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Freshwater snail dynamics focused on potential risk of using urine as fertilizer in Katiola, an endemic area of Schistosomiasis (Ivory Coast; West Africa)

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ABSTRACT

The aim of this study was to conduct a large-scale freshwater snail survey in Katiola to assess the malacological diversity and the larval trematode infections. We conducted 156 samples of snails and environmental parameters in 13 sampling sites in 3 localities. Nine species were identified among the 6049 collected snails, with four of human schistosome transmitting snails, *Bulinus forskalii*, *B. globosus*, *B. truncatus* and *Biomphalaria pfeifferi*. Although *B. pfeifferi* was the most largely distributed and none of *B. truncatus* and *B. forskalii* were found naturally infected by schistosomes. *B. globosus*, *B. pfeifferi* and *Lymnaea natalensis* shed parasites with low prevalence (respectively 12.17%, 1.77% and 10.71%). Physico-chemical parameters showed low variations except dissolved oxygen. Four hydrophytes (*Setaria longiseta*, *Ludwigia abyssinica*, *Polygonum salicifolium* and *Polygonum lanigerum*) out of thirteen are ubiquitous and influenced the distribution of snails. Our data showed the potential risk to public health in the use of urine as fertilizer in Katiola.

Keywords: Freshwater snail, Diversity, Larval trematode, Côte d'Ivoire.

1. Introduction

Artificial structures, such as dams, and tributary confluences are two major types of discontinuities in a river system [1, 2]. Despite the environmental impacts caused by dams and impoundments, dams continue to be constructed worldwide. In Ivory Coast, most of these infrastructures were especially built in Center and North of the country, where increased human demands on water resources because of hydrological deficit. Adverse effects of dams on river ecosystems have been widely recognized [3]. They pose a threat to aquatic biodiversity [4] and are responsible for the invasion of aquatic environments by freshwater snails. These invasions may have a negative impact on public health because many species, especially gastropods, may be intermediate hosts for waterborne parasites [5]. Therefore, endemic to schistosomiasis areas in Ivory Coast are located mainly in the north and center of the country. Settlements in these areas are characterized by informal housing without proper water reticulation and sanitation facilities. These circumstances give rise to the situation where the local residents are forced to make use of natural water for domestic and recreational purposes. These dams that are favorable habitats for the transmission of parasites [6] contribute to the survival of the population through the development of rice schemes. Also, in order to diversify crops, fertilizing with urine in irrigated rice is tested in the Katiola department (central northern). This new approach reduced input costs, but presents a risk because the area is endemic to schistosomiasis. It's necessary to provide, regularly, epidemiological data on prevalence and intensities of infection in humans, associated with malacological surveys and schistosome prevalence's in snails, because of the serious public health problems that schistosomiasis causes. Our study aimed to conduct a comprehensive freshwater snail survey in the different site tested, in order to assess the malacological diversity and the larval trematode infections with a focus to establish the potential risk to public health.

2. Materials and methods

2.1. Study area

Katiola is located in north-central Ivory Coast (Fig 1), approximately 430 km from Abidjan the economical capital between 8°00’ and 9°20’ N, and 4°43’ and 5°78’ N and an altitude of about 320 meters. The town covers 9420 km² and has three dams with irrigated perimeter Lopé, Nianra and Foro-Foro covering respectively 37, 76 and 29 hectares.

2.2. Environmental parameters

The sampling sites were characterized monthly by measuring *in situ* selected Physical parameters using a multi-parameter apparatus (*WTW 840i*). These were pH, conductivity (µS/cm), dissolved oxygen (mg/l), water temperature (°C) and redox potential (mV). Water depth and turbidity were measured respectively with a meter stick from the surface of the tiles to the water surface, and a Turbidimeter WTW Turb 430. Samples for chemical analysis were collected from each station using prewashed polyethylene bottles.

The water samples were kept at a temperature below 4 °C to stop all the activities and metabolism of the organisms in the water and were analyzed within 24 hours after collection. Afterward, the concentrations of nitrite, nitrate, ammonium, phosphorus, and Chemical Oxygen Demand (COD) were determined in the laboratory according to standard spectrophotometric methods by using a spectrophotometer SCHIMADZU UV 160A. Coordinates of each sampling station were determined with a GPS (*GARMIN eTrex*).

