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Original Article

Population dynamics of *Achatina fulica* in a peri-urban area adjacent to the Fiocruz Atlantic Forest Biological Station (EFMA), in Rio de Janeiro, Brazil, with report on *Angiostrongylus cantonensis* infection

Dinâmica populacional de *Achatina fulica* em área periurbana adjacente à Estação Biológica Fiocruz Mata Atlântica (EFMA), no Rio de Janeiro, Brasil, com relato de infecção por *Angiostrongylus cantonensis*

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Abstract

Achatina fulica is a species native to East Africa, considered one of the 100 worst invasive alien species in the world. The present study investigated the population of the snail, *A. fulica*, in a peri-urban area adjacent to the Fiocruz Atlantic Forest Biological Station (EFMA), in Jacarepaguá, Rio de Janeiro state, Brazil, focusing on population dynamics and the nematodes associated with this species. To this end, specimens were collected during four climatic seasons of the years 2021 and 2022 in three fixed 20 m × 10 m plots. The abundance of *A. fulica* in these areas was evaluated in relation to a set of environmental variables (temperature, relative humidity air, and soil pH and calcium). The abundance of snails infected by nematodes was also evaluated in relation to the season and body size of the specimens. The molluscs were found by active search, and standardized (15 minutes/three collections). Nematode larvae were extracted from the specimens by artificial digestion and identified by their external morphology and the sequencing of molecular markers. A total of 280 specimens of *A. fulica* were collected, with the highest abundances being recorded in the autumn and summer, although no significant relationship was found between the number of specimens collected and the environmental variables. Overall, 192 snails were infected by nematodes: *Angiostrongylus cantonensis*, *Cruzia tentaculata* and free-living nematodes, including *Caenorhabditis briggsae*. These findings demonstrate the epidemiological importance of the study area and the need to implement educational measures in the community, with the aim of controlling the local *A. fulica* population, thereby minimizing the risk of parasitic infection in the local human population.

Keywords: *Lissachatina*, nematódeos, parasites, intermediate host, Eosinophilic Meningitis.

Resumo

Achatina fulica é uma espécie nativa da África Oriental, considerada como uma das 100 piores espécies exóticas invasoras do mundo. O presente estudo investigou a população do caracol, *A. fulica,* em uma área periurbana adjacente à Estação Biológica Fiocruz Mata Atlântica (EFMA), em Jacarepaguá, Rio de Janeiro, Brasil, com foco na dinâmica populacional e os nematódeos associados a este molusco. Para isto, foram realizadas coletas em quatro estações climáticas, dos anos de 2021 e 2022 em três parcelas fixas de 20 m × 10 m. A abundância de *A. fulica* nessas áreas foi avaliada em relação a um conjunto de variáveis ambientais (temperatura, umidade relativa do ar, pH e cálcio do solo). A abundância de moluscos infectados por nematódeos também foi avaliada em relação à estação do ano e tamanho corporal dos espécimes. Os moluscos foram coletados por busca ativa, e padronizadas (15 minutos/três coletores). Larvas de nematódeos foram extraídas dos espécimes por digestão artificial e identificadas por sua morfologia externa e sequenciamento de marcadores moleculares. Um total de 280 espécimes de *A. fulica* foram coletados durante o presente estudo, com as maiores abundâncias sendo registradas no outono e verão, embora nenhuma relação significativa tenha sido encontrada entre o número de espécimes coletados e as variáveis ambientais. Ao todo, 192 moluscos estavam infectados por nematódeos: *Angiostrongylus cantonensis*, *Cruzia tentaculata*, e nematódeos de vida livre, incluindo *Caenorhabditis briggsae*. Esses resultados demonstram a importância epidemiológica da área de estudo e a necessidade de implementar medidas educativas na comunidade, com o objetivo de controlar a população local de *A. fulica*, minimizando assim o risco de infecção parasitária na população humana local.

Palavras-chave: *Lissachatina*, nematódeos, parasitos, hospedeiro intermediário, Meningite Eosinofílica.

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1. Introduction

The giant African snail, *Achatina* (*Lissachatina*) *fulica* Bowdich, 1822, is on the Invasive Species Specialist Group - ISSG (IUCN, 2015) list of the 100 world's Worst Invasive Alien Species. This species is native to Africa, but has been introduced into many other regions, including South America, where it was first recorded in Brazil in the 1980s (Kliks and Palumbo, 1992; Teles et al., 1997; Raut and Barker, 2002). This snail is now known to be present in all 27 Brazilian states (Arruda and Santos, 2022; Silva et al., 2022a), raising concerns due to its potential role as an intermediate host of nematodes that cause parasitosis in both humans and domestic animals, including Eosinophilic Meningitis (EM), which is also known as cerebral angiostrongyliasis (Sauerländer and Eckert, 1974; Carvalho et al., 2003; Caldeira et al., 2007; Thiengo et al., 2022). A number of studies have associated *A. fulica* with the spread of EM in Brazil and in other countries (Caldeira et al., 2007; Lima et al., 2009; Thiengo and Fernandez 2010, 2016; Kim et al., 2018; Valente et al., 2018). This zoonosis is endemic in Asia and the Pacific Islands, and has now spread to numerous other countries, with approximately 2800 cases being recorded overall, including some 40 confirmed and 84 suspected cases in Brazil (Wang et al., 2008; Morassutti et al., 2014; Andrade et al., 2018; Barbosa et al., 2020).

The nematode *Angiostrongylus cantonensis* (Chen, 1935) is the etiological agent of eosinophilic meningitis and it was originally described from China (Cowie et al., 2022). The distribution of *A. cantonensis* includes tropical and subtropical regions of Southeast Asia, the Pacific (including Hawaii), Australia, Japan, South America, the southeastern United States, the Caribbean, Africa, and the Canary and Balearic Islands (Cowie et al., 2022). The life cycle of *A. cantonensis* includes rodents (as definitive hosts) and non-marine gastropods (as intermediate hosts). Several species of terrestrial molluscs and one species of the freshwater genus *Pomacea* have been found infected by this nematode in Brazil (Morassutti et al., 2014; Valente et al., 2020; Thiengo et al., 2022), with many records in the state of Rio de Janeiro, in different municipalities (Morassutti et al., 2014; Oliveira et al., 2015; Thiengo et al., 2022).

