

Parasite-Driven host manipulation: The case of trematodes in Neotropical tadpoles

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ABSTRACT

Parasites can impact tadpole survival in both lethal and sublethal ways. Sublethal effects include alterations in morphology and behavior, reduced competitive ability, and increased vulnerability to predation, while lethal effects result in direct mortality. These impacts can have significant consequences at both individual and population levels, especially given that amphibians host various parasites and pathogens, which may contribute to population declines. This study investigated the influence of digenetic trematodes, specifically *Lophosicyadiplostomum* sp. and Echinostomatidae metacercariae – larval stages found in second intermediate hosts, on the development and behavior of *Physalaemus cuvieri* tadpoles. Behavioral experiments assessed tadpole activity (time and movement number), water column use, and food consumption rates using video recordings and image analysis. Swimming performance was evaluated through predation simulations, and tadpole morphology (i.e. body size and mass) was measured post-experimentally. Infections by these digenetic trematodes resulted in a notable reduction in tadpole activity. A negative correlation was observed between *Lophosicyadiplostomum* sp. parasite load and both tadpole activity duration and movement frequency, indicating a common response to trematode infection. This study underscores the need for further research on digenetic trematodes to determine whether these behavioral modifications represent host manipulation by the parasites, potentially optimizing their transmission to the final host.

1. Background

Most studies related to amphibians' behavior mediated by parasites have focused primarily on the response of tadpoles to the presence of the cercariae phase (free-swimming larval stage of digenetic trematodes), or associating this response to the predator's presence (usually fish) in the same environment (pre-infection) in temperate regions (Koprivnikar et al., 2014; Szuroczi and Richardson, 2012; Thiemann and Wassersug, 2000), rather than how parasite infection may affect tadpoles' behavior or physiology. This gap is particularly notable in studies involving digenetic trematodes, a diverse group of parasites with complex life cycles that often include amphibians as intermediate hosts. Furthermore, not only does the life cycle of digeneans remain poorly understood, but also their identification and morphology, especially in larval stages (Pinto and Melo, 2013); and their effects on the behavior and development of their final hosts (Goodman and Johnson, 2011; Queiroz

et al., 2020; Travassos, 1950). To address this knowledge gap, it is essential to understand the unique characteristics and complexity of the digenetic trematode life cycle.

Digeneans exhibit the most complex life cycle among the Neodermata clade and constitute the largest group of endoparasitic metazoans (Cribb et al., 2003; Olson et al., 2003). They can have one intermediate and one definitive host, but their life cycle can incorporate up to four different intermediate hosts in diverse ways, with the three-host life cycle being the most common (Esch et al., 2002; Olson et al., 2003; Travassos, 1950). Parasites that exploit a second intermediate host tend to exhibit greater infection success and a higher probability of completing their reproductive cycle compared to those with direct life cycles (Bush et al., 1997; Queiroz et al., 2020). Generally, a gastropod mollusk serves as the first intermediate host, where the cercarial stage develops; and the second intermediate host (e.g., insect larvae, annelids, tadpoles, and fish) is where the metacercariae phase

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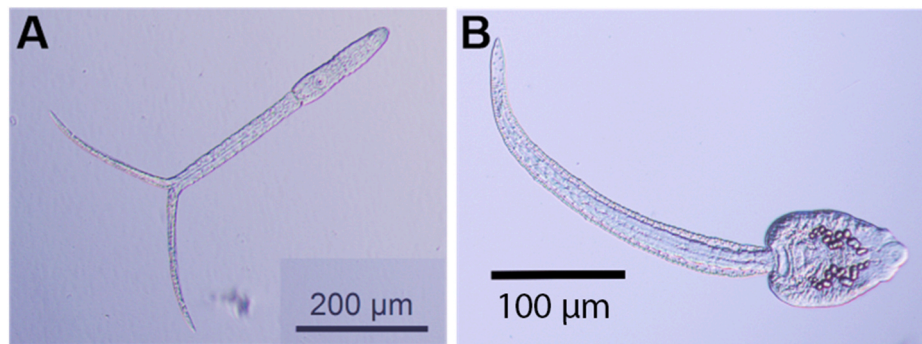


Fig. 1. Cercariae used in the experimental infections of *Physalaemus cuvieri* tadpoles. (A) *Lophosicyadiplostomum* sp.; (B) Echinostomatidae.

develop (Pinto and Melo, 2013). Metacercariae are transmitted to the final host from the second intermediate host, a process that rarely occurs passively. Many trematodes have the remarkable ability to change their hosts' morphology or behavior, a critical adaptation that enhances transmission success (Moore, 1984; Poulin, 2010).

When these changes benefit the parasites, they are referred to as "adaptive manipulation." According to Poulin (2010), this type of manipulation can occur through four distinct pathways. The first pathway involves the parasite inducing the host to remain close to ensure protection of the parasite's pupae until they emerge, after which the host typically dies. This behavior is observed in certain parasitoid insects, which manipulate the host to defend the pupae from predation (Grosman et al., 2008). The second pathway is characterized by the parasite altering the host's habitat, often moving it to an environment that is unsuitable for the host. In this scenario, the parasite must either exit the host on its own or release its propagules in the definitive host's environment. An example of this is parasitized ants, which are manipulated to climb to higher regions of shrubs or trees, thereby facilitating the dispersal of fungal spores (Andersen et al., 2009). The third form of host manipulation is observed in interactions between vectors and their parasites, particularly in relationships between vertebrate hosts and blood-sucking insects. Here, behavioral changes in the vector may reduce the duration of feeding, thereby increasing the number of host visits and enhancing the chances of parasite transmission (Moore, 1993). Finally, the fourth pathway is termed "trophic transmission", where the parasite is passed to its definitive host through predation of the intermediate host. This is achieved by making the intermediate host more visible or susceptible to predators, such as snails whose tentacles change in size, shape, and color, pulsing in response to light to attract predators (Moore, 2002). This ability to manipulate the host increases the likelihood of successful transmission to the definitive host.

