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Urban Malaria Transmission in Niamey, Niger: Species Diversity and Entomological Parameters of the *Anopheles gambiae complex*

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Abstract

A comprehensive understanding of vector diversity, spatio-temporal distribution, and malaria transmission dynamics is fundamental for establishing effective vector control strategies. This study assessed entomological parameters across six sites in Niamey. *Anopheles* mosquitoes were collected using indoor residual spraying and CDC light traps during three seasons (rainy, cold, and hot). Specimens were identified morphologically and by PCR. Diversity was evaluated using ecological indices, while blood meal origin and *Plasmodium* infections were also detected by PCR. In total, 18,141 females *Anopheles* mosquitoes belonging to six species were collected: *An. gambiae s.l.*, *An. rufipes*, *An. funestus*, *An. pharoensis*, *An. nili*, and *An. ziemanni*. *An. gambiae s.l.* was the predominant species complex, ranging from 96.62% to 99.68% across sites. Within the *An. gambiae* complex, two main species were identified: *An. coluzzii* (89.2%) and *An. arabiensis* (9.2%), along with 0.4% hybrids. These two anthropophagic species were the only ones found infected with *Plasmodium*, with infection frequencies varying by species, site, and season. The entomological inoculation rate (EIR) varied across sites, peaking during the rainy season (17.55 ib/p/m in Banigoungou versus 0.17 ib/p/m in Koira-Tegui) compared to lower values in the hot season (1.03 ib/p/m to 0.00). This study demonstrated that *An. coluzzii* and *An. arabiensis* are the principal malaria vectors in Niamey. Malaria transmission was found to be omnipresent, continuous, and particularly elevated during the rainy season and in riparian

sites along the Niger River.

Keywords

Urban Malaria, Vector Diversity, Seasonal Dynamics, Niamey-Niger

1. Introduction

Culicidae represent a major concern for both human and animal health. In addition to the nuisance they cause during both day and night, mosquitoes are involved in the transmission of numerous pathogens. According to the World Health Organization (WHO), vector-borne diseases account for more than 17% of all infectious diseases worldwide [1]. Malaria remains by far the deadliest disease globally. The WHO World Malaria Report documented 282 million cases and 610,000 deaths worldwide in 2024 [2]. The African region continues to bear the greatest burden, accounting for 94% of cases and 95% of deaths globally [2].

Niger, malaria remains a major public health problem. According to the latest WHO report (2025), Niger is among the four countries worldwide that together accounted for more than half of global malaria deaths, with 5.6% attributed to Niger alone [2]. Malaria transmission in Niger is highly heterogeneous, stratifying the country into three epidemiological zones based on transmission intensity: hyper-endemic, meso-endemic, and hypo-endemic [3]. *Anopheles gambiae s.l.* is the principal malaria vector in Niger, while *An. funestus* plays a secondary role due to its limited geographical distribution [4] [5]. Other less common species such as *An. pharoensis*, *An. nili*, and *An. ziemanni* have also been suspected of contributing to malaria transmission in Niger [6].

Despite progress achieved through interventions such as the large-scale distribution of long-lasting insecticidal nets (LLINs) and seasonal malaria chemoprevention (SMC), the Nigerien population continues to pay a heavy toll. Malaria affects the entire country, including urban areas such as Niamey, where transmission appears to be influenced by urbanization and its associated factors, including overpopulation and anthropogenic activities.

Urban malaria has been the subject of several studies, and the general consensus has been that urbanization tends to reduce malaria transmission compared to rural areas, where environmental and ecological conditions are more favorable for vector development [7]. However, malaria transmission persists in several African cities [8]-[10] and in some cases reaches higher levels than in rural areas [10]. Moreover, within the same city, malaria transmission is influenced by the degree of urbanization, micro-ecological conditions, and overall living standards [11]. According to some authors, this situation is largely driven by rapid and unplanned urbanization, accompanied by exponential growth of urban populations. Studies conducted in the sub-region have shown that urbanization, as it occurs in these countries, contributes to the creation of new types of breeding sites to which

Anopheles mosquitoes adapt [12] [13]. In addition, population growth is proportional to consumption needs, and to meet this increasing demand, communities are heavily engaged in urban agriculture. Unfortunately, while urban agriculture is promoted as a means to meet food needs and reduce poverty [14], it also contributes to sustaining malaria endemicity, even during unusual periods, by creating breeding sites favorable to vector development [15] [16]. Environmental modifications such as the establishment of irrigated or rice-growing perimeters may alter existing ecological settings and promote the reproduction of other mosquito species. At the local scale, this can lead to changes in the structure, diversity, distribution, and abundance of vectors [15] [17] [18].

The most recent entomological data on malaria transmission in Niamey date back more than a decade, with some studies conducted even before the period of major droughts, at a time when the city was smaller and less populated [6] [19] [20]. As urbanization continues and *Anopheles* vectors adapt to these changing environments, sustained surveillance and control of malaria in sub-Saharan Africa deserve particular attention in urban settings [7].

Against this backdrop of rapid and unplanned urbanization, the present study aimed to conduct entomological monitoring in order to: 1) assess the diversity, abundance, and spatio-temporal distribution of *Anopheles* species and their role in malaria endemicity and 2) evaluate malaria transmission across six sites in the city of Niamey, Niger.

2. Methodology

2.1. Study Design

This was a descriptive cross-sectional study, repeated every two months, aimed at assessing the diversity, abundance, and spatio-temporal distribution of *Anopheles* species and their involvement in the intensity of malaria transmission across selected sites in the city of Niamey.

2.2. Study Sites

This study was conducted throughout the year 2020 in Niamey, the capital city of Niger, located in the western part of the country (13˚30'49"N, 2˚06'35"E). The climate is tropical semi-arid, with an average annual rainfall of 540 mm occurring between June and October, peaking in August. Mean monthly temperatures vary considerably: January, the coolest month, records an average of 24.7˚C (min 17.1˚C, max 32.7˚C), while April, the hottest month, reaches an average of 34.8˚C (min 27.9˚C, max 46˚C).

Niamey is divided into two parts by the Niger River: the larger left bank and the right bank. In addition to the river, the city contains semi-permanent ponds. These water bodies provide opportunities for irrigated agriculture, particularly for riparian populations, in response to the growing demands of an exponentially increasing population. Agricultural practices vary across sites, including market gardening, arboriculture, and irrigated rice cultivation, thereby creating diverse

agro-ecosystems. Rice cultivation is practiced year-round, though with reduced intensity during the hot season, which coincides with the river's low water period. Irrigation, particularly for rice, is largely supported by motor pumps. During a single rice-growing cycle, plots are often cultivated asynchronously, resulting in fields at different developmental stages. These practices contribute to environmental modifications that increasingly favor mosquito proliferation, especially *Anopheles* species.

