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RESEARCH ARTICLE

FACTORS FAVOURING HELMINTHS PARASITES PREVALENCE IN PTYCHADENA MASCARENIENSIS FROM WETLAND AND FORESTS OF URBAN AND PERI-URBAN AREAS OF DALOA DEPARTMENT (IVORY COAST, WEST AFRICA).

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Abstract

The period from November 2018 to June 2020 was marked by the sampling of amphibians in wetlands and forests of the urban and peri-urban areas of Daloa department. Three types of habitats were explored: urban shallows; peri-urban shallows and peri-urbanizing forests. Ptychadena mascareniensis was the only one amphibian species found to be constant at the 14 sampling sites of the study area. It was therefore used to evaluate parasitic infections of amphibians in the study sites. Thus, 381 specimens of Ptychadena mascareniensis were examined and 124 were infected by helminths parasites. A total of 5 helminths species belonging to 3 classes were identified: three nematodes species: *Rhabdias bufonis*; *Cosmocerca ornata* and *Capillaria* sp.; one trematode: *Haplometroides eburnense*; one cestode: *Proteocephalus* sp. Globally, prevalence of helminths parasites in the study area was 32.54%. In urban shallows, peri-urban shallows and peri-urban forests; parasites prevalence was 36.08%, 34.09% and 17.46% respectively. There was a significant difference between the prevalence in shallows and forest area (Kruskal-Wallis and Mann-Whitney test; $p < 0.05$). In addition, parasites prevalence was favoured by two factors. They were firstly parameters with a strong positive correlation with parasites prevalence like soil cover by grasses, human pressure and water pH. Secondly parameters with a strong negative correlation with prevalence: air temperature, canopy, litter cover, number of woody plants and relative air humidity.

Introduction: -

Biodiversity is variability of living organisms from all sources (Wilson, 1988). It is undergoing a drastic loss through the world. Concerning amphibians, out of 8337 species, more than 43% are in decline (Amphibia Web, 2021). Forest ecosystems degradation represents an important cause of biodiversity reduction (FAO, 1997). It's not without consequences: emergence of parasitic diseases in humans and wildlife is linked at the alteration of ecosystems and the evolution of relationships between hosts and pathogenic parasites. This phenomenon is accentuated by anthropic pression on lands used to agricultural activities (use of pesticides), climate change and

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pollutions (Blaustein & Kiesecker, 2002; Corn, 2005; McKenzie, 2007; Rohr et al., 2008 a, b; Aisien et al., 2011). According to Bray et al. (2007), agricultural crops carried out in wetlands leaded to a high prevalence of parasites and a high rate of infections. In Ivory Coast, after independence period, the option of development through agriculture has resulted in the loss of most of its forest resources within three decades. In fact, the forest areas of this country decreased from 7.85 million hectares in 1986 to 5.09 million hectares in 2000, then to 3.6 million hectares in 2015 (SER-REDD + & FAO, 2017). Daloa is a town in the West- central of Ivory Coast. It belongs to the forest block of Upper Guinea. This forest contains an important colony of species of animals and vegetables (Brooks et al., 2001, Wieringga & Poorter, 2004). This forest contains also shallows usage for agriculture in tropical areas. Alongside agricultural activities, urbanization and other human activities is a factor in the degradation of forest ecosystems in large cities, particularly that of Daloa. In sum, we meet two important types of habitats that constitute refuges for amphibians: shallows; very exploited for market gardening and ricefield. There are also; fragments of forests located at the periphery. Therefore, for the sustainable management of terrestrial and aquatic ecosystems, regular evaluations of their ecological health status are necessary. Many biological communities are used as indicators of the level of conservation or an environment degradation. Amphibians, because of their high sensitivity to modifications in environmental conditions, are excellent indicators of the biotic integrity of ecosystems (Hillers et al., 2008). In addition, amphibians are hosts for a diversity of parasites (Prudhoe & Bray, 1982). However; studies relating to the parasitology of amphibians in Ivory Coast are insufficient (Maeder, 1969; Maeder, 1973; Oungbé et al., 2019a; Assemian, et al. 2016; Oungbé et al., 2019b; Oungbé, 2021). The only study carried out on Ptychadena mascareniensis in Daloa (Central-west of Ivory Coast) is this of Assemian et al. (2016) in four ricefield shallows of Daloa city. But this study only took into account Daloa city wetland. It did not take into account peri-urban areas. This present study aims to determine the environmental factors favoring the prevalence of helminths parasites in Ptychadena mascareniensis from shallows and forests in urban and peri-urban areas of Daloa department.