Following this, the specific behaviors that can be infection-induced differ depending on the identity of the species involved (Poulin and George-Nascimento, 2007). For example, trematodes can manipulate host behavior to reduce predation on intermediate hosts before the parasite completes its development within them (Parker et al., 2009; Poulin and Leung, 2011). Besides that, they can indirectly induce the host to increase food consumption to compensate for the cost of the immune response (Poulin and George-Nascimento, 2007), which, in tadpoles, is directly associated with a higher activity level (Bonneaud et al., 2016). Conversely, echinostomatid trematodes can also negatively affect the growth and development of anuran larvae, leading to malformations of the hind legs (Fried et al., 1997; Goren et al., 2014). Also, trematodes can cause morphological alterations in the spinal column, such as scoliosis and kyphosis (Pathirana et al., 2019). In addition, these parasites can reduce adult frog swimming performance by diminishing burst speed, distance, and endurance—traits that are critical predictors of host survival (Goodman and Johnson, 2011). Ultimately, these behavioral changes seem to enhance the likelihood of predation on intermediate hosts by the final hosts of digenetic trematodes (Lafferty and

Morris, 1996). Specifically, the altered behaviors can increase the visibility of tadpoles, making them easier targets for capture by vertebrate final hosts, as seen in killifish infected by *Euhaplorchis californiensis*, a digenetic trematode.

Herein, we conducted experimental investigations to assess the impact of two digenetic trematodes, *Lophosicyadiplostomum* sp. (Diplostomidae) and a species of Echinostomatidae family, on the behavior of *Physalaemus cuvieri* tadpoles, a widely distributed anuran species (Miranda et al., 2019). Both parasites utilize gastropods (Planorbidae) as their first intermediate host and are commonly found in ponds where anuran tadpoles develop (López-Hernández et al., 2023). The infection sites in tadpoles, their second intermediate hosts, differ between parasites. *Lophosicyadiplostomum* sp. metacercariae stage develops in the kidney's host but does not encyst (Pinto and Melo, 2013; Queiroz et al., 2020), while the species of the Echinostomatidae family we tested encyst between the skin and muscles in the tadpole's tail (Hamann and González, 2009). Also, the final hosts may differ between the parasites. *Lophosicyadiplostomum* sp. likely targets diurnal birds, but because the exact identity of the Echinostomatidae parasite is unknown, it is difficult to determine which groups of vertebrates this specific parasite infects (Pinto and Melo, 2013). We quantified the activity level, foraging efficiency, and swimming escape performance of experimentally infected *Physalaemus cuvieri* tadpoles. The choice of these response variables considered some of the most common effects of parasites on tadpole behavior (Goodman and Johnson, 2011; Koprivnikar et al., 2006; Robar et al., 2011; Thiemann and Wassersug, 2000).

Our hypothesis posited that tadpoles infected by *Lophosicyadiplostomum* sp. would exhibit increased activity levels, number of movements, and spend more time toward the water surface, along with a reduced ability to escape from predators. Thus they are predicted to be more susceptible to predation by diurnal birds, their likely final host. Additionally, we predict that *Lophosicyadiplostomum* sp. infected tadpoles will show an increase in feeding activity due to the energetic cost of the parasite, as they develop inside the host's kidney. Conversely, tadpoles infected with Echinostomatidae cercariae are expected to display a different behavioral pattern due to the specific site of infection (caudal muscle) and the uncertainty of a definitive vertebrate host associated with this parasite, which could be fish, reptiles, amphibians, birds, and mammals. Given the infection of the caudal muscle, we expect reduced tadpole mobility, which would result in decreased activity levels, fewer movements, more time spent at the bottom, and a reduced ability to escape predators. We predict that feeding consumption will not differ from the control group, as the parasites are encysted in the caudal muscle, which we anticipate will result in a low metabolic demand.

2. Methods

2.1. Species

We collected *Drepanotrema lucidum* (Pfeiffer, 1839) and

Drepanotrema depressissimum (Moricand, 1839) gastropods of the Planorbidae family, known as intermediate hosts for digenean trematodes (Pinto and Melo, 2013). These gastropods were collected between December 2019 and February 2020 from temporary water bodies along the riparian vegetation of Córrego da Véstia in the Selvíria municipality (20°23'43.4"S, 51°23'39.8"W), MS, Midwest Brazil. The gastropods were maintained in glass aquariums (35 × 20 × 25 cm) containing 4 L of dechlorinated water at 25 °C ± 1 °C under a controlled photoperiod (12 h in light/12 h in the dark) for 2 days.

To confirm trematode infection, a subset of these gastropods was individually placed in culture cell plates with potable water and exposed to 60 W incandescent light for 12 h. This procedure stimulates the emergence of cercariae from gastropods, allowing for visual confirmation of parasite presence via stereoscopic microscopy (Brasil. Ministério da Saúde, 2014).

We identified the type of cercariae via light microscopy (Fig. 1) using neutral red and Nile blue sulfate stains, according to Pinto and Melo (2013), and via electron microscopy using fresh slides, according to Kanev et al. (2009). Staining the cercariae allowed for the visualization of diagnostic structures, such as penetration glands, fin folds, and tail size and shape, enabling us to identify the cercariae involved in the infection. Fresh slides allow for counting the number of collar spines, which is especially useful for identifying members of the Echinostomatidae family. To ensure the occurrence of infection, we utilized active forms of cercariae, which were maintained in a limited space. Also, no more than 8 h have passed since the identification of the cercariae and the experimental infection.

The identity, final host, and life cycle of the Echinostomatidae species that we studied remain unknown. The identification of the species is currently unfeasible due to inherent difficulties in distinguishing the specimen. Although the cercarial collar of spines is widely recognized as a useful diagnostic feature, it proved insufficient for precise identification in this case. This challenge is frequently encountered in megadiverse regions such as Brazil. While existing studies suggest that the spine pattern remains consistent across the cercaria, metacercaria, and adult stages, the high biodiversity in Brazil complicates the ability to make definitive identifications based on this characteristic alone. Furthermore, there is a significant possibility that this specimen represents an undescribed taxon.