For this study, six sites were selected. Five were riparian sites along the Niger River: upstream sites (Tondibiah and Goudel), central sites (Lamordé and Gamkallé), and a downstream site (Banigoungou). The sixth site, Koira-Tegui, was located away from the river and represented a non-riparian area (Figure 1).

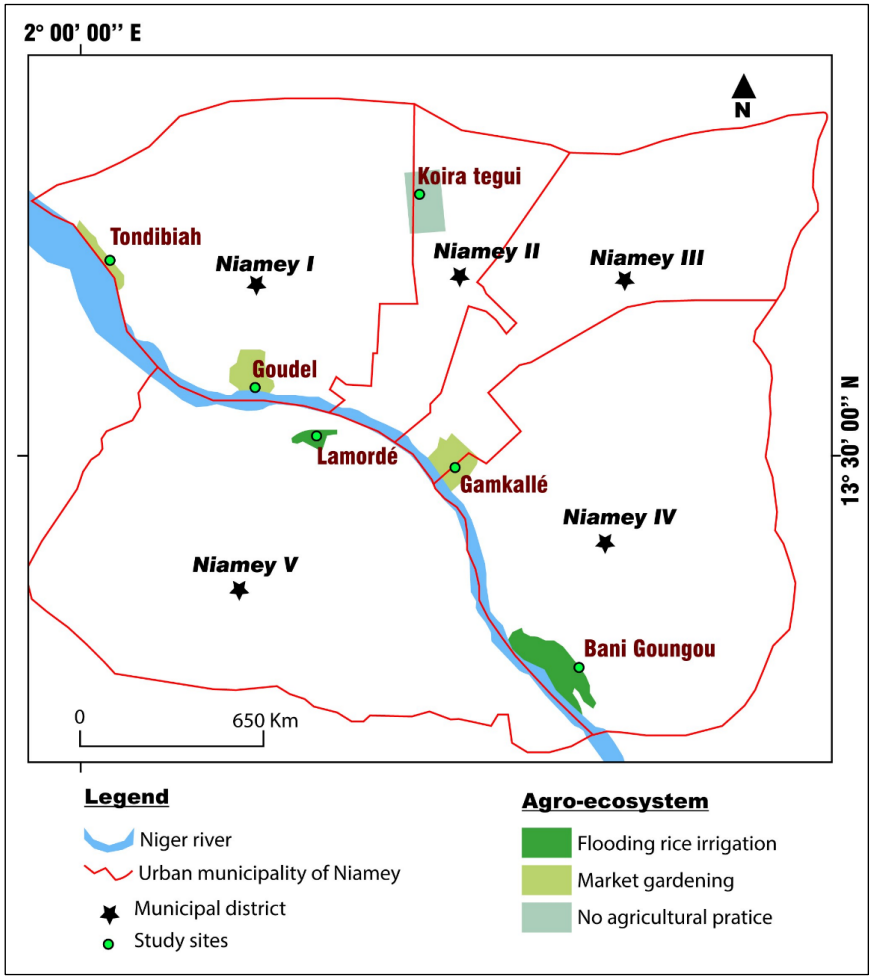

Figure 1. Map of study sites [21].

2.3. Collection and Processing of Adult Mosquitoes

2.3.1. Collection, Identification, and Preservation of Mosquitoes

The study protocol was based on longitudinal entomological monitoring conducted from January to December 2020. Adult mosquito collection was performed using two sampling techniques, every two months, over two consecutive days at

each study site. The first method involved pyrethrum spray collections (PSC) inside dwellings. This technique was applied in twenty randomly selected houses per site. Collections were carried out early in the morning, between 7:00 and 10:00 a.m., in designated rooms where white sheets were spread to cover the entire floor and beds. All openings were sealed, and the room was sprayed with pyrethroid-class insecticides, particularly targeting the ceiling where mosquitoes typically rest. After 15 minutes, the insecticide's action time, the sheets were carefully retrieved, and knocked-down mosquitoes were collected using forceps and placed into Petri dishes labeled by house and site [22]. The second technique used CDC light traps. Eight traps were deployed in four randomly selected houses, with prior consent from the occupants. In each house, traps were placed both indoors and outdoors, suspended at a height of 1.5 meters. Traps were activated at 6:00 p.m. (sunset) and operated until 6:00 a.m. During this period, room lights were generally turned off, and mosquitoes were attracted by the incandescent bulb, then drawn in by the trap's fan and deposited into the collection chamber [22].

At the end of each collection, the total number of sleepers present in each surveyed room was recorded. This information enabled us to assess aggressiveness as proposed by Williams and Pinto (see section 4: Data Analysis). The same houses were monitored throughout the study period, except in cases where a replacement was necessary due to unforeseen circumstances; in such cases, a neighboring house with similar structure was selected. Collected specimens were transported to the laboratory for identification. Using a stereomicroscope, mosquitoes were sorted by genus, sex, and species based on morphological criteria. Only female *Anopheles* mosquitoes were retained. Their physiological status was determined, and the proportion of blood-fed females was recorded during morphological identification [23]. Subsequently, unfed and gravid females were preserved in batches of ten in Eppendorf tubes containing silica gel and stored at −20˚C. Blood-fed females were individually preserved in 96-well plates until molecular analyses were performed.

2.3.2. Molecular Characterization

A subsample of 2243 *Anopheles gambiae s.l.* specimens was dissected into three parts: wings and legs for species identification within the *An. gambiae* complex; abdomen for determining the origin of the blood meal in engorged females; and head-thorax (along with whole specimens of other species) for detecting the presence of *Plasmodium*. DNA from all parts was extracted following the protocol described by Rudbeck and Dissing [24]. Species within the *An. gambiae* complex were identified using the SINE-PCR method, following the protocol established by Santolamazza *et al.* [25]. Infectivity was assessed using COX-I PCR, which targets the *Plasmodium* gene for sporozoite detection, as described by Echeverry *et al.* [26]. The origin of the blood meal was determined according to the protocol outlined by Kent and Norris [27]. The primers used for these PCR assays are listed in **Table 1**. At the end of each PCR reaction, products were electrophoresed on a

2% agarose gel, and DNA fragment sizes were visualized under ultraviolet light by comparing the resulting bands to a 100 bp molecular weight marker.

Table 1. Primers used for species identification, *Plasmodium* detection, and blood meal source determination by PCR.

Type of PCR	**Primers Name**	**Sequence (5' → 3')**
Species identification	200X6.1F	TCGCCTTAGACCTTGCGTTA
	200X6.1R	CGCTTCAAGAATTCGAGATAC
Plasmodium detection	COX-IF	AGAACGAACGCTTTTAAACGCCTG
	COX-IR	ACTTAATGGATATAAAGTCCATCCWGT
Blood meal origin	UnRev1025	GGTTGT/GCCTCCAATTCATGTTA
	Human741F	GGCTTACTTCTCTTCATTCTCTCCT

2.4. Data Analysis

Collected data were initially entered into Microsoft Excel and subsequently transferred to SPSS for statistical analysis. The sample size used for species identification within the *An. gambiae* complex and for infectivity assessment was determined using the OpenEpi online tool: (https://www.openepi.com/SampleSize/SSPropor.htm) with a 95% confidence interval.