Materials And Methods: -

Study area

This study has been carried out in the West- central of Ivory Coast; precisely in shallows and forests of urban and peri-urban areas of Daloa. The study area is located between 6°30' and 7°00′ North latitude and between 6°00′ and 6°30′ West longitude (Alla,1991).

Fourteen sampling sites were prospected. These are shallows that are used for market gardening and rice cultivation. The forests were secondary forests. These sites have been grouped into three types of ecosystems: S1: urban shallows; S2: peri-urban shallows and S3: peri-urban forests (Figure1).

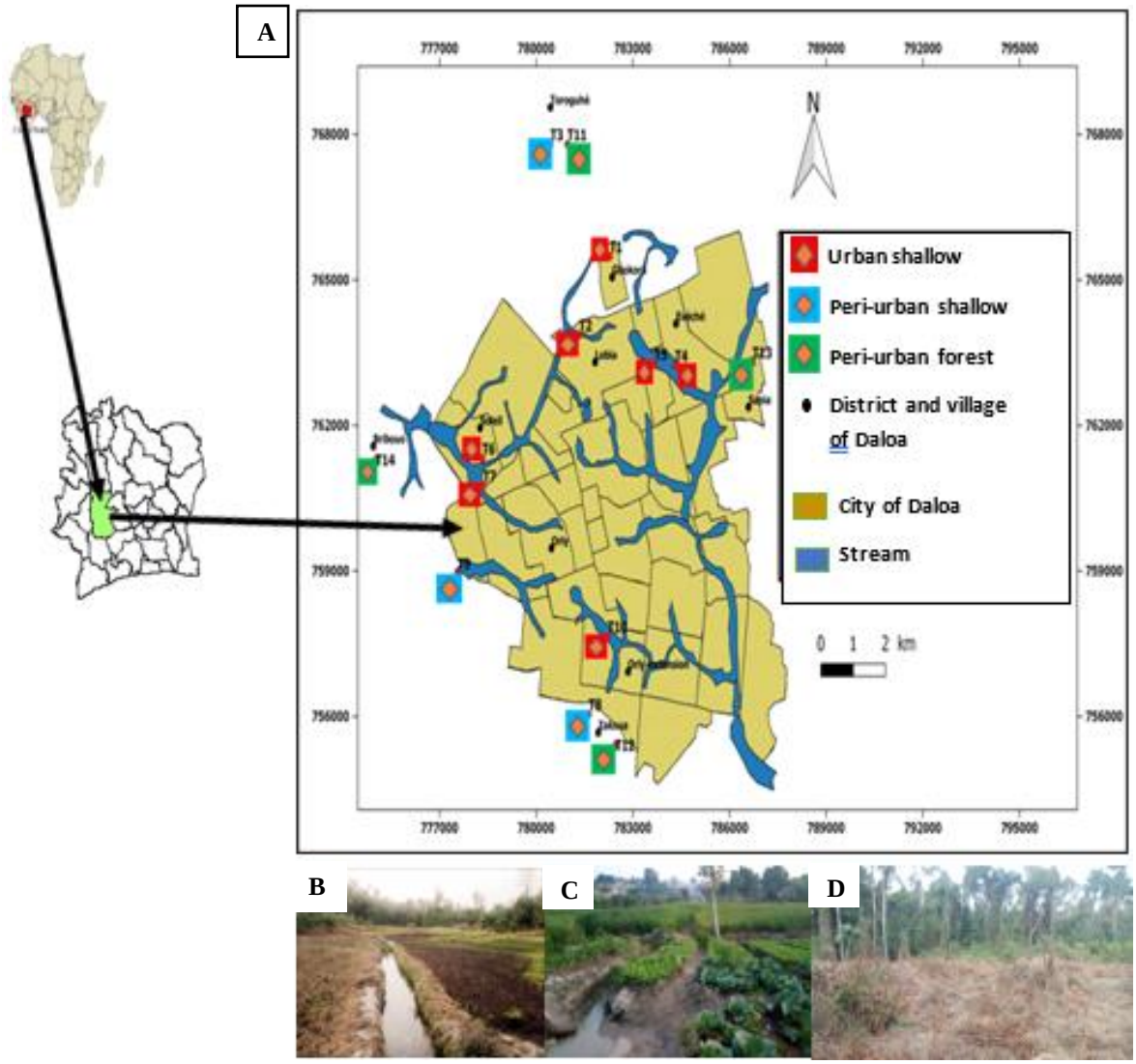

Figure 1: - Study area location (A) and Overview sampling sites: peri-urban shallow of Toroguhe (B) urban shallow of Lobia (C) and peri-urban forest of Sapia (D).