We obtained tadpoles from five *P. cuvieri* spawn collected between December 2019 and February 2020 from different ponds in Nova Aliança municipality, SP (21°07'84.0"S, 49°53'92.7"W), Southeast Brazil. Egg collection ensured that the tadpoles did not have any parasitic infections (Schotthoefer et al., 2003). Additionally, acquiring tadpoles from various spawns ensures genetic variability (Szuroczi and Richardson, 2012). The spawn and hatched tadpoles were maintained in aquaria (35 × 20 × 25 cm) at 28 °C ± 1 °C under a controlled photoperiod (12 h in light/12 h in the dark), mimicking the natural conditions of the range of *P. cuvieri* (Miranda et al., 2019).

Hatched tadpoles were fed ad libitum with commercial fish food based on algae and krill (Sera Micron®) until they reached developmental stage 30 (Gosner, 1960), which is the phase reported to have the lowest mortality rate due to Echinostomatidae (*Riberoia ondatrae*) parasitic infections (Schotthoefer et al., 2003). Gastropods and tadpoles were collected from locations approximately 150 km apart. Despite this distance, it is known that this anuran species reproduces in temporary marginal ponds where gastropods are collected (Queiroz et al., 2021). Gastropods and tadpoles were transported in plastic bags containing water from the ponds where they were collected and placed in isothermal expanded polystyrene boxes to prevent overheating.

2.2. Experimental infection

We first experimentally infected tadpoles with *Lophosicyadiplostomum* sp., subjected them to the experiments, and only after completing the necropsies did we start the experiment with Echinostomatidae. For

the experimental infection, we placed two groups of 20 tadpoles into each aquarium (30 cm × 22 cm × 7 cm) with a 3 cm water column. All tadpoles were in the same developmental stage (31–34) according to Gosner (1960), a period considered a phase of relative stability in terms of morphological changes leading up to metamorphosis. Unlike the methodology frequently employed in experimental infections, we chose to infect the tadpoles together due to the difficulty in obtaining more snails infected with the same parasite, despite our extensive efforts. For this reason, one gastropod infected with *Lophosicyadiplostomum* sp. was added to one of these aquariums. The tadpoles were then exposed to cercariae emerging from the gastropods for 30 h. After an additional 24 h, following the individualization of each tadpole into a glass aquarium and acclimation, we conducted two experiments aimed at assessing the influence of the infection on tadpole behavior.

After completing the behavioral experiments with the control and *Lophosicyadiplostomum* sp. infected tadpoles, the same procedure was used to infect the tadpoles with Echinostomatidae cercariae. In this case, three gastropods infected with Echinostomatidae cercariae were added to the aquarium containing 20 tadpoles due to the low number of cercariae released by each gastropod. The different number of gastropods added was due to the availability of parasitized gastropods at the collection site and the number of cercariae emerging from the infected gastropods. The tadpoles were exposed to cercariae emerging from the gastropods for 30 h. Another aquarium with 20 tadpoles without gastropods served as the control group, similar to the setup for the *Lophosicyadiplostomum* sp. experimental infection.

At the beginning of the experiments, 20 tadpoles were exposed to *Lophosicyadiplostomum* sp. cercariae, and 20 tadpoles were exposed to Echinostomatidae cercariae. However, due to mortality during the experiment, the final number of infected tadpoles was 11 for *Lophosicyadiplostomum* sp. and 9 for Echinostomatidae, reflecting the impact of individual deaths on the overall population. Similarly, the initial control group for each parasite group consisted of 20 tadpoles, but by the end, we had 12 individuals in the *Lophosicyadiplostomum* sp. control group and 9 individuals in the Echinostomatidae control group.

The experiments focused on evaluating the parasite's impact on tadpoles concerning (i) the use of the water column, time of activity, number of movements, and food consumption, and (ii) the performance of predator swimming escape. In each experiment, both the control and infected groups consisted of tadpoles of similar sizes and developmental stages (stages 31–34; Gosner, 1960). Video recordings of tadpoles were made using a Sony HDR-XR160 camera in both experiments.

2.2.1. Tadpole activity rate, use of a water column, and food consumption

To assess the influence of parasites on tadpole behavior, individuals were individually placed in glass aquariums (20 cm × 10 cm × 30 cm) filled with dechlorinated water to a depth of 15 cm at a temperature of 26 °C ± 1 °C under a controlled photoperiod (12 h in light/12 h in the dark). After 24 h of acclimation, tadpole behavior was recorded twice daily (at 9 a.m. and 7 p.m., corresponding to periods of heightened activity based on personal observations) for two days, with each recording lasting 5 min. The recording camera was positioned in front of each set of four glass aquariums at a distance of 60 cm, with the height adjusted using a tripod.

We utilized custom time quantification software developed exclusively for this experiment to determine the following response variables: (i) time of activity (percentage of total recorded time each tadpole spent swimming), (ii) number of movements, and (iii) water column position (near the surface or at the bottom) for the majority of the recorded time.

To assess the influence of parasites on food consumption rates, we followed the protocol of Venesky et al. (2013). A suspension of 40 mg/ml Sera Micron® and water was brushed onto one side of glass plates (15 cm × 7.5 cm). After drying for 24 h, one plate was placed in each aquarium daily between video recording periods (10 a.m.–6 p.m.) to avoid behavioral changes induced by feeding. Following tadpole feeding, the plates were allowed to dry naturally outside the water.