Humans are accidental hosts, although the life cycle of the parasite is not completed, given that first-stage larvae are not eliminated in the feces, as observed in rodents, which are the definitive hosts. (Thiengo and Fernandez, 2013). Humans may be infected by ingesting a mollusc or paratenic host infected with *A. cantonensis*, or through the consumption of poorly sanitized vegetables and fruits containing third-stage larvae (L_3) released in the mucus of the molluscs (Hwang and Chen, 1991; Slom et al., 2002; Morassutti et al., 2014). As these larvae pass through the meninges, they cause meningitis. Therefore, they do not reach the adult stage in accidental hosts (Almeida, 2013; Moreira et al., 2013).

Achatina fulica also causes economic impacts, as an pest of several different agricultural crops. In addition to these economic losses and risks for public health, the invasion of protected areas by this species may cause the loss of local biodiversity through increased competition with native molluscs, for example (Cowie and Robinson, 2003; Eston et al., 2006; Meyer III et al., 2008; Colley and Fischer, 2009; Leão et al., 2011). As a synanthropic invader, *A. fulica* is common in urban environments, where it is often found in close proximity to houses, principally in shady and humid areas, where garbage and other residues accumulate, and in association with drainage systems (Simião and Fischer, 2004; Fischer and Colley, 2005; Silva et al., 2020). This snail has been recorded in many urban areas in different countries, such as on the oceanic island - Christmas Island (Lake and O'Dowd, 1991), Venezuela (Martínez Escarbassiere and Martínez Moreno, 1997), Argentina (Valente et al., 2017). The availability of food and shelter may favor the persistence of populations, supporting high rated of infestation at many sites (Silva et al., 2022a).

In addition to its tolerance of adverse conditions, *A. fulica* has a very enhanced reproductive capacity. In Florida, in the United States, Roda et al. (2016) found that specimens six months old laying 100 eggs, with the number of eggs increasing progressing in subsequent years, reaching 1800, with the offspring having an enhanced dispersal capacity, typical of other terrestrial molluscs.

Infestations of *A. fulica* have been reported in many of the municipalities of the Brazilian state of Rio de Janeiro (Thiengo et al., 2007; Zanol et al., 2010, Rodrigues et al., 2016, 2022; Rangel et al., 2023), which emphasizes the importance of monitoring this species in this state, to mitigate the potential risks. It is especially important to implement measures such as the orientation of the local population regarding the risks of infection and alerting the local authorities to the need for the management of the problem, in particular the control of the species (Zanol et al., 2010). This snail is known to act as a host for different nematode species, and infections have been recorded in a number of regions of Brazil, including Rio de Janeiro (Ramos-de-Souza et al., 2021; Thiengo et al., 2022).

Other alien, synanthropic terrestrial molluscs have also been recorded in urban areas. *Leptinaria unilamellata* (d'Orbigny, 1835), *Subulina octona* (Brugüière, 1789), *Bradybaena similaris* (Férussac, 1821) (Lopes et al., 2012; Rodrigues et al., 2016; Alexandre et al., 2017) *Deroceras leave* (Rodrigues et al., 2016) are some examples. As these environments have been modified by human activities, the relative paucity or absence of predators and competitors may favor the invasion of these species, a process reinforced by the concentration of food sources and refuges, as well as humid microhabitats (Cowie, 1998; Zanol et al., 2010; ICMBio, 2022).

The present study investigated the population dynamics of *A. fulica* in a per-urban zone adjacent to the Fiocruz Atlantic Forest Biological Station (EFMA), a strictly protected area of the Atlantic Forest in Rio de Janeiro (Brasil, 2000). The specimens collected were examined for the detection of infection by nematodes, and the analysis of the possible influence of environmental factors on the characteristics of the study population and nematode infection patterns.

2. Material and Methods

2.1. Study area

The peri-urban area investigated in the present study is located within the Fiocruz Atlantic Forest campus (*Campus Fiocruz da Mata Atlântica* – CFMA), in Jacarepaguá, Rio de Janeiro state (Brazil). The CFMA includes the Fiocruz Atlantic Forest Biological Station (*Estação Biológica Fiocruz Mata Atlântica* – EFMA), which is an area of approximately 500 hectares inside the Pedra Branca State Park, PEPB (Figure 1), which is an environmental Conservation Unit located in the West Zone of the city of Rio de Janeiro, Brazil. CFMA is located at the interface between the urban zone and a recovered remnant of preserved Atlantic Forest that makes up part of which overlaps partially with PEPB. The PEPB is considered one of the largest urban forests in the world and the largest stretch of Atlantic Forest in an Urban Area in Brazil, measuring approximately 12,400 hectares (INEA, 2013). There has been intense urban development in the area surrounding the EFMA over the past decade, resulting in the expansion of local communities and commercial establishments in this area (Gentile et al., 2018).

2.2. Sampling

Specimens were collected during four days in 2021 and 2022, representing the region's different climatic seasons, that is, the austral autumn (June 9th, 2021), winter (August 24th, 2021), spring (December 6th, 2021), and summer (February 24th, 2022). Standardized samples were collected, following the methods of Silva et al. (2020), with appropriate adaptations, with specimens being obtained from three fixed plots, measuring 20 m × 10 m where they were inspected in the four campaigns. These plots were chosen based on information provided by the CFMA personnel on the areas most infested by *A. fulica*.

The three plots were in a peri-urban area with unpaved streets, and close to the forested area of the EFMA (Figure 1). Plot I (Figure 2A-C) with vegetation, including bushes and alien plants such as banana trees (*Musa* sp.), as well as piles of garbage and areas in which garbage had been incinerated. Plot II (Figure 2D-F) also had bushes and piles of garbage, as well as rubble from a demolished house and a wall located next to the plot. Plot III (Figure 2G-I) was on a sloping terrain next to a stream, with dense undergrowth, and tree trunks and piles of garbage. A direct observation of the soil was made, where it appeared to have a moist appearance, and sewage pipes from nearby houses were observed within the area, together with some dead specimens of *A. fulica*.

