Journal of Helminthology

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Research Paper

Cite this article: Nana PA, Tchakonté S, Pahane Mbiada M, Fotseu Kouam AL, Mouchili Palena RS, Bricheux G, Nola M and Sime-Ngando T (2024). Influence of tides on the dissemination and related health risks of intestinal helminths along the Kribi beaches (Atlantic Coast, Southern Cameroon). *Journal of Helminthology*, **98**, e10, 1–8 https://doi.org/10.1017/S0022149X24000026

Received: 26 November 2023
Revised: 03 January 2024
Accepted: 03 January 2024

Keywords:
Tide; Kribi beaches; faecal pollution; intestinal helminths; health risk exposure

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Influence of tides on the dissemination and related health risks of intestinal helminths along the Kribi beaches (Atlantic Coast, Southern Cameroon)

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Abstract

Kribi is a seaside town that welcomes thousands of tourists each year. However, the poor sanitation condition of its beaches along the Atlantic coast is not without risk for visitors. In this study, we used the formol-ether concentration technique to identify and quantify larvae or eggs of intestinal helminths in waters of three regularly visited Kribi beaches (Mpalla, Ngoyè, and Mboamanga). Results revealed that all identified larvae and eggs were cestodes (*Hymenolepis nana*) and nematodes (*Strongyloides* sp., *Ascaris* sp., *Ancylostoma duodenale* and *Trichuris trichiura*). All the helminth eggs and larvae showed high abundance at low tide during rainy seasons. *Ancylostoma duodenale* eggs, totally absent at Mpalla, were densely present at low tide at Ngoyè (301 ± 15 eggs/L). *Trichuris trichiura* eggs showed the lowest abundance (0 to 62 eggs/L) at all sites. Abiotic variables indicated that waters at the various beaches were basic (pH: 8.75–9.77), generally warmer (32.44°C at Mpalla in the Short Rainy Season), more oxygenated at low tide, and moderately mineralized at high tide. Positive and significant correlations were observed at Ngoyè at low tide between *Strongyloides* sp. larvae and dissolved oxygen ($P < 0.05$); and between *Ancylostoma duodenale* eggs and temperature ($P < 0.05$). The overall results indicated that the beaches studied are subjected to fecal pollution. This pollution is more accentuated during low tides than during high tides. Depending on tidal movements, swimmers risk exposure to helminth eggs and larvae known to be responsible for gastroenteritis.

Introduction

Microbiological pollution represents one of the major problems to which coastal and marine environments are subjected (Dang & Lovell 2016; Basili *et al.* 2021; Oduro *et al.* 2023). It refers to the presence of microbial organisms in these ecosystems, such as bacteria, viruses, or parasites, some of which may be pathogenic to humans or animals (Nimnoi & Pongsilp 2020). Although marine and coastal ecosystems are the natural environment for some microorganisms, those involved in microbiological contamination of coastal waters are of human or animal origin (Rodríguez *et al.* 2021; Manini *et al.* 2022). These are enteric microorganisms, i.e., from the intestines of humans or warm-blooded animals and brought into the environment via their excreta. Sources of this excreta include discharges of treated and untreated sewage on land and from ships' ballast water, livestock effluents (animal faeces), stormwater discharges, rainfall-runoff, and other diffuse sources (Assako Assako *et al.* 2010; Manini *et al.* 2022).

The concentration and dissemination rate of these organisms depends on tidal range, rainfall, turbidity, and hydrodynamics, among other factors (Di Biase & Hanssen 2021). Tides, approximately two highs and two lows per day, generate and influence ocean currents (Madani *et al.* 2020). In turn, these currents directly and indirectly affect the movement of aquatic fauna (seedlings, fish) and the dispersion of microbes. Like the tide, winds, underwater topography, and weather conditions influence the dispersal of microorganisms (Ferrarin *et al.* 2021; Kraus *et al.* 2022).

Bacteria and viruses introduced into the marine environment can affect bathing water quality and cause health impacts, which can lead to the closure of the affected areas if the contamination is significant and persistent (Bonadonna *et al.* 2019; Manezeu Tonleu *et al.* 2021). Helminthiasis is one of the most common infections in the world, disproportionately impacting the poorest and most disadvantaged communities (WHO 2006). They are transmitted by eggs in human excreta, which then contaminate soil where sanitation conditions are inadequate (Collender *et al.* 2015; Truscott *et al.* 2016; Walusimbi *et al.* 2023). Bathing in water of poor microbiological quality thus presents health risks and can lead to infections, mainly gastroenteritis caused by helminth eggs or larvae (WHO 2006; Bonadonna *et al.* 2019; Manezeu Tonleu *et al.* 2021). In rare cases, contaminated water can also lead to more serious infectious diseases such as typhoid fever, cholera, etc. (WHO 2019).

