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Article

Helminth's assemblage of *Trachemys dorbigni* (Testudines: Emydidae) in southern Brazil: implications of anthropogenic environments and host's genders

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ABSTRACT. The assemblage of helminths of *Trachemys dorbigni* was analyzed according two environments (rural and urban) and according to host's gender. Thus, the helminths found were: *Spiroxys contortus* (Rudolphi, 1819), *Falcaustra affinis* (Leidy, 1856), *Camallanus emydidius* Mascarenhas & Müller, 2017, *Dioctophyme renale* (Goeze, 1782) (larvae), *Eustrongylides* sp. (larvae) (Nematoda), *Telorchis corti* (Stunkard, 1915), *Telorchis achavali* Mañé-Garzón & Holcman-Spector, 1973, *Telorchis* spp. (Digenea), *Polystomoides rohdei* Mañé-Garzón & Holcman-Spector,1968 and *Neopolystoma* sp. (Monogenoidea). Parasitological indices suggests that *S. contortus*, *F. affinis*, *C. emydidius*, *T. corti* and *P. rohdei* are species common in helminth assemblage of *T. dorbigni* in southern Brazil. Infection by *Dioctophyme renale* is typical of the urban area and suggest relation with eutrophication process and feedback of parasitic cycle in the freshwater urban environment. Parasitological indices of *Neopolystoma* sp. and *T. achavali* suggest to be occasional infections; whereas infection by *Eustrongylides* sp. could be considered accidental. About Telorchiidae, there was no co-occurrence of the species of this group for the examined hosts. In relation to host's gender, it was observed that infections by *S. contortus*, *F. affinis*, *C. emydidius* and *T. corti* had higher rates in females, showing a more intimate association with the females of *T. dorbigni*. The results suggest that human impact on the environment could affect the infrapopulacional dynamics of parasite species influencing the presence and rates of infection of helminths or exacerbating heterogeneities related to host's gender.

KEYWORDS. Freshwater turtles, D'Orbigny's slider turtle, parasites, rural area, urban area.

RESUMO. Assembleia de helmintos de *Trachemys dorbigni* (Testudines: Emydidae) no sul do Brasil: implicações dos ambientes antrópicos e do gênero sexual dos hospedeiros. A assembleia de helmintos de *Trachemys dorbigni* foi analizada de acordo com dois ambientes (rural e urbano) e de acordo com o gênero sexual dos hospedeiros. Os helmintos encontrados foram: *Spiroxys contortus* (Rudolphi, 1819), *Falcaustra affinis* (Leidy, 1856), *Camallanus emydidius* Mascarenhas & Müller, 2017, *Dioctophyme renale* (Goeze, 1782) (larva), *Eustrongylides* sp. (larva) (Nematoda), *Telorchis corti* (Stunkard, 1915), *Telorchis achavali* Mañé-Garzón & Holcman-Spector, 1973, *Telorchis* spp. (Digenea), *Polystomoides rohdei* Mañé-Garzón & Holcman-Spector,1968 and *Neopolystoma* sp. (Monogenoidea). Os indices parasitológicos sugerem que *S. contortus*, *F. affinis*, *C. emydidius*, *T. corti* e *P. rohdei* são espécies comuns da assembleia de helmintos de *T. dorbigni* no extremo sul do Brasil. A infecção por *Dioctophyme renale* é tipica da area urbana e sugere estar relacionada com os processos de eutrofização e a retroalimentação do ciclo parasitário no ambiente aquático urbano. Os indices parasitológicos de *Neopolystoma* sp. e *T. achavali* sugerem que a infecção por estes seja occasional; a infecção por *Eustrongylides* sp. pode ser considerada acidental. Não houve co-ocorrência entre as espécies de Telorchiidae. Em relação ao gênero sexual, observou-se que as infecções por *S. contortus*, *F. affinis*, *C. emydidius* e *T. corti* apresentaram taxas mais elevadas nas fêmeas, mostrando uma associação mais íntima com as fêmeas de *T. dorbigni*. Os resultados sugerem que o impacto humano sobre o ambiente pode afetar a dinâmica das infrapopulações dos helmintos parasitos, influenciando a presença e os índices de infecção de helmintos ou exacerbando heterogeneidades relacionadas ao gênero sexual dos hospedeiros.

PALAVRAS-CHAVE. Tartarugas dulceaquícolas, tigre d'água, parasitos, área rural, área urbana.

Parasitism involves a homoplastic and historical condition in nearly every animal group (Bush *et al.*, 2001; Dobson *et al.*, 2008; Pizzi, 2009). This condition is ruled by selective processes of host-parasite coevolution (Combes, 1997; Dybdahl & Storfer, 2003). Several factors - that is, heterogeneities - of both host and parasite are important for parasite life cycles to be properly developed (Von Zuben, 1997; Wilson *et al.*, 2002). For the host, these heterogeneities involve gender, sexual dimorphism, reproductive condition, habitat use during the reproductive process, age, diet, and geographical distribution (Von Zuben, 1997; Wilson *et al.*, 2002; Klein, 2004). For parasites, these heterogeneities include size, quantity, fertility rate, reproduction mechanisms, and intra- or inter-host dispersion ability (Crofton, 1971; Von Zuben, 1997; Wilson *et al.*, 2002; Khokhlova *et al.*, 2010).

Concerning to *Trachemys dorbigni* (Duméril & Bibron, 1835) (Emydidae) helminths, there have been morphological studies including new species descriptions and host records, thus expanding knowledge on the geographical distribution of known species (Mañé-Garzón & Holcman-Spector, 1968a,b,1973; Mascarenhas & Müller, 2013, 2015a,b, 2017; Bernardon *et al.*, 2014).

