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Ecological and Genetic Insights into *Schistosoma haematobium* Transmission in Bamako, Mali: Snail Host Density, Cercarial Shedding, and Molecular Profiling

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Abstract: Background: Urogenital schistosomiasis remains a major public health concern in sub-Saharan Africa, particularly in West Africa. Although traditionally considered a rural disease, ecological conditions and human–water interactions facilitate its transmission in urban areas. This study aimed to assess the ecological and genetic factors influencing *Schistosoma haematobium* transmission in Bamako, the capital of Mali. **Methods:** Malacological surveys were conducted at two sites in the capital district, Taliko and Missabougou, over a 12-month period from July 2024 to June 2025. Snail abundance, relative density, and infection rates were determined. Water physicochemical parameters, including temperature, conductivity, and pH, were recorded. Genotypic analysis of cercariae shed daily (24 h) by the snails was performed using PCR. **Results:** *Bulinus truncatus* was the predominant intermediate host in Taliko, with high densities (up to 27 snails/collector/15 min) and infection rates ranging from 10% to 45%, peaking in May 2025. In Missabougou, snail densities and infection rates were very low (<1%). Cercarial shedding exhibited a diurnal pattern with a peak between 10:00 a.m. and 2:00 p.m. All genotyped cercariae were pure *S. haematobium* strains, indicating that there is no genetic variation associated with the time of shedding. Months with high snail infection coincided with elevated temperatures (27.9–30.6 °C), conductivity levels ⩾99 μS/cm, and pH values > 6, suggesting that the water's physicochemical conditions were suitable for snail survival and parasite development. **Conclusion:** The physicochemical parameters of water and ecology and geography influenced snail density and infection rates. These findings could guide snail control strategies, while longitudinal monitoring improves our understanding of schistosomiasis transmission dynamics across seasons.

Keywords: *Schistosoma haematobium*; water physicochemical parameters; *Bulinus truncatus*; cercariae genotype; Mali

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1. Introduction

Schistosomiasis remains one of the most widespread poverty-related neglected tropical diseases (NTDs) globally. It disproportionately affects individuals living in disadvantaged communities with limited access to potable water or adequate sanitation facilities [1,2]. An estimated 253.8 million people worldwide are affected, including 134.8 million school-aged children and 119 million adults [3]. According to WHO, disease-related mortality ranges between 1.7 and 4.5 million deaths [4]. It is further estimated that 91% of people requiring preventive chemotherapy for schistosomiasis reside in Africa [5]. Transmission is water-dependent and occurs following the infestation of humans or animals by flatworms of the genus *Schistosoma* [6]. The life cycle of the parasite involves two obligatory hosts: the definitive host (humans), where sexual reproduction occurs, and the intermediate host (freshwater snails), where asexual reproduction takes place, including the development and transformation of miracidia into cercariae. The latter are responsible for human infection after their release into freshwater [7].

In Africa, the intermediate host snail species of major medical importance involved in the transmission of human urogenital schistosomiasis belong to the family Planorbidae and the subfamily Bulininae, which comprises the single genus *Bulinus* spp. This genus includes 37 species classified into four groups: the *B. africanus* group (e.g., *B. globosus*), the *B. forskalii* group (e.g., *B. forskalii* and *B. senegalensis*), the *B. truncatus/tropicus* group (e.g., *B. truncatus*), and the *B. reticulatus* group (e.g., *B. reticulatus* and *B. wrighti*) [8]. Several studies in West Africa have demonstrated the involvement of *Bulinus truncatus* in the transmission of urogenital schistosomiasis in Senegal, notably in the Senegal River Basin and the Niakhar region [9–11], as well as in the Niger Valley (Niger). Other species such as *Bulinus senegalensis*, *B. globosus*, and *B. umbilicatus* have also been implicated in transmission in the Fatick–Niakhar region of Senegal, while *Bulinus forskalii* has been reported as a natural host in irrigated areas of Niger [12].

In Mali, schistosomiasis occurs in two forms: the intestinal form caused by *Schistosoma mansoni* and the urogenital form caused by *Schistosoma haematobium*. The latter is found across nearly the entire country, with an average prevalence estimated at approximately 38.3% [13–15], largely due to the widespread distribution of its two principal intermediate hosts, *Bulinus truncatus* and *B. globosus* [16,17]. Although *B. forskalii* and *B. senegalensis* have been collected (notably in the Dogon Plateau region and at the Office du Niger), they have not been shown to sustain active transmission [17,18]. The presence of *B. umbilicatus* has only been reported in Mopti, central Mali, in a pond named “Pague” adjacent to the town (*Dabo, oral communication*).

Several studies have demonstrated that abiotic parameters such as temperature, pH, conductivity, turbidity, and dissolved oxygen play a key role in the biology of *Bulinus* populations

and thereby in the distribution of *S. haematobium* [1,19]. Beyond these, the epidemiology of schistosomiasis is also strongly influenced by ecological and anthropogenic factors [20]. In Mali, the climate is characterized by an alternation between a rainy season (June–October) and a long dry season (November–May), which strongly influences the abundance and distribution of snail. Rainfall promotes the formation of temporary ponds, rivers, and lakes favorable to intermediate host proliferation; however, during the dry season, human–water contact is concentrated around permanent water bodies [21]. High temperatures recorded between March and June further exacerbate these risks, reinforcing the seasonality of *S. haematobium* transmission [9,10] through increased exposure of local populations to contaminated water.