The specimens were collected manually within the area of each plot by three collectors, who surveyed each plot for a standard period of 15 minutes. The specimens were collected during daylight hours, on one day in the latter half of each season, i.e., the austral autumn (June), winter (August), spring (December), and summer (February).

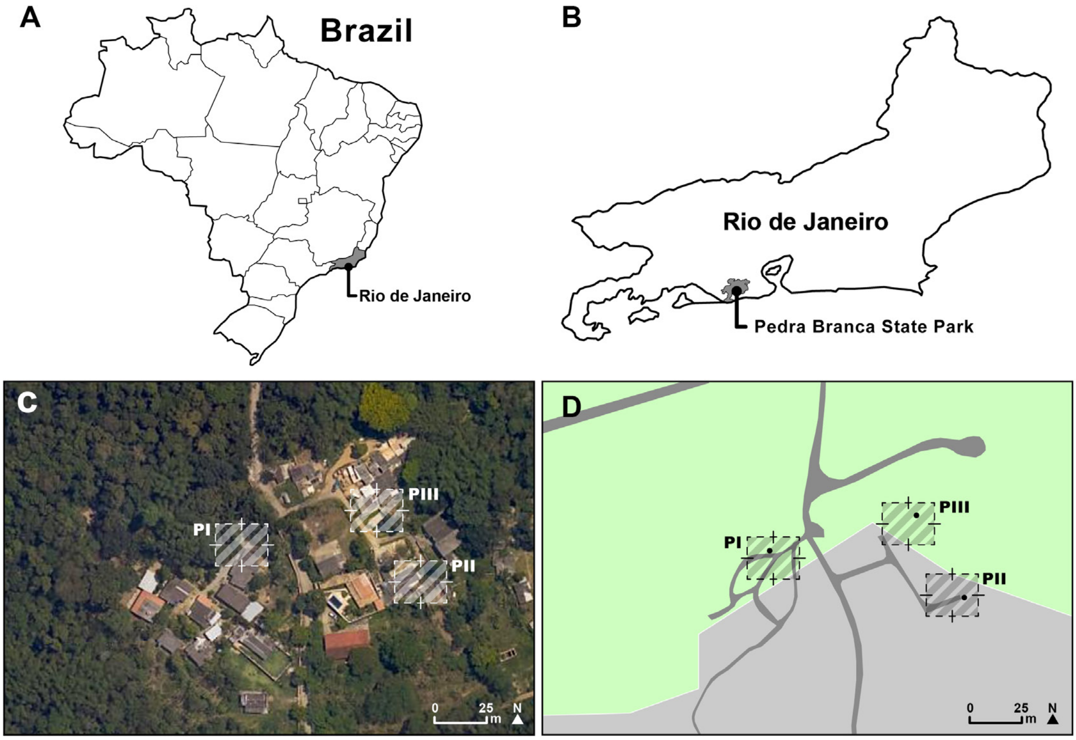

Figure 1. (A) Brazil, showing the state of Rio de Janeiro; (B) The state of Rio de Janeiro, showing the location of Pedra Branca State Park (PEPB); (C) Satellite image of the peri-urban area of the Fincão community adjacent to the Fiocruz Atlantic Forest Biological Station (EFMA), superimposed on the PEPB. The white squares represent the three sample plots (I, II, III) monitored in the present study; (D) The location of the plots within the peri-urban area, outlined by the dashed lines. Source: Google Maps (2023).

Figure 2. Plot: I. (A) Bushes; (B) Banana trees (*Musa* sp.); (C) Piles of garbage and garbage n incinerated. Plot II: (D) Demolished house and and piles of garbage, as well as rubble; (E) A wall located next to the plot; (F) Bushes. Plot III: (G) Dense undergrowth; (H) Tree trunks; (I) Piles of garbage.

2.3. Environmental data

Environmental parameters were also recorded during the surveys, including the presence of household refuse and debris within each plot, and the characteristics of its vegetation. Meteorological data (temperature, rainfall, and relative humidity air) were obtained from the website of the National Institute of Meteorology (INMET), based on the readings of the automatic and traditional meteorological stations located within the Jacarepaguá region. The mean values of these parameters were calculated for each study month, based on the daily records collected during the respective periods.

Once the snail specimens had been collected from each plot, a trowel was used to clear away the leaf litter for the collection of a sample of approximately 200 g of the soil, which was stored in labeled plastic bags. The samples were taken from the four corners of each plot and a central point at a depth of no more than 5 cm. The samples were taken to the laboratory, where they were refrigerated until being processed, when they were analyzed to determine their pH and total hardness of $CaCO_3$ following the method described by EMBRAPA (2017). These analyses were carried out at the Laboratory for the Evaluation and Promotion of Environmental Health (LAPSA) at the Oswaldo Cruz Institute in Rio de Janeiro.

2.4. Morphological and parasitological analyses of the molluscs

The specimens collected were analyzed at the National Reference Laboratory for Schistosomiasis–Malacology (LRNEM) at the Oswaldo Cruz Institute in Rio de Janeiro, where they were identified based on the analysis of the morphological characteristics of the shell and internal organs. Each specimen was assigned to one of four size categories, based on shell length, as defined by Silva et al. (2022b): separated by (shell length) defined by the methodology of infants (< 1.0 cm), juveniles (1.01-4.0 cm), young adult (4.01-7.0 cm) and adult (> 7.0 cm). Each specimen was then examined for infection by nematode larvae using Graeff-Teixeira and Morera (1995) artificial digestion technique with 0.7% HCL.

2.5. Morphological and molecular identification of the nematodes

The nematode larvae obtained during the parasitological examination were initially separated by superfamily, fixed in AFA solution (2% acetic acid, 3% formaldehyde, and 95% ethanol) for morphological analysis by Light Microscopy (LM) and Scanning Electron Microscopy (SEM). The species were identified based on Ash (1970), Thiengo et al. (2008, 2022), Ramos-de-Souza et al. (2021), and Rodrigues et al. (2022).

