UNIVERSITÉ DE LA MÉDITERRANÉE
FACULTE DE MÉDECINE DE MARSEILLE

UTILISATION DE DONNEES D'OBSERVATION DE LA TERRE PAR SATELLITE POUR L'EVALUATION DES DENSITES VECTORIELLES ET DE LA TRANSMISSION DU PALUDISME

T H È S E

Présentée et publiquement soutenue devant

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AVANT PROPOS

Le format de présentation de cette thèse correspond à une recommandation de la spécialité Maladies Infectieuses et Microbiologie, à l'intérieur du Master des Sciences de la Vie et de la Santé qui dépend de l'Ecole Doctorale des Sciences de la Vie de Marseille.

Le candidat est amené à respecter des règles qui lui sont imposées et qui comportent un format de thèse utilisé dans le Nord de l'Europe et qui permet un meilleur rangement que les thèses traditionnelles. Par ailleurs, la partie introduction et bibliographie est remplacée par une revue envoyée dans un journal afin de permettre une évaluation extérieure de la qualité de la revue et de permettre à l'étudiant de commencer le plus tôt possible une bibliographie exhaustive sur le domaine de cette thèse.

Par ailleurs, la thèse est présentée sur article publié, accepté ou soumis associé d'un bref commentaire donnant le sens général du travail. Cette forme de présentation a paru plus en adéquation avec les exigences de la compétition internationale et permet de se concentrer sur des travaux qui bénéficieront d'une diffusion internationale.

Prof. Didier Raoult

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PUBLICATIONS ET COMMUNICATIONS

Publications dans le cadre de la thèse

Machault V, Orlandi-Pradines E, Michel R, Pages F, Texier G, Pradines B, Fusai T, Boutin JP, Rogier C. **Remote sensing and malaria risk for military personnel in Africa.** *J Travel Med* 2008, **15:216-220.**

Pages F, Texier G, Pradines B, Gadiaga L, Machault V, Jarjaval F, Penhoat K, Berger F, Trape JF, Rogier C, Sokhna C. **Malaria transmission in Dakar: a two-year survey**. *Malar J* 2008, **7**:178.

Machault V, Gadiaga L, Vignolles C, Jarjaval F, Bouzid S, Sokhna C, Lacaux JP, Trape JF, Rogier C, Pages F. **Highly focused anopheline breeding sites and malaria transmission in Dakar.** *Malar J* 2009, **8:138.**

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Machault V, Vignolles C, Pages F, Gadiaga L, Gaye A, Sokhna C, Trape JF, Lacaux JP, Rogier C: **Spatial heterogeneity and temporal evolution of malaria transmission risk in Dakar, Senegal, according to remotely sensed environmental data**. *Malar J* 2010, **9**:252.

Machault V, Borchi F, Vounatsou P, Pages F, Vignolles C, Lacaux JP, Rogier C: **The use of remotely sensed environmental data in the study of malaria**. *Geospat Health*. Soumis.

Pages F, Machault V, Gadiaga L, Gaye A, Jarjaval F, Lacaux JP, Godefroy L, Trape JF, Sokhna C, Rogier C: **Entomological data in Dakar from 2007 to 2010**. En préparation.

Machault V, Vignolles C, Pages F, Gadiaga L, Gaye A, Sokhna C, Trape JF, Lacaux JP, Rogier C : **Mapping of spatial and temporal distribution of Anopheles larvae and adults in Dakar.** En préparation.

Autres publications

Orlandi-Pradines E, Rogier C, Koffi B, Jarjaval F, Bell M, Machault V, Pons C, Girod R, Boutin JP, Pages F: **Major variations in malaria exposure of travellers in rural areas: an entomological cohort study in western Cote d'Ivoire**. *Malar J* 2009, **8**:171.

Resseguier N, Machault V, Ollivier L, Orlandi-Pradines E, Texier G, Pradines B, Gaudart J, Buguet A, Tourette-Turgis C, Rogier C: **Determinants of compliance with malaria chemoprophylaxis among French soldiers during missions in inter-tropical Africa**. *Malar J* 2010, **9**:41.

Communications orales

Actualités du Pharo, septembre 2006.
Machault V, Orlandi-Pradines E, Michel R, Pages F, Texier G, Pradines B, Fusai T, Boutin JP, Rogier C
Etude des déterminants individuels, comportementaux et environnementaux du paludisme chez les militaires français effectuant une mission de courte durée en Afrique intertropicale

XXIIèmes Journées Scientifiques d'EPITER, Bellerive-sur-Allier près Vichy (Allier), 10 & 11 novembre 2006.
Machault V, Orlandi-Pradines E, Michel R, Pages F, Texier G, Pradines B, Fusai T, Boutin JP, Rogier C
Etude des déterminants individuels, comportementaux et environnementaux du paludisme chez les militaires français effectuant une mission de courte durée en Afrique intertropicale.

Space Show, Toulouse, 22-25 avril 2008.
Machault V, Vignolles C, Pages F, Lacaux JP, Rogier C
Evaluer et prédire la transmission du paludisme aux militaires en mission en Afrique intertropicale : travaux en cours.

Space Show, Toulouse, 8-11 juin 2010.
Machault V, Vignolles C, Pages F, Lafaye M, Lacaux JP, Rogier C
Use of remotely sensed environmental data for the evaluation of malaria transmission risk in urban settings.

DEROULEMENT DE LA THESE

Année universitaire 2005/2006 - Master 2 de recherche en Santé publique et management de la santé, Spécialité épidémiologie, Option pays en développement. Université Paris 6.

Février 2006/juin 2006 - Stage à Institut de Médecine Tropicale du Service de Santé des Armées, au Département d'Epidémiologie et Santé Publique, Marseille (ancien nom de l'actuel IRBA).

> Etude des facteurs de risque individuels, comportementaux et environnementaux des accès palustres chez des militaires français se rendant en Afrique intertropicale.
> Direction : Pr. Christophe Rogier, Dr. Rémy Michel, Pr. Jean-Paul Boutin.

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Février 2007/juin 2007 - Stage à MEDIAS-France, Groupement d'intérêt public, Toulouse.

> Télédétection et paludisme urbain.
> Direction : Pr. Jean-Pierre Lacaux, Dr. Cécile Vignolles, Mme Murielle Lafaye.

Travail de terrain à Dakar, sous forme de trois séjours pour la sélection et la délimitation des zones d'études, la supervision du travail de terrain, l'exécution des prospections larvaires et la mise en place des bases de données entomologiques et géographiques.

> Septembre 2007/octobre 2007 - Première mission à Dakar.
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Etant donné la possibilité donnée par l'Ecole Doctorale de présenter la thèse sous la forme d'articles, j'ai choisit cette option par rapport à l'écriture d'un document de thèse « classique » en français. Comme l'indique le détail du déroulement des travaux, deux stages ont précédés le début officiel de la thèse. Dès le premier stage, un article a été préparé. Ensuite, j'ai toujours tenté de publier les résultats au fur et à mesure, en accumulant le moins de retard possible, de façon à structurer mon travail. En effet, la soumission d'un article paraissait la meilleure solution pour finaliser une analyse et ne rien laisser « en chantier ». De plus, il est apparu pertinent de mettre à profit le temps à ma disposition pour aller le plus loin possible dans les analyses et la mise en place des cartes et non pas pour générer un document qui aurait repris des travaux déjà présentés et validés par des comités de lecture.
Les articles publiés, soumis ou en préparation contiennent la totalité du travail effectué pendant la thèse et le choix de présenter la thèse dans ce format n'a pas nui à la présentation exhaustive des travaux entrepris.
Bien sûr, ce format implique parfois un chevauchement de certaines parties d'introduction et de discussion mais j'ai essayé de clarifier le plus possible l'enchaînement logique et chronologique des articles grâce aux parties de liaisons constituant le « ciment » du document de thèse.

Concernant ma bibliographie, j'ai décidé de ne pas inclure dans le corps de la thèse la revue en français, publiée dans Médecine Tropicale. En effet, cette revue est préliminaire à la revue écrite en anglais (présentée en introduction de ce document) et est moins élaborée que la version anglaise. De plus, certains concepts n'y sont pas abordés, tel que le détail des analyses statistiques à mettre en place lors d'études associant des données environnementales à des indicateurs paludométriques. La revue française est présentée en annexe.

Deux articles ne sont pas inclus dans la thèse (**Major variations in malaria exposure of travellers in rural areas: an entomological cohort study in western Cote d'Ivoire**. *Malar J* 2009, **8**:171 et Resseguier N, Machault V, Ollivier L, Orlandi-Pradines E, Texier G, Pradines B, Gaudart J, Buguet A, Tourette-Turgis C, Rogier C: **Determinants of compliance with malaria chemoprophylaxis among French soldiers during missions in inter-tropical Africa**.). En effet, mon implication dans ces travaux n'entrait pas de façon logique dans le cadre des travaux de thèse.

Les travaux présentés dans l'article « **Malaria transmission in Dakar: a two-year survey** » ont été effectués avant mon arrivée dans le laboratoire. Mon implication a donc été mineure mais l'article a été intégré dans la thèse car il constitue la première étape des travaux entrepris à Dakar entrant dans le cadre de la thèse.

Concernant les six autres articles, mon implication a été importante, tant sur le plan de la collecte des données, du traitement des images, de l'analyse et de la rédaction.

SOMMAIRE

RESUME

Malgré les efforts nationaux et internationaux, le paludisme reste un problème de santé publique majeur dans de nombreux pays et les systèmes de santé ont des difficultés pour évaluer son poids réel, le risque de transmission des plasmodiums et leur répartition géographique. Pourtant, l'hétérogénéité spatiale du paludisme peut être importante et dans ce contexte de concentration du risque, la lutte contre la maladie peut gagner à être focalisée dans certains lieux et certaines périodes. D'une part, l'environnement est un déterminant majeur de la biodiversité du paludisme à cause du caractère vectoriel de la transmission et des préférences bioécologiques des vecteurs. D'autre part, les satellites en orbite peuvent fournir des données environnementales, climatiques et météorologiques qui ont déjà été utilisées pour l'étude de maladies infectieuses. La « télé-épidémiologie » a été définie comme une approche intégrée visant à associer des données médicales, épidémiologiques ou entomologiques de terrain à des données environnementales obtenues par satellites, en s'appuyant sur la compréhension et la mesure des mécanismes physiques et biologiques qui sont en jeu. Dans les villes, il a déjà été possible de mettre en évidence, en utilisant des données satellites à des échelles appropriés, des associations entre des éléments urbains cartographiés et des indicateurs paludométriques. Chez des voyageurs, dans l'objectif d'une évaluation du risque de contracter le paludisme ou de l'efficacité de mesures prophylactiques, il serait utile de pouvoir évaluer et prédire les niveaux d'exposition à la transmission dans les différentes localités parcourues. L'objectif général des travaux de la présente thèse était d'identifier des facteurs environnementaux mesurables par satellite et utilisables pour l'évaluation du risque de paludisme chez les voyageurs d'une part et en milieu urbain d'autre part.

Tout d'abord, des données de télédétection ont été utilisées pour évaluer les niveaux d'expositions au risque de paludisme d'une population de militaires, dans le cadre d'un travail sur l'estimation de leurs facteurs de risque d'accès palustre. Les résultats ont montré que, même en prenant en compte les facteurs de confusion de l'âge et de l'observance de la chimioprophylaxie, l'environnement était le principal facteur associé au risque de survenue d'accès palustres.

En parallèle, une large collecte de données entomologiques a été effectuée pendant cinq ans dans la ville de Dakar et a permis de mettre en évidence une très forte hétérogénéité spatiale et temporelle de la transmission du paludisme dans la ville. Les informations collectées ont été centralisées dans une base de données géoréférencée (SIG - Système d'Information

Géographique) contenant toutes les variables entomologiques, environnementales, météorologiques, biologiques et physiques relevées sur le terrain ou par satellites.

Puis un travail de modélisation du risque entomologique dans la capitale du Sénégal, basé sur les données collectées sur le terrain et sur des données environnementales issues d'images satellites a été mené. Une première étape a permis de mettre en évidence les évolutions des zones à risque de transmission et d'affirmer que le pourcentage de la population dakaroise à fort risque de transmission avait diminué entre 1996 et 2007. Une deuxième étape a conduit à l'élaboration de 1) une carte des gîtes larvaires accompagné d'un indice de productivité dans la ville de Dakar, 2) une carte des densités d'agressivité des anophèles adultes, puis 3) ces cartes étaient rendues dynamiques, c'est-à-dire que les variations temporelles liées aux variations de leurs déterminants météorologiques étaient prises en compte.

Les résultats des travaux de thèse ont montré que des données de télédétection associées à une grande quantité de données de terrain peuvent permettre l'ajustement de modèles prédictifs et la construction de cartes de risque entomologique, en milieu urbain ou pour des populations mobiles.

ABSTRACT

Despite national an international efforts, malaria remains a major public health in many countries and sanitary systems are hindered by the lack of information on the actual burden of malaria, on the plasmodium transmission risk and on their geographical distribution. Nevertheless, spatial heterogeneity can be important and in this context, malaria control could be improved if could be focused in place and time. On one hand, the environment is a major determinant of malaria biodiversity, because of the vectorial transmission and the vectors bioecological preferences. On another hand, orbiting satellites can provide environmental, climatic and meteorological data that already have been used for the study of infectious diseases. "Tele-epidemiology" has been defined as an integrated approach aiming at associating medical, epidemiological or entomological ground data, with remotely-sensed environmental data, based on the in depth comprehension and measurement of the involved physical and biological mechanisms. In cities, it has already been possible to highlight associations between mapped urban settings and malariometric indices, using satellite data at appropriate scales. Among travellers, in the objective to evaluate malaria risk or efficacy of prophylactic devices, it would be useful to evaluate and predict transmission levels in the visited places. The objective of the present thesis was to identify environmental factors that could be remotely-sensed and that could be used in the evaluation of malaria risk among travellers on one hand and in urban settings on the other hand.

First, remotely-sensed data have been used to evaluate levels of exposure to malaria risk of militaries, in the scope of a study on their risk factors for clinical malaria. Results have showed that, even when taking into account age and compliance to chemoprophylaxis as confusion factors, the environment was the factor the most strongly associated to clinical malaria risk.

In parallel, an extensive entomological study has been conducted during five years in Dakar and allowed demonstrating a strong spatial and temporal heterogeneity of malaria transmission in the city. Collected information were centralized in a georeferenced database (GIS - Geographic Information System) containing all entomological, environmental, meteorological, biological and physical data collected on the field or by remote sensing.

Finally, modelling of entomological risk in the capital city of Senegal was undertaken, based on data collected on the ground and environmental data issued from satellites. A first step showed the evolution of malaria transmission risk areas and allowed declaring that the fraction of human population that was at high risk for transmission decreased between 1996

and 2007. A second step led to the development of 1) a map of the breeding sites with a productivity indicator in Dakar city, 2) a map of aggressive adult *Anopheles* densities, and 3) a dynamic aspect was added to those maps, taking into account the variations of their meteorological determinants.

The results of the work undertaken in this thesis demonstrated that remotely-sensed information, associated with a large amount of ground data, allow to adjust predictive models and to draw entomological risk maps, in urban settings or for moving populations.

INTRODUCTION

Le paludisme est une infection parasitaire causée par un *Plasmodium* transmis à l'homme par la piqûre d'un moustique vecteur du genre *Anopheles*. Malgré les efforts nationaux et internationaux, le paludisme reste un problème de santé publique majeur dans de nombreux pays et les avancées dans la lutte contre la maladie se heurtent à de nombreux obstacles. Les systèmes de santé ont des difficultés pour évaluer le poids réel du paludisme, le risque de transmission des plasmodiums et leur répartition géographique. Pourtant, l'hétérogénéité spatiale du paludisme peut être importante, parfois à des échelles réduites.

Le contrôle du paludisme s'effectue simultanément à plusieurs niveaux, par la lutte contre les vecteurs, la diminution du contact Homme-vecteurs (*e.g.* à l'aide de moustiquaires imprégnées d'insecticides), les traitements préventifs intermittents des enfants et des femmes enceintes, la chimioprophylaxie chez les voyageurs en zone d'endémie palustre et le traitement rapide et efficace des cas de paludisme clinique. Les méthodes de lutte antivectorielle reposent sur la lutte antilarvaire, par exemple par la dispersion d'insecticides dans les gîtes larvaires d'anophèles, et la lutte anti-imago (*i.e.* moustiques adultes), par exemple en pulvérisant des insecticides dans les maisonnées.

L'environnement est un déterminant majeur de la biodiversité du paludisme à cause du caractère vectoriel de la transmission des plasmodiums et des préférences bioécologiques des vecteurs. La saisonnalité du climat, la distribution et la quantité des pluies, la température, l'humidité, l'altitude, la présence d'eau de surface ou de végétation, ainsi que des facteurs anthropogéniques, tels que les activités agricoles, l'irrigation, la déforestation, l'urbanisation, la construction de routes ou de barrages, sont associés à la transmission des plasmodiums. Ces facteurs peuvent déterminer l'apparition et la persistance des gîtes larvaires, la vitesse de développement des larves, la survie des adultes - et donc leur densité - ou la vitesse de développement du parasite chez les anophèles vecteurs (cycle extrinsèque). Ils peuvent donc déterminer la répartition spatiale et temporelle de la transmission des plasmodiums et du paludisme. La répartition géographique, la densité et la longévité des vecteurs, l'accès aux traitements antipaludiques efficaces et la sensibilité génétique et immunologique des populations humaines au parasite sont à l'origine de l'hétérogénéité du paludisme.

Dans ce contexte de concentration du risque de paludisme, la lutte contre la maladie peut être focalisée dans certains lieux et certaines périodes afin d'optimiser l'efficacité des méthodes de contrôle et de réduire leurs coûts. Cette focalisation de la lutte peut tirer avantage de la connaissance des déterminants écologiques de la répartition spatiale et temporelle de la transmission et de la maladie. L'obtention de cartes de risques de paludisme (entomologiques ou épidémiologiques) peut être considérée comme une étape clé dans le processus de lutte contre la maladie car elles peuvent permettre de diriger les équipes de terrain vers les lieux et périodes pour lesquelles le risque et le poids du paludisme sont les plus importants.

Depuis que des satellites d'observation de la terre sont en orbite, ils ont largement été utilisés pour répondre à des questions de santé, même s'ils ne sont pas spécifiquement dédiés à l'enregistrement de données dans ce domaine. De nombreux travaux ont tiré parti des informations environnementales fournies par les plateformes spatiales pour l'étude de maladies infectieuses, en utilisant les techniques de traitement d'images, SIG (Systèmes d'Information Géographique) et GPS (*Global Positioning System*) [1-6]. Parmi ces maladies, le paludisme a fait l'objet de nombreuses recherches et des cartes et modèles de risque ont été dressés à tous les niveaux du cycle de transmission pour évaluer ou prédire différents indicateurs paludométriques : présence de gîtes larvaires, densités larvaires, densités et agressivité vectorielles (nombre de piqûres reçues par individu et par unité de temps), taux d'inoculation entomologique (TIE, nombre de piqûres infectées reçues par individu et par unité de temps), taux de prévalence parasitaire, taux d'incidence des infections plasmodiales et de la morbidité palustre et mortalité attribuée au paludisme. De plus, les données de télédétection ont permis d'obtenir des cartographies de population humaines ce qui est un point essentiel dans l'évaluation du nombre de personnes à risque de paludisme et du poids de la maladie.

La « télé-épidémiologie » a été définie par le CNES (Centre National d'Etudes Spatiales) comme une approche intégrée visant à associer des données médicales, épidémiologiques ou entomologiques, de terrain à des données environnementales obtenues par satellites, en s'appuyant sur la compréhension et la mesure des mécanismes physiques et biologiques qui sont en jeu [7].

En pratique, les échelles spatiales et temporelles de la télé-épidémiologie sont déterminées par la nature des phénomènes biologiques, médicaux et épidémiologiques concernés. Pour ce qui est du paludisme, les phénomènes se réalisent à l'échelle de quelques centaines de mètres à une résolution temporelle d'une à quelques semaines. Les résolutions spatiale et temporelle utiles des données de télédétection dépendent de ces « échelles épidémiologiques ».

La population urbaine croît rapidement et les estimations prévoient que plus de 70% de la population mondiale et plus de 60% de la population africaine vivra dans les villes d'ici 2050 [8]. En milieu urbain, la transmission du paludisme a été rapportée à des niveaux en général plus bas que dans les zones périurbaines et rurales [9, 10]. Malgré cela, l'importance de la population à risque en fait une zone prioritaire dans le cadre de l'étude et de la lutte contre la maladie. De plus, la faible exposition des populations humaines aux parasites implique que l'immunité acquise est généralement faible chez les citadins. Ainsi, le paludisme urbain est considéré comme un problème de santé publique majeur [11].
Dans les villes, la transmission du paludisme est loin d'être homogène et elle peut être même très focalisée. Il a déjà été montré que le risque de transmission peut varier d'un quartier à l'autre [12]. D'autre part, l'hétérogénéité du couvert urbain implique certaines difficultés dans l'utilisation des images satellites pour sa cartographie. Cependant, en utilisant des données satellites à des échelles appropriés, il a été possible de mettre en évidence des associations entre des éléments urbains cartographiés et des indicateurs paludométriques dans les villes [13, 14]. Ainsi, même à l'échelle d'une cité, il est envisageable d'obtenir des cartes de risque qui seraient utilisables pour cibler les actions de lutte ou de prévention de la maladie.

En Europe, environ 10 000 cas de paludisme sont importés chaque année, par des voyageurs se rendant en zone tropicale. Chez les militaires, de nombreuses mesures de protections sont mises en œuvre (répulsifs cutanés, moustiquaires et vêtements longs imprégnés insecticides, lutte antivectorielle par insecticides). En comparaison avec les populations autochtones, l'incidence des cas cliniques de paludisme ou de séroconversion chez les voyageurs apparaît comme faible. Il est difficile de savoir si cela est dû à une différence d'exposition à la transmission (dépendant des lieux et des moments d'exposition aux anophèles infectés) ou à l'efficacité des mesures prophylactiques. Dans l'objectif d'une évaluation de l'efficacité de ces mesures ou de leur risque de contracter le paludisme, il serait utile de pouvoir évaluer et prédire les niveaux de transmission auxquels sont exposés les voyageurs, civils ou militaires. La disponibilité des données de télédétection et la mise en place de modèles de risque environnementaux peuvent permettre une évaluation de cette exposition.

L'objectif général des travaux de la présente thèse était d'identifier des facteurs environnementaux mesurables par satellite et utilisables pour l'évaluation du risque de paludisme chez les voyageurs d'une part et en milieu urbain d'autre part. Ce travail devait permettre le développement d'une méthodologie pour l'établissement de cartes de risque entomologique de paludisme s'appuyant sur trois niveaux :

- Niveau terrain : collecte de données entomologiques ou épidémiologiques suffisantes et pertinentes.
- Niveau télédétection : choix et traitement de produites satellites adaptés.
- Niveau analyse : application de méthodes statistiques appropriées au caractère géospatial des données.

La première partie rapporte les résultats de l'utilisation des données de télédétection pour l'évaluation des niveaux d'expositions au risque de paludisme d'une population de militaires, dans le cadre d'un travail sur l'estimation de leurs facteurs de risque d'accès palustre. Ensuite, trois articles décrivent le travail de collecte de données entomologiques effectué pendant cinq ans dans la ville de Dakar. Enfin, la dernière partie présente le travail de modélisation du risque entomologique dans la capitale du Sénégal, basé sur les données collectées sur le terrain et sur des données environnementales issues d'images satellites.

Ces trois parties sont précédées d'une revue de la littérature des méthodes et données de télédétection ayant été utilisées dans le domaine de l'évaluation ou de la prédiction du risque de paludisme.

REVUE 1

Machault V, Borchi F, Vounatsou P, Pages F, Vignolles C, Lacaux JP, Rogier C: **The use of remotely sensed environmental data in the study of malaria**. *Geospat Health*.

REVUE 1

The use of remotely sensed environmental data in the study of malaria

Machault V, Borchi F, Vounatsou P, Pages F, Vignolles C, Lacaux JP, Rogier C

Geospat Health (soumis).

Title

The use of remotely sensed environmental data in the study of malaria

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Abstract

Malaria is an infectious disease caused by a *Plasmodium* parasite that is transmitted among humans by the bites of an infected female *Anopheles* mosquito. Approximately half of the world's population is at risk of malaria, and the disease remains a major public health problem, causing an estimated one to three million deaths each year. The fight against malaria faces obstacles such as a persistent high degree of uncertainty regarding the annual number of malarial cases and their geographical distribution. However, it is of prime importance to evaluate the burden of malaria at a spatial and temporal level in order to inform decision-makers and to plan, organize and prioritize interventions when and where this burden is the heaviest. Risk maps drawn at appropriate scales can provide valuable information for this targeted control. In addition, anticipating future risk is a major issue in the fight against malaria.

The present review aims to provide the essential foundation for understanding the principles, methods, advantages and limitations of the use of remotely-sensed ecological, meteorological and climatologic data for the study of malaria. It first describes the determinants of the malaria burden and the impact of the environment on the malaria transmission cycle. After a brief introduction to remote sensing methods, the main remotely-sensed environmental indicators of interest in the study of malaria are described and a panel of analysis methods is provided. Next, the literature is reviewed, and examples of the use of environmental data in the study of different malariometric indices (vector breeding sites, vector densities, *Plasmodium* entomological inoculation rate, malaria prevalence, and morbidity and mortality) are presented. Special issues, including the detection of changes over time and urban malaria mapping, are addressed. Then, the use of remotely-sensed data in mapping human populations is described. Indeed, malaria risk becomes real only when infected competent vectors meet both a *Plasmodium* reservoir and a malaria-sensitive population. Finally, the limitations of the use of remotely-sensed data are highlighted, and the review is then concluded with a description of the strong interest that has arisen concerning the use of these data and possible development in the study of malaria.

Keywords

malaria, environment, remote sensing, satellites

Introduction

Malaria is an infectious disease caused by *Plasmodium* parasites transmitted among humans by the bites of infected female mosquitoes of the genus *Anopheles.* Approximately half the world's population is at risk of malaria and the disease causes an estimated one to three million deaths each year. Malaria remains a major public health problem in some parts of the world despite national and international endeavors.

The fight against malaria faces obstacles such as a persistent high degree of uncertainty regarding the annual number of malarial cases and their geographical distribution (Sullivan, 2010). Indeed, although efforts are made to collect and centralize the existing entomological, parasitological and epidemiological data (<http://www.map.ox.ac.uk/>; MARA/ARMA, 1998; Coetzee et al., 2000; Hay et al., 2000), the availability of field information is often limited and sparse. Entomological data are rarely collected continuously in a given area, and when field studies are undertaken, they can provide only partial time and space analysis of an actual situation. Few to no epidemiological or parasitological data are available in many areas where health information systems are weak or even nonexistent. Thus, vector control and sanitary interventions are hindered by a lack of continuous and long-term data. However, it is of prime importance to evaluate the burden of malaria at a spatial and temporal level in order to inform decision-makers and to plan, organize and prioritize interventions when and where this burden is the heaviest.

At the regional, national and international scales, the spatial distribution of malaria depends on several factors such as climate, presence of competent vectors, access to health care and sensitivity of the human populations. At the local scale, the concept of focal units of transmission has been described as a system where the breeding site of malaria vectors is the center of a transmission focus (Carter et al., 2000). As a result of this clustering, targeted interventions can be more effective than random control measures. For example, targeted management of the most productive breeding sites can lead to significant reductions not only in adult mosquito productivity but also in the incidence and prevalence of malaria (Gu and Novak, 2005). Thus, risk maps drawn at appropriate scales can provide valuable information for selective malaria control and for monitoring and evaluation of ongoing interventions.

With regard to the temporal distribution of malaria, anticipating future risk and incidence is also critical to the fight against the disease. The history of malaria early warning systems goes back to 1921 in India where the intensity and distribution of epidemics could have been forecasted using the combination of the absence of malaria in the previous five years, rainfall anomalies from July to August and the local price of wheat as a proxy for the current nutritional status of the population (Gill, 1923). In the 1950s, the introduction of insecticides and the launch of the worldwide malaria eradication program led some to believe the anticipation of epidemics was not longer needed. However, as the risk of epidemics grew with the loss of malaria immunity, an interest in early warning systems rose again.

The present review aims at providing the key knowledge for understanding the principles, methods, advantages and limitations of the use of remotely sensed ecological, meteorological and climatologic data for the study of malaria. It first describes the malaria burden determinants and the impact of the environment on the malaria transmission cycle. After having provided a brief introduction to remote sensing methods, it describes the main remotely sensed environmental indicators that can be of interest in the study of malaria and a panel of analysis methods is given. Then, a review of the literature is presented with examples of the use of environmental data in the study of different malariometric indices (vector breeding sites, vector densities, *Plasmodium* entomological inoculation rate, parasite prevalence, morbidity and mortality). Special issues of the detection of changes along time and the urban malaria mapping are addressed. Then, the use of remotely sensed data is described for mapping human population. Indeed, malaria risk become real only when competent vectors meet both *Plasmodium* reservoir and sensitive people. Finally, the limitations of the use of remotely sensed data are highlighted, before concluding with a description of the strong interest that has arisen concerning the use of these data and possible development in the study of malaria.

Determinants of malaria burden

Due to the vector transmission of malaria, the environment is clearly a determinant of malaria biodiversity (Guthmann et al., 2002; Ernst et al., 2009) and a key factor for vector species distribution.

Climate seasonality, rainfall patterns, air temperature, air humidity and the presence of vegetation or surface water can relate to each step of the malaria transmission cycle. In addition, anthropogenic activities such as agricultural activities, irrigation, deforestation, urbanization, population movements (e.*g.,* rural-urban migration), dam or roads constructions and wars are also connected to transmission levels and malaria epidemiology because they can impact one or more malariometric indices at each step of the transmission cycle

(Thomson et al., 1996; Beck et al., 2000; Martens and Hall, 2000; Patz et al., 2000; Patz et al., 2004; Ceccato et al., 2005; Afrane et al., 2007). Malariometric indices can be derived from entomological, parasitological and/or epidemiological measurements. These include the presence and persistence of *Anopheles* breeding sites, larval densities, the aggressiveness or human biting rate (HBR - number of *Anopheles* bites per person per unit of time), the prevalence of *Plasmodium* infection among *Anopheles* mosquitoes, the entomological inoculation rate (EIR - number of infected *Anopheles* bites per person per unit of time), the parasite prevalence (percentage of persons infected by a *Plasmodium* parasite), and malaria morbidity and mortality in the human population.

The basic requirement for completion of the malaria transmission cycle is the presence of *Anopheles* breeding sites, *i.e.,* water reservoirs containing *Anopheles* larvae. Surface water availability and its spatial and temporal distribution are driven by land-use and land-cover characteristics, climate seasonality, rainfall patterns, air humidity and air temperature. If water collections appear and persist over time, they can harbor *Anopheles* mosquito larvae if several conditions are met, such as low densities of predators and adequate water temperature, salinity, turbidity and sunlight. Determinants of the presence of larvae and larval densities depend on the specific *Anopheles* species. For example, *Anopheles gambiae s.l.* usually breeds in small, temporary, clear and shallow water, with small amounts of organic matter and surface vegetation (Gillies and De Meillon, 1968; Gillies and Coetzee, 1987), but it can also adapt to polluted water (Chinery, 1984; Sattler et al., 2005; Awolola et al., 2007). In the breeding sites, the entire development cycle from egg to emerging adult, which passes through four larval stages or instars, can be completed under favorable conditions. This cycle can range between one and three weeks, depending on the water and air temperatures, assuming sufficient food availability. For *Anopheles gambiae s.s.* under laboratory conditions, the rate of development from one immature stage to the next increases at higher temperatures to a peak around 28 °C, after which it declines. Adult emergence is optimized between 22 and 26 °C and is inhibited below 18 °C or above 34 °C (Bayoh and Lindsay, 2003). Throughout this process, adequate rainfall is required to maintain the appropriate conditions for the larval cycle to complete. Too little water can lead the water bodies to dry up before full completion of the cycle, whereas too much water can flush away larval habitats.

The adult mosquito survival rate is another key factor in the malaria transmission cycle because it affects the mosquito density and, thus, the biting risk for human populations. The survival rate is driven by climatic factors, such as temperature and air relative humidity, as well as by land-use and land-cover characteristics, such as vegetation that provides the necessary resting sites for adult mosquitoes. Depending on environmental conditions, vectors can survive from a few days to several months.

Dispersal relates to the flight range of mosquitoes, and it should also be considered an important determinant of malaria transmission (Killeen et al., 2003). Land-use and land-cover characteristics impact dispersal, which is generally lower (<300 m) in highly-populated urban settings (Sabatinelli et al., 1986; Trape et al., 1992; Manga et al., 1993; Robert et al., 1993; Baragatti et al., 2009) than it is in open rural areas, where it can reach several kilometers for some species (Charlwood and Alecrim, 1989).

The gonotrophic cycle occurs between two ovipositions of *Anopheles* female mosquitoes. It is a physiological process consisting of two steps: digestion of the blood-meal taken from a human or animal host and development of ovaries. The duration of this cycle depends on the *Anopheles* species and on the air temperature, and it usually ranges from two to three days in tropical regions (Mouchet et al., 2004). The duration of the gonotrophic cycle impacts the rate of increase in the mosquito population and, thus, the frequency of mosquito bites.

Ecological parameters also play a role in *Plasmodium* parasite development. The sporogonic cycle, also called the extrinsic cycle, occurs in the abdomen of the mosquito, and it is the process that allows the parasites to reach the sporozoite stage. Sporozoites migrate to the salivary glands of the female mosquito, which then inoculates them when feeding. The duration of this cycle depends on the parasite species and on the air temperature. High temperatures lead to a decrease in the length of the cycle. At 25 °C, the duration of the cycle is ten days for *Plasmodium vivax*, 13 days for *Plasmodium falciparum* and approximately 18-20 days for *Plasmodium ovale* and *Plasmodium malariae* (Mouchet et al., 2004). A reduction in the length of the sporogonic cycle implies a greater possibility for *Anopheles* females to transmit the parasite during its lifespan.

Finally, ecological factors are also important because they drive the spatial and temporal distribution of *Anopheles* species, and malaria epidemiology depends strongly on the involved vector species. Indeed, vector competence, a measure that indicates the ability of *Anopheles* to transmit the parasites, depends on the vector species. As a consequence, given the same *Anopheles* adult density, malaria risk could be very different depending on the vector species.

Thus, the relationship is strong between the ecological factors and the malariometric indices but it should be underlined it is weighted by several biotic factors. The human-vector contact is one of those factors and it depends on the way the population protect itself from mosquito bites. Immunity also moderates the link between environment and malaria transmission. It can be progressively acquired in humans who are regularly exposed to infected bites and it protects them from infection and clinical malaria attacks, or at least from severe malaria. In areas of low or rare transmission, this immunity is also low and epidemics can occur.

Currently, even if satellite sensors are not dedicated to recording data for the study of infectious diseases, remotely-sensed information can provide measures and evaluation of the geoclimatic, ecological and anthropogenic factors related to malaria transmission levels and patterns. Thus, since the first studies in 1970-80 (Cline, 1970; Hayes et al., 1985), public health has benefited from the growing availability of remotely sensed data.

Remote sensing: methods

The first civilian Earth observation satellites have been launched in the latter half of the 20th century and, since then, they provide imagery of Earth from space. Remote sensing refers to instrument-based techniques and knowledge employed to measure physical and biological characteristics without direct contact. Remotely-sensed data can be proxies for ground conditions. Sensors on board of satellites record electromagnetic radiation reflected or emitted by the Earth surface and this radiation is converted in measurable electric signal. The detection of the ecosystems lies on the differences in reflection and emission of the objects on the Earth. Passive sensors record natural radiation that is emitted or reflected by the objects whereas active remote sensing, such as radar, emits energy and measures the radiation that is reflected or backscattered from the target.

Spectral resolution relates to the number of frequency bands recorded by the sensor. A panchromatic image is made of only one band, a multispectral image contains several bands and a hyperspectral image is composed of a very high number of narrow and contiguous spectral bands (upwards of hundreds of bands). Thus, digital images are made of pixels containing the values of the recorded spectral bands. The size of a pixel relates to its spatial resolution (often simply named resolution). Spatial resolution of images of the same sensor can differ from one band to another, and this resolution is often higher for panchromatic images.

Temporal resolution describes the frequency of acquisition of images on a same area. For very high or high spatial resolution satellites (down to <1 m resolution), such as the optical WorldView-2 (down to 50 cm), Quickbird-2 (down to 61 cm), Ikonos-3 (down to 50 cm), SPOT-5 (*Satellites Pour l'Observation de la Terre,* down to 2.5 m), Landsat-7 (down to 15 m) or the radar platforms TerraSAR-X (down to 1 m) or RADARSAT-2 (down to 3 m), flyovers above the same area can be several weeks.

Low resolution platforms, such as Meteosat (5 km), MODIS (Moderate Resolution Imaging Spectroradiometer, 250 m), NOAA AVHRR (National Oceanographic and Atmospheric Administration Advanced Very High Resolution Radiometer, 1 km) can revisit the same place once or several times a day. Orbiting meteorological satellites produce two images per day of the Earth's entire surface, whereas geostationary platforms produce two images per hour to monitor weather systems.

As a consequence, environmental indicators necessitating precise spatial measurements can be derived from the very high resolution sensors, and applications can be local or regional. Indicators that require temporal long or middle term evaluation, such as vegetation or rainfall, can be derived from the low spatial resolution satellites. Related studies will preferably cover a country or a continent (Beck et al., 2000) as the spatial resolution could be insufficient for local applications. Thus, the choice of the images to be acquired for a given study depends on the study's objectives and on the spectral, temporal and spatial resolution criteria. In general, low spatial resolution imagery is free or inexpensive, in contrast to high and very high resolution images. A detailed list of past and present orbiting satellites is provided at: http://gdsc.nlr.nl/gdsc/information/earth_observation/satellite_database.