Ecological changes resulting from the presence of water bodies influence the survival, dispersal, distribution, and abundance of both cercariae and intermediate host snails. This is particularly true in the district of Bamako, in which numerous tributaries of the Niger River cross the city, including Sogoniko, Bankoni, Farako, and Woyowayanko [22]. Indeed, this site has already been the subject of several studies reporting overall prevalence levels of up to 18.2% for *S. haematobium*, with local prevalence reaching as high as 34.4% in Taliko [19,22]. Beyond their mere presence, the population dynamics of urogenital schistosomiasis intermediate host snails are strongly shaped by environmental, climatic, and anthropogenic factors, which in turn condition the risk of disease transmission. We hypothesized that, despite its urban context and ongoing control efforts, the transmission of urogenital schistosomiasis persists in Bamako due to favorable ecological conditions that sustain intermediate host snail populations and cercarial shedding. In this context, a better understanding of snail population dynamics, environmental drivers, and cercarial diversity is essential to inform targeted control strategies. In line with the World Health Organization (WHO) goal to eliminate schistosomiasis as a public health problem and interrupt transmission by 2030, this study aimed to assess (i) the seasonal and temporal variation in intermediate host snail populations of urogenital schistosomiasis and the physicochemical parameters of water, and (ii) the diversity of cercarial species shed in Bamako.

2. Methods

2.1. Study Site

The study was conducted in Missabougou (Commune VI) and Taliko (Commune IV). Bamako covers an area of 267 km^2 and is irrigated by the Niger River and several of its tributaries (Figure 1). The city lies within the north Sudanian climatic zone, characterized by two main seasons. The rainy season, extending from June to October, is marked by intense precipitation events, often in the form of thunderstorms and runoff at the beginning and end of the season, with an annual average rainfall of approximately 878 mm. The dry season, lasting from November to May, is characterized by high temperatures, with an annual average close to 35 °C. The neighborhoods of Taliko, in the western part of the city, and Missabougou, in the east, were chosen as study sites due to their endemicity for schistosomiasis [22]. Taliko is traversed by a tributary of the Niger River, Woyowayanako, while Missabougou is crossed by a canal diverted from the Niger to irrigate rice fields in Baguineda, a village located 30 km away. Both water bodies serve simultaneously as collectors of rainwater runoff and habitats for intermediate host snails (Figure 2).

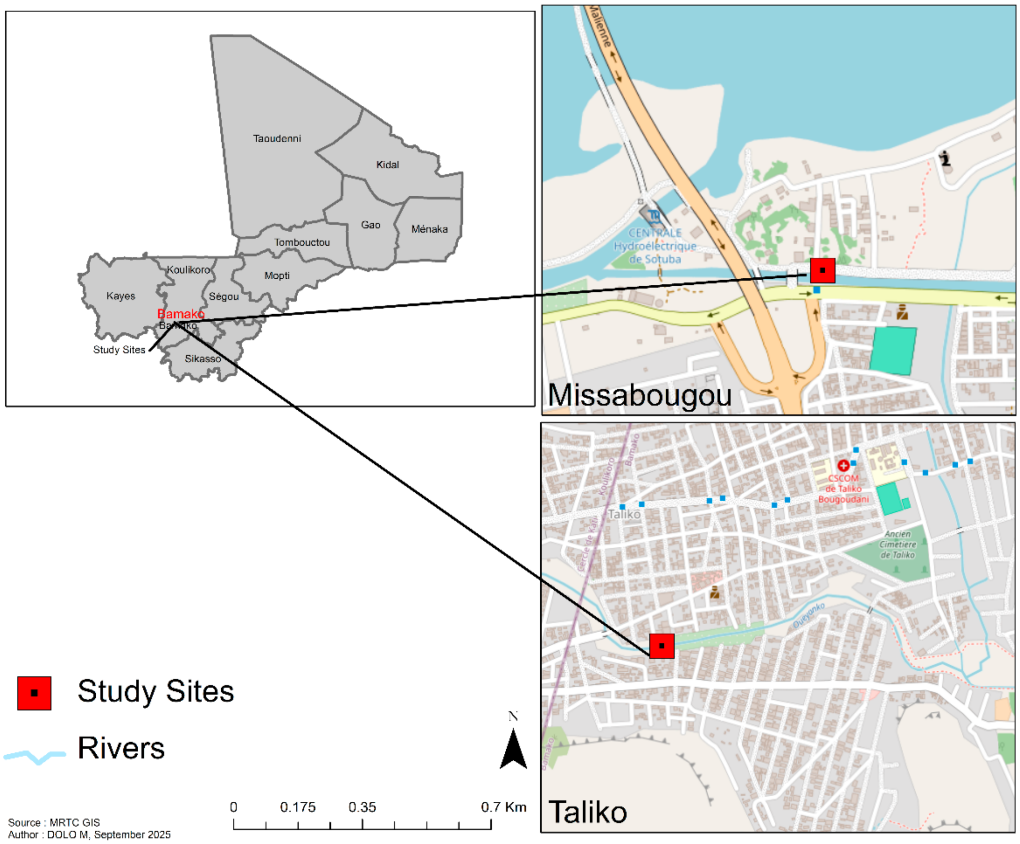

Figure 1: A map of Mali showing the localization of snail collection sites (MRTC_GIS, 2024).

Figure 2: Representative images of human–water contact sites and snail collection points. (**A**) Irrigation canal in Missabougou; (**B**) irrigation canal in Taliko.

2.2. Type and Period of the Study

A prospective study was conducted with monthly cross-sectional surveys over a 12-month period, from July 2024 to June 2025.

2.3. Sampling of Water and *Schistosoma haematobium* Intermediate Host Snails in Mali

Monthly sampling was conducted to collect both water for physicochemical measurements and urogenital schistosomiasis intermediate host snails in Taliko and Missabougou (Figure 1). Sampling was performed between 08:00 and 11:00 a.m., following previously described procedures [1]. Collected water samples were immediately covered with aluminum foil and transported to the Parasitology Laboratory of the Parasites & Microbes Research and Training Center (PMRTC) for analysis, although some parameters, such as temperature, were measured on-site. The variables recorded included water temperature (°C), conductivity (μS/cm), and pH, as well as the morphology of snails (genus *Bulinus*) using species identification keys. Water samples for physicochemical analyses were collected in 1.5 L plastic bottles and stored at 2–8 °C in a cooler box. All samples were processed in the laboratory on the same day of collection.