Ecological diversity indices were calculated to assess species diversity, including:

- Species richness (S)
- Relative abundance (Pi), where Pi = ni/N, with ni representing the number of individuals of a given species and N the total number of individuals across all species
- Shannon-Wiener diversity index (H), calculated as $H = -\Sigma Pi \times \log(Pi)$
- Evenness index (E), calculated as $E = H/Hmax$
- Simpson's diversity index (D), calculated as $D = 1/\Sigma Pi$

In accordance with the methodology proposed by Williams and Pinto [28], entomological indicators were also evaluated at all study sites, including: Anthropophily index.

- (Ia): Corresponds to the proportion of female mosquitoes that have taken a blood meal from a human subject.

$$Ia = \frac{\text{Total number of } \textit{Anopheles} \text{ that have taken a human blood meal}}{\text{Total number of engorged } \textit{Anopheles} \text{ analyzed}}$$

- Human biting rate (ma): Corresponds to the average number of bites received per person per unit of time.

$$ma = \frac{\text{Total number of engorged } \textit{Anopheles}}{\text{Total number of human sleepers}} \times Ia$$

- Sporozoite index (Is): Number of *Anopheles* mosquitoes tested positive for sporozoites.

$$\text{Is} = \frac{\text{Total number of sporozoite} - \text{positive } \textit{Anopheles}}{\text{Total number of } \textit{Anopheles} \text{ specimens analyzed}}$$

- Entomological inoculation rate (EIR), calculated as EIR = Is × ma.

For statistical comparisons, the Kruskal-Wallis test was used to analyze seasonal variations in abundance, while the Chi-square test was applied to compare infection rates and anthropophily levels.

3. Results

3.1. Mosquito Composition and Density

A total of 22,340 female mosquitoes belonging to three genera (*Anopheles*, *Culex*, and *Aedes*) were collected during the study period. *Anopheles* was by far the most abundant genus, accounting for 81% of the total collection, followed by *Culex* (18%) and *Aedes* (1%). Overall, the seasonal distribution of these genera showed that *Anopheles* was markedly more abundant during the rainy season (63.81%) compared to the cold season (26.64%) and was least abundant in the hot season (9.55%). In contrast, *Culex* was most abundant in the cold season (69.19%), followed by the hot season (5.53%) and the rainy season (25.28%). *Aedes* mosquitoes were collected almost exclusively during the hot season (99.12%). Spatial distribution revealed that *Anopheles* was relatively more abundant in Banigoungou, Goudel, and Lamordé, wherea *Culex* predominated in Gamkallé, Koira-Tegui, and Tondibiah. *Aedes* was collected in only two sites, with higher abundance in Gamkallé and presence also in Koira-Tegui (Figure 2).

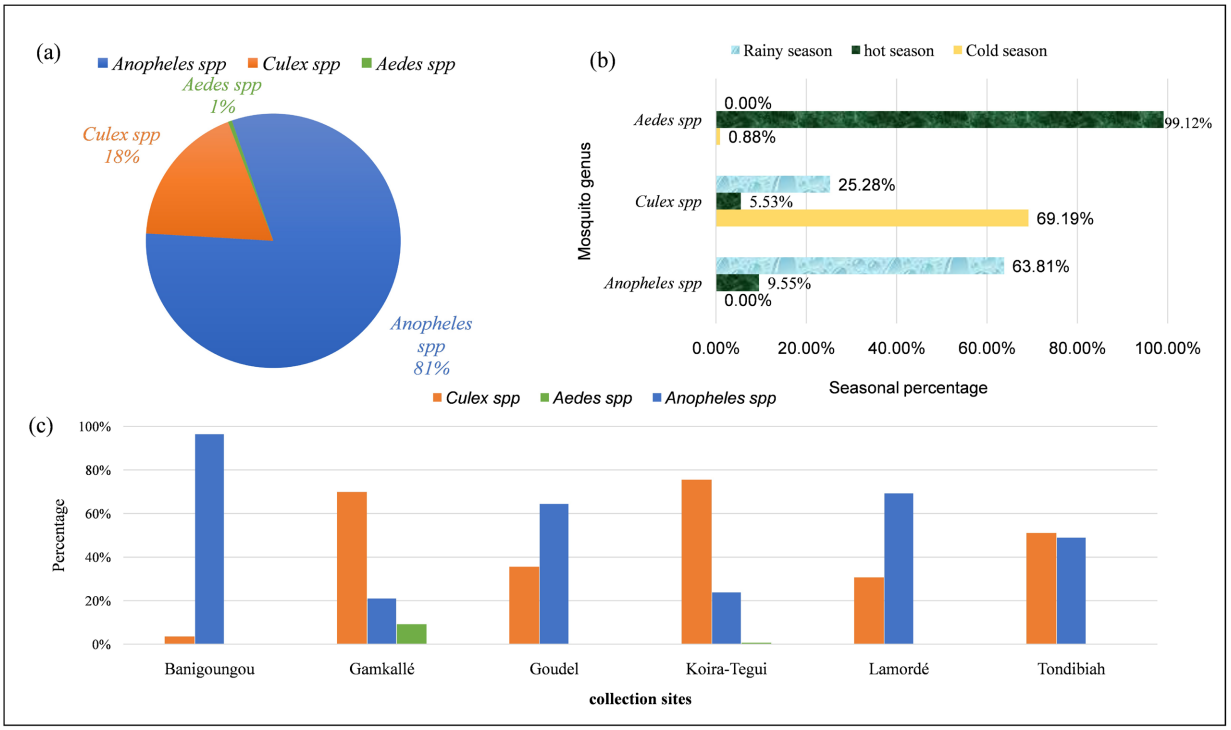

Figure 2. Distribution of mosquito genera: (a) Overall distribution of genera; (b) Seasonal distribution of genera and (c) Distribution across collection sites.