After acquisition, an image usually must be pre-processed for radiometric, atmospheric and geometric corrections. Next, the classification or calculation of indices can be undertaken, as explained below. Image classification aims to assign individual pixels of images to categories or classes to produce a thematic representation of an area on the ground. The process of automatic classification groups pixels according to certain criteria using automatic or semi-automatic algorithms (<http://www.ccrs.nrcan.gc.ca/glossary/index_e.php?id=47>). The classical techniques of supervised or unsupervised classification are pixel-based and rely on the spectral characteristics of each pixel. These techniques are based on conventional statistics (Matinfar et al., 2007). Recently, new forms of classification have been developed based on object-oriented techniques that are particularly well adapted for very high spatial resolution image analysis. The benefit of the object-based approach, moving from a pixel to object representation, is that it allows the incorporation of information of spatial neighborhood and not only information from a single pixel at once (Corcoran et al., 2010; Zhang et al., 2010). The calculation of indices combines the values of different spectral bands of a same pixel, using more or less complex mathematical operations with the objective of measuring one ecological characteristic (*e.g.*, vegetation or humidity). Some satellite products already contain processed information, such as rainfall quantity or temperature value. Other images are delivered in raw format and their pixels contain radiance data in different spectral bands. In this case, the images must be processed with appropriate tools and software (*e.g.,* ENVI or ERDAS IMAGINE).

GPS (Global Positioning System) technology allows the geolocation of epidemiological and entomological data collected on the ground. Next, geographic information systems (GIS) integrate all spatial data as layers, such as ground information, products of the classifications and calculation of indices, and non-satellite data (existing topographic, demographic, socio-economic or health maps). Within the GIS, data can be comprehensively displayed to facilitate understanding and communication of the distribution of spatial information. A wide range of analyses can then be performed between layers to highlight relationships, patterns or trends. Data can also be extracted from the GIS to statistical software programs to carry out analyses aimed at setting up risk maps and models.

Remotely sensed environmental indicators for the study of malaria

Temperature

Temperature is one of the main ecological factors affecting malaria epidemiology and burden. It impacts all steps of the malaria transmission cycle: persistence of the breeding sites, speed of larval development, adult mosquito survival rate and duration of the gonotrophic and sporogonic cycles.

Land surface temperature (LST) can be estimated from thermal infrared (IR) sensors. As examples, MODIS-Terra (<https://wist.echo.nasa.gov/api/>), Meteosat, GEOS (Geostationary Operational Environmental Satellite) and AVHRR provide day and/or night temperature information. LST correlates highly with air temperature, but the relationship can differ depending on land cover and humidity, atmospheric conditions, the study area and the period of the day that the image is recorded (Connor et al., 1997; Cresswell et al., 1999; Colombi et al., 2007; Vancutsem et al., 2010). A study comparing remotely-sensed LST with ground data interpolated from meteorological stations concluded that, in Africa, the annual mean LST corresponds to the air temperature +/-4 °C (Hay and Lennon, 1999). Another article showed, in Africa and Europe, a significant correlation between the LST and ground observations, with root mean square errors of around 2 °C (Green and Hay, 2002). Thus, the raw LST should be interpreted with care but can provide useful information for temperature comparisons.

Rainfall

Rainfall impacts mainly the temporal and spatial distribution of the breeding sites, as well as their persistence. It also can improve adult survival rates by creating a favorable humid microclimate for adult mosquitoes to rest.

Rainfall can be either directly measured or evaluated by indirect methods. The cold cloud duration (CCD) provides an estimate of rainfall based on the length of time for which a cloud top is below a threshold temperature. For example, CCD images can be derived from Meteosat Thermal Infrared images (Dugdale et al., 1991). A near-real-time direct measurement of rainfall in tropical areas is provided by the TRMM (Tropical Rainfall Measuring Mission) satellite using both passive microwave and active radar sensors (<http://trmm.gsfc.nasa.gov/>). Several studies showed that the TRMM grid value closely matched ground rain gage observations (Sharma et al., 2007; Han et al., 2010), even in urban areas (Hand and Shepherd, 2009). Nevertheless, the influence of location, climate, topography, time period, cloud types, and rainfall types were found to be factors affecting accuracy (Barros et al., 2000) and satellite rainfall data from TRMM can be overestimated during the pre-monsoon season and in arid regions but underestimated during the monsoon season and in humid regions as it has been showed in Bangladesh (Islam and Uyeda, 2007).

Elevation

Elevation closely relates to temperature and rainfall. Elevation generally correlates positively with precipitation and negatively with temperature. As a result, it can be used as a surrogate for these two factors.

Several digital elevation models (DEMs) provide information at different resolutions. Among the available forms of data, gtopo30 provides a 30 arc-second (about 1 km) (<http://eros.usgs.gov/#/Find_Data/Products_and_Data_Available/gtopo30_info>) worldwide DEM, while the SRTM (Shuttle Radar Topography Mission) provides maps at 90 m resolution (<http://www2.jpl.nasa.gov/srtm/>). In addition to elevation, several pieces of information can be extracted from DEMs, such as the wetness index, curvature and heat load index (Li et al., 2006). Wetness index represents soil moisture and is calculated by ln(draining area/tan(slope of location)). Heat load index is a substitute for solar radiation. Curvature measures the convexity or concavity of the land surface and is an indicator for the possible accumulation of water.

Land use and land cover (LULC)

The LULC is related to the mapping of the natural environment and to the impact of human activities on the landscape. Despite their differences, land cover and land use are often mapped together. Land cover refers to characteristics of the biophysical earth surface, such as water, vegetation, bare soil or artificial structures (Sarma et al., 2008). Land use could be viewed as a description of how the land surface is used by people. It reflects human activities such as agriculture, forestry or urban development.

The LULC can link to malaria burden through its impact on the presence and persistence of breeding sites and on the adult mosquito survival rate and dispersal.

Maps of LULC can be derived from images after classification processes, or can be directly available thanks to various global mapping projects, such as Corine Land Cover in Europe (<http://www.eea.europa.eu/themes/landuse/interactive/clc-download>).

Surface water and soil moisture

Among the numerous land covers, water is the one of the most important to map because the presence of surface water is the basic requirement for malaria transmission. Water can be detected on optical images, but microwave sensors also have a high potential to detect surface water and humidity. After the water bodies are detected, breeding sites can be described as specific objects, with their ecological characteristics making them more or less favorable for the development of vectors (Lacaux et al., 2006; Vignolles et al., 2009). In the study of malaria, direct detection may be impossible when breeding sites are too small or covered by surface vegetation or surrounded by trees. In such cases, soil moisture can be used as a surrogate for the presence of water.

The near infrared (NIR) and short wave infrared (SWIR) bands are of particular interest for water and moisture mapping as they are very sensitive to the humidity contained in vegetation and soils. Several satellites, such as SPOT-5 or Landsat, contain spectral information in these wavelengths that can be used independently or in combination with other bands in order to calculate indices, such as the NDWI (Normalized Difference Water

Index) for which different versions have been set up [NDWI Gao (Gao, 1996; McFeeters, 1996), NDWI McFeeters (McFeeters, 1996), NDWI modified (Xu, 2006)].

Air humidity

The relative air humidity primarily has an impact on the presence and persistence of breeding sites and on the adult mosquito survival rate. This indicator can be extracted from other meteorological factors as detailed in (Beugnet et al., 2009), but it should be used with care. Indeed, air humidity is mainly a function of air temperature, so it can change significantly over the course of the day. In addition, it strongly depends on the elevation.

Vegetation

First, vegetation plays an important role in vector larval development. Indeed, surface vegetation or surrounding vegetation (providing shade on the water collection) impacts the development of larvae, depending on the *Anopheles* species. In addition, vegetation impacts adult mosquito survival by providing resting sites. Finally, it can also be used as a surrogate for precipitation in certain periods and areas (Justice et al., 1991).

Numerous vegetation indexes exist, but the normalized difference vegetation index (NDVI) is the most commonly used index for human health applications. The NDVI was defined in the year 1970 (Rouse et al., 1973; Tucker, 1979). It integrates the combined effects of temperature, humidity, rainfall, sunshine, altitude, land use and land cover (Britch et al., 2008). Its calculation takes advantage of the fact that active vegetation reflects near infrared radiation (NIR) and catches red radiation (R). Its formula is as follows: NDVI= (NIR – R) / (NIR + R). The result can range from –1 to +1. Because the value of the NDVI depends not only on the land cover but also on the atmospheric conditions, no strict threshold can be described. Nevertheless, a value superior to 0.2 usually corresponds to a vegetated area, and increasing values reflect increasing density. A negative value can correspond to water, areas of infrastructure or asphalt.

Several low resolution satellites provide NDVI values (MODIS (<https://wist.echo.nasa.gov/api/>), AVHRR), but the NDVI can also be calculated using any high or very high resolution image containing the appropriate spectral bands. Hyperspectral images can also be useful for vegetation mapping because the calculation of vegetation indicators can take advantage of their numerous spectral bands.

"Inventive use" of satellite indicators

In addition to the direct detection of environmental characteristics, satellite imagery can serve as a proxy for socioeconomic factors, housing type or quality. Indirectly, those characteristics can relate to the level of access to antivectorial protective devices or to health care. For example, in a study of malaria in South Africa, the distance to the Mozambique border was a proxy for migration from this country, where no malaria control has been implemented (Kleinschmidt et al., 2001). Another example is the brightness of the remotely-sensed night time lights data that is a robust proxy for assessing poverty in Africa, which relates closely to health status (Noor et al., 2008).

Analysis methods

Once all relevant spatial information has been extracted in a GIS (*e.g.,* surface of a given land cover class in a buffer; distance to a feature such as vegetation or water; or the mean, maximum or minimum value of an index), analyses can be carried out to evaluate the associations between environmental factors and malariometric indices (presence and persistence of breeding sites, larval and adult *Anopheles* densities, EIR, parasite prevalence, malaria incidence, and morbidity or mortality). Different statistical and non-statistical methods can be used to analyze these relationships.

Principal Component Analysis (PCA), which allows summarization of the data, can be used. Indeed, PCA transforms a number of possibly-correlated variables into a reduced number of variables, called principal components. As environmental variables are often correlated (*e.g.,* the temperature decreases when the altitude increases), PCA allows all variables to be included in the analysis without having to exclude correlated information and risk losing part of the information (Lawpoolsri et al., 2010). When the initial variables have been "summarized" in principal components, environmental classes can be investigated because they may be either positively or negatively related to malariometric indices. Algorithms such as hierarchical classification or K-means clustering can generate those classes (Chamaille et al., 2010). Because the principal components are linear combinations of initial variables, the interpretation of groups is easy and intuitive.

Discriminant analysis is also effective in the field of spatial epidemiology (Beck et al., 1994; Beck et al., 1997). It relates closely to PCA but has the advantage of permitting the prediction of new observations. Partial Least Square (PLS) regression (Wold et al., 1984) can also be considered when using collinear variables and ENFA (Ecological Niche Factor Analysis), which introduces habitat suitability notions (Hirzel et al., 2002). ENFA can be calculated with Biomapper (<http://www2.unil.ch/biomapper/index.html>) and is a presence-only model to be used when no absence data are available. Other presence-only models exist, such as maximum entropy or genetic algorithms (De Meyer et al., 2010), and these are also of interest because they do not require independence of covariates.

Statistical models such as linear, logistic, Poisson or negative binomial regressions can also be fitted to model the relationships between environmental factors and the malariometric indices. The choice of regression depends on the type of data to model (dichotomous or continuous outcome, proportion or rate), and each implies a different mathematical formula that links the environmental factors and the malariometric indicators. Inverting the formula of the regression model allows risk models and maps to be drawn. The advantage of these statistical analyses relative to PCA lies in their prediction possibilities. Indeed, once a model is fitted, the results can be extrapolated to predict the outcome for unsampled observations by the inversion of the regression formula.

The basic requirement for using classical statistics is the independence of observations. In a spatial context, this prerequisite is not always fulfilled because the observations that are close in space can be more similar than those that are distant. Neglecting this spatial autocorrelation in the analysis tends to underestimate the standard deviations of the estimates and can lead to an overestimation of the strength of the associations and the detection of spurious correlations (Thomson and Connor, 2000). Indeed, there are actually fewer independent observations than are assumed. In Kenya, 87% of the variability in tsetse density were explained with remote sensing data, but after taking into account the spatial autocorrelation, these results were no longer significant (Kitron et al., 1996).

Spatial autocorrelation among the outcomes means the presence of any systematic pattern in the spatial distribution of the variable. This autocorrelation can be assessed using Moran’s I (Moran, 1950) or Geary’s C statistics (Geary, 1954). Moran’s I is a global indicator, whereas the Geary coefficient is more sensitive to differences in small neighborhoods. The spatial variations can also be investigated due to the use of the nonparametric D statistic (point pattern analysis), which is defined as the average absolute difference in ranks of the data, taken over all possible pairs of adjacent spatial units. Because the weights favor pairs of areas in close proximity, a low D value implies the presence of spatial correlation (Walter, 1994).

A number of modeling approaches can be used to take into account spatial dependence. Location-specific covariates and error terms (random effects) can be introduced to describe geographical differences in the mean of the outcome and to take into account spatial correlation respectively. On one hand, multilevel models can be employed to analyze correlation in malariometric indices observed at individuals nested within larger units, such as persons within households or households within villages (Matthys et al., 2006b; Vanwambeke et al., 2006; Baragatti et al., 2009). On the other hand, spatial models, such as geostatistical (Diggle and Tawn, 1998), conditional and simultaneously autoregressive ones incorporate explicitly spatial correlation as a function of distance or neighbor structure of the data. They allow assessment of disease determinants in the presence of spatial correlation, as well as smoothing and prediction. Spatial models can be formulated within the framework of Generalized Linear Mixed Models (GLMM) (Kleinschmidt et al., 2001). However model fit is complicated by the large number of parameters (Gemperli and vounatsou, 2004; Gosoniu et al., 2006). Thus, GLMM spatial models have been applied in malaria mapping by Bayesian formulations to overcome parameter estimation by Markov chain Monte Carlo (MCMC) simulation. Predictions at unsampled locations are enabled by Bayesian kriging based on geostatistical regression models. The use of Bayesian models in mapping malaria and other tropical diseases grew over the last years (Clements et al., 2006; Gosoniu et al., 2006; Li et al., 2006; Steinmann et al., 2007; Silue et al., 2008; Raso et al., 2009; Haque et al., 2010; Riedel et al., 2010). Appropriate software, such as OpenBUGS (<http://www.openbugs.info/w/>) are available for fitting spatial Bayesian regressions.

For analyzing temporal data, several techniques have been used in malaria epidemiology , such as Fourier analysis to assess seasonality (Hay et al., 2006), Autoregressive Integrated Moving Average (ARIMA) (Abeku et al., 2002) or derivatives (ARIMAX (Wangdi et al., 2010)) to estimate temporal patterns and obtain short term forecasts. Finally, it should be noted that some results have been produced using other methods such as fuzzy logic (Snow et al., 1998) or neural networks (Kiang et al., 2006).

The uncertainty of the models and of the predictions must be taken into account, or even controlled for. In classical statistics, accuracy can be measured in several ways. The model assessment refers to the estimation of the precision of the model, and it expresses how well the model fits the data. In classical statistics, this precision is provided by the standard error (Atkinson and Graham, 2006). In this context, Bayesian modeling has the great advantage of being able to estimate the parameters and the predictions as posterior distributions rather than as single values (Brooker, 2007). Thus, for each predicted observation, the upper and lower Bayesian credible limits are known, allowing one to draw maps with the prediction error in the case of spatial models. To compare model-based predictions to the “real” values (*i.e.,* the Gold Standard), measurements of the agreement can be given by the overall accuracy, sensitivity and specificity, positive and negative predictive values, area under the ROC (Receiver Operating Characteristics) curve or the correlation coefficient. When there is no Gold Standard but only two sets of values to compare, the Kappa statistic can be used to provide an estimate of the level of agreement, taking into account the agreement that could be obtained only by chance. A list of accuracy metrics is provided in (Rogers, 2006). In Bayesian statistics, the predictive ability of models can be assessed using a Bayesian "p-value" analogue calculated from the predictive posterior distribution (Gosoniu et al., 2006).

Remote sensing and malaria

The study of several infectious diseases has taken advantage of the growing availability and precision of remotely-sensed data (Thomson et al., 1997; Hay et al., 1998; Manguin and Boussinesq, 1999; Kitron, 2000; Randolph and Rogers, 2000; Ceccato et al., 2005). With regard to mapping malaria, remotely-sensed environmental data have been linked to a wide range of malariometric indices: larval and vector densities, aggressiveness, EIR, parasite prevalence and malaria incidence.

Breeding sites

Since 1972 and the launch of Landsat-1 satellite with a 30-m spatial resolution, research has been undertaken to spatially map malaria risk, relying on the **identification, characterization and mapping of the favorable environments for anopheles breeding**. Satellites can provide information on breeding sites. It has been shown that remotely-sensed data in Thailand could have delivered environmental data as reliable as those one collected on the ground. Indeed, the presence of *Anopheles minimus*, *Anopheles maculatus* and *Anopheles barbirostris* in water habitats was similarly predicted by regression models including environmental data from Landsat (30 m) and those using field data (Vanwambeke et al., 2007). On one hand, the mapping of breeding sites may rely on prior knowledge of the environments favorable for larval presence and development. Then, risk is deducted from the mapping of those ecosystems. For example, in Burkina Faso, potential high- and low-risk malaria areas at the village level could have been predicted from satellite data (SPOT-5 at 2.5 m). Known potential land covers producing anopheles were mapped following a supervised classification, and each village was classified in terms of malaria risk based on its surroundings (Dambach et al., 2009). On the other hand, mapping can be attempted on the basis of collected entomological field data. In several countries, associations have been drawn between the presence of larval habitats and the results of image classifications at a range of different spatial resolutions (from QuickBird at 0.6 m to Landsat at 30 m) to map favorable ecosystems (Rejmankova et al., 1998; Sithiprasasna et al., 2005a; Mushinzimana et al., 2006; Stoops et al., 2008; Mutuku et al., 2009). Finally, remote sensing can even bring new knowledge on breeding habitats, such as in Thailand, where differences in the habitat preferences of *Anopheles minimus A* and *C* were characterized using remotely-sensed ecological data (Rongnoparut et al., 2005).

An interesting application of the mapping of the larval habitats was undertaken in Korea Republic around two military camps, using a **decision support system** (Claborn et al., 2002). The processing of Landsat images allowed the retrieval of larval habitat surfaces that needed to be treated with larvicides. The derived cost was then calculated and compared with the cost of providing antimalarial chemoprophylaxis to the population of both camps. Thus, the choice between the two control methods could have been driven by this comparison.

Another methodology aims to **directly detect water collections and breeding sites** on satellite images. In Korea, photointerpretation of Landsat and Ikonos images, undertaken by trained operators, has allowed the mapping of rice fields and other potential breeding sites for *Anopheles sinseni* around two military camps (Masuoka et al., 2003). In this case, some pools were better detected with the very high resolution Ikonos images, even if no SWIR band was available. In Kenya highlands, which are a very heterogenic ecosystem, image classification errors can be important. In this context, the photointerpretation of Ikonos images (1 m) allowed the detection of 41% of the water collections, without providing information on the larval status, whereas the photointerpretation of Landsat images (30 m) did not provide any useful information (Mushinzimana et al., 2006). Indeed, the authors state that object detection is feasible only when the object size is at least 1.5 times larger than the pixel size. In this study, visual detection results could have been coupled with statistical results to improve detection. Sometimes, classification methods provide better results than does photointerpretation (Mutuku et al., 2009).

In addition to optical satellite images, **radar imagery** has also been exploited for breeding sites mapping, taking advantage of radar's capacity to detect water and humidity and to overcome the acquisition problems caused by cloud cover. In Mali, a study highlighted the relationship between radar images and the development of rice plants, which in turn were coupled with anopheles larval densities (*Anopheles gambiae s.l*, *Anopheles pharoensis*, *Anopheles rufipes* and *Anopheles funestus)*. Eight ERS-2 SAR (12.5 m) allowed researchers to draw temporal profiles of rice fields and, thus, to monitor the abundance of vectors in nearby villages (Diuk-Wasser et al., 2006).

Some studies have also focused on **temporal predictions**. One of the first papers published in the field of vector diseases and remote sensing was on a study done in California where the most productive *Anopheles freeboni* rice fields were mapped two months before larval density peaked (Wood et al., 1991). Predictors included extensive vegetation at the beginning of the season, measured on a Landsat image, and livestock proximity, measured by mapping the pastures using infrared aerial photography.

Vector densities

Larval mapping and adult vector density mapping are closely related. Indeed **maps of larval habitats** can be exploited **for evaluation or prediction of vector densities**. Breeding sites are the source of adult mosquitoes and vector density can, thus, be mapped including additional predictors, *i.e.,* the distance between a sampling point and the breeding sites, the mosquito flight range or the type of land cover surrounding the breeding site. In Belize, *Anopheles albimanus* larval habitats are located where water and sparse vegetation meet, and these habitats could be mapped using the classification of a SPOT image (20 m). Villages were then separated into two risk classes for *Anopheles albimanus* adult densities, depending on their distance to the mapped larval source (Rejmankova et al., 1995). In the same country, the presence of *Anopheles pseudopunctipennis* in houses was predicted by processing a SPOT image (20 m) and topographic maps on which were extracted the maps of rice fields, the distance to rice fields, as well as the difference of elevation or the presence of forest between the rice fields and the houses. Even if images were not contemporary to the entomological field data collection, the appropriateness of their use was verified before undertaking this work (Roberts et al., 1996). In both studies, the predictions were validated thanks to adult mosquito ground collection. In Camargue, a marshy area in the south of France, larval- and adult-stage *Anopheles hyrcanus* have been mapped using a Landsat (30 m) image (Tran et al., 2008). Each pixel contained a probability of the presence of larvae, depending on the biotope, the distance to the first rice field and any larvicide intervention. Here, the flight range was not predefined but was deducted from research on the best correlation between the ground-measured adult densities and larval probabilities in a range of different size buffers. **Applications** of vector densities mapping can rely on the definition of areas that require indoor insecticide spraying (Rakotomanana et al., 2007).

A reverse methodology consists of **measuring adult mosquito densities to define the ecosystems favorable for larval development**. In Mexico, a Landsat (30 m) image allowed identification of ecosystems associated with the adult *Anopheles albimanus* density around 40 villages (Beck et al., 1994).

Finally, remote sensing data processing even produced **information on the distribution of *Anopheles* species or molecular forms**. In Mali, the presence of *Anopheles gambiae s.s.* Mopti has been correlated with low rainfall in the study month or the previous study month (Touré et al., 1994) or with low values of the NDVI (Thomson et al., 1997). In the same country, Bayesian modeling allowed maps of spatial distribution of *Anopheles gambiae s.s*. and *Anopheles arabiensis* (Sogoba et al., 2007) or of chromosomal forms of *Anopheles gambiae* (Sogoba et al., 2008) to be drawn, based on climatic and environmental factors (rainfall, minimum and maximum temperature, the NDVI, soil water storage index, distance to water bodies, and suitability to transmission). On a **large scale**, satellite imaging has been used to predict the distribution of 5 of the 6 *Anopheles gambiae* complex species that are responsible for much of the malaria transmission in Africa (Rogers et al., 2002).

Plasmodium entomological inoculation rate and parasite prevalence

Following the same principle of researching correlations between larval and adult densities, some studies have investigated the **associations between ecosystems and *Plasmodium* EIR** to weight the biting risk with the percentage of infected mosquitoes. In Kenya, *Anopheles gambiae* and *Anopheles funestus* aggressiveness and EIR have been predicted using land cover and soil features and a soil moisture model of surface-water availability that combined multiple weather parameters (Patz et al., 1998). In The Gambia, *Anopheles gambiae s.l.* EIR was estimated in villages using breeding site areas and distance mapped on Landsat (30 m) images (Bogh et al., 2007). In the same country, a model was fitted using the EIR in villages and a map of breeding sites issued from a SPOT (20 m) classification. Extrapolation of the model to other villages allowed the prediction of transmission levels (Thomas and Lindsay, 2000). Here, parasite prevalence in the villages correlated with the EIR. Indeed, whereas EIR can be linearly linked to larval and adult densities, the relationship between vector populations and the incidence or prevalence of plasmodial infections is more complex. It depends not only on transmission levels but also on the use of antivectorial devices, on acquired immunity and on the access to antimalarial drugs (Rogers et al., 2002).

As a result, a few studies have aimed to **define parasite prevalence levels from the presence of breeding sites or the vector densities**. In Thailand, no association was found between the presence of malaria cases in houses and the distance to streams extracted from an Ikonos (1 m) image (Sithiprasasna et al., 2005b). In contrast, in a village of Cambodia, an increased distance to the forest (extracted from the Cambodia Reconnaissance Survey Digital Data, derived from maps, aerial and satellite photographs and field verifications) was a significant protective factor for *Plasmodium* infection (Incardona et al., 2007).

Most of the **predictive models for malaria cases have been built from geo-climatic indicators**. In The Gambia, children parasite prevalence was predicted in 65 villages using the following explanatory variables: age, use of impregnated bednets and the NDVI. The NDVI was included as the area under a temporal curve and served as a proxy for the length of the transmission season. The fitted model allowed a prediction of the effects of changes in bednet use on transmission (Thomson et al., 1999).

From the first phases of research in the field of remote sensing applied to health themes, the need for **malaria risk maps on the scale of the entire African continent** has been underlined (Snow et al., 1996). To meet this goal, the MARA/ARMA project (Mapping Malaria Risk in Africa/*Atlas du Risque de Malaria en Afrique*) was a collaborative work that compiled all published or unpublished malariometric data in Africa. Models including geo-climatic factors allowed data to be modeled where they were not available (Snow et al., 1996; MARA/ARMA, 1998). In addition, stable transmission limits were defined for stable malaria transmission in the continent (Craig et al., 1999). More recently, MAP (Mapping Malaria Project) was born with the objective of gathering worldwide parasite prevalence data and making them freely available (<http://www.map.ox.ac.uk/>; Guerra et al., 2007). Again, a model of the spatial limits and endemicity levels of *Plasmodium falciparum* and *Plasmodium vivax* relied on these data and on the specific associations of the *Anopheles* species with temperature and humidity (Guerra et al., 2008).

On the **regional or national level**, several articles have been published on this topic. In Afghanistan, a *Plasmodium vivax* prevalence map was drawn using the NDVI and LST (8-km resolution) (Brooker et al., 2006). In West and Central Africa, *Plasmodium falciparum* prevalence maps have been completed based on MARA/ARMA field data and a seasonality model based on the NDVI, temperature and rainfall (Gemperli et al., 2006a). In Mali, a prevalence risk map was based on the distance to water, the mean NDVI during the rainy season, the lowest temperature during the three months preceding the rainy season and the number of months of rainfalls more than 60 mm (Kleinschmidt et al., 2000). In Kenya, Uganda and Tanzania, predictions of the parasite prevalence were based on temperature, rainfall, humidity, the NDVI and altitude and corroborated historical maps (Omumbo et al., 2002). Later, these maps were further improved by adding human density and urbanization data as well as the presence of water collections and ecological environment data (Omumbo et al., 2005).

In addition to the use of these risk maps to **evaluate malaria burden**, another application could lie in the **evaluation of the risk of re-emergence** of malaria in countries where it has been eliminated. In a rice field region of Spain, remotely-sensed ecological and climatic features, together with other malariometric variables, were found to be associated with the possible risk of transmission (Sainz-Elipe et al., 2010). In the south of France, *Anopheles hyrcanus* at the larval and adult stages were mapped using remote sensing imagery in the scope of a research project on the risk of malaria re-emergence (Tran et al., 2008).

Finally, **mathematical epidemiological models** allow overcoming difficulties in the comparability of non standardized and non overlapping surveys in the field of malaria mapping (Gemperli et al., 2006b). In those models, remotely-sensed data can also be included as it has been done in Mali where the NDVI was included in a temporal model of transmission to forecast the evolution of malaria epidemiology (Gaudart et al., 2009).

Morbidity and mortality

As has been done for the study of parasite prevalence, **environmental and climatic indicators** have been used in models explaining **malaria morbidity and mortality**. In Bangladesh, remotely-sensed temperature and vegetation indexes correlated with the number of malaria cases admitted to hospitals, especially during the rainy season. The NDVI and surface temperature were calculated as the deviation from their minimum and maximum values in order to take into account meteorological fluctuations rather than long-term climatic variables (Rahman et al., 2006). A study was also carried among non-immune travelers who may be briefly exposed to malaria. Among French military members in a short-duration mission in sub-Saharan Africa, staying in areas with an average NDVI higher than 0.35 was the main risk factor for clinical malaria, before age or compliance with chemoprophylaxis (Machault et al., 2008). In Afghanistan, a model took into account the fact that malariometric indices were autoregressive (*i.e.,* infection during one month depends on the infections during the previous month) and showed that the NDVI and LST (from MODIS at 1 km) predicted the average total cases for 6 months. Results showed that predictions exceeded the actual cases by only 8.9% (Adimi et al., 2010).

As an important objective is to predict outbreaks in areas of unstable malaria transmission, modeling can be of great interest for **anticipating epidemics** (Myers et al., 2000). The interaction between climatic factors and their biological influence on mosquito and parasite life cycle is a key factor in the association between weather and malaria. Thus, weather should be considered in the development of malaria early warning system (Teklehaimanot et al., 2004). In Kenya, an NDVI superior to 0.35-0.40 in a given month predicted that hospital admissions for severe malaria in the following month would reach at least 5% of the total annual admissions (Hay et al., 1998). In Burundi, a model including the NDVI (from AVHRR), ground measured temperature and rainfall and number of malaria cases in a given month predicted malaria incidence for the following month (Gomez-Elipe et al., 2007). An early warning system in Eritrea was based on rainfall forecasts and allowed prediction of monthly clinical malaria incidence anomalies with a lead time of 2-3 months (Ceccato et al., 2007). On the **scale of the African continent**, an early warning system for malaria epidemics was developed and is available on the internet (Grover-Kopec et al., 2005). This system relies on the association between epidemics and decade-level rainfall anomalies.

Detection of changes

Image processing allows for the detection of environmental changes over time that can be associated with malariometric indices. Changes can be taken into account in the study of the spatial distribution of *Anopheles* breeding sites. In a Kenyan village, the presence of *Anopheles arabiensis* larval habitats have been shown to be associated with ecological changes over time (Jacob et al., 2007). Indeed, the proportion of water collections positive for larvae was higher in areas that had changed between 1988 and 2005, especially in lands that became fallow. In urban settings, land cover changes have also been associated with breeding sites. In two Kenyan cities, Multi-spectral Thermal Imager and Thermal Imager images, lagging 14 years, allowed changes to be mapped between urban areas, non-urban areas and water areas. Results showed that the presence, abundance and spatial distribution of larval sites were related to this evolution of urbanization (Jacob et al., 2003).

In Dakar, *Anopheles arabiensis* aggressiveness was predicted and mapped using the built-up area extracted from the classification of two SPOT (2.5 m and 20 m) images. Evolution was explored on a decade-long time scale, between 1996 and 2007. The results highlighted the benefits of urbanization in the city; while the total population increased, the proportion of the population at higher risk for malaria transmission greatly decreased (Machault et al., 2010).

In Paraguay, the mapping of land cover changes, particularly from forest to non-forest (evaluated using time series of the NDVI derived from the Global Inventory Modeling and Mapping Studies) showed an association with malaria case rates (Wayant et al., 2010).

Urban malaria mapping

Urbanization is occurring at a rapid pace and the United Nations forecasts that, by 2030, nearly 60% of the world's population will live in cities (Nations, 2003). Inescapably, these changes have consequences for the health of local populations. With regard to malaria, many studies have reported the existence of transmission in urban areas, even if levels are usually lower than those in peri-urban and rural places (Robert et al., 2003; Keiser et al., 2004). The epidemiology of malaria in cities is specific, and the urban form of the disease is considered to be an emerging health problem of major importance in Africa (Donnelly et al., 2005).

The urban malaria burden, as well as its spatial and temporal distribution, is closely related to a wide range of factors, such as the degree and type of urbanization, the density of the human population, vector control measures, access to health care (Robert et al., 2003; Wang et al., 2005a) and adaptation of the vector to new or polluted breeding sites (Chinery, 1984; Sattler et al., 2005; Awolola et al., 2007; Omlin et al., 2007). Urbanization has a considerable impact on the composition of the vector system and malaria transmission dynamics (Antonio-Nkondjio et al., 2005). Moreover, in urban settings, blood meal sources are abundant, dispersion of the vectors is low and malaria transmission is driven primarily by the proximity of breeding sites (Trape et al., 1992; Staedke et al., 2003). Malaria risk is heterogeneous over small distances, and transmission can vary among different districts of the same city, as shown in Brazzaville (Trape and Zoulani, 1987) and Dakar (Machault et al., 2009). The consequence of this situation is that good results can be expected from environmental control, including vegetation clearance, modification of river boundaries, draining swamps and insecticide application to open water bodies. Nevertheless, in this context of high heterogeneity, the choice of the spatial scale of the data and images to use is of utmost importance.

In Dar-Es-Salaam, Tanzania, the **direct identification of breeding sites** based on aerial photos allowed focusing larval control in the city (Caldas de Castro et al., 2004). Other work has not been as successful, such as in Malindi and Kisumu, Kenya, where photointerpretation of breeding sites for *Anopheles gambiae s.l.*, *Anopheles funestus* and *Anopheles merus* was attempted using Multi-spectral Thermal Imager (MTI, 5 m and 20 m) images. Even an experienced operator could detect only 6% of the larval habitats (Jacob et al., 2005). Urban breeding sites are usually small, and further work will likely benefit from the growing availability of very high resolution images that should allow improved direct detection.

As has been done in rural settings, **environmental proxies** can also be of interest for malaria mapping in urban settings. In the same Kenyan cities, the NDVI, re-sampled at 270x270 m, was correlated with a low density of dwellings and, as a result, the high presence of *Anopheles* breeding sites (Eisele et al., 2003). In Ouagadougou, a high prevalence of malaria antibodies and a high prevalence of infection among children were associated with urban environmental characteristics: unplanned and sparsely built-up areas mapped on a SPOT-5 image and cadastral maps (Baragatti et al., 2009).

The importance of **urban agricultural activity** on malaria has also been reported in several African countries, such as Côte d'Ivoire and Ghana (Afrane et al., 2004), where irrigation has led to the emergence of larval habitats (Afrane et al., 2004; Matthys et al., 2006a) and higher malaria prevalence (Klinkenberg et al., 2005; Wang et al., 2005b). Irrigated vegetable fields near a French military camp in Abidjan have been suggested as the source of the unexpectedly high number of adult *Anopheles* found there (Girod et al., 2006). In other cities, such as Malindi in Kenya, no relationship was found between household-level urban agriculture practices and the distribution of water bodies (Keating et al., 2004). In addition, market gardens likely provide resting sites to *Anopheles* rather than increase the number of breeding sites, as was previously demonstrated in Ghana (Klinkenberg et al., 2008). As a result, mapping of urban agricultural areas is one of the key elements in malaria risk mapping in cities. In Antananarivo, the capital city of Madagascar, the rice field surface area, together with altitude, temperature, rainfall and population density, were investigated as potential risk factors for confirmed malaria cases (Rakotomanana et al., 2010).

Malaria mapping and risk

Human population mapping

In the field of global malaria mapping, remote sensing data have been exploited to generate population grids that are a key tool for providing information on populations at risk for the disease. Indeed, malaria occurs only where and when an infected competent vector meets a human sensitive population. A recent review has focused on the use of global population distribution data for estimating malaria morbidity and mortality (Balk et al., 2006).

Clear identification, characterization and classification of urban ecotypes is sometimes weak, and this weakness impedes the evaluation of actual malaria burden, as well as the extrapolation of results to other cities (Siri et al., 2008). In this context, work is being done to map and model human urban population using remote sensing (Tatem and Hay, 2004). The definition of an urban area is not standardized and finding accurate global maps of urban settings can be problematic. As a result, remote sensing images have been used to delineate these areas using more or less complex geostatistical analysis. Radar, optical or DMSP (US Air Force Defense Meteorological Satellite Program) night-light imagery have been exploited (Tatem and Hay, 2004). Even if the latest should be quantitatively interpreted with care due to the diffusion of light in neighboring pixels (Hay, 2005), it has been used in a large population mapping initiative: GRUMP (Global Rural Urban Mapping Project). On a worldwide scale, GRUMP urban areas have been shown to match up with descriptions of urban settings provided in malaria papers (Tatem et al., 2008). On a national scale, semi-automated mapping of urban areas with images at middle spatial resolution in Kenya have been shown to provide satisfactory results (Tatem et al., 2005). However, this type of global source could fail to be linked to malariometric indicators, especially where population density is low (Tatem et al., 2008). On the local scale, an operational tool for rapid urban mapping based on the joint use of radar and optical sensors has shown to delineate urban zones better than do isolated radar or optical images (Corbane et al., 2008). Unsupervised and supervised pixel-based classifications of a Landsat 7 image have also provided accurate estimates of population density in census areas of Besançon, a French city (Viel and Tran, 2009). Finally, the detection of changes and quantification of urbanization growth have also been undertaken using radar and optical images in Douala, Cameroon (Onana et al., 2005).

Risk, hazard and vulnerability

The risk areas should be defined as the areas where hazard and vulnerability overlap. Hazard represents the “potential risk”, *e.g.,* the vector distribution, and vulnerability relates to the distribution, sensitivity and exposure of human populations. Some studies have taken into account the superimposition of potential risk and vulnerability to estimate risk.

In Kenya, RADARSAT-1 (25 m) images allowed mapping of the areas favorable to the presence of anopheline vector breeding sites that also intersected with human populated areas. Risk zones were defined as the overlaying surface of those two areas (Kaya et al., 2002). In Dakar, the non-populated areas, mapped using SPOT images (2.5 m and 20 m) were masked when predicting malaria risk (Machault et al., 2010). Because it is known that the peak of anopheline aggressiveness occurs in the middle of the night in Dakar (Machault et al., 2009), and because evening and night activities are expected to take place mainly in or around dwellings, areas without infrastructure were excluded from predictions. On a worldwide scale, a recent study provided a map of the global spatial extent of *P. vivax* malaria, together with estimates of the human population at risk of any level of transmission (Guerra et al., 2010).

