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Natural diet and feeding habits of the royal spiny lobster, *Panulirus regius* de Brito Capello 1894 in the Gulf of Guinea off Côte d'Ivoire

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Abstract
This study aimed to determine the diet and feeding habits of this species from the eastern coast of Côte d'Ivoire. The specimens were sampled from commercial catches between September 2019 and August 2020. The diet analysis has concerned the stomach of 64 males and 96 females sampled in cold seasons and 72 males and 99 females during the warm seasons. Of the total number of examined stomachs (331), 272 were empty (Coefficient of vacuity: Cv = 82%). This food index (Cv) was higher in male individuals (86.03%) than in female specimens (79.49%). The diet this species consisted mainly of fish, crustaceans, molluscs and detritus (DE). Based on the composition of its diet, the palinurid spiny lobster, *P. regius* could be considered to be an omnivorous species with a preference for animal materials and showed a specialist feeding strategy.

Keywords: Gulf of guinea, feeding habits, spiny lobsters, Palinuridae, *Panulirus regius*

Introduction

Spiny lobsters (Decapoda: Achelata: Palinuridae), one of the most commercially significant groups of decapod crustaceans are found throughout tropical and temperate oceans and supporting subsistence in most areas [1, 2]. The family of Palinuridae, Latreille, 1802 comprises 54 extant species/subspecies arranged in 12 genera [3, 4], with the most speciose being *Panulirus* White, 1847 most diversified with 24 species/subspecies, followed by *Jasus* Parker, 1883 and *Palinurus* White, 1847, each with six species [5, 6, 4, 7, 1]. Geographically, *Palinurus* species are restricted to south east Africa and the north-eastern Atlantic, and generally occur at depths greater than 100 m, whereas those of *Jasus* are distributed exclusively in cold waters of the southern hemisphere [8, 9]. Finally, the species of *Panulirus* are found in shallow tropical and subtropical waters at a depth of less than 100 m of both hemispheres, where the diversity of habitats may have promoted a greater radiation of this genus [10, 11, 12].

The royal spiny lobster, *Panulirus regius* De Brito Capello 1864 is a tropical crustacean and considered by [13] as an Atlantic-Mediterranean species. It is encountered in the western Mediterranean and in the south of the Strait of Gibraltar from Morocco to Angola including the Cape Verde Islands [14, 15]. It lives in rocky areas with agitated and continuously stirred coastal waters. It is most abundant at depths between 8 and 10 m [16] and weakly concentrated at 25-30 m depth [17]. From an economic perspective, this is recognized as the most important crustacean species in the Gulf of Guinea [18, 19, 20].

Little is known about the natural diet of *P. regius* in the Gulf of Guinea [21], but until now, no study has been investigated in this spiny lobster off Côte d'Ivoire. Knowledge of the natural diet in an animal species is essential for studies on its nutritional requirements, its interactions with other organisms and its potential for culture [22, 23]. This work aimed to study the natural diet of the spiny lobster, *P. regius* to better understand the general feeding habits of this commercially valuable resource.

Materials and Methods

Study area

The Ivorian coastal waters are influenced by Guinea current [24, 25, 26, 27] with a short cold season (SCS) occurring from January to February (minor upwelling), a long warm season (LWS) between March to June, a long cold season (LCS) from July to October (major upwelling) and a short warm season (SWS) in November to December. The specimens of *P. regius* used in this work were sourced from the artisanal fisheries operating in the exclusive economic zone (EEZ) off Assinie. This city (5°7'25" N and 3°16'40") is located 80 Km east of Abidjan along the coast of the Gulf of Guinea (Figure 1). The city of Assinie is chosen since specimens of *P. regius* are regularly present in the landings throughout the year.

Spiny lobster Sampling

Lobsters used in this work were monthly catched in the Ivoirian Exclusive Economic Zone (EEZ) off Assinie from September 2019 to August 2020. Fishing generally was carried out by a group of four to five fishermen with one artisanal boat. Once a month, the nets were set around 6 p.m., and remained in the water for around 10 hours. Captured lobsters (25 individuals) were removed from the nets around 4 a.m, and kept in storage nets for conservation. Then, they were brought back by fishermen at the Assinie stage around 6 and 8 a.m. depending on weather conditions. At the landing stage, lobsters sampled were kept in a cooler filled with ice and rapidly transported to the laboratory of the Oceanological Research Center (CRO) for processing. A total of 331 of lobsters were collected during the study period. For each individual, we recorded: Total weight (TW ± 0.1 g), carapace length (CL ± 0.1 cm) and Sex. They were sexed based on the 3rd pereiopods for the females and 5th pereiopods for the males. Under ice, the flank of each animal was opened and the stomach cut at the level of the pylorus. Stomach was weighed to the nearest gram and preserved in 5% formaldehyde for later analysis.