Trachemys dorbigni occurs in Brazil, Uruguay and Argentina (Rhodin *et al.*, 2017). In Brazil, it is one of the most abundant freshwater turtles in Rio Grande do Sul State (Bujes *et al.*, 2011); in addition, it can usually be found in heavily anthropogenic urban environments, such as sewage systems, as well as in rural environments, as rice crop water drainage channels with pesticide residues (Bujes & Verrastro, 2008; Fagundes *et al.*, 2010).

Thus, the lack of knowledge on *T. dorbigni* helminths biodiversity in anthropic environments have justified the development of this study, which analyzes helminth's assemblage of *T. dorbigni* in southern Brazil according to urban and rural environments and host's gender.

MATERIALS AND METHODS

Sixty *T. dorbigni* were collected between July 2010 and December 2012 in two distinct areas, an urban and a rural, in the state of Rio Grande do Sul, Brazil. The sampling period encompassed spring and summer months in the southern hemisphere. Only two hosts were sampled in July 2010.

Twenty-eight freshwater turtles (13 males: 15 females) were collected in four ponds in the rural area from at the *Centro Agropecuário da Palma* from the *Universidade Federal de Pelotas* (UFPel) located in Capão do Leão county (31°48'01.1"S - 52°30'48.6"W) (Fig. 1A). Another 32 hosts (14 males: 18 females) were collected in channels in the urban area of the city of Pelotas (31°46'16.9"S - 52°18'45.9"W) (Fig. 1B). Average weigh and average linear carapace length of females and males was 1494.7 grams; 207.6 mm, and 825 grams; 175.7 mm, respectively. The sexing was done during necropsy with the examination of the reproductive system.

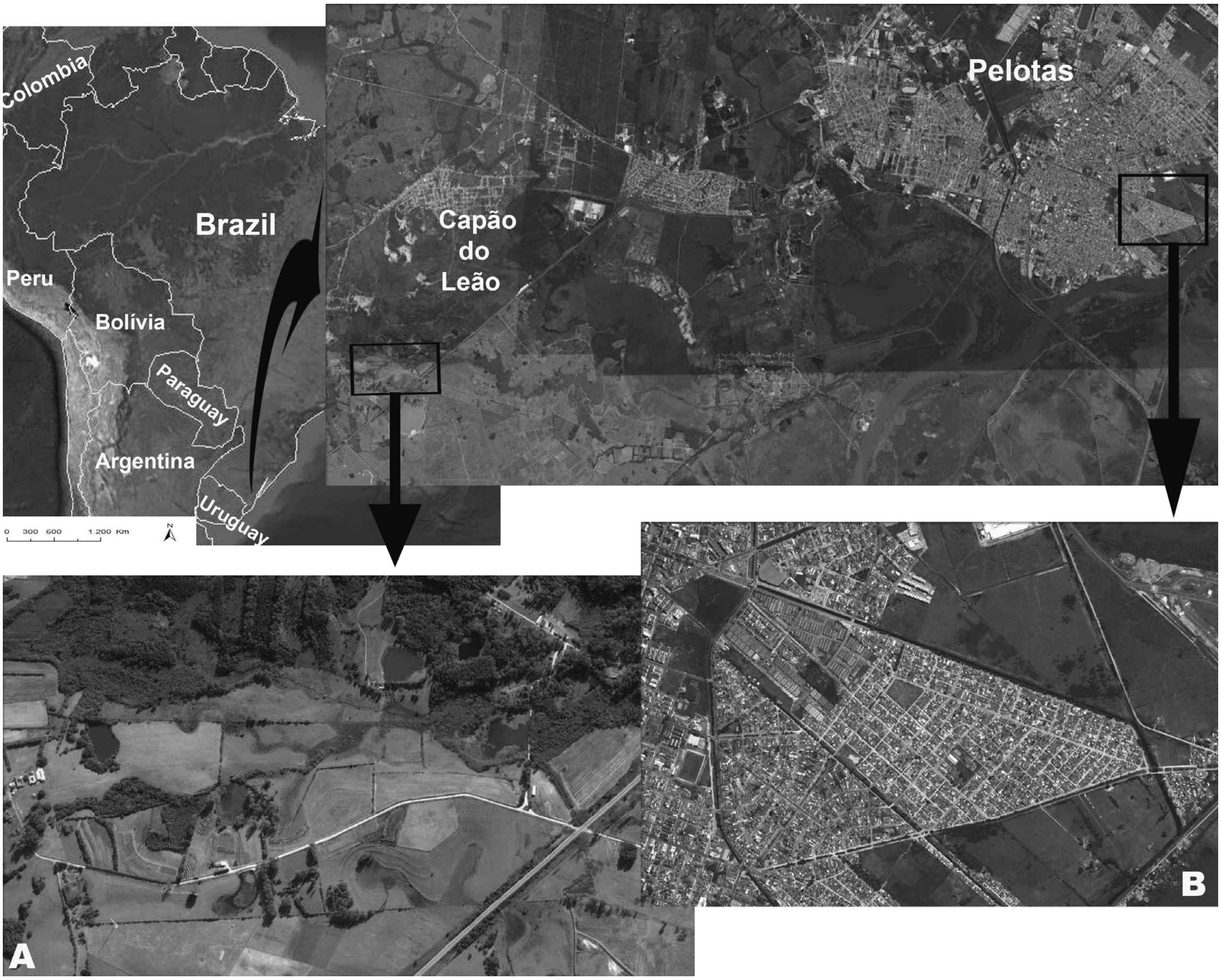

Fig. 1. Overall layout of the collection environments in the study of helminth assemblage of *Trachemys dorbigni* in southern Brazil: A, detail of the *Centro Agropecuário da Palma* (UFPel), rural area of Capão do Leão, State of Rio Grande do Sul, Brazil; B, detail of the urban área of Pelotas, Rio Grande do Sul, Brazil. Source: extracted and modified the site Google® Earth (©2014 Google – Images ©2014 Digital Globe).

