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PRESSÃO PARASITÁRIA DE *Bucephalus* sp. SOBRE *Leukoma pectorina*: IMPLICAÇÕES ECOLÓGICAS E DE SAÚDE EM UM ESTUÁRIO

PARASITIC PRESSURE OF *Bucephalus* sp. ON *Leukoma pectorina*: HEALTH AND ECOLOGICAL IMPLICATIONS IN A TROPICAL ESTUARY

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Abstract - The present study investigated the infection of the parasite *Bucephalus* sp. in the gonads and digestive acini of the bivalve *Leukoma pectorina* (Lamarck, 1818) in the estuary of Village Camará, Marapanim, Pará, Brazil, highlighting its reproductive and ecological impacts. Sampling was conducted from July 2008 to June 2009, with 633 individuals subjected to histological processing. The analysis revealed an average prevalence of 3.8%, with no significant seasonal variation, although parasitic intensity was higher during the dry season. The infection compromises gonadal function, causing follicular compression and infertility, particularly in females. Histological observations showed that the parasite predominantly develops in gonadal tissue, interfering with gamete production. In response to infection, mollusks activate their immune system, encapsulating sporocysts with hemocytes, but this response is insufficient to contain parasitic proliferation. The impact of *Bucephalus* sp. infection extends beyond individual damage, potentially affecting the population structure of *L. pectorina*. The reduction in reproductive capacity may lead to population decline, impacting the ecological chain and local economy, especially in communities dependent on the commercialization of these mollusks. Continuous monitoring is essential to understand the dynamics of the infection and its long-term effects.

Keywords: Bucephalosis; Bivalve; Immune response; Cercaria, Histology.

Resumo - O presente estudo investigou a infecção pelo parasita *Bucephalus* sp. nas gônadas e ácinos digestivos do bivalve *Leukoma pectorina* (Lamarck, 1818) no estuário da Vila Camará, Marapanim, Pará, Brasil, destacando seus impactos reprodutivos e ecológicos. As amostras foram coletadas de julho de 2008 a junho de 2009, com 633 indivíduos submetidos a processamento histológico. A análise revelou prevalência média de 3,8%, sem variação sazonal significativa, embora a intensidade parasitária tenha sido maior na estação seca. A infecção compromete a função gonadal, causando compressão folicular e infertilidade, especialmente em fêmeas. Histologicamente, o parasita se desenvolve predominantemente no tecido gonadal, interferindo na produção de gametas. Em resposta à infecção, os moluscos ativam a imunidade, encapsulando os esporocistos com hemócitos, mas essa resposta não é suficiente para conter a proliferação parasitária. O impacto da infecção vai além de danos individuais, podendo afetar a estrutura populacional de *L. pectorina*. A redução da capacidade reprodutiva pode levar ao declínio populacional, impactando a cadeia ecológica e a economia local, especialmente em comunidades dependentes da comercialização desses moluscos. Monitoramento contínuo é essencial para entender a dinâmica da infecção e seus efeitos a longo prazo.

Palavras-Chave: Bucefalose; Bivalve; Resposta immune; Cercária; Histologia.

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INTRODUCTION

Bivalves feed through filtration, which makes them susceptible to miracidia penetration through the tegument, raising concerns for populations that consume these mollusks (Bulantová et al. 2011). The sanitary conditions of these animals are highly correlated with the levels of water contamination in their habitat, as demonstrated by evidence showing that many microorganisms ingested by bivalves survive their digestive process (Cook, 1984).

Understanding the ecology of diseases is essential for assessing variability in abundance and, consequently, managing commercially exploited marine species (Stentiford et al. 2012). There is clear evidence that pathogen prevalence impacts population fluctuations, often leading to population declines (Ward & Lafferty, 2004; Hershberger et al. 2016). Parasites establish an intimate association with their hosts, causing harm and leading to diseases. Lauckner (1983) defines disease as a negative deviation from the normal (healthy) state whether functional or structural resulting in impairments that reduce the host's ecological potential.

The primary disease-causing agents in marine bivalves belong to the groups of bacteria, protozoa, and digenean trematodes. Mollusks serve as preferred hosts for trematodes, acting as both first and second intermediate hosts (Lauckner, 1983). They are easily infected by free-living propagules through their feeding activity and provide various tissue niches for parasite development (Montaudouin et al. 2009). However, the impact on the host population depends on two factors: the pathogenicity of the parasite and its prevalence (i.e., the percentage of infected hosts) and/or abundance (the number of parasites per individual host) (Magalhães et al. 2015).