3.2. Diversity and Spatial Distribution of *Anopheles* Mosquitoes

A total of 18,141 females *Anopheles* specimens belonging to six species, *An. gambiae s.l.*, *An. rufipes*, *An. funestus*, *An. pharoensis*, *An. ziemanni*, and *An. nili*, were collected across all study sites during the survey period. Pyrethrum spray collections (PSC) yielded 91% (n = 16,485) of the total catch, significantly higher than CDC light traps, which accounted for 9% (n = 1656) (p = 0.007) (Table 2). Among the six *Anopheles* species captured, *An. gambiae s.l.* was the most common and dominant across all sites, representing 99.61% (n = 18,071) of the total *Anopheles* catch, followed by *An. rufipes* at 0.26% (n = 47). The remaining species were more site-specific and included *An. funestus* (0.07%, n = 12), *An. pharoensis* (0.04%, n = 7), *An. nili* (0.01%, n = 2), and *An. ziemanni* (0.01%, n = 2). *Anopheles* abundance was highest in the peripheral districts of Banigoungou and Tondibiah, accounting for 80% (n = 14,520) and 7.8% (n = 1412) of the total catch, respectively. Lamordé ranked third with 6.0% (n = 1082), while Koira-Tégui recorded the lowest *Anopheles* abundance at 0.6% (n = 103). Relative abundance and species richness varied slightly across sites. Banigoungou exhibited the highest *Anopheles* species richness, with all six species present. *An. gambiae s.l.* was dominant at 99.7% (n = 14,473), followed by *An. rufipes* at 0.3% (n = 38). Goudel ranked second in species richness, with five species detected, including *An. gambiae s.l.* (99.1%, n = 765) and *An. rufipes* (0.4%, n = 3). Lamordé and Gamkallé showed the lowest species richness, each with two species and a strong predominance of *An. gambiae s.l.* (99.8%, n = 1080 and 99.6%, n = 251, respectively). Analysis of ecological indices including Shannon-Wiener diversity, evenness, and Simpson's index revealed consistently low values across all sites, confirming the dominance of *An. gambiae s.l.* throughout the study area. However, some variation was observed, with the highest diversity indices recorded at Koira-Tégui, where non-*gambiae* species accounted for 3.88% of the catch, and the lowest at Lamordé, where *An. gambiae s.l.* was nearly exclusive (Table 3).

Table 2. Distribution of *Anopheles* species according to capture methods.

Capture Method	*An. gambiae*	*An. pharoensis*	*An. rufipes*	*An. funestus*	*An. nili*	*An. ziemanni*	Total per Method
Resting fauna	16,441	5	31	7	1	0	16,485
Light trap	1630	2	16	5	1	2	1656
Total per species	18,071	7	47	12	2	2	18,141
P-value	0.006**	0.396ns	0.000***	0.046*	0.205ns	0.025*	0.007**

*: significant test; **: very significant; ***: highly significant; ns: no significant.

Table 3. Diversity and spatial distribution of *Anopheles*.

Species	Banigoungou	Gamkallé	Goudel	Koira-Tegui	Lamordé	Tondibiah
	ni (Pi)	ni (Pi)	ni (Pi)	ni (Pi)	ni (Pi)	ni (Pi)
An. gambiae s.l.	14,473 (99.68)	251 (99.6)	765 (99.09)	99 (96.12)	1080 (99.82)	1403 (99.36)

Continued

An. pharoensis	3 (0.02)	0 (0)	2 (0.26)	0 (0)	0 (0)	2 (0.14)
An. rufipes	38 (0.26)	1 (0.4)	3 (0.39)	2 (1.94)	2 (0.18)	1 (0.07)
An. funestus	3 (0.02)	0 (0)	1 (0.13)	2 (1.94)	0 (0)	6 (0.42)
An. nili	1 (0.01)	0 (0)	1 (0.13)	0 (0)	0 (0)	0 (0)
An. ziemanni	2 (0.01)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Total	14,520 (100)	252 (100)	772 (100)	103 (100)	1082 (100)	1412 (100)
Species richness (S)	6	2	5	3	2	4
Shannon index (H')	0.011	0.002	0.004	0.017	0.001	0.003
Simpson index (D)	0.006	0.008	0.018	0.075	0.004	0.013
Evenness index (E)	0.013	0.006	0.006	0.035	0.003	0.005

Ni: total number of individuals, Pi: percentage of individuals;

3.3. Density and Seasonality of *Anopheles* Species across Sites

Across all sites, the overall abundance of *Anopheles* varied according to season. The Kruskal-Wallis test revealed that the seasonal variation in the abundance of *An. gambiae s.l.* was significant (p = 0.000). As shown in Table 4, in the majority of sites (4/6), *An. gambiae s.l.* was more abundant during the rainy season. Furthermore, its density decreased progressively from the rainy season to the cold season and was lowest in the hot season. Specifically, in Banigoungou, its density was 65.92% in the rainy season compared to 24.26% in the cold season (p = 0.6) and 9.82% in the hot season (p = 0.000). In Tondibiah, *An. gambiae s.l.* accounted for 68.14% in the rainy season, 20.88% in the cold season (p = 0.03), and 10.98% in the hot season (p = 0.000). In Goudel, its density was 63.53% in the rainy season, 33.86% in the cold season (p = 0.6), and 2.61% in the hot season (p = 0.000). At Koira-Tegui, *An. gambiae s.l.* reached 88.89% in the rainy season compared to 6.06% in the cold season (p = 0.07) and 5.05% in the hot season (p = 0.07). Conversely, in Lamordé and Gamkallé, *An. gambiae s.l.* was more abundant in the cold season than in the rainy season, with 53.15% versus 36.30% (p = 0.000) and 63.75% versus 34.66% (p = 0.1), respectively. Across all sites, *An. gambiae s.l.* persisted but remained underrepresented in the hot season, ranging from 1.59% in Gamkallé to 10.97% in Tondibiah. Similarly, *An. rufipes* was collected at all sites, with relatively higher density in Banigoungou and greater abundance in the rainy season compared to the cold and hot seasons (50% vs. 24.25% and 18.42%, respectively). The Kruskal-Wallis test revealed significant differences between its abundance in the hot and cold seasons (p = 0.02) and between the hot and rainy seasons (p = 0.000). However, no significant difference was detected between the cold and rainy seasons. Other species such as *An. funestus*, *An. pharoensis*, *An. nili*, and *An. ziemanni* were collected at very low densities, and their seasonal variation was not numerically clear. Nevertheless, the Kruskal-Wallis test showed that *An. pharoensis*, unlike *An. funestus*, *An. nili*, and *An. ziemanni*, varied significantly according to season (p = 0.016) (Table 4).

Table 4. Composition, distribution, and seasonal density of *Anopheles* species by site.