Seagrass beds were made with hydrophytes of different stations and then identified at Botanical Laboratory of the University of Cocody (Abidjan, Ivory Coast).

Fig 1: Map showing the three sampling sites in the study area.

2.3 Malacological surveys

Snails were collected monthly from April 2009 to April 2010 in 13 sites situated in 3 localities (geographical positions in Table 1), by removing them from vegetation and mud using long-handled sieves (mesh size<1 mm). Each site has been regularly visited for a variable period of time depending on snail density, but always by the same collector during the same time period. Specimens were sorted, stored in plastic boxes and transferred to the laboratory alive. Snails were identified with morphological characters according to [7] and anatomy of the reproductive system [8]. To screen for emergence of cercariae, snails were kept separately in test tubes each containing 10 ml filtered habitat water and exposed to light. A cotton plug prevented the snails from leaving the water. The water in each of the test tubes was separately decanted into Petri dishes after a 2-h shedding period. Snails were then rinsed in filtered water from the habitat and transferred to identical clean test tubes also containing 10 ml of filtered habitat water. Observation and identification of parasites under a stereomicroscope commenced directly after each 2 hours shedding period and was discontinued after the last 2 hours period during which no cercariae were observed.

2.4. Statistical analysis

In order to determine whether the observed differences between sampling sites were significant, data were subjected to the non-parametric comparison tests (Kruskal-Wallis test and Mann-Whitney test) and presented by boxplots. All statistical analyses were carried out by the software Paleotological Statistic (PAST) version 2.17c [9].

3. Results.

3.1 Environmental parameters

Spatial and temporal variations in physical and chemical parameters are shown in Figure 2. pH shows low variations between sites (5.8-8.2) and a seasonal trends. Dissolved Oxygen varies significantly only from one site to another (2-6 mg/l), while measured temperatures are representative of the region, whatever the season (28-35 °C). Conductivity means are essentially stronger in Lope and Foro Foro (48.2 to 198 µS/cm), with significant variability from one site to the other and no seasonal trends. Redox potential shows little spatial fluctuations (-64 to 64 mV), but a significant variation between seasons (-64.3 to 78.6 mV).

Table 1: Distribution and geographical positions of the sites.

Localities			Geographical positions		
	Nb	**Type of site**	**Latitudes**	**Longitudes**	**Altitudes (m)**
Nianra	5	Lake	8°06'N	4°96'W	226
		Lake	8°07'N	4°97'W	231
		River	8°08'N	4°95'W	211
		Pond	8°09'N	4°94'W	220
		Canal	8°09'N	4°94W	195
Lopé	5	Lake	8°12'N	5°12'W	173
		Lake	8°11'N	5°12'W	297
		Pond	8°12'N	5°12'W	288
		Canal	8°11'N	5°11'W	249
		Pond	8°11'N	5°10'W	277
Foro-Foro	3	Lake (dam)	7°96'N	5°04'W	260
		Pond	7°96'N	5°05'W	239
		Pond	7°97'N	5°06'W	265

Nitrite, nitrate and COD are chemical parameters that have significant spatial variability (respectively 2.22 to 77.7 mg/l; 6.342 to 1395 mg/l and 10,148 to 319.662 mg/l). They show no significant trends from one season to another. Phosphorus showed no significant variations while ammonium shows seasonal fluctuations (5.8 et 2088 µg/l), and turbidity varies significantly in space and time (1 et 73 UNT).