In other words, the effect of parasites on their hosts is related to their parasitic stage. Typically, as metacercariae, parasites exhibit high prevalence but are reported to have low pathogenicity (Magalhães et al. 2015). According to Cheng (1978), digenean trematodes are of great interest in the pathology of bivalve mollusks, as they are recognized as intermediate hosts. Sporocysts and cercariae of these parasites have been found in the digestive gland, gonads, and gills of bivalves, while fish serve as intermediate or definitive hosts (Marchiori, 2008; Winstead et al. 2004). Infestation by sporocysts (or rediae) often causes severe impacts on the host due to the strong interaction between the parasite and vital organs, such as the gonads, digestive tract, or gills (Magalhães et al. 2015).

Among the bivalves exploited in Brazil, the species *Leukoma pectorina* (Lamarck, 1818), commonly known as "sarnambi," stands out (Teixeira & Papavero, 2006). According to Borcem et al. (2011), in the communities of Marapanim, in the state of Pará, harvesting these bivalves is considered a source of income and subsistence for the local population.

Given the above, there is a need to study and address the issue of parasitosis in mollusks in the Amazon region, as they are part of the traditional diet of Amazonian populations. Therefore, the objective of this study was to report the occurrence of the parasite *Bucephalus* sp. in the host *L. pectorina* and characterize the parasite-host relationship, analyzing the effects of seasonality and parasite size.

MATERIAL AND METHODS

Study Area and Sampling

Sampling was conducted in the municipality of Marapanim, which belongs to the Salgado microregion and is part of the Northeast Pará mesoregion. The municipality's climate is humid tropical, with annual rainfall reaching up to 3,000 mm, an average temperature of 27.7 °C, and humidity levels ranging between 80% and 85%. The highest rainfall occurs between January and June, while the driest months extend from July to December (Furtado, 1978; Berrêdo et al. 2008).

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Samples were collected monthly in Village Camará, at coordinates 00°35'35.40" S and 47°41'26.60" W, in the state of Pará (Figure 1), during both the dry and rainy seasons of the Amazon. Sampling took place at low spring tide from June 2008 to July 2009. Due to the tidal regime, the maximum possible number of individuals was captured without standardization across months. A total of 633 individuals were collected using a scarifier (gardening fork) to dig and extract them from the substrate. One specimen of the mollusk *L. pectorina* was deposited in the UFRA reference ichthyology collection with the record CIRUFRA- 1506. The parasite identified as *Bucephalus* sp. is found in the helminthological collection of the Laboratory of Cell Biology and Helminthology Prof. Dr. Reinalda Marisa Lanfredi, Institute of Biological Sciences, UFPA.

