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Seasonal and Temporal Distribution of Anopheles Melas in Djegbadji, a Coastal Lagoon Village of Southwestern Benin

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Abstract

Background: The complex *Anopheles gambiae* includes the most effective vectors of malaria in the world. The identification of these vector species and their seasonal and temporal distribution are essential for effective control of malaria. In Benin, this complex *Anopheles gambiae* is represented by *Anopheles coluzzii*, *Anopheles gambiae*, *Anopheles arabiensis* and *Anopheles melas*-which occur near the lakes and lagoons of southern Benin. The present study provides information on the seasonal and temporal variation of *Anopheles melas*, the predominant malaria vector in Djegbadji, a coastal lagoon town of Benin.

Methods: Sampling of adult mosquitoes was performed at Djegbadji by night inside and outside of homes using human landing catches (from May 2013 to February 2015) and morning indoor residual spray catches (from January 2014 to December 2014). The collected mosquitoes were identified morphologically and *Anopheles melas* specimens were separated from other vectors by the palps biometrics technique.

Results: The results showed that the collected mosquitoes belonged to 5 genera and 8 species. It included: *Anopheles gambiae sensu lato*, *Aedes aegypti*, *Culex quinquefasciatus*, *Culex decens*, *Culex nebulosus*, *Culex fatigans* and *Mansonia africana*. Among the different species identified, *Anopheles gambiae sensu lato* had the highest abundance. At Djegbadji, *Anopheles melas* was the predominant malaria vector in dry season (90%), in rainy season (73.43%) and during flood periods (76.47%). In times of recession, there is a reduction of Anopheles melas density by 30.76% with a predominance of *Anopheles gambiae sensu stricto* (69.24%). The two methods used for the collection of *Anopheles melas* gave similar results (p = 0.35).

Conclusion: This study allowed us to update the data on malaria vector species in coastal lagoon areas of Benin. It also demonstrated that the predominant species of malaria vectors in this area was *Anopheles melas*. These findings will guide planning strategies against malaria vectors in the coastal lagoon areas of Benin.

Keywords

Anopheles melas; *Anopheles gambiae stricto sensu*; Vector; Predominant; Malaria; Djegbadji; Benin

Introduction

The knowledge of disease vectors biology and ecology is an essential pre-requisite to all vectors control strategies [1]. But, Plasmodium transmission and its vector biology were described more than 50 years ago [2]. Previously, the description and identification of vector species was based on morphological criteria and subdivisions called subspecies, varieties races and forms, were reported on the basis of geographical, behavioral criteria and the level of malaria transmission or a small morphological differences at a given stage of development of Anopheles.

Recently, Coetzee and colleagues [3] distinguished eight sibling species in the complex *Anopheles gambiae*. Four of the species are found in West Africa and differ in their biological behavior and vector capacity. The species include *Anopheles gambiae*, *Anopheles arabiensis* and *Anopheles coluzzii* which are well known for their ecological plasticity, their efficiency in disease transmission, their distribution, and anthropophily. In addition there is *Anopheles melas*, a salt-tolerant species mostly found in coastal environments, the mangrove ecosystem where its larvae develop in brackish water. It has low anthropophily and has reduced longevity which makes it a wrong malaria vector [4-6].

These species can be identified irrespectively of their physiological status by biometrics palps and polymerase chain reaction (PCR) techniques. The biometric palps technique can identify freshwater species (*Anopheles gambiae sensu stricto*) and brackish species (*Anopheles melas*). The identification of *Anopheles melas* by the biometric method is based on the ratio between the 4th, 5th and 3rd segments of palps length. This ratio, also called palps index (IP) is a distinguishing character between *Anopheles melas* and other fresh water species of *Anopheles gambiae* complex [7,8].

In Benin, previous studies have shown that that *Anopheles gambiae sensu stricto* plays a secondary role in malaria transmission [9,10]. In areas where *Anopheles gambiae sensu stricto* is absent, *Anopheles melas* plays a critical role in malaria transmission [9,10]. The composition of the mosquito fauna is potentially dynamic and closely dependent on the weather and the seasons. This observation showed the necessity to update regularly data on malaria vectors. In addition, the study of the diversity of malaria vectors species and their seasonal distribution, are the pre-requisites for designing sound management strategies aimed at controlling the vectors.