Limitations

In the field of spatial modeling and risk mapping, caution must be taken to ensure the validity of the results. In addition to the usual requirement to validate any model and to take care in extrapolating the models, which should not exceed their intrinsic possibilities, special issues need to be addressed with geospatial modeling.

The choice of satellite images must not only be driven by logistical constraints. Images have to be specifically selected depending on the scale of the biological phenomenon under study. As reported in some studies, an analysis of scale and spatial resolution needs to be undertaken (Atkinson and Graham, 2006). Very high resolution images can be considered as the best choice, but depending on the topic, other types of images may lead to identical or even better results (Hay, 2005). In addition, images that are contemporary to the field work are not always available. The consequences of any distortion between the times of collection of remotely-sensed data and ground data must be analyzed and discussed.

Remotely-sensed environmental indicators can be proxies for ground conditions. Nevertheless, their capability of approximating field data must always be discussed, depending on the location, climate or topography.

The present review has shown that remote sensing provides a great deal of information for malaria mapping. Nevertheless, it may only partially explains the phenomenon of malaria transmission, as in the Baltic States where land cover and climatic variables explained only 55% of spatial variation in tick-borne encephalitis in the period from 1993-98 (Sumilo et al., 2006). Indeed, variability can also exist even in geographically homogeneous areas, and other factors could improve modeling, such as socio-economic variables (Kreuels et al., 2008). In addition, malaria interventions can confound the relationship between the environment and the disease. This is apparent, in the analyses of recent malaria indicator survey data for Zambia or Angola (Gosoniu et al., 2010; Riedel et al., 2010), that no relationship was observed between remotely-sensed data and malaria risk.

Conclusion

The present review has demonstrated how earth-orbiting satellites can measure or evaluate many environmental, climatic and meteorological indicators that are the determinants of malaria biodiversity and of the levels and patterns of malaria transmission. Remote sensing has become one of the tools to help reach the challenging objectives of evaluating malaria burden, modeling malaria temporal and spatial distribution and planning malaria control. Remote sensing provides a huge source of data to be used for modeling and mapping malaria risk at entomological, parasitological and epidemiological levels.

In addition to research interests, one key issue is to implement operational systems to facilitate real-time monitoring of human health. To attain this objective, collection of appropriate field data is the basic requirement. Next, an informed choice of remote sensing product should be made in light of all available spatial, temporal and spectral resolutions. The analysis methods must be chosen based on the type of data to avoid as much bias as possible. The mechanisms of the relationships between environmental data and each step of the transmission cycle should be carefully understood.

On a spatial level, risk maps can and should be drawn to model each step of malaria transmission. For example, an initial map of water collections could be drawn using appropriate optical and/or radar indicators. Then, this map could be updated in an *Anopheles* breeding sites map if the environmental factors leading to the presence of larvae are well known for the region under study and the *Anopheles* species, and if they can be mapped from images. Next, the emergence of adult mosquitoes could be evaluated depending on meteorological factors and their dispersion could be mapped with knowledge of the land use and land cover around the breeding sites. Finally, the risk map for the presence of *Anopheles* adults could be superimposed on the human density map to generate the malaria risk map for the population. These maps could become dynamic if the temporal association with environmental and climatic factors is well understood and if continuous remotely-sensed data or meteorological forecasting is available. In this case, early warning systems can be set up to anticipate epidemics. Of course, this process can be completed only if the biology of the vectors in the studied area is very well understood, due to field work.

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PARTIE I - Risque de paludisme chez les militaires français en mission en Afrique intertropicale

Les travaux présentés dans cette première partie constituent un travail préliminaire au développement de cartes et de modèles de risque des voyageurs (en particulier aux militaires) pendant leurs séjours en zone d'endémie palustre. Il s'agissait de démontrer que la télédétection pouvait être utilisée pour évaluer le risque pour des populations mobiles alors qu'elle n'avait été employée jusque là que pour des populations résidant dans des lieux fixes. Les militaires suivis dans cette étude avaient un niveau d'immunité antipalustre faible ou nul, comme une partie importante des populations vivant en milieu urbain dans les zones d'endémie. Le risque de paludisme clinique chez les populations urbaines, comme chez les voyageurs, est ainsi étroitement lié à l'exposition aux piqûres d'anophèles infectés.

Dans ce contexte, l'objectif du travail était l'étude des facteurs de risque individuels, comportementaux et environnementaux des accès palustres chez des militaires se rendant en mission en Afrique intertropicale. Alors que les variables comportementales étaient relevées par questionnaires, les facteurs environnementaux étaient pris en compte indirectement par une mesure des niveaux de végétation, obtenus pas télédétection, auxquels étaient exposés quotidiennement chacun des individus. Les compagnies étant suivies dans cinq pays différents et, au sein de chaque pays, dans des régions distinctes, les images MODIS (1 km de résolution spatiale) permettaient d'obtenir une information globale, à une résolution spatiale suffisante pour étudier les contrastes entre les positions occupées par les individus suivis. Les résultats ont montré que, même en prenant en compte les facteurs de confusion de l'âge et de la mauvaise observance de la chimioprophylaxie, l'environnement était le facteur de risque le plus important pour la survenue d'accès palustres. Ainsi, la prise en compte de l'environnement permettrait de standardiser des études sur l'efficacité des mesures prophylactiques et pourrait contribuer à la compréhension des variations spatiales et temporelles de l'incidence du paludisme observées chez les militaires en opération en Afrique intertropicale.

ARTICLE 1

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ARTICLE 1

Remote sensing and malaria risk for military personnel in Africa

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Remote Sensing and Malaria Risk for Military Personnel in Africa

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Background. Nonimmune travelers in malaria-endemic areas are exposed to transmission and may experience clinical malaria attacks during or after their travel despite using antivectorial devices or chemoprophylaxis. Environment plays an essential role in the epidemiology of this disease. Remote-sensed environmental information had not yet been tested as an indicator of malaria risk among nonimmune travelers.
Methods. A total of 1,189 personnel from 10 French military companies traveling for a short-duration mission (about 4 mo) in sub-Saharan Africa from February 2004 to February 2006 were enrolled in a prospective longitudinal cohort study. Incidence rate of clinical malaria attacks occurring during or after the mission was analyzed according to individual characteristics, compliance with antimalaria prophylactic measures, and environmental information obtained from earth observation satellites for all the locations visited during the missions.
Results. Age, the lack of compliance with the chemoprophylaxis, and staying in areas with an average Normalized Difference Vegetation Index higher than 0.35 were risk factors for clinical malaria.
Conclusions. Remotely sensed environmental data can provide important planning information on the likely level of malaria risk among nonimmune travelers who could be briefly exposed to malaria transmission and could be used to standardize for the risk of malaria transmission when evaluating the efficacy of antimalaria prophylactic measures.

Nonimmune civilians and military personnel traveling in malaria-endemic areas are exposed to transmission and may experience clinical malaria attacks during or after their travel. In the French armies, individual malaria control is based on the use of diethyltoluamide-based insect repellents, clothing that covers the arms and legs (long clothes), deltamethrin-impregnated bed nets, and the intake of chemoprophylaxis (doxycycline 100 mg/d).[1]

According to the vectorial transmission of the disease, environment plays an essential role in the epidemiology of this disease. It has long been understood that geoclimatic factors determine the presence of breeding sites, the density and longevity of anopheles, and their effectiveness at parasite transmission. Remote sensing techniques provide a great amount of environmental information that is available from various earth observation satellites. Among these data, the Normalized Difference Vegetation Index (NDVI) is an index that quantifies the green leaf vegetation coverage. It has been used in several studies as an indicator of environments conducive to malaria vector abundance[2] and has also been associated with transmission of the disease[3] and parasite prevalence.[4–6] To our knowledge, it had not yet

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been tested as an indicator of malaria risk among nonimmune travelers.

Methods

This prospective longitudinal study was carried out in 10 French military companies traveling for a short-duration mission (about 4 mo) in sub-Saharan Africa from February 2004 to February 2006. Six companies were based in Côte d'Ivoire, one in the Central African Republic, one in Chad, one in Senegal, and one in the Republic of Djibouti. The dependent variable was the incidence rate (IR) of clinical malaria attacks occurring during the mission or within 16 weeks after travelers return to France and defined as any clinical signs or symptoms associated with biological confirmation of plasmodial infection, either by microscopy or by rapid diagnostic tests. Clinical data were collected by means of individual questionnaires, medical records, and a military epidemiological surveillance system. No later than 15 days after their return to France, a self-administered questionnaire containing behavioral items was filled out by each traveler and validated by a member of the research team. The protocol used has been approved by the ethical committee of Marseille 2.

The geographic coordinates of the locations visited during the mission were recorded using the Global Positioning System. Images from the Terra satellite, part of the National Aeronautics and Space Administration Earth Observing System (http://edcimswww.cr.usgs.gov/pub/imswelcome/), were downloaded to extract NDVI values. The spatial resolution was 1 km. The NDVI was calculated by measuring the wavelengths and intensity of visible and near-infrared light reflected by the land surface backup into space. Its value ranges between −1 and +1.[7] A value ranging from 0 to 0.2 corresponds to bare soils, from 0.2 to 0.6 corresponds to green vegetation, and a negative value corresponds to aquatic zones.[8] To avoid possible problems due to missing data from cloud cover,[9] the NDVI was chosen as the maximal available value over 1 month. The NDVI in the preceding month[10] was used in this study (NDVI lagged 1 mo). The independent variable was the average NDVI to which each subject was exposed, regarding all visited places using 9 km^2 squares around the actual geographic position weighted by the time spent in each place.

The IR of clinical malaria attacks was analyzed using Poisson regression models. In the cohort, some intragroup correlations could exist due to the sampling design (by company). Thus, analyses were carried out using random effect mixed models. The variables associated with the IR of clinical malaria attacks in univariate analysis with a p value <0.25 were retained for multivariate analysis. A backward stepwise selection procedure was applied in the final model to keep variables with a p value <0.05. All analyses were performed with STATA 9.0 (StataCorp LP, College Station, TX, USA). The unit of the IRs for clinical outcomes was 100 persons for 4 months, also called persons-missions (PM) in this paper. Among the 1,189 subjects included in the statistical analysis, 31 clinical malaria attacks (no severe cases) occurred among 29 persons (IR 7.64 for 100 persons-years or 2.55 for 100 PM). The mean duration of the mission was 124.7 days, ranging from 54 to 150 days (median 124 d). The mean age at inclusion was 25.6 years, ranging from 18 to 47 years (median 25 y). Men accounted for 98.6% of the population. The interquartile extent of the average NDVI in a company was dependent on the mobility of the subjects during the mission.

Results

The results of the univariate analysis are presented in Table 1. An age of at least 30 years old was statistically associated with clinical episodes for junior ranks and noncommissioned officers (JRs–NCOs) only [relative risk (RR) 5.98, 95% confidence interval (CI) 2.73–13.11, p < 0.001]. For higher ranks [officers and warrant officers (Os–WOs)], no clinical episodes were experienced among the subjects of at least 30 years old. Nevertheless, taking the Os–WOs of any age as the reference group, JRs–NCOs of at least 30 years old had a significantly higher risk of malaria (RR 11.72, 95% CI 3.21–42.75, p < 0.001), while JRs–NCOs under 30 years did not (RR 1.92, 95% CI 0.56–6.56, p = 0.299). A self-declared lack of compliance with chemoprophylaxis was significantly associated with clinical episodes (RR 4.20, 95% CI 1.98–8.92, p < 0.001). In contrast to the expected outcome, the self-declared use of protective anti-mosquito measures (mosquito repellent, mosquito nets, and long clothes) was a risk factor in univariate analysis (RR 2.35, 95% CI 1.01–5.47, p = 0.048). An average NDVI superior or equal to 0.35 was significantly associated with an increased risk of clinical malaria (RR 6.15, 95% CI 2.00–18.96, p = 0.002). There were differences in the IR of clinical malaria attacks according to the companies or countries visited, but they were no longer significant when controlling for the NDVI effect in multivariate analysis. The adjusted effects of age, rank,

Table 1 Incidence rate of clinical malaria attacks and estimations from univariate and multivariate analysis of environmental, behavioral, and individual variables (n = 1,189, except other indication). Random effect Poisson regression model

	Number of subjects	Number of clinical malaria attacks	Number of person-days in mission	Incidence rate per 100 person-mission	Crude RR	95% CI	p Value	Adjusted RR	95% CI	p Value
Average NDVI							0.002			<0.001
Inferior to 0.35	511	3	62,777	0.58	1			1		
Superior or equal to 0.35	678	28	85,459	3.83	6.15	2.00–18.96		14.20	4.20–47.94	
Age and rank							<0.001			<0.001
Os–WOs, all ages	271	3	33,932	1.08	1			1		
JRs–NCOs, 18–29 y	811	17	100,981	2.05	1.92	0.56–6.56	0.299	1.68	0.49–5.77	0.407
JRs–NCOs, 30–47 y	107	11	13,323	10.05	11.72	3.21–42.75	<0.001	8.92	2.46–32.33	0.001
Allegated intake of the chemoprophylaxis							<0.001			<0.001
Regular	729	12	91,009	1.61	1			1		
Not always regular	460	19	57,227	4.04	4.20	1.98–8.92		4.20	2.00–8.83	
Tolerance to the chemoprophylaxis							0.081			
Very bad or bad	141	6	17,419	4.19	1					
Good or very good	1048	25	130,817	2.33	0.44	0.18–1.11				
Knowledge of the recommended duration of chemoprophylaxis after returning to France							0.282			
No	209	7	25,903	3.29	1					
Yes	980	24	122,333	2.39	0.62	0.26–1.47				
Allegated use of antivectorial devices							0.048			
Never to rarely	796	12	97,803	1.49	1					
Often to always	393	19	50,433	4.59	2.35	1.01–5.47				
Perceived frequency of mosquito bites							0.620			
Few	389	9	48,193	2.27	1					
A lot	800	22	100,043	2.68	1.22	0.55–2.70				
Perceived personal attraction of mosquitoes compared to the soldiers of the same company							0.460			
Inferior	936	26	116,662	2.71	1					
Equivalent or superior	253	5	31,574	1.93	0.69	0.26–1.83				
Perceived personal risk of malaria compared to the soldiers of the same company							0.152			
Inferior or equivalent	934	20	115,954	2.10	1					
Superior	255	11	32,282	4.15	1.75	0.81–3.76				
Perceived gravity of malaria							0.905			
Not or mildly serious	750	19	93,541	2.47	1					
Serious	439	12	54,695	2.67	0.96	0.46–1.98				
Tobacco consumption							0.200			
Nonsmoker	529	10	65,924	1.85	1					
Smoker	660	21	82,312	3.11	1.64	0.77–3.50				
Bedding time during the mission (n = 1,045)							0.246			
Before midnight	886	16	108,449	1.80	1					
At midnight or after	159	5	19,671	3.09	1.83	0.66–5.07				

CI = confidence interval; NDVI = Normalized Difference Vegetation Index; RR = relative risk; Os–WOs = officers and warrant officers; JRs–NCOs = junior ranks and noncommissioned officers.

chemoprophylaxis, and NDVI estimated in the multivariate model are presented in Table 1.

Discussion

An age of at least 30 years old was associated with an increased risk for non-officer personnel only. Preliminary results from a previous survey that we conducted in the French army suggested that bad compliance with malaria preventative measures could be acquired during previous numerous stays in endemic areas by lower ranks aged 30 years or more. The 30-year-old threshold was then tested in the present study. The increased risk of malaria attacks among the oldest soldiers may be due to weariness in applying prophylactic measures associated with a false feeling of invulnerability as a result of their escape from clinical attacks during and after their previous exposure to malaria transmission.

Poor compliance with the chemoprophylaxis regimen was a risk factor, in agreement with the studies on its efficacy.[11] The use of antimosquito measures was not found to be protective in our study. Subjects who used these protective devices were those who stayed in environments with an average NDVI superior or equal to 0.35, possibly because of a higher degree of nuisance mosquito biting. The self-declared use of protective antimosquito measures appeared to be a risk factor in univariate analysis but was not significant in multivariate analysis controlling for the NDVI effect. Environmental factors may have confounded the relationship between the use of antimosquito measures and malaria risk. The collection of behavior data by questionnaires could lead to information bias, but such a bias did not mask the association between compliance with the chemoprophylaxis regimen and the incidence of malaria.

Persons exposed to an average NDVI superior or equal to 0.35 had a significantly higher risk of clinical malaria. This NDVI threshold is consistent with those found in previous papers. We show that the results from previous studies on populations living in endemic areas can potentially be applied to travelers.

Conclusion

Remotely sensed environmental data can provide important planning information on the likely level of malaria risk among nonimmune travelers who could be briefly exposed to malaria transmission and could be used to standardize for the risk of malaria transmission when evaluating the efficacy of antimalaria prophylactic measures. Additional investigations are necessary to complete this model and should include other environmental data known to be associated with malaria risk, remotely sensed or not, such as rainfall, humidity, quality of water, land use, and land cover.

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Declaration of Interests

The authors state that they have no conflicts of interest.

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PARTIE II - Etude entomologique des conditions de la transmission du paludisme en milieu urbain à Dakar

La transmission du paludisme a été rapportée dans de nombreuses villes africaines [9, 10]. Concernant la zone urbaine de Dakar, la transmission avait été démontrée ou suggérée dans plusieurs études faites à Pikine, une des villes satellites de la capitale du Sénégal [15, 16]. Dans les quartiers plus centraux de la capitale, plus éloignés des zones marécageuses et des zones maraîchères de la presqu'île, des anophèles avaient été capturés à des densités assez faibles, des cas de paludisme étaient soupçonnés d'être autochtones, mais aucun anophèle infecté n'avait été capturé [17-19]. Pourtant, des conditions favorables semblaient être réunies pendant la saison des pluies, de juillet à octobre, pour que la transmission soit possible dans Dakar. Des migrations de population humaines en provenance des zones rurales pouvaient importer des parasites, le régime des pluies permettait l'apparition de gîtes potentiels pour les larves d'anophèles, l'augmentation de la température et de l'humidité de l'air pouvaient permettre une survie suffisante des anophèles femelles adultes.

Dans ce contexte, notre démarche a consisté à mener une importante étude entomologique de terrain afin d'identifier les vecteurs locaux, de mesurer la transmission du paludisme à Dakar, et de documenter son hétérogénéité. Il s'agissait aussi de décrire et d'identifier les déterminants de la répartition spatiale et temporelle des gîtes larvaires et des densités d'anophèles adultes. Ce travail a tout d'abord été entrepris dans deux quartiers dans lesquels stationnent des militaires français (décrit dans l'article 2), puis a été étendu à 45 quartiers de Dakar et de sa proche banlieue, pour une durée totale de cinq ans. L'article 3 décrit les résultats des collectes de données dans 10 zones pendant la saison des pluies 2007. L'article 4 étend la description des résultats aux zones suivies de 2008 à 2010 et reprend en partie les résultats de 2007 afin de permettre des comparaisons selon les années et l'augmentation de la puissance des analyses statistiques. La totalité des données collectées de 2007 à 2010 devaient permettre d'entreprendre une modélisation du risque de paludisme dans Dakar. Ainsi, une attention particulière a été portée à la mesure des facteurs environnementaux et climatiques qui pouvaient être évalués par télédétection. L'article 4, en préparation, doit donc être considéré comme un article d'introduction à l'article 6 sur la modélisation du risque entomologique à partir de données télédétectées.

Les résultats obtenus ont permis tout d'abord de documenter la transmission du paludisme dans la ville de Dakar et sa proche banlieue grâce à des collectes de données entomologiques. *Anopheles arabiensis* était l'espèce majoritaire à Dakar mais des proportions élevées d'*Anopheles melas* ont été mesurées dans certains quartiers, allant jusqu'à 40% des populations d'anophèles adultes. De rares specimens de *Anopheles gambiae s.s.* ont aussi été collectés. L'hétérogénéité temporelle de la transmission était attendue, de part le caractère très saisonnier du climat. Une très forte hétérogénéité spatiale a aussi été démontrée. En effet, alors que certains quartiers étaient totalement exempts ou quasi-exempts d'anophèles adultes, l'agressivité a pu atteindre jusqu'à 250 piqûres par personne par nuit en moyenne pendant la saison des pluies dans d'autres quartiers centraux. Ainsi à Dakar, des données moyennes de transmission ne permettent pas de dresser un portrait réel de la situation entomologique et l'évaluation du risque de paludisme doit être faite à des échelles fines. A cette étape du travail de thèse, une base de donnée géoréférencée a été créée et un SIG a été construit, rassemblant toutes les variables entomologiques, environnementales, climatiques, biologiques et physiques relevées sur le terrain.

Les trois articles suivant décrivent les travaux entomologiques. L'article 4 n'a pas encore été soumis.

ARTICLE 2

Pages F, Texier G, Pradines B, Gadiaga L, Machault V, Jarjaval F, Penhoat K, Berger F, Trape JF, Rogier C, Sokhna C: **Malaria transmission in Dakar: a two-year survey**. *Malar J* 2008, **7**:178.

ARTICLE 3

Machault V, Gadiaga L, Vignolles C, Jarjaval F, Bouzid S, Sokhna C, Lacaux JP, Trape JF, Rogier C, Pages F: **Highly focused anopheline breeding sites and malaria transmission in Dakar**. *Malar J* 2009, **8**:138.

This article has been highly accessed in Malaria Journal.

ARTICLE 4 - En préparation

Pagès F, Machault V, Gadiaga L, Gaye A, Jarjaval F, Lacaux JP, Godefroy L, Trape J.F., Sokhna C, Rogier C: **Conditions of malaria transmission in Dakar from 2007 to 2010**.

ARTICLE 2

Malaria transmission in Dakar: a two-year survey

Pages F, Texier G, Pradines B, Gadiaga L, Machault V, Jarjaval F, Penhoat K, Berger F, Trape JF, Rogier C, Sokhna C

Malar J 2008, **7**:178.

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Malaria transmission in Dakar: A two-year survey

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Abstract

Background: According to entomological studies conducted over the past 30 years, there was low malaria transmission in suburb of Dakar but little evidence of it in the downtown area. However; there was some evidence of local transmission based on reports of malaria among permanent residents. An entomological evaluation of malaria transmission was conducted from May 2005 to October 2006 in two areas of Dakar.

Methods: Mosquitoes were sampled by human landing collection during 34 nights in seven places in Bel-air area (238 person-nights) and during 24 nights in five places in Ouakam area (120 person-nights). Mosquitoes were identified morphologically and by molecular methods. The *Plasmodium falciparum* circumsporozoïte indexes were measured by ELISA, and the entomological inoculation rates (EIR) were calculated for both areas. Molecular assessments of pyrethroid knock down resistance (*Kdr*) and of insensitive acetylcholinesterase resistance were conducted.

Results: From May 2005 to October 2006, 4,117 and 797 *Anopheles gambiae s.l.* respectively were caught in Bel-air and Ouakam. Three members of the complex were present: *Anopheles arabiensis* (> 98%), *Anopheles melas* (< 1%) and *An. gambiae s.s.* molecular form M (< 1%). Infected mosquitoes were caught only during the wintering period between September and November in both places. In 2005 and 2006, annual EIRs were 9,5 and 4, respectively, in Bel-air and 3 and 3, respectively, in Ouakam. The proportion of host-seeking *An. gambiae s.l.* captured indoors were 17% and 51% in Bel air and Ouakam, respectively. Ace 1 mutations were not identified in both members of the *An. gambiae* complex. *Kdr* mutation frequency in *An. arabiensis* was 12% in Bel-air and 9% in Ouakam.

Conclusion: Malaria is transmitted in Dakar downtown area. Infected mosquitoes were caught in two subsequent years during the wintering period in two distant quarters of Dakar. These data agree with clinical data from a Senegalese military Hospital of Dakar (Hospital Principal) where most malaria cases occurred between October and December. It was the first detection of *An. melas* in Dakar.

Background

In the sixties, the relative seriousness of malaria and the seasonal transmission of *Plasmodium falciparum* by *Anopheles gambiae s.l.* in Dakar were reported in different studies [1-4]. Immunity was acquired quite slowly during the first 20 years of life [5]. Malaria-infected individuals came from rural areas to Dakar and contributed to the start of transmission, which culminated in October-November after the end of the rainy period [6]. In the eighties, an entomological survey proved that malaria transmission occurred in Pikine in the suburb of Dakar and that *Anopheles arabiensis* was the main vector in this area [6]. In the nineties, malaria transmission persisted in Pikine and in the surrounding villages of Dakar, always with *An. arabiensis* as the vector [7]. During the same period, two parasitological and entomological studies conducted in two sanitary districts of Dakar showed that the prevalence of malaria was very low; a few *An. arabiensis* were caught, and none of them were infected by *P. falciparum* [8-10]. Based on these results, many practitioners thought that there was no malaria transmission in Dakar *intra-muros* (*i.e.* in the down-town area) and that the infections occurred in the suburbs or inland. Nevertheless, human malaria cases were reported in autochthonous people who had not been outside Dakar for at least one year, suggesting that malaria was transmitted in Dakar [10]. At the same time, French military doctors were confronted with malaria cases in expatriates, who were visiting a malaria endemic country for the first time, but had never left downtown Dakar. To assess the reality of malaria transmission in Dakar, an entomological evaluation was conducted at the French military bases of Dakar from May 2005 to October 2006, over two winters.

Materials and methods

Location

Located at 14°40'20" North, 17°25'22" West (the westernmost point of Africa), Dakar, the capital city of Senegal, has 1,030,594 inhabitants and covers the major part of the Cap Vert Peninsula. The altitude does not exceed 104 m. The population of the Dakar area is estimated to be 2.45 million people, representing 20% of the Senegalese population. The estimated population density is 12,233 inhabitants/km^2.

The study was conducted in two districts of Dakar: Bel-air in the east of the city and Ouakam in the west (Figure 1). Bel-air is a residential district with luxuriant vegetation and with many market gardens and water wells along a railway, which crosses the area. The French military camp is bordered by the sea on three sides and by the railway on the other side. Ouakam is also a residential district with individual and collective houses. The French military camp of Ouakam is bordered by the sea on the west side and by houses on the other sides. It is a dry area with little vegetation. Between the camp and the sea lie a market gardens area and two water wells. The waste water of the French camp flows into a network that irrigates all the market gardens.

Climate

The Cap Vert Peninsula is located in the Atlantic Sudan zone. Two distinct seasons exist: a hot and wet season from June to November (maximum average temperature 28.2°C in October) and a cool and dry season from December to May (minimum average temperature 20.4°C in February). The first rains generally occur at the end of June or the beginning of July, and the last occur at the beginning of October. In 2005 and 2006 (the period covered by the study), the average rainfall was 525 and 350 mm, respectively.

Field mosquito processing

Sampling by human landing of malaria vectors was carried out both indoors and outdoors. Collectors gave prior informed consent and received anti-malaria prophylaxis and yellow fever immunization. Collectors were organized in teams of two for each collection point. Replacement of workers within a team was done every two hours from 7:00 PM to 7:00 AM. Teams of collectors were rotated among the collection points on different collection nights to minimize sampling bias. Landing catches were performed at seven points (two places indoors and five outdoors) in two periods, from May 2005 to May 2006 and from September to October 2006, during 34 nights in Bel-air (i.e. 238 person-nights). In Ouakam, human landing collections were performed at five points (two indoor and three outdoor locations) for two periods, from July 2005 to May 2006 and from September to October 2006, during 24 nights (i.e. 120 person-nights).

Mosquitoes were recorded by the location and hours of capture. They were sorted by genera, and anopheline mosquitoes were identified morphologically following the Gillies and Coetzee keys and by software from Hervy *et al* [11,12]. Culicinae were identified morphologically following the Edwards keys [13]. All mosquitoes were stored individually in numbered vials with desiccant and preserved at -20°C until processing at the Medical Entomology Unit of the Institute for Tropical Medicine (IMTSSA), Marseille (France).

Laboratory mosquito processing

Heads and thoraces of anopheline females were tested by enzyme-linked immunosorbent assay (ELISA) for *P. falciparum* circumsporozoite protein (CSP)[14]. All females belonging to the *A. gambiae* complex caught during the dry season, a random sample of females caught during the rainy season, together with all CSP-positive anopheline, were identified by polymerase chain reaction (PCR) at the

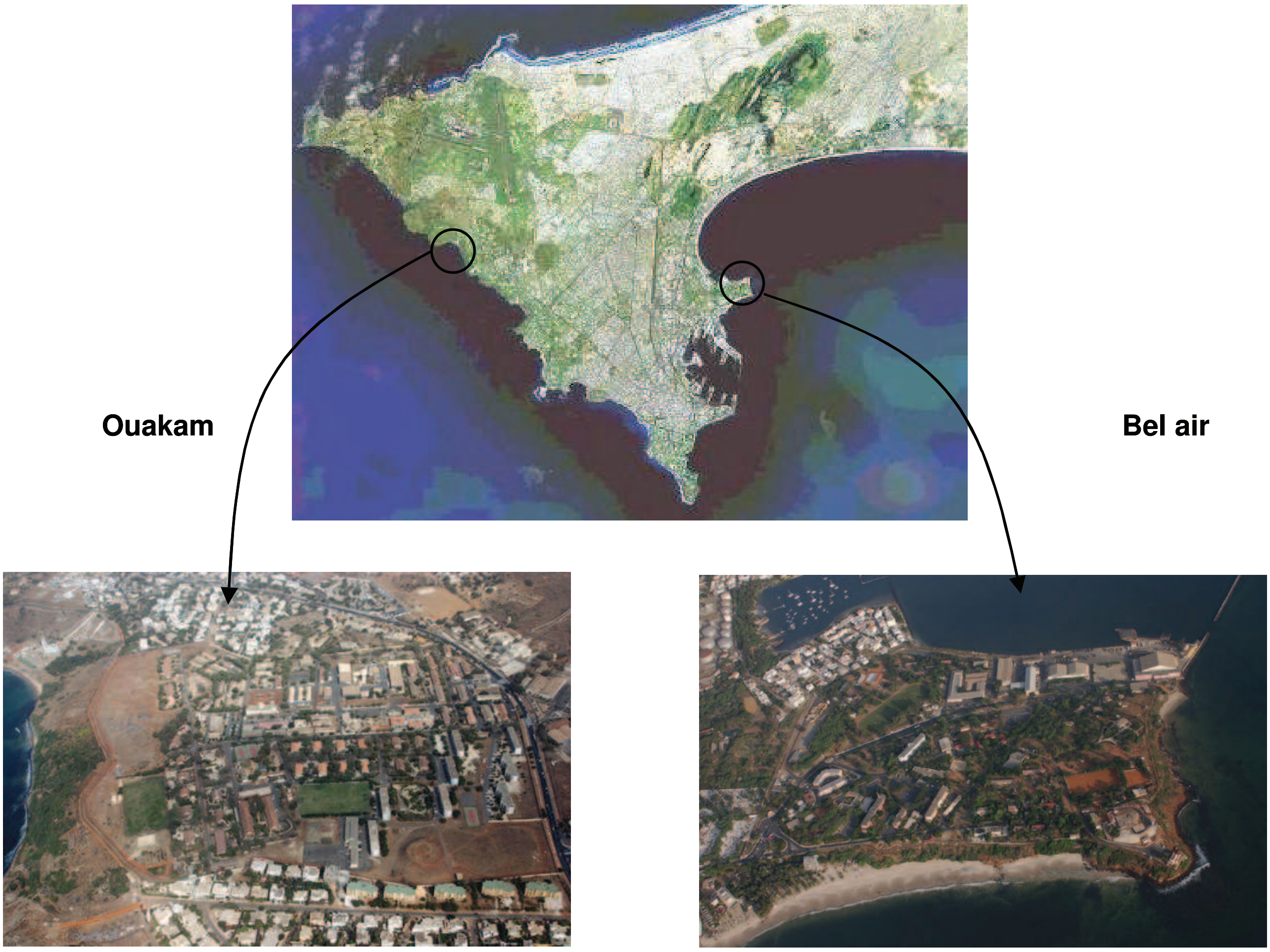

Figure 1
Localization and surroundings of the French military camps in Dakar.

species and molecular forms levels [15]. Molecular characterizations of the *Kdr* and *Ace1* mutations were carried out on these mosquitoes as previously described [16,17].

Data analysis
The human biting rate (HBR) was expressed as the number of female anopheline bites per human per night. The CSP index was calculated as the proportion of mosquitoes found to be positive for CSP. The entomological inoculation rate (EIR) was calculated as the product of the HBR and the CSP index of mosquitoes collected on humans. The *An. gambiae s.l.* biting activity is sufficient for transmission only during the end of the rainy period; the EIR calculated for this period will be considered as the annual EIR. Conformity of *Kdr* and *Ace1* frequencies with Hardy-Weinberg expectations was tested using a Pearson chi-square test considered significant when $P < 0.05$. Endophagic rates were compared using a chi-square test.

Weather data
Rainfall data were graciously provided by the National Weather Agency.

Results
Mosquito collection
A total of 69,082 mosquitoes were caught (76.4% *Culex quinquefasciatus*, 13.9% *Culex tritaeniorynchus*, 7.1% *An. gambiae s.l.*, 2.2% *Aedes aegypti*; Tables 1 and 2).

Biting rates and biting behaviour of An. gambiae s.l
The biting activity at the two sites from May 2005 to May 2006 is shown in Figure 2. *An. gambiae s.l.* was present throughout the year in Dakar, but most of the specimens (98%) were caught between July and December. The peak of biting occurred in September and October, at the end of the winter period (rainy season): 67% and 87% of mosquitoes were caught during these two months in Ouakam

Table 1: Mosquitoes collected in the French military camps of Dakar from May 2005 to May 2006: distribution by species, camps, periods and place of capture (indoor or outdoor).

	From May 2005 to May 2006				
	Bel-air		**Ouakam**		TOTAL
	Indoor	Outdoor	Indoor	Outdoor	
Anopheles gambiae s.l.	346	2703	242	347	3638
Anopheles pharoensis	2	17	0	1	20
C. quinquefasciatus	12024	26407	1480	3568	43479
C. tritaeniorynchus	419	5230	71	1585	7305
Aedes aegypti	113	964	26	256	1359
Aedes metallicus	2	81	4	82	169
Aedes vitattus		7			7
Mansonia sp		4			4
Aedeomyia sp				2	2

and Bel-air, respectively. During this period in 2005, in Bel-air, the average biting rate for *An. gambiae s.l.* was 112 bites per person per night, with a peak of 181 bites per person per night; in Ouakam, the average biting rate for *An. gambiae s.l.* was 19.7 bites per person per night, with a peak of 37.2 bites per person per night. During September-October in 2006, the average rates in Bel-air and Ouakam were 19.1 and 10.4 *An. gambiae s.l.* bites per person per night, respectively. The distribution of *An. gambiae s.l.* bites by hour is shown in Figure 3. Indoors over 70% of biting occurred between 1:00 a.m. and 6:00 a.m. in Ouakam and between 2:00 a.m. and 7:00 a.m. in Bel-air; outdoors, over 70% of biting occurred between 1:00 a.m. and 6:00 a.m. in both locations.

The average number per catching point of host-seeking *An. gambiae s.l.* caught indoor and outdoor were 121 and 115, respectively in Ouakam and 115 and 540, respectively in Bel air. The proportion of host-seeking *An. gambiae s.l.* captured indoors were 51% and 17% in Ouakam and Bel air, respectively. ($P < 0.0001$, RR = 2.92 [2.37; 3.59], indicating that this species was more endophagic in Ouakam.

CSP and EIR

In Bel-air, 3,049 *An. gambiae s.l.* collected by human landing catches were processed by ELISA for *P. falciparum* antigen detection in 2005, and 1,084 were processed in 2006. Assuming that malaria transmission could occurred only in the end of the rainy season, CSP index has been calculated only from the end of September to December. From May 2005 to the middle of September 2005, none of the 1,546 mosquitoes were positive for CSP. From the end of September to December, seven specimens among 1,503 were CSP positive. The CSP index was 0.46% (CI95% = 0.19–0.96). From December 2005 to May 2006, none of the 16 mosquitoes were positive for CSP. In September and October 2006, three specimens among 1,068 were positive. The CSP index was 0.28% (CI95% = 0.06–0.82).

Infected mosquitoes were identified only between the end of the rainy season. In 2005 and 2006, annual EIRs were

Table 2: Mosquitoes collected in the French military camps of Dakar from September to October 2006: distribution by species, camps, periods and place of capture (indoor or outdoor).