2.4. Water Physicochemical Properties

Three physicochemical parameters were measured monthly in three water samples collected at each site: water temperature, pH, and conductivity. Water temperature was measured on-site. In the laboratory, the pH of water samples was measured using a Jenway 3520 pH Meter (Bibby Scientific Ltd., Stone, UK). Electrical conductivity of water samples was measured using a YSI EC300F Conductivity Meter (Yellow Springs, OH, USA). The data on water physicochemical parameters were gathered simultaneously with snail surveys. These data were used to determine the effect of climatic factors, water physicochemical parameters, seasons, and habitats on snail density, as well as infection rates.

2.5. Snail Sampling and Identification

Snails were collected at human–water contact points (HWCPs) where the population engage in recreational and domestic activities, including watering of animals. Freshwater snail sampling was carried out by two experienced and well-trained field collectors using long-handheld scoops, forceps, and handpicking for 15 min. As the scoop was pushed through vegetation, snails were picked out of the scoops by hand using gloves and forceps and were placed in plastic containers with water and vegetation from the same habitat to prevent friction against container walls. They were then transported in a cooler with moistened towels to the PMRTC mollusquarium for further processing [23]. The geographic locations of collection sites were mapped using portable differential GPS units (Trimble Navigation Ltd., Sunnyvale, CA, USA) with an estimated accuracy of ± 1 m. Data were downloaded with differential correction into a GPS database using GPS Pathfinder Office 2.8 (Trimble Navigation Ltd., Sunnyvale, CA, USA), and spatial analyses were performed with ArcView version 9.2 (Environmental Systems Research Institute, Inc., Redlands, CA, USA). In the laboratory, snails were cleaned to remove algae and other debris. They were identified morphologically at the species level using the standard identification keys according to the *Field Guide to African Freshwater Snails* from the Danish Bilharziasis Laboratory [24]. Differentiation between *Bulinus truncatus* and *B. globosus* was based on shell truncation and microsculpture observations.

2.6. Determination of Relative Density (RD)

After sorting snails by species, the relative density of *Bulinus* spp. was calculated using the following formula [23]:

$$RD = \frac{Number\ of\ snails\ collected\ in\ 15\ minutes}{Number\ of\ collectors}$$

2.7. Determination of Natural Infection Rate (NIR) of Snails

The natural infection rate of snails was determined using the cercarial shedding method. The snails from positive groups were then placed individually into small cups (10 cm × 5 cm) filled to one-third with well water or water from their habitat. The cups containing snails were exposed to sunlight for 1–4 h. During exposure, the water in each cup was examined every 10 minutes under a stereomicroscope (Motic led-SMOS0181, Motic China Group Co., Ltd., Xiamen, China) to detect the first emerging cercariae. Once a positive snail was identified, exposure was terminated, and the snail was returned to the mollusquarium in a covered tank lined with black fabric for subsequent chronobiological assays. Schistosome cercariae were identified by their bifurcated tails curved backward and their rapid, vigorous vertical swimming motion.

The natural infection rate was calculated using the formula [23]:

$$NIR\,(\%) = \frac{Number\ of\ snails\ positive\ for\ *Schistosoma\ spp*100}{Total\ number\ of\ snails\ exposed}$$

Snails that did not shed on the first exposure were kept and re-exposed to sunlight after 48 h to induce cercariae shedding. If they still did not shed cercariae, they were transferred to their original habitats.

2.8. Cercarial Chronobiology

Cercarial chronobiology is defined as the rhythm of cercarial emergence from snails at specific time intervals or "time slots." In this study, chronobiology was assessed by counting the number of cercariae released by infected snails at two-hour intervals. Snails were exposed to light for 24 h, divided into 12 consecutive 2-h intervals. After each interval, the water from the cups was filtered through a Nytrel filter, which was then placed on a microscope slide and covered with a drop of Lugol's iodine. Cercariae were counted under a light microscope at 40× magnification [23]. The cercarial emission rate per snail was calculated for each time interval as the proportion of cercariae released during the interval relative to the total number of cercariae released over all 12 intervals.

2.9. Molecular Analyses

Cercariae were individually collected according to their site of origin under a stereomicroscope using a P10 micropipette with 3 µL of water. Care was taken to ensure that only a single cercaria was pipetted at each collection. Collected cercariae were deposited onto FTA cards (pre-gridded, 5 mm × 6 mm), which allow for DNA preservation at room temperature. For each snail, a total of fifteen (15) cercariae were collected and deposited onto FTA cards (GE Healthcare Life Sciences; Amersham, UK) and stored at ambient temperature in moisture-proof zip-lock bags until molecular analyses [25]. Genomic DNA was extracted individually from FTA® card discs using a Chelex-based protocol, as previously described [25]. Genetic profiling of the cercariae was performed by targeting the mitochondrial *Cox1* marker using Rapid Diagnostic (RD)-PCR and the nuclear *ITS/18S* markers using the Amplification Refractory Mutation System (ARMS)-PCR. The final parasitic profile was obtained by combining the results from the different molecular markers [23]. Indeed, RD-PCR generates two profiles (Sh and Sb/Sc) based on band sizes (120 bp or 260 bp) on agarose gel. It should be noted that RD-PCR does not allow for discrimination between *Sb* and *Sc*. In contrast, ARMS-PCR produces more complex profiles (4 to 6 bands) and enables discrimination among *Sc*, *Sb*, *Sh*, as well as all hybrid combinations. The combination of species assignment based on RD-PCR profiles (mitochondrial gene) and ARMS-PCR profiles (nuclear genes) allowed for the identification of either pure genotypes (*Sh_Sh* × *Sh*, *Sc_Sc* × *Sc*, *Sb_Sb* × *Sb*) or hybrid genotypes (*Sb_Sb* × *Sh*, *Sb_Sb* × *Sc*, *Sh_Sh* × *Sb*, *Sh_Sh* × *Sc*, *Sc_Sc* × *Sh*, and *Sc_Sc* × *Sb*).