Data on malaria vectors are very important as they provide information on the levels of malaria transmission which helps in understanding the vectorial capacities of *Anopheles gambiae* complex species. This study reports the seasonal and temporal distribution of *Anopheles melas* in Djegbadji, a coastal community where its presence was observed few years ago [9]. The study identified periods when this vector could become very active in the transmission of malaria for a better planning vector control strategies against *Anopheles melas*.

Study Species: *Anopheles melas* Theobald, 1903

Anopheles melas is found on the west coast and extends all along the Atlantic coastline from the mouth of the Senegal River to Benguela in Angola. However, in contrast to its East African "twin

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(*Anopheles merus*)", it does not penetrate far inland. The degree of salinity tolerated by the larvae ranges from 5 to 37g of Cl Na/l. It grows in mangrove of Avicenna not Rhizophora, its development is synchronized with the monthly high tides of great amplitude which leave residual pools of water. Waves of *Anopheles melas* invade local villages every two weeks following the high tides in Gambia [11,12]. During these almost ideal conditions, rainfall also plays an important role in this mosquito's development. In Senegal, the "tales", or stripped salt plates which fill with water during the rains also provide excellent larval habitats for *Anopheles melas* [5]. On the coastline of the Gulf of Guinea where there is heavy rainfall, the rain reduces the salinity of water pools and the relationship between *Anopheles melas* and *Anopheles gambiae sensu stricto* is greatly disturbed, especially in Benin [13].

Methods

Study area

Adult mosquitoes were collected at Djegbadji, a village in the lagoon coastal areas of southwest Benin (2° and 6° 19'14,52"N 4'22,44"E) located along the lagoon of Ouidah. Djegbadji is a salt production area. It is a village characterized by an agricultural economy: fishing, salt mining and pig farming in some concessions. The water in the lagoon is brackish during any season [9]. Salinization of water in these localities facilitates colonization, by Anopheles melas which prefers brackish deposits. At Djegbadji, mangrove stands out with a great extension by mangrove trees areas and ferns. Djegbadji being located at a lagoon environment, the ecosystem is strongly influenced by rising water levels. Thus, apart from the classical periods of the dry season (January-February) and rainy season (May to July), Djegbadji is also characterized by a flood period (September-October) and a period of flood recession (November-December) (Figure 1).

Sampling mosquitoes

The study was based on the method of separation of freshwater and brackish species in systematic members of the complex *Anopheles gambiae*. It consisted of updating inventory of the existing mosquito fauna by collecting adult mosquitoes at Djegbadji and highlighting the seasonal variation of the *Anopheles melas* and *Anopheles gambiae sensu stricto* species. Two main methods of sampling were used go ahead and provide the names of the methods that were used before detailing them.

Collections by human landing catch

Human landing catches were performed from May 2013 to February 2015 inside and outside 02 selected houses, one in the periphery (20 meters from the boundary of the village) and the other in the center of the village. The collectors are volunteers who gave prior informed consent. They received prophylaxis against malaria and have been vaccinated against yellow fever. Every collection was done from 9:00 pm to 5:00 am and included four successive nights. Mosquitoes were collected by four collectors, two inside and two outside of houses using mouth aspirators and flashlights, collected all landing mosquitoes on their feet.

Mosquito collections with pyrethrum spray catches

The number of mosquitoes resting indoor and outdoor was estimated from January 2014 to December 2014 by pyrethrum spray catches in 10 houses during each of the four seasons (dry season, rainy season, flood season and flood recession period). For the selection of homes, we used systematic sampling, this means that there is an interval between each selected home that is included in the sample. According to the General Census of Population and Habitation in Benin in 2002, Djegbadji has 1266 houses. The sampling interval (K) was determined by dividing the number of houses by the chosen sample size (10 houses), K = 127. The first house included (n°120) was selected between 1 and 127. The 9 others houses was selected at each 127 (K) interval.