	From September to October 2006				
	Bel-air		**Ouakam**		TOTAL
	Indoor	Outdoor	Indoor	Outdoor	
Anopheles gambiae s.l.	78	990	23	185	1276
Anopheles pharoensis	2	4			6
C. quinquefasciatus	858	7713	252	475	9298
C. tritaeniorynchus	43	2100	3	159	2305
Aedes aegypti	2	156	8	34	200
Aedes metallicus					0
Aedes vitattus		3			3
Mansonia sp		11			11
Aedeomyia sp		0			0

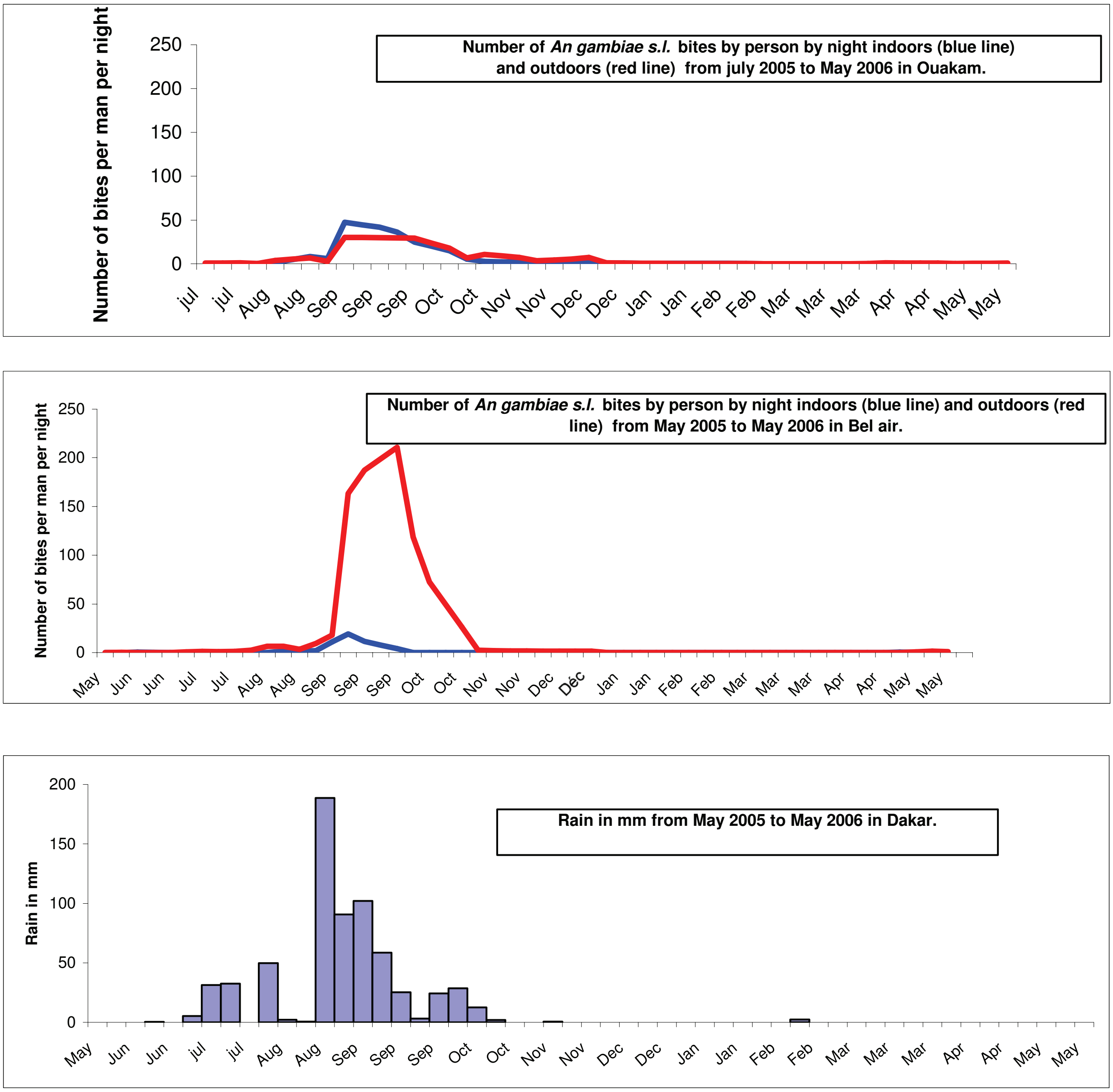

Figure 2
Outdoor and indoor aggressiveness of *An. gambiae s.l.* in Ouakam and Bel air from May 2005 to May 2006, and rainfall.

9.5 and 4 infective bites for a person without protection, respectively.

In Ouakam, 589 *An. gambiae s.l.* collected by human landing catch were processed by ELISA for *P. falciparum* antigen detection in 2005, and 217 were processed in 2006. From July to the middle of September 2005, none of the 279 mosquitoes were positive. From the end of September to December, two specimens among 310 were positive. The CSP index was 0.64% (CI95% = 0.08–2.30). From December 2005 to May 2006, none of the 9 mosquitoes were positive. In September and October 2006, two specimens among 1,068 were positive. The CSP index was 0.96% (CI95% = 0.12–3.40). In 2005 and 2006, annual

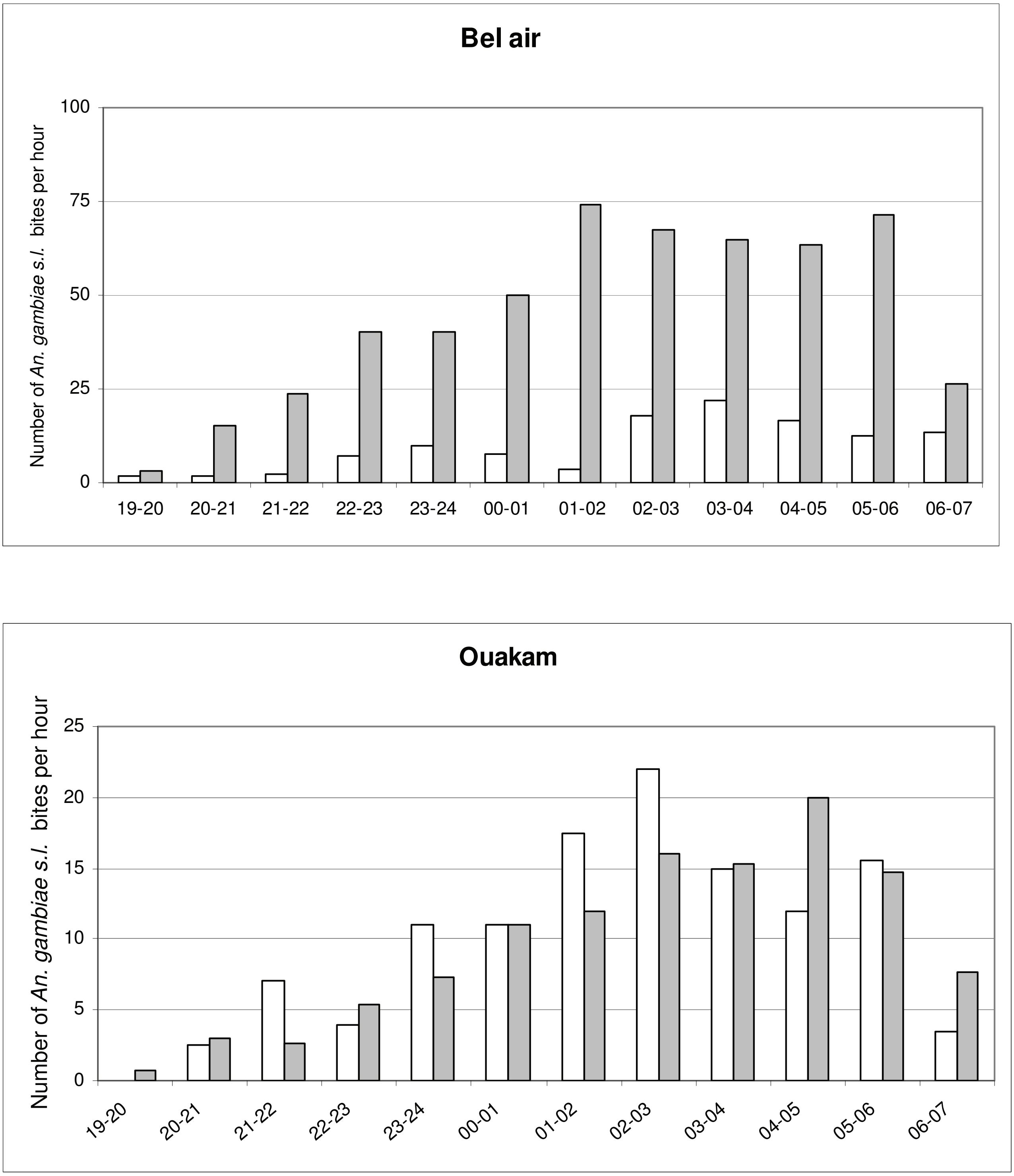

Figure 3
Distribution by hours of *An. gambiae s.l.* bites indoors (white bars) and outdoors (gray bars) in Dakar, May 2005 to May 2006.

EIRs were 3 and 3 infective bites for a person without protection, respectively.

Molecular identification of **An. gambiae s.l**

Using rDNA-PCR, all specimens of *An. gambiae s.l.* captured before September 2005 (373 from Bel-air, and 82 from Ouakam), a random sample of the mosquitoes caught from September to December 2005 (346 from Bel-air and 216 from Ouakam), a random sample of the mosquitoes caught in September and October 2006 (137 from Bel-air and 134 from Ouakam), and all CSP-positive specimens were identified at the species level. In Bel-air, three members of the complex were present in 2005: *An. arabiensis* (97.8%), *Anopheles melas* (2.0%) and *An. gambiae s.s.* molecular form M (0.2%). In 2006, only *An. arabiensis* (98.5%) and *An. gambiae s.s.* molecular form M (1.5%) were present. In Ouakam, and only *An. arabiensis* was present in 2005. In 2006, one specimen of *An. melas* was caught. Emerging adults and all CSP-positive specimens were *An. arabiensis*.

Kdr-w *and Ace1 mutation frequencies in* **An. gambiae s.l**

All CSP-positive mosquitoes per site and a random sample of 100 PCR-identified mosquitoes were tested for the *Kd-wr* and *Ace1* mutations. Ace 1 mutations were not identified in any members of the *An. gambiae* complex either in Ouakam or in Bel-air. The *Kdr-w* mutation frequency in *An. arabiensis* was 12% in Bel-air and 9% in Ouakam (not a significant statistical difference). The genotypic frequencies are shown in Table 3. The populations were not at Hardy-Weinberg equilibrium at either site for kdr ($P < 0.0001$).

Discussion

Malaria transmission is a reality in Dakar. *P. falciparum*-infected Anopheles have been caught during two rainy periods consecutively in two quarters of the town. All infected *An. gambiae s.l.* were caught only during the rainy period. The seasonal transmission occurs in the end of the rainy season from September to November. In August 2005, very heavy rains (278 mm of water in six days) flooded many parts of the city. Many potential breeding sites were created, and the *An gambiae s.l.* aggressiveness was high, with a peak of almost 200 bites per person per night in September. This abnormal situation in Dakar, due to an exceptional climatic event, could have led to an exceptional malaria transmission. However, the catch of infected Anopheles during the 2006 winter proved that malaria transmission is not exceptional and occurred each year during this period. These data agree with clinical data from a Senegalese military Hospital of Dakar (Hospital Principal), where most malaria cases occur between October and December.

Table 3: Genotypic frequencies for *Kdr* loci of two samples of *An. arabiensis* collected in the French military camps of Bel air and Ouakam from May 2005 to May 2006.

	Bel-air	Ouakam
RR	11 (0,10)	5 (0,05)
RS	4 (0,04)	9 (0,09)
SS	94 (0,86)	90 (0,86)
Total	109	104

Only specimens of *An. arabiensis* were infected. *Anopheles arabiensis* is the main member of the gambiae complex in Dakar and, according to its abundance and to the results of this study, is the main malaria vector. The presence of *An. melas* was detected in Dakar for the first time in the present study. Nevertheless, this vector was not found to be infected with *P. falciparum*. Until now, *An. melas* had been reported only in the mangrove swamps of the Delta's Saloum (south Senegal) and in the Senegal River delta (north Senegal) [18,19]. In addition, neither *Anopheles pharoensis* nor *An. gambiae* molecular form M caught in Dakar were infected with *P. falciparum*. The aggressiveness of *An. arabiensis* was higher in Bel-air than in Ouakam, but EIRs were very high in the two quarters. *An. arabiensis* is more endophagic in Ouakam. This behavior allows for an easier access to a human blood meal that may explain the similar EIRs observed for the two quarters.

The seasonal character of transmission allows us to calculate mean annual EIR for the two years of study. In Bel-air, the mean annual EIR fell from 9.5 in 2005 to 4 in 2006. In Ouakam, a similar mean annual EIR of 3 was estimated for 2005 and 2006. Our observations are consistent with the results of a meta-analysis of studies of malaria transmission in sub-Saharan Africa, which found a mean annual EIR of 7.1 in the city centers, with more than two-thirds of the studies reporting EIRs < 4/year [20]. In 1996, the number of infective bites per person was estimated to 0.05/year for the central area of Dakar, i.e., one infective bite every 20 years [10]. This work was conducted on a very large area with 13 study sites but with only two catching points per site and human landing collections performed once every month. Ten years later, the risk of malaria transmission seemed to be 60 to 80 times higher. The peak of *An. gambiae s.l.* aggressiveness lasts for a little longer than one month (Figure 3). With a monthly rhythm of capture, the previous study could have missed this peak. Nevertheless, a modification of the entomological situation over ten years cannot be excluded. There is some evidence that anopheline species may be adapting to urban ecosystems. Adaptation of *An. gambiae s.s.* to urban aquatic habitats, such as water-filled domestic containers, has been observed in Accra, Ghana [21]. In addition, adaptations of the anopheline vectors to new breeding sites (tree holes, polluted water) are reported from many urban areas in Africa [22-24]. The impact of urbanization on the composition of the vector system and malaria transmission dynamics has been highlighted in many studies [25,26]. Urban farming provides ample aquatic habitats for mosquitoes, which are responsible for the persistence of anopheline populations in many African towns [27-29]. In Ouakam and Bel-air, as in other places in Dakar, market gardens are present with or without water wells (cement wells or traditional wells called "ceanes"). The urban area of Dakar contains more than 5,000 market-garden wells, which provide permanent sites for mosquito larvae, in particular *An. Arabiensis* [30]. The increase of the *An. arabiensis* population size during the 2005 winter is an argument to highlight the major role of temporary pools in malaria transmission in Dakar. Further specific study is necessary to understand the impact wells and urban farming on anopheline density and malaria transmission.

This situation questions the origins of these anopheline populations. Are there autochthonous populations from Dakar growing during the rainy season, or are they coming in from other areas of Senegal? Populations of *An. arabiensis* in West Africa are considered to be continuous throughout the year, with many individuals surviving through the dry season, perhaps in a physiologically altered state rather than through extinction or a severe bottleneck during the dry season, followed by re-colonization by a few individual survivors or immigrants in the subsequent rainy season [31]. In Barkedji, Senegal, Simard *et al* did not detect any difference in measures of genetic diversity and linkage disequilibrium between the dry and rainy seasons [32]. They concluded that, despite extreme minima in local density, malaria transmission in this area was due to autochthonous population of *An. arabiensis*. They also found a low differentiation between two populations, which were 250 Km apart, suggesting extensive gene flow across this distance. These results suggest that *An. arabiensis* maintains a large permanent deme over a large area. The situation in Dakar seems similar to that in Barkedji: a long dry season where no *An. arabiensis* (or only a few) are caught [33]. Further genetic studies will be necessary to confirm the hypothesis that malaria transmission is due to an autochthonous *An. arabiensis* population, and to assess the gene flow between the urban and rural populations of Senegalese *An. arabiensis*.

Pyrethroids are the main insecticide used in malaria vector control, including indoor residual spraying and impregnated materials (bednets, curtains, plastic sheeting). Pyrethroids have the advantage of acting very rapidly as insecticides, with both knockdown and lethal effects at dosages under the threshold of mammalian toxicity [34]. Since 1970s, pyrethroids have been extensively used in urban areas and for agricultural purpose in rural areas. Detected in the 1990s, knock-down resistance (*Kdr*) to pyrethroids and DDT of *An. gambiae s.l* is an increasing problem. Two mutations at the same locus in the voltage-gated sodium channel are known to confer knock-down resistance to a wide range of pyrethroids and DDT [35-37]. These mutations were previously described in west and east Africa (*Kdr-w* and *Kdr-e*) in *An. gambiae s.s.* as well in *An. arabiensis* [38-41]. There are few studies of *An. arabiensis* insecticide susceptibility in the area of Dakar. In 1987, bioassays were conducted in two places in the suburbs of Pikine and of Thiaroye [42], where in vivo resistance to DDT was observed. The agricultural use of DDT in market gardens was incriminated. In 1999, a normal susceptibility of *An. arabiensis* was found in Dakar [43]. Our data show a *Kdr-w* frequency of 0.12 in Bel-air and 0.09 in Ouakam. The populations were not at Hardy-Weinberg equilibrium for *Kdr* in either site ($P < 0.0001$). This disequilibrium could be due to local selection pressure by the agricultural use of pyrethroids in market gardens.

The *Kdr* mutation has been shown to be closely associated with DDT and pyrethroid resistance in several *An. gambiae* populations (particularly the molecular S form) [35-37]. However, the role of *Kdr* in conferring resistance in *An. arabiensis* remains unclear [44,45] As a result, insecticide susceptibility tests should be carried out to assess physiological resistance levels in *An. arabiensis* in Dakar. However, this is the first report of the *Kdr-w* mutation in *An. arabiensis* in Dakar. Resistance to carbamate and organophosphate insecticides is also widespread in West Africa. The presence of an insensitive acetylcholinesterase in populations of *An. gambiae s.s.* of both forms was revealed by biochemical assays. The ACE-1-R mutation has also been detected in the two molecular forms of *An. gambiae s.s* in many West African countries [17,46]. This mutation has not been detected in *An. arabiensis* at present. Our results are consistent with these data.

In terms of effective vector control at the military camps and in town, choice of insecticide should depend on the results of susceptibility tests on *An. arabiensis*.

Malaria prevalence is very low in Dakar and its urban periphery [7,8,10]. Nevertheless, cerebral malaria is the first etiology of neuromeningeal diseases in Dakar [47]. Extensive genetic diversity was observed in *P. falciparum* isolates collected in Dakar [48-50]. Significant linkage disequilibrium was observed with microsatellite loci in urban parasites. Two non-exclusive hypothesis could explain the situation in Dakar: (i) a global non-panmictic structure of Dakar malaria population due to a high predominance of selfing; (ii) a structuration in subpopulations of several malaria foci in Dakar. The entomological findings of the present study are consistent with seasonal transmission leading to an increase of cases in the October end of the rainy season. There is a concern about the origin of *Plasmodium falciparum* infections observed in Dakar during the rainy season. Are they due to autochthonous parasites transmitted during the winter or to parasites imported from the suburbs by commuters, as suggested by Vercruysse? [6] In Senegalese people employed by the French army, the first malaria cases are diagnosed in commuters at the beginning of the rainy season, and cases in residents occur later in the season. The movement of populations from the suburbs or rural areas to Dakar must be considered to understand malaria epidemiology in Dakar [51,52].

Urban malaria is considered to be an emerging problem in Africa. In 2003, 39% of Africa's people lived in urban settings; by 2030, 54% of Africans are expected to do so [53]. With the increase of people living in urban dwellings, it's important to develop and validate new approaches for rapid appraisal of malaria risk. The rapid urban malaria appraisal (RUMA) methodology has been develop to provide a cost effective tool to conduct assessment of the malaria situation in urban sub-Saharan Africa and to improve the understanding of urban malaria [54]. The only entomological point required is in this evaluation is the mapping of breeding sites. Considered very time-consuming, this task has been done only in Ouagadougou [27].

The results of this study confirm that the vector complex situation in African towns is always changing, with the description of *An melas* and *An gambiae s.s.* in Dakar. Entomological studies are long, difficult and require time and special expertise but they are indispensable to understand the dynamics of malaria transmission in urban settlements and to monitor the increase of the insecticide resistance in urban mosquitoes. The study showed a difference in host finding behaviour between the two quarters: *An. arabiensis* are more endophagic in Ouakam than in Bel air. This difference of behaviour has an impact on the malaria transmission level. With a lower biting activity during the transmission season, the Annual EIR in Ouakam remains as high than in Bel air. Indoor vector control measures will probably not have the same impact in the two quarters. The misuse of impregnated bed nets during this period is probably riskier in Ouakam.

Malaria is transmitted in Dakar. This seasonal transmission occurs only during the two last months of the rainy season. The transmission level could be very high. Further studies have to be conducted in other parts of Dakar to assess the risk of transmission, to understand the role of permanent and temporary pools, the impact of urban farming and to discover the origin of the Dakar anopheline populations.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

FP was responsible for the study design, supervision of data collection, analysis, interpretation and production of the final manuscript and revisions. GT contributed to the supervision of data collection, the data analysis, interpretation and production of final manuscript. BP contributed to the supervision of data collection, to the data analysis, interpretation and production of final manuscript. LG contributed to the supervision of data collection, to the data analysis, interpretation and production of final manuscript. VM contributed to the data analysis and to the preparation of the final manuscript. FJ contributed to the supervision of data collection, to the data analysis. KP contributed to the supervision of data collection, to the data analysis. FB contributed to the supervision of data collection, to the data analysis. JFT was contributed to overall scientific management, analysis, interpretation and preparation of the final manuscript and revisions. CR was contributed to overall scientific management, analysis, interpretation and preparation of the final manuscript and revisions. CS was responsible for overall scientific management, analysis, interpretation and preparation of the final manuscript and revisions. All authors read and approved the final manuscript.

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ARTICLE 3

Highly focused anopheline breeding sites and malaria transmission in Dakar

Machault V, Gadiaga L, Vignolles C, Jarjaval F, Bouzid S, Sokhna C, Lacaux JP, Trape JF, Rogier C, Pages F

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Highly focused anopheline breeding sites and malaria transmission in Dakar

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Abstract

Background: Urbanization has a great impact on the composition of the vector system and malaria transmission dynamics. In Dakar, some malaria cases are autochthonous but parasite rates and incidences of clinical malaria attacks have been recorded at low levels. Ecological heterogeneity of malaria transmission was investigated in Dakar, in order to characterize the *Anopheles* breeding sites in the city and to study the dynamics of larval density and adult aggressiveness in ten characteristically different urban areas.

Methods: Ten study areas were sampled in Dakar and Pikine. Mosquitoes were collected by human landing collection during four nights in each area (120 person-nights). The *Plasmodium falciparum* circumsporozoite (CSP) index was measured by ELISA and the entomological inoculation rates (EIR) were calculated. Open water collections in the study areas were monitored weekly for physico-chemical characterization and the presence of anopheline larvae. Adult mosquitoes and hatched larvae were identified morphologically and by molecular methods.

Results: In September-October 2007, 19,451 adult mosquitoes were caught among which, 1,101 were *Anopheles gambiae s.l.* The Human Biting Rate ranged from 0.1 bites per person per night in Yoff Village to 43.7 in Almadies. Seven out of 1,101 *An. gambiae s.l.* were found to be positive for *P. falciparum* (CSP index = 0.64%). EIR ranged from 0 infected bites per person per year in Yoff

Village to 16.8 in Almadies. The *An. gambiae* complex population was composed of *Anopheles arabiensis* (94.8%) and *Anopheles melas* (5.2%). None of the *An. melas* were infected with *P. falciparum*. Of the 54 water collection sites monitored, 33 (61.1%) served as anopheline breeding sites on at least one observation. No *An. melas* was identified among the larval samples. Some physico-chemical characteristics of water bodies were associated with the presence/absence of anopheline larvae and with larval density. A very close parallel between larval and adult densities was found in six of the ten study areas.

Conclusion: The results provide evidence of malaria transmission in downtown Dakar and its surrounding suburbs. Spatial heterogeneity of human biting rates was very marked and malaria transmission was highly focal. In Dakar, mean figures for transmission would not provide a comprehensive picture of the entomological situation; risk evaluation should therefore be undertaken on a small scale.

Background

Malaria and urbanization

Urbanization has a significant impact on the health of local populations. It is estimated that by 2025, 800 million people will live in African cities and urban malaria is considered to be an emerging health problem of major importance in Africa. Urban malaria should be seen as a specific public health issue and assessment, understanding and control should not simply reproduce initiatives taken in rural communities [1,2].

In urban settings, malaria risk heterogeneity is recorded over small distances due to diversity in the degree and type of urbanization, density of human population, quality of water and waste management, vector control measures, household factors and access to health care [1,3], or human migration patterns that might import parasites from rural areas [4]. Urbanization has a great impact on the composition of the vector system and malaria transmission dynamics [5]. In regard to breeding requirements, there is evidence of adaptation of anopheline species to urban settings and several examples of polluted breeding habitats or new types of breeding habitats have been brought to light [6-9]. The importance of urban agricultural activity on malaria has also been reported in several African cities, such as in Côte d'Ivoire and Ghana [10], where irrigation leads to the creation of larval habitats [10,11] and higher malaria prevalence [12,13].

Finally, variations in *Anopheles* densities play a major role in the spatial and temporal heterogeneity of malaria risk. In cities, where blood meal sources are abundant, dispersion of the vectors is low and malaria transmission is focal and highly driven by the proximity of breeding sites [14,15]. Thus, an understanding of transmission heterogeneity requires a good knowledge of the geographical localization of breeding sites. Characterizing and mapping these habitats will help to spatially rank malaria risk in urban settings and focus control activities on a small scale [16].

Clinical malaria in Dakar

In Dakar, the capital city of Senegal, some malaria cases are recognized to be autochthonous [17] but parasite rates and incidences of clinical malaria attacks in the city and its nearby periphery have been recorded at low levels compared to continent-wide level [14,17,18]. Nevertheless, malaria should not be neglected, as severe cases have been reported among Dakar residents with little acquired malaria immunity [19]. In some health facilities, up to 65% of patients diagnosed with malaria present severe forms of the disease [20]. In Dakar, a high prevalence of severe anaemia was found in young children between 1990 and 1996 [21] and placental malaria infections have been associated with pre-eclampsia in pregnant women with poor malaria immunity [22]. In the nearby suburbs, it has been found that 10% of delivering women were positive for *Plasmodium* parasites in the placenta and 44% of placentas showed chronic infection, associated with low birth weight [23].

Malaria transmission in Dakar

In this clinical context, local malaria transmission has been studied for several decades. In Pikine, a suburban area of Dakar, transmission was demonstrated in 1979–80, with anopheline aggressiveness peaking at more than 100 *Anopheles arabiensis* bites per person per night (*Plasmodium falciparum* sporozoïte rate up to 1.14%) and an Entomological Inoculation Rate (EIR) of 43 infective bites per person per year [24]. Less than 10 years later in the same city, *An. arabiensis* was still the main anopheline species captured but the estimated annual EIR did not exceed 0.382 [14].

In the south and central sanitary districts of Dakar, in 1994–95 and 1996–97 respectively, *An. arabiensis* aggressiveness was low, with less than one bite per person per night and no infected *Anopheles* collected [17,25]. In 2005–2006, malaria transmission was assessed in two vegetated areas of downtown Dakar during the wintering periods; the recorded aggressiveness peak was close to 200 bites per person per night and the EIR was up to 9.5 infective bites per year [26].

The results underline possible changes in the entomological situation in the Dakar region and suggest the need for larger entomological investigations, in order to assess the current malaria transmission risk in the area.

Breeding sites

Every *Anopheles* species has its preferred water bodies for oviposition, depending on climate, physical geography and human activities. Breeding sites can be natural or man-made, of various sizes, located in running or stagnant waters, shaded or sunny, permanent or temporary.

The main anopheline species found in the Cap-Vert peninsula are members of the *Anopheles gambiae* complex. *Anopheles arabiensis* is the major malaria vector and usually breeds in small, temporary, clear and shallow water, with small amounts of organic matter and surface vegetation [27]. In 2005, the following species were also found in Dakar [26]: *Anopheles melas*, a salt-water species, and one specimen of *An. gambiae s.s*.

In and around Dakar, temporary breeding sites can appear during the rainy season in tyres, step tracks, puddles, ditches and garbage cans, or in debris on construction sites. Anopheline larvae have also been sampled in permanent water collection sites, such as permanent swamps created by the rise of the water table, which are known locally as "niaye" [14], or permanent wells, called "céanes," that usually lack cemented walls and are used for the watering of market-gardens [28,29].

To assess the heterogeneity of malaria transmission risk in Dakar, a clear understanding of current ecological requirements for the persistence of productive breeding habitats is necessary.

Methods

Study site

Dakar (14°40'20" North, 17°25'22" West), the capital city of Senegal, is located in the Cap-Vert peninsula at the westernmost point of Africa. The estimated population was 1,030,594 inhabitants in 2005, amounting to about 20% of the country's population. The population density is 12,233 inhabitants per km^2. The altitude peaks at 104 m above sea level (Mamelles). The study was conducted in ten different areas of downtown Dakar and Pikine, one of its satellite city.

Site selection was done on the basis of a SPOT-5 (*Satellite Pour l'Observation de la Terre*) satellite image (CNES 2006, Distribution Spot Image SA) acquired in October 2006 (Figure 1) and classified using a supervised technique which allowed to affect each pixel of the image to a land cover. Result of this process provided a map of vegetation, water, bare soils and different types of urban areas. Based on this land cover map, the study areas were sampled in order to cover as many different environments as possible, in terms of type of urbanization and presence of vegetation. Each site was delimited on the ground to cover an area of about 200 × 200 m, depending on the technical and logistical limitations presented by the landscape (Figure 1). Geographic coordinates are given for the centre of each study area.

One study site was located in Pikine (14°45'30"N, 17°23'56"W), an underprivileged satellite city of Dakar. About half of the area is covered with marshland (locally called "niaye"), vegetation and market-gardens whose wells (locally called "céanes") are not reinforced with cement. In the remainder of this area, buildings are individual or collective, structured around a network of unpaved sand roads.

The other study sites were located in the city of Dakar. "Almadies" (14°44'42"N, 17°30'38"W) is located in a privileged residential area. About half of the study area is covered with vegetation and a pond. In the other half, houses are big, air-conditioned and surrounded by large private gardens. The primary road network is paved and the secondary one is unpaved.

"Université" (14°41'22"N, 17°27'49"W) is located on the campus of Dakar Université. Most of the area is covered with low vegetation, trees and a pond. The two existing buildings housing student dormitories are about 70 m long and 4–5 stories high and served by paved asphalt pathways.

"Hann Maristes" (14°43'54"N, 17°25'57"W) is mostly covered by a large park with tall trees and a lake. Outside of the park, the recently-built collective high-rise buildings are spaced out and the road network consists of unpaved sand roads.

"Ouest Foire" (14°44'41"N, 17°28'17"W), close to the airport, is half-covered with low-lying vegetation on sandy ground. On the other half of the site, there are individual houses or small collective buildings, new or under construction. The roads are unpaved. The centre of the area is located in a depression.

"Gibraltar" (14°41'3"N, 17°26'41"W) is in a well-urbanized area, the one closest to the city centre, with medium-size collective buildings and asphalt roads. Vegetation is limited to some trees bordering the main roads.

"Yarakh" (14°42'56"N, 17°26'7"W) was in an area consisting of spontaneous dwellings (huts) built near the railroad and surrounded by an industrial neighbourhood.

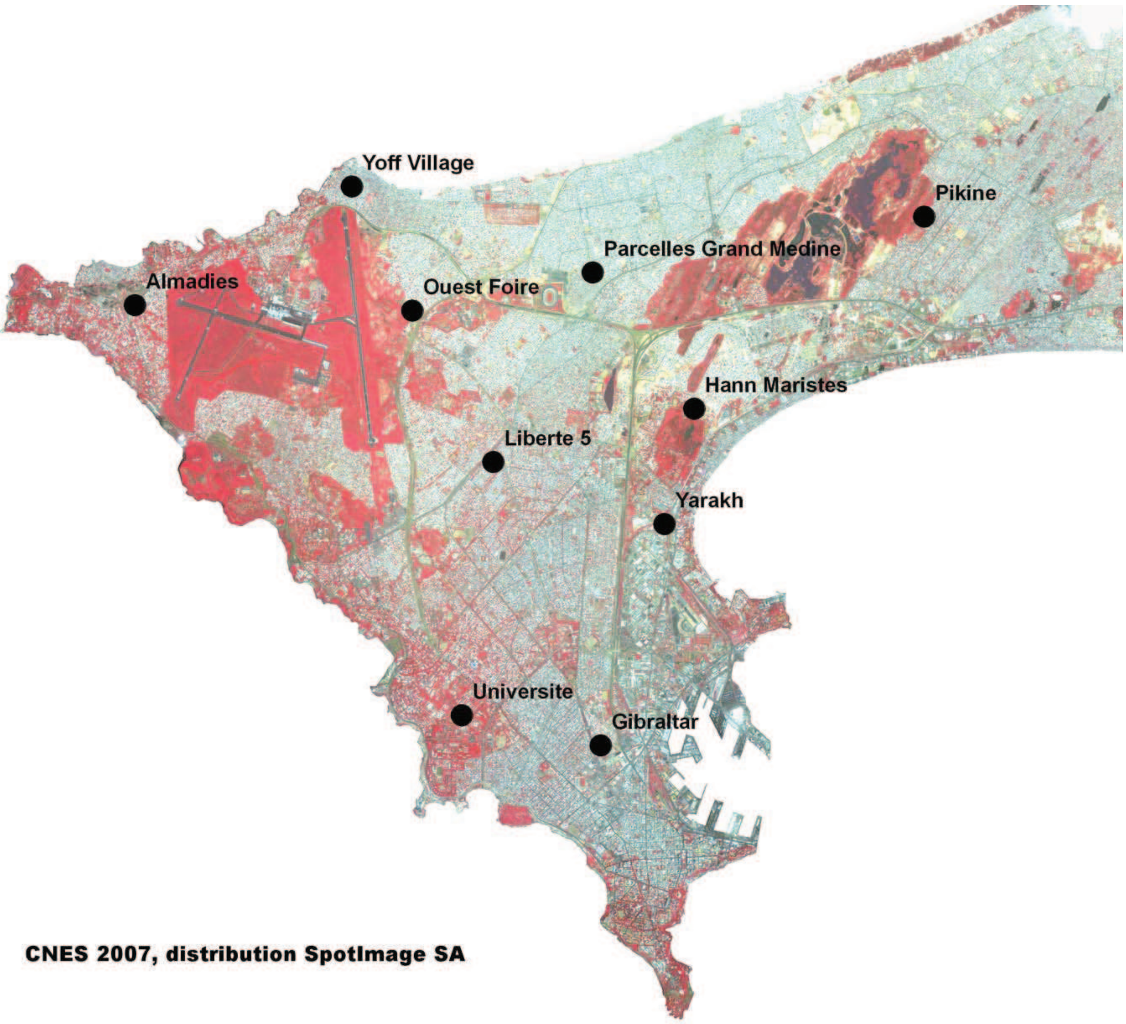


Figure 1
Cap-Vert peninsula and localization of the ten study areas.

About half of the area was composed of market-gardens, watered using either "céanes" or cemented wells.

"Liberté 5" (14°43'23"N, 17°27'36"W) is in a well-urbanized residential area, with individual houses of medium size, sometimes with small private gardens. The road network is asphalted. Vegetation is limited to trees bordering main roads and inside the gardens.

Half of the "Parcelles Grand Medine" site (14°44'58"N, 17°26'42"W) is located in a well-planned, urbanized area built on cleaned-up swamps. Individual and collective buildings have two or three storeys and roads are sandy. The other half of the site is in a crowded area, with very narrow sandy pathways. Individual houses are small, often with cemented yards. Nearly no vegetation was found in this part of the study area.

The "Yoff Village" site (14°45'36"N, 17°28'49"W) is a former fisher village, urbanized with small individual and collective buildings of about two storeys, near the seashore. Roads are narrow and sandy. Nearly no vegetation was found in this study area.

Climate and study period
The Cap-Vert peninsula has a mild sahelian climate. The hot and wet season lasts from June to November, with average temperatures between 24 and 30°C. The cool and dry season lasts from December to May, with average temperatures between 19 and 25°C. The first rains generally occur at the end of June or the beginning of July, and the last ones at the beginning of October. In 2005, 2006 and 2007, the average annual rainfalls were 525, 350 and 248 mm, respectively (data from the Tropical Rainfall Measuring Mission [TRMM] – NASA – http://trmm.gsfc.nasa.gov/

). As a majority of the rain falls in August and September, the study period was chosen to last from September through October 2007, in order to catch the peak of malaria transmission.

Adult mosquito field sampling

Adult mosquito sampling was carried out once every two weeks during the study period. Human landing catch of adult mosquitoes was conducted both indoors (one catching point) and outdoors (two catching points) in each of the ten study areas, for a total of four nights of capture in each place. Indoor captures were conducted with the window or door slightly ajar. The three catching points were located around the centre of each study area. Within each area, distance between each of the three catching point was about 30 meters. Collectors gave prior informed consent and received yellow fever immunizations and anti-malarial chemoprophylaxis consisting of 100 mg doxycycline per day for the duration the study and one month thereafter. Two collectors were contracted for each catching point to work from 8:00 p.m. to 7:00 a.m., with each one resting every two hours. Collectors were rotated among the catching points on different collection nights to minimize sampling bias.

The mosquitoes were recorded by catching point, date and hour of capture and they were sorted by genera.

Biting patterns and sporozoite rates

The heads and thoraces of all adult anopheline females caught on human bait were tested by enzyme-linked immunosorbent assay (ELISA) to detect the presence of *P. falciparum* circumsporozoite protein (CSP) [30].

The human biting rate (HBR), also termed aggressiveness, was expressed as the number of female anopheline bites per person per night, averaged for both outdoor and indoor catching points. The CSP index was calculated as the proportion of mosquitoes positive for CSP. Differences between the CSP indices of the various study areas were tested using the Fisher exact test.

The entomological inoculation rate (EIR) was the product of the HBR and the CSP index of mosquitoes collected on humans. Data on seasonal transmission in sahelian climates [31] and previous results [25,26] indicate that *An. gambiae s.l.* biting activity is compatible with malaria transmission occurring only at the end of the rainy season. Consequently, the annual EIR was considered equivalent to the September-October EIR. Thus, annual EIR was calculated as the product of the EIR multiplied by 60 days.

Adult mosquito species identification

The anopheline mosquitoes were identified morphologically following the Gillies and Coetzee keys [27]. *Culicinae* were identified morphologically following the Edwards keys [32]. All anopheline mosquitoes were stored individually in numbered vials with desiccant and preserved at -20°C in the Medical Entomology Unit of the Institute for Tropical Medicine (IMTSSA), Marseille (France), until processing.

Depending on the number of *Anopheles* caught by site, all specimens or a random sample of a maximum of 100 specimens belonging to the *An. gambiae* complex were selected from each study site for identification to species by polymerase chain reaction (PCR) [33]. All CSP-positive anopheline mosquitoes were also tested. Differences in the distribution of species between the study areas were tested using the Fisher exact test.

Field larval sampling

Each yard within the study areas was searched for open water collection sites. The study areas were visited every week during the eight weeks of the study period, except for the "Université", "Hann Maristes" and "Gibraltar" areas, which were only monitored during the last six weeks of the study period. All the water collections in the ten study areas were examined for larvae. Larvae and pupae were sampled using a standard dipping method [34]. When anopheline specimens were found, larval density was calculated as the number of larvae (all instars) and pupae (further emerged and identified at the laboratory) per dip and recorded for each water collection site. Temporal synchronism in larval density was examined within each area to assess whether the peaks of larval density were synchronized across the breeding sites within each study area. The presence of *Culicinae* larvae was also recorded.