In sub-Saharan Africa in general and in Cameroon in particular, microbiological data and standards on beach water are rare and almost non-existent, yet these environments constitute high contamination sites due to their high frequency of use year-round. This poses an ecological and public health problem on Cameroonian beaches in general and those of Kribi in particular. This study investigates the influence of tidal cycles on the diversity and abundance of intestinal helminth larvae and eggs in the waters of Kribi beaches. We hypothesized that dispersion of intestinal helminths would be influenced by the tidal cycle. To evaluate the impact of the tidal cycle on the dissemination of intestinal helminths in the waters of the city of Kribi, Southern Cameroon Region, we qualitatively and quantitatively compared pathogen concentrations at high and low tide on three Kribi beaches (Mpalla, Ngoyè, and Mboamanga).

Materials and methods

Study area

The study was conducted from April to December 2021 on the three most frequented beaches of the city of Kribi, in the Ocean Division, southern Atlantic coast, Cameroon (Figure 1). This area is subject to a Guinean-type equatorial climate, characterized by four seasons: Long Dry Season (LDS) from December to February, Short Rainy Season (SRS) from March to May, Short Dry Season (SDS) from June to July, and Long Rainy Season (LRS) from August to November (Olivry 1986). Four sampling campaigns were conducted: April (SRS), July (SDS), September (LRS), and December (LDS), respectively. At the level of each beach, one sampling station

