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Parasitic Infections of *Hoplobatrachus occipitalis* (Günther, 1858) from Pond and Shallow in Daloa City (Côte D'ivoire)

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ABSTRACT

The parasitic infections of *Hoplobatrachus occipitalis* from ponds and shallows in Daloa city (Côte D'Ivoire) were investigated. A total of 55 specimens of *H. occipitalis* were examined and parasites recovered included *Clinostomum complanatum*, *Enterobius vermicularis* and *Rhabditis* sp. Results obtained show a high prevalence and mean infection intensity of *Clinostomum complanatum* at the *H. occipitalis* in the ponds. It should be noted that several parameters could explain these observations, namely the variation of the temperature to which the amphibians are sensitive, the acidity of the water in the ponds. Furthermore, the parasites were found in the large intestine, small intestine and stomach of the captured individuals.

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INTRODUCTION

Anurans are the most diverse order of amphibians in the world [1]. They are also of fundamental interest to agriculture because of their diet. Indeed, they consume invertebrates in general and insects in particular [2]. They play a very important role in the reduction of the levels of insect pests in agriculture and help increase agricultural yields. In addition, some species of anurans are an important source of animal protein, notably the species *H. occipitalis*. Indeed, the consumption of this frog has increased considerably in recent years, which has earned them an important place in the dietary habits of the Ivorian population, more specifically those of Daloa [3]. This frog is therefore subject to heavy predation by humans to satisfy their high demand for animal protein. However, the consumption of frogs by certain human populations is considerably reducing their abundance [4-5]. Faced with the overexploitation of natural stocks, it is necessary to consider production to prevent the decline of *H. occipitalis* in regions with high human consumption [6]. In this context, it is essential to control all parameters related to the captivity of *H. occipitalis* frogs. In addition to reproduction and feeding, health and epidemiological monitoring is essential to the success of any breeding process.

While parasites can be used to estimate the level and source of pollution in an environment [7] and to determine the impact of eutrophication [8], they can be detrimental to the frogs and also to the people who eat frogs. Furthermore, according to [9], parasites can harm their hosts in a number of ways, causing mechanical injuries such as chafing, tissue atrophy, limb deformities and worse, causing stunted growth, skeletal deformities, impaired eyesight, reduced host fecundity, etc. In addition, several species of parasites are known to inhibit the absorption of nutrients by host anurans, thereby altering their physiology, feeding and behavior [10]. These threats can lead to the death or even the decline of the population of this amphibian species. Thus, the presence of parasites in amphibians could constitute a public health problem if they are found alive or dead in their hosts intended for human consumption [11]. This is because amphibians are intermediate hosts of certain parasites in humans in the same way as aquatic molluscs [8]. They are therefore carriers of certain human parasites. In Daloa, parasitic infections of amphibians are little studied [12-13]. Moreover, no study has been done on the parasitosis of the species *H. occipitalis*. Our aim was to fill this gap by contributing to the knowledge of the parasites of this amphibian species who lives in two environments in the city. The objective of

the present study was to investigate the prevalence and mean intensity of infection of the parasites of *H. occipitalis* in two different environments (ponds and shallows).

MATERIAL AND METHODS

Study area

Daloa city is located in the Central-west of Ivory Coast (6°53’ N and 6°27’ W). Covered by dense evergreen forest, this region is now disturbed due to expensive cacao and coffee plantations. Furthermore, the department is watered by the Sassandra River and its tributary, the Lobo flood all localities.

In these areas, the climate is equatorial characterized by four seasons: the long dry season runs from December to March, the long rainy season from April to July, the short rainy season lasts from October to November and the short dry season from August to September [14].

The survey sampling was conducted from 13 November 2020 to 23 January 2021 at the fish farm of the *Association Pisciculture et Development Rural en Afrique Tropicale Humide Côte d'Ivoire* (APDRACI). The structure is located in the town of Daloa. The choice of the sampling site was determined in order to evaluate the effect of the level of anthropisation on the frog *Hoplobatrachus occipitalis*. This site was chosen in an urban environment. It is a lowland rice field located in the Fatiga neighborhood of the city of Daloa (Figure 1). The geographical coordinates are: 06°52'30" North latitude and 07°34'30" West longitude with an altitude of 243.400 m. This site is surrounded by houses. The various ponds and rivers in this site are murky and contain rubbish, animal and human waste. The depth of the various water bodies varies between 10 and 30 cm and the dominant vegetation consists mainly of grasses such as *Megathyrsus maximus* (synonym of *Panicum maximum*, Poaceae).