Measure of environment parameters

Measure of temperature and relative humidity of air in our study area was done by a thermo-hygrometer. A multiparameter was used to read pH and water temperature (T°Cwater). Other environmental parameters like canopy (Canop %), ground cover by herbaceous plants (CoSHe %), ground cover by litter (CoSLi %) and human pression (HP %) were estimated according to Rödel et al. (2004) and Assemian (2009) methods. Number of woody plants was obtained by counting the plants.

Amphibians sampling and parasites extraction

Amphibians sampling was realized according to standard techniques of Heyer et al. (1994); Rödel & Ernst (2004). This activity lasted 12 months. It was consisted of applying acoustic and visual surveys. There was also the capture of specimens met during habitats exploration.

Then, captured specimens were identified using the determination keys of Rödel (2000), Frost (2016), Frétey et al. (2011). Ptychadena mascareniensis is the only specie sampled over all sites of our study area. His specimens were first measured and weighed. They were then sacrificed for parasitic investigation. The remaining species were stored in 70% ethyl alcohol. Using a pair of scissors, an incision along the medial-ventral line (from muzzle to anus) allowed to remove the digestive tract and its appendages. These are: the esophagus, stomach, lungs, liver, gall

bladder, bladder and small intestine. Each organ was put in a petri dish and was opened longitudinally. The internal cavity was rinsed with water. Water collected was examined with a binocular magnifying glass.

After observation, parasites were photographed using a camera microscope. The captured image was used to identify the parasite. Parasites identified were transferred to boxes then fixed and stored in 70% ethyl alcohol. The references (host number, host capture site, date of harvest, and location of the parasite in the host) were noted on each box.

Parasite index

Parasite index used in this study is the prevalence rate (P) proposed by Anderson (1993) and by Blahoua et al. (2015). Prevalence (P) is the ratio between the number of hosts infested (ni) by a species of parasite and the number of hosts examined (N). It is defined as the percentage :

$P = (ni / N) x100$

Statistical analysis

Kruskal-Wallis and Mann-Whitney tests were used to compare parasite prevalence of sampling sites. The correlation test was also used to characterize the link which exists between two parameters (A) and (B) of environment. The correlation coefficient (r) quantifies the importance and the link that could exist between these two parameters. Indeed when :

$r > 0.5$: high positive correlation between A and B (progress in the same direction);
$r > - 0.5$: high negative correlation between A and B (change in the opposite direction);
$r < 0.5$: low positive correlation between A and B (regression in the same direction);
$r < - 0.5$: low negative correlation between A and B (regression in opposite direction);
$r = 0$: The two parameters A and B are independent;
$r = 1$, the slope of the line is increasing

These tests were realized with Statistica 7.1 software.

Results:-

The amphibian host

In this study, specimen of *Ptychadena mascareniensis* (Ptychadenidae) from Daloa shallows and forests were amphibian host. Males measured reached 43-57 mm SVL and females reached 42-59 mm SVL. Their belly varied from white to yellow with sometimes black speckles at the throat. Males have pair of lateral gray vocal sacs in superior position. Pedious webs are extended: presence of three distinct pairs of dorsal folds; presence of dark task on the shoulder (Figure 2).

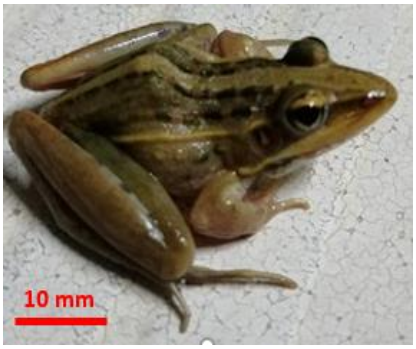

Figure 2: - Adult male *Ptychadena mascareniensis* from Daloa area (Photo by Goly,2021).