These collections were conducted between 06.45 and 08.00 hours and consisted of covering all exposed surfaces white sheets

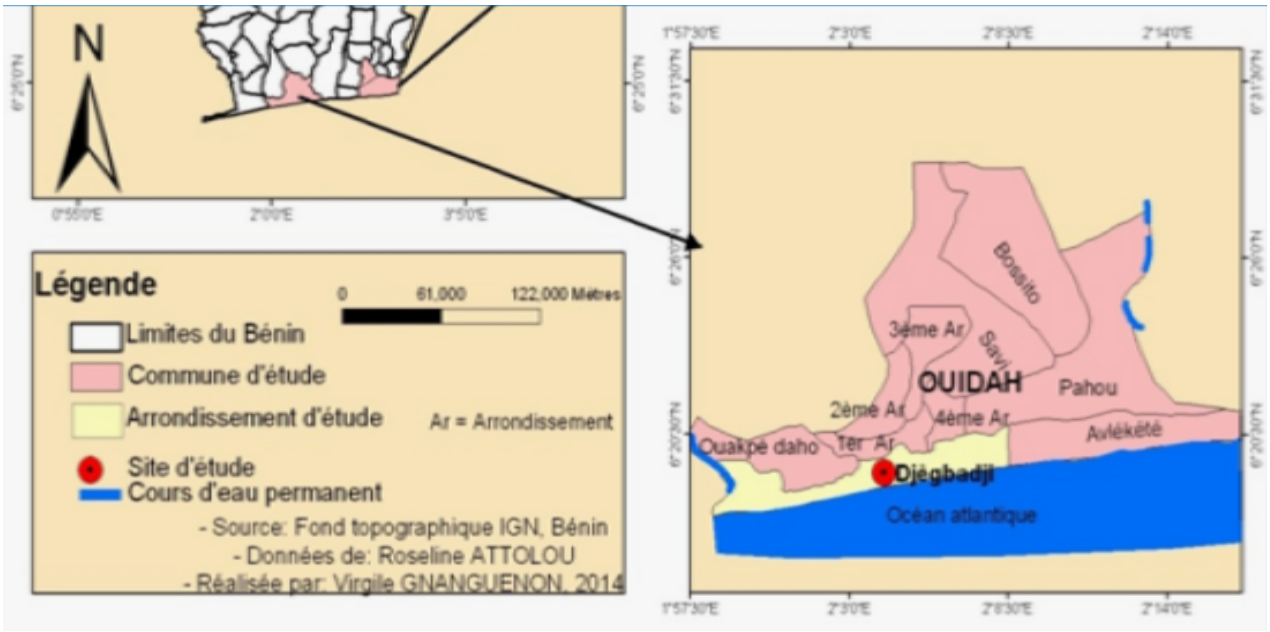

Figure 1: Map of southern Benin with the study locality, Djegbadji.

and collecting all fallen mosquitoes after spraying the room. After collection, mosquitoes were counted and morphologically identified. Collected *Anopheles gambiae sensu lato* were stored individually in labeled Eppendorf tubes with desiccant until laboratory processing. No windows trap was used for outdoor resting mosquito collection due to logistic constraints.

Morphological identification of mosquitoes

The collected mosquitoes were morphologically identified using taxonomic identification keys of Gillies & De Mellon [14]. All *Anopheles gambiae sensu lato* specimens were separated from other mosquitoes and the heads were cut and used to separate the freshwater from brackish water species using biometric palps method.

To identify the species of complex *Anopheles gambiae* with biometrics palps, the palps of the heads of *Anopheles gambiae sensu lato* specimens cut were whitened in chloral-lacto phenol for 48 hours. These palps were then mounted between slide and cover in a drop of phenolbalsame. The measurements were done using an optical microscope at 10x objective with an eyepiece micrometer. A palpal index (PI) of 0.81 was used as a threshold for separation of freshwater and brackish species. Freshwater species have palp index less than or equal to 0.81. An IP of greater than 0.81 is associated with brackish species in this case (*Anopheles melas*).