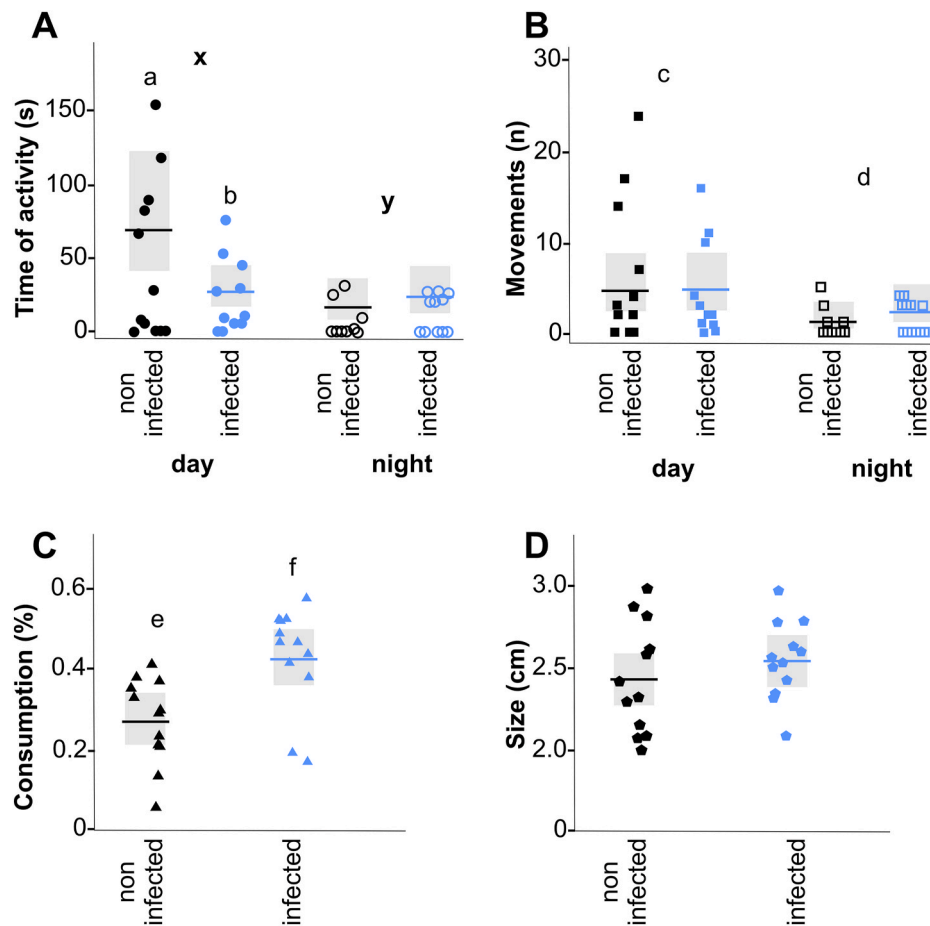


Fig. 2. (A) Time of activity (in seconds); (B) number of movements; (C) proportion of food consumed; and (D) size (in centimeters) of *P. cuvieri* tadpoles noninfected and infected by *Lophosicyadiplostomum* sp. We measured the duration of activity and number of movements during the day and night. The bold letters “x” and “y” indicate significant differences between the day and night periods. The regular letters (“a” and “b” in figure A; “c” and “d” in figure B; and “e” and “f” in figure C) indicate significant differences between the infected and noninfected groups. The lower and higher limits of the gray boxes represent the 95% confidence intervals of the mean values for each group (the bars inside the boxes).

Subsequently, we captured images of the plates and analyzed them in ImageJ software (Hartig, 2013) to quantify the percentage of food removed, assessing the (iv) consumption rate for each tadpole.

2.2.2. Tadpole swimming performance experiment

To assess the effect of parasites on tadpole swimming performance, we utilized the same individuals from the activity rate, water column use, and food consumption experiments, a day after the end of the video records. Tadpoles were individually placed inside a glass canal (50 cm × 2 cm × 5 cm) filled with 1 cm of water. The chosen water volume was tailored to the tadpole size to limit their response to two-dimensional space (Egea-Serrano and Tejedo, 2014). Predation simulations were conducted by gently touching the tadpole’s caudal region using a metal stem (Bridges, 1997; Dayton et al., 2005; Egea-Serrano and Tejedo, 2014). Each individual was stimulated three times, and all trials were used for statistical analysis.

Video records, analyzed using Kinovea visual tracking software (Charmant, 2019), allowed quantification of the average (i) burst speed (the distance covered by a tadpole during the 0.20 s after being touched with the metal stem), (ii) total distance covered (cm), and (iii) time in seconds from the beginning of the escape swimming until the end of the caudal movements (Bridges, 1997; Egea-Serrano and Tejedo, 2014; Wilson et al., 2000).

After the experiment, pictures of all individuals were taken to measure the length and width of the caudal muscle, as these variables can directly influence tadpole swimming performance (Dayton et al., 2005;

Wilson et al., 2000). Additionally, we determined the tadpole’s total length, a variable incorporated into the analyses to investigate whether food consumption was related to individual size (Audo et al., 1995). All these measures described above were made in ImageJ software (Hartig, 2013). Post-experiment, the tadpoles were anesthetized in a 2% lidocaine solution until the heart stopped beating. Subsequently, they underwent necropsy to determine parasite load as a continuous variable and to confirm the absence of infection in the control group.

It is crucial to note that the metacercariae of Echinostomatidae encyst inside the second intermediate host, while the metacercariae of *Lophosicyadiplostomum* sp. remain unencysted within the tadpole host.

2.3. Data analysis

We employed generalized linear models (GLMs) and generalized linear mixed models (GLMMs) to model all response variables based on the experimental treatments. Model fit was assessed through the visual inspection of randomized quantile residuals (Dunn and Smyth, 1996). Analysis of deviance was used in all cases to test the effect of treatments.

To evaluate the influence of parasites on tadpole behavior, we tested the effect of group treatments (infected and noninfected) on (i) time of activity, (ii) number of movements, and (iii) water column position. These were modeled by zero-inflated gamma, negative binomial, and binomial distributions, respectively. Day period (daylight and night) served as a fixed predictor (covariable) as an interactive variable, and tadpole identity was included as a random predictor. Additionally, we

Table 1

Response variables, predictors (Group: infected vs. non-infected, Period: day vs. night, and the interaction between Group and Period), χ^2 values, degrees of freedom (df) and p values for tadpoles of *Physalaemus cuvieri* infected with *Lophosicyadiplostomum* sp and Echinostomatidae.