Study site	Season	Number and percentage (%) of collected *Anopheles* species						
		An. gambiae	*An. pharoensi*	*An. rufipes*	*An. funestus*	*An. nili*	*An. ziemanni*	Total per site/season
Banigoungou	Cold	3511 (24.26)	3 (100)	12 (31.58)	1 (33.33)	-	2 (100)	3529 (24.32)
	Rainy	9540 (65.92)	-	19 (50)	1 (33.33)	1 (100)	-	9561 (65.84)
	Hot	1422 (9.82)	-	7 (18.42)	1 (33.33)	-	-	1430 (9.84)
Total		**14,473 (100)**	**3 (100)**	**38 (100)**	**3 (100)**	**1 (100)**	**2 (100)**	**14,520 (100)**
Gamkallé	Cold	160 (63.75)	-	-	-	-	-	160 (63.49)
	Rainy	87 (34.66)	-	1 (100)	-	-	-	88 (34.92)
	Hot	4 (1.59)	-	-	-	-	-	4 (1.59)
Total		**251 (100)**	**-**	**1 (100)**	**-**	**-**	**-**	**252 (100)**
Goudel	Cold	259 (33.86)	1 (50)	3 (100)	1 (100)	1 (100)	-	265 (34.33)
	Rainy	486 (63.53)	1 (50)	-	-	-	-	487 (63.08)
	Hot	20 (2.61)	-	-	-	-	-	20 (2.59)
Total		**765 (100)**	**2 (100)**	**3 (100)**	**1 (100)**	**1 (100)**	**-**	**772 (100)**
Koira-Tegui	Cold	6 (6.06)	-	-	-	-	-	6 (5.83)
	Rainy	88 (88.89)	-	-	-	-	-	88 (85.44)
	Hot	5 (5.05)	-	2 (100)	2 (100)	-	-	9 (8.73)
Total		**99 (100)**	**-**	**2 (100)**	**2 (100)**	**-**	**-**	**103 (100)**
Lamordé	Cold	574 (53.15)	-	1 (50)	-	-	-	575 (53.14)
	Rainy	392 (36.30)	-	-	-	-	-	392 (36.23)
	Hot	114 (10.55)	-	1 (50)	-	-	-	115 (10.63)
Total		**1080 (100)**	**-**	**2 (100)**	**-**	**-**	**-**	**1082 (100)**
Tondibiah	Cold	293 (20.88)	2 (100)	-	3 (50)	-	-	298 (21.10)
	Rainy	956 (68.14)	-	1 (100)	3 (50)	-	-	960 (68.0)
	Hot	154 (10.98)	-	-	-	-	-	154 (10.90)
Total		**1403 (100)**	**2 (100)**	**1 (100)**	**6 (100)**	**-**	**-**	**1412 (100)**
Overall total		**18,071**	**7**	**47**	**12**	**2**	**2**	**18,141**

3.4. Specific Composition and Distribution of Subspecies of the *An. gambiae s.l.* Complex

Out of 2243 specimens of *An. gambiae s.l.* examined for specific identification of complex members by PCR, 89.2% (n = 2000) were identified as *An. coluzzii*, 9.2% (n = 206) as *An. arabiensis*, 0.4% (n = 8) as hybrids, and 1.2% (n = 29) were not amplified. Figure 3 shows that the predominance of *An. coluzzii* extended across all study sites and throughout all seasons of the year (χ^2 = 112.33; df = 20; p < 0.001). However, regardless of site, *An. arabiensis* was more abundant during the rainy season, with significantly higher predominance observed in Goudel (χ^2 = 16.22; df = 6; p = 0.013) and Tondibiah (χ^2 = 22.31; df = 8; p = 0.004) (Figure 3).

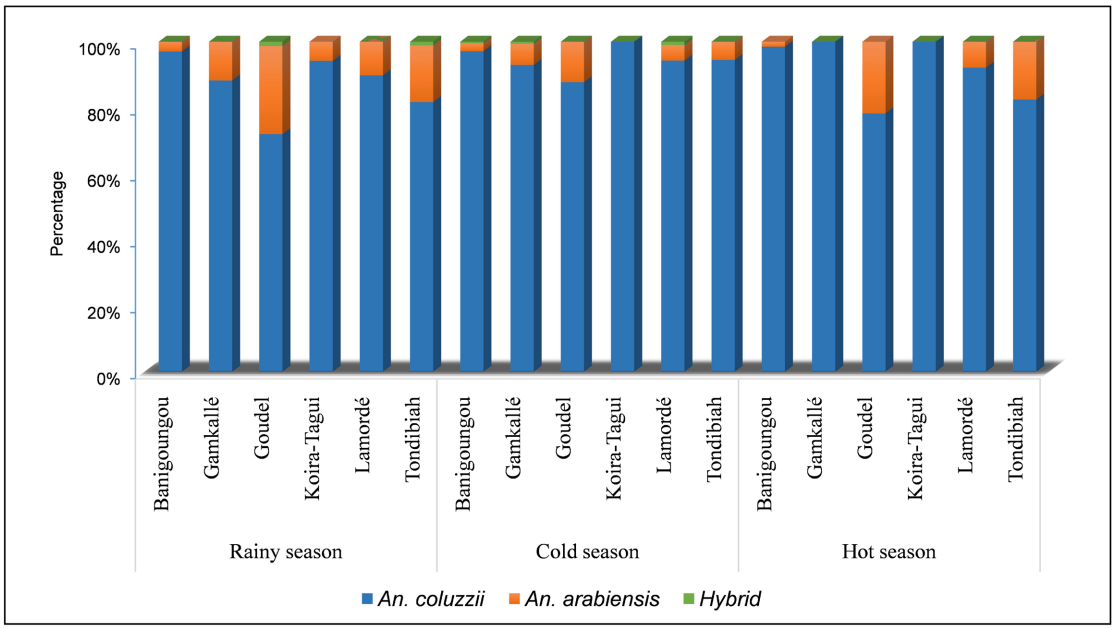

Figure 3. Spatio-temporal distribution of species within the *An. gambiae* complex.

3.5. Spatio-Temporal Assessment of Entomological Parameters Related to Malaria Transmission

3.5.1. Infection Rate of *Anopheles* with *Plasmodium* spp.

Table 5. Spatio-temporal distribution of *Plasmodium falciparum* infection rates in *An. gambiae s.l.*

Site	Rainy season			Cold season			Hot season		
	Tested (n)	Positive (n)	IR % [95% CI]	Tested (n)	Positive (n)	IR % [95% CI]	Tested (n)	Positive (n)	IR % [95% CI]
Tondibiah	176	9	5.1 [2.4 - 9.5]	145	3	2.1 [0.4 - 5.9]	118	2	1.7 [0.2 - 6.0]
Goudel	160	9	5.6 [2.6 - 10.4]	164	3	1.8 [0.4 - 5.3]	69	1	1.4 [0.0 - 7.0]
Lamordé	150	7	4.7 [1.9 - 9.3]	196	6	3.1 [1.1 - 6.5]	105	2	1.9 [0.2 - 6.7]
Gamkallé	85	3	3.5 [0.7 - 10.0]	156	3	1.9 [0.4 - 5.5]	4	0	0
Banigoungou	207	11	5.3 [2.7 - 9.3]	212	7	3.3 [1.3 - 6.7]	197	3	1.5 [0.3 - 4.4]
Koira-Tagui	88	2	2.3 [0.7 - 9/6]	6	0	0 [0.0 - 45.9]	5	0	0 [0.0 - 52.2]
Total	866	41	4.7 [3.5 - 6.5]	879	22	2.5 [1.6 - 3.8]	498	8	1.6 [0.7 - 3.1]

(n): nombre; IR = Infection Rate; CI = Confidence Interval.