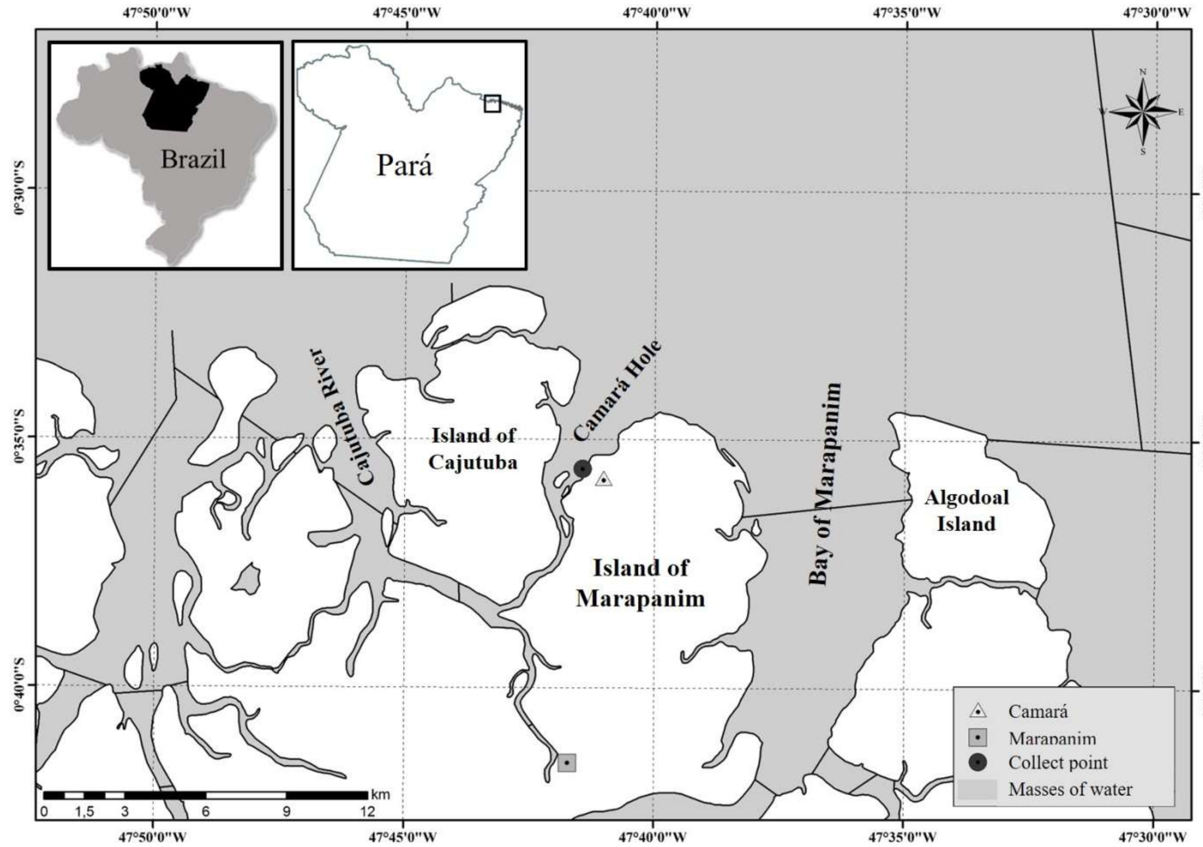

Figure 1. Location of the municipality of Marapanim, in the state of Pará, Brazil, highlighting the collection point and Village Camará.

Simultaneously with the collection of specimens, environmental parameters were measured. Water temperature was recorded using a mercury thermometer with a precision of 0.1 °C, while salinity was determined using a portable refractometer with a scale of 1/100. Precipitation data were provided by the Pará Meteorology District. After collection, individuals without shells were fixed in Davidson's solution for 24 hours and subsequently preserved in 70% ethanol until they were processed using a modified histological protocol based on Junqueira & Junqueira (1983). The specimens were dehydrated in a series of ethanol and xylene solutions, embedded in paraffin, sectioned at a thickness of 5 µm, and stained with Harris hematoxylin and eosin (H&E).

Histological sections were examined under a light microscope to detect the presence of parasites. If an individual tested positive, the pathological alterations caused by the parasites were assessed and described. Parasites were identified according to Gibson et al. (2008), and parasitic prevalence and mean intensity were calculated following the methodology of Bush et al. (1997).

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Histological images of infected individuals were captured using a Motic 5.0 camera at 400x magnification, and parasites were quantified using Photoshop 6.0 software, allowing for the estimation of mean parasitic intensity. Sporocysts were classified as primary or secondary according to the description by Bogitsh et al. (2018). Only in female hosts were the width and length of trematodes and sporocysts measured, with the latter also having its wall thickness assessed (Figure 2), using the Image Tool 3.0 software. This allowed us to characterize the space occupied by the parasite in the infected regions.