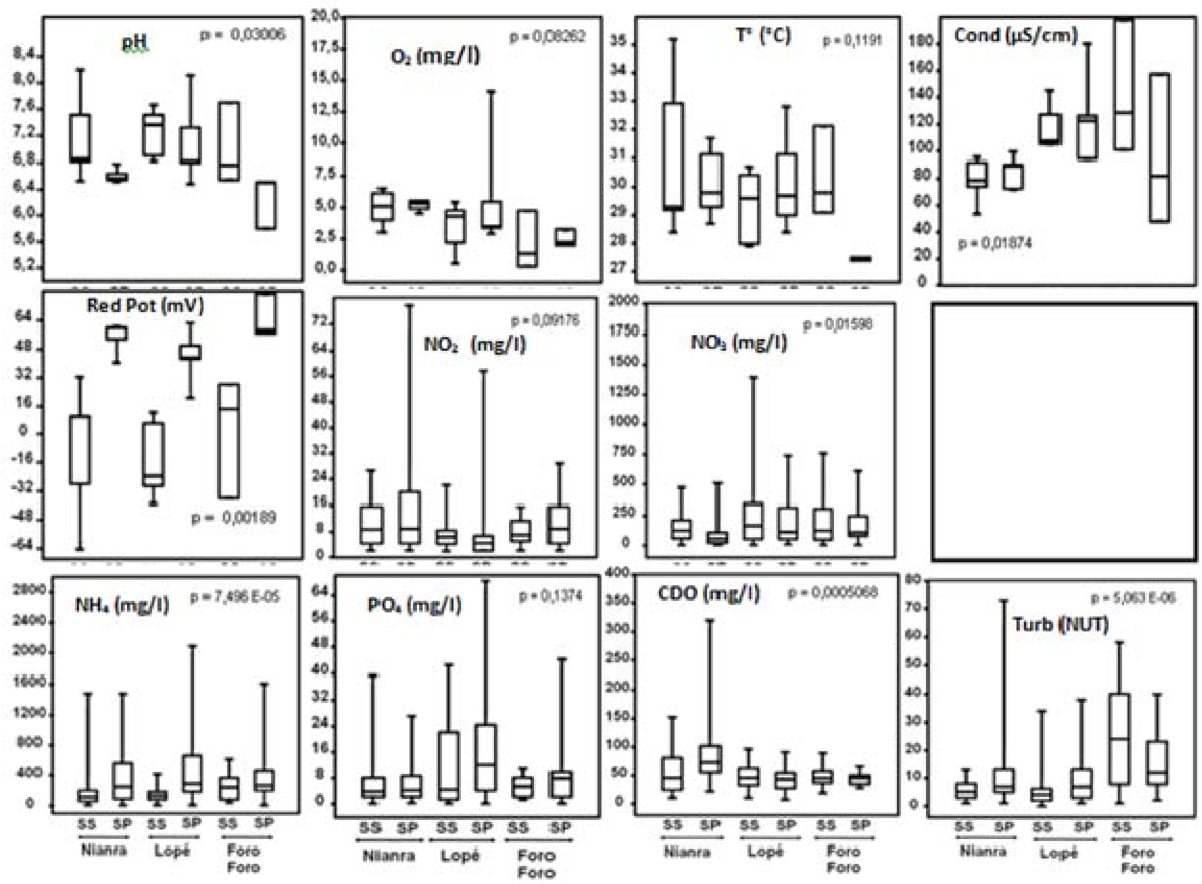

Fig. 2: Box plots of the spatial and temporal variation of environmental parameters.

A total of 13 species of hydrophytes belonging to 9 families were identified in the sampling sites. Indeed, we have the following families: Onagraceae (*Ludwigia abyssinica*, *Jussiaea repens*), Polygonaceae (*Polygonum lanigerum*, *P. salicifolium*), Nymphaeceae (*Nymphaea lotus*), Marantaceae (*Thalia welwitschii*), Poaceae (*Sacciolepis africana*, *Setaria longiseta*), Cyperaceae (*Cyperus exaltatus*, *Rynchospora corymbosa*), Pterydophite (*Cyclosorus dentatus*), Salviniaceae (*Salvinia nymphellula*) and Thyphaceae (*Typha australis*). They are distributed unevenly within the perimeters, areas and sampling sites (Table 2).

Table 2: Inventory and spatial distribution of hydrophytes in the sampling sites. + = present, **-** = absent.