The freshwater turtles were anesthetized with a combination of 10% ketamine and 2% xylazine; after analgesia, a 2% lidocaine hydrochloride intrathecal injection was administered, as recommended by Resolution 1000/2012 of the Conselho Federal de Medicina Veterinária (2012). This study was licensed by the *Instituto Chico Mendes para Conservação da Biodiversidade* (23196-ICMBio) and was approved by the Ethics and Animal Experimentation Committee of the *Universidade Federal de Pelotas* (3026 - CEEA/UFPel).

The nasal and oral cavity, eyes, carapace and plastron, nails, skin folds, skin-carapace and plastron-skin insertions were examined. At necropsy, the plastron was removed by osteotomy of its lateral processes and circumferential integument section for general observation and splanchnological screening. The viscera were isolated anatomically according to system, *i.e.* respiratory, digestive, reproductive and urinary systems. After that, the splanchnological systems were lacquered and divided into their anatomical and functional parts: esophagus and stomach together and separately, as well as the small and large intestines. The same procedure was applied to the other splanchnological systems, according to their anatomical and functional divisions. Host voucher specimens were deposited in the *Coleção Herpetológica do Museu de Ciências Naturais Carlos Ritter* (UFPel), Rio Grande do Sul, Brazil (numbers 78-79).

Helminths were fixed in AFA (70°GL ethanol, 37% formaldehyde, glacial acetic acid). Nematoda taxa were preserved in 70°GL glycerin ethanol and then mounted on semi-permanent slides with Amann's lactophenol. Digenea and Monogenoidea were preserved in 70°GL ethanol, stained with Langeron's carmine or Delafield hematoxylin, and assembled in Canada balsam (Amato *et al.*, 1991). Helminths were identified based on morphological and morphometric characteristics according to Hedrick (1935), Mace & Anderson (1975), Measures & Anderson (1985), Baker (1986), Measures (1988), Moravec & Vargas-Vázquez (1998) and Mascarenhas & Müller (2017) to Nematoda; Mañé-Garzón & Holcman-Spector (1973) and MacDonald & Brooks (1989) to Digenea; Mañé-Garzón & Holcman-Spector (1968a) and Pichelin (1995) to Monogenoidea. Vouchers were deposited in *Coleção Helmintológica do Instituto Oswaldo Cruz* (CHIOC), Rio de Janeiro, Brazil; *Coleção de Helmintos do Laboratório de Parasitologia de Animal Silvestres* (CHLAPASIL-UFPel), Pelotas, Rio Grande do Sul State, Brazil, and *Coleção de Invertebrados do Museu de La Plata* (MLP), La Plata, Argentina (Tab. I).

The *T. dorbigni* helminth assemblage was analyzed by Prevalence (P%), Mean Intensity of Infection (MII), Mean Abundance (MA) (Bush *et al.*, 1997). Parasitological indices were calculated for all sampled hosts as well as for

Tab. I. Helminths of *Trachemys dorbigni* (Duméril & Bibron, 1835) (Emydidae) (n=60) on the southern Brazil and their site of infection, parasitological indices (P%, Prevalence; MII, Mean Intensity of Infection; MA, Mean Abundance) and Range (RA) (CHIOC, Coleção Helmintológica do Instituto Oswaldo Cruz; MLP, Coleção de Invertebrados do Museu de La Plata; CHLAPASIL, Coleção de Helmintos do Laboratório de Parasitologia de Animais Silvestres da Universidade Federal de Pelotas).

Helminths	Site of infection	P (%)	MII	MA	RA	Vouchers
NEMATODA						
Spiroxys contortus	Heart; esophagus; stomach, small intestine; large intestine	68.33	70.17	47.95	1 - 1184	35875-35876 (CHIOC) 6740-6744 (MLP) 472-476 (CHLAPASIL)
Falcaustra affinis	Heart; stomach; large intestine; kidney	85	92.39	78.53	1 - 472	35873-35874 (CHIOC) 6745-6747 (MLP) 477-480 (CHLAPASIL)
Camallanus emydidius	Lung; stomach; small intestine; large intestine	100	30.31	30.31	1 - 242	38507-38512 (CHIOC) 523-527 (CHLAPASIL)
Dioctophyme renale (larva)	Coelomic cavity; muscles; stomach (serous); mesentery; surface of various organs: heart, lung, liver, pancreas, spleen and intestines	50	13.03	6.51	1 - 129	519-521 (CHLAPASIL)
Eustrongylides sp. (larva)	Stomach (serous)	1.66	1	0.016	1	522 (CHLAPASIL)
DIGENEA						
Telorchis corti	Small intestine	36.66	25.36	9.3	1 - 130	37846-37850 (CHIOC) 6748-6749 (MLP) 481-504 (CHLAPASIL)
Telorchis achavali	Small intestine	1.66	3	0.05	3	505-507 (CHLAPASIL)
Telorchis spp.	Small intestine	10	2.16	0.21	1 - 6	516-517 (CHLAPASIL)
MONOGENOIDEA						
Polystomoides rohdei	Oral cavity	81.66	16.81	13.73	1 - 132	530-544 (CHLAPASIL)
Neopolystoma sp.	Urinary bladder	1.66	2	0.03	2	528-529 (CHLAPASIL)

host gender (Male: M; Female: F) and environment (Rural: R; Urban: U). Thus, P% and MII were compared in four different ways: 1) between hosts from rural (RM + RF) and urban (UM + UF) environment; 2) between host's genders (RM + UM) x (RF + UF); 3) between males and females from the same environment (RMxRF; UMxUF); and, 4) between individuals of the same gender from different environments (RMxUM; RFxUF). Prevalence indices were compared by using exact Fisher's test *"F"* ($p \leq 0.05$) and MII indices were compared by using Bootstrap's confidence interval (BC_{α}, $p \leq 0.05$) in Quantitative Parasitology (QP 3.0) software (Reiczigel *et al.*, 2019).