Analysis of environmental parameters and parasite prevalence at study sites

Table1 shows that parameters such as canopy (canopy %) and number of woody plants (N) are almost inexistent in shallows (S1 and S2). No streams were encountered in the peri-urban forests (S3), so water pH and temperature (T°Cwater) are not considered. In addition, litter cover (CSoLi %) is low in shallows and almost average in forests. Herbaceous cover (CSoHe %) is abundant in the shallows and low in the forests. Human pressure (HP %) is high in urban areas (S1) and lower in peri-urban areas (S2 and S3), temperature of air (T °C air) is around 29 °C and relative humidity of air (Hum %) is high (around 72%) at all the study area. Finally, the mean parasite prevalence is higher in urban areas than in peri-urban areas and higher in shallows than in forests.

Table 1: - Mean values of parasite prevalence and environmental parameters.

Sites	Habitats	T°C air	Canopy (%)	CSoLi (%)	CSoHe (%)	N	Hum (%)	HP (%)	pH of water	T °C water	Prévalence (%)
S1	wet	29.43	0	8	56	1	71	80	6.85	25.56	36.08
		29.38	0	3	64	0	72	70	6.78	25.41	
		29.15	0	15	80	0	71	50	6.54	25.59	
		29.22	0	4	76	0	71	60	6.68	25.28	
		29.23	0	4	75	1	71	60	6.81	25.21	
		29.8	0	8	56	0	71	60	6.66	25.34	
		28.9	0	23	56	1	76	63	6.92	25.34	
S2	wet	29.48	0	23	50	0	73	70	6.52	25.52	34.09
		29.27	0	21	56	1	71	48	6.39	25.25	
		30.16	0	11	44	2	74	58	6.67	25.33	
S3	dry	29.51	50	28	48	10	74	41	-	-	17.46
		29.97	69	41	40	2	74	44	-	-	
		29.35	56	55	34	5	72	41	-	-	
		29.85	50	53	36	9	74	33	-	-	

T°C air: Temperature of air; CSoLi (%): Soil cover by litter; CSoHe (%): Soil cover by herbaceous plants; N: Number of woody plants; Hum (%): Relative humidity of air; HP (%): Human pression; T°C water: Temperature of water.

Spatial distribution of Ptychadena mascareniensis specimens collected and their infestation rate at study sites. During this study, 381 specimens of Ptychadena mascareniensis (urban shallows = 230 specimens, peri-urban shallows = 88 specimens, peri-urban forests = 63 specimens) were collected and dissected. 124 were infected with helminth parasites. In urban shallows (S1), 230 specimens were dissected and 83 were parasitized; in peri-urban shallows (S2), 88 specimens were dissected and 30 were parasitized; in peri-urban forest (S3); 63 specimens were dissected and 11 were parasitized. In all the study area; the prevalence of endoparasites in Ptychadena mascareniensis specimens was 32.54%. Prevalence of specimens from urban shallows was 36.08%; that from peri-urban shallows was 34.09% and that of peri-urban forests was 17.46%. There was a significant difference between prevalences in shallows and that of forests (Mann-Whitney test; $P < 0.05$).

Inventory and prevalence of helminths parasites collected from specimens of Ptychadena mascareniensis.
Dissection of specimens revealed 5 helminths parasites species divided into 3 classes (nematodes, trematodes and cestodes). Three nematodes species were identified: Rhabdias bufonis, Cosmocerca ornata and Capillaria sp. One trematode was found in alimentary canal: Haplometroides eburnense and one cestode Proteocephalus sp. was identified (Figure 3). In the study area, the prevalence of nematodes was 27.03%; that of trematodes was 4.72% and finally cestodes prevalence was 0.78%. In all the study sites, the prevalence of nematodes is higher (27.03%) than that of trematodes (4.72%). Trematodes had higher prevalence than cestodes (0.78%) (Table 2)

Table 2: - Distribution of *Ptychadena mascareniensis* specimens and prevalence of helminths parasites.

	Urban shallows (S1)	Peri-urban shallows (S2)	Peri-urban forests (S3)	Total
Number of *Ptychadena mascareniensis* specimens dissected	230	88	63	381
Total Number of parasitized specimens	83	30	11	124
Number of specimens parasitized by *Rhabdias bufonis*	62	24	10	96
Number of specimens parasitized by *Cosmocerca ornata*	5	1	0	6
Number of specimens parasitized by *Capillaria* sp	1	0	0	1