Table 5 shows the *Plasmodium* infection rate detected in *Anopheles* across study sites during the three seasons of the year. Only *An. gambiae s.l.* was found infected with *Plasmodium* sporozoites throughout the study period. Overall, the *Plasmodium* infection rate in *An. gambiae s.l.* was 3.2% (n = 71; 95% CI: 2.5 - 4.0). This rate varied significantly by season, being highest during the rainy season at 4.8% (n = 42; 95% CI: 3.5 - 6.5), compared to 2.5% (n = 22; 95% CI: 1.6 - 3.8) in the cold season and 1.6% (n = 8; 95% CI: 0.7 - 3.2) in the hot season ($\chi^2 = 12.6$; df = 2; p = 0.002). By site, variability in infection rates was not significant, ranging from

3.4% (n = 21/616; 95% CI: 3.1 - 4.4) in Banigoungou, where the rate was highest, to 2.0% (n = 2; 95% CI: 0.7 - 8.6) in Koira-Tegui, where it was lowest (χ^2 = 1.01; df = 5; p = 0.9). Similarly, seasonal variation across sites showed that infection rates during the rainy season ranged from 5.6% (n = 9/160; 95% CI: 0.2 - 10.4) in Goudel to 2.3% (n = 2/88; 95% CI: 0.7 - 8.0) in Koira-Tegui. In the cold season, the highest infection rate was observed in Banigoungou at 3.3% (n = 7/212; 95% CI: 1.3 - 6.7), while no infections were detected in Koira-Tegui. During the hot season, the highest infection rate was recorded in Lamordé at 1.9% (n = 2/105; 95% CI: 0.2 - 6.7), whereas no infections were detected in Gamkallé and Koira-Tegui. Overall analysis of infection rates within the *An. gambiae* complex showed that the infection rate was significantly higher in *An. coluzzii* compared to *An. arabiensis* (χ^2 = 13.47; df = 4; p = 0.009).

3.5.2. Trophic Preference: Anthropophilic Rate in *An. gambiae s.l.*

The anthropophilic index was determined from a total of 1449 blood-fed abdomens of *An. gambiae s.l.*. As shown in Table 6, overall and across all sites and seasons, *An. gambiae s.l.* was significantly more anthropophagic (72.6%; n = 1052; 95% CI: 61.8 - 85.7) than zoophagic (27.4%; n = 397; 95% CI: 23.6 - 31.2) (χ^2 = 120.09; df = 4; p < 0.001). The anthropophilic rate varied significantly by season. It was lower during the cold season (67.2%; n = 305/454; 95% CI: 59.8 - 82.4) compared to the hot season (79%; n = 282/357; 95% CI: 68.6 - 86.7) and the rainy season (72.9%; n = 465/638; 95% CI: 62.5 - 86.6) (χ^2 = 120.1; df = 4; p < 0.001). This significant seasonal variation was observed across all sites: Tondibiah (χ^2 = 80.14; df = 4; p < 0.001), Goudel (χ^2 = 47.60; df = 4; p < 0.001), Lamordé (χ^2 = 57.74; df = 4; p < 0.001), Gamkallé (χ^2 = 14.52; df = 4; p = 0.006), Banigoungou (χ^2 = 45.46; df = 4; p < 0.001), and Koira-Tegui (χ^2 = 80.14; df = 4; p < 0.001). Overall analysis of blood meal origin within the *An. gambiae s.l.* complex showed that *An. coluzzii* was significantly more anthropophagic than *An. arabiensis*, which exhibited a stronger tendency toward zoophagy (χ^2 = 37.12; df = 8; p < 0.001).

Table 6. Evaluation of the anthropophilic rate in *An. gambiae s.l.* according to sites and seasons.

Site	Rainy season			Cold season			Hot season		
	Tested (n)	SH	Ia % [95% CI]	Tested (n)	SH	Ia % [95% CI]	Tested (n)	SH	Ia % [95% CI]
Tondibiah	145	91	62.8 [54.3 - 70.6]	49	29	59.2 [44.2 - 73.0]	69	49	71 [58.8 - 81.3]
Goudel	100	91	91.0 [83.6 - 95.8]	45	38	84.5 [70.5 - 93.5]	22	22	100 [84.5 - 100]
Lamordé	91	78	85.7 [76.8 - 92.2]	57	45	78.9 [66.1 - 88.6]	69	49	90.8 [81.0 - 96.5]
Gamkallé	33	32	97.0 [84.2 - 99.9]	91	80	87.9 [79.4 - 93.8]	0	0	0
Banigoungou	207	114	55.1 [48.2 - 62.0]	212	113	53.3 [46.3 - 60.2]	197	162	82.2 [76.1 - 87.3]
Koira-Tagui	62	59	95.1 [86.5 - 99.0]	0	0	0	0	0	0
Total	638	465	72.9 [62.5 - 86.6]	454	305	67.2 [59.8 - 82.4]	357	282	79.0 [68.6 - 86.7]

(n): Number of blood-fed females examined, SH: Human blood meals detected, Ia: Anthropophilic index (%), 95% IC: 95% confidence interval.

3.5.3. Entomological Inoculation Rate (EIR)

Table 7 presents the variation in the entomological inoculation rate (EIR). On average, the EIR ranged from 3.52 infective bites per person per month (ib/p/m) during the rainy season to 0.62 ib/p/m and 0.22 ib/p/m during the cold and hot seasons, respectively. Across study sites, the highest EIR was recorded in Banigoungou with 17.55 ib/p/m, followed by Goudel with 1.46 ib/p/m and Tondibiah with 1.07 ib/p/m. The lowest EIR was observed in Koira-Tegui with 0.17 ib/p/m. During the cold and hot seasons, Banigoungou and Lamordé recorded the highest transmission rates, with 2.72 ib/p/m and 0.44 ib/p/m in the cold season, and 1.03 ib/p/m and 0.12 ib/p/m in the hot season, respectively.

Table 7. Seasonal variation of entomological inoculation rates (EIR) of *An. gambiae s.l.*.

Sites	Rainy season			Cold season			Hot season		
	An. gambiae s.l.			*An. gambiae s.l.*			*An. gambiae s.l.*		
	ma	IR	EIR/month	ma	IR	EIR/month	ma	IR	EIR/month
Banigoungou	11.01	0.05	17.55	2.74	0.03	2.72	2.26	0.02	1.03
Gamkallé	0.19	0.04	0.20	0.47	0.02	0.27	0.00	0.00	0.00
Goudel	0.87	0.06	1.47	0.32	0.02	0.18	0.13	0.01	0.06
Koira-Tegui	0.25	0.02	0.17	0.00	0.00	0.00	0.00	0.00	0.00
Lamordé	0.41	0.05	0.58	0.48	0.03	0.44	0.20	0.02	0.12
Tondibiah	0.69	0.05	1.07	0.20	0.02	0.12	0.25	0.02	0.13
Seasonal mean EIR	2.24	0.05	3.52	0.70	0.02	0.62	0.47	0.01	0.22

ma: aggressiveness (mean biting rate per person per night), *IR*: infection rate, EIR/month: entomological inoculation rate per month.

