the helminths, the organs were fixed in 70% alcohol at 60°C, and preserved in 70% alcohol for further screening, counting and identification. After necropsy, the
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RESULTS

We found a total of 91 helminths in *Pseudopaludicola pocoto*, and the mean intensity of infection (MII) was 1.78 ± 0.18 parasites per host. We recorded at least one helminth for 51 hosts, representing 54% of total prevalence.

The host population was infected by three nematode species: *Cosmocerca parva* Travassos, 1925 (13 males and 6 females) (*Cosmocercidae*), *Physaloptera* sp. (8 larvae) (*Physalopteridae*), and *Oxyascaris* sp. (37 females) (*Oxyascarididae*) (Tab. I). In most of the infected hosts (86%), we found only one species of helminth, with no host harboring all three species simultaneously. The highest prevalence of helminths was in the digestive tract, mainly in small intestine (38%), followed by large intestine (14%) and stomach (12%). The small intestine was parasitized by *Oxyascaris* sp. (64%), *C. parva* (27%) and *Physaloptera* sp. (9%), the stomach by *C. parva* (67%) and *Physaloptera* sp. (33%), and the large intestine only by *Oxyascaris* sp. Only one individual of *Physaloptera* sp. was found in body cavity, and no lungs or gonads were infected. Regarding mean intensity of infection by site, large intestine was more infected (MII = 1.69; n = 13) than small intestine (MII = 1.53; n = 36) and stomach (MII = 1.09; n = 11) (Tab. I).

Concerning the infection patterns along the time, 56% of hosts were infected in rainy season with a mean intensity of infection of 1.38 ± 0.22, while in the dry season the prevalence was 53% with mean intensity of infection of 1.92 ± 0.16. In relation to intersexual differences, 61% of females (SVL 13.19 ± 0.14 mm) and 49% of males (SVL 11.81 ± 0.21 mm) were infected, with a mean intensity of infection of 2.04 ± 0.26 for females and 1.53 ± 0.10 for males. The overall parasite load was not influenced by body size nor sampling period, and the minimal adequate model had only host sex as the unique significant parameter (GLM - Z = -2.37; p = 0.02; Fig. 1).

Tab. 1. Helminth assemblage associated with *Pseudopaludicola pocoto* (Anura, Leptodactylidae) (n = 51 frogs) from the municipality of Assú, Rio Grande do Norte State, Brazil. HN = total number of hosts per parasite species; PN = total number of nematode parasites; TPR = total prevalence rate (%); MII = mean intensity of infection, MA = mean abundance and IS = infection site (St = stomach; SI = small intestine; LI = large intestine; Cav = body cavity).

<table>
<thead>
<tr>
<th>Nematoda</th>
<th>HN</th>
<th>PN</th>
<th>TPR(%)</th>
<th>MII</th>
<th>MA</th>
<th>IS</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Cosmocerca parva</em></td>
<td>16</td>
<td>19</td>
<td>17.02</td>
<td>1.19</td>
<td>0.20</td>
<td>St, SI</td>
</tr>
<tr>
<td><em>Oxyascaris</em> sp.</td>
<td>23</td>
<td>37</td>
<td>24.47</td>
<td>1.61</td>
<td>0.39</td>
<td>SI, LI</td>
</tr>
<tr>
<td><em>Physaloptera</em> sp.</td>
<td>7</td>
<td>8</td>
<td>7.45</td>
<td>1.14</td>
<td>0.08</td>
<td>St, SI, Cav</td>
</tr>
</tbody>
</table>