2.10. Statistical Analysis

Descriptive statistics, including means and standard deviations, and the construction of graphs were performed using Microsoft Excel (version 2007) and SPSS (IBM, version 25.0). All physicochemical measurements were conducted in triplicate. Non-parametric analyses (Kruskal–Wallis or Wilcoxon tests) were performed in R software to compare mean values of the different physicochemical parameters across months and sampling sites. Percentages of infected snails per human–water contact site and cercariae emitted per hour per snail were calculated. Snail infection rates were computed using SPSS (IBM, version 25.0). A significant threshold of $p < 0.05$ was applied.

3. Results

3.1. Water Physicochemical Parameters

The water parameters varied across months and between sites. The water temperature ranged from 18.2 ± 0.42 °C to 31.2 ± 0.35 °C at both sites, with the lowest temperatures recorded in January 2025 and the highest in July 2024 ($p = 0.81$) for the study period. The pH varied between 5.1 ± 0.14 and 9.1 ± 0.14, with the minimum observed in July 2024 and the maximum in October 2024 at both sites. Water conductivity ranged from 60.6 ± 0.51 µS/cm to 159.2 ± 0.76 µS/cm. The lowest values were observed in December 2024 in Taliko (65.5 ± 0.78 µS/cm) and in February 2025 in Missabougou (52.7 ± 0.42 µS/cm) ($p = 0.52$), while the highest values were recorded in February 2025 in Taliko (159.2 ± 0.76 µS/cm) and in May 2025 in Missabougou (159.2 ± 0.76 µS/cm) ($p = 0.006$) (Table 1).

Table 1: Monthly variation in water physicochemical parameters at Taliko and Missabougou sites.

	Temperature (°C)			pH			Conductibility (µS/cm)		
Month	**Taliko**	**Missabougou**	***p***	**Taliko**	**Missabougou**	***p***	**Taliko**	**Missabougou**	***p***
July 2024	30.5 ± 0.71	31.2 ± 0.35	0.81	5.1 ± 0.14	5.9 ± 0.21	0.52	100.3 ± 0.64	95.7 ± 0.42	0.006
August	28.5 ± 0.71	28.2 ± 0.42		6.1 ± 0.14	6.1 ± 0.14		95.1 ± 1.01	90.3 ± 1.06	
September	25.0 ± 0.50	24.9 ± 0.14		9.0 ± 0.07	8.9 ± 0.21		96.4 ± 0.57	80.0 ± 0.28	
October	24.0 ± 1.48	24.4 ± 092		9.0 ± 0.07	9.1 ± 0.14		79.6 ± 0.57	80.5 ± 0.71	
November	20.5 ± 0.50	19.0 ± 0.07		7.9 ± 0.14	8.1 ± 0.14		70.5 ± 0.71	62.0 ± 1.41	
December	18.7 ± 0.49	18.8 ± 0.26		7.1 ± 0.14	6.1 ± 0.14		65.5 ± 0.78	60.6 ± 0.51	
January 2025	18.6 ± 0.14	18.2 ± 0.42		6.3 ± 0.14	6.1 ± 0.35		109.0 ± 1.48	57.7 ± 0.52	
February	18.9 ± 0.21	19.0 ± 0.07		6.9 ± 0.31	6.2 ± 0.14		159.2 ± 0.76	52.7 ± 0.42	
March	30.6 ± 0.47	28.0 ± 0.07		6.9 ± 0.36	6.5 ± 0.71		99.3 ± 0.67	62.7 ± 0.58	
April	29.7 ± 0.64	29.0 ±0.64		7.1 ± 0.07	6.8 ± 0.25		100.4 ± 0.85	60.1 ± 1.10	
May	27.9 ± 0.32	27.2 ± 0.76		7.7 ± 0.28	7.8 ± 0.28		100.2 ± 0.76	99.3 ± 0.64	
June	24.4 ± 0.51	23.9 ± 0.14		6.4 ± 0.14	6.6 ± 0.14		91.1 ± 1.01	89.5 ± 0.50	

3.2. Density of Snail and Human Schistosome Intermediate Hosts in Mali

The relative density of snails varied across months and between sites. The mean density was 27 snails/person/15 min in Taliko and less than 1 snail/person/15 min in Missabougou. Densities ranged from 0 to 124 snails/person/15 min, with the highest recorded in February 2025 in Taliko. Two snail species were collected at both sites over the 12-month study period. Bulinus truncatus was the predominant species, with 476/645 individuals in Taliko and 5/8 in Missabougou. The highest snail densities were observed in February 2025 at both sites (Table 2).

Table 2: Monthly distribution of snail species in Taliko and Missabougou.