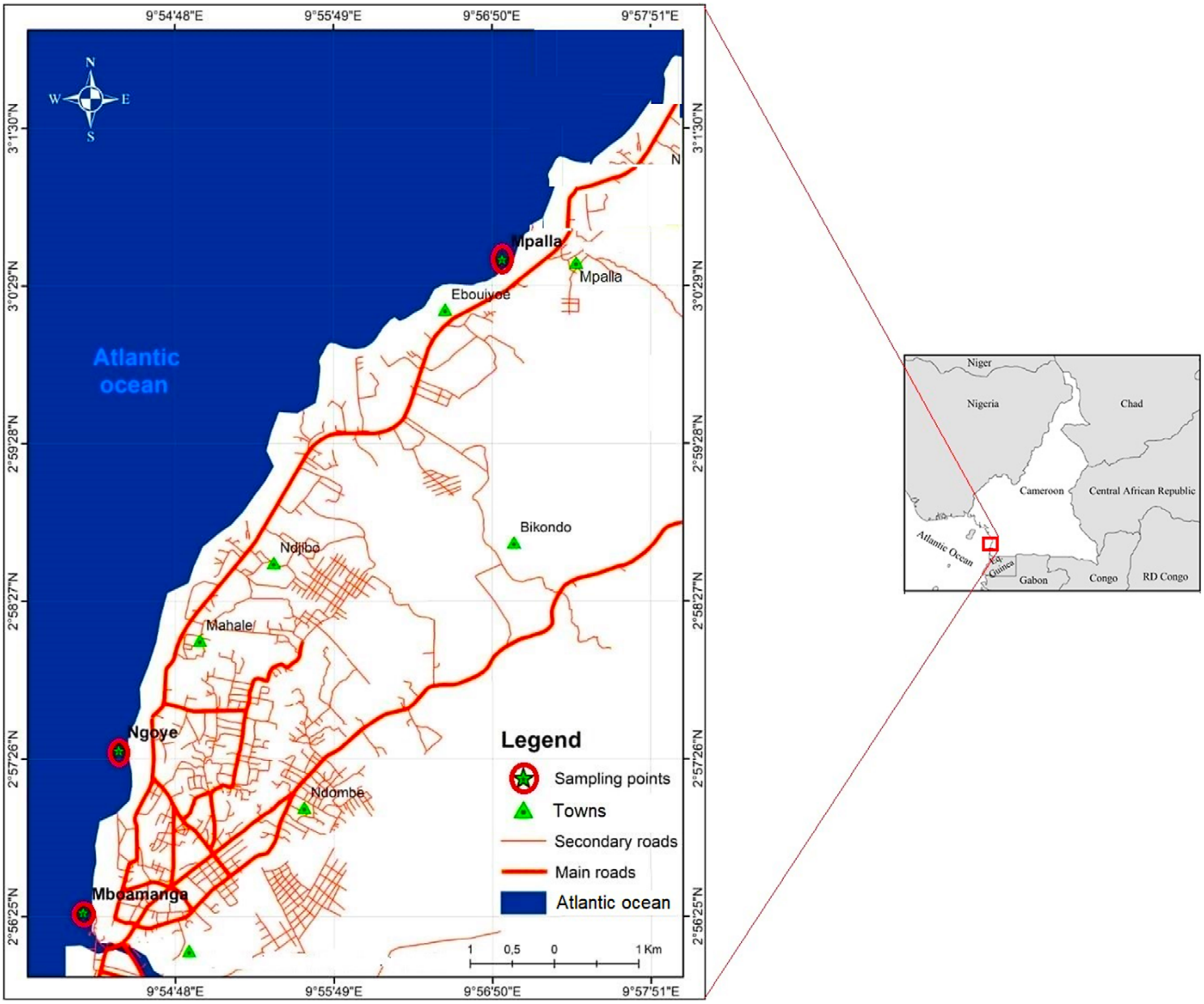

Figure 1. Location map showing sampling points.

was surveyed based on its accessibility and frequentation. Station 1 is located at Mpalla beach (3∘00’29”N–0009∘56’54.5”E) and characterized by a gray sandy substrate. Station 2 is situated at Ngoyè (2∘57’26.6”N–0009∘54’36.9”E), 4 km from Mpalla, and characterized by a black sandy substrate. Located 9 km from Ngoyè, station 3 on Mboamanga beach (02∘56’22.4”N–0009∘54’12.3”) is characterized by a sandy clay gray substrate.

Measurement of hydrodynamic and abiotic parameters

At each tidal cycle, water depth was recorded using a Plastimo ECHOTEST II (Lorient Cedex - France) handheld depth sounder. Current velocity was assessed by float gauging using a limnimetric scale, float, chronometer, and decameter (Ngoma & Wang 2018).

Physicochemical parameters were analyzed according to Rodier *et al.* (2009) and APHA (2017) standard methods. At each campaign and each sampling station, eight physico-chemical parameters were measured *in situ*, in triplicate during each tide period (low and high tide), using a hand-held multiparameter (HANNA/HI98494Tanneries Cedex - France). These variables included pH, temperature (°C), salinity (PSU), dissolved oxygen content (mg/L), total dissolved solids (g/L), electrical conductivity (mS/cm), resistivity (Ω/cm), and pressure (mbar).

Collection and treatment of biological samples

At each station, for each season, water samples were collected at high and low tide in 1000 mL sterile polyethylene bottles. In the laboratory, for the identification and enumeration of helminths, the samples were first left for 24 h at room temperature in the sedimentation flasks. After sedimentation, the supernatant was decanted and the muddy deposit obtained was then measured, homogenized, and distributed in test tubes. The formol-ether concentration technique enabled us to concentrate the helminth eggs or larvae to guarantee better enumeration (Suwansaksri *et al.* 2002; Collender *et al.* 2015). Therefore, in each test tube, 1 mL of 10% formalin, 5 mL of distilled water, and 2–3 drops of Lugol were added. The tubes were then centrifuged at 500 rpm for 5 min using a centrifuge (Medifriger, Barcelona - Spain). Each time, the entire pellet was recovered and placed on slides for direct observation and enumeration of eggs or larvae under an optical microscope (Olympus Model CK2, Hamburg - Germany) (Ajeagah & Fotzeu Kouam 2019). The helminth eggs or larvae were identified using the WHO manual (2019) and the Thivierge workbook (2014). The number of eggs or larvae contained in 1 L of sample was obtained by the following formula, proposed by Ajeagah *et al.* (2014):

$$X = \frac{y.Vx}{Vy}$$

X = number of parasites, Vx = volume of the pellet of 1 L of sample, Vy = volume of the pellet used for observation, y = number of parasites observed in Vy

Data analysis

As the sample concentrations had a normal distribution, the linear correlation coefficient r (Pearson) was used to calculate the dependency between the quantitative variables (biotic and abiotic). SPSS software version 16.0 allowed us to perform correlation tests. P values were used to assess the significance of the correlation between abiotic and biotic parameters. The safety threshold was 5% ($P < 0.05$).

Results

Hydrodynamic and abiotic variables

At the different sites surveyed, the average water depth varied between 0.42–0.83 m at low tide and 1.81–2.10 m at high tide (Table 1). Concerning current velocity values were globally higher at low tide than at high tide. The lowest average current velocity (0.89 m/s) was recorded at high tide, at Ngoyè.