Larval mosquito data analysis

A random sample of the larvae and all the pupae were taken to the laboratory for growth and emergence. The neonate *Anopheles* were identified morphologically following the Gillies and Coetzee keys [27], stored by date and breeding site in numbered vials with desiccant and preserved at -20°C at the Medical Entomology Unit of the Institute for Tropical Medicine (IMTSSA), Marseille (France), until processing.

All anopheline larvae that were collected in study areas where *An. melas* adults had been caught on human bait and that emerged in the laboratory were identified by species following the same PCR protocol.

Characterization of open water collection sites

Physical, biological and chemical characteristics of the open water collection sites were recorded by the same person, in order to maintain consistency in visual classifications.

Habitat type of all bodies of water were categorized as ditches or puddles, swamp areas, marshes, ponds or lakes, "céanes," cemented wells or basins, man-made water collection sites, waterproof containers or canals. A water collection site was considered temporary when it was found to be dry at least once during the follow-up or during one field visit undertaken at the end of the 2008 dry season, before the first rains. Otherwise, it was recorded as permanent.

Identification of predators was limited to all types of larvivorous fishes, such as guppies, *Gambusia* or *Tilapia*, which are larval predators. Larvivorous fishes were introduced in Dakar in the 1930s and their presence in market-garden wells is recommended by the National Hygiene Service. Their presence was assessed visually.

The perimeter of each body of water was measured using a centimetre for small pools of water (perimeter <5 metres) and estimated using the number of strides (gauged at one metre each) for large bodies of water (perimeter ≥ 5 metres). The area of each body of water was evaluated by approximating the shape as a square, a rectangle, a circle or an ellipse.

The temperature of the water was measured with a mercury-in-glass thermometer immerged for 60 seconds.

Turbidity was estimated by using a graduated transparent bottle with black letters written on the bottom. The bottle was filled with water from the collection site and turbidity was evaluated by the graduation that the water reached before the letters were no longer visible. Graduations ranged from 0 to 26 cm, starting from the top of the bottle, so that a higher value indicated greater turbidity.

The proportion of the water surface covered by vegetation was estimated visually. The vegetation was not classified further, but included water lettuce, water lentils and grass.

The proportion of the water surface exposed to sunlight was estimated visually by assessing the proportion of the water surface shadowed at midday.

Because of recent reports of *An. melas* in Dakar [26], salinity was measured for a sub-sample of the observed water collection sites. Two drops of chloroform were added to water samples, which were then transported to the laboratory (Laboratoire des Moyens Analytiques, IRD Bel Air, Dakar) in a cool box containing ice, for analysis with a conductivity meter (Symphony™ SB70C, VWR International®) within hours after their collection.

Statistical analysis

The statistical analyses of the larval collections aimed to identify: 1) the determinants of the presence/absence of anopheline larvae in water collection sites, and 2) the factors associated with the density of anopheline larvae in the breeding sites.

Continuous independent variables were dichotomized at the median. The statistical unit was the weekly measurement of variables for a given water collection site.

In longitudinal studies, some correlation could exist between observations made on the same water collection site. To take into account this interdependence of observations, GEE population-averaged models were used. The within-group correlation structure was chosen as autoregressive of order 1, corresponding to the one-week delay between two observations of the same site.

Similarities could exist between water collection sites in the same study area. Thus, a dummy variable corresponding to the study sites was forced in all univariate and multivariate analyses to take into account the fact that several water collection sites belonged to the same study area.

The presence/absence of larvae in the water collection sites was analysed using a logistic regression model. The larval density by breeding site was analysed using a negative binomial regression model. The dependant variable was the number of larvae per dip minus 1, in order to overcome the exclusion of zero-values and take into account the number of dips sampled at each water collection site.

The variables associated with the presence or the density of larvae with a p-value < 0.25 in univariate analysis were retained for multivariate analysis. A backward stepwise selection procedure was applied in the final model to keep variables with a p-value < 0.05.

Adult densities were estimated at the study site level by the total number of *Anopheles* caught indoors and outdoors during one night of capture. The larval density index at each study site was estimated as follows. The product of the larval density multiplied by the estimated water surface was calculated for each breeding site within one week before the night of mosquito capture. The sum of these products over all the water collection sites was considered as the larval density index for the study area. Correlations between larval density index and adult densities were then examined for each study area. Correlations were also researched between raw larval densities and adult densities and between the estimated water surface and adult densities. All analyses were performed with STATA 9.0 (Stata-Corp LP).

Results

Adult mosquito collection

A total of 19,451 mosquitoes (74.18% *Culex quinquefasciatus*, 15.47% *Culex tritaeniorynchus*, 5.66% *An. gambiae* *s.l.*, 4.22% *Aedes aegypti*, 0.05% *Anopheles pharoensis*) were caught during 120 person-nights of collection on human bait. A total of 1,101 *An. gambiae s.l.* were collected (Table 1).

Biting behaviour of An. gambiae s.l

The total number of *An. gambiae s.l.* caught during the 12 person-nights of collection in each of the study areas ranged from one in Yoff Village to 524 in Almadies (Table 2). Among the 1,101 *An. gambiae s.l.* caught on human collectors during the eight weeks of follow-up in the 10 study areas, 870 were caught outdoors (two catching points for each night of capture) and 231 were caught indoors (one catching point for each night of capture) (Table 2). Using the number of outdoor bites averaged for one catching point only, it has been found that 35% of all bites were received indoors. Considering all ten study areas together, the peak biting time was between 1:00 a.m. and 5:00 a.m. both outdoors (Figure 2a) and indoors (Figure 2b). *Anopheles gambiae s.l.* caught during this time range accounted for 65% and 61% of the total number caught outdoors and indoors, respectively, during the whole night.

Molecular identification of An. gambiae s.l. *caught on human bait*

Depending on the total number of specimens collected in each study area, the random sample selected for molecular identification of species represented 19% to 100% of the total adult *An. gambiae s.l.* caught. Among the 496 specimen tested by PCR, the *An. gambiae* complex population was composed of *An. arabiensis* (94.8%) and *An. melas* (5.2%). The detailed percentages of *An. arabiensis* and *An. melas* per study area are presented in Table 3. Differences among areas were significant (exact Fisher test; $p < 0.001$).

HBR, CSP and EIR

HBR was calculated as the number of *Anopheles* bites received per person per night, taking into account figures for both indoor and outdoor bites, averaged over the eight weeks of follow-up. *An. gambiae s.l.* HBR ranged from 0.1 bites per person per night in Yoff Village to 43.7 in Almadies. The highest recorded HBR was 211 bites per person per night, outdoors at the end of September in Almadies. The highest aggressiveness was recorded in the second half of September (Figure 3), when 42% of all adult *An. gambiae s.l.* were caught.

All of the 1,101 *An. gambiae s.l.* were processed by ELISA for *P. falciparum* antigen detection and seven were found to be positive. None of the *An. melas* were found to be infected with *P. falciparum*. The infected *An. arabiensis*. were caught outdoors in Almadies, Pikine, Ouest Foire and Yarakh, in September or in the first fortnight of October. The mean CSP index was 0.64% (95% CI = 0.19% – 0.96%). No significant differences were found in CSP indices between the study areas (exact Fisher test; $p = 0.790$). Thus, EIR, annual EIR and the calculated period (in days) between two *An. arabiensis* infective bites were calculated using the mean CSP index. Annual EIR ranged from 0 infective bites in Yoff Village to 16.8 in Almadies. HBR and EIR figures for *An. arabiensis* are presented in Table 4. Differences between *An. arabiensis* annual EIR and *An. gambiae s.l.* annual EIR were found mainly for Pikine were it decreased form 7.3 to 5.8 taking *An. arabiensis* HBR only.

Larval sampling

A total of 54 open bodies of water were monitored weekly, during four to eight weeks. Several types of open water collection sites were found: 23 ditches or puddles, seven swamp areas, marshes, ponds or lakes, 16 "céanes," cemented wells or basins, three man-made water collection sites, four waterproof containers and one canal.

Of the water collection sites, 34 (63%) were temporary (23 ditches or puddles, four swamp areas, marshes, ponds or lakes, three man-made water collection sites and four waterproof containers) and 20 (37%) were permanent

Table 1: Distribution by genus and species of adult mosquitoes collected on humans in the ten study areas of Dakar in September-October 2007; there were two outdoor catching points (80 person-nights collection) and one indoor catching point (40 person-nights collection) for each site.

	Outdoors (2 catching points per study site)	Indoors (1 catching point per study site)	% Indoors*	Total	% of total population
Anopheles gambiae s.l.	870	231	35%	1101	5.7%
Anopheles pharoensis	9	1	18%	10	0.05%
Culex quinquefasciatus	9393	5035	52%	14428	74.2%
Culex tritaeniorhynchus	2606	404	24%	3010	15.5%
Aedes aegypti	770	51	12%	821	4.2%
Aedes metallicus	1	0	0%	1	0.01%
Mansonia sp	62	18	37%	80	0.4%
Total	13711	5740	42%	19451	100%

* Percentages of indoor bites were calculated using the number of outdoor bites averaged for one catching point.

Table 2: Distribution of adult *An. gambiae s.l.* collected on humans in two outdoor catching points (8 person-nights collection per study site) and one indoor catching point (4 person-nights collection per study site) in the ten study areas of Dakar in September-October 2007.

Study sites	Outdoors (2 catching points per study site)	Indoors (1 catching point per study site)	Total
Almadies	478	46	524
Pikine	150	78	228
Université	111	27	138
Hann Maristes	35	29	64
Ouest Foire	32	17	49
Gibraltar	24	19	43
Yarakh	31	11	42
Liberté 5	6	2	8
Parcelles Grand Medine	2	2	4
Yoff Village	1	0	1
Total	870	231	1101

(one canal, three swamp areas, marshes, ponds or lakes, 16 "céanes," cemented wells or basins). Most (79%) of 34 temporary collection sites and many (40%) out of 20 permanent collection sites were observed to be habitats for anopheline larvae at least once during follow-up.

More than half (33, 61.1%) of the bodies of water were found to be breeding sites for anophelines on at least one observation during the follow-up period but only six (11.1%) harboured larvae for the whole duration of the follow-up. No breeding habitats were found in Liberté 5 and Yoff Village. In Parcelles Grand Medine, one breeding site was found outside of the 200 × 200 m area. In the other study areas, the number of water collection sites ranged from four to eight. Most of the positive collection sites for mosquitoes were located in Almadies, where every body of water was observed to be a breeding site at least once during follow-up. In breeding habitats, the density of larvae and pupae ranged from 0.05 to 35 per dip (mean = 6.05, 95% CI = 4.85 – 7.25).

No temporal synchronism was observed in the larval density of breeding sites within each area and the peaks in larval density were not synchronized within each study area.

Identification of An. gambiae s.l. *reared from larval samples*

Identification of species by PCR amplification showed that 388 adult *An. gambiae s.l.* specimens reared from larval samples were *An. arabiensis*. No *An. melas* mosquitoes were identified.

Characterization of open water collections

A total of 389 observations of water collection sites were recorded, among which 130 (33.4%) were positive for *Anopheles* in immature stages (*i.e*,. larvae or pupae), 196 (50.4%) were negative and 63 (16.2%) corresponded to a water collection site that had dried up. The percentage of observations in which the water collection site had dried up increased from 0 to 31.5% over the course of follow-up. Only the 326 observations of sites containing water (and not the observations of dried-up sites) were taken into account in the following analysis.

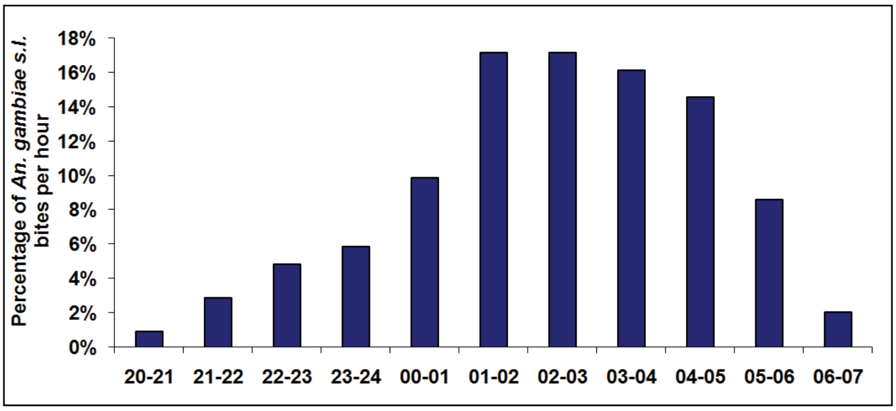
Larvivorous fishes were found in 180 (62%) observations of 42 water collection sites that were negative for larvae and pupae and 112 (48%) observations of 36 water collection sites that were positive. Description of the quantitative physical, biological and chemical parameters recorded for the open bodies of water are presented in Table 5. Based on 99 observations, mean salinity was 1.34 g/l (95% CI = 1.07 – 1.6]) and ranged from 0 to 6.8 g/l.

Determinants of the presence/absence of larvae and larval density

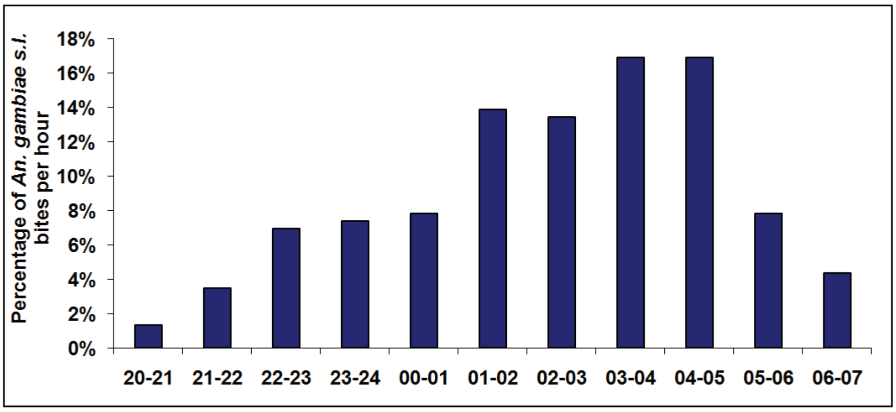
Three water collection sites were observed only once because they dried up after the first week of observation, although all three harboured larvae when they were observed. The 19 observations corresponding to these locations were excluded from the statistical analysis, as the fit of GEE models with an autoregressive correlation matrix required at least two observations of the same water collection site.

Thus, 323 observations with known breeding status and 123 observations of breeding sites with known larval density were considered for the following analysis.

Tables 6 and 7 provide the results of univariate analyses for the presence/absence of *Anopheles* larvae and larval density, respectively. The total number of observations may differ from the one in Table 5 because of the restric-



2a



2b

Figure 2
Hourly distribution of *An. gambiae s.l.* bites outdoors (Figure 2a) and indoors (Figure 2b) in the ten study areas of Dakar in September-October 2007.

tion introduced by the fit of the GEE statistical model. Analyses for the presence/absence of *Anopheles* larvae were completed based on observations for which the larval status was known, even if the larval density was unrecorded. Thus, the total number of observations may differ between Tables 6 and 7.

Table 8 provides the results of the multivariate analyses for the presence/absence of *Anopheles* larvae and for larval densities. Salinity was not included in the multivariate analysis because of the small number of observations for which this parameter was recorded.

Temporary nature, habitat type, perimeter, water temperature, percentage of surface vegetation and co-occurrence of *Culicinae* larvae were significantly associated with the presence/absence of larvae in bivariate analysis (taking into account the study area effect). The only variables remaining in the model after applying the backward stepwise selection were the presence of *Culicinae* larvae, the habitat type and the study area.

Larval density was significantly associated with habitat type, water temperature, percentage of surface vegetation and co-occurrence of *Culicinae* larvae in bivariate analysis (taking into account the study area effect). These four parameters remained significant in the multivariate analysis.

Correlation between larval and adult densities

Figure 4 shows the temporal variations in adult density and larval density index the week before the adult captures, in the study districts for which the larval and adult densities were high enough for comparison. Liberté 5, Parcelles Grand Medine and Yoff Village are not represented, as no breeding sites were found within the 200 × 200 m study area. In Université, Hann Maristes and Gibraltar, only three values are available for larval density

Table 3: Proportions of *An. arabiensis* and *An. melas* among the *An. gambiae s.l.* collected on humans in the ten study areas of Dakar in September-October 2007.

	Number of *An. gambiae s.l.* processed by PCR	Number of *An. arabiensis*	Proportion of *An. arabiensis*	Number of *An. melas*	Proportion of *An. melas*
Almadies	102	102	100%	0	0%
Pikine	99	78	79%	21	21%
Université	93	93	100%	0	0%
Hann Maristes	62	58	94%	4	6%
Ouest Foire	44	44	100%	0	0%
Gibraltar	43	43	100%	0	0%
Yarakh	41	41	100%	0	0%
Liberté 5	7	7	100%	0	0%
Parcelles Grand Medine	4	3	75%	1	25%
Yoff Village	1	1	100%	0	0%
Total	496	470	95%	26	5%

because the follow-up lasted six weeks. In Pikine and Ouest Foire, one value for larval density was missing.

The graphical examination highlights a very close parallel between larval and adult densities in six out of seven study areas. Graphical comparisons of the temporal variations of the adult density with the raw larval density on one hand and the surfaces of the water collections on the other hand did not show this close parallel.

Discussion

Heterogeneity in local malaria transmission

The results provide evidence of malaria transmission in downtown Dakar and its nearby suburb. The rate of infection of the *An. gambiae s.l.* caught on human bait at the end of the 2007 rainy season was 0.64%.

Spatial heterogeneity of human biting rates was very marked, with HBR up to 400 times higher in one area than in other areas located a few kilometres away. HBR ranged from 0.1 bites per person per night in Yoff Village to 43.7 in Almadies. Heterogeneity of the CSP index could not be demonstrated, as no significant differences were found in the rates of infection among the study areas. Nevertheless, the high heterogeneity in anopheline aggressiveness led to a high heterogeneity in the risk of malaria transmission between inhabitants of different study areas. Annual EIR could be calculated using the figures for September and October, as the majority of *Anopheles* bites are received during this period of the year [25,26]. EIR ranged from 0 infective bites per person per year in Yoff Village to 16.8 in Almadies (one infective bite every four days during the transmission season). Thus, in Dakar, no mean figures for transmission would provide a comprehensive picture of the situation; risk evaluations should be conducted on a local scale.

The highest HBR (43.7) and the highest annual EIR (16.8) of the present study were recorded in Almadies. In one of the outdoor catching points, the HBR reached 211 bites

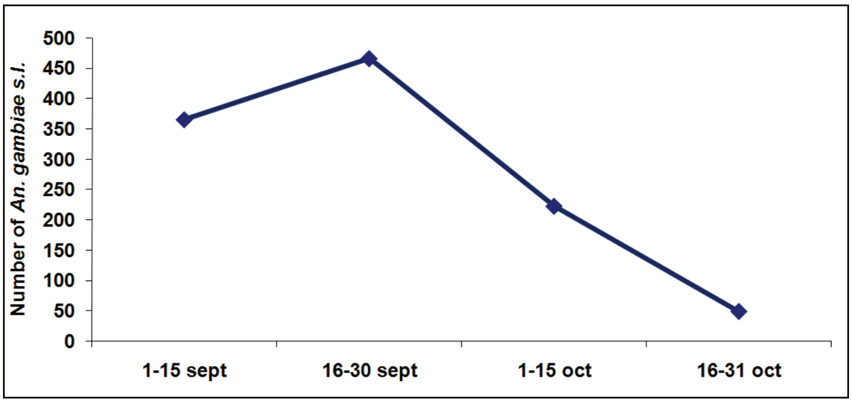


Figure 3
Temporal distribution of adult *An. gambiae s.l.* collected in two catching points outdoors and one catching point indoors in the ten study areas of Dakar in September-October 2007.

Table 4: HBR, EIR, annual EIR and calculated period in days (1/EIR) between two infected bites by *An. Arabiensis* in the ten study areas of Dakar in September-October 2007 (mean CSP index = 0.64%).

Zone	HBR	EIR	Annual EIR	Calculated period (in days) between 2 infected bites
Almadies *	43.7	0.28	16.8	4
Pikine *	15.0	0.10	5.8	10
Université	11.5	0.07	4.4	14
Hann Maristes	5.0	0.03	1.9	31
Ouest Foire *	4.1	0.03	1.6	38
Gibraltar	3.6	0.02	1.4	43
Yarakh *	3.5	0.02	1.3	45
Liberté 5	0.7	0.00	0.3	223
Parcelles Grand Medine	0.2	0.00	0.1	694
Yoff Village	0.1	0.00	0.0	1563

* Study areas in which infected *Anopheles* have been caught.

per person in one night at the end of September. This high HBR could be explained by the large number of productive breeding sites found in this study area, and the high density of larvae in these habitats. The large amount of vegetation in this area could also favour the survival of adult mosquitoes. Furthermore, the population density in the area was low and the type of housing in this privileged residential area meant that most of the population slept in air-conditioned rooms, which could lead to a concentration of bites on the few night watchmen sleeping outdoors.

Annual *An. arabiensis* EIRs in Pikine, Université, Hann Maristes and Ouest Foire ranged from 1.6 to 5.8 and were consistent with the results of the meta-analysis by Robert *et al* [3], which showed that mean annual EIR was 7.1 in sub-Saharan African city centres and that more than two-thirds of the studies reported an EIR <4 infective bites per

Table 5: Description of the quantitative physical, biological and chemical parameters recorded for the open water collection sites in the ten study areas of Dakar, depending on breeding status.

Parameters		Anopheline larvae and pupae absent	Anopheline larvae and pupae present
Perimeter (metres)	n collection sites = 54	43	36
	n observations = 326	196	130
	Range	0.3 – 579.3	0.4 – 1973.6
	Mean and 95% CI	36.3 [26.0–46.7]	148.4 [76.6–220.2]
	25–50–75 percentiles	6.3 – 16.7 – 39.5	9.8 – 29.7 – 75.6
Temperature (°C)	n collection sites = 54	40	34
	n observations = 248	152	96
	Range	25.0 – 39.0	25 – 40
	Mean and 95% CI	29.5 [29.1–29.9]	31.7 [30.9–32.5]
	25–50–75 percentiles	28 – 29 – 30	28 – 31 – 34
Turbidity	n collection sites = 54	35	31
	n observations = 225	135	90
	Range	0 – 26	0 – 26
	Mean and 95% CI	9 [7-10]	16 [14-17]
	25–50–75 percentiles	0 – 4 – 17	8 – 19 – 23
Surface vegetation (%)	n collection sites = 54	43	36
	n observations = 324	194	130
	Range	0 – 100	0 – 90
	Mean and 95% CI	32 [27-36]	18 [13-22]
	25–50–75 percentiles	2 – 10 – 60	0 – 5 – 20
Sunlight (%)	n collection sites = 54	42	34
	n observations = 319	193	126
	Range	0 – 100	0 – 100
	Mean and 95% CI	63 [59–67]	69 [64–74]
	25–50–75 percentiles	50 – 70 – 95	50 – 70 – 100

Table 6: Factors related to the presence/absence of anopheline larvae.

Habitat characteristics	Number of obs.	Number of obs. positive for anopheline larvae	% obs. anopheles positive	OR	95% CI	p-value
Zone						**< 0.001**
Yarakh	92	3	3%	1		
Parcelles Grand Medine *	7	1	14%	2.51	0.06 – 97.52	**0.623**
Gibraltar	4	1	25%	7.71	0.34 – 173.14	**0.198**
Pikine	91	29	32%	11.83	2.31 – 60.62	**0.003**
Ouest Foire	36	23	64%	41.99	6.96 – 253.38	**<0.001**
Université	21	15	71%	65.20	8.70 – 488.74	**<0.001**
Hann Maristes	23	18	78%	68.69	9.11 – 517.73	**<0.001**
Almadies	49	38	78%	95.02	15.33 – 589.07	**<0.001**
Liberté 5	0	0	0%	-		
Yoff Village	0	0	0%	-		
Habitat type						**<0.001**
"Céanes", cemented weels or basins	128	5	4%	1		
Man-made water collections	18	5	28%	2.45	0.29 – 20.83	0.412
Canals	8	4	50%	3.02	0.21 – 43.62	0.417
Waterproof containers	15	6	40%	4.43	0.48 – 40.62	0.188
Swamp areas, marshes, ponds or lakes	44	24	55%	7.82	1.40 – 43.73	0.019
Ditches or puddles	110	84	76%	24.82	4.63 – 130.82	<0.001
Period						**0.793**
Weeks 1 – 4	162	62	38%	1		
Weeks 5 – 8	161	66	41%	1.09	0.58 – 2.03	
Temporary collection						**0.058**
No	155	22	14%	1		
Yes	168	106	63%	2.67	0.97 – 7.37	
Perimeter (metres)						**0.168**
<20 m	157	54	34%	1		
>= 20 m	166	74	45%	0.59	0.27 – 1.25	
Water temperature (n = 243)						**0.077**
<30°C	120	31	26%	1		
>= 30°C	123	62	50%	1.96	0.93 – 4.13	
Turbidity (n = 222)						**0.808**
<13 (clear)	106	26	25%	1		
>= 13 (turbid)	116	62	53%	1.09	0.54 – 2.21	
Surface vegetation (%, n = 320)						**0.078**
<20%	195	93	48%	1		
>= 20%	125	35	28%	0.44	0.18 – 1.10	
Sunlight (%, n = 317)						**0.974**
<80%	202	73	36%	1		
>= 80%	115	52	45%	0.99	0.40 – 2.45	
Presence of predator (n = 287)						**0.641**
No	132	55	42%	1		
Yes	155	54	35%	0.84	0.41 – 1.74	
Presence of *Culicinae* larvae (n = 293)						**<0.001**
No	211	48	23%	1		
Yes	84	58	69%	5.46	2.55 – 11.66	

Adjusted (by study area) odds ratio (OR) estimated by GEE logistic regression model. Bivariate analysis: 323 observations of water collection sites unless otherwise indicated.
* Water collections found outside of the 200 × 200 m study area

year. In those areas, figures were also consistent with measurements conducted in two areas of Dakar in 2005–2006, which found EIRs of 3 to 9.5 infective bites per person per year [26]. Breeding habitats were found in these four areas and the large amount of vegetation may have favoured adult mosquito survival.

In Gibraltar, the annual EIR was 1.4 but none of the small water collection sites of the area provided a breeding habitat for more than one week during follow-up. Moreover, Gibraltar is a well-urbanized area with very little vegetation, which is probably not favourable for adult mosquito longevity. Extended investigations are needed to detect the breeding sites of the adult *Anopheles* caught on human bait in this area.

The annual EIR was 1.3 in Yarakh, but few breeding sites were located within the study area. The lack of a parallel between adult aggressiveness and the larval densities in this setting also suggests that the adult mosquitoes caught in this study area had their breeding site in the neighbourhood. The large amount of vegetation in the area also probably favoured the survival of adult mosquitoes.

In Liberté 5, Parcelles Grand Medine and Yoff Village, annual EIRs were below one infective bite per person per year. No infected specimens where caught in these areas, but even when applying the mean CSP index, the EIR was not compatible with transmission due to the low aggressiveness. Although no breeding sites were detected in Liberté 5, HBR was not nil. This could be explained by the presence of trees bordering the main roads and inside the gardens, which could constitute resting sites for adult *Anopheles* coming from sources located outside the study perimeter.

In Parcelles Grand Medine and Yoff Village, the EIR was close to zero. No breeding sites were detected in the 200 × 200 m area and vegetation was very sparse, consistent with the very small number of specimens caught on human bait.

In an ecological point of view, areas where the presence of vegetation was important showed the highest vector densities (ex: Almadies, Pikine), favouring the presence of larval habitats and probably of resting places. On the contrary, areas where percentage of urbanization was high showed a lower number of adult *Anopheles*. Type of soil also had an importance as sand and asphalt (ex: Parcelles Grand Medine and Yoff Village) did not favour persistence of water, compared to mud and swamp areas (ex: Almadies and Yoff).

Among the ten study areas, four (Université, Liberté 5, Gibraltar, Yarakh) were located close to the areas studied by Diallo *et al* in 1994–95 and 1996–97 [17,25]. The HBRs measured in the present study were higher than those recorded during those past studies. This difference must be interpreted with caution, as the sites of mosquito captures were not exactly identical. Furthermore, the peak of *An. gambiae s.l.* aggressiveness lasts for little longer than one month, with a monthly frequency of capture, the previous studies could have missed this peak. The previously published results did not show any infected *Anopheles*, whereas the present study shows that transmission exists.

Transmission was demonstrated in Pikine 30 years ago [14,24] and the present study provides new evidence of local transmission (annual EIR = 5.8) at an intermediate level compared to the results published in 1979–80 (annual EIR = 43) and 1987–88 (annual EIR = 0.38).

Malaria transmission has not been recently studied in the other study areas of the project, so the present study provides new entomological data for Dakar's districts.

Endo/exophagic behaviour

Important differences existed between the study areas in terms of the endo/exophagic behaviour of *An. gambiae s.l.* but results should be interpreted carefully. Large differences were recorded between outdoor catching sites in the same study area during the same night. As logistical constraints limited the number of indoor catching points to one per site, the experimental design probably did not allow for results representative of adult *An. gambiae s.l.* behaviour in each study area. Using more outdoor and indoor catching points would allow for representative estimates of HBRs in each study site.

Anopheline species

Among the total female mosquitoes caught on human bait, 5.71% were *Anopheles* (5.66% *An. gambiae s.l.*, and 0.05% *An. pharoensis*). This percentage was higher than that recorded in 1994–95 in the south district of Dakar, where the percentage of *Anopheles* was 0.7% [25], and in 1996–97 in the central district, where this proportion was 1.5% [17]. In Pikine in 1987–88, this percentage rose to more than 20% when collections were done on human bait [14].

In the present study, *An. arabiensis* and *An. melas* were the only representatives of the *An. gambiae* complex caught on human bait. No *An. gambiae s.s.* was found, as had been the case in Dakar in 2005–2006 [26]. *Anopheles arabiensis* was the main species identified during September and October 2007 and it accounted for 94.2% of the *An. gambiae s.l.* caught. *Plasmodium falciparum* infection was detected only in *An. arabiensis* specimens. The predominance of *An. arabiensis* was consistent with previous results from the Cap-Vert peninsula [14,24,25,35].

Table 7: Factors related to anopheline larval density.

Habitat characteristics	Number of obs.	Number of dips	Total number of anopheline larvae	Anopheline larval density (per dip)	RR	95%CI	p-value
Zone							**0.028**
Hann Maristes	18	209	208	1.00	ref.		
Pikine	27	140	357	2.55	1.66	0.77 – 3.60	**0.198**
Université	14	54	246	4.56	2.23	0.91 – 5.46	**0.080**
Yarakh	2	18	110	6.11	2.28	0.41 – 12.76	**0.348**
Ouest Foire	22	93	578	6.22	3.09	1.38 – 6.95	**0.006**
Almadies	38	179	1309	7.31	4.09	1.98 – 8.45	**<0.001**
Gibraltar	2	9	160	17.78	8.10	1.48 – 44.39	**0.016**
Parcelles Grand Medine *	0	-	-	-			
Liberté 5	0	-	-	-			
Yoff Village	0	-	-	-			
Habitat type							**<0.001**
"Céanes", cemented weels or basins	8	53	126	2.38	ref.		
Waterproof containers	5	17	30	1.76	2.56	0.45 – 14.72	0.291
Swamp areas, marshes, ponds or lakes	22	116	467	4.03	14.76	3.76 – 57.88	<0.001
Ditches or puddles	80	488	2137	4.38	15.96	4.44 – 57.36	<0.001
Man-made water collections	4	16	82	5.13	17.45	2.62 – 116.14	0.003
Canals	4	12	126	10.50	22.50	3.90 – 129.71	<0.001
Period							**0.762**
Weeks 1 – 4	57	328	1775	5.41	ref.		
Weeks 5 – 8	66	374	1193	3.19	0.94	0.63 – 1.40	
Temporary collection							**0.292**
No	23	134	379	2.83	ref.		
Yes	100	568	2589	4.56	1.44	0.73 – 2.85	
Perimeter (metres)							**0.755**
<20 m	49	226	1078	4.77	ref.		
>= 20 m	74	476	1890	3.97	0.93	0.59 – 1.46	
Water temperature (n = 93)							**<0.001**
<30°C	33	228	475	2.08	ref.		
>= 30°C	60	307	1611	5.25	3.34	1.97 – 5.68	
Turbidity (n = 88)							**0.592**
<13 (clear)	28	142	700	4.93	ref.		
>= 13 (turbid)	60	392	1261	3.22	0.87	0.52 – 1.46	
Surface vegetation (%)							**0.001**
<20%	89	504	2596	5.15	ref.		
>= 20%	34	198	372	1.88	0.41	0.24 – 0.70	
Sunlight (%, n = 120)							**0.674**
<80%	72	447	1194	2.67	ref.		
>= 80%	48	244	1665	6.82	1.13	0.64 – 2.00	

Table 7: Factors related to anopheline larval density. *(Continued)*

Presence of predator(n = 104)							**0.865**
No	48	308	1326	4.31	ref.		
Yes	56	286	1245	4.35	0.96	0.59 – 1.56	
Presence of *Culicinae* larvae (n = 106)							**0.016**
No	51	291	1259	4.33	ref.		
Yes	55	321	1033	3.22	1.71	1.10 – 2.64	

Adjusted (by study area) risk ratio (RR) estimated by GEE negative binomial regression model. Bivariate analysis; 123 observations of anopheline breeding sites, unless otherwise indicated.
* Water collections found outside of the 200 × 200 m study area

In 1979–80, in Pikine, one *An. melas* was caught out of 92 *An. gambiae s.l.* adult females [24]. *Anopheles melas* was then captured for the first time in downtown Dakar in 2005–2006 and accounted for 2% of the *An. gambiae s.l.* population [26]. In the present study, the percentage of *An. melas* was 5.2% on average, and the peak was greater than 20% in Pikine. As it is known that *An. melas* is not as good a vector as *An. arabiensis* [36], the relationship between aggressiveness and related malaria transmission risk must be interpreted carefully. This relationship will depend on the proportion of *An. melas* and also on the geographic areas. In the present study area of Pikine, the annual EIR calculated on *An. gambiae s.l.* aggressiveness was 7.3 infective bites, but the EIR calculated on *An. arabiensis* aggressiveness was only 5.8 infective bites.

In the breeding habitats, classification down to the species level of the sampled anopheline larvae identified only *An. arabiensis*. The places where *An. melas* were caught were those closest to the large marshy area locally called "niaye." In this area close to the Atlantic Ocean, the water table is high. In the eighties, Vercruysse *et al* [24] noted that this water table in Pikine was not brackish and that the first salty water collections were observed more than 10 km away from the study's capture points. Thirty years later, the situation could have changed so that the water table now provides habitats for *An. melas* larvae.

However, no *An. melas* larvae were found in the breeding sites we surveyed. There is no report in the literature of *An. melas* larval habitats in Dakar or Pikine. Tolerance of *An. melas* larvae for salinity ranges from 5 to 37 g/l [37]. The maximum salinity recorded in the present study was 6.8 g/l, which would have been favourable for *An. melas* breeding. Further investigations are needed to detect larval habitats that might have been located outside of the present study areas.

Anopheles pharoensis accounted for only 0.05% of the total anopheline population and were captured mainly in Hann Maristes. *Anopheles pharoensis* had comprised 4% of the adult anopheline population in Pikine in 1979–80 [24] but only one specimen was caught in the present study in 2007, perhaps indicating a change in the mosquito population. Even though *An. pharoensis* has been suggested as a significant vector in the Senegal River basin [38], it is generally not of epidemiological significance as a malaria vector in Senegal [39]. The low density measured in the present study confirms this fact in the capital.

Anopheles ziemanni was previously caught on human bait in Senegal, but in very low numbers [40]. In the present study, no adult specimens were caught on human bait. *Anopheles ziemanni* larvae have already been reported in Pikine [24] and accounted for 14% of the sampled immature stage mosquitoes in the céanes of Dakar [28]. In the present study, no *An. ziemanni* was found among the sampled anopheline larvae. The population of *An. ziemanni* could have changed but the malaria risk would not be affected, as the human blood index of this species'is usually low and it is known only as a secondary or incidental vector [27].

Larval habitats

Among the 54 water collection sites monitored during the present study, several factors were found to be associated with the occurrence and abundance of anopheline larvae. The presence of larvae and the larval density were strongly associated with the study areas, demonstrating high spatial heterogeneity. This was consistent with the selection of the study areas, which aimed to cover maximal ecological diversity. Thus, adjusting further statistical models for the study area would make it possible to take into account factors that were not measured in the water collection sites but that could be related to their geographical localization.

In bivariate analysis, a higher probability of presence of anopheline larvae was found for water collection sites that were temporary and those with perimeter <20 m, with surface vegetation covering less than 20% of the total area, with a temperature >= 30°C, or with a co-occurrence of *Culicinae* larvae. Anopheline larvae were also mainly found in ditches, puddles (all of them being temporary) and swamps, ponds or lakes (about half of them being temporary), highlighting the importance of temporary

Table 8: Factors related to the presence/absence of larvae and larval densities, adjusted by study area.