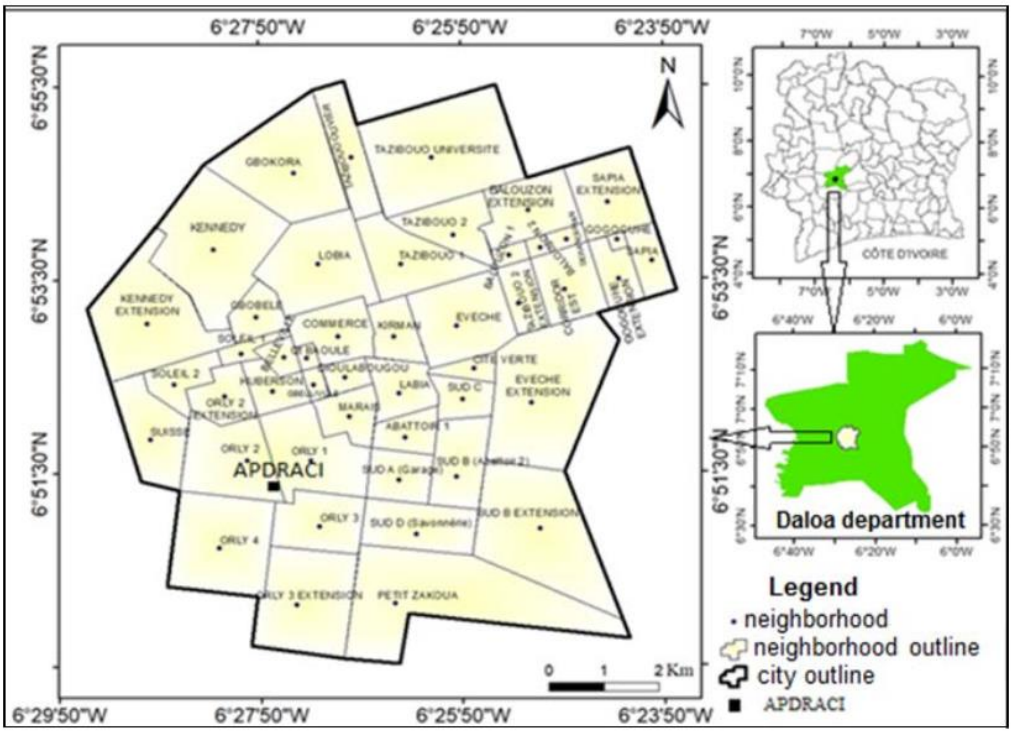

Figure No. 1: Geographical location of Daloa city and the fish farm of the APDRACI [15].

Data collection. We sampled the target species *Hoplobatrachus occipitalis* (Günther, 1858) using visual and acoustic surveys in two types of habitats (Ponds and shallows) applying standard techniques by [16] and [17]. Due to its nocturnal activities, this species was captured between 7 hour and 10 hour PM. The target species is listed as Least Concern in view of its very wide distribution, its tolerance of a broad range of habitats and its presumed large population [18].

Snout-Vent Length (SVL) of Frog specimens captured was measured (with dial calipers) to determine individual size. This species has a wide distribution, inhabiting a broad range of habitats. It was often locally very abundant [19-20-21]. Target species is widespread in different savannah types and disturbed forest habitats [19-22-23-24].

H. occipitalis (Dicroglossidae) is a sizable flat frog manifesting protruding eyes, numerous dorsal warts, and a minute inner metatarsal tubercle. This anuran exhibits complete webbing between all the toes and fingers. This large dorso-ventrally flattened frog features warty skin, with eye and nostril dorsal placement. Viewed from above, the eyes are completely contained

within the outline of the frog's head. Owing to the presence of numerous glands, the exterior skin is extremely slippery [19]. The color of the body and limbs is a yellow-green, olive or drab brown. Large dark green to blackish spots occasionally form rows, are exhibited on the dorsum. Spots of this same dark green to black present on the upper lip and on the extremities. A light green to yellow transverse line behind the eyes is exhibited in juvenile frogsand often in adults as well. The outer elements of the thighs are marbled. The venter is white, occasionally punctuated with black spots.

Representative specimens were collected and dissected thereafter in laboratory. The esophagus, stomach, small intestine and large intestine/rectum were examined for parasites. The lungs, liver/gall bladder, urinary bladder and the body cavity of the amphibians were also examined for parasites. Parasites specimens were fixed and preserved in 70% ethanol after observation on a microscope slide. Helminths were identified by observation and measurement of morphological characteristics based on descriptions from literature [25-26-27-28-7-29]. Parasite vouchers and frogs' carcases were kept at Department of Ecology, Biodiversity and Evolution at Jean Lorougnon Guédé University (Côte D'Ivoire).