Response variables	Predictors	<i>Lophosicyadiplostomum</i> sp.			Echinostomatidae		
		χ^2	df	p value	χ^2	df	p value
Time of activity (s)	Groups	2437	1	0,118	3875	1	0,049 ^a
	Period	4338	1	0,037 ^a	9255	1	0,002 ^a
	Groups*Period	3,89	1	0,048 ^a	0,146	1	0,701
	Parasite load	6309	1	0,012 ^a	1665	1	0,196
	Period	0,11	1	0,739	5021	1	0,025 ^a
	Parasite load*Period	5035	1	0,024 ^a	0,762	1	0,382
Number of movements (n)	Groups	0,378	1	0,538	4,12	1	0,042 ^a
	Period	6681	1	0,009 ^a	21,193	1	>0,001 ^a
	Groups*Period	0,665	1	0,414	0,011	1	0,915
	Parasite load	0,029	1	0,836	3712	1	0,054
	Period	6404	1	0,011 ^a	14,492	1	>0,001 ^a
	Parasite load*Period	5757	1	0,016 ^a	2,08	1	0,149
Water column position (deep vs. surface)	Groups	0,561	1	0,453	0,731	1	0,786
	Period	0,105	1	0,745	0,848	1	0,356
	Groups*Period	1076	1	0,299	1,38	1	0,24
	Parasite load	0,011	1	0,916	0,885	1	0,346
	Period	0,475	1	0,49	0,008	1	0,925
	Parasite load*Period	0,83	1	0,362	0	1	0,999
Consumption (%)	Groups	9911	1	0,001 ^a	2089	1	0,148
	Parasite load	2927	1	0,087	0,396	1	0,529
Total length (mm)	Groups	0,954	1	0,328	4227	1	0,039 ^a
	Parasite load	0,875	1	0,349	0,133	1	0,715

^a Statistically significant.

tested the effect of the treatment group on (i) the food consumption rate and (ii) the total length of tadpoles, modeled by beta-binomial and Gaussian distributions, respectively. These same response variables were analyzed as a function of parasite load in infected tadpoles, where variables i, ii, and iii included the day period as a fixed predictor and tadpole identity as a random predictor.

To assess the influence of parasites on tadpole swimming performance, we tested the effect of group treatments on (i) burst speed, (ii) total distance traveled, and (iii) swimming time. A Gaussian distribution was used for the burst speed response variable, and a zero-inflated gamma distribution was used for the other two response variables. Caudal muscle width and length were considered fixed covariables. Additionally, we tested for the effect of parasite load on these response variables, considering only individuals from the infected group (supplementary material). Gosner stage was not included in the statistical analysis, as 86% of the tadpoles were at the same stage (Gosner stage 31), and only 14% were distributed across stages 32–34. All analyses were performed using R software (R Core Team, 2022 – version 4.2.1).

3. Results

3.1. Influence of *Lophosicyadiplostomum* sp. on the behavior of tadpoles

A total of 23 tadpoles were tested in this experiment, 11 of them being infected with *Lophosicyadiplostomum* sp. and 12 noninfected individuals. The mean abundance of the metacercariae was 12.7, and the maximum range was 41. Infected tadpoles had less time active than noninfected tadpoles, only during daylight (Fig. 2A, Table 1). Both infected and noninfected tadpoles had greater number of movements during daylight than at night, and neither group was affected by the presence of a parasitic infection (Fig. 2B–Table 1). Infected tadpoles consumed more food than noninfected tadpoles (Fig. 2C), but this greater food consumption did not result in a significant increase in tadpole size, although infected tadpoles exhibited a nonsignificant trend toward having a larger body size (Fig. 2D–Table 1).

When considering only tadpoles from infected group, individuals with a greater parasitic load spend less time active and exhibited fewer movements than those with smaller parasite loads (Fig. 3, Table 1)

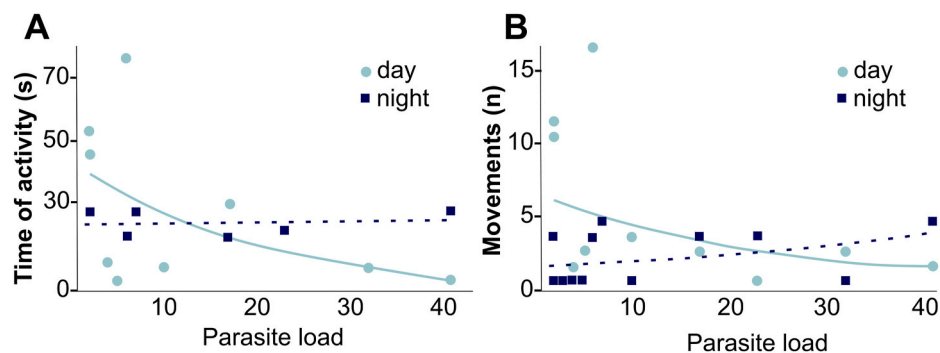


Fig. 3. (A) Time of activity (in seconds) and (B) number of movements of *Physalaemus cuvieri* infected by *Lophosicyadiplostomum* sp. as a function of parasite load during the day and night.

Table 2
Predator swimming response variables, predictors (Group: infected vs. non-infected, Period: day vs. night, and the interaction between Group and Period), χ^2 values, degrees of freedom (dfs) and p values of tadpoles of *Physalaemus cuvieri* infected by each species of trematode parasite.