Presence/absence of anopheline larvae (n = 293), GEE logistic regression model			
Habitat characteristics	Odds Ratio	95% CI	p-value
			<0.001
Study areas			
Parcelles Grand Medine	1		
Pikine	1.96	0.08 – 49.21	0.682
Yarakh	2.10	0.05 – 93.07	0.702
Hann Maristes	9.19	0.35 – 238.37	0.182
Ouest Foire	13.88	0.51 – 378.05	0.119
Almadies	35.56	1.47 – 862.54	0.028
Université	46.69	1.50 – 1449.39	0.028
Habitat type			
Man-made water collections	1		
Waterproof containers	1.51	0.09 – 24.72	0.771
Canals	1.53	0.06 – 41.92	0.801
"Céanes", cemented weels or basins	3.65	0.23 – 58.63	0.360
Swamp areas, marshes, ponds or lakes	10.14	1.14 – 90.28	0.038
Ditches or puddles	33.47	4.05 – 276.48	0.001
Presence of Culicinae larvae			<0.001
No	1		
Yes	8.24	3.26 – 20.86	
Larval density (n = 86), GEE binomial regression model			
Habitat characteristics	Risk Ratio	95% CI	p-value
			<0.001
Study areas			
Hann Maristes	ref.		
Université	1.15	0.29 – 4.54	0.838
Pikine	2.00	0.86 – 4.66	0.107
Ouest Foire	5.03	2.09 – 12.53	0.001
Almadies	5.48	2.37 – 12.68	<0.001
Yarakh	10.98	1.46 – 82.75	0.020
Habitat type			
Waterproof containers	ref.		
Canals	3.78	0.61 – 23.53	0.155
"Céanes", cemented weels or basins	9.53	1.08 – 83.91	0.042
Ditches or puddles	10.82	2.83 – 41.41	0.001
Swamp areas, marshes, ponds or lakes	23.29	5.16 – 105.04	<0.001
Man-made water collections	48.16	4.55 – 509.71	0.001
Water temperature			<0.001
<30°C	ref.		
>= 30°C	3.13	1.67 – 5.84	
Surface vegetation (%)			0.007
<20%	ref.		
>= 20%	0.36	0.17 – 0.75	
Presence of *Culicinae* larvae			0.019
No	ref.		
Yes	2.29	1.14 – 4.60	

Multivariate analysis with GEE logistic regression model or negative binomial regression model.

water collections in larval presence. In multivariate analysis, habitat type and presence of *Culicinae* larvae remained significant.

Higher larval densities were associated with water temperature >= 30°C, surface vegetation covering less than 20% of the total surface area, co-occurrence of *Culicinae* larvae and habitat type. All four parameters remained significant in multivariate analysis.

These results are consistent with the known preference of An. *gambiae s.l.* for breeding in temporary pools [27,41]. Co-occurrence of *Anopheles* and *Culicinae* larvae was previously reported in the literature [11]. Low-floating vegetation was also previously found as a determinant of the presence of anopheline larvae [11,42].

The other physico-chemical parameters measured in the water collection sites were not significantly associated either with the occurrence or the abundance of larvae. Concerning turbidity, conflicting results were found in previous studies. Higher turbidity has been associated positively [42] or negatively [7] with the presence of anopheline larvae. In Dakar, Robert *et al.* found a prefer-

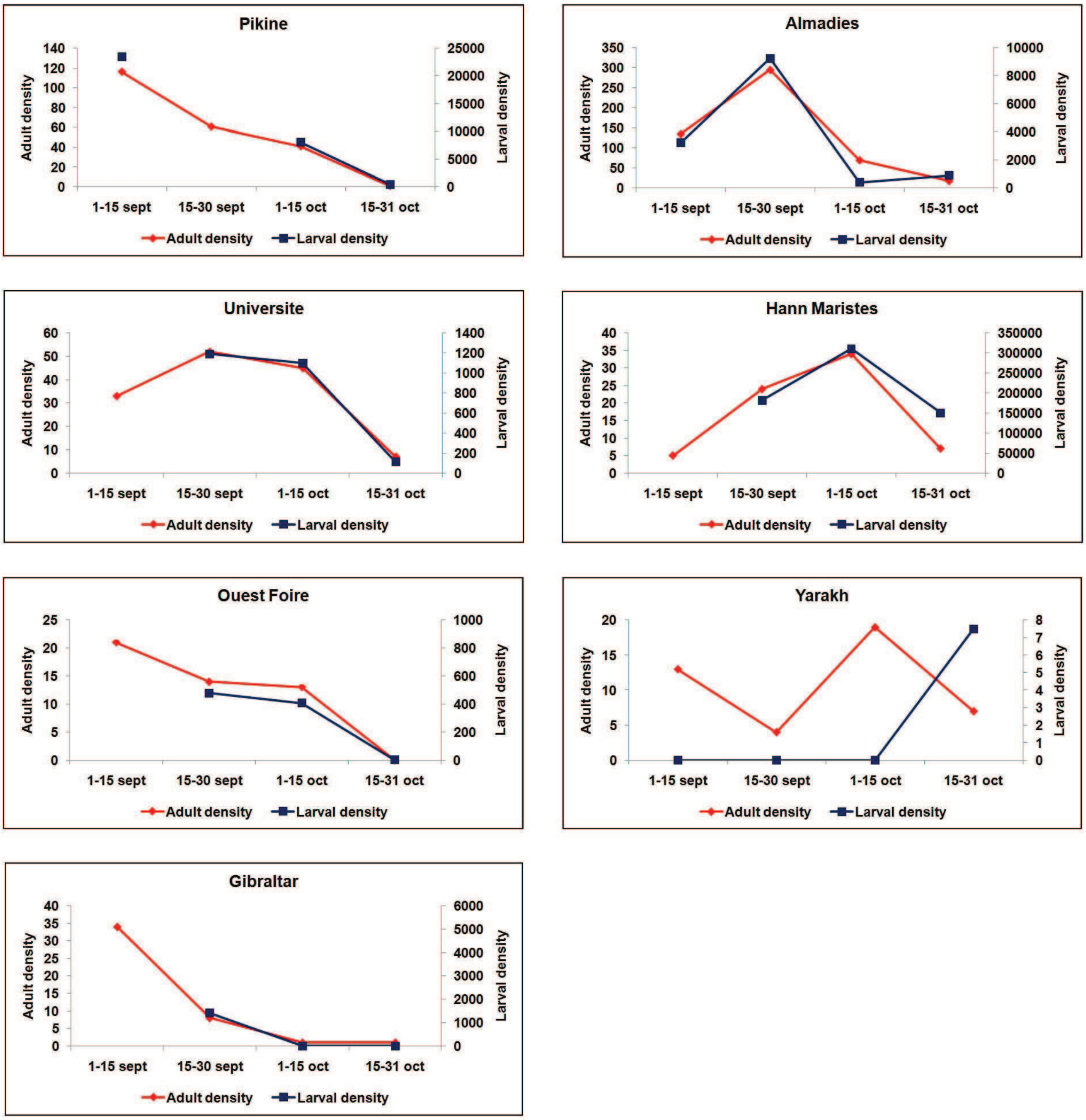


Figure 4
Graphical representation of *An. gambiae s.l.* aggressiveness (left scale, total number of Anopheles caught indoors and outdoors) and larval densities observed one week before adult catch and ponderated by the breeding habitats surface (right scale, larval density × surface water for all the breeding sites), for seven out of ten study areas of Dakar in September-October 2007.

ence for breeding in clear water in the "céanes" [28]. As turbidity can be an indicator of particulate matter in suspension that could be food for larvae or polluting agents, its effect remains unclear. Even though water collection sites exposed to sunlight are known to provide breeding places for *An. gambiae s.l.* [27,41], the present study could not show any association with this parameter, probably because of a lack of contrast (*i.e.*, very few water collection sites with no sunlight exposure) and the consequent lack of analysis power.

The presence of predator fish was not associated either with a lower probability of larvae or lower larval density. Finally, the period of prospection was associated neither with the presence of larvae nor with larval density.

One possible limitation of the data interpretation may have been the use of the dipping method, which could have failed to provide repetitive measures of larval densities even though the sampling location and the person collecting the samples were consistent throughout the study. The presence/absence of larvae was probably recorded correctly with this technique, especially for small habitats.

Adaptation to urban settings

The data recorded from water collection sites did not aim to measure pollution but anopheline larvae were sampled in bodies of water that, based on visual examination, appeared polluted. In urbanized environments such as the ones in the present study areas, it cannot be excluded that *Anopheles* can adapt to new conditions, as was previously shown in some studies. In Accra, *An. gambiae s.l.* evolution over a few decades led to a rise in breeding in domestic water and polluted water [6]. In Dar Es Salaam, *An. gambiae s.l.* bred in organically polluted habitats [7]. *Anopheles gambiae s.s.* larvae have been found in water polluted with heavy metals and oil in Lagos [8].

Urban agriculture

The importance of urban agricultural activity on malaria has been reported in several African cities, such as in Côte d'Ivoire and Ghana [10], where irrigation led to the emergence of larval habitats [10,11] and higher malaria prevalence [12,13]. It has been suggested that irrigated vegetable fields around a French military camp in Abidjan could have been the source of the unexpectedly high number of adult *Anopheles* caught there [43]. In other cities such as Malindi in Kenya, no relationship has been found between household-level urban agriculture and the occurrence of bodies of water [44].

Two of the areas in the present study (Pikine and Yarakh) sustained urban agricultural activities, but no irrigation systems were in place. Watering was done manually every morning, with water coming from the "céanes." In both areas, infected adult *Anopheles* were caught on human bait. This was consistent with results in 2005–2006 where infected *Anopheles* have been caught in Dakar (district of Ouakam), close to market gardens [26].

Among the studied "céanes," only three (37%) harboured larvae and the larval densities were very low. These results were consistent with those reported by Robert *et al* in 1998, in which only 33% of 48 "céanes" harboured anopheline larvae, with low densities [28]. Even though the presence of mosquito-eating fishes was not significantly associated with the presence/absence of larvae and larval density in the statistical analysis, these low larval densities could be partly linked to the systematic presence of larvivorous fishes, only allowing larvae to grow if they are hidden in the floating vegetation. These fishes were introduced in Dakar in the 1930s for larval control and their presence in the wells is recommended by the National Hygiene Service. In 1998, Awono-Ambéné *et al* [29] confirmed their utility, indicating that predation in the "céanes" was probably mainly due to fishes (*Gambusia* and *Tilapia*). Other factors which were not measured in this study, such as the use of pesticides, could also lead to low larval density in the "céanes". Furthermore, it is possible that in the rainy season temporary breeding sites were more attractive for breeding than the "céanes," as higher larval densities in the céanes were recorded at the end of the dry season but not during the rainy season [29].

In Pikine, some temporary and permanent breeding sites which were not linked to market-gardening were present, so it was difficult to measure the link between the agricultural activity and the adult *Anopheles* density. In contrast, water collections were highly related to urban agriculture in Yarakh but larval densities were low as previously described in Dakar [28], so it is probable that market-gardens provided resting sites to *Anopheles* rather than increased number of breeding sites, as was previously demonstrated in Ghana [45].

Spatial scale of malaria transmission

In the present study, a very close parallel was found between larval density index and adult densities. In six out of ten areas, it was possible to superimpose temporal variations in larval and adult densities. This correlation is consistent with the hypothesis of a malaria transmission system that is contained within the limits of the study areas. The larval habitats available in each area could be sources of adult mosquitoes, or at least were representative of the habitats available in a larger area providing the adult specimens caught on human bait. The parallel between larval density index and adult densities was not found by taking into account all areas together, as no information on population and building densities were available to balance the associations.

The present results were consistent with the low dispersion (<300 metres) of *Anopheles* from their breeding habitats. In rural areas, dispersion can reach several kilometres, and it is highly reduced in urban settings, due to the high density of houses and the proximity of readily available hosts for blood meals. Several studies have highlighted the low dispersion of *Anopheles* in urban settings, its consequences in terms of heterogeneity of malaria transmission levels and its implications for the incidence of clinical malaria. In Pikine, a gradient of *Anopheles* density and malaria prevalence was shown on a 910-metre transect, going from the marshland to the city centre. Most of the *An. arabiensis* were caught at <285 m from the marshland [14].

Similar examples also exist in other African cities. In Ouagadougou, Burkina-Faso, most of the *An. gambiae s.l.* females were collected within 300 m of the breeding sites located along a water reservoir [46] and the *P. falciparum* infections where concentrated in the human population living within 200 m of the hydrographic network [47]. In two cities in Cameroon, *Anopheles* densities recorded on hilly slopes (around 40 m high) were zero at 200 and 250 m from the swampy valleys where the breeding sites are concentrated [48]. In Edea, also in Cameroon, the EIR varied from 0 to 86 infective bites per person per year between houses within 200 m of each other, depending on their proximity to the breeding sites [49]. In Brazzaville, Congo, great heterogeneity of transmission was recorded between districts, ranging from more than 100 infective bites per person per year to less than one infective bite per person every three years [50]. Maps of malaria transmission intensity in Brazzaville showed that districts with very different malaria risk levels could be adjacent to each other [51]. In Uganda, proximity to the breeding habitats has been recognized as a risk factor for clinical malaria episodes at scales of a few hundred metres [15].

The method used in the present study for the measurement of larval densities accounted for all larval stages including pupae. The actual productivity of the breeding sites would have been better estimated by accounting for stage IV and pupae only, as the dynamics of larval mortality could differ depending on the type of breeding habitat [52]. To obtain such estimates, the larval sampling effort should be much greater. However, this is not a limitation in the present study, as the larval and adult densities were compared temporally within each study area. Since the figures were relative and not absolute, the comparisons were valid.

Temporal dynamics of malaria transmission

A very close parallel was found between the adult density and the larval density index but not between the adult density and the raw larval density if the latest was not adjusted on breeding sites surfaces. As water collection surfaces are driven by rainfall amounts and frequency of rainfall events, relationship between meteorological data and *Anopheles* larval and adult densities should be further investigated.

Implications for malaria control

Since the beginning of the 21st century, there has been renewed interest in larval control as part of an integrated malaria control strategy (including ITNs, indoors residual insecticide spraying and health care access) [53]. In urban settings, the human population density relative to the number of breeding sites is very high, so larval control could be effective [54].

Focusing on larval control in African urban areas could lead to satisfactory results, as was the case in Palestine/Israel, Italy and the United States, where the modification or elimination of aquatic habitats was applied extensively and contributed significantly to the eradication of malaria transmission, especially in urban settings [55]. Regarding the *An. gambiae* complex, several reports of successful control efforts have been reported. In Ethiopia, environmental management led to a 49% reduction in *An. arabiensis* adult density [56]. In the city of Dar Es Salaam, the killing of larvae succeeded in significantly reducing malaria transmission and morbidity [57]. In Djibouti City, malaria has been controlled by larval control using larvivorous fishes [58]. In Brazil, *An. arabiensis* invaded the North-East region in the thirties and led to a ten-year malaria epidemic [59]. Focus on larval control made it possible to eradicate the vector and the disease [60] from the area while the proportion of temporary breeding sites was high, just as it is in Dakar.

In areas where transmission is low or moderate, focusing malaria control activities in limited areas should greatly improve their efficacy and their cost-effectiveness [16]. Thus, a good knowledge of mosquito dynamics and of the ecological requirements leading to the presence of breeding sites is crucial. A deep understanding could even help to target larval control to the most productive habitats, thus enhancing the efficacy of control [61]. The present study has shown that transmission in Dakar is highly focal, at scales of a few hundred metres, and that there is a high degree of correlation between larval and adult densities within each area. Furthermore, transmission is temporally focal and lasts only a few weeks. Even if productive habitats in Dakar show a great variety, they could be spatially and temporally identified; in this context, malaria control in Dakar could benefit from larval management.

Conclusion

In Dakar, malaria transmission exists and is highly focal. In order to spatially focus malaria control in the areas that are at greater risk, precise mapping of malaria transmission levels should be conducted. With new technologies such as Geographic Information Systems and Remote Sensing, associated with entomological and epidemiological work, the possibility of mapping the malaria risk in Dakar exists and should be further explored.

Competing interests
The authors declare that they have no competing interests.

Authors' contributions
VM was responsible for the study design, supervision of data collection, analysis, interpretation, production of the final manuscript and revisions. LG contributed to the supervision of data collection, data analysis, interpretation and production of the final manuscript. CV contributed to the study design, analysis and interpretation. FJ contributed to the data analysis. SB contributed to the data analysis. JPL contributed to overall scientific management, analysis, interpretation, preparation of the final manuscript and revisions. JFT contributed to overall scientific management, analysis, interpretation, preparation of the final manuscript and revisions. CR was responsible for overall scientific management, analysis, interpretation, preparation of the final manuscript and revisions. FP was responsible for overall scientific management, analysis, interpretation, preparation of the final manuscript and revisions. All authors read and approved the final manuscript.

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ARTICLE 4

Conditions of malaria transmission in Dakar from 2007 to 2010

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En préparation

Conditions of malaria transmission in Dakar from 2007 to 2010

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Abstract

Background

Previous entomological studies in some areas of Dakar have highlighted the spatial heterogeneity of *Anopheles gambiae s.l.* biting rates according to the distribution of the breeding sites. In order to improve the knowledge of the determinants of malaria transmission in this city, the present study reports the results of an extensive entomological survey conducted in 45 areas in Dakar and its suburbs from 2007 to 2010.

Methods

Open water collections in the studied areas were monitored weekly for physico-chemical characterization and the presence of anopheline larvae was researched. Mosquitoes sampled by human landing collection were identified morphologically and by molecular methods. The *Plasmodium falciparum* circumsporozoïte (CSP) indexes were measured by ELISA, and the entomological inoculation rates (EIR) were calculated for the 45 areas. Adult mosquitoes and hatched larvae were identified morphologically and by molecular methods.

Results

Factors playing a role in the presence or absence of larvae and on the density of larvae in water collections were identified, such as soil temperature, vegetation and trees, soil moisture, type and density of urbanization, temporary or permanent collections. A total of 496,310 adult mosquitoes were caught among which, 44 967 were *Anopheles gambiae s.l.* According to the studied areas, *An. gambiae s.l.* human biting rate ranged from 0.1 bites per person per night to 248.9 during the rainy seasons (September-October). The annual CSP index was 0.64% in 2007, 0.09% in 2008-2009 and 0.12% in 2009-2010. The averaged EIR ranged from 0 to 17.6 infected bites per person during the transmission season, according to the studied areas. Three members of the *An. gambiae* complex were present: *An. arabiensis* (93.14%), *An. melas* (6.83%) and *An. gambiae s.s.* form M (0.03%). *An. arabiensis* and *An. melas* were found CSP positive in Dakar.

Conclusion

The spatial and temporal heterogeneity of *An. gambiae s.l.* larval density, adult aggressiveness and malaria transmission in Dakar has been confirmed, and environmental factors associated

with this heterogeneity have been identified. The results of the present study pave the way to the building of malaria risk maps and to a focused anti-vectorial malaria control strategy in the city of Dakar.

Introduction

Urban malaria is considered as an emerging problem in Africa as the populations of most of African big cities are growing exponentially since 30 years [1]. In 2003, 39% of the African population lived in urban settings; by 2030, 54% are expected to do so [2, 3].

In African cities, urban malaria is considered to be a focal disease and transmission appears in areas or places that are favourable for malaria vectors [4-7]. In urban settings, malaria risk heterogeneity is recorded over small distances due to diversity in degrees and types of urbanization, density of human population, quality of water and waste management, vector control measures, household factors and access to health care [8, 9] or human migration patterns which could import parasites from rural areas [10]. The occurrence of malaria in African towns has also been linked to agricultural practices [11-14], to the distance from breeding sites [15-19] and to the vegetation cover [19]. A meta-analysis of studies concerning malaria transmission in sub-Saharan Africa found a negative relationship between the level of malaria transmission and the level of urbanization: transmission decreased from rural to peri-urban areas and from peri-urban areas to urban centres [9].

As malaria transmission in town is focal, it could be appropriate to guide the vector control interventions on the most vulnerable populations and in the transmission areas only, in order to optimise the use of the human and financial resources of the national programs for disease control. Entomological maps of larval or adult mosquito spatial and temporal distribution, drawn at appropriate scales can provide valuable information for targeted malaria control and selective allocation of resources. On one hand, drawing such map could be based on exhaustive entomological studies covering the whole city during several years. However, entomological studies can be costly and difficult to set up in large and complex urban areas. On the other hand, the identification of the environmental factors associated with malaria transmission focus and their remotely-sensed measurement open the way to a more practical malaria risk mapping. Indeed, remote sensing (RS) and geographic information systems (GIS) became, from several decades, tools to evaluate the environmental, meteorological and climatic factors leading to malaria risk geographical and temporal distribution [20-25]. In Dakar, entomological date have already been collected in several areas from 2005 to 2007 [5, 7] and part of them allowed conducting a preliminary step towards malaria risk mapping, by setting up two risk maps for years 1996 and 2007 [7, 26]. They were based on the fitting of models on vector aggressiveness field data collected in a limited number of areas during one year, with remotely-sensed urbanization level as the only explanatory variable. In the perspective of a more accurate and precise malaria risk mapping, the objective of the present study was to in-depth investigate the environmental conditions associated with malaria transmission in 45 studied areas of Dakar and its suburbs from 2007 to 2010.

Methods

Study site

Dakar (14°40'20" North, 17°25'22" West), the capital city of Senegal, is located in the Cap-Vert peninsula at the westernmost point of Africa. The estimated population was 1 030 594 inhabitants in 2005, amounting to about 20% of the country's population. The population density is 12 233 inhabitants per km². The altitude peaks at 104 m above sea level (Mamelles). The study was conducted in 45 different areas of downtown Dakar, as well as in Pikine, Thiaroye and Guediawaye, three of its satellite cities. The choice of the studied zones was done in order to cover as many diverse environments as possible in terms of type of urbanization, road network, vegetation and socio-economic level. Each site was delimited on the ground to cover an area of about 200 x 200 m, depending on the technical and logistical limitations presented by the landscape. The studied zones are presented and listed in Figure 1, each point being the centre of the area.

1	Almadies
2	Gibraltar
3	Grand Médine
4	Hann (IRD)
5	Liberté 5
6	Ouest Foire
7	Pikine
8	Université
9	Yarakh
10	Yoff
11	Grand Yoff
12	Médina
13	Parcelles
14	Patte d'Oie (1)
15	Roi Baudoin
16	BA 160
17	BA Ouakam
18	BIMA
19	Bourguiba
20	Cafetériat
21	Cambérène
22	Dial Diop
23	Fana
24	Golf
25	Karack
26	Pikine Est
27	Potou
28	Réservoir
29	Sandial
30	Zone A
31	Castor
32	Dalifort
33	Doro Aw
34	HLM
35	Liberté 6 Extension
36	Mamelles
37	Maristes
38	Nord Foire
39	Patte d'Oie (2)
40	Point E
41	Pointe des Almadies
42	Sacré Coeur
43	Thiaroye Mairie
44	Touba Thiaroye
45	Virage

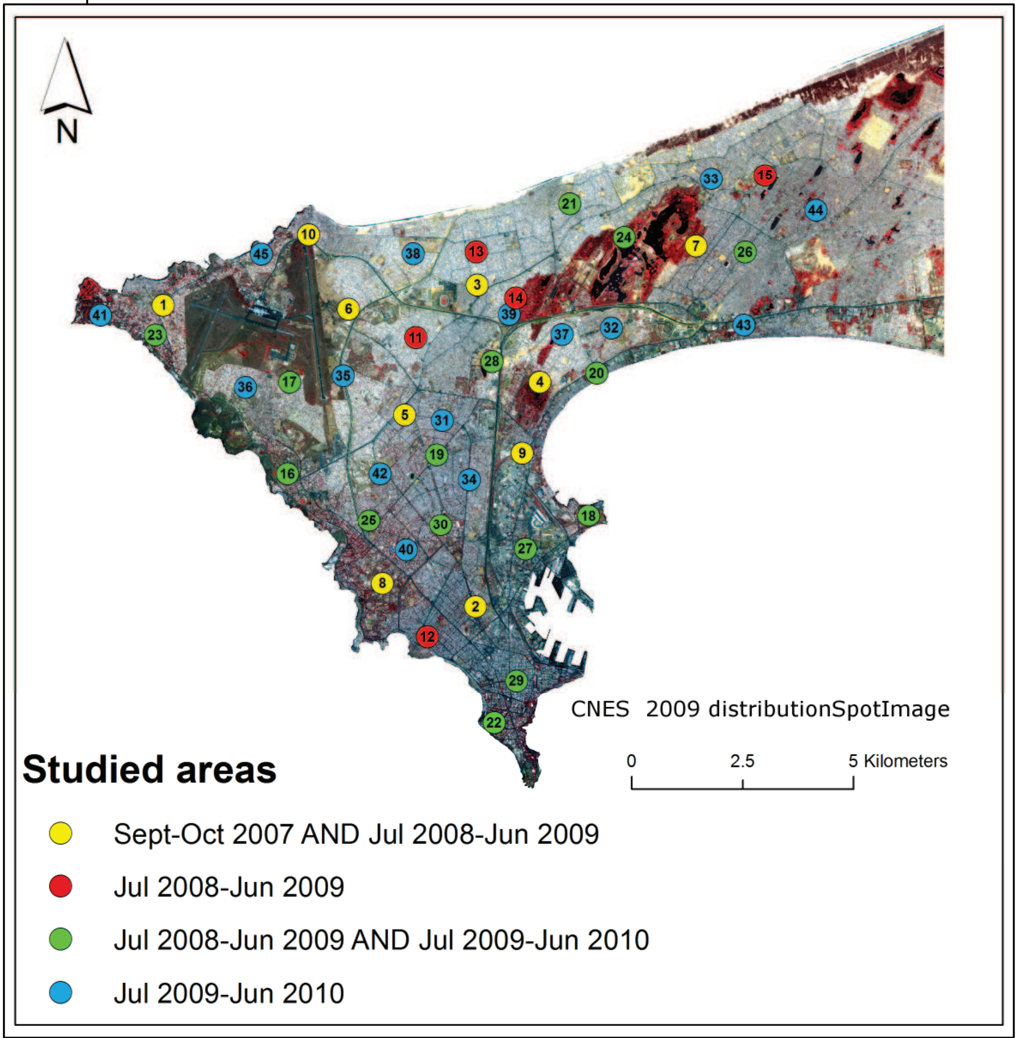

Figure 1. Spatial distribution of the 45 studied areas and their period of study.

Climate and study period

The Cap-Vert peninsula has a mild sahelian climate. The hot and wet season lasts from June to November, with average temperatures between 24 and 30°C. The cool and dry season lasts from December to May, with average temperatures between 19 and 25°C. The first rains generally occur at the end of June or the beginning of July, and the last ones at the beginning of October. In 2007, 2008 and 2009, the annual rainfall was respectively 178 mm, 510 mm and 565 mm (data from the national weather forecast).

Field study was undertaken in September and October 2007 and from July 2008 to June 2010 so three rainy seasons and two dry seasons were covered. In summary, 10 zones were studied in September-October 2007 (see [6]), 30 zones in July 2008-June 2009 and 30 zones in July 2009-June 2010. Each zone was followed during one or two years. The studied periods are presented in Figure 1. Two of those 45 zones (Bima and BA 160) where also studied in 2005 and 2006 but followed a slightly different sampling methodology (see [5]).

Adult mosquito

Field sampling

Adult mosquito sampling was carried out once every two weeks during September-October 2007 and during both extended 2008 and 2009 wet seasons (July to December), and once every month during both 2009 and 2010 dry seasons (January to June). Human landing catches of adult mosquitoes were conducted both indoors and outdoors in each of studied areas. For every zone, one catching point was indoors and two were outdoors. For Point E and Nord Foire, the three catching points were outdoors respectively from mid-October 2009 (and the 10 following catching nights) and end-September 2009 (and the 10 following catching nights). Indeed, it has not been possible in those two areas to find a family or administration that allowed the regular visit of the project team in their house or their office.

Indoor captures were conducted with the window or door slightly ajar. The three catching points were located around the centre of each studied area. Within each area, distance between each of the three catching point was about 30 meters. Collectors gave prior informed consent and received yellow fever immunizations and anti-malarial chemoprophylaxis for the duration the study and one month thereafter. Two collectors were contracted for each catching point to work from 8:00 p.m. to 7:00 a.m. or 7:00 p.m. to 7:00 am, depending on the hours of dawn and dusk in Dakar, with each one resting every two hours. Collectors were rotated among the catching points on different collection nights to minimize sampling bias. Five teams of seven persons (six collectors and one supervisor) were handling mosquito collections.

Species identification

The mosquitoes were recorded by catching point, date and hour of capture and they were sorted by genera. The anopheline mosquitoes were identified morphologically following the Gillies and Coetzee keys [27]. *Culicinae* were identified morphologically following the Edwards keys [28]. All anopheline mosquitoes were stored individually in numbered vials with desiccant and preserved at -20°C until processing. Part of the mosquitoes was stored in IRD in Dakar (Senegal) and part was sent to the Medical Entomology Unit of the Institute for Biomedical research of the French Forces (IRBA), in Marseille (France),
Depending on the number of *Anopheles* caught by studied area, all specimens or a random sample of a maximum of about 100 specimens belonging to the *An*. *gambiae* complex were selected from each study site for identification to species by polymerase chain reaction (PCR) [29]. All CSP-positive anopheline mosquitoes were also tested.

Biting patterns and sporozoïtes rates

The human biting rate (HBR), also termed aggressiveness, was expressed as the number of female *Anopheles* bites per person per night, averaged for both outdoor and indoor catching points. For 2007, 2008 and 2009, averaged values over September-October, which was the period of the peak of *Anopheles* densities (4 collection nights in each studied area), provided a HBR for the rainy season (further named RHBR). Values were also averaged from November 2008 to August 2009 and from November 2009 to June 2010 in order to calculate the HBR for the dry seasons (further named DHBR). The heads and thoraces of a nearly exhaustive sample of the adult *Anopheles* females caught on human bait were tested by enzyme-linked immunosorbent assay (ELISA) to detect the presence of *P. falciparum* circumsporozoite protein (CSP) [30]. In the present study, all the calculated HBRs took into account the number of *An. gambiae s.l.* bites received per person per night.

The annual CSP index was calculated as the proportion of positive mosquitoes out of the total number of ELISA processed *An. gambiae s.l.* The 2007, 2008 and 2009 annual CSP indexes were respectively calculated with the *Anopheles* caught in September-October 2007, July 2008-June 2009 and July 2009-June 2010. The entomological inoculation rate (EIR) was the product of the HBR and the CSP index. It provided the number of infected *Anopheles* bites per person per night. The EIR for the rainy seasons (further named REIR) was derived from the RHBR and the annual CSP index. The REIR was calculated in two ways: using the annual CSP index averaged for all areas or using the annual CSP index actually evaluated in each area, taking into account the number of infected mosquitoes caught in each area. The REIR was multiplied by 60 to provide the total number of infected *Anopheles* bites that could be received during the peak of transmission (September and October).

Mosquito larval sampling

Identification of water collections in the studied areas

Each yard within the studied areas was searched for open water collections by a team of two operators. The studied areas were visited every 10 days during the three rainy seasons and every month during the two dry seasons. Water collections were followed during September-October 2007 and from June 2008 to April 2010 without any interruption. All records on water collections described below were registered using a paper form in 2007 or a digitized form and a Tablet PC in 2008 to 2010. Physical, biological and chemical characteristics of the open water collections were recorded. For visual classifications, the consistency was maintained by the fact that the same team undertook the field work during the full duration of the project (two persons out of four were rotating for field work). All the collected information was transferred in an Excel database.

Characterization of open water collection

Habitat type of all water bodies were categorized as ditches or puddles, swamp areas, marshes, ponds or lakes, “céanes” (non cemented wells used in the market gardens), cemented wells or basins, waterproof containers or canals. A water collection was considered temporary when it was found to be dry at least once during the follow-up. Otherwise, it was recorded as permanent. The presence of larvivorous fishes, such as guppies, *Gambusia* or *Tilapia*, assessed visually. In 2007, the temperature of the water was measured with a mercury-in-glass thermometer immerged for 60 seconds. From 2008 to 2010, a waterproof pH meter (PHSCAN 30, Hanna) provided the temperature, the pH and the conductivity. Turbidity was estimated by using a graduated transparent bottle with black letters written on the bottom. The bottle was filled with water from the collection site and turbidity was evaluated by the graduation that the water reached before the letters were no longer visible. Graduations ranged from 0 to 26 cm, starting from the top of the bottle, so that a higher value indicated greater turbidity. The proportion of the water surface covered by vegetation was estimated visually. The vegetation included water lettuce, water lentils, grass or algae. The proportion of the shadow on the water at midday was estimated visually, depending on the surrounding. The salinity of the water varies with the conductivity. As *An. melas* breeds in brackish water, significant difference of conductivity was researched between *An. melas* and *An. arabiensis* larval habitats.

Field larval sampling

All the water collections in the 45 studied areas were examined for larvae. Larvae and pupae were sampled using a standard dipping method [31] (minimum of 20 dips for small collections, up to 100 for large water bodies). When anopheline specimens were found, larval density was calculated as the number of larvae (all instars) and pupae (further emerged and identified at the laboratory) per dip and recorded for each water collection. The presence of *Culicinae* larvae was also recorded. Results have already been presented for 2007 [6] but all data from 2007 to 2010 have been taken into account in the analysis to increase statistical power.

Larval mosquito data analysis

A random sample of the anopheline larvae found during prospecting were stored by date and breeding site in numbered vials filled with 70% alcohol. All larvae collected in the studied areas where *An. melas* have been caught were identified by species following the same PCR protocol, in the Medical Entomology Unit in Marseille. The PCR were done on pools of larvae, each pool corresponding to a sample of larvae collected in one breeding site and one date. PCR had been undertaken for pools collected in September-October 2007 and between July and September 2008.

Larval sampling inside private properties

A transversal field study was undertaken in September 2009 for prospecting larval collection inside private properties. In each of the 45 studied zones, a random geographical sampling was done in a GIS in order to choose the private properties to visit. Very high resolution images from Google Earth were used as maps to guide the field work. The sampling scheme included 30 private properties in each zone, numbered from 1 to 30. On the ground, the private properties were visited in the order of their numbers. When a private property was closed or if the access was denied, the following one was tried, until a maximum of about 10 private properties. In every acceded private property, the gardens, the courtyards and the flat roofs were searched for presence of water. The description of the detected water collections was recorded. The presence of larvae was researched visually for small collections or with the dipping method. The *Anopheles* or *Culicidae* were visually differentiated in the water collection or the dip.

Correlation between larvae and adults densities

The adult density fraction was estimated at the study site level by the total number of *Anopheles* caught indoors and outdoors during one decade, divided by the total number of *Anopheles* caught during the studied year. The larval density fraction at each study site was estimated as follows: the product of the larval density multiplied by the estimated water surface was calculated for each breeding site for a decade. Then, the sum of these products over all the water collection sites was considered as the larval density index for the studied area. It was then divided by the total larval densities summed for the whole studied year. Correlations between larval density fraction and adult density fraction were then graphically examined.

Statistical analysis

For each studied area that was followed during more than one rainy season the CSP indexes estimated each year were compared using Chi² test.

The statistical analyses for the larval study aimed to identify: 1) the determinants of the presence/absence of anopheline larvae in water collection sites, and 2) the factors associated with the density of anopheline larvae in the breeding sites. Continuous independent variables were dichotomised at the mean or median. The statistical unit was the weekly measurement of variables for a given water collection. In longitudinal studies, some correlation could exist between observations made on the same water collection. To take into account this interdependence of observations, random effect models were used.

The presence/absence of larvae in the water collection sites was analysed using a logistic regression model. The larval density by breeding site was analysed using a negative binomial regression model. The dependant variable was the number of larvae per 100 dip. The variables associated with the presence or the density of larvae with a p-value <0.25 in univariate analysis were retained for multivariate analysis. A backward stepwise selection procedure was applied in the final model to keep variables with a p-value <0.05. All analyses were performed with STATA 9.0 (Stata-Corp LP).

Results

Adult mosquito collection

A total of 496,310 mosquitoes were caught between 2007 and 2010, during 3,096 person-nights of collection on human bait (1,012 person-nights indoor and 2,084 person-nights outdoor). A total of 44,967 *An. gambiae s.l.* were collected (Table 1).

Table 1. Distribution by genus and species of adult mosquitoes collected on humans in the studied areas of Dakar.

	Sept-Oct 2007 (10 zones)		Jul 2008-Jun 2009 (30 zones)		Jul 2009-Jun 2010 (30 zones)		Total	
	Nb	% of total population	Nb	% of total population	Nb	% of total population	Nb	% of total population
Anopheles gambiae s.l.	1 101	5.66%	20 773	11.42%	23 093	7.83%	44 967	9.06%
Anopheles pharoensis	10	0.05%	298	0.16%	95	0.03%	403	0.08%
Anopheles ziemani			33	0.02%	93	0.03%	126	0.03%
Culex quinquefasciatus	14 428	74.18%	136 001	74.78%	257 947	87.44%	408 376	82.28%
Culex tritaeniorynchus	3 010	15.47%	19 475	10.71%	10 665	3.62%	33 150	6.68%
Aedes aegypti	821	4.22%	4 289	2.36%	1 839	0.62%	6 949	1.40%
Aedes metallicus	1	0.01%	23	0.01%			24	0.00%
Aedes vitatus					8	0.00%	8	0.00%
Aedes sp.			11	0.01%	1	0.00%	12	0.00%
Mansonia sp.	80	0.41%	964	0.53%	1 251	0.42%	2 295	0.46%
Total	19 451		181 867		294 992		496 310	

Biting behaviour of *An. gambiae s.l.*

Among the 44,967 *An. gambiae s.l.* caught, 32,403 (72%) were caught outdoors and 12,564 (28%) were caught indoors (Table 2). Considering the 45 studied areas together, the peak biting time was between 1:00 a.m. and 5:00 a.m. (Figure 2) and 77.4% of the bites occurred after midnight.

Table 2. Outdoors and indoors distribution of adult *An. gambiae s.l.* collected on human bait.