Statistical analysis

For seasonal and temporal distribution of species of the complex Anopheles gambiae in the coastal lagoon areas of Ouidah, we calculated the confidence interval of the proportion of each species of the complex using the exact binomial method (small sample size) and the normal approximation method to a binomial distribution (large sample size) [15]. The workforce confidence interval was calculated using the exact method of distribution Gamma [16]. Comparisons of proportions were performed using X^2 test and the multiple comparisons of proportions. We used the R-Software version R 2.15 for analyzing the data (reference) software was used for processing and analyzing data and seasonal and temporal distribution of *Anopheles melas* by collection methods.

Results

Mosquito diversity

A total of 4,949 mosquitoes including 4,542 collected by human landing catch (Table 1) and 407 by pyrethrum spray catches (Table 2) were collected. The collected mosquitoes belong to 5 genera and 8 species. The mosquitoes identified include: *Anopheles gambiae sensu lato*, *Aedes aegypti*, *Culex quinquefasciatus*, *Culex gr decens*, *Culex nebulosus*, *Culex fatigans* and *Mansonia africana*. Among the different species identified *Anopheles gambiae sensu lato* was most abundant representing respectively 79.79% and 50.61% of human landing and pyrethrum spray collections. There was a seasonal variation in the *Anopheles gambiae sensu lato* density. The rainy season was identified as the period of high density of *Anopheles gambiae sensu lato* with a percentage of 45.23 for human landing and 72.33 pyrethrum spray collections. The trend was similar (p=0,35) between the two sampling techniques with a high proportion of *Anopheles gambiae sensu lato* the rainy season representing 45.23% of the mosquito collected only in May with human landing catches, versus 45.95 % for all the rainy season with pyrethrum spray catches.

Seasonal and temporal distribution of *Anopheles melas*

The separation of the complex *Anopheles gambiae* species by biometrics palps method included 3,291 females with 2,683 from human landing catches and 608 from the pyrethrum spray collections.

Seasonal and temporal distribution of the aggressiveness of *Anopheles melas* on human: Out of a total of 2,683 *Anopheles gambiae sensu lato, Anopheles melas* represented 52.93%. *Anopheles melas* was predominant during the dry season (P<0.05) (February 2014, April 2014 and February 2015) (Figure 2). The density of this malaria vector is reduced during flood period while density of *Anopheles gambiae sensu stricto* increased (Figure 2). During the rainy season (May 2013) and during floods (October 2013), *Anopheles gambiae sensu stricto* was predominant (P<0.05). A similar result is observed between the biting behavior of *Anopheles melas* and Anopheles *gambiae sensu stricto* in July (P>0.05) (Figure 2).

Seasonal and temporal distribution of *Anopheles melas* endophilic: Out of a total of 198 *Anopheles gambiae sensu lato* identified, 144 were *Anopheles melas* representing 72.73%. In the dry season, *20 Anopheles gambiae sensu lato* were collected by pyrethrum spray catches and included 18 *Anopheles melas* (90%) versus 143 *Anopheles gambiae sensu lato* in the rainy season including 105 *Anopheles melas* (73.43%) (Figure 2). During floods, *Anopheles gambiae sensu stricto* represented 23.53% (n=four) of the mosquitoes collected versus 76.47% (n=13) of *Anopheles melas*. However, during recession, Anopheles *gambiae sensu stricto* represented 69.23%

Table 1: Diversity and abundance of fauna aggressive culicidienne night.

Species	May-13	July-13	October-13	February-14	April-14	June-14	February-15	Total
Anopheles gambiae sensu lato	1639	254	586	237	294	386	228	3624
Aedes aegypti	5	0	4	4	1	0	2	16
Culex quinquefasciatus	32	66	33	67	26	4	56	284
Culex gr decens	42	16	81	106	184	35	65	529
Culex nebulosus	22	0	7	3	3	0	1	36
Culex fatigans	0	0	0	0	2	0	0	2
Mansonia africana	20	16	8	0	0	5	0	49
Uranotaenia bilineata	2	0	0	0	0	0	0	2
Total	1762	352	719	417	510	430	352	4542

Table 2: Endophilic morning culicidienne fauna (January 2014-December 2014).