RESULTS

Considering all sampled hosts: (RM+RF) + (UM+UF). All examined hosts (n = 60) were parasitized by helminths (Nematoda, Digenea, and Monogenoidea). The Nematoda found included *Spiroxys contortus* (Rudolphi, 1819) (Gnathostomatidae), *Falcaustra affinis* (Leidy, 1856) (Kathlaniidae), *Camallanus emydidius* Mascarenhas & Müller, 2017 (Camallanidae), *Dioctophyme renale* (Goeze, 1782), *Eustrongylides* sp. (Dioctophymatidae); Digenea consisted of *Telorchis corti* (Stunkard, 1915), *Telorchis achavali* Mañé-Garzón & Holcman-Spector, 1973 and *Telorchis* spp. (Telorchiidae), while Monogenoidea included *Polystomoides rohdei* Mañé-Garzón & Holcman-Spector,1968 and *Neopolystoma* sp. (Polystomatidae).

Dioctophyme renale and *Eustrongylides* sp. occurred only as third-stage larvae (one larva per cyst) (Tab. I). *Telorchis* spp. were represented by immature specimens. There were no co-infections between *T. achavali*, *T. corti* and immature *Telorchis* spp., that is, Telorchiidae species occurred in different hosts. *Eustrongylides* sp., *T. achavali* and *Neopolystoma* sp. infections showed low parasitic indices, suggesting that these infections are occasional or incidental for this host. Helminths found and their respective infection sites, prevalence, mean intensity of infection, and mean abundance are shown in Tab. I.

Comparison of the helminth assemblage of *T. dorbigni* in relation to environment: (RM + RF) x (UM + UF). Table II shows the parasitological indices of helminths by comparing rural and urban environments. The results showed a species-specific fluctuation of these indices between the two studied environments. Among the Nematoda species found, only two showed statistical differences for prevalence. *Spiroxys contortus* presented a higher prevalence (85.7%) in the countryside, differing from urban area (53.1%), whereas *D. renale* showed higher prevalence (87.5%) in the urban environment as opposed to rural area (7.1%). As to mean intensity of infection, only *C. emydidius* showed differences between rural and urban areas, with 16.46 and 42.44 helminths/host, respectively. Among the Nematoda species, *C. emydidius* was the most prevalent (100%) in both environments.

Considering Digenea, *T. corti* was the only species that occurred in both rural and urban areas. This species only showed prevalence differences between the environments studied, which were higher in urban area hosts (65.6%) in contrast to rural area, which were 3.6%. Monogenoidea species, *P. rohdei*, showed an occurrence pattern similar to that of *T. corti*, occurring in both environments. However, this species was more prevalent in rural area (100%) than for urban area (65.6%). Mean abundance and range basically reinforce the differences observed for *S. contortus*, *C. emydidius*, *D. renale*, *T. corti* and *P. rohdei*. Prevalence differences occurred in species of parasites with greater mean abundance and range fluctuations between environments. Mean abundance and range of *S. contortus* showed that the parasitic loads were higher in the rural environment. *Dioctophyme renale* and *C. emydidius* showed mean abundance and range with expressive values in the freshwater turtles from urban environment. Among species occurring in both urban and rural areas, only *F. affinis* showed no prevalence or mean intensity of infection differences between the two areas. *Telorchis achavali* and *Telorchis* spp. occurred only in rural hosts. On the other hand, *Eustrongylides* sp. and *Neopolystoma* sp. were present only in urban hosts (Tab. II). Low prevalence and mean intensity of infection values for *Eustrongylides* sp., *T. achavali* and *Neopolystoma* sp. for each environment as well as for the whole sample reinforced the hypothesis that these infections in *T. dorbigni* may be accidental or occasional.

Comparing helminth assemblage of *T. dorbigni* between host's gender independently of sampled environments: (RM + UM) x (RF + UF). On comparing helminth assemblage of male and female, it was observed that infection by *F. affinis*, *S. contortus*, and *T. corti* was more prevalent in female hosts, with values of 93.9%, 81.8%, and 48.5%, respectively. Likewise, *C. emydidius* mean intensity of infection was significantly higher in female hosts (42.12 helminths/host). Another observation made was that only *T. dorbigni* females showed infection by *Eutrongylides* sp., *T. achavali* and *Neopolystoma* sp., with each species occurring isolatedly in a different female host, with extremely low parasitological indices (Tab. III). The other parasites occurred in both genders of the *T. dorbigni*.

Comparing helminth assemblage of *T. dorbigni* between host's genders from the same environment: RMxRF; UMxUF. With reference to prevalence of helminths of male and female hosts from the same environment, it was observed that infection by *S. contortus* and *F. affinis* was more prevalent in females (100% and 100%, respectively) than in males in rural area. This pattern was also observed to *T. corti* in urban area, where this species showed higher prevalence (83.3%) in females (Tab. IV). As to mean intensity of infection, there were differences between host's genders for infection of *C. emydidius* in urban area, where female hosts showed significantly higher values for this Nematoda than males. These analyses reinforce the comparisons between helminth assemblage in the male and female hosts (RM + UM) x (RF + UF) listed above. For these helminths, the analyses seem to suggest the occurrence of a dominant-female host pattern.

Tab. II. Prevalence (P%), Mean Intensity of Infection (MII) and Mean Abundance (MA) and Range (RA) of helminths of *Trachemys dorbigni* (Duméril & Bibron, 1835) (Emydidae) (n=60) from rural and urban environments on the southern Brazil [n, number of sampled hosts; a, b, differences between the environments (exact Fisher's test "F", p≤0.05, for P% and Bootstrap's confidence interval, p≤0.05, for MII)].