The larvae recovered from the snail specimens were stored in 1.5 mL Eppendorf tubes containing Phosphate Buffered Saline Solution (PBS), for acid neutralization, and stored at -20 °C, prior to the molecular diagnosis for the confirmation of the species identification. Thermal shock using liquid nitrogen, the Standard Operating Procedure of the LRNEM, was employed for the extraction and amplification of the genomic DNA of the metastrongyloid larvae. This DNA was then used to sequence the mitochondrial Cytochrome Oxidase Subunit I (COI) gene, using primers and protocol described by Prosser et al. (2013), with some modifications. In this case, the Polymerase Chain Reaction (PCR) was run in a total volume of 25 µl containing 2 µL of dNTP mix (0.2 mM), 2.50 µL of $MgCl_2$ (2.5 mM), 0.5 µL of each primer (0.01 µM), 0.25 µL of GoTaq G2 Hot Start Taq polymerase - Promega (1.25 U), 5 µl of Flexi buffer (1X), 5 µl of the genomic DNA, and ultrapure water to complete the 25 µl reaction volume.

For the other nematode species, which have larger larvae that are less prone to tissue lysis, the genomic DNA was extracted using the DNeasy Blood & Tissue kit (Qiagen), following the manufacturer's recommendations. In this case, the species were identified through the amplification of Internal Transcribed Spacer 2 of the Ribosomal DNA (ITS2), using the primers NC1 and NC2, as described by Gasser et al. (1993), and using their thermal cycling conditions, with some adaptions. Here, the PCR was run in a final volume of 25 µL following the work of Each PCR reaction containing 2 µL of dNTP (0.2 mM), 2.50 µL of $MgCl_2$ (2.5 mM), 0.75 µL of each primer (0.3 µM), 0.25 µL of GoTaq G2 Hot Start Taq polymerase – Promega (1.25 U), 5 µL of buffer (1X), and 5 µL of the genomic DNA, with ultrapure water to complete the reaction volume.

The amplified DNA was purified using the Illustra GFX PCR DNA and gel band purification kit (Cytiva, Little Chalfont, UK), following the manufacturer's instructions. The purified samples were sequenced in both directions (forward and reverse) in an ABI 3730xl automatic sequencer (Applied Biosystems), available on the Genomics and DNA Sequencing platform (RPT01A - http://plataformas.fiocruz.br/site) at the Oswaldo Cruz Institute.

The contigs of these sequences were assembled and edited in Seqman v7.0, and then used to search for similar sequences in GenBank (www.ncbi.nlm.nih.gov/genbank/) with the Basic Local Alignment Search Tool, or BLAST (Altschul et al., 1990). The sequences obtained in the present study were deposited in GenBank (*A. cantonensis*, OR048336-OR048344; *C. briggsae,* OR714544; *C. tentaculata*, OR713896 and OR713897).

2.6. Statistical analysis

The data were analyzed using the Statistical Package for Social Sciences (SPSS) 22.0, with the results being presented in absolute and relative frequencies. The relationship between the number of *A. fulica* specimens collected in each month and the environmental variables recorded in the month (rainfall, relative humidity air, and soil pH and hardness of $CaCO_3$) was evaluated using Pearson's correlation coefficient (Collis and Hussey, 2005; Lopes, 2016).

The normality of the numerical data was determined using the Kolmogorov-Smirnov test (Berger and Zhou, 2014) to determine whether the variables were parametric (homogeneous distribution) or non-parametric (heterogeneous distribution). As neither the categorical variable (*A. fulica* being positive or negative for some nematode) nor the continuous variable (shell length) satisfied the criteria for a parametric analysis, the Kruskal-Wallis test (McKight and Najab, 2010) was applied.

Pearson's Chi-Square was applied to the analysis of the categorical data, such as the occurrence of a given nematode species in the *A. fulica* specimens (positive or negative) by season (autumn, winter, spring or summer), the occurrence of nematode species (*Cruzia tentaculata*, *Angiostrongylus cantonensis*, free-living nematodes, Metastrongyloidea) by season, and the occurrence of nematode species in *A. fulica* specimens of different sizes (infants, juveniles, young adult or adult). A standard confidence interval of 95% was used to represent the statistical significance of the data ($p<0.05$).

3. Results

The occurrence of *A. fulica* was confirmed in all three study plots, with a total of 280 specimens being collected. The largest number of specimens (136, 48.5% of the total) was collected in plot II, followed by plots I (n = 106; 38%) and III (n = 38; 13.5%). The largest numbers of *A. fulica* specimens were collected in the summer (n = 120; 42.8% of the total) and autumn (n = 81; 28.9%), while the smallest numbers were registered in the winter (n = 28; 10%) and spring (n = 51; 18.2%).

3.1. Abundance of the snails and environmental variables

The number of *A. fulica* specimens collected during the study period did not correlate significantly ($p > 0.05$) with rainfall (Figure 3A), the mean temperature (Figure 3B) or relative humidity air (Figure 3C) recorded during the different seasons. Similarly, no significant relationship was found between these variables and the percentage of specimens infected with nematodes (Table 1). However, mean rainfall was higher in the summer (6.62 mm; Figure 3A), when most *A. fulica* specimens were collected (n = 120), and the second highest infection rate was found (30.7%; n = 59). The mean temperature was also highest in the summer (25.7 °C; Figure 3B), although relative humidity peaked in the autumn (80.6%; Figure 3C), when the second highest number of snails was collected (n = 81) and the highest infection rate was recorded (35.4%; n = 68).

The number of molluscs collected was correlated with the physicochemical parameters of the soil for each season, with no significant being demonstrated between pH values and total hardness of calcium CaCO$_3$ ($p > 0.05$) (Table 1; Appendix 1).