SPECIES	DRY SEASON (JANUARY- APRIL)	RAINY SEASON (MAY- JULY)	FLOOD SEASON (SEPTEMBER-OCTOBER)	FLOOD RECESSION SEASON (NOVEMBER-DECEMBER)	TOTAL
ANOPHELES GAMBIAE SENSU LATO.	20	149	17	20	206
AEDES AEGYPTI	41	1	12	8	62
CULEX GR DECENS	0	1	4	8	13
CULEX NEBULOSUS	8	6	6	1	21
CULEXQUINQUEFASCIATUS	35	27	28	11	101
MANSONIA AFRICANA	0	3	1	0	4
TOTAL	104	187	68	48	**407**

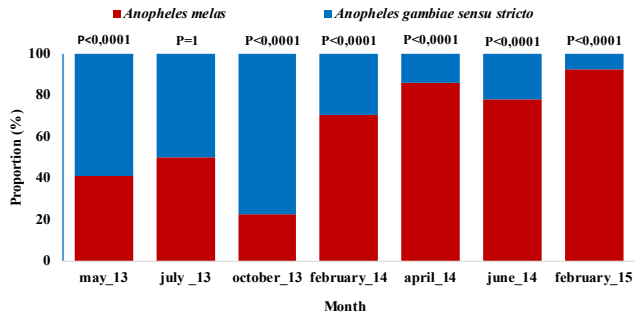

Figure 2: Distribution of seasonal and temporal aggressiveness *Anopheles melas* on man.

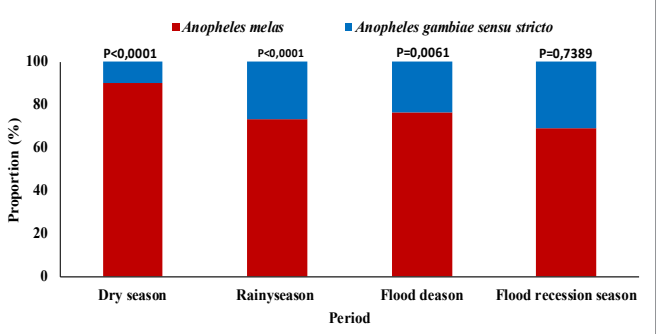

Figure 3: Distribution of seasonal and temporal endophilic *Anopheles melas*.

(n=18) of the mosquitoes collected versus 30.76% (n=eight) of *Anopheles melas*. *Anopheles melas* was strongly represented in the dry season, rainy season and flood period (P<0.05). However, in times of recession, Anopheles melas was found in similar proportion with *Anopheles gambiae sensu stricto* (P=0.7389) (Figure 3).

Seasonal and temporal distribution of *Anopheles melas* by collection methods: A comparison of the probability of catching *Anopheles melas* using the two methods show that probability to collect *Anopheles melas* was similar for both methods (P=0.35) and *Anopheles melas* was similarly distributed between the two methods (P=0.349).

Discussion

The study showed that mosquito fauna of Djegbadji is rich and diverse. The observed results indicate the presence of different species of mosquitoes during different times of the year. The results show that *Anopheles gambiae* complex species is the dominant species found in the study area. The Biometrics palps method allowed identification of Anopheles melas specimen involved in malaria transmission in the study area. We were able to separate Anopheles melas from *Anopheles gambiae sensu stricto* using the Biometrics palps method. The practical utility of this technique was demonstrated by Petrarca et al. [17] and evaluated by Akogbeto et al. [18].

The results show that the density of *Anopheles gambiae sensu lato* population is high during the rainy season, a period when the frequency of rainfall favours the proliferation of mosquito breeding sites. The high abundance of *Anopheles gambiae sensu lato* at Djegbadji is also related to the favourable conditions created by human activities such as vegetable farming.