Helminths	Environments							
	Rural (n=28)				Urban (n=32)			
	P%	MII	MA	RA	P%	MII	MA	RA
NEMATODA								
Spiroxys contortus	85.7^{a}	112.58	96.5	6 - 1184	53.1^{b}	10.29	5.46	1 - 41
Falcaustra affinis	85.7	85.42	73.21	1 - 305	84.4	98.59	83.18	2 - 472
Camallanus emydidius	100	16.46^{a}	16.46	1 - 54	100	42.44^{b}	42.44	1 - 242
Dioctophyme renale (larva)	7.1^{a}	1.5	0,1	1 - 2	87.5^{b}	13.86	12.12	1 - 129
Eustrongylides sp. (larva)	-	-	0	-	3.1	1	0.03	1
DIGENEA								
Telorchis corti	3.6^{a}	130	4.64	130	65.6^{b}	20.38	13.37	1 - 120
T. achavali	3.6	3	0.1	3	-	-	0	-
Telorchis spp.	21.4	2.16	0.46	1 - 6	-	-	0	-
MONOGENOIDEA								
Polystomoides rohdei	100^{a}	19.93	19.93	1 - 132	65.6^{b}	12.67	8.31	1 - 46
Neopolystoma sp.	-	-	0	-	3.1	2	0.06	2

Tab. III. Prevalence (P%), Mean Intensity of Infection (MII) and Mean Abundance (MA) and Range (RA) of helminths in males and females of *Trachemys dorbigni* (Duméril & Bibron, 1835) (Emydidae) (n=60) on the southern Brazil [n, number of sampled hosts; a, b, differences between the host's gender (exact Fisher's test "F", p≤0.05, for P% and Bootstrap´s confidence interval, p≤0.05, for MII)].

Helminths	Host's gender							
	Male (n=27)				Female (n=33)			
	P%	MII	MA	RA	P%	MII	MA	RA
NEMATODA								
Spiroxys contortus	51.9^{a}	42.64	22.11	1 - 338	81.8^{b}	84.44	69.09	1 - 1184
Falcaustra affinis	74.1^{a}	62.1	46	2 - 472	93.9^{b}	111.94	105.15	1 - 427
Camallanus emydidius	100	15.89^{a}	15.89	1 - 73	100	42.12^{b}	42.12	1 - 242
Dioctophyme renale (larva)	48.1	14	6.74	2 - 57	51.5	12.29	6.33	1 - 129
Eustrongylides sp. (larva)	-	-	0	0	3.03	1	0.03	1
DIGENEA								
Telorchis corti	22.2^{a}	16.83	3.74	1 - 59	48.5^{b}	28.56	13.85	1 - 130
T. achavali	-	-	0	0	3.03	3	0.09	3
Telorchis spp.	7.4	1	0.07	1	12.1	2.75	0.33	1 - 6
MONOGENOIDEA								
Polystomoides rohdei	74.1	13.15	9.74	1 - 79	87.9	19.34	17	1 - 132
Neopolystoma sp.	-	-	0	0	3.03	2	0.06	2

Comparing helminth assemblage of *T. dorbigni* for the same host's gender between environments: RMxUM; RFxUF. By comparing parasitological indices for parasites between males from rural and urban areas, prevalence differences were found only for *P. rohdei*, which was higher in rural area males (100%). As to female host infection in both rural and urban areas, differences in prevalence for *S. contortus*, *D. renale* and *T. corti* were found; females from rural area showed a higher prevalence for *S. contortus* (100%), whereas females from urban area presented higher prevalence for *D. renale* (83.3%) and *T. corti* (83.3%). Concerning to mean intensity of infection, differences between female hosts from the two environments were only observed for *C. emydidius*, which was higher in females from urban area (61.67 helminths/host). Other parasite species either did not differ or could not be analyzed due to restrictions related to host's gender, as shown in previous analyses. With reference to host's gender, *T. achavali* occurred in only one RF, while *Eustrongylides* sp. and *Neopolystoma* sp. occurred isolatedly in one UF. These helminths showed extremely low parasitological indices (Tab. IV), as previously mentioned.

Tab. IV. Prevalence (P%), Mean Intensity of Infection (MII) and Mean Abundance (MA) and Range (RA) of helminths in males and females of *Trachemys dorbigni* (Duméril & Bibron, 1835) (n=60) (Emydidae) on the southern Brazil according to rural and urban environments [n, number of sample hosts; a, b, differences between the host's gender of the same environments; ■,■■ differences for the same gender between site of study (exact Fisher's test "F", p≤0.05, for P% and Bootstrap's confidence interval, p≤0.05, for MII)].