Abiotic parameters varied according to two aspects: time and tidal cycles (Figure 2). At both high and low tide, waters were strongly basic at all beaches across the study period. The highest pH value (9.77) was recorded at Mboamanga in SRS, whereas the lowest (8.75) was recorded in LDS at Mpalla, during high tide (Figure 2A). At high tide, the water temperature varied from 28.16°C in LDS at Ngoyè to 31.75°C in SRS at Mboamanga. At low tide, temperature ranged from 29.34°C in LDS at Mboamanga to 32.44°C in SRS at Mpalla (Figure 2B). At all stations, the warmest waters were recorded at low tide. Spatial and temporal variation of salinity did not differ significantly for any tidal cycle. The minimum salinity (30.87 PSU) was recorded in SRS, at low tide at Ngoyè, and the maximum value (36.19 PSU) in SDS, at high tide at Mboamanga (Figure 2C). The waters of Mpalla and Ngoyè beaches were poorly oxygenated at high tide compared to low tide throughout the study. Maximum values (0.70 mg/L) of dissolved oxygen were recorded in SRS, at high and low tides (Figure 2D). Total dissolved solids changed from 26.39 to 27.45 g/L at high tide and from 24.24 to 27.25 g/L at low tide (Figure 2E).

With values of electrical conductivity ranging from 48.28 µS/cm in LDS to 47.88 µS/cm in LRS at low tide, Ngoyè appeared to be the less mineralized beach (Figure 2F). Concerning resistivity, values were higher at Ngoyè than at other stations, and the maximum value (21 Ω/cm) was recorded at low tide in SRS (Figure 2G). Thus, the more mineralized the waters of the studied beaches were, the more concentrated the ions were, and, consequently, the higher

Table 1. Some hydrodynamic characteristics of the surveyed sites

		Mpalla	Ngoyè	Mboamanga
Depth of water at low tide (m)	Min.	0.32	0.42	0.25
	Max.	0.65	0.94	0.54
	x̄ ± σ	0.61 ± 0.01	0.83 ± 0.01	0.42 ± 0.01
Depth of water at high tide (m)	Min.	1.11	1.33	1.41
	Max.	1.95	2.84	2.64
	x̄ ± σ	1.81 ± 0.06	2.03 ± 0.05	2.10 ± 0.04
Current velocity at low tide (m/s)	Min.	1.88	1.42	1.73
	Max.	2.23	2.59	2.80
	x̄ ± σ	2.02 ± 0.03	1.99 ± 0.02	2.01 ± 0.05
Current velocity at high tide (m/s)	Min.	0.98	0.68	0.97
	Max.	1.34	1.23	1.69
	x̄ ± σ	1.21 ± 0.01	0.89 ± 0.01	1.30 ± 0.02

Min.: minimum; Max.: maximum; x̄: average; σ: standard deviation; N=8 (for each sampling site)