3.2. Nematodes isolated from the snails

The parasitological analysis revealed that 192 (68.5%) of the *A. fulica* specimens were infected by at least one larval nematode morphotype. *Angiostrongylus cantonensis* (Figure 4A) was recovered from 11 (5.7%) specimens. Free-living nematodes (which included rhabditiforms) were the most common parasites (Figure 4D), being found in 137 (71.3%) of the infected *A. fulica* specimens, although only one species was identified – *Caenorhabditis briggsae* (Dougherty & Nigon, 1949). Other nematode identified was *Cruzia tentaculata* (Rudolphi, 1819) (Figure 4B and 4C), which was found in 122 (63.5%) of the infected specimens.

Just over a third (n = 68; 35.4%) of the infected *A. fulica* specimens were collected in the autumn, with slightly fewer (n = 59; 30.7%) being captured in the summer, 45 (23.4%) in the spring, and only 20 (10.4%) in the winter. In the autumn, most of the infected molluscs (n = 58; 85.2%) had been parasitized by free-living nematodes. In the winter, 12 (60.0%) of the infected molluscs had been parasitized by *C. tentaculata*, while in the spring, 36 (80.0%) had been infected by this nematode. In the summer, once again, most of the infected *A. fulica* specimens (n = 41; 69.5%) were infected by free-living nematodes (Table 2).

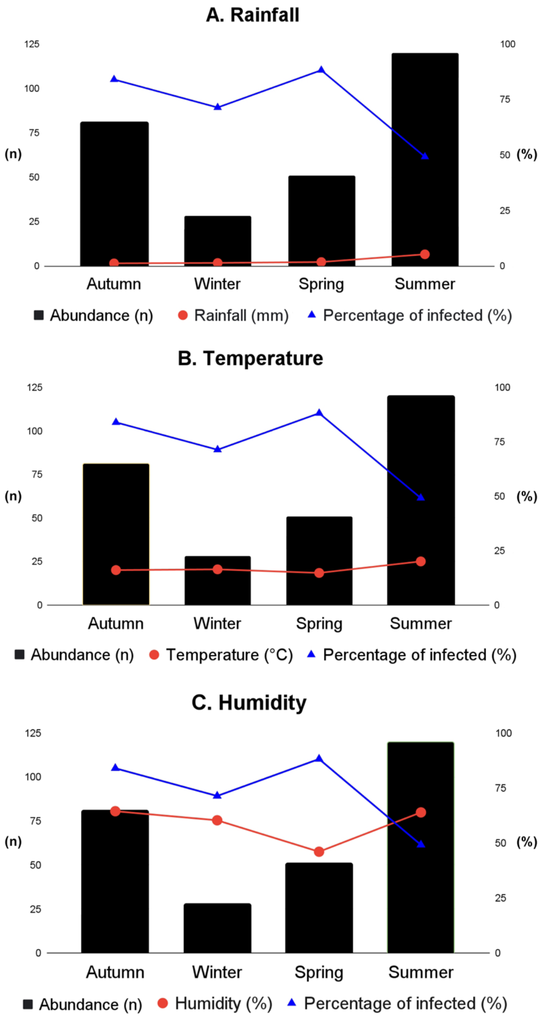

Figure 3. The number of *Achatina fulica* specimens and the percentage of infected individuals recorded in each season, in comparison with (A) rainfall (mm), (B) temperature (°C), and (C) air relative humidity (%).

Table 1. Results of the correlation of the number of *Achatina fulica* with the variables soil pH and Cálcio, rainfall (mm), temperature (°C) and Humidity (%), in Rio de Janeiro 2023. r = Correlation strength, p = Pearson correlation.

CORRELATION		**Variables**					
		***Achatina fulica* Number**	**pH (Soil)**	**Cálcio (Soil)**	**Temperature (°C)**	**Humidity (%)**	**Rainfall (mm)**
***Achatina fulica* Number**	**r**	1.000	-0.181	0.938	0.804	0.493	0.837
	p	-	0.819	0.062	0.196	0.507	0.163
Ph (Soil)	**r**	-0.181	1.000	-0.426	0.167	0.454	0.333
	p	0.819	-	0.574	0.833	0.546	0.667
Cálcio (Soil)	**r**	0.938	-0.426	1.000	0.785	0.738	0.711
	p	0.062	0.574	-	0.215	0.262	0.289
Temperature (°C)	**r**	0.804	0.167	0.785	1.000	0.674	0.949
	p	0.196	0.833	0.215	-	0.326	0.051
Humidity (%)	**r**	0.493	-0.454	0.738	0.674	1.000	0.416
	p	0.507	0.546	0.262	0.326	-	0.584
Rainfall (mm)	**r**	0.837	0.333	0.711	0.949	0.416	1.000
	p	0.163	0.667	0.289	0.051	0.584	-