4. Discussion

This prospective longitudinal study assessed the diversity of *Anopheles* mosquitoes, their spatio-temporal distribution, and malaria infection rates across five riparian sites along the Niger River and one non-riparian site within the city of Niamey. The study highlighted the seasonal and relative abundance of *Anopheles* species and evaluated their role in malaria transmission within the six sites of Niamey.

The results demonstrated the presence of three mosquito genera *Anopheles*, *Culex*, and *Aedes*, collected across all study sites. *Anopheles* was the most abundant genus and was recorded throughout all seasons of the year, with site-specific variations in abundance. This finding contrasts with entomological studies conducted in other African cities, which reported relatively higher densities of Culicinae compared to Anophelinae [29]. The predominance of *Anopheles* fauna in Niamey may account for the high malaria transmission observed in the city. *Culex* was the second most abundant genus. This result differs from those reported by Labbo *et al.* [30] who, in a study conducted in two districts of Niamey, found *Culex* to be more abundant. Such discrepancies may be explained by differences in

the geographical positioning of collection sites, which likely represent distinct ecological systems depending on whether they are located in the city center or peripheral areas. In contrast, our findings are consistent with those of Kwi *et al.* [31] and Traoré *et al.* [32]. *Aedes* was the least represented genus. Its low abundance may be partly attributed to the capture time intervals and collection techniques employed, which may not have been optimal for *Aedes* sampling, as well as to the agro-ecosystems selected for the present study. Nevertheless, the detection of *Culex* and *Aedes* mosquitoes should alert public health authorities, as these genera are responsible for the transmission of arboviral diseases such as yellow fever, dengue, and Rift Valley fever, which are frequently misdiagnosed as malaria.

Entomological monitoring identified six *Anopheles* species: *An. gambiae s.l.*, *An. rufipes*, *An. funestus*, *An. pharoensis*, *An. nili*, and *An. ziemanni*. Across all sites, *An. gambiae s.l.* was the predominant species. Previous entomological studies conducted in Niamey reported the presence of these species and confirmed the predominance of *An. gambiae s.l.* [5] [6] [30]. This strong predominance of *An. gambiae s.l.* has also been documented in other countries of the sub-region [32]-[39]. Species distribution analysis showed that only *An. gambiae s.l.* and *An. rufipes* were present across all sites. *An. funestus*, once abundant and considered an efficient malaria vector, disappeared from Niamey in the 1970s following the destruction of its larval habitats under the combined influence of drought and agricultural intensification [6]. The species reappeared in 2004 [40] and was later reported with particularly high abundance in certain localities of the country [41] but it has struggled to re-establish in Niamey despite the presence of favorable ecological conditions. Banigoungou was the only site where all six species were recorded, and it exhibited particularly high abundance of all species, followed by Tondibiah and Lamordé. The lowest *Anopheles* density was observed in Koira-Tegui, a peripheral non-riparian site. This finding corroborates Julvez *et al.* [6], who noted that, unlike Sudanian cities, peripheral districts of Niamey located far from the river tend to record relatively low *Anopheles* densities. Overall, the diversity, species distribution, and variation in relative abundance of *Anopheles* mosquitoes appear to result from the interplay of specific ecological factors in each district [31] [37] [38] [42]. Among these ecological drivers, anthropogenic activities such as urban agriculture, particularly irrigated rice cultivation, are frequently cited [37] [43]. In addition to ecological factors influencing the development of immature stages, several authors have highlighted the role of housing characteristics in adult mosquito abundance. Indeed, rudimentary human dwellings have been shown to harbor more *Anopheles* than modern housing structures [44] [45].

This study demonstrated that the diversity and abundance of *Anopheles* species varied significantly across seasons. Overall, the highest abundance of *Anopheles* was recorded during the rainy season, while the lowest was observed in the hot season. This finding corroborates results obtained by Salako *et al.* in Benin [46], and Zogo *et al.* in Côte d'Ivoire [36], who reported a decline in *Anopheles* density from the rainy to the dry season. The increased abundance of *Anopheles* during

the rainy season can be attributed to the availability, abundance, and particularly the quality of larval habitats. Indeed, breeding sites of this species are known to increase in number and productivity during the rainy season but tend to decrease during the dry season [38]. Our observations are consistent with those reported by [37] [38]. However, when rainfall becomes excessive, some breeding sites may become flooded, washed out, and larvae carried away by water currents [47]. This phenomenon may explain the higher abundance of *An. gambiae s.l.* recorded during the cold season in Lamordé and Gamkallé compared to the rainy season. In contrast, the densities of *An. rufipes* decreased during both the cold and hot seasons relative to the rainy season.

The largest proportion of all *Anopheles* species was collected through indoor residual spraying (90.87%). This yield contrasts with the findings by [30] [34], who reported higher efficiency using CDC light traps. The reduced effectiveness of light traps observed in our study may be attributed to a shift in mosquito resting behavior, from exophilic to endophilic, as noted by Thabet *et al.* in Nigeria [48]. These results support the potential relevance of applying indoor residual spraying (IRS) as a vector control strategy in Niamey.

This study also showed that the *An. gambiae* complex was represented by two species, *An. coluzzii* and *An. arabiensis*, along with a few hybrids across all sites. *An. coluzzii* was the predominant species at all sites and during all seasons of the year, whereas *An. arabiensis* was relatively more abundant during the rainy season. No *An. gambiae* sensu stricto was detected in any season across the study sites. The predominance of *An. coluzzii* has previously been reported in Niger [4] and in other countries of the sub-region [35] [37] [48] [49]. This predominance is thought to be linked to the species' preference for specific breeding habitats, most often large anthropogenic permanent or semi-permanent sites such as irrigated rice fields [50]. Moreover, *An. coluzzii* appears to have a strong adaptive capacity, as studies have shown that the species can colonize polluted water bodies during the dry season in Niger [42]. This adaptability may explain its consistent presence across all seasons. In contrast, the relatively low frequency of *An. arabiensis* may be due to ecological conditions unfavorable to its reproduction. Indeed, *An. arabiensis*, like *An. gambiae*, has long been considered a species of arid zones [48] typically exploiting temporary sunlit natural habitats dependent on rainfall for reproduction [35] [50]. The scarcity, irregularity, and decline of rainfall, combined with high temperatures, likely limit the persistence of such natural habitats in Niamey, thereby hindering the development of these species. This may explain the progressive regression of *An. gambiae*, which is closely associated with these habitats, in favor of *An. coluzzii*, which shows increasing adaptability [4]. Several other studies have reported the disappearance of *An. gambiae*, notably in Chad [51] and northern Nigeria, which share a climate similar to that of Niger [52]. Furthermore, detailed studies conducted in Mali have demonstrated that the adaptability of *An. coluzzii* is associated with specific chromosomal inversions that appear to confer traits enabling the exploitation of both flooded or irrigated

areas and arid environments [53]. According to these authors, the persistence of *An. coluzzii* during the dry seasons (cold and hot) is strongly linked to this adaptive factor. Finally, the failure of PCR amplification in some samples was likely due to misidentification of mosquitoes at the morphological level and/or degradation of DNA quality associated with storage [54].