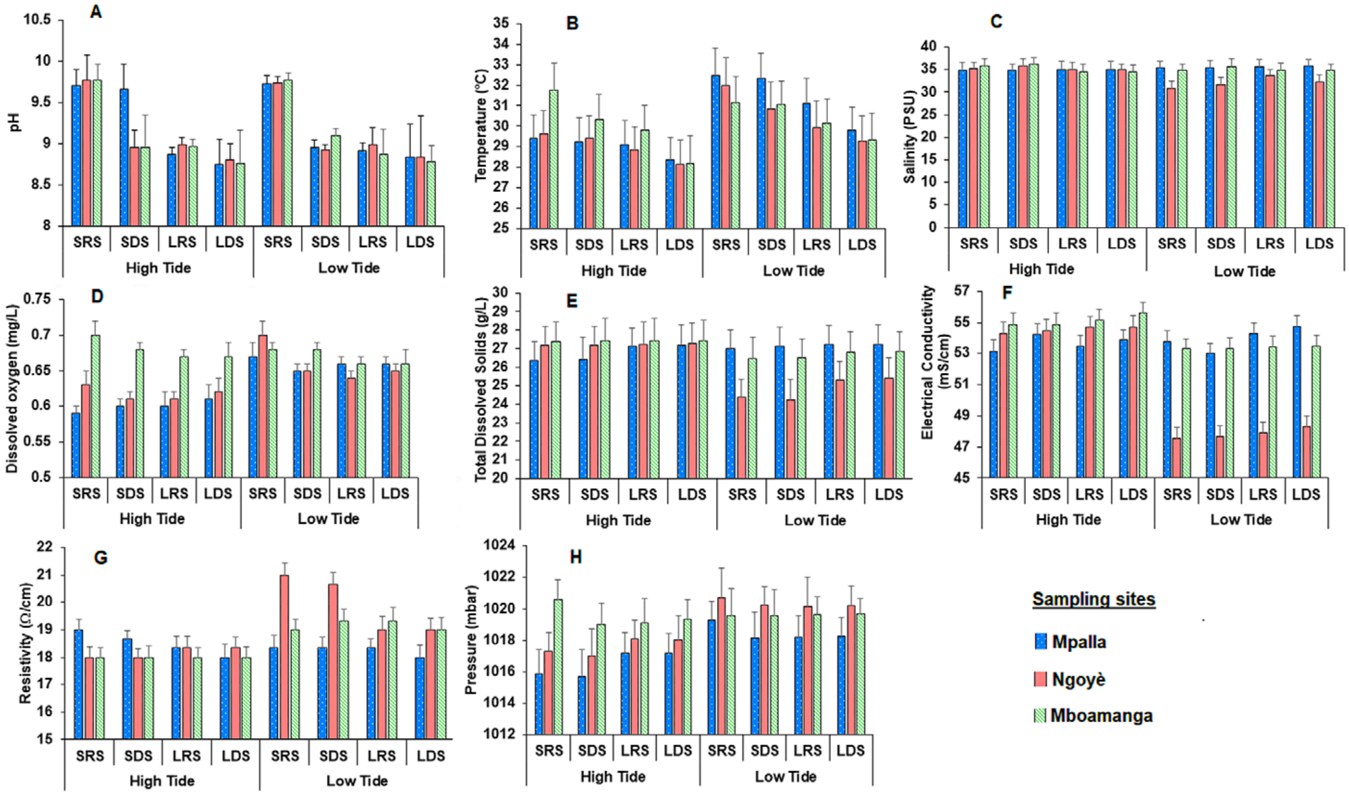

Figure 2. Physicochemical variables according to seasons and tidal cycles. SRS: Short Rainy Season; SDS: Short Dry Season; LRS: Long Rainy Season; LDS: Long Dry Season.

electrical conductivity was. At the same time, the resistivity of these waters was low. Overall, the pressure was slightly lower at high tide than at low tide. Nevertheless, the lowest value (1015.73 mbar) was recorded at high tide in Mpalla in SDS (Figure 2H).

Diversity, distribution, and abundance of helminths

In this study, five species of intestinal helminths were identified in the waters of Kribi beaches. They belong to the Cestode class (*Hymenolepis nana*) and Nematode phylum (*Strongyloides* sp., *Ascaris* sp., *Ancylostoma duodenale* and *Trichuris trichiura*). Their abundances varied from one station to another and especially with tidal cycles (Table 2).

The eggs of *H. nana* were identified at all sites during low tide, while at high tide they were scarce. Maximum concentrations were recorded in LDS at low tide (18 eggs/L) and in SDS at high tide (35 eggs/L) at Ngoyè (Figure 3A). Mboamanga beach was the least contaminated with *H. nana*. Larvae of *Strongyloides* sp. were identified at all the sites sampled. On the beaches of Mpalla, Ngoyè, and Mboamanga, we counted, on average, 15, 34, and 13 *Strongyloides* sp. larvae per liter of water at high tide versus 21, 44, and 26 larvae/L at low tide, respectively. Regardless of the tidal cycle, the waters of Ngoyè had a high concentration of *Strongyloides* sp. larvae in contrast to the other beaches (Figure 3B). Across the study period, the abundance of *Ascaris* sp. eggs was three times higher at low tide than at high tide (Figure 3C). Across all sites, an average of 17 eggs/L was noted at high tide, against 50 eggs/L counted at low tide. During the study period and regardless of the tidal cycle, no *A. duodenale* eggs were identified in Mpalla (Figure 3D). Ngoyè beaches were the most contaminated, with an average of 18 eggs/L counted at low tide and 75 eggs/L at high tide. Unlike the other intestinal helminths, *T. trichiura* showed very low abundance at all the sites surveyed (Figure 3E). At Mpalla beach, a mean value of 3 eggs/L was recorded at high tide against 16 eggs/L at low tide. At Mboamanga, *T. trichiura* eggs were only identified in LDS at low tide (3 eggs/L).

Correlation between physicochemical and biological variables

At all the beaches surveyed, significant correlations were revealed between certain physicochemical and microbiological parameters. At Mpalla, a positive and significant correlation was observed, at low tide, between concentration of *T. trichiura* eggs and total dissolved solids ($r = 0.821$, $P = 0.047$). At Ngoyè, positive and significant correlations were observed, at low tide, between concentrations of *Strongyloides* sp. larvae and dissolved oxygen ($r = 0.781$, $P = 0.039$, and $r = 0.728$, $P = 0.042$); and between concentrations of *A. duodenale* eggs and temperature ($r = 0.836$, $P = 0.041$, and $r = 0.735$, $P = 0.036$). At Mboamanga, at low tide, concentrations of *Strongyloides* sp. and *Ascaris* sp. were positively correlated with temperature ($r = 0.738$, $p = 0.040$, and $r = 0.733$, $P = 0.039$). In contrast, at high tide, concentration of *T. trichiura* eggs was negatively correlated with pH ($r = -0.738$, $P = 0.041$).