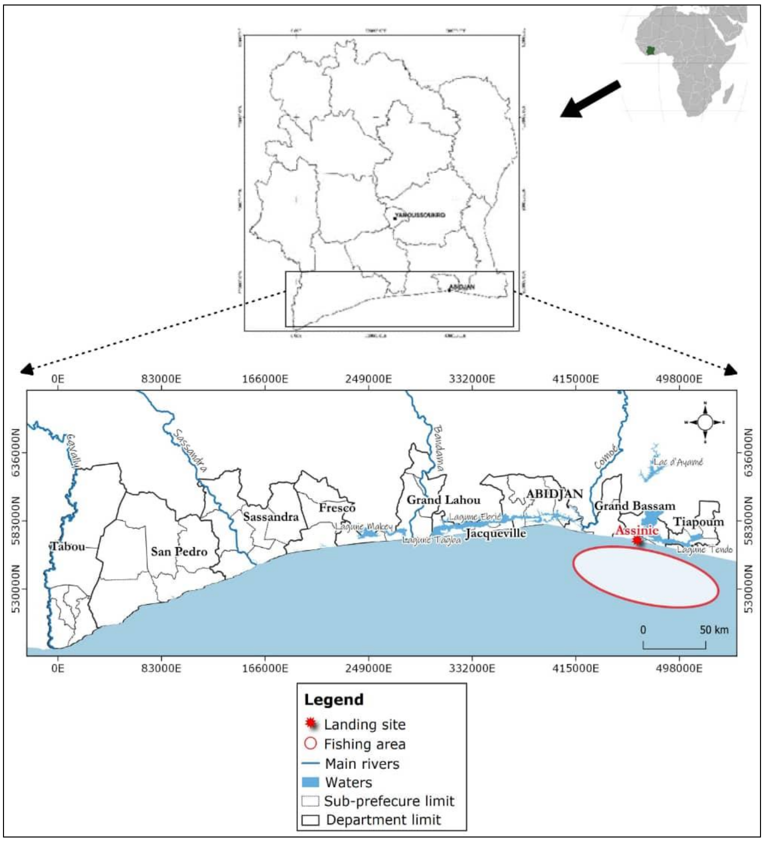

Fig 1: Geographical location of the study area

Stomach content analysis

In the laboratory, full stomach of each specimen of *P. regius* was weighted with a 0.1 g precision balance, dissected and its content was emptied into a Petri dish. Individuals whose stomachs are full or partially empty were retained after an analysis of the stomach repletion degree [28, 29]. Food items removed from each individual stomach were identified using an optical microscopy. A qualitative analysis of the stomach content of each individual that consists to group food items by gross taxa representing a few food categories because of their fragmentation and partial digestion [23]. In this work, feeding activity rate through the stomach coefficient of vacuity (Cv) and a few food indexes were provided:

Coefficient of vacuity

The stomach coefficient of vacuity (Cv) corresponds to the percentage of empty stomachs compared to the total number stomachs examined [30]:

$$\%\,Cv = \frac{\text{Number of the empty stomachs}}{\text{Total number of examined stomachs}} \times 100$$

Index of specific abundance

The index of specific abundance (% Si) is estimated by the percentage of each prey only in the stomach where it is found [31]:

$$\%\,Si = \frac{\text{Weight of prey category i}}{\text{Weight of all stomaches contents containing the prey i}} \times 100$$

Frequency of occurrence

This food index indicates the importance of each group of prey in the diet and also food preferences of the species [32]. It is expressed as the number of stomachs containing prey i compared to the number of full stomachs examined [33]:

$$\% \text{ O} = \frac{\text{Ni}}{\text{Nt}} \text{ X } 100$$

With Ni = number of stomachs containing prey i, and Nt = number of full stomachs examined.