Helminths	Parasitological indices	Environments			
		Rural		Urban	
		Host's gender		Host's gender	
		Male (n=13)	Female (n=15)	Male (n=14)	Female (n=18)
NEMATODA					
Spiroxys contortus					
	P%	69.2^{a}	100$^{b;\blacksquare}$	35.7	66.7$^{\blacksquare\blacksquare}$
	MII	64.33	141.53	3.6	13.08
	MA	44.54	141.53	1.29	8.72
	RA	6 - 338	8 - 1184	1 - 7	1 - 41
Falcaustra affinis					
	P%	69.2^{a}	100^{b}	78.6	88.9
	MII	50.33	106.47	71.73	117.06
	MA	34.85	106.47	56.36	104.06
	RA	4 - 191	1 - 305	2 - 472	8 - 427
Camallanus emydidius					
	P%	100	100	100	100
	MII	13.92	18.67$^{\blacksquare}$	17.71^{a}	61.67$^{b;\blacksquare\blacksquare}$
	MA	13.92	18.67	17.71	61.67
	RA	3 - 44	1 - 54	1 - 73	1 - 242
Dioctophyme renale (larva)					
	P%	-	13.3$^{\blacksquare}$	92.9	83.3$^{\blacksquare\blacksquare}$
	MII	-	1.5	14	13.73
	MA	0	0.2	13	11.44
	RA	0	1 - 2	2 - 57	1 - 129
Eustrongylides sp. (larva)					
	P%	-	-	-	5.6
	MII	-	-	-	1
	MA	0	0	0	0.06
	RA	0	0	0	1
DIGENEA					
Telorchis corti					
	P%	-	6.7$^{\blacksquare}$	42.9^{a}	83.3$^{b;\blacksquare\blacksquare}$
	MII	-	130	16.83	21.8
	MA	0	8.67	7.21	18.17
	RA	0	130	1 - 59	1 - 120
T. achavali					
	P%	-	6.7	-	-
	MII	-	3	-	-
	MA	0	0.2	0	0
	RA	0	3	0	0
Telorchis spp.					
	P%	15.54	26.7	-	-
	MII	1	2.75	-	-
	MA	0.15	0.73	0	0
	RA	1	1 - 6	0	0

Tab. IV. Cont.

Helminths	Parasitological indices	Environments			
		Rural		Urban	
		Host's gender		Host's gender	
		Male (n=13)	Female (n=15)	Male (n=14)	Female (n=18)
MONOGENOIDEA					
Polystomoides rohdei					
	P%	100▪	100	50▪▪	77.8
	MII	15.77	23.53	8.29	14.86
	MA	15.77	23.53	4.14	11.56
	RA	1 - 79	2 - 132	1 - 25	1 - 46
Neopolystoma sp.					
	P%	-	-	-	5.6
	MII	-	-	-	2
	MA	0	0	0	0.11
	RA	0	0	0	2

DISCUSSION

Anthropic environmental implications on helminth assemblage of *T. dorbigni*. Windsor (1998) and Dobson *et al.* (2008) have argued that most of the world's species are parasites, upon considering the relation between the number of parasite species and a single host species. This supremacy in the number of parasitic species can be explained by the plasticity of certain parasite groups, such as Nematoda, in the face of selection pressure of the environment. Jairajpuri (2005) discussed the plasticity of Nematoda considering the high irradiation of the group, and the consequent diversity, to different environments and life conditions. On considering *T. dorbigni* parasite groups and the two anthropic environments studied, parasitological indices relating to Nematoda (*S. contortus*, *F. affinis* and *C. emydidius*) reveal this (co-evolutionary) plasticity, which is typical of the group in the face of the environmental diversity which they may be exposed. *Falcaustra affinis* specifically showed high parasitological indices in both urban and rural environments, proving to be the most successful Nematoda for the host species in both environments. *Falcaustra affinis* success can be proved by its high infection as compared to other Nematoda found, such as *S. contortus*, *C. emydidius* and *D. renale*. Mean intensity of infection and mean abundance observations for *S. contortus*, *C. emydidius* and *D. renale* suggest that these parasites have not had the same infection success in the host species in both environments.

The same plasticity pattern between the two environments studied can be extended to Digenea and Monogenoidea. Digenea species that showed greatest plasticity was *T. corti*, while for Monogenoidea was *P. rohdei.* Thus, it can be said that parasite loads suggest that these species may be commonly found in the helminth assembly of *T. dorbigni* in the study area, regardless of the sampled environment.

On the other hand, considering parasitological indices of *Eustrongylides* sp., *T. achavali* and *Neopolystoma* sp., other interpretations can be suggested, such as an accidental infection by *T. dorbigni*. Parasitological indices are tools that demonstrate host-parasite fitness (Rósza *et al.*, 2000; Reiczigel *et al.*, 2005). This host-parasite evolutionary fitness constitutes a historical relationship and indices such as prevalence are commonly used to predict and estimate the parasite load or parasitic pressure on a particular host species (Gregory & Blackburn, 1991). In this context, the accidental infection hypothesis can be more easily understood in relation to *Eustrongylides* sp., since species of this genus occur in waterfowl (definitive hosts) that acquire the infection by ingesting fish (second intermediate and/or paratenic host), which in turn become infected by ingesting the third-stage larvae present in freshwater Oligochaeta (intermediate host) (Anderson, 2000). Therefore, the finding of only one third-stage larva of *Eutrongylides* sp. in a single host, in addition to helminth biology, may signal that the infection was accidentally acquired and that *T. dorbigni* is an unlikely host for this Nematoda. Occurrence of *Neopolystoma* sp. and *T. achavali* at low parasite loads may be related to a number of factors, such as environmental characteristics (for both), presence of a suitable intermediate host (*T. achavali*), and reproductive characteristics (for both). Such factors may influence the development of free and parasitic life forms (intermediate hosts) jeopardizing the success of the species outside the definitive host (freshwater turtle). Nevertheless, the lack of information on *Neopolystoma* sp. and *T. achavali* biology compromises conclusions on the low parasitic indices found in *T. dorbigni.*

The composition of the helminth assemblage of *T. dorbigni* in southern Brazil showed a significant relationship between the two sampled anthropogenic environments - urban and rural. The relevance of this relationship includes environmental characteristics (quality and flow of water) of the sites analyzed as an important factor for the parasite Taxa. Both flow and quality of water may have influenced helminth assemblage composition and parasitic loads in the two environments, especially in relation to the intermediate host community for the helminths found in *T. dorbigni*.