Data analysis. The prevalence rate (P) and mean intensity of infection (MII) were calculated according to [30]. Prevalence rate was calculated as a percentage of the number of a particular host species infected with a specific helminth parasite divided by the total number of host examined. The mean intensity of infection refers to the number of parasites per host (calculated only for the infected hosts examined). The Kruskal-Wallis and Mann- Whitney non-parametric tests were used to determine differences in the parasite load in the target species. All these correlations and tests were performed with Statistica 7.1.

RESULTS

In this study, specimen of *Hoplobatrachus occipitalis* from Daloa city ponds and shallows were amphibian host (Figure 2). In the study site, SVL of the frog specimens captured in ponds was comprised between 65 and 112 mm, and in shallows between 64 and 97 mm. Males measured reached 64-75 mm SVL and females reached 72-112 mm SVL.

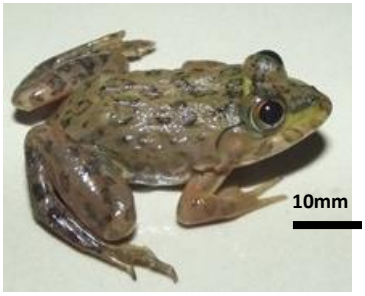

Figure No. 2: **Adult male of *Hoplobatrachus occipitalis* from Daloa city ponds and shallows (Photo by Assemian N.E., 2021).**

A total of 55 specimens of *Hoplobatrachus occipitalis* were examined, 25 in captivity (ponds) and 30 in a natural environment (shallows). However, 16 specimens were infected in the ponds and 10 in the shallows (Table 1). The helminth parasites encountered consisted of *Clinostomum complanatum*, *Enterobius vermicularis* and *Rhabditis* sp.(Figure 3).

Table No. 1: Abundance of *Hoplobatrachus occipitalis* parasites in each habitat type.

	Hoplobatrachus occipitalis		**Parasites**		
Habitat	**Examined**	**Infected**	***Clinostomum complanatum***	***Enterobius vermicularis***	***Rhabditis* sp.**
Ponds	25	16	25	3	5
Shallows	30	10	15	4	0

Figure No. 3: A **specimen of each helminth parasite recovered in *Hoplobatrachus occipitalis* from ponds and shallows of Daloa city: *Clinostomum complanatum* (a), *Enterobius vermicularis* (b), *Rhabditis* sp. (c)**

The prevalence and average intensity of infection by parasite species are presented in Figure 4. The prevalence of *Clinostomum complanatum* (P= 44%) in the ponds was higher than in the shallows (P= 23.33%). In contrast, *Enterobius vermicularis* prevalence were higher in the shallows (P= 10%) than in the ponds (P= 08%). No *Rhabditis* sp. parasites were found in *Hoplobatrachus occipitalis* specimens in the shallows.

With regard to the average intensity of infection, the highest values of intensity of *Enterobius vermicularis* infections (IMI= 0.4) were observed in the shallows. The average intensity of *Clinostomum complanatum* infection was almost the same in both habitats (IMI ≈ 1.5).

None of these differences were significant (Mann-Whitney test, $p \geq 0.05$).