Number of specimens parasitized by *Haplometroides eburnense*	13	4	1	18
Number of specimens parasitized by *Proteocephalus* sp	2	1	0	3
Overall parasites prevalence (%)	36,08	34,09	17,46	32,54
Prevalence of *Rhabdias bufonis* (%)	26,96	27,27	15,87	27,03
Prevalence of *Cosmocerca ornata* (%)	2,17	1,14	0	
Prevalence of *Capillaria* sp (%)	0,43	0	0	
Prevalence of *Haplometroides eburnense* (%)	5,65	4,54	1,59	4,72
Prevalence of *Proteocephalus* sp (%)	0,87	1,14	0	0,78

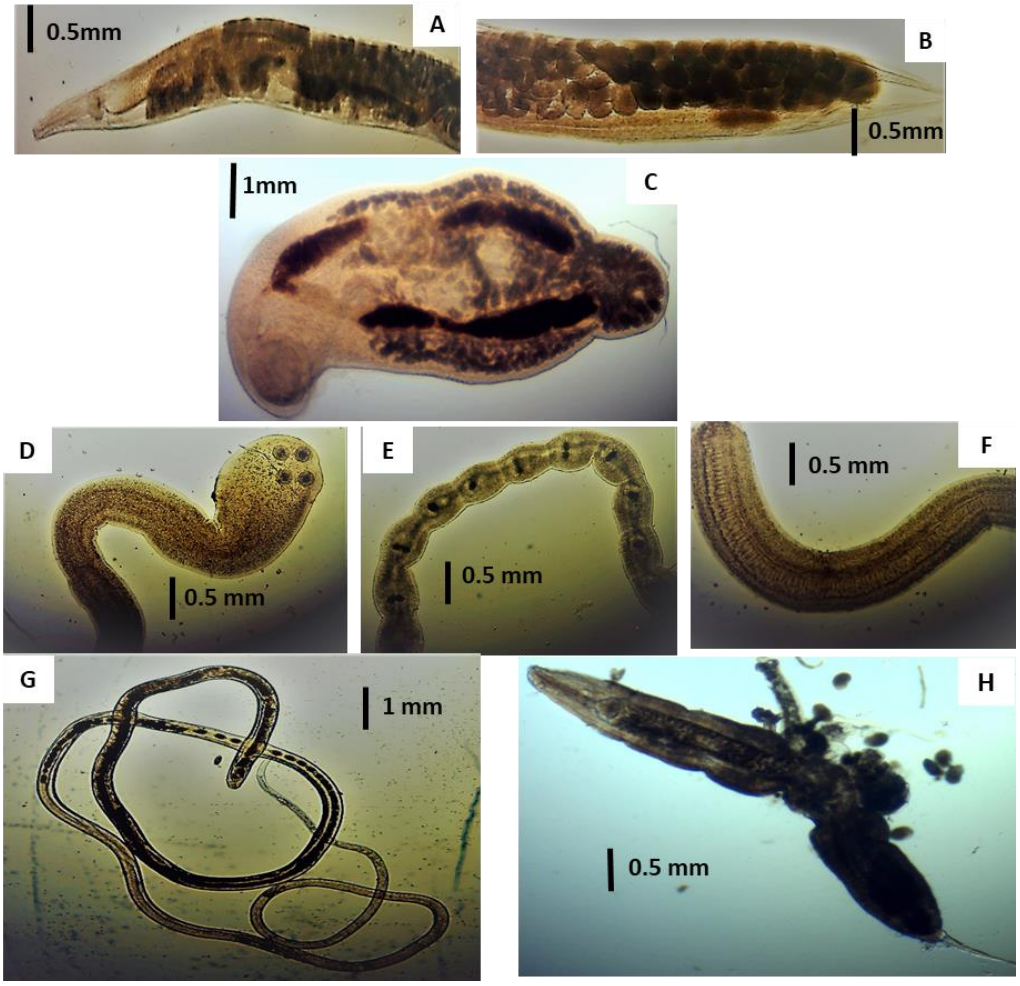

Figure 3: - Helminths parasites from digestive tract and appendages of *Ptychadena mascareniensis* from shallows and forests of Daloa urban and peri-urban areas.
A: Top half and B: back half of *Rhabdias bufonis*; C: *Haplometroides eburnense*; D: head; E: trunk and F: tail of *Proteocephalus* sp; G: *Capillaria* sp; F: *Cosmocerca ornata*.

Correlation between parasite prevalence and parameters of the environment.
In the study environment, there is a high positive correlation between parasite prevalence and parameters such as the ground cover by herbaceous plants (CSoHe%); human pression (HP%) and water pH. However, water temperature (T°Cwater) is not high but only positive correlation with prevalence (Table3). In addition, there is high negative

correlation between parasite prevalence and temperature of air (T°Cair); canopy (canopy%); Ground cover with litter (CSoLi%); number of woody plants (N) and relative humidity of air (Hum%) (Table3).