	Sept-Oct 2007 (10 zones)		Jul 2008-Jun 2009 (30 zones)		Jul 2009-Jun 2010 (30 zones)		Total	
	Number of person-nights	Number of *An. gambiae s.l.*	Number of person-nights	Number of *An. gambiae s.l.*	Number of person-nights	Number of *An. gambiae s.l.*	Number of person-nights	Number of *An. gambiae s.l.*
Outdoors	80	870	936	14 443	1 068	17 090	2 084	32 403
% outdoors	67%	79%	67%	70%	68%	74%	67%	72%
Indoors	40	231	468	6 330	504	6 003	1 012	12 564
% indoors	33%	21%	33%	30%	32%	26%	33%	28%
Total	120	1 101	1 404	20 773	1 572	23 093	3 096	44 967

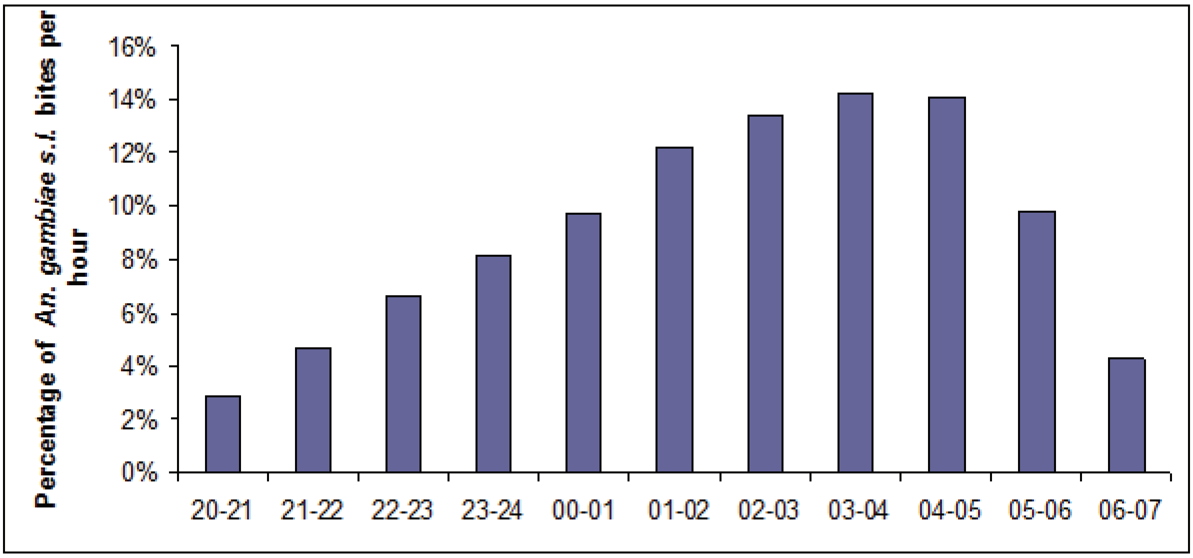

Figure 2. Hourly distribution of *An. gambiae s.l.* bites in the 45 studied areas of Dakar, from 2007 to 2010.

Molecular identification of *An. gambiae s.l.*

Among the 3,775 specimen caught between 2007 and 2010 and successfully tested by PCR, the *An. gambiae* complex population was composed of 3,516 *An. arabiensis* (93.14%), 258 *An. melas* (6.83%) and 1 *An. gambiae s.s.* form M (0.03%). The detailed percentages of species per studied area are presented in Table 3. The most important presence of *An. melas* was found in Hann (IRD) and Golf during the season 2008-2009 and 2009-2010 where it accounted for more than 40% of the *An. gambiae s.l.* population.

HBR, CSP and EIR

The HBR was calculated as the number of *An. gambiae s.l.* bites received per person per night. Both indoor and outdoor figures were taken into account. The Figure 3 provides the *An. gambiae s.l.* densities recorded for each year and averaged for all the studied areas. The highest measured aggressiveness was 713.5 bites of *An. gambiae s.l.* per person per night, outdoors the 17 September 2008 in Zone A. The Figure 3 also represents every rainfall event. The peak of aggressiveness occurs between 15 to 20 days after the peak of precipitations. It points out the very high temporal heterogeneity in the HBR during the year, with a very marked peak of aggressiveness during the rainy season. In 2007, *An. gambiae s.l.* RHBR ranged from 0.1 bites per person per night in Yoff to 43.7 in Almadies. In 2008, *An. gambiae s.l.* RHBR ranged from 0.3 bites per person per night in Grand Medine to 248.9 in Zone A. In 2009, *An. gambiae s.l.* RHBR ranged from 0 bites per person per night in Castor to 244.8 in Zone A. Additional files provide the *An. gambiae s.l.* densities recorded each year in each studied areas. The importance of the *An. gambiae s.l.* populations in the different studied areas of Dakar during the rainy season was clearly linked to the level of rainfall.

	2007			2008/2009				2009/2010		
Studied areas	*An. Arabiensis* (% of *An. arabiensis*)	*An. melas* (% of *An. melas*)	Total number of specimens processed	*An. Arabiensis* (% of *An. arabiensis*)	*An. melas* (% of *An. melas*)	*An. gambiae s.s form M* (% of *An. gambiae s.s form M*)	Total number of specimens processed	*An. Arabiensis* (% of *An. arabiensis*)	*An. melas* (% of *An. melas*)	Total number of specimens processed
ALMADIES	102 (100%)		102	99 (100%)			99			
PIKINE	78 (78.8%)	21 (21.2%)	99	71 (72.4%)	27 (27.6%)		98			
UNIVERSITE DAKAR	93 (100%)		93	110 (100%)			110			
HANN IRD	58 (92.1%)	4 (6.4%)	62	68 (59.1%)	47 (40.9%)		115			
OUEST FOIRE	44 (100%)		44	119 (96%)	5 (4%)		124			
YARAKH GARE	41 (100%)		41	105 (92.1%)	9 (7.9%)		114			
GIBRALTAR	43 (100%)		43	104 (100%)			104			
LIBERTE V	7 (100%)		7	31 (100%)			31			
GRAND MEDINE	3 (75%)	1 (25%)	4	6 (100%)			6			
YOFF	1 (100%)		1	7 (100%)			7			
PARCELLES				2 (100%)			2			
GRAND YOFF				8 (100%)			8			
MEDINA				9 (100%)			9			
CAMBERENE				11 (68.8%)	5 (31.2%)		16			
BOURGUIBA				21 (100%)			21			
SANDIAL				62 (100%)			62			
BA 160				93 (100%)			93			
DIAL DIOP				98 (99%)	1 (1%)		99			
ROI BAUDOUIN				101 (100%)			101			
KARACK				96 (99%)	1 (1%)		97			
BIMA				91 (97.8%)	2 (2.2%)		93			
RESERVOIR				67 (100%)			67			
PIKINE EST MAIRIE				81 (100%)			81	1 (100%)		1
BA OUAKAM				99 (100%)			99	1 (100%)		1
POTOU				94 (95.9%)	4 (4.1%)		98	1 (100%)		1

	2007			2008/2009				2009/2010		
Studied areas	*An. Arabiensis* (% of *An. arabiensis*)	*An. melas* (% of *An. melas*)	Total number of specimens processed	*An. Arabiensis* (% of *An. arabiensis*)	*An. melas* (% of *An. melas*)	*An. gambiae s.s form M* (% of *An. gambiae s.s form M*)	Total number of specimens processed	*An. Arabiensis* (% of *An. arabiensis*)	*An. melas* (% of *An. melas*)	Total number of specimens processed
HOTEL FANA				99 (100%)			99	25 (100%)		25
PATTE D'OIE				110 (80.9%)	25 (18.4%)	1 (0.7%)	136			
YARAKH CAFETERIAT				102 (91.9%)	9 (8.1%)		111	4 (100%)		4
GOLF				108 (69.7%)	47 (30.3%)		155	3 (60%)	2 (40%)	5
ZONE A				103 (73.6%)	37 (26.4%)		140	3 (100%)		3
CASTOR*								0	0	0
NORD FOIRE								6 (100%)		6
DORO AW								4 (100%)		4
VIRAGE								28 (100%)		28
PATTE D'OIE 2								33 (100%)		33
HLM								13 (76.5%)	4 (23.5%)	17
LIBERTE 6 EXTENSION								55 (100%)		55
MARISTES								33 (94.3%)	2 (5.7%)	35
SACREE CŒUR								84 (97.7%)	2 (2.3%)	86
MAMELLES								128 (100%)		128
POINTE								92 (100%)		92
TOUBA THIAROYE								33 (100%)		33
POINT E								103 (97.2%)	3 (2.8%)	106
THIAROYE MAIRIE								103 (100%)		103
DALIFORT								118 (100%)		118

Table 3. Proportions of *Anopheles* species among the *An. gambiae s.l.* collected on humans in the 45 studied areas of Dakar in Sept-Oct 2007, Jul 2008-Jun 2009 and Jul 2009-Jun 2010.

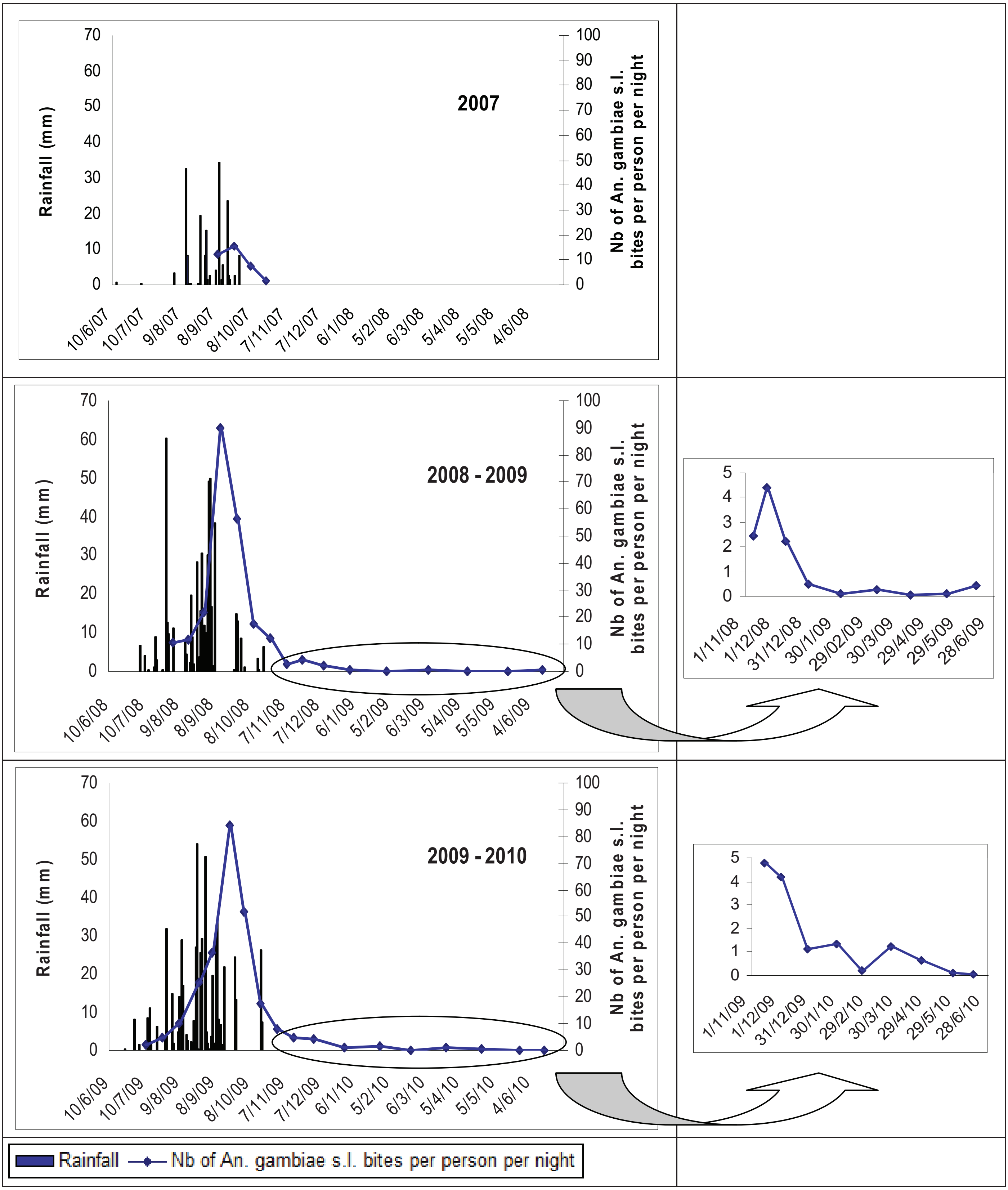

Figure 3. Number of *An. gambiae s.l.* and rainfall events for 10 studied areas in 2007, 30 studied areas in 2008 - 2009 and 30 studied areas in 2009 - 2010.

The Figure 4 provides the total RHBR for every studied zones and the Figure 5 shows the spatial representation of those RHBR. The figure 6 provides the total dry HBR for each studied zone. It shows that *Anopheles* aggressiveness remained significant during the dry season in some areas like in Golf, with more than 15 bites per person per night.

For the full duration of the field work, a total of 44,628 *An. gambiae s.l.* were processed by ELISA for *P. falciparum* antigen detection and 54 were found to be positive (52 *An. arabiensis* and 2 *An. melas*). Seven *An. arabiensis* were found to be infected in September-October 2007. During the 2008-2009 field study, 19 *An. arabiensis* were found positives, between August and December 2009. During the 2009-2010 field study, 26 *An. arabiensis* were found to be infected, between September 2009 and June 2010 and two *An. melas* were found to be infected with *P. falciparum,* in the beginning of the dry season in January 2010 in Golf. Table 4 presents the zones and dates of the caught infected *Anopheles*. The annual CSP index averaged for all studied areas was 0.64% in 2007, 0.09% in 2008-2009 and 0.12% in 2009-2010. For all the studied sites followed during more than one year, the CSP indexes were not statistically significant different between two consecutives years. In 2008-2009 and 2009-2010, the annual CSP index was close to the CSP index calculated only with the mosquitoes caught during Sept-Oct. In consequence, the CSP index calculated in Sept-Oct 2007 was comparable to the annual CSP index of 2008-2009 and 2009-2010. Table 5 provides the annual CSP index of each studied area, the annual CSP index averaged for all studied areas and the rainy EIR (for Sept-Oct) for each studied area, for *An. gambiae s.l.* figures from 2007 to 2010. The EIR for 2007, 2008-2009 and 2009-2010 were respectively 3.5, 2.3 and 3.4 infected bites that may have been received during the peak of transmission in September-October (from 0 to 17.6 depending on the studied areas and years).

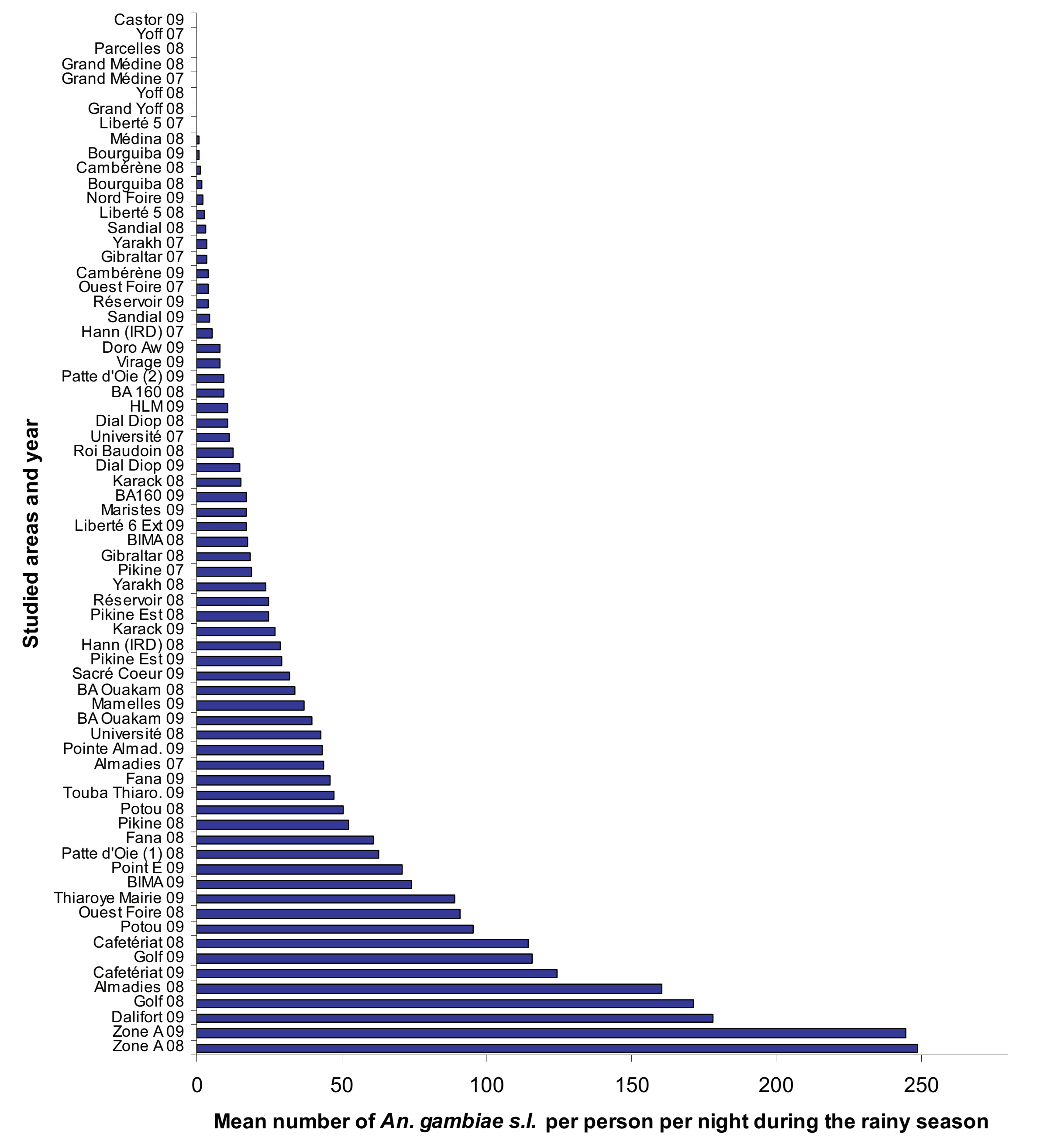

Figure 4. Mean Rainy HBR for 45 studied areas in Dakar, for 2007, 2008 and 2009 rainy seasons (Sept-Oct).

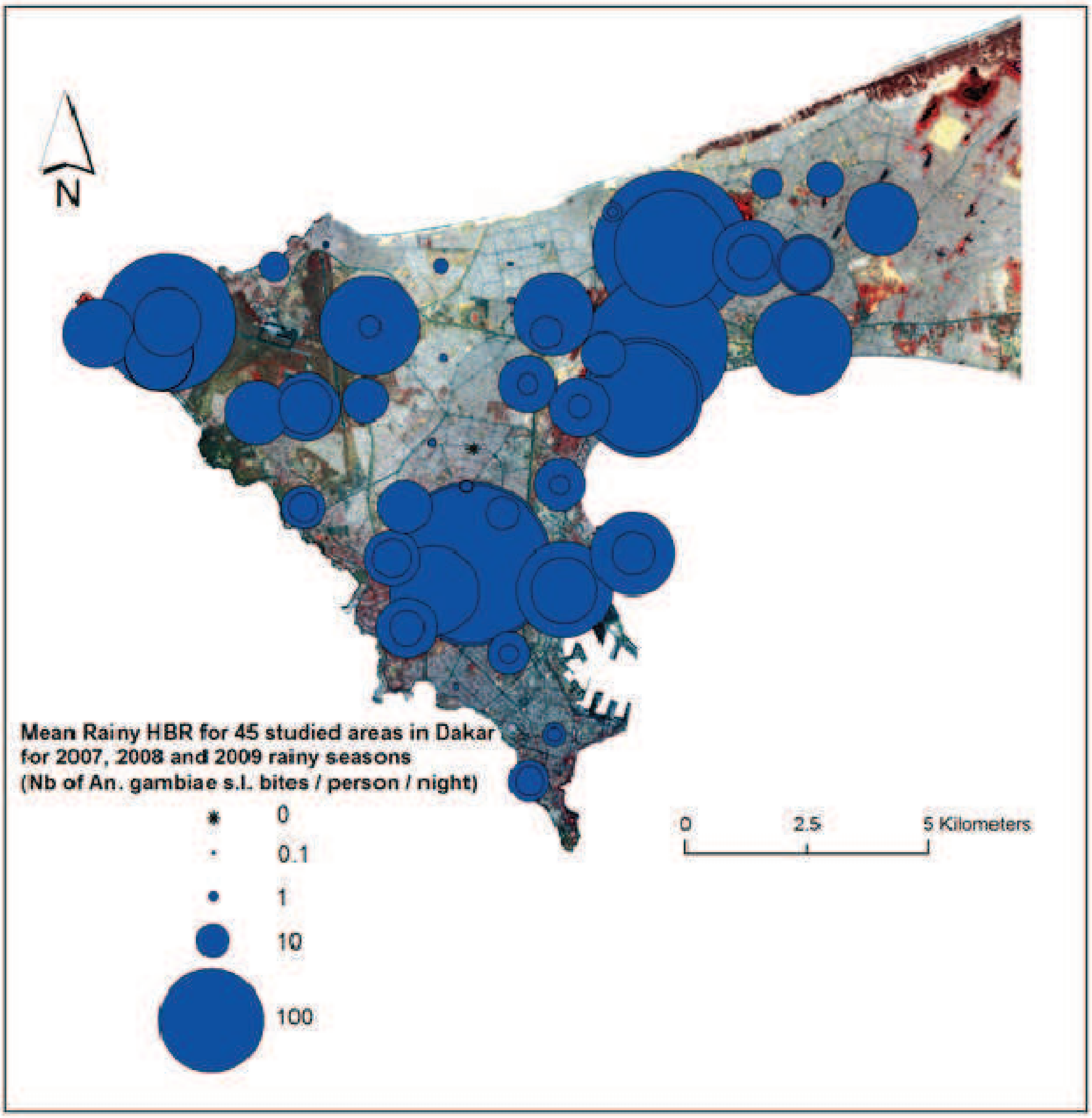

Figure 5. Mean Rainy HBR for the 45 studied areas in Dakar, for 2007, 2008 and 2009 rainy seasons (Sept-Oct), spatial distribution.

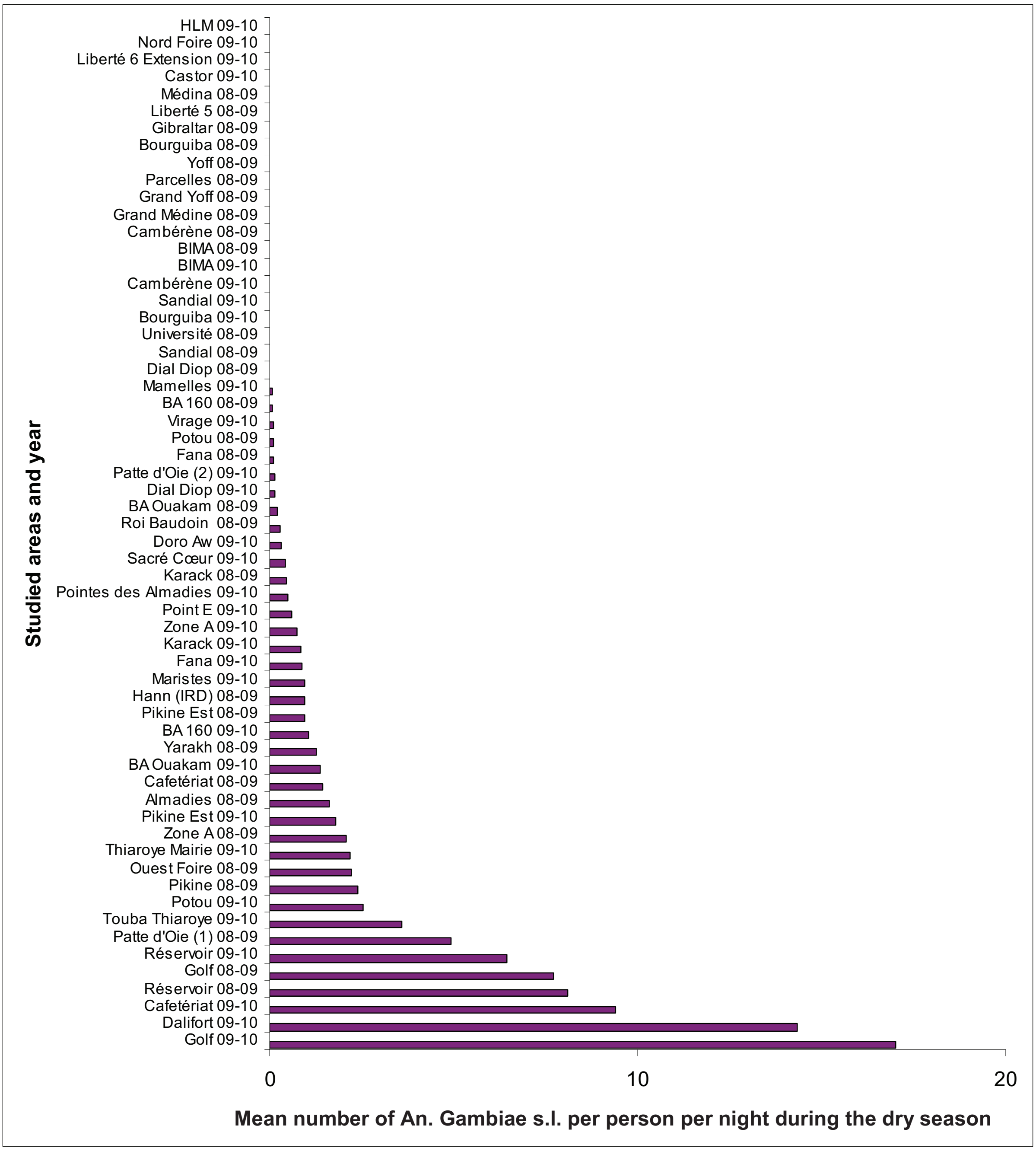

Figure 6. Mean Dry HBR for the studied areas in Dakar, for 2008 and 2009 dry seasons (Nov-Jun).

	Studied period* *Rainy/Dry*	Jul	Aug	Sept	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Tot
Almadies	2007			3	1									4
Almadies	2008/09		1	1	1									3
Hann (IRD)	2008/09				1		1							2
Ouest Foire	2007				1									1
Ouest Foire	2008/09			3	1									4
Pikine	2007			1										1
Yarakh	2007			1										1
Patte d'Oie (1)	2008/09			2										2
BA Ouakam	2008/09		1											1
BA Ouakam	2009/10			1	1									2
Cafetériat	2009/10				5									5
Fana	2009/10				1									1
Golf	2008/09			1										1
Golf	2009/10							3			1		1	5
Karack	2008/09				1									1
Pikine Est	2008/09				1									1
Pikine Est	2009/10				1									1
Potou	2009/10				1									1
Réservoir	2008/09			1		1	1							3
Zone A	2008/09			1										1
Zone A	2009/10			1	3									4
Dalifort	2009/10			1				1						2
Liberté 6 Extension	2009/10				1									1
Mamelles	2009/10				1									1
Point E	2009/10				1									1
Sacré Cœur	2009/10			1		1								2
Thiaroye Mairie	2009/10				1									1
Touba Thiaroye	2009/10				1									1
Total			2	18	24	2	2	4			1		1	54

Table 4. Temporal and spatial distribution of the *P. falciparum* infected *An. arabiensis* and *An. melas* caught in 2007 to 2010 in Dakar.

	2007					2008/2009					2009/2010				
Studied areas	Rainy HBR	Annual CSP index for each area	Rainy EIR for each area	Annual CSP index averaged for all areas	Rainy EIR averaged for all areas	Rainy HBR	Annual CSP index for each area	Rainy EIR for each area	Annual CSP index averaged for all areas	Rainy EIR averaged for all areas	Rainy HBR	Annual CSP index for each area	Rainy EIR for each area	Annual CSP index averaged for all areas	Rainy EIR averaged for all areas
Almadies	43.7	0.76%	19.93	0.64%	16.78	160.8	0.09%	8.68	0.09%	8.68					
Pikine	19	0.44%	5.02	0.64%	7.3	52.3	0%	0	0.09%	2.82					
Université	11.5	0%	0	0.64%	4.42	43.1	0%	0	0.09%	2.33					
Hann (IRD)	5.3	0%	0	0.64%	2.04	28.8	0.37%	6.39	0.09%	1.56					
Ouest Foire	4.1	2.04%	5.02	0.64%	1.57	90.7	0.30%	16.33	0.09%	4.90					
Yarakh	3.5	2.38%	5	0.64%	1.34	23.8	0%	0	0.09%	1.29					
Gibraltar	3.6	0%	0	0.64%	1.38	18.5	0%	0	0.09%	1.00					
Liberté 5	0.7	0%	0	0.64%	0.27	2.5	0%	0	0.09%	0.14					
Grand Médine	0.3	0%	0	0.64%	0.12	0.3	0%	0	0.09%	0.02					
Yoff	0.1	0%	0	0.64%	0.04	0.6	0%	0	0.09%	0.03					
Patte d'Oie (1)						62.8	0.18%	6.78	0.09%	3.39					
Roi Baudoin						12.6	0%	0	0.09%	0.68					
Médina						0.7	0%	0	0.09%	0.04					
Grand Yoff						0.6	0%	0	0.09%	0.03					
Parcelles						0.2	0%	0	0.09%	0.01					
Cambérène						1.2	0%	0	0.09%	0.06	3.9	0%	0	0.12%	0.28
Bourguiba						1.8	0%	0	0.09%	0.10	0.8	0%	0	0.12%	0.06
Sandial						3.3	0%	0	0.09%	0.18	4.5	0%	0	0.12%	0.32
BA 160						9.6	0%	0	0.09%	0.52	17.3	0%	0	0.12%	1.25
Dial Diop						10.8	0%	0	0.09%	0.58	14.8	0%	0	0.12%	1.07
Karack						15.2	0.41%	3.74	0.09%	0.82	27.2	0%	0	0.12%	1.96
BIMA						17.6	0%	0	0.09%	0.95	74.1	0%	0	0.12%	5.34
Réservoir						24.8	0.48%	7.14	0.09%	1.34	4.1	0%	0	0.12%	0.30
Pikine Est						25	0.25%	3.75	0.09%	1.35	29.3	0.18%	3.16	0.12%	2.11
BA Ouakam						34	0.23%	4.69	0.09%	1.84	39.9	0.32%	7.66	0.12%	2.87
Potou						50.8	0%	0	0.09%	2.74	95.6	0.08%	4.59	0.12%	6.88
Fana						61.1	0%	0	0.09%	3.30	46.1	0.14%	3.87	0.12%	3.32
Cafetériat						114.5	0%	0	0.09%	6.18	124.4	0.26%	19.41	0.12%	8.96

Golf						171.6	0.03%	3.09	0.09%	9.27	115.8	0.21%	14.59	0.12%	8.34
Zone A						248.9	0.03%	4.48	0.09%	13.44	244.8	0.12%	17.63	0.12%	17.63
Castor											0	0%	0	0.12%	0.00
Nord Foire											2.3	0%	0	0.12%	0.17
Doro Aw											8	0%	0	0.12%	0.58
Virage											8.3	0%	0	0.12%	0.60
Patte d'Oie (2)											9.5	0%	0	0.12%	0.68
HLM											10.8	0%	0	0.12%	0.78
Liberté 6 Extension											17.3	0.43%	4.46	0.12%	1.25
Maristes											17.3	0%	0	0.12%	1.25
Sacré Coeur											32	0.45%	8.64	0.12%	2.30
Mamelles											37.3	0.20%	4.48	0.12%	2.69
Pointe des Almadies											43.3	0%	0	0.12%	3.12
Touba Thiaroye											47.4	0.12%	3.41	0.12%	3.41
Point E											71.1	0.10%	4.27	0.12%	5.12
Thiaroye Mairie											89.3	0.05%	2.68	0.12%	6.43
Dalifort											178.3	0.06%	6.42	0.12%	12.84
All areas	9.2			0.64%	3.5	42.9			0.09%	2.3	47.2			0.12%	3.4

Table 5. Annual CSP index of each studied area, annual CSP index averaged for all studied areas and rainy EIR (for Sept-Oct) for each studied area. All figures for *An. gambiae s.l.* from 2007 to 2010.

Larval sampling

A total of 325 water collections were followed. Depending on the persistence of the water bodies and on their characteristics of draining, emptying or drying out, they were visited between 1 to 37 times. Several types of open water collections were found: 203 ditches or puddles, 24 swamp areas, marshes, ponds or lakes, 70 "céanes", cemented wells or basins, 11 large artificial holes (type underpinning), 9 small waterproof containers (tyres or buckets) and 8 canals. Of the water collections, 261 (80%) were temporary and 64 (20%) were permanent.
A large part (228 out of 325, 70%) of the water bodies were found to be breeding sites for *Anopheles* for at least one observation during the follow-up period. Among temporary collections, 191 (73%) were habitats for anopheline larvae at least once during follow-up. Among permanent collection, 37 (58%) were *Anopheles* breeding sites at least once. Several studied areas did not harbour any breeding site during the full duration of their follow-up: Grand Yoff, HLM, Nord Foire and Point E. In breeding habitats, the density of *Anopheles* larvae and pupae ranged from 0.01 in two large permanent collections (Golf and Hann IRD) to 42 per dip in a concrete basin of an abandoned market-garden in Yarakh in 2008.

Identification of *An. gambiae s.l.* reared from larval samples

In 2007, the PCR amplification did not highlight the presence of any *An. melas.* For the 2008 rainy season, a total of 164 pools of larvae were identified for species using PCR amplification. The identification of species by PCR amplification allowed identifying 147 pools of *An. arabiensis*, 15 pools of mixed *An. arabiensis* and *An. melas* and two pools of *An. melas* only. All the pools containing *An. melas* were collected in Cafetériat, Pikine or Zone A. Due to laboratory constraints, no pools where tested for Bima, Cambérène, Golf, Potou. In the other areas, no *An. melas* was found. In Cafetériat, *An. melas* was found in two different water collections. In one small artificial basin in a private garden, three pools were tested at different dates and only one showed the presence of *An. melas,* mixed with *An. arabiensis*. In a very large inundated area, six pools where tested at different dates, three were *An. arabiensis*, two were *An. melas* and one was mixed. In Pikine, two *An. melas* breeding sites where detected. In the large marshy area (called Niaye), two out of seven pools where mixed *An. arabiensis* and *An. melas*. In a ceane communicating with the marshy area, two pools out of two where mixed *An. arabiensis* and *An. melas*. In Zone A, three breeding sites were exclusively found with mixed *An. arabiensis* and *An. melas* pools. Among those three larval habitats, two were puddles and one was a large permanent hole of an abandoned building underpinning. In addition, another puddle was found to contain mixed *An. arabiensis* and *An. melas* at two dates and *An. arabiensis* alone at one date. In Zone A, two pools containing *An. melas* could not be related to their breeding site due to sample storage problems. For the three studied areas, none of the breeding sites was an exclusive *An. melas* breeding site during the survey. Indeed *An. arabiensis* was always found in at least one of the tested pool for each larval habitat at one time during the survey. In the breeding sites containing *An. melas*, the conductivity was 5.03 (range: 0.3 to >20; 95% CI: 1.43 - 8.62). In the breeding sites that did not contain *An. melas*, the conductivity was 1.75 (range: 0.06 to 12.03; 95% CI: 1.40 - 2.09). The difference was significant ($p<0.0001$) so the salinity in the *An. melas* larval habitats was higher than in the *An. arabiensis* habitats.

Characterization of the water collections

A total of 2 903 observations were recorded in October-September 2007 and between July 2008 and April 2010, in collection sites filled with water. The status concerning *Anopheles* larvae presence/absence was known for 2 683 observations (1 015 positives for larvae: 38%) and the status concerning *Anopheles* larval density was known for 1 008 observations. The results of the univariate analysis for the presence / absence of *Anopheles* larvae and for the *Anopheles* larval densities are presented in Table 6. The results of the multivariate analysis for the presence / absence of *Anopheles* larvae and for the *Anopheles* larval densities are presented in Table 7. The multivariate analysis excluded variables which has too few observations (conductivity and number of continuous decades of water persistence).

	Presence/absence of *Anopheles* larvae				*Anopheles* larval density			
	Nb of observations	OR	95% CI	p-value	Nb of observations	IRR	95% CI	p-value
All water collections								
Surface	2 671				1 008			
<2 000 m^2		1				1		
>=2 000 m^2		1.09	0.58 - 2.02	0.795		0.73	0.62 - 0.87	0.0005
Water temperature (°C)	2 287				857			
<30°C		1				1		
>=30°C		1.69	1.31 - 2.16	<0.0001		1.31	1.16 - 1.47	<0.0001
Turbidity (from 0: clear, to 26: turbid)	2 576				975			
Clear (<10)		1				1		
Turbid (>=10)		1.30	1.01 - 1.67	0.042		1.03	0.92 - 1.16	0.574
pH	2 594				980			
<8		1				1		
>=8		1.89	1.46 - 2.45	<0.0001		1.27	1.13 - 1.43	0.0001
Conductivity (not recorded in 2007)	1 494				557			
<3		1				1		
>=3		0.80	0.59 - 1.10	0.167		0.91	0.78 - 1.06	0.228
Shade (%)	2 676				1 005			
<20%		1				1		
>=20%		0.62	0.41 - 0.94	0.023		0.82	0.71 - 0.94	0.004
Surface vegetation (%)	2 651				1 008			
<20%		1				1		
>=20%		1.68	1.22 - 2.32	0.001		0.92	0.82 - 1.04	0.196
Presence of *Culicinae* larvae	2 653				993			
No		1				1		
Yes		13.57	9.94 - 18.53	<0.0001		0.94	0.84 - 1.06	0.342
Presence of larvivorous fishes	2 366				882			
No		1				1		
Yes		0.35	0.22 - 0.54	<0.0001		0.63	0.50 - 0.79	0.0001
Season	2 683				1 008			
Dry (Nov to Jun)		1				1		
Wet (Jul to Oct)		3.20	2.43 - 4.21	<0.0001		1.48	1.27 - 1.73	<0.0001
Persistence of water collection	2 683				1 008			
Permanent		1				1		
Temporary		10.25	5.63 - 18.63	<0.0001		1.52	1.29 - 1.79	<0.0001
Number of continuous decades of water persistence	1 079				575			
For every decade		1.21	1.12 - 1.31	<0.0001		1.00	0.96 - 1.03	0.734

	Presence/absence of *Anopheles* larvae				*Anopheles* larval density			
	Nb of observations	OR	95% CI	p-value	Nb of observations	IRR	95% CI	p-value
Type of water collection (puddle vs others)	2 683							
Other		1				1		
Ditch or puddle		3.42	2.01 - 5.83	<0.0001		1.40	1.22 - 1.60	<0.0001
Muddy bottom	2 675				1 007			
No		1				1		
Yes		2.57	1.50 - 4.40	0.0006		1.24	1.07 - 1.44	0.003
Water collection located in market-garden	2 683				1 008			
No		1				1		
Yes		0.09	0.05 - 0.17	<0.0001		0.73	0.59 - 0.89	0.003
Water collection located in highly urbanized area	2 683				1 008			
No		1				1		
Yes		0.08	0.02 - 0.33	0.001		0.83	0.40 - 1.71	0.613

Table 6. Factors associated with presence/absence of *Anopheles* larvae and the *Anopheles* larval density in the open water collections studied in 45 studied areas of Dakar in October-September 2007 and between July 2008 and April 2010. Logistic regression and Binomial negative regression with water collection random effect. Univariate analysis.