Month	Taliko			Missabougou		
	Total Snails Collected	*B. truncatus*	*B. globosus*	Total Snails Collected	*B. truncatus*	*B. globosus*
July 2024	0	0	0	0	0	0
August	0	0	0	0	0	0
September	0	0	0	0	0	0
October	1	1	0	0	0	0
November	0	0	0	0	0	0
December	0	0	0	0	0	0
January 2025	96	69	27	0	0	0
February	248	186	62	8	5	3
March	84	66	18	0	0	0
April	84	71	13	0	0	0
May	124	76	48	0	0	0
June	8	7	1	0	0	0
Total	645	476	169	8	5	3

3.3. Natural Infection Rate (NIR) of Snails

The natural infection rates (NIRs) of *Bulinus* spp. varied monthly, ranging from 10% to 45.2%. Naturally infected snails were collected from March to May 2025 in Taliko, with the highest NIR observed in May (45.2%). In Missabougou, only one snail was infected out of eight collected, corresponding to an NIR of 12.5% in February 2025 (Table 3).

Table 3: Natural infection rates (NIRs) of *Bulinus* spp. collected in Taliko and Missabougou.

Month	Taliko		Infected *Bulinus*			Missabougou		Infected *Bulinus*		
	Total *Bulinus*	*Bulinus* spp.	*B. truncatus*	*B. globosus*	*TIN (%)*	Total Bulinus	*Bulinus* spp.	*B. truncatus*	*B. globosus*	*TIN (%)*
July 2024	0	0	0	0	0	0	0	0	0	0
August	0	0	0	0	0	0	0	0	0	0
September	0	0	0	0	0	0	0	0	0	0
October	1	0	0	0	0	0	0	0	0	0
November	0	0	0	0	0	0	0	0	0	0
December	0	0	0	0	0	0	0	0	0	0
January 2025	96	0	0	0	0	0	0	0	0	0
February	248	0	0	0	0	8	1	1	0	12.5
March	84	8	6	2	9.5	0	0	0	0	0
April	84	8	5	3	9.5	0	0	0	0	0
May	124	56	39	17	45.2	0	0	0	0	0
June	8	0	0	0	0	0	0	0	0	0
Total	645	72	50	22	11.1	8	1	1	0	13

3.4. Physicochemical Parameters and Snail Dynamics

The physicochemical parameters associated with the natural infection rates (NIRs) of snail species varied across months and sites. Infected snails were observed during March, April, and May in Taliko, when water temperatures ranged from 27.9 °C to 30.6 °C. In Missabougou, infected snails were recorded in February, when the temperature was around 19 °C. High water conductivity ($\geqslant$99 µS/cm) and elevated pH values (>6) were also observed during months with higher NIRs (Table 4).

Table 4: Effects of monthly variation in physicochemical parameters on natural infection rates (NIRs) of snails in Taliko and Missabougou.

Passage	Taliko						
	Temperature (°C)	pH	Conductibility	Total Bulinus	*B. truncatus*	*B. globosus*	*TIN*
July 2024	30.5	5.1	100.3	0	0	0	0
August	28.5	6.1	95.1	0	0	0	0
September	25	9	96.4	0	0	0	0
October	24	9	79.6	1	0	0	0
November	20.5	7.9	70.5	0	0	0	0
December	18.7	7.1	65.5	0	0	0	0
January 2025	18.6	6.3	109	96	0	0	0
February	18.9	6.9	159.2	248	0	0	0
March	**30.6**	6.9	99.3	84	6	2	9.5
April	**29.7**	7.1	100.4	84	5	3	9.5
May	**27.9**	7.7	100.2	124	39	17	45.2
June	24.4	6.4	91.1	8	0	0	0
Total				645	50	22	11.1
	Missabougou						
July 2024	31.2	5.9	95.7	0	0	0	0
August	28.2	6.1	90.3	0	0	0	0
September	24.9	8.9	80	0	0	0	0
October	24.4	9.1	80.5	0	0	0	0
November	19	8.1	62	0	0	0	0
December	18.8	6.1	60.6	0	0	0	0
January 2025	18.2	6.1	57.7	0	0	0	0
February	19	6.2	99.3	8	1	0	12.5
March	28	6.5	62.7	0	0	0	0
April	29	6.8	60.1	0	0	0	0
May	27.2	7.8	52.7	0	0	0	0
June	23.9	6.6	89.5	0	0	0	0
Total				8	1	0	13

3.5. Cercarial Emission Patterns

Figure 3A–C show the mean daily peaks of cercarial emissions (circadian rhythm) of *Bulinus* spp. snails collected in Taliko (Bamako). Cercarial shedding occurred at different times across the months. A diurnal emission pattern was observed only for snails collected during March, April, and May 2025, with a peak occurring at midday. Two variants of diurnal emission were identified: *(i)* a diurnal pattern with minor late emissions in March, and *(ii)* a diurnal pattern without late emissions in April and May 2025. Minor late emissions (7%) were observed between 4 and 6 p.m. In March, snails emitted cercariae between 8 a.m. and 6 p.m. (Figure 3A), with a peak of 37.4% from 10 a.m. to 12 p.m., followed by a second peak of 32.7 % from 12 to 2 p.m. Emission levels remained low ($\leqslant$15%) during the early morning (8:00–10:00) and late afternoon periods (4–6 p.m.), with minor late emissions (7%) recorded between 4 and 6 p.m. In April, emissions occurred between 8 a.m. and 4 p.m., with a peak of 35.8% from 10 a.m. to 12 p.m., followed by another peak of 30.2% from 12 to 2 p.m. (Figure 3B). Outside these peak periods, emission levels were comparatively low, not exceeding 20%. In May 2025, emissions were observed between 08 a.m. and 2 p.m., with a single peak of 53.8% from 10 a.m. to 12 p.m. (Figure 3C). However, the emission rates observed from 8 to 10 a.m. and from 12 to 2 p.m. were less than or equal to 30%. No early-morning (6–8 a.m.) or evening (6–8 p.m.) cercarial emissions were observed in any for the *Bulinus* spp. samples collected during these three months in Bamako. No snails in our study released cercariae between 8 p.m. and 06 a.m. The only snail that shed cercariae in Missabougou died immediately after the cercarial shedding test (prior to chronobiology

assessment). Therefore, we were unable to perform the chronobiology test or molecular genotyping of cercariae from Missabougou.