To investigate the factors affecting the parasite load, we performed a model selection by simplification of a full generalized linear model (GLM), considering a Poisson error distribution of the response variable (parasite load; i.e. the total number of parasites per host, including zeros) and all interactions between explanatory variables: snout-vent length (in mm), sampling period (rainy and dry season), and sex (female or male). To evaluate the possible negative effects of parasite load on reproductive investment, we first searched for influence of host body size (SVL) on the response variables (log transformed number of oocytes and raw testis volume) using Simple Linear Regressions (SLR). When a significant relationship was found (only in males, see results), we extracted the residuals from the regression model for further analysis. So, we made one sided Spearman’s Correlations (SC) between parasite load (the total number of parasites per host, including zeros) and gonads’ parameters (log transformed number of oocytes for females, and residuals from testis volume–SVL regression model for males). Prior to analysis, we evaluated the datasets regarding premises of homoscedasticity and error distributions. Whenever possible, we transformed the response variable for the use of parametric tests. All statistical analyses were conducted in R environment v.3.6.1 (R Core Team 2019), considering a significance level α = 0.05, and using the package “beeswarm” v0.2.3 (EKLUND, 2016) for graphical purposes. Descriptive characterization of variables was provided as mean ± one standard error.
Regarding influence of parasite load on reproductive investment, we considered 32 females and 34 males for which number of oocytes and testes volume were respectively quantified. The number of oocytes was not influenced by body size (SLR - $R^2 = 0.01; p = 0.60$), and the parasite load was not negatively correlated with the number of oocytes ($SC - r = 0.03; p = 0.57$). The mean testes volume was positively affected by body size (SLR - $R^2 = 0.11; p = 0.05$), but the parasite load was not negatively correlated the testis volume after removed the effects of body size ($SC - r = -0.15; p = 0.20$).

**DISCUSSION**

Nematode parasites are most commonly found in frogs of the Leptodactylidae (CAMPÃO et al., 2014), with eight species previously known to parasitize Pseudopaludicola pocoto (SILVA et al., 2018a). We recorded three species of Nematoda parasitizing P. pocoto in Assú municipality, Rio Grande do Norte state. Cosmocerca parva is widely distributed in South American anurans (CAMPÃO et al., 2014) and has previously been reported to parasitize other Caatinga amphibians (OLIVEIRA et al., 2019), including P. pocoto (SILVA et al., 2018a). Oxyascaris sp. and Physaloptera sp. recorded by us in P. pocoto could not be identified at the species level, as there were only females of Oxyascaris sp. (diagnostic characters in males – (BACHER & VAUCHER, 1985) and larvae of Physaloptera sp. (anurans are intermediate hosts - BOQUIMPANI-FREITAS et al., 2001). The total prevalence observed in Assú population was similar to that found for another population of P. pocoto located 400 km away (50% in SILVA et al., 2018a and 54% in the present study). However, we observed a higher mean intensity of infection in the study area ($MII = 1.8 \pm 1.3$ parasites/host) than that previously recorded for another population ($MII = 1.0 \pm 0.5$ parasite/host) (SILVA et al., 2018a). As other species of Oxyascaris and Physaloptera have also been reported to parasitize P. pocoto (SILVA et al., 2018a), our results suggest that the helminth fauna composition in P. pocoto may not vary substantially between populations.

The prevalence and mean intensity of infection in P. pocoto did not differ between the rainy and dry seasons. In semi-ard regions, it is expected that the activity of most frogs is restricted to the short rainy period of the year, when reproduction is concentrated (ABE, 1995; GARDA et al., 2017). We expected that the number of parasites per host would be greater during the reproductive period (FILHO et al., 2016; OLIVEIRA et al., 2017), when the majority of anurans are active and, consequently, they would be more exposed to infection by parasites (POULIN & MORAND, 2000). However, as observed in another population (SILVA et al., 2018a), we also recorded some P. pocoto individuals active in the first months of dry period next to remaining water bodies. In this sense, it is known that anuran species that have a prolonged period of activity tend to have prevalence and infection rates more distributed over time (MADELAIRE et al., 2020). Thus, although the reproductive activity of Pseudopaludicola species are considered explosive, it is possible that P. pocoto behaves in an opportunistic way, taking advantage of fortuitous favorable conditions between years according to rainfall distribution. If this is true, this strategy could explain the low variation observed in the parasitological parameters of P. pocoto between the rainy and dry periods.