To determine the involvement of vector species and characterize malaria transmission levels across all sites, female *Anopheles* mosquitoes were individually analyzed by PCR for the detection of *Plasmodium* sporozoites. The results showed that only the *An. gambiae* complex (*An. coluzzii* and *An. arabiensis*) was implicated in malaria transmission in the study sites. Thus, *An. gambiae s.l.* emerges as the principal malaria vector in Niamey. Previous studies conducted in Niger have similarly identified *An. gambiae s.l.* as the main malaria vector [4] [41] in line with findings from other countries in the sub-region [35]-[37]. Across all sites, specimens of *An. coluzzii* and *An. arabiensis* were found infected with sporozoites, with infection rates varying significantly between the two species of the *An. gambiae* complex. Studies conducted in the sub-region have documented the involvement of both species in malaria transmission in areas where they occur in sympatry [48] [55]. Furthermore, investigations in Niger and Nigeria have shown that *Plasmodium* infection rates are significantly higher in *An. coluzzii* than in *An. arabiensis*, a difference likely attributable to the predominance of *An. coluzzii* over *An. arabiensis* [4] [48]. However, a study conducted in Burkina Faso, in an area where both species were equally abundant, demonstrated no significant difference in infection rates between them [55]. Between sites, *Plasmodium* infection rates were highest in Banigoungou, followed by Lamordé and Tondibiah, while the lowest was recorded in Koira-Tegui. Overall, infection rates peaked during the rainy season and were lowest in the hot season, although this difference was not statistically significant. This non-significant variation suggests that vector competence was relatively homogeneous across sites and that infection rates were simply proportional to vector abundance, corroborating the observations of Zogo *et al.* [36]. Notably, neither *An. funestus*, *An. pharoensis*, nor *An. rufipes* tested positive for infection. Yet, *An. funestus* and *An. pharoensis* have previously been incriminated in malaria transmission in certain localities of Niger [41]. Elsewhere, *An. funestus* has been identified as a major vector due to its strong anthropophilic behavior, which enhances its efficiency in malaria transmission [56] [57]. In contrast, *An. rufipes* has never been implicated in malaria transmission in Niger. This species has long been suspected of playing no role in malaria transmission, largely due to its predominantly zoophilic rather than anthropophilic behavior [58]. However, recent studies have demonstrated its vectorial role, notably in Cameroon [59] and Zambia [56].

The analysis of host preference revealed that, regardless of site, *An. gambiae s.l.* exhibited a tendency toward anthropophily (preference for feeding on humans). This tendency was significantly higher during the hot season and in sites other than Banigoungou and Tondibiah. Such variation may be attributable to species

behavior or host accessibility. Indeed, the peak of anthropophily coincides with the hot season, when populations tend to remain outdoors for extended periods and/or sleep outside, thereby increasing host accessibility. A study conducted in Côte d'Ivoire demonstrated that *An. gambiae s.l.* species live longer during the dry season than in the rainy season [37]. This increased longevity may partly explain the elevated anthropophily observed in the hot season, as older females are more numerous and more active in seeking blood meals [60]. The lower anthropophily rates observed in Banigoungou and Tondibiah may be explained, on the one hand, by the greater availability of alternative hosts, particularly large ruminants such as cattle present in these sites [61]. On the other hand, the high level of individual protection among populations in these areas, exposed to persistent Culicidae aggressiveness, through the use of bed nets during the rainy season may also account for the reduced anthropophily. Within the *An. gambiae* complex, *An. coluzzii* was found to be more anthropophagic than *An. arabiensis*, which displayed a stronger tendency toward zoophagy. This observation supports our findings and may further explain the differences in infection rates previously mentioned between the two species. The highly anthropophagic behavior of *An. coluzzii* observed in this study corroborates results already reported in Niger [4] and Ghana de [47].

Similar to infection rates, the entomological inoculation rate (EIR) varied according to seasons and sites. Across all sites, the highest EIR was recorded during the rainy season, while the lowest was observed in the hot season. Between sites, Banigoungou, Tondibiah, and Lamordé exhibited the highest EIR values, whereas Koira-Tegui recorded the lowest. These findings indicate that malaria transmission in Niamey remains perennial, although it is more intense during the rainy season and varies spatially across neighborhoods. The variation in malaria transmission observed between sites in Niamey may be explained by differences in the abundance of *An. gambiae s.l.*, the principal vector, driven by the bio-ecological characteristics previously described. Sites with the highest EIR also recorded relatively higher abundances of *An. gambiae s.l.*. Similar observations have been reported in Burkina Faso and Côte d'Ivoire [35] [38]. This pattern also aligns with studies on urban malaria, which established a gradient of transmission depending on whether neighborhoods are peripheral or centrally located [11]. According to Klinkenberg *et al.* [62] sites located near agricultural zones are more exposed to *Anopheles* bites than those farther away, thereby presenting a higher risk of malaria infection, with irrigated rice cultivation being particularly associated with transmission [43]. Indeed, Doannio *et al.* [63] demonstrated that malaria transmission rates in their study area were synchronized with the rice growth cycle. Furthermore, seasonal variation in transmission may be influenced by environmental temperature, which during the cold or hot seasons may fall below or exceed the optimal range for malaria transmission [64] contributing to the observed seasonality [38].

5. Conclusion

This study revealed the presence of six *Anopheles* species in Niamey: *An. gambiae*

s.l., *An. rufipes*, *An. funestus*, *An. pharoensis*, *An. ziemanni*, and *An. nili*. *An. gambiae s.l.* was the most abundant vector, although subject to spatio-temporal variability. Malaria transmission was continuous throughout the year, with varying intensities depending on sites and seasons. Transmission was essentially sustained by *An. coluzzii*, the predominant species, and *An. arabiensis*, the only member of the *An. gambiae s.l.* complex was identified in this study. These vector species were present year-round, with particularly high abundance during the rainy season and in riparian sites along the Niger River, especially peripheral and rice-growing areas of the city. These findings underscore the need to adapt and strengthen vector control strategies in such high-risk locations.

Authors' Contributions

NMS and AD designed the study. NMS, WH, ADS, SA, and IIA collected the data. NMS, MD, and WH analyzed the data, and NMS drafted the manuscript. AD, KMA, MIS, and IML critically revised the document.

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Conflicts of Interest

The authors declare no conflict of interest.

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