Response variables	Predictors	Lophosicyadiplostomum sp.			Echinostomatidae		
		χ^2	df	p value	χ^2	df	p value
Burst speed (cm/s)	Groups	0,439	1	0,507	7091	1	0,007 ^a
	Muscle length	0,967	1	0,325	0	1	0,978
	Muscle width	4536	1	0,033 ^a	2454	1	0,117
	Parasite load	1,71	1	0,19	1,71	1	0,104
	Muscle length	5646	1	0,017 ^a	5646	1	0,168
	Muscle width	8,97	1	0,002 ^a	8,97	1	0,481
	Groups	0,64	1	0,423	1236	1	0,266
	Muscle length	2685	1	0,101	1148	1	0,276
	Muscle width	2368	1	0,123	2186	1	0,139
Distance (cm)	Parasite load	0,263	1	0,607	0,241	1	0,622
	Muscle length	3688	1	0,054	1235	1	0,266
	Muscle width	2932	1	0,086	0,055	1	0,813
	Groups	1921	1	0,165	0,033	1	0,854
	Muscle length	1066	1	0,301	0,409	1	0,522
	Muscle width	0,008	1	0,972	0,123	1	0,725
	Parasite load	0,339	1	0,559	0,077	1	0,781
	Muscle length	0,002	1	0,957	0,007	1	0,913
	Muscle width	0,188	1	0,664	0,039	1	0,842

^a Statistically significant.

during the day period. Additionally, both the time of activity and the number of movements were greater during the day than during the night, but only for tadpoles with low parasite loads (Fig. 3, Table 1).

Infection by *Lophosicyadiplostomum* sp. cercariae did not affect the performance of tadpoles, specifically burst speed, distance, or time of escape swimming. Additionally, there was no relationship between these response variables (burst speed, distance, and time of escape swimming) and the parasite load. Burst speed was affected only by muscle length and width (Table 2).

3.2. Influence of Echinostomatidae on the behavior of tadpoles

A total of 18 tadpoles were tested in this experiment, 9 of them being infected with Echinostomatidae and 9 noninfected individuals. The mean abundance of the metacercariae was 9.9, and the maximum range was 29. Tadpoles infected by Echinostomatidae cercariae were active for a shorter time and exhibited fewer movements than noninfected tadpoles during both the day and night periods (Fig. 4A and B; Table 1). Infected tadpoles displayed a nonsignificant trend toward increased food consumption (Fig. 4C; Table 1) and a significant increase in body size (Fig. 4D; Table 1). An inverse pattern was observed for the response variables consumption and body size for tadpoles infected with *Lophosicyadiplostomum* sp.

When considering only tadpoles from infected group, both the time of activity and the number of movements were greater during the day

than at night (Fig. 5A and B; Table 1). Although there appears to be a trend between parasite load and the variables of activity time and number of movements during the day period, no significant relationship was found. The graph shows that this trend can be explained by the presence of the point with the highest parasite load. The only observed difference in this variables were between day and night periods (Fig. 5B; Table 1).

Tadpoles infected by Echinostomatidae cercariae exhibited a greater burst speed than noninfected tadpoles (Fig. 6; Table 2). However, the distance and duration of swimming response did not differ between infected tadpoles, regardless of the parasite load (Table 2).

4. Discussion

We have shown that tadpoles infected with *Lophosicyadiplostomum* sp. exhibited a reduced duration of activity time during the day compared to uninfected tadpoles. A similar result was observed in tadpoles infected with Echinostomatidae metacercariae. In addition to daytime activity, Echinostomatidae metacercariae infection also reduced tadpole activity at night and led to fewer movements overall. According to our hypothesis, this response was expected for Echinostomatidae metacercariae infected tadpoles — as we supposed that caudal muscle cysts would reduce mobility — but not for the *Lophosicyadiplostomum* sp. infected tadpoles, in which we expected increased activity, due to the final host being visually oriented.

Activity reduction behavior is known as an important response to escape from predators (Brown et al., 2001; Mogali et al., 2020), as it reduces the chance of being found (Koprivnikar et al., 2006, 2012; Szuroczi and Richardson, 2012; Van Buskirk et al., 2011). Consistent with this idea and our findings for Echinostomatidae metacercarial cysts, Parker et al. (2009) reported that trophic-transmitted parasites can manipulate host behavior to decrease predation on intermediate hosts before the parasite completes its development.

Most parasites require a certain period of development within the intermediate host before becoming infective to their definitive host, enhancing transmission success and increasing parasite fitness (Poulin, 2010). As well, our results for Echinostomatidae metacercarial cysts can be aligned with this hypothesis, considering that cysts are formed to avoid adverse conditions and for protection (Aguilar-Díaz et al., 2011). Moreover, increased tadpole survival enhances the likelihood of completing metamorphosis and transitioning to terrestrial environments, where they may serve as prey for birds and other vertebrate predators—the definitive hosts of Echinostomatidae. (D’Heursel and Haddad, 1999; Fitzpatrick, 1980; Fried and Toledo, 2009; Martins et al., 2021).

In addition, as expected, the reduction in activity observed in Echinostomatidae-infected tadpoles may be necessary for survival in the typical habitat of the second intermediate host. *Physalaemus cuvieri* tadpoles normally inhabit the shallow margins of water bodies (Prado et al., 2009), and the observed reduction in movement may reflect a strategy to reduce exposure to predators—a behavior potentially influenced by parasite infection. This behavior may serve as a response to the presence of vertebrates using the water body as a drinking source, thereby reducing vulnerability to predation.

The reduced activity time in *Lophosicyadiplostomum* sp.-infected tadpoles during the day suggests a similar transmission strategy. This behavioral similarity between both parasites may reflect a general response to infection, helping tadpoles mitigate predation risks despite being infected. Conversely, the number of tadpole movements was not related to infection—this was a surprising finding, as we initially predicted that the infection would cause tadpoles to increase their exposure, given that birds are one of the parasite’s final hosts.