Discussion

In Kribi, rivers and beaches are used extensively by the populations (Batanga, Ngoumba, Mabéa, etc.) for bathing, washing dishes, laundry, fishing, and even for traditional ceremonies (Assako

Table 2. Helminth eggs or larvae abundance recorded in the different sites

		Mpalla		Ngoyè		Mboamanga	
		High Tide	Low Tide	High Tide	Low Tide	High Tide	Low Tide
Hymenolepis nana (eggs/L)	Min.	0	15	0	10	0	10
	Max.	5	24	18	35	1	24
	x̄ ± σ	2 ± 1	18 ± 4	11 ± 2	22 ± 2	1 ± 0	14 ± 4
Strongyloides sp. (Larvae/L)	Min.	5	10	20	22	3	11
	Max.	22	48	60	63	32	47
	x̄ ± σ	15 ± 3	31 ± 4	34 ± 3	44 ± 4	13 ± 2	26 ± 2
Ascaris sp. (eggs/L)	Min.	5	28	0	28	0	23
	Max.	37	72	35	75	32	58
	x̄ ± σ	21 ± 5	59 ± 7	21 ± 3	59 ± 8	10 ± 2	34 ± 4
Ancylostoma duodenale (eggs/L)	Min.	0	0	11	42	2	12
	Max.	0	0	28	108	11	32
	x̄ ± σ	0 ± 0	0 ± 0	18 ± 4	76 ± 11	5 ± 1	18 ± 3
Trichuris trichiura (eggs/L)	Min.	0	4	0	4	0	0
	Max.	7	24	1	5	0	3
	x̄ ± σ	3 ± 1	16 ± 2	1 ± 0	5 ± 2	0 ± 0	1 ± 0

Min.: minimum; Max.: maximum; x̄: average; σ: standard deviation; N=8 (for each sampling site)

Assako *et al.* 2010). However, poor management of these aquatic ecosystems can make the water resource dangerous, exposing populations to health risks (Nana *et al.* 2023a).

Globally, helminthiasis is one of the most common infections in the world, disproportionately impacting the poorest and most disadvantaged communities (WHO 2019). These infrections are transmitted by eggs in human excreta, which contaminate soil and water where sanitation conditions are inadequate (Schiefke *et al.* 2006; Walusimbi *et al.* 2023). All the helminth species identified in this study are potentially responsible for human disease. *H. nana* is a cestode responsible for hymenolepiasis. It manifests itself through abdominal pain, nausea, slight emaciation, and anemia in case of massive infestation (Ikumapayi *et al.* 2019; Coello Peralta *et al.* 2023). *Strongyloides* sp. is a nematode responsible for Strongyloidosis or anguillulosis or "cutaneous larva migrans". It causes skin lesions at the point of larval entry, possible inflammatory pulmonary reaction with dry cough during larval migration, enteritis with abdominal pain, and diarrhoea (Schär *et al.* 2013; Lupia *et al.* 2023). *Ascaris* sp. is a nematode, responsible for ascariasis. At the beginning, the disease is manifested by respiratory disorders with fever at 38°C, dry cough, sometimes productive coughing, and breathing difficulties. Later on, digestive disorders, nausea, vomiting, abdominal pain, loss of appetite, and diarrhoea can emerge (Silver *et al.* 2018; Holland *et al.* 2022). *A. duodenale* is a nematode, responsible for hookworm disease or Ankylostomiasis. It manifests itself through itchy skin, followed by a skin rash on the feet and hands, and then bronchitis with coughing fits. It later evolves towards the chronic form with notable digestive and nervous disorders and anemia (Kucik *et al.* 2004; Walusimbi et al. 2023). *T. trichiura* is a nematode responsible for trichuriasis. It is more often benign or asymptomatic. If the parasite is abundant, colic (abdominal pain, diarrhoea) can be complicated by rectal hemorrhaging (Badri *et al.* 2022; Guilavogui *et al.* 2023). These species have been identified on Kribi beaches where tidal conditions had an impact on the quality and abundance of the helminth larvae or eggs. Contamination is favored at low tides by low flows, due to less dilution of water (Madani *et al.* 2020). At the shoreline level, periods of spring tides, due to high tidal coefficients, would be the most favorable for the dilution of pollution (Kraus *et al.* 2022; Nana *et al.* 2023b). These conditions would favor high concentrations of parasites in the water at low tide. Whether in Mpalla, Ngoyè, or Mboamanga, the highest concentrations of *Strongyloides* sp. larvae and *Ascaris* sp. eggs are linked to the great capacity of these parasites to adapt to variations in environmental conditions (Fotseu Kouam & Ajeagah 2019; Manezeu Tonleu *et al.* 2021). In contrast, the low abundance of *T. trichiura* eggs in all the sampled sites could be related to the high salinity of the waters.