Table 3: - Correlation coefficients between parasite prevalence and parameters of study area.

Correlation coefficients between parasite prevalence and parameters of the study area	Parameters of the study area
- 0.66	Air temperature (T°Cair)
- 0.99	Canopy (%)
- 0.98	Ground cover with litter (CSoLi%)
0.85	Ground cover by herbaceous plants (CSoHe%)
- 0.99	Number of woody plants (N)
- 0.91	Relative humidity of air (Hum%)
0.99	Human pression (HP%)
0.65	Water pH
0.08	Water temperature (T°Cwater)

Discussion:

During this study, we dissected 381 specimens of Ptychadena mascareniensis. Among these specimens, 124 were parasitized by helminths i.e. a prevalence of 32.54% in study areas. Prevalence of helminths parasites was 36.08% on the urban shallows. Peri-urban shallows and peri-urban forests were respectively 34.09% e 17.46%. Compared to the results of Oungbe work (2021), our results (focused on a single taxon) show a high parasitic infestation in the study area. Indeed, for the author, the examination of 28 amphibian taxa revealed an overall parasite prevalence of 63.63% in three agro-industrial zones in the southeast of Ivory Coast.

Infestation rate of Ptychadena mascareniensis specimens was higher in shallows than in forests sites. Similar results were found by Fournet et al. (2002). These authors having worked on urinary schistosomes of Daloa (Ivory Coast), revealed a prevalence more important among rice farmers working in the shallows. In addition, this study revealed the presence of three classes of parasites in Daloa city wetland. These helminths were nematode, trematode and cestode parasites. Assemian et al (2016) have done similar work in Daloa urban ricefields. They revealed presence of nematode, trematode and acanthocephale classes in Ptychadena mascareniensis population. Indeed, contrary to Assemian et al (2016), our results indicate the absence of Acanthocephalans. This would be due to the disappearance of this class of parasite in the study area. This view is in line with the results of the work of Hadrien (2020). According to this author, Acanthocephalans depend on several viable host communities in the ecosystem. They are therefore more sensitive to changes in their environment. Thus, the slightest impact on one of their hosts would compromise their entire life cycle.

Two environmental conditions were favorable to the parasite prevalence in our study area:
-Parameters with a high positive correlation with the prevalence. It was about: ground cover by herbaceous plants; human pression and water pH.
-Parameters with a high negative correlation with the prevalence. These parameters were: temperature of air; canopy; Ground cover with litter; number of woody plants and relative humidity of air.

These declarations corroborate the OMS (1982) point of view. Indeed, speaking of the prevalence of parasites in humans, the OMS asserted that the global prevalence and intensity of human intestinal protozoan and helminth infestions showed considerable variations in distribution and seasonal occurrence due to geographic; climatic factors and human activities (changes in the environment; improvement of sanitation etc…). The same observation was made by Sahibi and Rhalem (2007). These authors have shown that several factors influenced the survival and development of ticks (ectoparasites), in particular the temperature of ambient environment, the wind, the relative humidity of air and the vegetable cover. Another similar opinion was given by Hulme (2017). According to this latter, the development of a direct transmission parasite depends on the characteristics of the environment like temperature, relative humidity of air, pH, quality of microbiota and pollution.
In addition, our work revealed that the pH of the various streams existing in the shallows was close to neutrality (oscillated between 6,39 and 6,85). This result is in agreement with those of EL GUAMRI et al (2007): their results also revealed that wastewater with a substantially neutral pH favored a high concentration of helminth eggs.

Conclusion:

Prevalence of endoparasites from our study area was 32,54%. The collection sites presented these following prevalences: urban shallows: 36,08%; peri-urban shallows: 34,09% and peri-urban forests: 17,46%. Our results revealed the presence of nematode parasites; trematode parasites and cestode parasites in the study area. Rhabdias bufonis having parasitized more specimens (26,96%).

Two types of environmental factors favored the prevalence of endoparasites in Ptychadena mascareniensis:
- Parameters with a high positive correlation with the prevalence: ground cover by herbaceous plants; human pression and pH water.
- Parameters with a high negative correlation with the prevalence: temperature of air; canopy; ground cover with litter; number of woody plants and relative humidity of air.

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