With respect to intermediate host, the quality of water of continental freshwater ecosystems is relevant to the expression of the species that live in it from an abiotic point of view (Gamboa *et al.*, 2008). These species are distributed among various trophic levels, including the different hosts a parasite species may need to complete its life cycle. These hosts, intermediate (primary and/or secondary) or paratenic, may be represented by macroinvertebrates. Heteroxenic cycle parasites such as *S. contortus*, *C. emydidius*, *F. affinis*, *D. renale* and *Telorchis* spp. may have their parasitological indices influenced by anthropic impacts, which reflects on the availability of intermediate and/or paratenic hosts of these helminths. These impacts may in turn be either directly or indirectly reflected in the success of intermediate and/or paratenic hosts to withstand the resulting environmental changes. The effect of anthropogenic environmental changes on parasites infracommunities is known, especially in fish species (Lafferty, 1997; Lafferty & Kuris, 1999; Sures, 2008) and such observations and records can be extended to other groups of vertebrate hosts. In the case of *T. dorbigni*, results suggest that *S. contortus* and *P. rohdei* may have their cycles and infection success related to less impacted environments, such as rural. In contrast, species such as *D. renale* and *T. corti*, have their cycles and infection success more closely related to impacted environments, such as urban. Thus, *S. contortus* and *P. rohdei* seem to be more demanding towards environmental quality.

The presence of third-stage *D. renale* larvae in freshwater turtles in the urban area of the city of Pelotas seems to be linked to the occurrence of infected domestic dogs (definitive hosts), which eliminate Nematoda eggs in the urine recontaminating aquatic urban environments such as ditches and domestic and rainwater sewage or drainage channels, where intermediate hosts (freshwater oligochaetes) and freshwater turtles coexist. The process of eutrophication caused by the demand for organic matter and other household waste in these aquatic urban environments may have favored an increased oligochaeta population, thus promoting the development of the infective form of *D. renale*. High densities of oligochaetes are usually found in altered aquatic environments due to an increase in food resources and a decrease in oxygen supply, and the ensuing decrease of predators and/or competitors (Martins *et al.*, 2008). Indirect adverse effects of eutrophication on piscivorous birds (Ardeidae) mortality rates due to an increased infrapopulation of *Eustrongylides ignotus* (Jägerskiöld, 1909) have been reported by Spalding *et al.* (1993). These results support the hypothesis that channels waters in the urban area of the city of Pelotas, due to their eutrophication by urban waste and the constant supply of urine of dogs infected by *D. renale*, contributed the cycle of this Nematoda. Rappeti *et al.* (2017) diagnosed 81 cases of dioctophymatosis in domestic animals in Pelotas. Mascarenhas & Müller (2015a) addressed the aspects related to eutrophication as well as the role of *T. dorbigni* in the *D. renale* cycle, pointing to the difficulty of this new host to contribute to the completion of the life cycle of this parasite in a significant way.

Diet studies may support helminth fauna investigation and vice versa. Studies of food webs suggest that approximately 75% of food chain links involve some sort of parasite (Dobson *et al.*, 2008). Ferguson & Smales (2006), evaluated the helminth assemblage of *Emydura macquarii* (Gray, 1830) (Chelidae), having identified 11 species (Nematoda, Digenea, Aspidogastrea, Monogenoidea and Amphilinidae); these authors concluded that dietary strategies significantly contributed to the variety of helminth species, once most of these species are transmitted through food webs interactions involving intermediate hosts. *Trachemys dorbigni* is an opportunistic omnivorous species according to studies conducted in Rio Grande do Sul State, Brazil, by Bujes *et al.* (2007), Hahn *et al.* (2014), Mascarenhas & Coimbra (2013) and Silveira *et al.* (2019). After the collection of helminths of present study, Silveira *et al.* (2019) analyzed the diet of the specimens and identified several items such as: gastropod molluscs, crustaceans (Cladocera, Decapoda), Blattodea oothecae, dipteran larvae and pupae, ants, filamentous algae. The omnivorous diet of *T. dorbigni* corroborates the information on the life history of *S. contortus*, *C. emydidius*, *F. affinis*, *D. renale* and *Telorchis* spp. (Anderson, 2000; Font & Lotz, 2008). In this context, the helminth assemblage composition and differences in parasite loads of *S. contortus*, *C. emydidius*, *F. affinis*, *D. renale* and *T. corti* between individuals from rural and urban environments may be related to the availability of intermediate and/or paratenic hosts, which should be ingested to continue the helminth cycle. Esch *et al.* (1979), upon analyzing the diversity of intestinal helminth *Trachemys scripta* (Thunberg in Schoepff, 1792) (Emydidae) in six habitats thermally influenced by a nuclear reactor noted greater diversity as to chemical and physical characteristics in more stable places, suggesting that diversity may result from the complexity of the parasite's life cycle. In order to evaluate diet influence on helminth acquisition, one should also take into account that the diet of freshwater turtles can vary in relation to maturity and gender (Teran *et al.*, 1995; Souza, 2004; Brasil *et al.*, 2011; Hahn *et al.*, 2014).