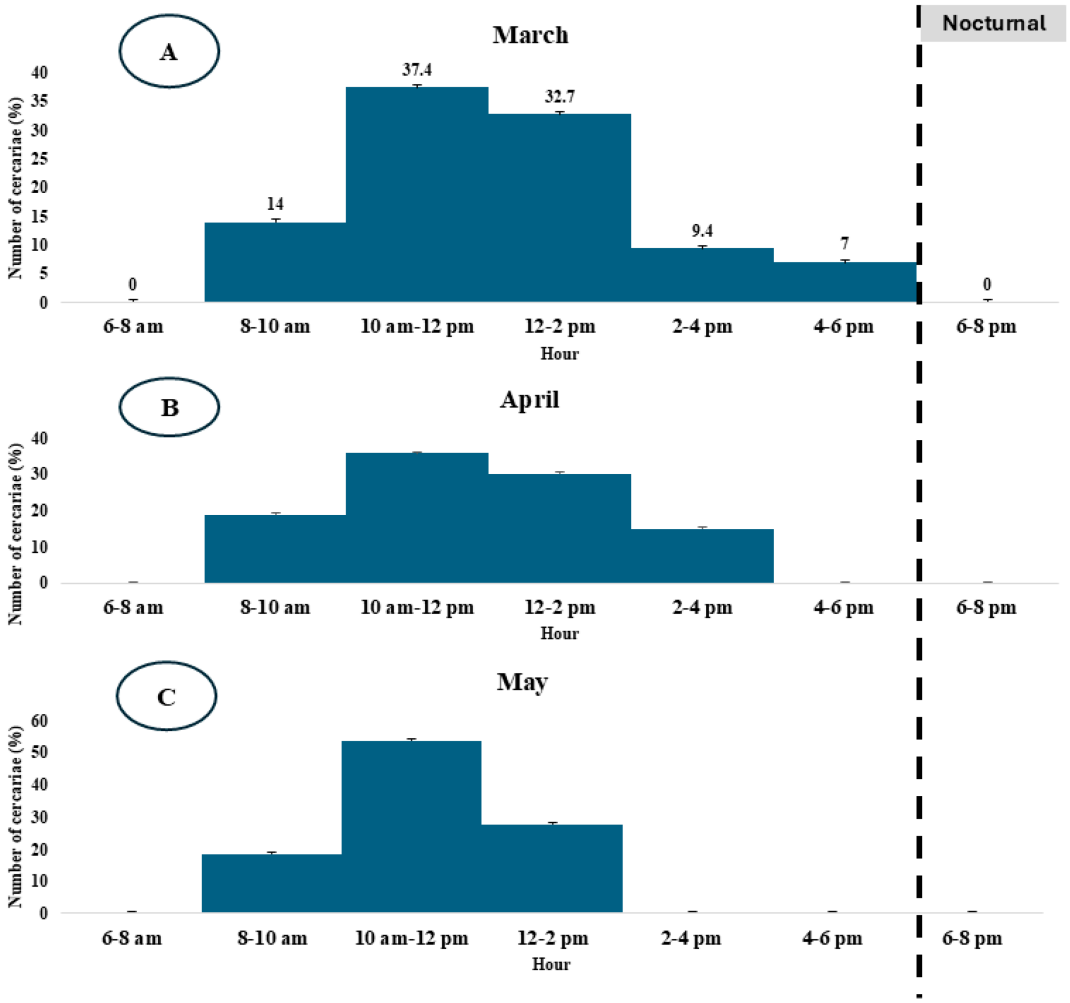

Figure 3: Cercarial emission patterns of *Schistosoma haematobium* from naturally infected *Bulinus* spp. in Taliko, shown according to month (March, April, and May 2025). Images representing the different months of chronobiology are labeled with the letters (**A**) (March), (**B**) (April), and (**C**) (May).

3.6. Genetic Profile of Cercariae Emitted by *Bulinus* spp.

The genetic profiles of cercariae emitted by snails during the three months (March, April, and May 2025) are presented in Table 5. Only pure *S. haematobium* profiles (*Sh_Sh* × *Sh*) were observed regardless of the emission time or month in Taliko. The highest number of genotyped cercariae was recorded in May 2025 (57.1%) (p = 0.0001).

Table 5: Proportion of pure *Schistosoma haematobium* cercariae (*Sh_Sh* × *Sh*) emitted by *Bulinus* spp. in the Taliko neighborhood of Bamako in 2025.

Month	*Sh_Sh* × *Sh*
March	10 (14.3)
April	20 (28.6)
May	40 (57.1)
Total	70

4. Discussion

This study aimed to understand the temporal variation in the populations of snails, which are the intermediate hosts involved in the transmission of urogenital schistosomiasis in Mali. Our results showed both spatial and temporal variations in the physicochemical parameters of the water from the Niger River tributaries in Bamako. The water temperature ranged from 18.2 to 31.2 °C, with a mean of 24.5 °C across the two sites over the twelve-month study period. These temperatures were slightly lower than those reported in Benin [1]. The pH ranged from 5.1 in July 2024 to 9.1 in October 2024, with an overall mean of 7.05. The pH values observed in our study were higher than those reported in Benin [1,26], confirming the basic nature of the waters in the Niger River tributaries.