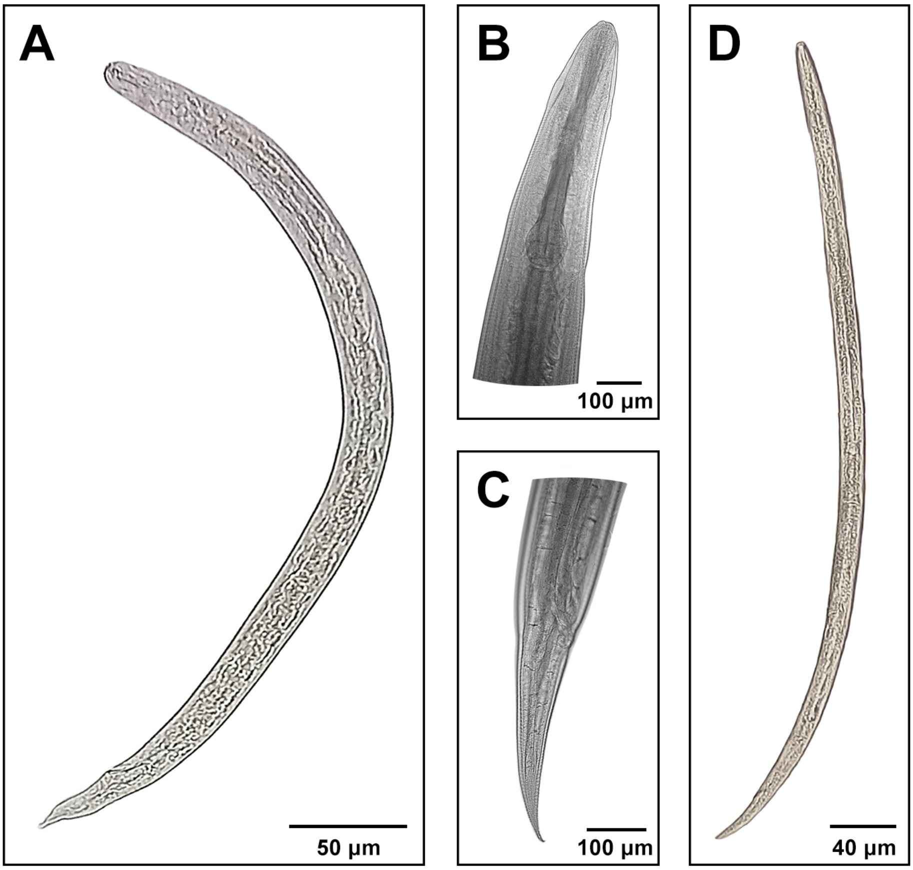

Figure 4. Larvae of (A) *Angiostrongylus cantonensis*; (B, C) *Cruzia tentaculata*; (D) A free-leaving nematode.

Table 2. Nematode species identified infecting the *Achatina fulica* specimens collected in three plots located near the Fiocruz Atlantic Forest Biological Station. The species were identified based on their morphological features and molecular sequences. The plots (I, II, and III) in which the species were recorded are also identified. n= number of infected specimens.

Seasons	Plots	Examined n (%)	Infected n (%)	*Cruzia tentaculata* n (%)	*Angiostrongylus cantonensis* n (%)	*Caenorhabditis briggsae* n (%)	Free living Nematodes n (%)	Double infection n (%)	Triple infection n (%)
Autumn	I	40	35 (87.5)	23	-	-	29	16	1
	II	24	22 (91.6)	12	1	1	19	10	-
	III	17	11 (64.7)	3	1	-	10	3	-
Winter	I	9	5 (55.5)	2	-	-	2	-	-
	II	13	9 (69.2)	5	-	-	5	3	-
	III	6	6 (100)	5	-	-	1	-	-
Spring	I	25	24 (96)	22	2	-	12	12	-
	II	21	19 (90.5)	13	1	-	17	11	1
	III	5	2 (40)	1	1	-	1	2	-
Summer	I	32	25 (68.7)	21	5	-	15	13	1
	II	78	26 (33.3)	7	-	-	22	4	-
	III	10	8 (80)	8	-	-	4	4	-
Total		280	192 (68.5)	122 (63.5)	11 (5.7)	1 (0.5)	137 (71.3)	78 (40.6)	3 (1.5)

A large proportion (40.6%, n = 78) of the infected specimens were co-infected with two different larval forms, and three individuals (1.5% of those infected) had three different types of larvae. Double infection was observed in 29 specimens (42.6% of the infected individuals) in the autumn, three (15%) in the winter, 25 (55.5%) in the spring, and in 21 individuals (35.6%) in the summer. Triple infection was observed in both the autumn (1.4% of those infected) and the spring (2.2%), and in one individuals in the summer (1.7%) (Table 2).

Almost half (46.4%, n = 89) of the infected snails were found in plot I, where most (76.4%, n = 68) of the individuals were parasitized by *C. tentaculata* (Table 2). A slightly smaller (39.6%, n = 76) of the snails collected in plot II were infected, and in most cases (83%, n = 63), the parasites were free-living nematodes. Only 27 (14%) of the infected snails were collected from plot III, and most (63%, n = 17) of these specimens were parasitized by *C. tentaculata*.

3.3. Size of the snails

The shells of the *A. fulica* specimens varied in length from 0.5 cm to 10.5 cm, with a mean length of 4.8±2.4 cm (Table 3). Most of the specimens were either young adults (46.4%, n = 130 specimens) or juveniles (29.6%, n = 83), while less than a fifth were adults (19.6%, n = 55), and only 12 (4.2%) were infants. In plot I, 64 specimens were young adults, representing 60.3% of the specimens collected (shells ranging from 4 to 7 cm in length). In plot II, most individuals were either juveniles (49.3%, n = 67) or young adults (37.5%, n = 51), while in plot III, 14 (36.8%) individuals were classified as young adults and were also the principal category in this plot. Although the adults were not the most frequent category, they were present in all four seasons, i.e., in the autumn (7 specimens, 8.6% of the total in this season), winter (1 specimen, 3.5%), spring (18 specimens, 35.3%) and summer (29 specimens, 24.1%). Infant specimens were only collected in the summer, when they represented 10.0% of the snails retrieved from the plots. The largest snails were collected from plot III in the spring, when mean shell length was 7.6±3.4 cm (Table 3), while the smallest snails (mean shell length = 2.4±2.0 cm) were found in plot II in the summer.

3.4. Shell length vs. infection by nematodes

The Kruskal-Wallis test showed that the mean shell length of individuals who were infected was greater than that of the smaller individuals analyzed (Figure 5). The greater the length of the snail, the greater the probability of the individual being infected by at least one larval nematode morphotype (p = 0.000; Figure 5). The presence of nematodes was significantly higher in young adult snails (p=0.000) (Table 4), which presented a higher frequency of free-living nematodes, present in 84 (64.6%) of the specimens, followed by *C. tentaculata*, whose larvae were recovered in 60 (46.1%) snails, while 24 superfamily Metastrongyloidea, which, in one case, was identified as *Angiostrongylus cantonensis*.

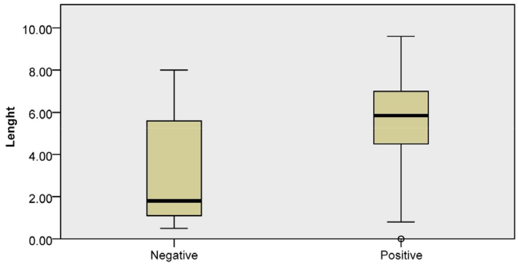

Figure 5. Graphical representation showing the difference between the length of the mollusc and the positivity of infection by nematodes (Kruskal-Wallis test).