For both parasites, reduced host activity could be a strategy to conserve energy, potentially reallocating it to processes such as enhancing the immune response (Altman et al., 2016). Alternatively, this reduction in activity may reflect the physiological toll of infection,

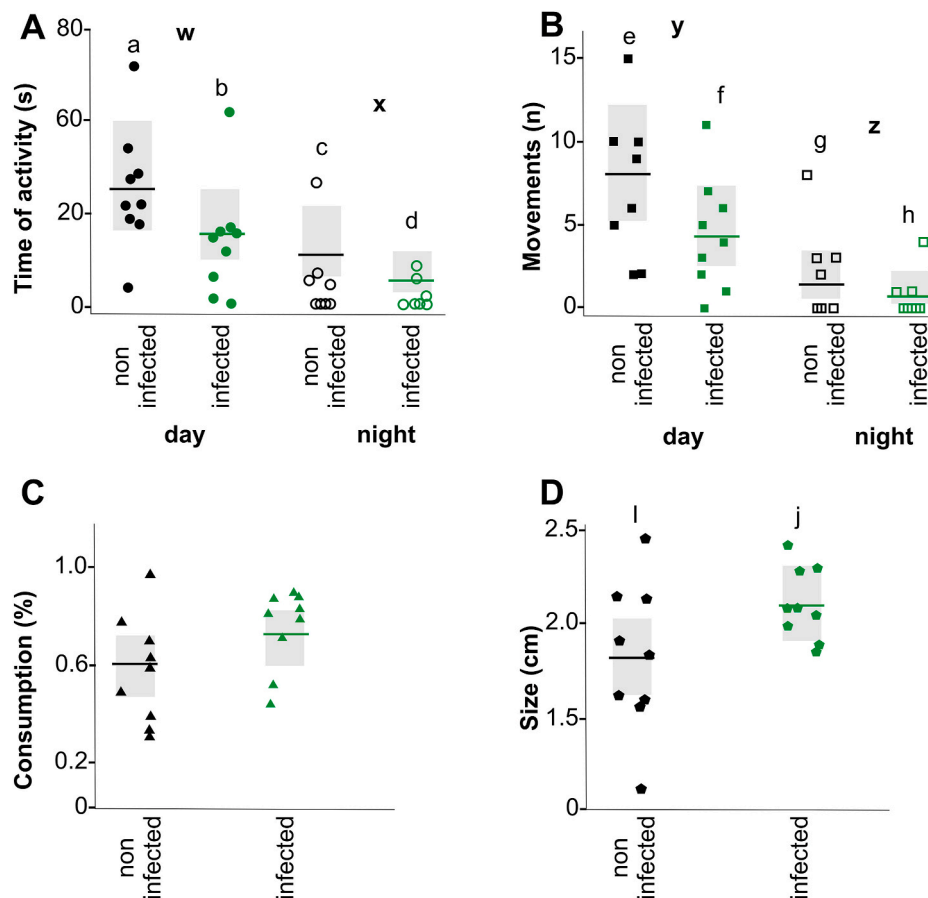


Fig. 4. (A) Time of activity (in seconds); (B) number of movements; (C) proportion of food consumed; and (D) size (in centimeters) of *P. cuvieri* tadpoles noninfected and infected by Echinostomatidae. We measured the time of activity and the number of movements during the daytime and night periods. The bold letters (“w” and “x” in figure A; “y” and “z” in figure B) indicate significant differences between the day and night periods. The regular letters (“a”, “b”, “c” and “d” in figure A; “e”, “f”, “g” and “h” in figure B; and “i” and “j” in figure D) indicate significant differences between the infected and noninfected groups. The lower and higher limits of the gray boxes represent the 95% confidence intervals of the mean values for each group (the bars inside the boxes).

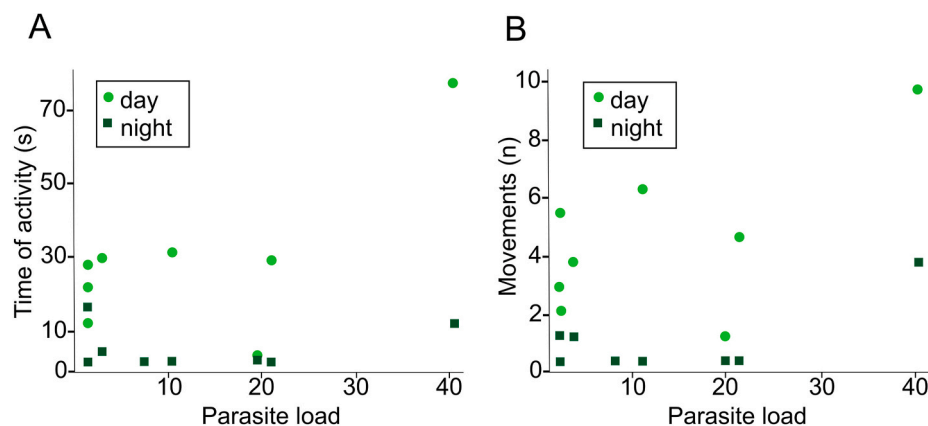


Fig. 5. (A) Time of activity (in seconds) and (B) number of movements of *Physalaemus cuvieri* infected by Echinostomatidae as a function of parasite load during the day and night.

manifesting as morbidity that limits the host’s normal behavior (Goren et al., 2014). Responses to trematode parasites can exhibit complexity, with patterns still awaiting comprehensive understanding, as they may vary based on the infection site and be host-species specific (Koprivnikar et al., 2012).

The considerable knowledge gap regarding the life cycles of digenetic trematodes, particularly in the Neotropical region, poses challenges in determining whether these observed behavioral changes in

tadpoles represent host manipulation by the parasite. Host manipulation involves alterations in the host phenotype induced by a parasite, conferring fitness benefits to the parasite (Poulin, 2010). In the context of altered host behavior, this typically implies that infected hosts behave in ways that enhance the transmission or dispersal of the parasite, contributing to the completion of its life cycle (Poulin, 2010).

The impact of *Lophosicyadiplostomum* sp. cercariae on tadpole behavior differed concerning parasite load. *Lophosicyadiplostomum* sp.