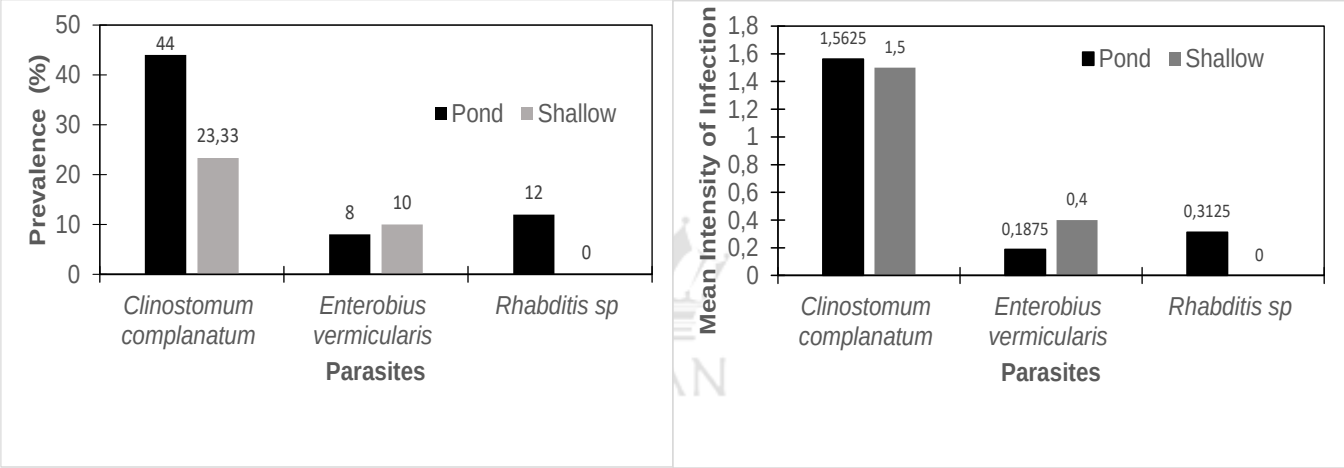

Figure No. 4: Prevalence rate and average infection intensity of parasites of *Hoplobatrachus occipitalis* from pond and shallow of Daloa city.

The sites of parasite infestation in the host are noted in Table 2. Indeed, the stomach, small intestine and large intestine are the preferred organs of the three parasite species identified in the two types of habitats.

Table No. 2: Parasites of *Hoplobatrachus occipitalis* and their target organs in the ponds and shallows of Daloa city.

Habitat	Parasites	Target organ
Ponds	*Clinostomum complanatum*	Large intestine, Stomach, Small intestine
	Enterobius vermicularis	Stomach,Small intestine
	Rhabditis sp.	Large intestine, Stomach, Small intestine
Shallows	*Clinostomum complanatum*	Large intestine, Stomach, Small intestine
	Enterobius vermicularis	Large intestine, Stomach

DISCUSSION

This study found three species of parasites which are *Clinostomum complanatum, Enterobius vermicularis* and *Rhabditis* sp. However,[12] found other parasites (*Centrorhynchus* sp., *Cosmocercoides variabilis* and *Haplometroideseburnense*) in the *Ptychadena mascareniensis* frog species in the shallows of Daloa. In this city, [31] also found in the *Ptychadena mascareniensis* from urban and peri-urban areas five helminth parasites species: *Rhabdias bufonis*, *Haplometroideseburnense*, *Proteocephalus* sp., *Capillaria* sp; *Cosmocerca ornate*. These different results are linked to human activities that differ from one site to another. In addition, the difference between host species could explain these results.

Our results indicate that the prevalence rate of *Clinostomum complanatum* is higher in both study environments. This would be justified by the fact that this parasite proliferates rapidly in the soil and could be present in all amphibian habitats and would be parasitic to several other aquatic species. Frogs sometimes feed on fry, which could be a vector for these parasites. Indeed, a study by [32] on freshwater fish parasites in Iran, showed that fish in the Shirnoud River were heavily contaminated with the digenean parasite.

Our results showed that *H. occipitalis* specimens were infected with more parasites in captive environments than in natural environments. The environmental characteristics of these sites could explain these differences. According to [33], the sensitivity of amphibians to thermal fluctuations in ponds would make possible the rapid transmission of pathogens compared to

shallows. Also, the acidity of pond water could be the basis of the high parasite rate in captive frogs according to [34].

Our results also show that the parasites were located in the small intestine, large intestine and stomach. Similar results have already been demonstrated by several authors [35-29].

CONCLUSION

At the end of the different dissections of *H. occipitalis* specimens, three helminth parasites taxa (*Clinostomum complanatum*, *Enterobius vermicularis* and *Rhabditis* sp.) were inventoried in the ponds and two (*Clinostomum complanatum* and *Enterobius vermicularis*) in the shallows. The prevalence study showed a high infestation rate of *Clinostomum complanatum* (44%) on anuran specimens in the ponds. In fact, most of the parasites were found in the large intestine, small intestine and stomach of the captured individuals.

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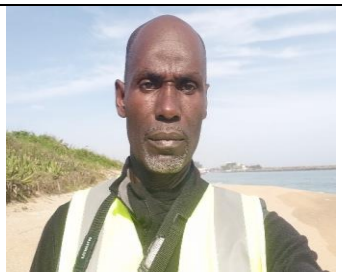	**ASSEMIAN N'Guessan Emmanuel – *Corresponding Author*** *University Jean Lorougnon Guédé (UJLoG), Laboratory of Biology and Tropical Ecology (BioEcoTrop), UFR Environment,* *BP 150 Daloa, CÔTE D'IVOIRE* *Professor-researcher at the UJLoG*
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