It is possible to identify several causes of contamination that can explain the arrival of these faecal microorganisms on the Kribi beaches in lower vs. higher quantities. The contamination from human beings is mainly related to a total absence of wastewater treatment systems in this seaside town. Indeed, the city of Kribi, which has experienced a demographic boom in recent years, has no real wastewater and sewage sludge treatment system (Assako Assako *et al.* 2010). Wastewater treatment plants are non-existent. Naturally, any absence or defect in the collective sanitation system can lead to the discharge of untreated water into the aquatic environment, resulting in the introduction of potentially pathogenic micro-organisms into the natural environment (Basili *et al.* 2021; Rodríguez *et al.* 2021; Oduro *et al.* 2023). Non-sewage facilities can also generate contamination if they do not comply and discharge untreated effluent into the natural environment (Mohammed *et al.* 2012; Soto-Varela *et al.* 2021). Other activities practiced on the Kribian coast could lead to the input of helminths from humans into the environment, particularly recreational activities, especially when boats are inhabited and do not have a sewage recovery system. Faecal pollution due to these activities is mainly located in marinas and fishermen's camps. Contamination linked

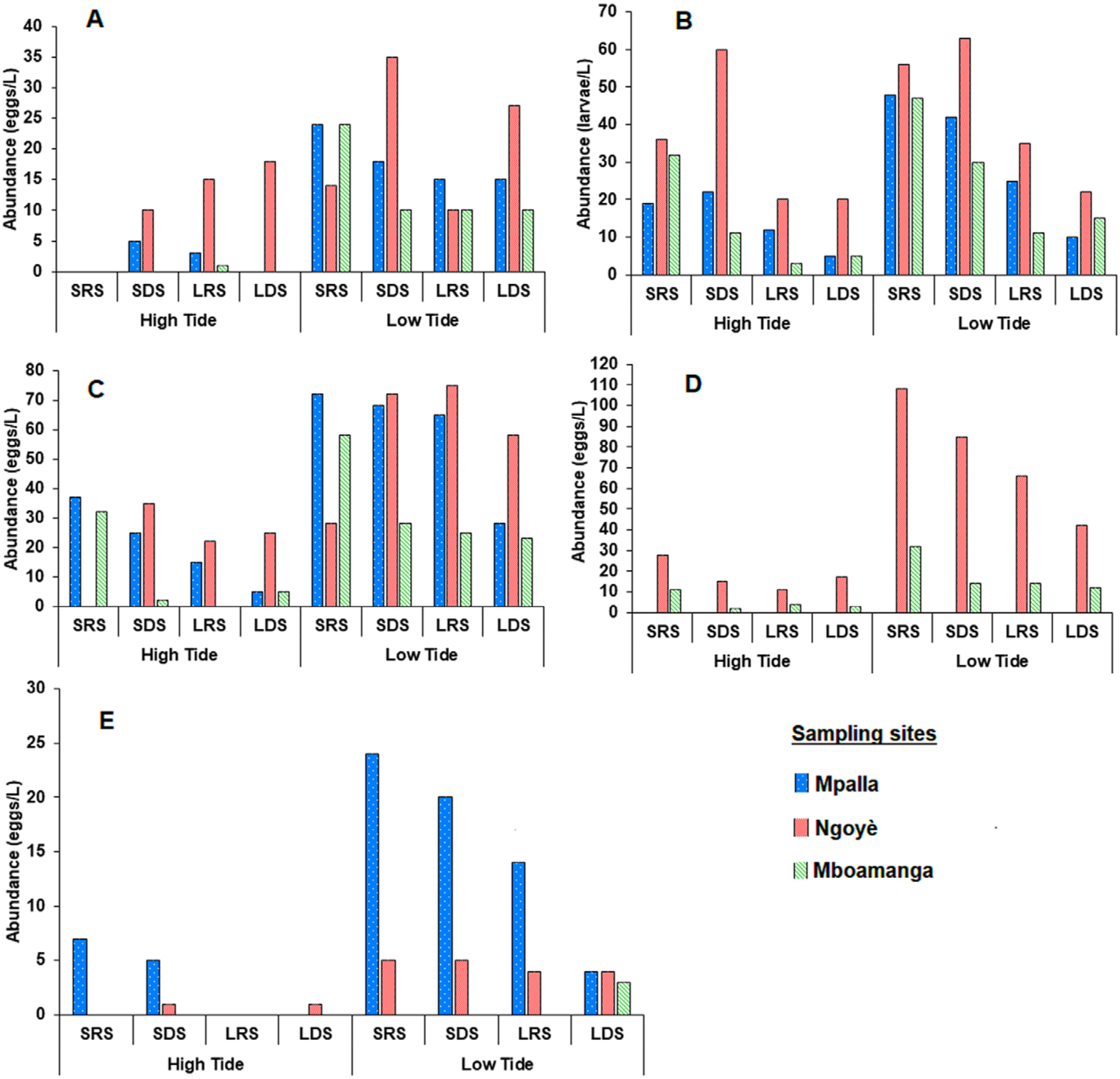

Figure 3. Helminth eggs or larvae abundanceaccording to seasons and tidal cycles. (A) *Hymenolepis nana*; (B) *Strongyloides* sp.; (C) *Ascaris* sp.; (D) *Ancylostoma duodenale;* (E) *Trichuris trichiura.* SRS: Short Rainy Season; SDS: Short Dry Season; LRS: Long Rainy Season; LDS: Long Dry Season.

to recreational activities (swimming, restaurants) remains secondary (Weiskerger *et al.* 2019; WHO 2021).

Rainfall would thus constitute one of the main vectors for the dissemination of helminth eggs or larvae (Manz *et al.* 2017; Di Biase & Hanssen 2021). These contaminations could also be due to industries, especially agri-food industries, if their effluents are not properly treated, or to wildlife, especially poultry (Yahya *et al.* 2017; Edge *et al.* 2021). Indeed, the lack of a solid and liquid waste collection and treatment system in the city of Kribi is conducive to the deposition of sediment, which would promote the development of microorganisms that would be evacuated to the beaches during rainy episodes.