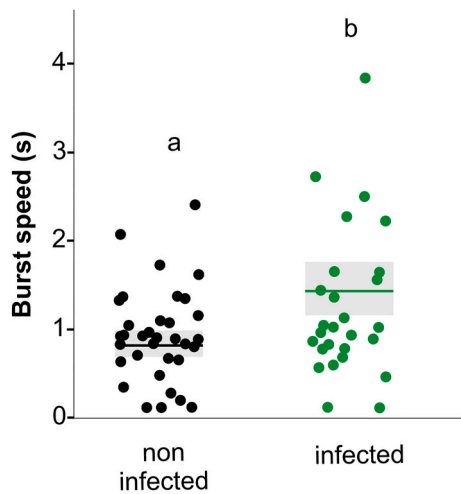


Fig. 6. Burst speed escape response in *Physalaemus cuvieri* tadpoles infected and not infected by Echinostomatidae parasites. The letters “a” and “b” represent significant differences between the infected and noninfected groups. All trials were used for statistical analysis.

significantly reduced tadpole activity time and movement number as the parasite load increased, whereas no such relationship was observed in tadpoles infected with Echinostomatidae cercariae. This finding was surprising, as we initially predicted that the infection would lead to increased exposure for the tadpoles, given that birds, being visually oriented, serve as one of the parasite’s final hosts. Typically, parasites exert minor effects on activity levels, but they can have substantial impacts on microhabitat choice, sometimes doubling the time an animal spends in a specific microhabitat (Friesen et al., 2018).

Both parasites caused a similar pattern of growth in their infected tadpoles. Notably, despite not leading to significant growth, tadpoles infected by *Lophosicyadiplostomum* sp. consumed more food, as we expected, and tended to have greater bodies than those in the control group. This result suggests a potential elevation in metabolic rate, as parasites utilize host resources, creating an energy deficit. Despite no significant difference in food consumption, Echinostomatidae-infected tadpoles achieved larger sizes compared to those in the control group. This result was unexpected, but it supports our hypothesis that the encystment of metacercariae may lead to a reduced metabolic demand, allowing the infected tadpoles to allocate more energy toward growth (Robar et al., 2011).

Another possible explanation for our hypothesis is that both types of cercariae might influence the size of the intermediate host, with larger individuals being less prone to predation in the aquatic environment (Gill, 2003), thereby increasing the likelihood of successfully completing metamorphosis (Johnson et al., 2010) and emerging to the terrestrial environment, where they might become more vulnerable to predation by the final host. Also, we acknowledge that variations in tadpole body mass could introduce a source of noise to our data. However, despite this potential confounding factor, we were able to identify consistent patterns.

Contrary to our hypothesis, the improved escape performance observed in tadpoles infected by Echinostomatidae cercariae, coupled with their larger size, supports the hypothesis that Echinostomatidae might enhance the success of metamorphosis. Burst speed and greater size are pivotal behaviors in avoiding predation (Badets et al., 2010; Dayton et al., 2005; Sears et al., 2015), and transmission to the final host, according to the results that we observed, may be anticipated to occur in the terrestrial environment.

5. Conclusions

The intricate interplay between digenetic parasites and tadpoles reveals a multifaceted relationship characterized by nuanced behavioral adaptations and potential host manipulation. Our study revealed a spectrum of responses among tadpole species infected with *Lophosicyadiplostomum* sp. and Echinostomatidae metacercariae, shedding light on the complexity of parasite-induced alterations in host behavior. While reductions in tadpole activity are typically interpreted as a predator avoidance strategy, the observed discrepancies in response patterns suggest a more intricate dynamic influenced by factors such as infection site and host species specificity. Moreover, the contrasting effects of *Lophosicyadiplostomum* sp. and Echinostomatidae cercariae on tadpole growth and activity underscore the potential for parasite-mediated manipulation of the host phenotype to enhance transmission success. The observed trends, including increased food consumption and altered growth patterns in infected tadpoles, suggest underlying mechanisms that optimize parasite fitness. Furthermore, the implications of these behavioral modifications extend beyond the aquatic environment, potentially influencing tadpole survival during metamorphosis and subsequent vulnerability to predation by final hosts. However, the complexity of these interactions is compounded by significant knowledge gaps regarding the life cycles of digenetic trematodes, particularly in the Neotropical region. Future research endeavors aimed at elucidating the mechanisms driving these behaviors and unraveling the intricacies of parasite-host dynamics are essential for advancing our understanding of ecological processes in aquatic ecosystems.

CRediT authorship contribution statement

Paula Maria Rosa: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Murilo de Souza Queiroz:** Writing – review & editing, Resources, Methodology. **Rodolfo Mei Pelinson:** Writing – review & editing, Formal analysis, Data curation. **Luciano A. Anjos:** Writing – review & editing, Validation, Supervision, Resources. **Denise de Cerqueira Rossa-Feres:** Writing – review & editing, Validation, Supervision, Resources, Funding acquisition, Conceptualization.

Availability of data and materials

https://zenodo.org/records/11034485?token=eyJhbGciOiJIUzUxMiJ9.eyJpZCI6ImYwZGh1OWU0LT12OTgtNGVmYi05MmQ0LWZiZWUxNjBjZDdmMSIsImRhdGEiOnt9LCJyYW5kb20iOiJlMDIyMzFkNzBkZTBiZjg5ZGYyMGF0ZWZjMzI0ODI1ZCJ9.ZM9RMLAuCj0jHYgCMQ42i0NPE_v7P-KVPPJcqX7SBsm0lJVp-Rf5Zh73JqbqKD_Zw47B8uvUFaGV420FvFpMsw.

Ethics approval

All procedures were performed with previous authorization (ICMBio #18163-1 to Denise de C. Rossa-Feres and CEUA-205/2019 to Paula M. Rosa).

Consent for publication

Not applicable.

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Declaration of No conflict of interest

The authors, Paula Maria Rosa, Murilo de Souza Queiroz, Rodolfo Mei Pelinson, Luciano A. Anjos, Denise de Cerqueira Rossa-Feres, hereby declare that we have no conflicts of interest related to the publication of this manuscript.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ijppaw.2024.101023>.

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