Another issue that should be considered in helminth studies associated with freshwater organisms refers to water flow influence on helminth infection. Lentic environments are represented by ponds and lakes, which can be classified as polymictic or monomictic according to the mix layer dynamics in the water column, inasmuch as lotic environments (*e.g.* rivers), have a steady stream of water (Margalef, 1983). It is believed that differences in water flow between rural ponds and urban channels can influence freshwater turtle helminth assemblage. In periods of heavy rain, urban channels in the city of Pelotas have an intense water flow and are characterized as lotic environments, once the rainwater collected is poured into the São Gonçalo Channel. In this context, stands out infections by Monogenoidea, which do not require intermediate hosts to complete their life cycles. Parasites monogenean of fish have been studied so as to assess environmental impact under different approaches such as effluent and heavy metal concentrations and dam building (Dušek *et al.*, 1998; Bayoumy *et al.*, 2008; Madi & Ueta, 2009; Acosta *et al.*, 2013). With regard to the influence of the lentic or lotic environment in the presence of parasites monogenean of fish, Acosta *et al.* (2013) found a higher prevalence and mean abundance of *Steindachnerina insculpta* (Fernández-Yépez, 1948) monogeneans in a lentic environment. Similarly, monogenean *P. rohdei* was found to be significantly more prevalent in rural hosts. *Polystomoides* species also show peculiar reproductive characteristics as to egg production (Price, 1939). Paul (1938) has reported that *Polystomoides oris* (Paul, 1938) laid two or three eggs/day and Pichelin (1995) has reported that *Polystomoides australiensis* (Rohde & Pearson, 1980) laid about six eggs/day. Therefore, it is likely that the water flow in urban area channels, added with the reproductive features of this group as to egg production, hampers the encounter between larvae (oncomiracidia) and hosts, thus playing a negative role on *P. rohdei* infection success in the freshwater turtles population in that environment. On the other hand, lentic environments such as rural ponds would act positively in *P. rohdei* colonization of hosts.

Freshwater turtle's gender implications on helminth's assemblage of *T. dorbigni.* In the study the helminth parasite assemblage of *T. dorbigni* presented a dominant-female host pattern concerning to heteroxenic parasites *S. contortus*, *F. affinis*, *C. emydidius* and *T. corti* whenever infections were compared between genders either considering the same environment or all hosts. Such gender-dependent differences (or heterogeneities related to gender) detected may be related to two main hypotheses: a) differences in qualitative and quantitative food demands; b) differences neuroendocrine in relation to host's gender.

With regards to qualitative and quantitative food differences, the likely dominant-female host pattern of *T. dorbigni* helminth infections could be related to differences in reproductive energy demands (vitellogenesis and development of the amniote egg) as well as to derived sexual dimorphism in larger females. As in this study, Bager *et al.* (2010), in southern Brazil, have reported that females were greater than males of the *T. dorbigni*. The diet of some freshwater turtle species may vary qualitatively and quantitatively between males and females, as observed for *T. dorbigni* (Hahn *et al.*, 2014; Silveira *et al.*, 2019); these authors found significant gender differences, with females having a diet which included a greater diversity of food items than males. Hahn *et al.* (2014) reported that animal origin items (both invertebrate and vertebrate) accounted for 16.4% of the total food items in females as contrasted to 8.3% in males. As to invertebrate consumption, Hahn *et al.* (2014) observed that females fed on 16 different groups (Gastropoda, Crustacea, Insecta, Arachnida and Hirudinea), of which crustaceans were the most frequent. On the other hand, males had the diet that comprised of four different food groups belonging to Gastropoda, Insecta and Hirudinea, the most frequent of which were insects. Teran *et al.* (1995) have suggested that *Podocnemis unifilis* (Troschel, 1848) (Podocnemididae) females eat more fish and molluscs to meet their calcium needs for the production of eggs.

In this context, the importance of crustaceans and gastropods as intermediate hosts of various groups of helminths, among which Nematoda (*e.g.* Camallanidae and *S. contortus*), which use copepod crustaceans (Hedrick, 1935; Anderson, 2000), and Digenea (*e.g. Telorchis* spp.), which use gastropod molluscs with intermediate hosts (Font & Lotz, 2008) is emphasized.

The parasitic indices of helminth parasites of *T. dorbigni* may also have been influenced by processes related to neuroendocrine differences between genders. Klein (2004) argues that the male tends to undergo an immunosuppression process both related to testosterone and its derivatives as it matures sexually, and to species with marked reproductive seasonality. On the other hand, Morales-Montor *et al.* (2004) have pointed out that the female supremacy paradigm in the face of a parasitic infection constraint should be carefully considered, taking into account the heterogeneities of host and parasite species. Additionally, Wirsing *et al.* (2007) have pointed out that the understanding of parasitic loads (= parasitic indices) relationships should be species-specifically developed in the host-parasite relationship, as research tends to investigate and analyze metacommunities, as discussed by Wirsing *et al.* (2007). Thus, immune dimorphism meanings can be discussed within their peculiarities. Studies with lizards have demonstrated that males show higher parasitic loads with reference to nematode infections (Sousa *et al.*, 2007; Pereira *et al.*, 2012). As to freshwater turtles, host gender has been little explored in studies of the helminth fauna. Zelmer & Platt (2009), upon analyzing *Chelydra serpentina* (Linnaeus, 1758) (Chelydridae) infracommunity similarities, produced results suggesting differences in helminth abundance between males and females host. However, these same authors call attention to host's gender sampling discrepancies (higher for males) and limited themselves to pointing out differences in similarity indices between environments with reference to *C. serpentina* males only.

Another important immunosuppressive factor which may exacerbate gender's heterogeneities refers to host-acting pollutants. Different studies have shown that pollutants can affect both host and parasite health (MacKenzie *et al.*, 1995; Lafferty *et al.*, 2004) and, within this context, there is a pollutant-host-parasite feedback along the lifespan of the different species involved. Also, both parasites and pollutants can trigger important hormonal, sexual (=gender-related) and stress changes (Sures, 2008) by triggering a complex neuroendocrine network of homeostatic interaction (Nava-Castro *et al.*, 2011). So, pollutants can exacerbate and feedback heterogeneity influences on *T. dorbigni* females regarding reproductive physiological pressures.

In short, the results obtained suggest that the gastrintestinal helminths have the a positive association with females, that related to different heterogenic factors of the *T. dorbigni*, such as derived sexual dimorphism for larger females, probably leading to differences in energy investment between genders (mainly in vitellogenic processes), and resulting in a qualitative and quantitative diet of primary and secondary helminth hosts according to the impact of negative interactions in human environments. However, immunosuppressive factors that may be present in the urban habitat cannot be discarded.

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