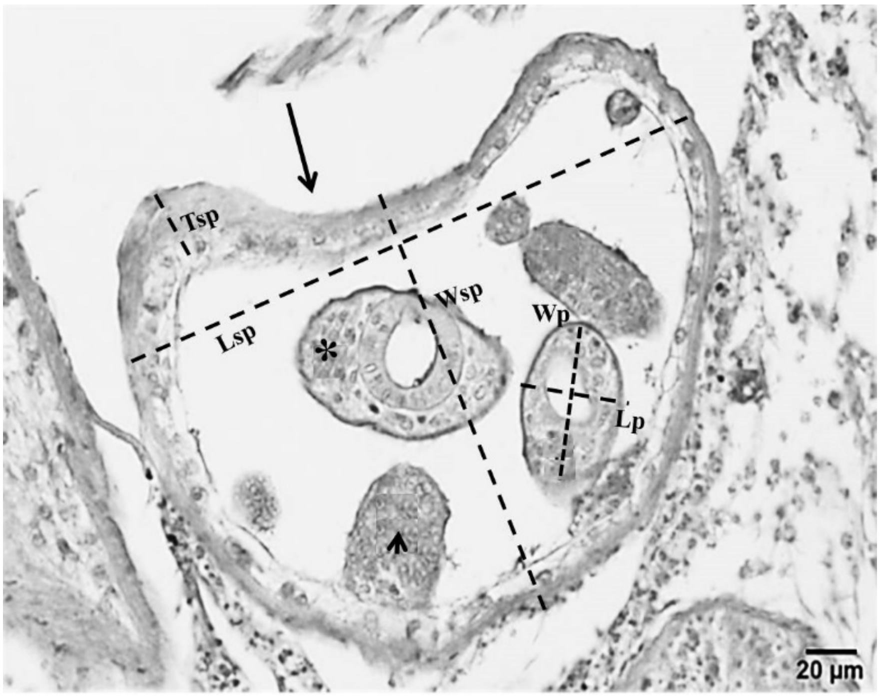

Figure 2. Diagram of measurements of the sporocyst and the trematode. Arrow indicating the late-stage sporocyst with cercariae in the late stage (*) and early stage (head of the arrow). Esp - Thickness of the sporocyst wall; Csp - Length of the sporocyst; Lsp - Width of the sporocyst; Cp - Length of the parasite; Lp - Width of the parasite.

Statistical Analysis

To assess differences in parasitic prevalence and intensity indices between males and females, as well as between juveniles and adults, we employed the chi-square test. To mitigate statistical test errors, we applied Yates' correction (1934). To determine whether these indices varied between the dry and rainy seasons, we used Student's t-test. To identify differences in the sizes of juvenile and late-stage sporocysts and cercariae across infestation sites (gonads and gonadal acini), we applied the non-parametric Mann-Whitney test. All analyses were conducted using the Past 4.03 software, with a significance level set at 0.05.

RESULTS

During the study period, temperature ranged from 28°C to 29°C, salinity varied between 4 and 36, and precipitation showed the greatest fluctuation, ranging from 0 mm to 412 mm. Microscopic examination revealed trematode parasitosis, with parasites located in the gonads and among the digestive glands. In both locations, cercariae and primary and secondary sporocysts were

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observed, surrounded by numerous cells, which appear to be part of the host's immune defense response.

In the gonads, trematodes were attached to the follicular wall and located between ovarian follicles or testicular tubules. These sites were the most preferred for sporocyst development, as 100% of the positive samples exhibited infestations in the gonads. In contrast, in the digestive glands, sporocysts were found between the digestive acini (Figure 3).