In the city of Kribi, wastewater disposal is generally done individually. That which is produced by households is discharged into the environment. Apart from a few homes that have modern cesspits (about 15%), most people pour their wastewater into the yard or into poorly equipped traditional latrines in the open. The flatness of the land and the presence of multiple geomorphologic depressions result in that domestic wastewater being discharged into nature, obviously without any treatment, creating numerous stagnation points where a string of pathogenic microorganisms, parasites, and infectious disease vectors develop (Assako Assako *et al.* 2010). As for hotels, generally located on the coast, their septic tanks are emptied by private emptying companies from Douala. This waste is taken to the Bois des Singes wastewater dump in Douala, although it is not impossible that some of these trucks are emptied into the rivers (Nyong, Kienké) that cross the Kribi-Douala road (Assako Assako 2005).

Bathing in water of poor microbiological quality, such as that of the Kribi beaches, thus presents health risks and can lead to infections, mainly gastroenteritis, with varying severity depending on the helminths involved and the concentration of helminth eggs or larvae in the medium (WHO 2006; Saingam *et al.* 2020). In rare cases, contaminated water can also lead to more serious infectious diseases such as typhoid, cholera, etc. (WHO 2006; 2021). In addition to the health issue, water contamination by helminth eggs or larvae is also an economic issue, since it can lead to the downgrading, or even the closure, of bathing or recreational fishing areas and thus impact tourism to a greater or lesser extent (Martínez *et al.* 2007).

Conclusion

The main objective of this study was to evaluate the influence of tides on the dissemination of intestinal helminths in the waters of the seaside town beaches of Kribi. It was found that the waters of the beaches surveyed are subject to microbiological pollution because they concentrate large quantities of helminth larvae or eggs. This high concentration, which is more pronounced at low tide than at high tide, has potential public health significance. If the developed countries have been able to set up sanitation systems that are still to be perfected, it should be noted that in African cities, in particular Kribi (Cameroon), the issue of efficient waste management in general and wastewater in particular should represent a major concern for the authorities and the populations. To limit microbiological and parasitic pollution of the Kribi beaches, it is urgent that municipal authorities define and implement an efficient plan for the collection and treatment of solid and liquid waste.

Acknowledgments. Paul Alain Nana thanks Professor Ousmane Traoré of Clermont-Ferrand University Hospital Centre for his logistical support.

Financial support. This research received no specific grant from any funding agency, commercial or not-for-profit sectors.

Competing interest. The authors declare none.

Ethical standard. The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional guides on the care and use of laboratory animals.

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