Percentage by number

The percent number (% N) is a good descriptor for determining the importance of prey exploited by a species [34, 35]. It is expressed as the ratio of the number of individuals of a specific prey i to the number of preys ingested, expressed as a percentage:

$$\% \text{ N} = \frac{\text{Number of individuals of prey i}}{\text{Total number of ingested preys}} \times 100$$

Percentage by Weight (% P)

The weight percentage consists of determining the weight of each prey category for the entire sample in relation to the total weight of all items and expressed as follow [36]:

$$\% P = \frac{Pi}{Pt} \times 100$$

With

% P = Weight percentage of a prey, P_i = total Weight of item i, Pt = total weight of all items.

Index of Feed relative importance

The Index of Feed Relative Importance (IRA) was used to evaluate the contribution of each category of item in the diet of the species [37]. It provides more accurate interpretation of feeding habits, minimizing the skews caused by each of these percentages [38]. The indexes (% O,% N and% P) are combined to minimise the bias from each index and gives a better interpretation of the diet [39].

$$\% \text{ IRA} = \frac{\%\text{O}+\%\text{N}+\%\text{P}}{\sum_1^s(\%\text{O}+\%\text{N}+\%\text{P})} \text{ x } 100$$

with, Numerical percentage (% N), Occurrence percentage (% O), Weight percentage (% P) and Index of Feed relative importance (IRA), s = total number of food items.

The value of frequency classifies the different groups of preys into three categories expressing the predator's affinity to the prey [37].

-% IRA $\geq$ 50%: Preferential prey

- 10% $\leq$ % IRA $<$50%: Secondary prey

-% IRA $<$10%: Incidental or accidental prey

Schoener's index (D)

The Schoener's index (D) was calculated to understand the overlap in spiny lobster diet between the marine seasons and between the sexes. This index assesses the degree of dietary similarity between the seasons and sexes. Dietary similarity is biologically significant if D is greater than 0.6.

$$\text{D} = 1 - 0{,}5 \sum_{i=1}^{n} |Pi - Qi|$$

Pi: proportion of type i prey in a group.

Qi: proportion of type i prey in other group.

N: number of prey types;

D: Varies from 0 (no overlap) to 1 (total overlap).

Feeding strategy analysis

Feeding strategy was determined according to [31] modified from the method of [40]. It is based on a two-dimensional representation of prey-specific abundance and frequency of occurrence of the different prey types in the diet (Figure 2). The Chi-Square Test (χ^2) ($\alpha < 0.05$) was used to compare the vacuity coefficient according to marine seasons, sexes and sexual maturity.

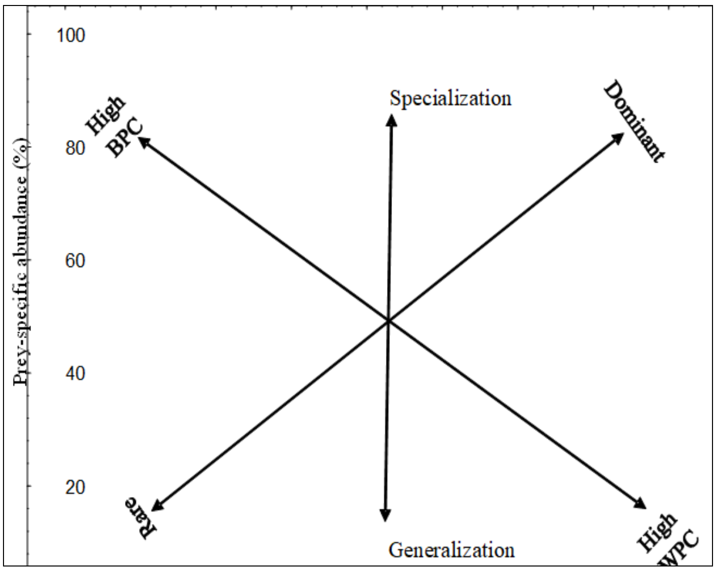

Fig 2: Description of the diagram of feeding strategy [31]; BPC = between phenotype component; WPC = within-phenotype component.