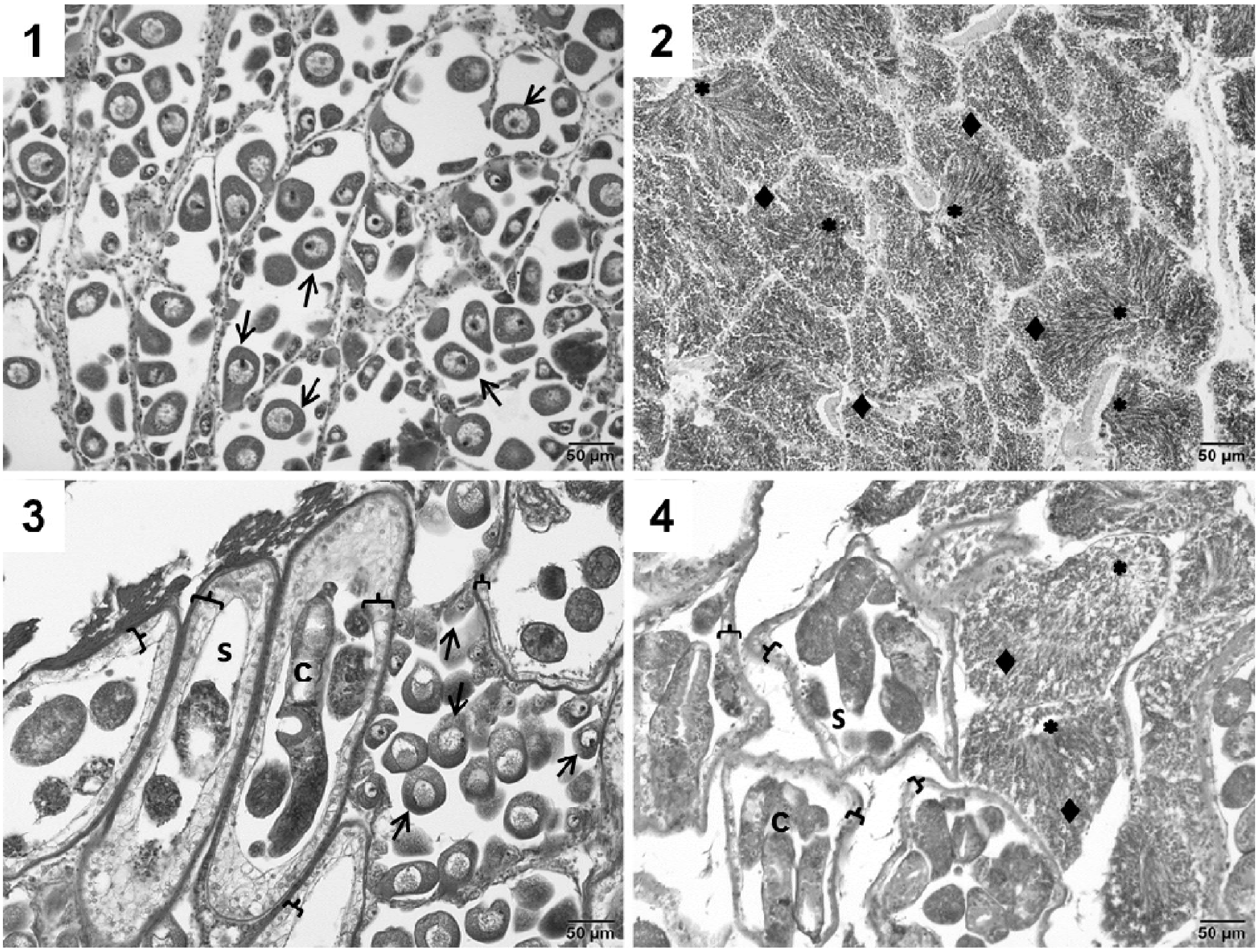

Figure 3. Photomicrographs of cross-sectional histological cuts of gonads of *Leukoma pectorina*. 1) Non-parasitized female gonad, where numerous vitellogenic oocytes (→) are observed; 2) Non-parasitized male gonad, where early (♦) and advanced (*) stages of germ cell development are observed; 3) Parasitized female gonad, where numerous vitellogenic oocytes (→) and cercariae (c) are developing inside sporocysts (s) bounded by the sporocyst wall (brackets); 4) Parasitized male gonad, where early (♦) and advanced (*) stages of germ cell development are observed, along with cercariae (c) developing inside sporocysts (s) bounded by the sporocyst wall (brackets).

The numerous sporocysts compressed the ovarian follicles or testicular tubules, exerting mechanical pressure on them, which led to the inhibition of gonadal maturation and hypertrophy of germ cells. In highly infected individuals, infertility was observed, occurring exclusively in females (Figure 4).

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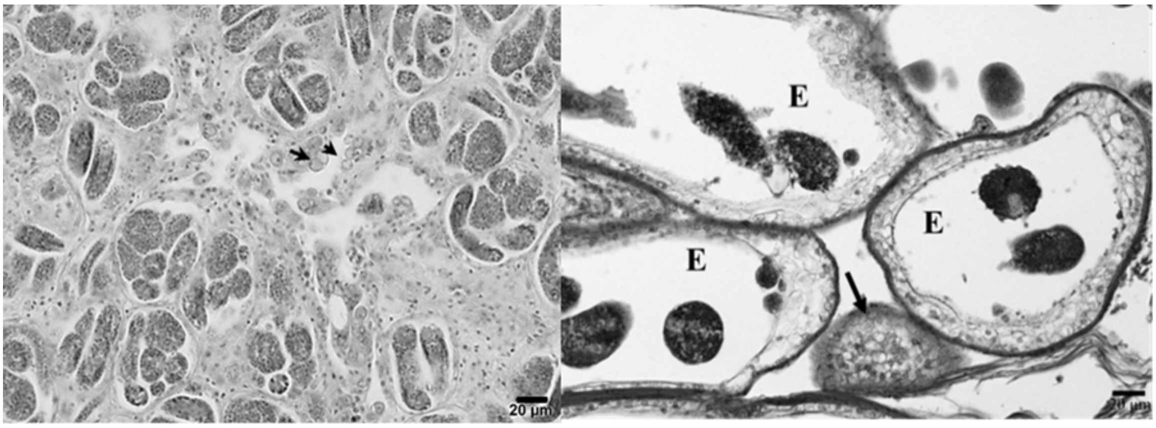