Electrical conductivity ranged from 52.7 μS/cm in Missabougou to 159.2 μS/cm in Taliko in February 2025, indicating a variation in the mineral content of freshwater in Bamako. These values fall within the low to moderate range typically observed for non-saline surface waters and were comparable to regional measurements. Analyses of the Niger River in Bamako have highlighted substantial spatial and seasonal variability in water quality, with conductivity influenced by urban inputs and seasonal flow [26]. The value observed in Missabougou (52.7 μS/cm) reflects low mineralization, consistent with waters relatively unaffected by dissolved inputs, whereas the value in Taliko (159.2 μS/cm) falls within the range reported for sites subjected to moderate anthropogenic influence or local mineral inputs (≈150–200 μS/cm) in field studies across West Africa [27,28]. These local differences may result from a combination of factors, including geological variations, domestic or agricultural effluents, and hydrodynamic differences between sampling points.

The relative density of snails, which serve as intermediate hosts of urogenital schistosomiasis in Mali, exhibited temporal and spatial variation. The mean densities observed in Taliko and Missabougou were 27 snails/person/15 min and less than 1 snail/person/15 min, respectively, with a minimum of 0 and a maximum of 124 snails/person/15 min recorded in Taliko. These contrasting patterns are consistent with observations reported elsewhere in West Africa (Niger, Mauritania, and Côte d'Ivoire), where the density of *Bulinus* and other intermediate host snails varies greatly between sites and across seasons. Studies using similar sampling techniques have reported both very-low-density sites and "hotspots" with high densities [6,29,30].

In Niger, systematic surveys along the Niger River Valley have reported substantial collections of *Bulinus* at certain sites during monthly sampling, reflecting local aggregations that could sustain transmission with significant proportions of infected snails [6]. Similarly, in Côte d'Ivoire and Mauritania, follow-up studies in urban and peri-urban contexts have shown that snail counts per collector/15 min could vary according to domestic inputs, habitat availability (aquatic vegetation, substrate), and hydrological fluctuations [29,30].

These analogies support the conclusion that Taliko represents a site with relatively high abundance at the time of sampling (indicating high local transmission potential), whereas Missabougou presents less favorable conditions for host aggregation. Our findings confirm that schistosomiasis, classically described as a rural disease, is also present in urban areas of Bamako, consistent with reports of expanding transmission foci in rapidly urbanizing cities [19]. The marked heterogeneity of snail densities between Taliko and Missabougou illustrates the existence of

micro-hotspots, as previously reported in surveys conducted across the six municipalities of Bamako, where urinary schistosomiasis was found to be endemic in over 70% of schools. The immediate proximity of snail breeding sites and human activities constitute major risk factors, explaining the persistence of localized foci despite the area being in an urban environment [19].

In our study, *Bulinus truncatus* was predominantly collected in Taliko (476/645) and was extremely scarce in Missabougou (5/8), with continuous presence from January to May 2025 in Taliko, whereas in Missabougou, snails were detected only in February. These results are consistent with observations from N'Djamena (Chad), where *B. truncatus* exhibited marked seasonal fluctuations, with higher densities during the dry season or immediately following short rains [31]. Similarly, in Tiko (Cameroon), *Bulinus* spp., including *B. truncatus*, showed greater abundance during low-flow periods, while their density decreased during rainy or flood seasons [32].

These consistent patterns suggest that Taliko provides prolonged favorable ecological conditions for *B. truncatus* over several months, including suitable substrate, water availability, vegetation, and limited disturbances, while Missabougou appears to offer a less favorable habitat for snail development. This could be explained by the development of the banks of the Niger River in Missabougou (Figure 2A). In contrast to the predominance of *B. truncatus* observed in our study, *Bulinus globosus* plays a major role in South Africa (Ingwavuma, KwaZulu-Natal), where it accounted for nearly half of the collected snails and was widely distributed across sites [33]. These inter-regional differences highlight the importance of the ecological and climatic context in shaping intermediate host snail populations. Indeed, in Mali, hydrological and physicochemical conditions appear to favor *B. truncatus*, whereas in southern Africa, *B. globosus* occupies a dominant ecological niche and contributes more substantially to the transmission of *S. haematobium*.

The natural infection rate (NIR) observed in our study, ranging from 10 to 45%, with a peak of 45% in May 2025, falls within the ranges reported in several studies across West Africa. In Senegal, a study conducted in Niakhar reported infection rates of 15–42% for *B. senegalensis* and *B. umbilicatus*, depending on the locality [34]. In contrast, a meta-analysis covering multiple African countries reported lower mean infection rates for *Bulinus* spp., approximately 12% for *B. globosus* and 5–6% for *B. truncatus*, noting that these studies used different detection techniques (molecular assays or cercarial shedding tests) [35]. This contrast suggests that ecological conditions in Taliko in May 2025, including high snail abundance, favorable temperature, water availability, and human–water contact, were particularly conducive to infection. The meta-analysis also showed that detection by PCR revealed substantially higher infection rates (26.7%) compared to that found using simple cercarial shedding observation (4.5%) [35]. The use of molecular methods in our context likely contributed to the very high NIR observed. The difference between our elevated rate and lower rates reported elsewhere underscores the importance of seasonality, host density, and aquatic vegetation in the local dynamics of transmission.

In our study, variations in the infection of *Bulinus* spp. appeared to be strongly influenced by the physicochemical parameters of the water. Infected snails were mainly observed during the warmest months in Taliko (March–May, temperatures ranging from 27.9 to 30.6 °C) and, more rarely, in Missabougou in February at a lower temperature (19 °C). This observation is consistent with several studies showing that temperature not only influences snail population dynamics but also affects the development of intermediate hosts of *Schistosoma* [36]. Indeed, elevated temperatures (25–30 °C) favor *Bulinus* reproduction and cercarial shedding, whereas temperatures below 20 °C slow down the parasite's life cycle [37].