Results

Coefficient of vacuity (Cv)

The diet analysis included 331 specimens of *P. regius*, 136 males (size and weight range from 48 to 115 mm CL and 54.71 - 400 g) and 195 females (size and weight range from 52 -159 mm CL and 61.94 -1263.77 g). Of the total, 64 male and 96 female lobsters were caught during the cold seasons, with 72 males and 99 females during the warm seasons. The degree of replenishment of *P. regius* stomachs is shown in Figure 3. Out of a total of 331 samples examined, 272 (82%) had empty stomachs while the remaining 59 (18%) stomachs contained food. Emptiness coefficients (Cv) are higher in males (86.03%) than in females (79.49%). The Cv is low (80.12%) during the warm seasons compared to the cold seasons (82.5%). In mature females (80.65%) and immature females (77.44%) no difference was observed (p= 0.798); the same was true in males (immature Cv: 84.4 6% and mature Cv: 84.85%).

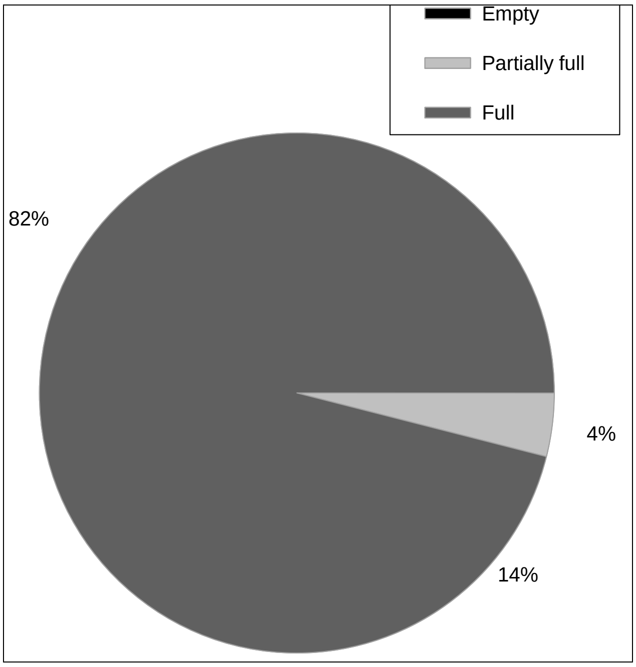

Fig 3: The percentages of individuals with empty, partially full and full stomachs.

Stomach repletion degree of *P. regius* sampled from the study area (331 individuals)

Composition of diet of *P. regius*

All of the stomach contents of *P. regius* consisted of fish (FI), crustaceans (CR), mollusks (MO), grains of sand (GS), nylon cables (NC) and detritus (DE) (Table 1 and Table 2).

Table 1: Food items found in stomach of *P. regius* in this study

Food items	% N	% O	% P	IRA
Fish (FI)	15.63	15.25	3.56	11.29
Crustaceans (CR)	23.44	23.42	37.78	29.4
Floats (FL)	1.56	1.69	0.41	1.2
Grains of sand (GS)	3.13	3.39	1.17	2.52
Nylon cables (NC)	26.56	28.81	4.89	19.75
Detritus (DE)	9.4	8.47	0.88	6.14
Mollusks (MO)	20.31	22.03	51.31	30.7

Where% N is the numeric percentage,% O is the frequency of occurrence,% P is the percentage by Weight, and IRA is the Index of Feed relative importance.

Table 2: Stomach contents in *P. regius* according to marine seasons

	Cold seasons				Warm seasons			
Food items	% N	% O	% P	IRA	% N	% O	% P	IRA
Fish (FI)	20.69	17.86	4.8	14.45	11.43	11.76	1.89	8.36
Crustaceans (CR)	6.9	7.14	1.5	5.18	37.14	38.24	86.18	53.85
Detritus (DE)	3.45	3,57	0.93	2.65	14.28	11.76	0.81	8.95
Mollusks (MO)	37.93	39.28	88.48	55.23	5.71	5.88	1.72	4.44

Where% N is the numeric percentage,% O is the frequency of occurrence,% P is the percentage by Weight, and IRA is the Index of Feed relative importance.