Figure 4. Infestation of sporocysts (E) compressing the ovarian follicles, causing infertility in females, arrow indicating hypertrophied follicle and oocytes. Magnification of 1000X.

The overall parasitic prevalence for the study period was 3.8%, with the lowest rates observed in June 2008, February 2009, May 2009, and July 2009, while the highest prevalence was recorded in August 2008 at 8.2%. Meanwhile, the mean parasitic intensity for the entire period was 450.8, with the highest intensity observed in November 2009, reaching 687 (Table 1). Parasitic prevalence did not differ significantly between the dry and rainy seasons ($p = 0.44$); however, parasitic intensity was significantly higher during the dry season ($p < 0.01$).

Table 1. Temporal variation of prevalence and mean parasitic intensity in *L. pectorina*.

Seasonality	Month	Prevalence (%)	Intensity
Dry	June/08	0	0
	August/08	8.2	605.3
	September/08	4.8	684.3
	October/08	2.6	647
	November/08	5.6	687
	December/08	2.2	675
Rainy	January/09	3.8	181.5
	February/09	0	0
	March/09	5.7	276.3
	April/09	6	146
	May/09	0	0
	July/09	0	0
	Mean	**3.5**	**491.9**

Although female hosts exhibited higher parasitic prevalence, there was no significant difference in infection levels between sexes ($p < 0.05$). The mean parasitic intensity was 198.30 in females and 171 in males, with no statistically significant difference ($p < 0.05$). However, adults were identified as the preferred hosts (Table 2).

Table 2. Chi-square test for the significant difference in mean intensity and parasitic prevalence between sexes and juvenile and adult stages of *L. pectorina*.

Index	Female	Male	χ^2	Yates Correction

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Prevalence (%)	2.8	0.6	1.4	0.85
Intensity	198.3	171	2.02	1.08

Index	Juvenile	Adult	χ^2	Yates Correction
Prevalence (%)	0.3	3.2	2.4*	8.69*
Intensity	640	485	2.02*	1.08

* significant at p= 0.05.

A total of 742 sporocysts were measured, of which 578 were juveniles located in the gonads. Juvenile sporocysts in the gonads displayed considerable morphological variation, with lengths ranging from 10.29 µm to 220.49 µm, widths from 2.44 µm to 135.82 µm, and wall thicknesses from 1.14 µm to 48.7 µm. In contrast, late-stage sporocysts exhibited lengths from 31.02 µm to 216.44 µm, widths from 16.45 µm to 69.32 µm, and wall thicknesses from 1.38 µm to 15.06 µm.

Juvenile sporocysts found in the gonadal acini showed length variations between 24.13 µm and 320.99 µm, widths ranging from 22.77 µm to 135.82 µm, and wall thicknesses between 1.95 µm and 13.47 µm. In the late stage, their lengths ranged from 51.51 µm to 240.43 µm, widths from 24.42 µm to 76.04 µm, and wall thicknesses from 2.49 µm to 11.9 µm. Juvenile sporocysts found in the gonadal acini exhibited significantly greater length ($p < 0.01$), width ($p < 0.01$), and wall thickness ($p < 0.01$) compared to those located in the gonads (Table 3). However, no significant size differences were observed between late-stage sporocysts from the gonads and those from the gonadal acini ($p > 0.05$).

Table 3. Length, width and wall thickness of sporocysts and cercariae measured in the gonad (G) and gonadal acinus (GA) region in the juvenile (J) and late (L) stages in females of *L. pectorina*.