Table 3. Mean shell length of the *Achatina fulica* specimens collected from a peri-urban area near the Fiocruz Atlantic Forest Biological Station, in each study plot, by season.

Plots	Autumn 2021	Winter 2021	Spring 2021	Summer 2022
	Length (Mean ± sd)	Length (Mean ± sd)	Length (Mean ± sd)	Length (Mean ± sd)
I	5.9 ± 1.03	5.6 ± 1.4	6.8 ± 1.8	6.8 ± 1.8
II	4.6 ± 1.4	2.8 ± 1.2	5.8 ± 1.2	2.4 ± 2.0
III	4.1 ± 1.9	4.7 ± 1.08	7.6 ± 3.4	7.4 ± 1.8

sd = standard deviation.

Table 4. Number of infected *A. fulica* specimens in relation to size (size category).

Size categories	Number of specimens infected (n)	(%)
Infant (< 1.0 cm)	01	0.5
Juvenile (1.01-4.0 cm)	38	19.8
Young adult (4.01-7.0 cm)	105	54.7
Adult (> 7.1 cm)	48	25.0

On the other hand, 45 (54.2%) of the juvenile snails were negative for nematodes, while 25 (30.1%) were infected by free-living nematodes, 18 (21.7%) were infected by *C. tentaculata*, and two by metastrongyloids. Overall, 32 (58.1%) of the adult snails were infected by *C. tentaculata* and 30 (54.5%) by free-living forms, whereas six (11%) were negative, four (7.2%) were infected by metastrongyloids, and one (1.8%) was contained *A. cantonensis*. Infant snails were only encountered during the summer, and only one (8.4%) of the specimens collected was positive, in this case, for free-living nematodes.

4. Discussion

The highest abundance of snails was recorded in the summer (n = 120 specimens collected) and autumn (n = 81 specimens). This variation may be related to climatic variables, relative humidity in these seasons, i.e., 79.9% in the summer and 80.6%, in the autumn. Like other terrestrial molluscs, *A. fulica* prefers humid environments, and tends to remain hidden during the day, sheltering under trunks, leaves, and other substrates (Raut and Barker, 2002; Costa, 2011; Silva et al., 2020). Albuquerque et al. (2009) indicated the humidity variable as significant in relation to the length and weight of *A. fulica* specimens, in Lauro de Freitas, Salvador/BA based on simple regressions. The presence of these substrates in the study plots likely contributed to the abundance of *A. fulica* recorded here. The activity of terrestrial molluscs is known to be influenced by ambient temperatures, relative humidity, and the humidity of the substrate (Raut and Barker, 2002; Silva et al., 2020). Fischer and Colley (2005) noted that the largest numbers of *A. fulica* specimens tend to be found in the spring and autumn, coinciding with the breeding seasons of this species. This may be one of the reasons why, in the present study, a larger number of young adult snails (shell length of 4.01-7.0 cm) were collected in these seasons. Silva et al. (2022b) concluded that young adults may be more active and tend to move more frequently within a given area, in search of reproductive partners. In the United States, Roda et al. (2016) examined a total of 23,890 snails, with shell lengths of 25-131 mm, which were measured and dissected, and when eggs were observed, they were counted. As eggs were found in snails with shells of 48-128 mm, it seems likely that the smaller individuals – classified as young adults here – are sexually mature.

The lowest abundance of individuals of *A. fulica* was recorded in the winter, when the study plots had dry soil, while daytime temperatures were high and relative humidity was low. *A. fulica* may guarantee its survival in unfavorable environmental conditions by using strategies such as estivation in dry periods, which reduces activity and saves energy for other functions, in particular, the reproduction (Raut and Barker, 2002; Almeida, 2013). Bhattacharyya et al. (2014) in their bibliographic review report that the temperature range between 15 and 25 °C is ideal for land molluscs reproduction, in addition relative air humidity of over 80% during the dark, as ideal conditions for the development and reproduction of land molluscs.

We observed some common features in the study plots such as proximity of residential buildings, and the presence of garbage, debris, and alien plants. This is consistent with the findings of Silva et al. (2020), who observed that *A. fulica* occurs primarily in the plots in which garbage is present, given that the snails often use solid residues as shelters. Fischer and Colley (2005) and Albuquerque et al. (2008) found that the accumulation of garbage and rubble in many anthropogenic habitats may facilitate the reproductive and defensive strategies of *A. fulica*, guaranteeing the persistence of individuals and, in particular, the success of the invasions of new areas. Silva et al. (2020) found that plots with garbage were five times more likely to have resident *A. fulica*. In the present study, in plot II, which had the highest abundance of *A. fulica* specimens (136 specimens, 48.5% of the total), the snails were invariably found under rubble, garbage or other substrates that provided shelter.

By contrast, the lowest abundance of snails was recorded in plot III, where a total of 38 (13.6%) specimens were collected. This plot was located in an area with relatively reduced amounts of debris, where the residents not only disposed of their garbage and other residues correctly, but also removed snails from the areas surrounding their properties. These differences may have determined the lower abundance of *A. fulica* specimens collected from this plot. Human activities may play a major role in the regulation in populations of this species, influencing the number of individuals found in a given area (Silva et al., 2020). The regular maintenance and cleaning of areas surrounding homes by residents can impede the accumulation of refuse and debris which, in turn, can help to reduce the risk of invasion by *A. fulica*, or at least, minimize its population density (Fischer and Gang, 2020).

Banana (*Musa* sp.) trees were present in plots I and III, this alien plant can provide food, shade, moisture, and shelters for *A. fulica*. This snail is known to feed on approximately 500 plant species (Teles et al., 2004), and is considered to be a pest due to its habit of attacking ornamental plants, gardens, vegetable plots, and even small-scale farms (Thiengo et al., 2007). In this case, some of the snails found on the banana trees in plots I and III were likely foraging for food. However, the snails found in the vicinity of the banana trees were either young adults (4.01-7.0 cm) or adults (>7.0 cm). In Sergipe, Silva et al. (2022b) found adult and young adult *A. fulica* in areas with highly variable soil pH (4.5-8.2), and that these individuals had allometric growth (shell > body weight) even in areas with acidic soils. These authors also reported that young adult and adult snails can use substrates other than the ground, such as fallen tree trunks or walls, as a resting place or as a defensive strategy against adverse conditions present in the soil, allowing the species to proliferate in the environment.