Previous studies show a general a lack of data on the seasonal distribution of *Anopheles gambiae* complex members in the coastal lagoon areas in southern Benin. In this study, we successfully used the biometric palps method to identify two major vector sibling species of malaria (*Anopheles gambiae sensu stricto* and *Anopheles melas*). Human landing catches show that Anopheles melas was predominant at Djegbadji. This vector was very predominant in the dry season (February, April) and early in rainy season (June). The prevalence dominance of *Anopheles gambiae sensu stricto* was observed during floods (October).

Indoor mosquito collections showed the dominance of *Anopheles melas* in the dry season, the rainy season and floods. In times of recession, there was a reduce density of Anopheles melas that was found in equal proportion with *Anopheles gambiae sensu stricto*. This could be due to the decrease in salinity of the lagoon that probably affects the development of *Anopheles melas* which prefer brackish water.

The probability to collect *Anopheles melas* was similar between the two collections methods (P=0.35) with a similar distribution of *Anopheles melas* collected by human landing catches and pyrethrum spray catches. Between the two methods, none was specific for the collection of *Anopheles melas*.

An important factor related to the development of *Anopheles melas* is the relationship between the larval ecology of this species and the salinity of its breeding sites [9]. It is this phenomenon which justifies the decrease of *Anopheles melas* between October and December while density of *Anopheles gambiae sensu stricto* increased. Indeed, the last quarter of the year is the period of flood and recession. Thus, the lagoon of Ouidah is filled with a large amount of fresh water mainly from the northern Benin between September and October that modifies *Anopheles melas* larvae ecology. The desalination results in a general decrease in mosquito density [9] especially *Anopheles melas*. During recession (November-December), the deposits left by the

Table 3: Seasonal and temporal distribution of *Anopheles melas* based catch methods.

Method	*Anopheles gambiae sensu lato*	*Anopheles melas*	%	*Anopheles gambiae sensu stricto*	%	P (Wald-test)	P(LR-test)
HLCs	697	483	69,3	214	30,7	0.35	0,349
PSC	198	144	72,73	54	27,27		

P (wald-test): p-value of the coefficients of null Wald test;
P (LR-test): p-value of the likelihood ratio test.
HLCs: Human Landing catches;
PSC: Pyrethrum spray catches

withdrawal of water are favorable for *Anopheles gambiae sensu stricto* and explains the high density of *Anopheles gambiae sensu stricto* observed during this period. In February, with the increase in salinity of the lagoon of Ouidah, we recorded 92.18% of Anopheles melas.

The seasonal and temporal variation of *Anopheles melas* density in our study area was similar to that observed by Akogbeto [19]. The results show a high density of *Anopheles melas* in the dry and rainy season. Similar results were observed in Ghana where Anopheles melas was predominant in the dry and rainy season [20].

The presence of a relatively high density of *Anopheles gambiae sensu stricto* may also be due to the urbanization of the traditional lagoon area and the reduction of mangrove (mangrove wood exploitation for the production of salt), natural habitat of *Anopheles melas*. The transformation of the traditional environment favourable to ecology Anopheles melas creates an imbalance compared to the initial populations of vectors and results in a decrease of Anopheles melas and an increase in *Anopheles gambiae sensu stricto* density. The salinization of the lagoon of Ouidah is also progressive, and helps *Anopheles gambiae sensu stricto* adaptation to high salinity. A laboratory study could be considered to specify the tolerance limits of both species.

Conclusion

In this study we showed the diversity of mosquito fauna and seasonal and temporal variation of *Anopheles melas* at Djegbadji, a village of the coastal areas of southern Benin. Our study shows that mosquito fauna at Djegbadji was mostly represented by *Anopheles gambiae sensu lato* principal malaria vector. *Anopheles melas* and *Anopheles gambiae sensu stricto* were the most common species of the complex *Anopheles gambiae* found in the study. Their seasonal and temporal variation was not uniform. *Anopheles melas* was predominant in the dry season and the beginning of the rainy season, but in the flood period, its density decreased while *Anopheles gambiae sensu stricto* increased. In consequence, this area is at high risk of a malaria outbreak. Therefore, permanent control measures, particularly in the flood period, should be taken to prevent malaria.

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