The food items most consumed by the study species was crustaceans (CR) with a numerical percentage of 23.44% followed by mollusks (MO) with a numerical percentage of 20.31%. The food index (IRA) did not indicate any preferential prey, but CR (IRA = 29.40%) and MO (IRA= 30.70%) are the most ingested food items. In cold seasons, MO were the preferred preys (IRA = 55.23%), FI were secondary food items (IRA = 14.45%) and incidental items consisted of CR (IRA = 5.18%) and DE (2.65%) (Table 2). In warm seasons, *P. regius* mainly consumed CR (IRA = 53.85%) and accidentally MO (IRA = 4.44%), FI (IRA =8.36%) and DE (8.95%). The contribution of the other items was 24.39%. The Schoener's index (D = 0.90) showed no diet variations between cold and warm seasons.

Feeding strategy of *P. regius*

The food index shows no food preference between sexes (Table 3). Both consumed the same types of prey (CR, MO, FI and DE) but at different proportions. The Schoener index showed that diet did not differ between sexes (D = 0.99). *P. regius* has a diet specialized in the consumption of MO, CR, FI and DE. The percentage of occurrence values are respectively 25.42%, 22.03%, 15.25% and 8.47% for CR, MO, FI and DE. The specific abundance ($\% Si$) presented increasing values of 95.60%, 99.76%, 100% and 100% (Figure 4).

Discussion

The vacuity coefficient (Cv) recorded in *Panulirus regius* during this study was 82% compared to a Cv value of 29% for the same species off Nigeria where the specimens were collected daytime [21]. The high Cv value recorded in our work could be related to the spiny lobster fishing season. Spiny lobsters are described as animals that are more active at night in search of food or for breeding [41]. Since fishing of lobsters took place at night, this could explain the high number of individuals with empty stomachs. According to data presented in this study, the Cv in *P. regius* could be influenced by sex, marine seasons and maturity stages. The availability of food linked by the two upwellings occurring in the Gulf of Guinea [24] can explain the Cv variations. The fact that the Cv was higher in mature females than in immature females could indicate that energy reserves are stored prior the reproduction period.

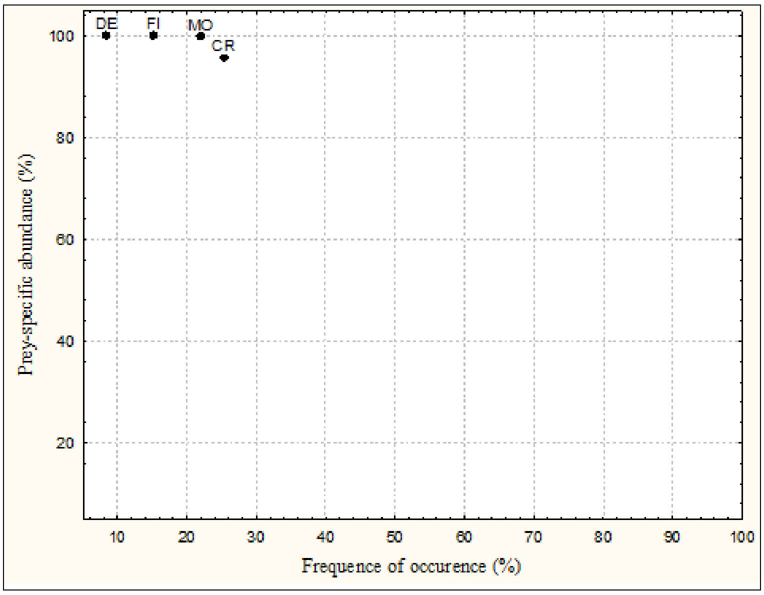

Fig 4: Food strategy of the spiny lobster *P. regius* in the study area.

In the present work, we did not find whole prey, but pieces of them in the stomachs of the specimens examined. This fact was also reported by [21] when studying the diet of *P. regius* sampled off the coast of Nigeria. Our results of the numeric indicators showed that this palinurid spiny lobster feeds mostly on crustaceans (CR) (% N = 23.44;% O = 23.42 and% P = 37.78), followed by mollusks (MO) (% N = 23.31;% O = 22.03 and% P = 51.31) and fish (FI) (% N = 15.63;% O = 15.25 and% P = 3.56). These findings are in agreement with [42] who noted that CR and MO are usually dominant in the diet of the species of the genus *Panulirus*.