Species	Nianra	Lopé	Foro Foro
Ludwigia abyssinica	+	+	+
Polygonum lanigerum	+	+	+
Nymphaea lotus	-	+	+
Polygonum salicifolium	+	+	+
Thalia welwitschii	+	-	-
Sacciolepis africana	+	-	-
Setaria longiseta	+	+	+
Cyperus exaltatus	+	-	-
Rhynchospora corymbosa	+	-	-
Cyclosorus dentatus	+	-	-
Jussiaea repens	-	+	+
Salvinia nymphellula	-	+	+
Typha australis	-	-	-

3.2 Freshwater snail diversity

Nine species were found among the 6049 collected snails in the 13 visited sites during the 156 surveys (Table 3). Abundances are higher at Lopé (3457), Nianra (2020) compared to Foro Foro (572). At Lopé, we note a high abundance in ponds (2493) while at Foro-Foro and Nianra, lakes have provided the largest number of specimens with respectively 1236 and 432 individuals. At specific level *B. pfeifferi* (2140) and *M. tuberculata* (1743) are best represented and constitute more than half of the specimens collected in contrast to *B. truncatus* (14) and *A. marmorata* (80) which are poorly represented. We have noted that *P. africana* is subservient to the ponds and is harvested only at Nianra and Lopé. Among them, four species of human schistosome transmitting snails were collected in all sites: *Biomphalaria pfeifferi* (2140), *Bulinus globosus* (410), *B. forskalii* (184) and *B. truncatus* (14). *B. pfeifferi* was the most largely distributed. Five species were non-human schistosome transmitting snails and belong to five different families: Planorbidae with *Indoplanorbis exustus* (318), Lymnaeidae with *Lymnaea natalensis* (776), Physidae with *Aplexa marmorata*. (80), Ampullaridae with *Pila africana* (384), and Thiaridae with *Melanoides tuberculata* (1743). The most represented species were *M. tuberculata* and *L. natalensis*

Table 3: Species and number of snails collected in 13 sampling sites.

Species	Nianra			Lopé			Foro Foro			Total
	Lake	pond	canal	Lake	pond	canal	Lake	pond	canal	
Human schistosome transmitting snail										
Biomphalaria pfeifferi	779	282	94	233	435	16	272	11	18	2140
Bulinus globosus	73	22	3	175	11	21	103	0	2	410
Bulinus forskalii	3	15	4	2	49	8	10	61	32	184
Bulinus truncatus	0	0	0	0	11	3	0	0	0	14
Non-human schistosome transmitting snail										
Indoplanorbis exustus	211	19	4	27	40	6	11	0	0	318
Aplexa marmorata	1	5	12	0	39	0	9	6	8	80
Lymnaea natalensis	148	130	23	102	293	52	27	0	1	776
Pila africana	12	124	42	0	167	39	0	0	0	384
Melanoïdes tuberculata	9	3	2	4	1448	276	0	0	1	1743
Total	**1236**	**600**	**184**	**543**	**2493**	**421**	**432**	**78**	**62**	**6049**
TOTAL	**2020**			**3457**			**572**			**6049**

3.3 Prevalences of larval trematode infections

The results of the prevalences of schistosomes and other digeneans in snails are presented in Table 4. None of the following species, *Bulinus truncatus* and *B. forskalii* were found naturally infected. Among the five species tested, *Bulinus globosus*, *Biomphalaria pfeifferi* and *Lymnaea natalensis* shed parasites. Only low intensity shedding by 36.04% of the snails was observed and the prevalence of infected snails was overall 4.63% (101 infected snails out of 2180 tested). The corresponding values were respectively 12.17%, 1.77% and 10.71% for *Bulinus globosus Biomphalaria pfeifferi and Lymnaea natalensis*. It is clear, this result confirm that the area is endemic to parasitic diseases.

Table 4: The total number of snails tested during our experience and the number of each species that were infected.

Species	Snails tested	Snails infected	Natural prevalence (%)
Biomphalaria pfeifferi	1409	25	1,77
Bulinus globosus	189	23	12,17
Bulinus forskalii	75	0	0,00
Bulinus truncatus	12	0	0,00
Lymnaea natalensis	495	53	10,71
Total	**2180**	**101**	**4,63**

4. Discussion

This study provides a large-scale analysis on environmental parameters and freshwater snail diversity in Katiola.