Our results also show that periods of snail infection coincided with relatively high conductivity values ($\geqslant$99 µS/cm) and pH levels above 6. This is consistent with observations from Benin and Côte d'Ivoire, where *Bulinus* proliferation was correlated with slightly mineralized waters exhibiting sufficient conductivity and mildly alkaline pH [1,38,39]. Similarly, in southern Africa, a study reported that moderate conductivity and pH values provide favorable conditions for snail survival and parasite transmission [33]. The monthly and spatial variations observed in Bamako may be explained by an interaction between local climatic conditions (seasonal temperature) and water physicochemical

characteristics, which together influence both snail population dynamics and their susceptibility to *Schistosoma* infection.

In accordance of the significant impact of water conductivity on snail development, previous laboratory and field studies have shown that conductivity can influence the survival and reproduction of snails, the intermediate hosts of schistosomiasis, as well as the development of *Schistosoma* parasites [32,40–42]. Higher conductivity values may indicate greater nutrient availability for snails, promoting their growth and reproduction [40]. The conditions observed in this study suggest an environment conducive to the development of intermediate hosts and *Schistosoma*, which could increase the risk of schistosomiasis transmission in the Niger River tributaries in Bamako.

Cercarial shedding patterns of *Bulinus* spp. collected in Taliko (Bamako) showed a diurnal peak between 10 a.m. and 2 p.m., with lower emissions observed between 4 and 6 p.m. This contrasts with the findings of Sidibé et al. (2024) in western Mali, who reported peaks in the early morning (6–8 a.m.) and late afternoon (6–8 p.m.) [23]. Our results are consistent with Fryer and Probert (1988), who observed similar cercarial shedding patterns in *Bulinus* infected with *Schistosoma haematobium* in Nigeria [43]. These findings suggest that cercarial shedding patterns can vary depending on local conditions and human–water interactions specific to the site. The observed differences may be attributed to factors such as local water use habits, site ecological characteristics, and the snail species.

The genetic profiles of collected cercariae showed the presence of pure *S. haematobium* (*Sh_Sh* × *Sh*) regardless of site, time, or month of shedding. The majority of genotyped cercariae were observed in May 2025 (57.1%, $p = 0.0001$), suggesting a seasonal intensification of transmission, possibly related to more favorable environmental conditions for snails and parasite development. These results are consistent with Sidibé et al. (2024) in Mali, where cercarial density and shedding patterns varied according to human–water interactions and seasonal timing, with midday peaks for pure *S. haematobium* [23].

The absence of genetic variation according to shedding time indicates that, unlike hybrid schistosomes (*S. bovis/S. curassoni* × *S. haematobium*) [23,44], the transmission of pure *S. haematobium* strains may be less influenced by circadian rhythm and more by environmental factors such as temperature, pH, and water conductivity, which favor snail survival and infection [40].

Thus, the combination of genetic and ecological data highlights that seasonality and water physicochemical conditions are key determinants of the transmission dynamics of urogenital schistosomiasis in Bamako. These insights are crucial for effectively targeting control interventions, particularly during peak cercarial shedding months. However, this study has certain limitations. First, it was conducted at only two sampling sites, which limits the generalization of the findings to all urban and peri-urban areas of Mali. Second, the observation period, restricted to twelve months, does not account for interannual climatic variability that may influence snail population dynamics and schistosomiasis transmission. Finally, the absence of parasitological analyses on a broader range of snail species and the lack of complementary molecular tests may have limited the detection of potential co-infections or schistosome hybrids.

Interestingly, only pure genotypes of *Schistosoma haematobium* were identified in this study, with no evidence of hybrid forms, despite the increasing reports of *Schistosoma haematobium* × *S. bovis* or *S. curassoni* hybrids in West Africa. Several hypotheses may explain this observation. First, ecological factors specific to Bamako, such as the limited contact between livestock and water at the sampled sites, may reduce opportunities for interspecific transmission and hybridization. Second, the sampling design, which targeted specific sites and periods, may have limited the detection of rare hybrid genotypes. Third, although the combination of RD-PCR and ARMS-PCR is robust, it may have limited sensitivity for detecting rare or complex hybrid forms when present at low prevalence or resulting from successive hybridization events.

However, this study has certain limitations. Our sampling strategy followed standard malacological survey protocols (15 min timed collections per collector), but no formal sample size calculation or statistical power analysis was performed prior to the study. In addition, the number of sampling

sites was limited to Bamako. Therefore, the findings should be interpreted with caution in terms of representativeness, as they may not fully capture the heterogeneity of all transmission settings in Bamako or other urban areas. Nevertheless, repeated monthly sampling over a 12-month period strengthens the reliability of the temporal trends observed in this study. A major limitation of this study is the absence of parasitological or epidemiological data on human infection. As a result, it was not possible to directly correlate snail infection rates and cercarial shedding profiles with the prevalence or intensity of infection in the human population. Such data are essential to confirm transmission intensity and to establish epidemiological links between environmental dynamics and human infections.

5. Conclusions

This study provides data on the comprehensive assessment of ecological and genetic factors influencing the transmission of urogenital schistosomiasis in Bamako, Mali. Our results show that *Bulinus truncatus* is the predominant intermediate host, with marked spatial and temporal variations, infection rates reaching up to 45%, and diurnal cercarial shedding patterns. Water conductivity significantly influenced snail populations and parasite development, while genetic analysis confirmed the presence of pure *S. haematobium* strains. The integration of ecological and molecular data highlights the importance of seasonality and local environmental conditions in shaping transmission dynamics. These findings provide essential information for effectively targeting control interventions, particularly during periods and at sites of peak transmission, to reduce the burden of urogenital schistosomiasis in urban settings. In-depth studies on snail population dynamics over multiple seasons will allow a better description of the impact of variations in mollusk density and infestation in the context of climate change.

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