Most of the snails collected in the present study were either young (n = 130; 46.4%) or young adults (n = 83; 29.6%). This age structure is similar to that recorded by Silva et al. (2022a) in Sergipe. In the present study, young adult snails predominated in the autumn (n = 59; 72.8%) and spring samples (n = 33; 64.7%), whereas young individuals were most common in the winter (n = 17;

60.7%) and summer (n = 51; 42.5%). Shell length did not vary noticeably among the plots, with most individuals being classified as young adults in all cases. Civeyrel and Simberloff (1996) described three phases in the establishment of *A. fulica* in new areas: (i) the initial, exponential phase, characterized by the presence of large and robust molluscs, (ii) the second, establishment phase, during which the population expands, and (iii) the final, decline phase, which is dominated by small molluscs with fragile shells. The findings of the present study are consistent with those of Silva et al. (2022a), who classified the population in the second phase, with young individuals and young adults, which is the establishment phase.

Although environmental variables did not have significant influence on the abundance of *A. fulica*, relative humidity fluctuated considerably between survey days and was highest in the autumn and winter. Humidity was relatively high in the autumn, with a mean of 80.60%, then declining through the winter (75.45%) and spring (57.6%), before increasing again in the summer (79.94%). Mean rainfall was highest in the summer (6.62 mm), which is the rainy season in the study region, and the heavy rains typical of this period provide a favorable environment for *A. fulica*, when the snails become more active, sheltering on surfaces where moisture accumulates, including walls and roofs, trees, leaf litter and the soil, and debris and garbage (Teles and Fontes, 2002; Zanol et al., 2010; Boaventura et al., 2011). Terrestrial gastropods tend to be more active during rainy periods, when the air and soil are relatively humid, creating conditions that facilitate interactions among individuals (Perez et al., 2008).

The parasitological analysis revealed the presence of nematodes in 192 *A. fulica* specimens. A high infection rate that may reflect the susceptibility of this snail to different types of nematodes (Mead, 1961; Ramos-de-Souza et al., 2018; Silva et al., 2022a; Thiengo et al., 2022). The largest proportion of infected individuals was recorded in the autumn, which may be related to the high humidity recorded during this period. Takeda and Ozaki (1986) reported that *A. fulica* may become more active when relative humidity is over 50%. Larger snails also tend to be more active overall, which favors exposure and contact time with plants, animals, and feces that may be infected with nematodes (Alicata, 1965; Almeida, 2014).

Free-living nematodes were the most frequently in the *A. fulica* specimens examined in the present study. These nematodes are typically found in humid environments with decomposing organic matter (Campos et al., 2002), and *Caenorhabditis briggsae*, in particular, is a nematode found in the soil (Jovelin et al., 2003). A lack of adequate sanitation may dampen the soil and favor the establishment of vegetation, creating an ideal environment for both *A. fulica* and for free-living nematodes, and, in turn, increasing the potential for infection.

Even though *A. fulica* is considered the main intermediate host species of *Angiostrongylus cantonensis* in Brazil (Carvalho et al., 2012; Ramos-de-Souza et al., 2018; Silva et al., 2022a; Thiengo et al., 2022), this nematode was recorded less frequently in the present study, in relation to others carried out in Brazil where the percentage of nematode infection was higher (Oliveira et al., 2015; Cardoso et al., 2020). Perhaps, this lower frequency is related to the fact that it is close to the transition area of the Atlantic Forest, considering that the other studies mentioned had samples in completely urbanized areas.

On the other hand, *C. tentaculata*, parasite of large intestine of marsupials of the genus *Didelphis* (opossum) (Anderson et al., 2009), was the second most common nematode recorded in this study. The presence of this nematode has previously been reported for this area, being the most abundant and prevalent species parasitizing the black-eared opossum *Didelphis aurita* (Boullosa et al., 2021), being an area with a favorable environment for this didelphid. In addition, Ramos-de-Souza et al. (2021) recovered *C. tentaculata* larvae from a native snail, acting as intermediate host, *Thaumastus taunaisii* (Férussac, 1821), found in the Pedra Branca State Park. *C. tentaculata* larvae have been found frequently in *A. fulica* (Ramos-de-Souza et al., 2018, 2021; Silva et al., 2020; Rodrigues, 2020).

Although it was not the most prevalent, snails infected with *A. cantonensis* were collected in all plots and in the following seasons: autumn, spring and summer. Cowie et al. (2022) note that neuroangiostrongyliasis is an emerging but neglected disease and that the range of *A. cantonensis* continues to expand, with recent cases being reported from areas where it did not previously occur, such as Europe. The authors also alert that, unfortunately, few medical professionals are familiar with *A. cantonensis* and the zoonosis it causes.

Overall, then, the results of the present study confirm behavior patterns described previously in *A. fulica*, such as its preference for anthropogenic environments in which garbage and rubble have accumulated. The availability of these environments in the study area facilitates its occupation by *A. fulica*, as well as favoring the occurrence of potentially harmful nematodes such as *A. cantonensis*, which increases the risk of human infection. The confirmation of the occurrence of *A. cantonensis* in this peri-urban area highlights the need for the implementation of controls and preventive measures to restrict the expansion of *A. fulica* in the area. These measures would not only promote the health of the local human population, but also contribute to the conservation of the native snails found in the Fiocruz Atlantic Forest Biological Station, which is adjacent to the study area.

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Supplementary Material

Supplementary material accompanies this paper.

Appendix 1. Chemical properties (hardness and pH) of the soil samples collected from the three study plots (PI, PII, and PIII) investigated in the CFMA in Jacarepaguá, Rio de Janeiro, Brazil.

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