The body size of P. pocoto was not related to the parasite load. However, it is known that the body size of the host is in general positively related to the richness and abundance of parasites, both because large species have a greater capacity for ingesting prey, and because they have a larger surface area and body volume for colonization by helminths (CAMPÃO et al., 2015; TOLEDO et al., 2018). Our results suggest that the low variation in body size of P. pocoto individuals is not enough to enable a significantly higher number of parasites in larger hosts. It is noteworthy that the widely known pattern of sexual dimorphism for anurans, in which females are larger than males (DUELLMAN & TRUEB, 1994), is not observed in P. pocoto (SILVA et al., 2018b), contributing to explain the lack of relationship between body size and parasite load. In fact, Pseudopaludicola species are recognized by their small body size, a characteristic that can restrict the parasite load independent of intraspecific variation in body size (DURÉ et al., 2004).

Sex significantly influenced the parasite load in P. pocoto, with the number of parasitized females 10% higher than those of males and with females harboring 30% more parasites than males. Although in general amphibians have low vagility compared to other vertebrates (WELLS, 2007), females occasionally travel longer distances than males (WELLS, 2007; WANG et al., 2019). This displacement may be related to greater foraging/feeding intensity to offset...
the greater energy demands for oocyte production (Price, 1990; Muths, 2003). Thus, it is possible that females of *P. pocoto* are doubly more susceptible to infection by helminths, both because of the possible greater vagility in search of prey (cutaneous infection route – Poulin, 2007), and because they feed on a greater diversity of prey (infection oral route – Poulin, 2007; Leivas et al., 2018). Since the only study on the foraging ecology of *P. pocoto* did not assess the intersexual differences in diet (see Silva et al., 2018a), we recommend future studies on this topic to test the aforementioned predictions.

Hosts with a higher parasite load may present reproductive contingencies (Minchella, 1985; Hurd, 2001; Bower et al., 2018), but the reproductive investment of *P. pocoto* was not affected by the parasite load. It is known that anuran species with small body size tend to live shorter than larger ones (Duellman & Trueb, 1994; Stark & Merri, 2018). Consequently, despite the trade-off between growing and reproducing, short-lived species are expected to have a relatively higher reproductive investment (Scharsel et al., 2015). Thus, it is possible that the species of *Pseudopaludicola*, among the smallest representatives of the Leptodactylidae (Giaretta & Kokobum, 2003; Andrade et al., 2016, 2020; Pansanato et al., 2016), have a low longevity and may present a high reproductive investment independent of their parasitic interactions (Silva et al., 2018a,b).

Although parasitic infections are often related to the damage of hosts (Agnew et al., 2000; Galdino et al., 2014; Moretti et al., 2017), this pattern did not occur in a *P. pocoto* population from Caatinga. As an environment with a short and unpredictable rainy season, the semiarid is known to exert an even greater contingency for the reproduction of amphibians, and may frame specific ecological and behavioral strategies (Madeira & Gomes, 2016). For example, it has been suggested in last years that amphibians from semiarid regions have a relatively more intense immune response, contributing so that parasitic diseases do not cause substantial damage to reproduction (Madeira et al., 2017). In conclusion, keeping in mind that the patterns of the host’s life history and their relationships with the environment can act as mediators of physiological responses to parasitism (Minchella, 1985), our findings contribute to the understanding of the consequences of parasitic interactions in amphibians, revealing the nature of parasitism by nematodes in a small frog from a semiarid environment.

Acknowledgments. We thank Anyssa Oliveira, Eduarda Lima-Alves, Nilton Aquino and Paulo Cunha for assistance in the fieldwork and laboratory analysis; Camila Kamblicicuvić for help to parasites identification. We are grateful to Castiele Bezerra and José Luis Novaes for valuable suggestions to a prior version of this manuscript. P.B.S. thanks the Universidade Federal Rural do Semi-Árido – UFERSA and the Conselho Nacional de Desenvolvimento Científico e Tecnológico - CNPq for undergraduate scholarship of scientific initiation.

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RESOLUÇÃO DO CONSELHO FEDERAL DE BIOLÓGIA - CFBio n°.301/2012, de 8 de dezembro de 2012.