Table 3: Stomach contents in *P. regius* according to sexes

	Male individuels				Female individuels			
Food items	**% N**	**% O**	**% P**	**IRA**	**% N**	**% O**	**% P**	**IRA**
Fish (FI)	14.28	15.79	0.96	10	16.28	17.5	4.15	12.33
Crustaceans (CR)	14.28	15.79	18.62	15.68	27.91	30	42.18	32.55
Detritus (DE)	19.05	21.05	1.02	13.24	4.65	5	0.84	3.41
Mollusks (MO)	14.29	15.79	73.49	33.35	23.26	25	46.22	30.72

Where% N is the numeric percentage,% O is the frequency of occurrence,% P is the percentage by Weight, and IRA is the Index of Feed relative importance.

According to [23], most food items found in the stomachs of *Panulirus* spp were not related to true capture. In fact, the palinurid spiny lobsters are well-known as nocturnal foragers and also described as a scavenging species scouring the seafloor in search of wounded or dead animals. But, these lobsters can capture and consume alive small animals [43]. The detritus (DE) and grains of sand (GS) found in the stomachs are accidental prey items. We can assume that this species could consume occasionally DE to meet their energy needs. The GS found in the stomach could be related to the scraper habits of this species [23]. This work has highlighted the presence of floats (FL) and nylon cables (NC) resulting from the activities of fishermen in the stomach of specimens. This accumulation of these emerging pollutants [44] in *P. regius* could be a potential threat to public health.

During the cold seasons, the spiny lobster, *P. regius* principally fed on mollusks (MO) (preferential prey), secondarily fish (FI) and accidentally crustaceans (CR) and detritus (DE). This marine species mainly consumed CR and accidentally MO, FI and DE in warm seasons. As noted by [45], the preponderance of CR and MO in the diet showed that these preys are main sources of calcium and minerals for the construction of exoskeletons during moulting cycle. Seasonal changes in diet were showed for *P. interruptus* [46, 47] and *P. homarus* [48] explained by the changes in environmental factors [43] and by the dietary plasticity observed in the spiny lobsters [49].

The results presented herein showing that *P. regius* had a specialized diet feeding mainly on animal matter. Our findings agree with that reported in *Panulirus* spp [21]. The palinurid spiny lobsters so far studied [43, 23] are known as omnivorous organisms feeding on CR, MO and FI for proteins and lipids needs, while plants and plant-like protists as algae serve as sources of carbohydrates [50, 47]. Among omnivorous species, we can cite the european *P. elephas* occuring in the Northeast Atlantic and Mediterrnean [51], the scalloped lobster *P. homarus* predominantly along the East coast of India [52] and the pink lobster, *P. mauritanicus* inhabiting Mauritanian waters [53]. The absence of plant and algae in the stomach of this species may result in a pronounced hydrolyse of these sources of carbohydrates. Indeed, plant material is showed to be important in the diets of spiny lobsters [54]. Since marine DE includes plant matter [55] and found in the stomach of *P. regius*, this can be considered as an omnivorous species.

Conclusion
The present study is the first to determine the natural diet of the spiny lobster *P. regius* sampled off the eastern coast of Côte d'Ivoire in the Gulf of Guinea. In the area, this species that consumes fish, crustaceans, mollusks and detritus, can be considered an omnivorous species. Although these findings present some ecological information on the feeding habits of palinurid spiny lobsters *P. regius* in its natural habitat in the Gulf of Guinea, further investigations are need to characterize the trophic environment of this species in order to refine our knowledge of the food choices of this decapod. Moreover, these results could be used to formulate experimental diets for *P. regius* in shrimp farming. In our study, plastic materials containing flame retardants as polybrominated diphenyl ethers (PBDEs) were found in the stomachs of the specimens. The presence of the BDEs in marine environment has become of increasing concern and a study may be conducted to determine if these xenobiotic compounds detected in the study are of concern to human health and the aquatic environment.

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Conflict of Interest
The authors declare that there are no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Authors Contribution
The authors' responsibilities were as follows: Ida Akissi KONAN: Methodology, Data analysis and Writing. Olivier Assoi ETCHIAN: Conceptualization, Supervision, Writing-original draft. Basile Kouakou KOUAME: Resources, Writing, Editing. Angelina Gbohono LOUKOU: Data collection, data analysis. Yao N'GUESSAN: Methodology, Data analysis. Jean-Noel YAPI: Methodology, Data analysis. All authors read and approved the final manuscript.

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