Regarding environmental parameters, they show little seasonal variations. The pH is near neutral in accordance with studies conducted by [10, 11, 12]. And the values are slightly higher compared to those measured in small dams in northern Côte d'Ivoire [13]. This is due to nitrogen fertilizers used on the perimeters and watershed dams. The temperature varies between 27.4 and 35.2 °C as in Nigeria [11] and there is more than 35 °C in small dams in northern Côte d'Ivoire [14]. Temperatures are measured on the surface of the water and therefore are strongly influenced by those of air generally observed in this region. The waters are poorly oxygenated according to measurements made by [14] in the north, south-east [8, 15] and Nigeria [11]. The conductivity is relatively low in lakes and ponds compared to channels, due to the load of dissolved salts of lotic environments while crossing [16]. They are weak as in small dams in the north (<120 µS/cm) and show no trace of salinisation [15]. The redox potential indicates low recirculation of reducing compounds [8]. Study sites have a wide range of nutrient concentrations (nitrite: 2-16 mg/l; nitrate: 6-250 mg/l; ammonium: 5400 mg/l and phosphorus: 0.11-10 mg/l). Overall, these concentrations are relatively low compared to the high exposure to anthropogenic effects. These environments are small, shallow and extremely susceptible to various noise sources [13].

Aquatic vegetation consists of thirteen species of which four are ubiquitous (*Setaria longiseta*, *Ludwigia abyssinica*, *Polygonum salicifolium* and *Polygonum lanigerum*). These hydrophytes influence the distribution of snails [8, 12] and have a direct relationship with abundance of pulmonates [17, 18, 19, 20]. [21, 6, 11] showed that *Ludwigia* indicate the presence of *B. globosus*. In Senegal, [18] reported that *B. truncatus* and *B. forskalii* are often associated with *Nymphaea sp*. They are most often the source of food, nesting sites, potential refuges against predators and supports for snails.

Nine species were found of which four are known as human schistosome transmitting snails: *B. forskalii*, *B. globosus*, *B. truncatus* and *B. pfeifferi*. Thus, we collected 3524 snails intermediate hosts of parasites representing 58.26% of the total identified snails. At the specific level, *B. pfeifferi* is the most ubiquitous and the most abundant (60.73% of total), in contrast to results obtained by [14] in small dams in the north. Bulinids are less abundant (17.25%), because of competition observed between *B. globosus* and *B. truncatus* [22]. Among them, *B. globosus*, is dominant (67.43%) and *B. truncatus* most rare (2.30%). *Lymnaea natalensis*, is the second species in abundance with 22.02%, it colonizes all permanent water bodies especially lakes and ponds.

At Katiola, our results showed a prevalence of 4.63% in accordance with the values reported by [14] in three northern small dams. It's slightly higher than 1.06% prevalence measured by [23] in Cameroon. However, it is still low compared to those (22-46%) obtained by [18] in Senegal. These low rates are due to the fragility of infected snails [24]. Note that *B. globosus* issued cercariae with the highest prevalence (12.17%), while *B. pfeifferi* has a low rate (1.77%) despite its high density. These results put Katiola as a whole at risk for all the *Schistosoma* species belonging to the *haematobium* group. The health risk is also for animals because the prevalence of parasites was higher in *L. natalensis* (10.71%) totaling the second largest abundance.

However, none of *Bulinus forskalii* and *B. truncatus* collected were found naturally infected by *Schistosoma* larvae. The intervention *B. forskalii* in the transmission of human schistosomiasis has not been shown in Côte d'Ivoire [14]. This lack of cercariae in *B. forskalii* was also observed in Cameroon [25]. *B. truncatus* is a cosmopolitan species with a preference for stagnant and sunny environments [14]. The lack of issuance of parasite is related not only to its low density but also its irregular presence and his temporary habitat.

5. Conclusion

In conclusion, this paper presents a large-scale freshwater snail survey and focal contemporary information on larval trematode infection in Katiola. The health risk exists in all seasons, except Foro-Foro which consist on temporary ponds and artificial lakes. Further researches are needed to get a large-scale schistosome survey in humans and to identify the active transmission sites in order to build strategies for control activities. Also, it is important to know the real impact of ecological sanitation on the transmission of parasites in this endemic area.

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