	Infestation location	Stage	Length (µm)	Width (µm)	Wall thickness (µm)
Sporocyst	G	J	47.23±23.78	31.21±12.96	4.80±3.65
		L	82.75±49.31	37.16±14.33	5.66±3.72
	GA	J	99.15±53.21	62.94±23.71	7.1±2.74
		L	108.61±62.57	42.81±13.94	6.39±3.06
Cercaria	G	J	34,16±17.39	11.92±11.33	-
		L	51.72±34.88	17.97±9.65	-
	GA	J	28.16±15.61	6.35±4.10	-
		L	62.68±29.70	21.09±4.47	-

A total of 1,120 cercariae were measured, of which 846 were juveniles located in the gonads. The dimensions of the parasites in the cercarial stage, both juvenile and late-stage, also exhibited significant variability. In the gonads, juvenile cercariae had lengths ranging from 3.45 µm to 101.45 µm and widths from 1.38 µm to 77.49 µm, while late-stage cercariae exhibited lengths between 14.14 µm and 155.19 µm and widths from 2.78 µm to 49.7 µm. Regarding juvenile cercariae found in the gonadal acini, their lengths varied from 1.72 µm to 70.77 µm, and widths from 0.98 µm to 41.58 µm. In the late stage, lengths ranged from 20.49 µm to 122.12 µm, and widths from 14.89 µm to 32.21 µm. Juvenile cercariae found in the gonadal region had significantly greater length ($p < 0.01$) and width ($p < 0.01$) compared to those found in the gonadal acini (Table 3). Conversely, late-stage cercariae located in the gonadal acini exhibited significantly greater width than those found in the gonads ($p < 0.01$).

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DISCUSSION

The gonad is the most frequent site of trematode infection in bivalve mollusks and is consistently found in parasitized organisms, as reported by Cremonte et al. (2005), Boehs & Magalhães (2004), Valderrama et al. (2004), Sabry et al. (2007), and Boehs et al. (2010). According to Boehs et al. (2010) and Lauckner (1983), this parasite is associated with follicular destruction, leading to castration, as well as the infiltration of sporocysts into digestive glands, which was observed in this study.

Bower et al. (1994) stated that sporocysts reduce glycogen content in tissues and impair circulatory system efficiency, leading to disruptions in gametogenesis and eventual castration. Furthermore, gonadal infestation reduces the synthesis of metallothioneins (MTs), proteins capable of binding physiological or xenobiotic elements, providing defense against stressors such as trace metals or parasites, which are stimulated by gametogenesis. By causing host castration, this process consequently decreases MT synthesis in mature cockles and increases their vulnerability (Magalhães et al. 2015).

Winstead et al. (2004) reported that sporocyst infestation in digestive glands interferes with food absorption in the host, reducing its condition index through mechanical action on the tissue.
The encapsulation of sporocysts by layers of fibers and cells (hemocytes) observed in this study represents a host reaction attempting to limit the parasite's mechanical effects. The recruitment of hemocytes in response to infectious agents is considered the first step in the phagocytosis process (Boehs et al. 2010; Soudant et al. 2013; Allam & Raftos, 2015).

The parasitic prevalence in *L. pectorina* was considered low (3.8%) and did not vary seasonally, similar to findings by Silva et al. (2002) for the bivalve *Perna perna* infected by the trematode *Bucephalus* sp., as well as for *A. brasiliana, I. brasiliana*, and *M. guyanensis* studied by Boehs et al. (2010). Both studies were conducted in tropical zones, and Boehs et al. (2010) suggest that the lack of seasonal variation in prevalence may be due to the narrow range of temperature fluctuations and the absence of salinity and precipitation variation patterns throughout the year.

Since the parasite was present throughout most of the study period, it can be characterized as an endemic infection in the "sarnambi" population, as Neves et al. (2005) define endemism as the constant presence of a disease within a population. The higher prevalence of parasitism in adults may be related to their greater ease of nutrient absorption, as adult organisms continuously invest energy in the maturation of germ cells, making it easier for the parasite to capture glycogen and carbohydrates.

This reinforces that, despite the mechanical and physiological impact of trematodes on infected individuals, the low prevalence suggests that the sarnambi population is not at significant risk of collapse due to parasitism. The infertility observed in females may be a long-term concern, but its low abundance suggests that the infection does not drastically affect the overall reproduction of the population. The recommendation to cook before consumption is essential because, even though trematodes do not pose a direct threat to the sarnambi population, they may have implications for other organisms, including potential definitive hosts in the food chain. This safety measure helps minimize any risk of zoonotic transmission.

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