	Presence/absence of *Anopheles* larvae			*Anopheles* larval density		
	OR	95% CI	p-value	IRR	95% CI	p-value
	2 273 observations		<0.0001	852 observations		<0.0001
Water temperature						
<30°C				1		
>=30°C				1.15	1.02 - 1.30	0.026
pH						
<8	1			1		
>=8	1.79	1.35 - 2.37	<0.0001	1.31	1.15 - 1.49	<0.0001
Presence of larvivorous fishes						
No	1			1		
Yes	0.52	0.34 - 0.83	0.006	0.70	0.55 - 0.89	0.003
Season						
Dry (Nov to Jun)	1			1		
Wet (Jul to Oct)	2.94	2.20 - 3.92	<0.0001	1.35	1.15 - 1.59	<0.0001
Surface vegetation (%)						
<20%	1					
>=20%	2.34	1.67 - 3.29	<0.0001			
Persistence of water collection						
Permanent	1					
Temporary	7.21	3.83 -13.58	<0.0001			
Water collection located in highly urbanized area						
No	1					
Yes	0.05	0.02 - 0.19	<0.0001			

Table 7. Factors associated with presence/absence of *Anopheles* larvae and the *Anopheles* larval density in the open water collections studied in 45 studied areas of Dakar in October-September 2007 and between July 2008 and April 2010. Logistic regression and Binomial negative regression with water collection random effect. Multivariate analysis.

Correlation between larvae and adults density fractions

Figure 7 shows the temporal variations in adult density fraction and larval density fraction. The fractions were estimated for each year. The graphical examination highlighted a very close parallel between larval and adult densities fractions for the three studied years. The correlation between adult density percentage and larval density percentage was 0.87. No lag time allow improving this correlation. Indeed, as the percentages were already averaged for 10 days, taking into account the larval density percentage lagged one decade decreases the correlation coefficient.

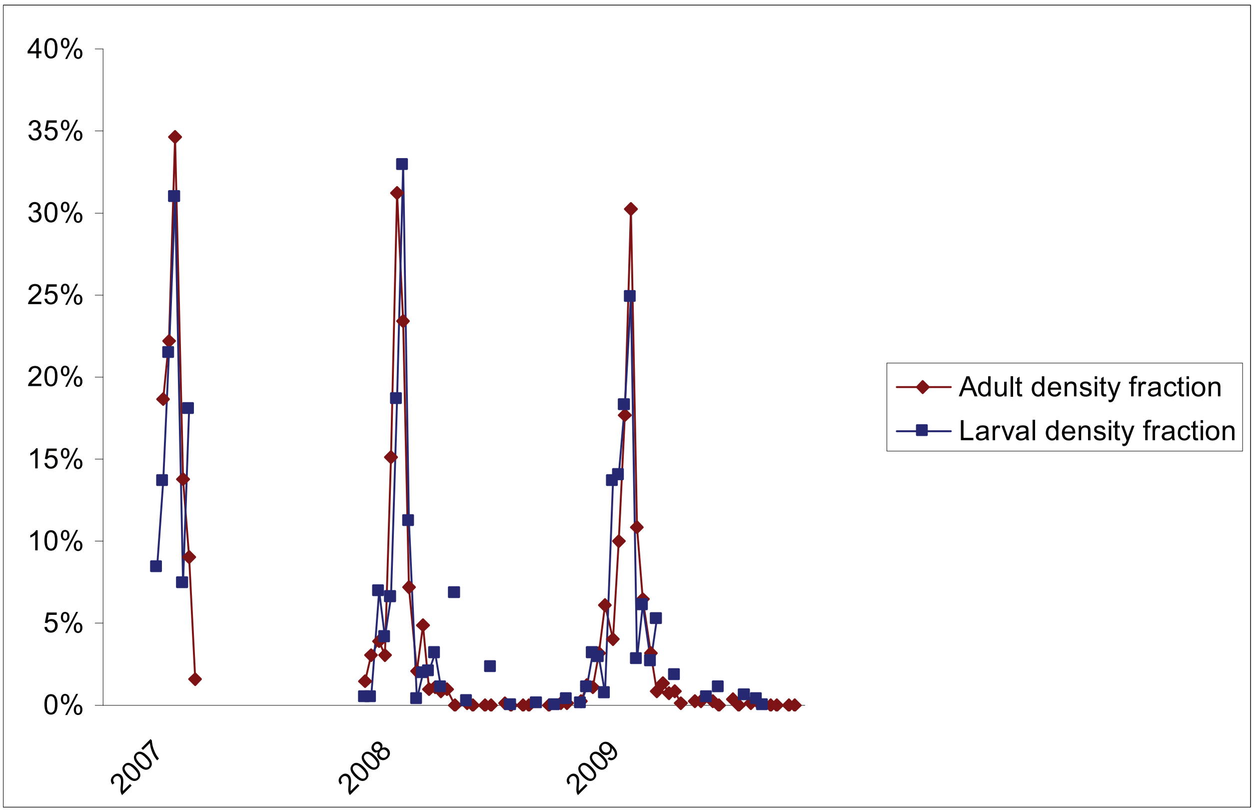

Figure 7. Temporal variations in adult density fraction and larval density fraction.

Larval sampling inside private properties

Depending on the studied areas, between five to 12 private properties or buildings were visited for a total of 355 private properties in the 45 studied areas. In 153 private properties, no water collection or water container was found. In the remaining 202 private properties, water was found in small containers (buckets, bowls, saucepans, cans…), in pools or basins, or in puddles located in the gardens, flat roofs or balconies. Small water containers were more common but they were not suitable for *Anopheles* breeding. *Culicidae* larvae were found in 80 private properties, located in 27 studies areas. *Anopheles* larvae were found in 11 private properties, located in 8 studies areas. In Cafetériat, larvae were found in a courtyard, in a puddle. In BA Ouakam, two puddles in gardens were anopheline breeding sites. In those two areas, the breeding sites were joined to the breeding sites located outside of the private properties (*i.e.* belonging to the same water collection). In Zone A and Dial Diop, the Anopheles bred in large puddles located the courtyards of two schools that were nearly empty due the holidays period. In Liberté 6 Extension, larvae were observed in the garden of a private propertie, in large cans and in a water tank. In Thiaroye Mairie, two breeding sites were detected: one unused old bathroom located in the field of a garage, and one in a bucket located on the flat roof of a private propertie. In point E and Karack, larvae were located in two unused swimming pools. In Karack in another garden, an unused large basin was also a breeding site. Those results show that very few larval habitats were located inside of the private sectors in comparison with the public sector. Whatever the type of housing and the socio-economic level, the inhabitants generally did not let potential *Anopheles* breeding sites to appear and persist.

Discussion

Malaria transmission in Dakar

The present results provide new evidences of malaria transmission in downtown Dakar and its nearby suburbs. Spatial heterogeneity of human biting rates was very marked, with an averaged Human Biting Rate ranging from 0.1 to nearly 250 bites per person per night, according to the studied areas.

The rate of infection of the *An. gambiae s.l.* caught on human bait was 0.64% in 2007, 0.09% in 2008-2009 and 0.12% in 2009-2010 and the averaged EIR ranged from 0 to 17.6 infected bites per person during the transmission season. From 2007 to 2010, *An. gambiae s.l.* infected with *P. falciparum* have been caught in 22 of the 45 studied zones. Considering also the investigations conducted in 2005 and 2006 [5], infected *An. gambiae s.l* have been caught in 24 of the 45 studied zones. In the 25 areas where investigations have been conducted during two consecutive rainy seasons, infected *An. gambiae s.l.* have been caught in 15 areas but infected anopheles have been found for both years only in five areas. Among those 15 areas, no statistical difference has been highlighted in the annual CSP indexes from one year to another. In BA 160 and BIMA where investigations have been conducted during four rainy seasons, infected anopheles have been caught only in 2005 and 2006 when the schedule of catches allowed a more powerful sampling, *i.e.* with a higher number of catching points. In those two studied areas, no statistical difference in the yearly CSP indexes (2005, 2006, 2008 and 2009) was found.

In one area (Golf), infected mosquitoes were caught all along the dry season, indicating that a permanent malaria transmission is possible in this area of Dakar city, around the large marshy area.

Anopheline species

Five anopheline species were encountered from 2005 to 2010 in the 45 studied areas of Dakar: three of them belonged to the *An. gambiae* complex and the two others were *An. pharoensis* and *An ziemanni*. *Anopheles ziemanni* has been previously caught on human bait in Senegal, but in at very low levels [32] and if *Anopheles ziemanni* larvae have already been reported in Pikine [33] and accounted for 14% of the sampled immature stage mosquitoes in the "céanes" of Dakar [14], no adult specimen have been caught on human from that time in Dakar. *Anopheles ziemanni* is a zoophilic specie that is considered as a secondary or an incidental vector [27]. Due to its low affinity to human blood and its low density even in the studies areas where it was present, *An. ziemanni* could not be significantly involved in malaria transmission in Dakar. *Anopheles pharoensis* is considered as a secondary vector of malaria and is clearly involved in malaria transmission in North Senegal [34] but it is generally not of epidemiological significance as a malaria vector in other parts of Senegal [35]. Due to its low density even in the zones where it was present, *An. pharoensis* could not be significantly involved in malaria transmission in Dakar.

Three members of the *An. gambiae* complex have been encountered in Dakar since the preliminary studies conducted in 2005. From 2007 to 2010, they were distributed as follows: *An. gambiae s.s. molecular form M* (0.03%), *An. melas* (6.83%) and *An. arabiensis* (93.14%). The presence of *An. gambiae s.s. molecular form M* was reported in 2005, 2006 and 2008 but its density was very low and it could not be significantly involved in malaria transmission in Dakar. From 2007 to 2010, in the 45 studied sites, 54 *An. gambiae s.l.* were found infected by *P. falciparum*: 52 *An. arabiensis* and 2 *An. melas*. Both infected *An. melas* were caught in 2010 in Golf and it was the first time that *An. melas* was proved to be infected in Dakar.

An. melas was described for the first time in downtown Dakar in 2005 and accounted for 2 % of the *An. gambiae s.l.* populations. In 2007, the percentage of *An. melas* was 5.2% on average but it was present essentially in two areas (Pikine and Hann IRD) that were those closest to the large marshy area locally called Niaye, with a peak greater than 20% in Pikine. From 2007 to 2010, *An. melas* has been reported in 18 zones where it represented between 1% to 40.9% of the *An. gambiae* complex according to the area and to the year. Considering that *An. melas* is not as good as vector as *An. arabiensis* [36], it could be implied in malaria transmission in Dakar only in areas where it is very abundant. So according to the 2007-2010 field data, *An. melas* could be involved in Pikine, Hann IRD, Golf, Zone A and Patte d'Oie.

In areas where data allowed a comparison of the *An. melas* proportions collected at different years, a large difference was found only in Hann (IRD). The proportion of *An. melas* was 6.4% in 2007 and 40.9% in 2008-2009. As no important changes have been recorded in the studied area, this rise could be related to higher rainfall in 2008 leading to an elevation of the salty water table level in this low elevation area.

In 2007, no *An. melas* larvae were found during the breeding sites survey. In 2008, the PCR amplification highlighted the presence of *An. melas* in 15 pools of mixed *An. arabiensis* and *An. melas* and two pools of *An. melas* only. *An. melas* larvae were found in artificial or natural breeding sites such as "céanes", Niaye, or temporary flooded areas. The salinity in the *An. melas* larval habitats was significantly higher than in the *An. arabiensis* habitats. This confirmed the previously published tolerance of *An. melas* larvae for salinity that could range from 5 to 37 g/l [37]. None of the breeding sites was an exclusive *An. melas* breeding site during the survey. Indeed *An. arabiensis* was always found in at least one of the tested pool for each larval habitat at one time during the survey. This was possibly due to variations in the level of salinity of the breeding sites that could allow, along the year, the development of the two species successively or at the same time. This supposes that *An. arabiensis* larvae have also a tolerance for low level of salinity. The variations of salinity in a collection could be due to a dilution by rainfall or to a concentration by evaporation.

Endo/exophagic and biting behaviours

In 2007, 2008-2009 and 2009-2010 respectively 34%, 42% and 46% of *An. gambiae s.l.* have been caught indoors. When comparing the endo/exophagic behaviour of *An. gambiae s.l.* between studied areas, between dry and rainy seasons for a same area and between years, it was not possible to define a specific behaviour according to the season or to the studied area. As logistical constraints limited the number of indoor catching points to one per site and as the characteristics of the rooms could be different between areas (private properties or public offices of different size), the experimental design may have now allowed for the results to be representative of the adult *An. gambiae s.l.* behaviour in each studied area. One can only say that malaria transmission can occur in Dakar both outdoors and indoors.

No difference was recorded in the hours of activity of *An. gambiae s.l.* between the different studied sites both indoors and outdoors. In consequence, a global description of the 45 sites was provided. Most of *An. gambiae s.l.* bites (85%) were recorded after 11 p.m. This finding highlighted the interest of the use of insecticide impregnated bednets to control malaria transmission in Dakar. Nevertheless, 15% bites occurred in the first part of the night when people are generally not asleep and out of mosquito nets. The use of others mosquito control devices like repellents or mosquito coils could be proposed to increase the protection during the first part of the night for people living in Dakar.

Dynamics of An. gambiae s.l. populations and malaria transmission

An. gambiae s.l. was present throughout the year in Dakar, but most of the specimens (98%) were caught between July and December. The biting peak occurred in September-October, at the end of the wintering period (rainy season). The abundance of *An. gambiae s.l.* was clearly dependant of the rainfall. The peak of the *An. gambiae s.l.* biting rate followed the peak of rainfall with a two weeks lag, whatever the year. The densities decreased quickly after the end of the rains. After a last peak of activity in December, only isolated specimens were caught until the beginning of the new rainy season in July. When comparing the human biting rates in the same areas between 2007, a year with low rainfall, and 2008, a year with heavy rainfall, the peaks of *Anopheles* bites were in average six fold higher in 2008 than in 2007. This was consistent with the fact that rainfall is closely related to the presence of surface water and to the larval productivity and adult densities, the two laters being highly correlated.

Heavy rainfall was not only increasing the number and size of temporary water collections and the filling level of the permanent collections. They were responsible of inundations in a lot of quarters of Dakar. In Golf, the persistence of flooded areas along the dry season could have been responsible in 2010 of an exceptional persistence of malaria transmission during the dry season. This situation could be encountered in other flooded parts of the city and floods could modify the patterns of malaria transmission in Dakar. Health practitioners have to be informed of this possibility.

Larval habitats

Among the 325 water collections monitored from 2007 to 2010, anopheline larvae were mainly found in ditches, puddles and swamps, ponds or lakes. Among temporary collections, 73% were habitats for anopheline larvae at least once during follow-up. Among permanent collection, 58% were *Anopheles* breeding sites at least once. Several factors were found to be associated with the occurrence and abundance of anopheline larvae.

In bivariate analysis, a higher probability of presence of anopheline larvae was associated with numerous factors that can classify in four categories: climatic factors, environmental factors, morphological factors and “biological” factors. Climatic factors (CF): the presence of anopheline larvae was positively linked to the wet season and to the number of continuous decades with water persistence that depends of the soil type and of the intensity and distribution of rainfall. Environmental factors (EF): the presence of larvae in an open water collection was less probable when this collection was located in a market garden or a highly urbanized area or when shade covered 20% or more of the surface of the water collection. Morphological factors (MF): permanent open water collections and collections other than ditches and puddles were negatively associated with the presence of larvae. Some biological factors (BF: factors linked to the biological or physical quality of water in the collection that represent the suitability of water to the development of larvae) were positively associated with the presence of anopheline larvae: co-occurrence of culicinae larvae, presence of surface vegetation covering more than 20% of the surface of the water collection, turbid water, pH>=8, muddy bottom and water temperature>=30°C. Another BF, the presence of larvivorous fishes, was negatively associated to the presence of larvae.

In multivariate analysis, pH>=8, wet season, surface vegetation>=20%, temporary collections were positively associated with the presence of larvae while presence of larvivorous fishes and high level of urbanization were negatively associated.

Higher larval densities were associated with the same factors than the presence of larvae, except the turbidity, percentage of surface vegetation, co-occurrence of culicinae larvae, number of continuous decades of water persistence and highly urbanized areas that was not significantly associated. In addition, the surface of the water collection was negatively associated with the larval density. Only four factors remained significant in multivariate analysis: water temperature>=30°C, pH>=8 and wet season were associated with higher densities of anopheline larvae while the presence of larvivorous fishes was associated with lower densities.

These results were consistent with the known preference of *An. gambiae s.l.* for breeding in temporary pools and in water collections exposed to sunlight [27, 38]. In addition, a study conducted in Kenya showed that in larval habitats located in forested areas, only 4-9% of first-instar larvae of *An. arabiensis* developed into adults and the development length exceeded 20 days against a larval-to-adult survivorship of 65-82% in deforested areas with a larval-to-adult development time shortened by 8-9 days [39]. The present results were consistent with those findings. In multivariate analysis, the water temperature >=30°C was not linked to the presence of anopheline larvae but to the density of larvae in a collection. The water temperature is perhaps not a factor playing a role in the laying of eggs by parous females but it has an impact on the development of *An. gambiae* immature stages. Co-occurrence of *Anopheles* and *Culicinae* larvae was previously reported in the literature [12]. Low-floating vegetation was previously found as a determinant of the presence of anopheline larvae [12, 40]. In the present study, the level of floating vegetation was positively associated with the presence of larvae. This difference could be explained by the difficulties to measure on the field the surface of vegetation covering a water collection. One could also consider the presence of vegetation as a factor suitable for life and so as predictor of the possibility of occurrence of larvae. Concerning the other physico-chemical parameters measured in the water collections, the pH was significantly associated either with the occurrence or the abundance of larvae. This factor could be considered as an indicator of the presence of food for larvae. The existence of a muddy bottom around the water collection was favourable to the presence of larvae. This was consistent with the habits of *An. gambiae* to lay its eggs on soil around puddle larval habitats [41]. Moist mud around water collection constitutes suitable habitat for *An. gambiae* eggs. Eggs can develop and larvae can emerge on mud to further crawl actively to reach water or to be passively displaced by flowing rainwater [41, 42]. In addition, muddy bottom could be a proxy for an environment suitable for providing food for the development of larvae, at the difference of asphalt or sand. The presence of predator fishes was associated either with a lower probability of larvae or lower larval density as it has already been described [43-45]. In Dakar, Awono-Ambéné et al. [46] had confirmed in 1998 their utility, indicating that predation in the "céanes" was probably mainly due to fishes (Gambusia and Tilapia) that have been introduced in Dakar in the 1930s for larval control and that are recommended by the National Hygiene Service. The results of the present survey confirmed the actual interest in the use of larvivorous fishes to control malaria vectors [47]. One possible limitation of the data interpretation may have lied in the use of the dipping method, which could provide weakly reproducible estimations of larval densities even though the sampling location and the person collecting the samples were consistent throughout the study. The presence/absence of larvae was probably accurately detected by this technique, especially for small water collections.

Impact of urban agriculture

Many works have been conducted about the impact of urban agriculture on malaria transmission [11-15, 48, 49] and most of them have shown that it was associated with a higher level of malaria transmission in the surroundings areas. Urban agricultural activities provide breeding sites and resting sites for malaria vectors. In the preliminary larval study in Dakar, it had been considered that market-gardens provided resting sites to *Anopheles* rather than increased number of breeding sites, as was previously demonstrated in Ghana [48]. This issue has been comforted by the result of the present analysis: water collections located in market-gardens harboured less frequently anopheline larvae and when anopheline larvae were present, the densities were lower than in surroundings places. This was mainly due to the presence of larvivorous fishes in the traditional wells in the market garden and to the frequent perturbations of water due to the watering but one cannot exclude an effect of the use of pesticide by the urban farmers [50, 51].

Spatial scale of malaria transmission and risk maps

In the present study, a very close parallel between larval density and adult density was showed, as in the 2007 study [7]. As several studies have highlighted the low dispersion of *Anopheles* in urban settings due to the high density of houses and the proximity of readily available hosts for blood meals, most of the *An. gambiae s.l.* caught on human bait in an area are probably coming from eggs laid in a breeding site in the same area or neighbouring area [15-19]. Most of the factors associated with the presence or absence of larvae and with the density of larvae in water collections are measurable or detectable, directly or indirectly, by remote sensing: soil temperature, vegetations and trees able to provide shade, moisture of the ground, type and density of urbanization, persistence of water collections during the dry season. In consequence, Remote Sensing and Geographic Information Systems can provide useful information for mapping the *Anopheles* breeding sites and it has been already showed in numerous studied [52]. The close relationship between larval density and adult aggressiveness suggests that anopheline aggressiveness maps could be deducted from maps of *Anopheles* breeding sites.

Implications for malaria control

The suitable conditions for malaria transmission were usually present only during the rainy season from July to December. The human biting rates of *An. gambiae s.l.* were increasing with the intensity of rainfall in most of the studied areas. Nevertheless, the probability to encounter an infected malaria vector was not the same among the different areas. In answer to this highly focused risk of malaria transmission, a focused approach of vector control responses has to be chosen. In a context of limitation of the financial, material and humans resources, the available resources must be concentrated on the more vulnerable populations (*e.g.* children under five years, pregnant women) but also on the populations living in areas with higher risks of transmission. As it would not possible to conduct entomological studies in the entire city of Dakar, an effort should be done to construct risk maps in order to focus the anti-vectorial activities of the national malaria control programme.

Conclusion

The spatial and temporal heterogeneity of *An. gambiae s.l.* larval density, adult aggressiveness and malaria transmission in Dakar has been confirmed, and environmental factors associated with this heterogeneity have been identified. The results of the present study pave the way to the building of malaria risk maps using Geographic Information Systems and Remote Sensing technologies, and to a focused anti-vectorial malaria control strategy in the city of Dakar.

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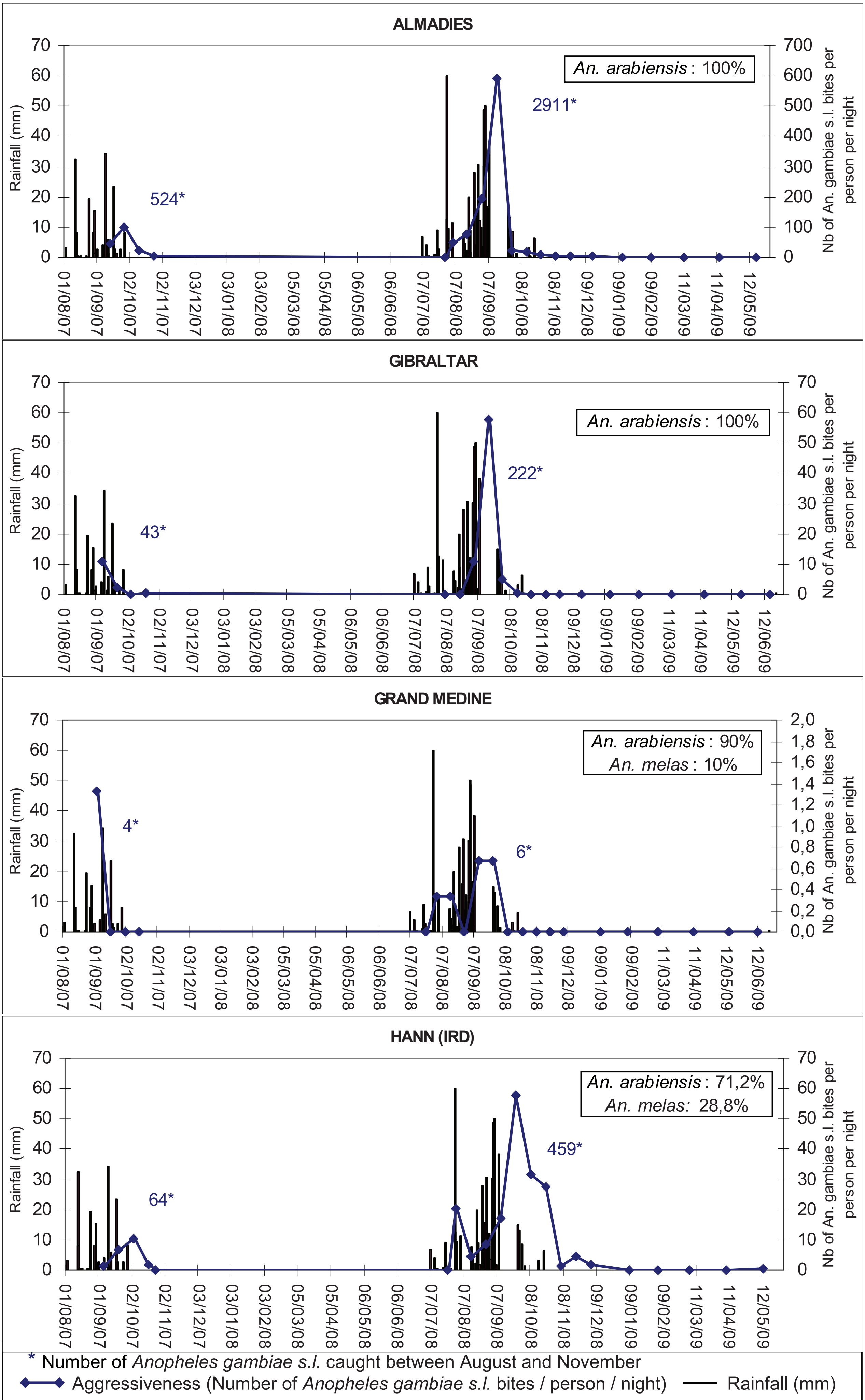
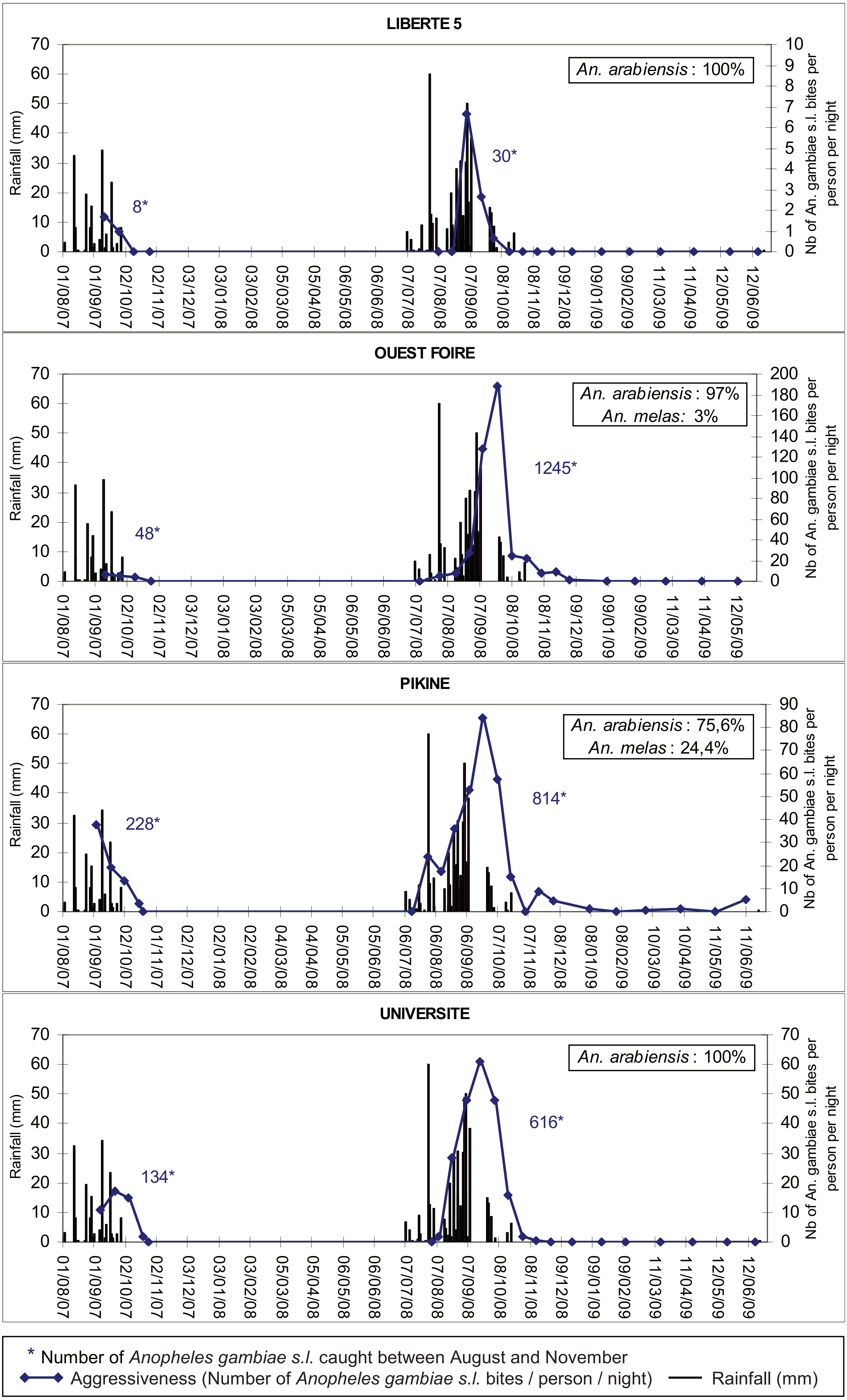
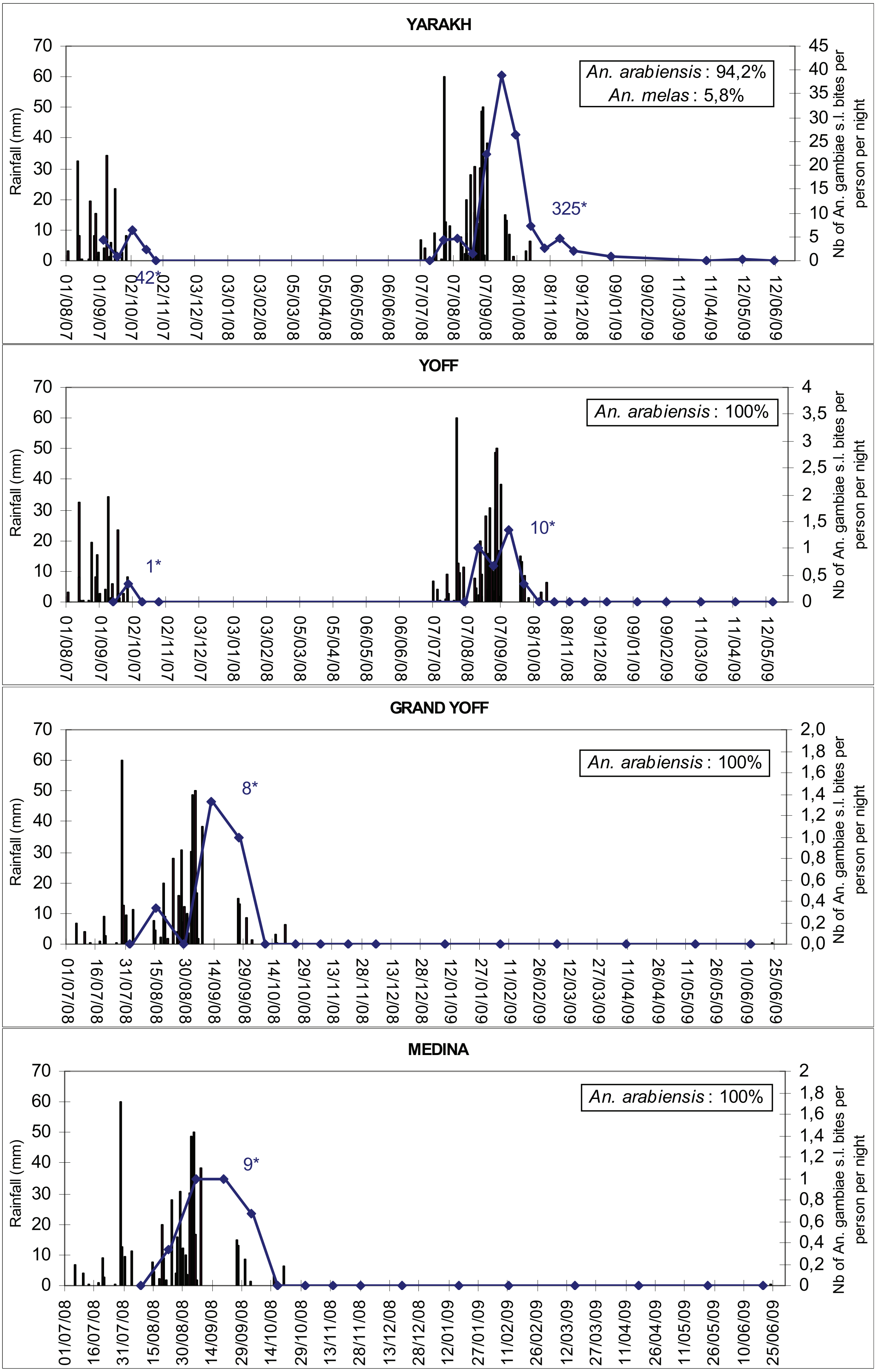
Additional files


ALMADIES
Rainfall (mm)
Nb of An. gambiae s.l. bites per person per night
An. arabiensis : 100%
524*
2911*
GIBRALTAR
An. arabiensis : 100%
43*
222*
GRAND MEDINE
An. arabiensis : 90%
An. melas : 10%
4*
6*
HANN (IRD)
An. arabiensis : 71,2%
An. melas: 28,8%
64*
459*
* Number of Anopheles gambiae s.l. caught between August and November
Aggressiveness (Number of Anopheles gambiae s.l. bites / person / night)
Rainfall (mm)

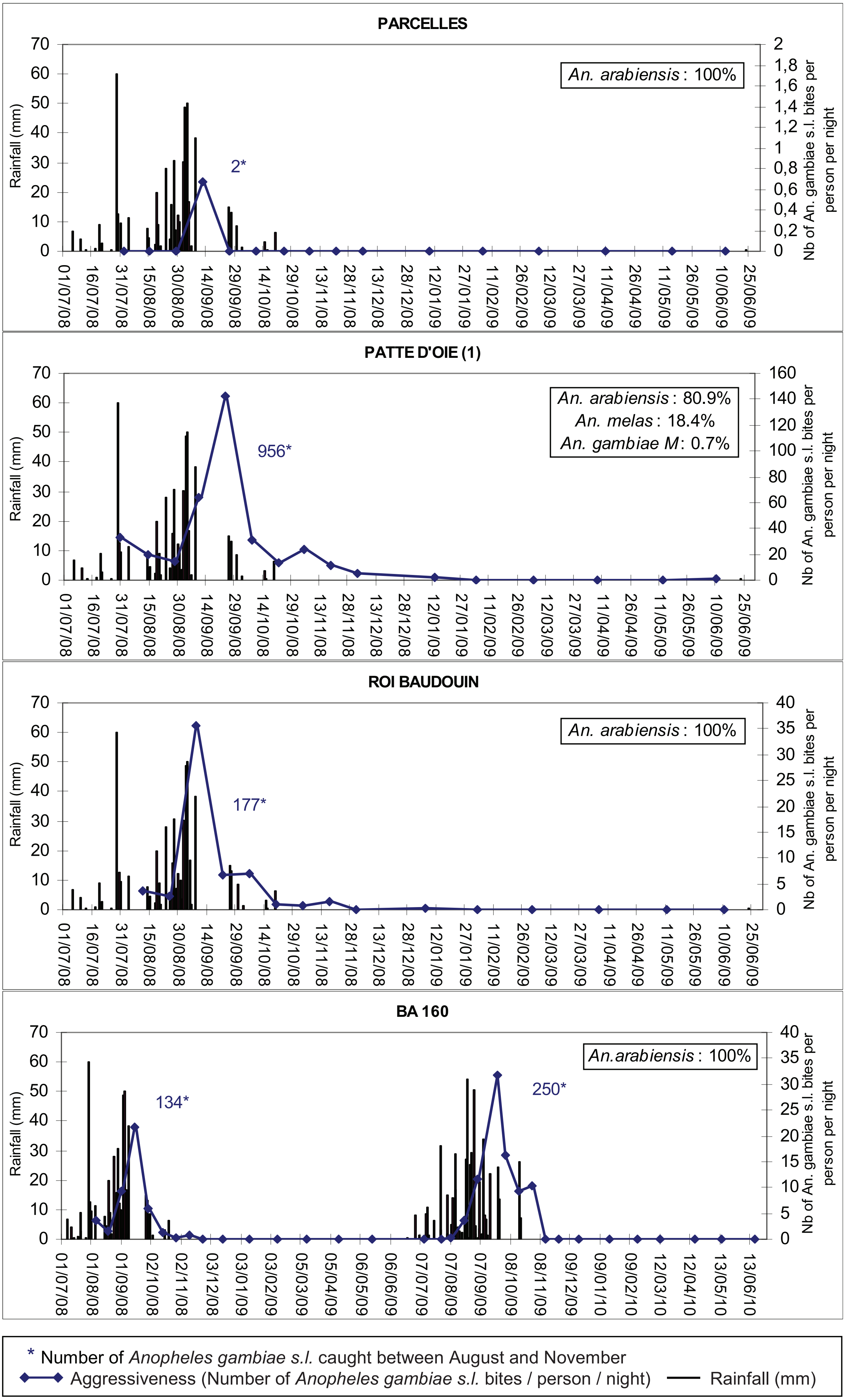


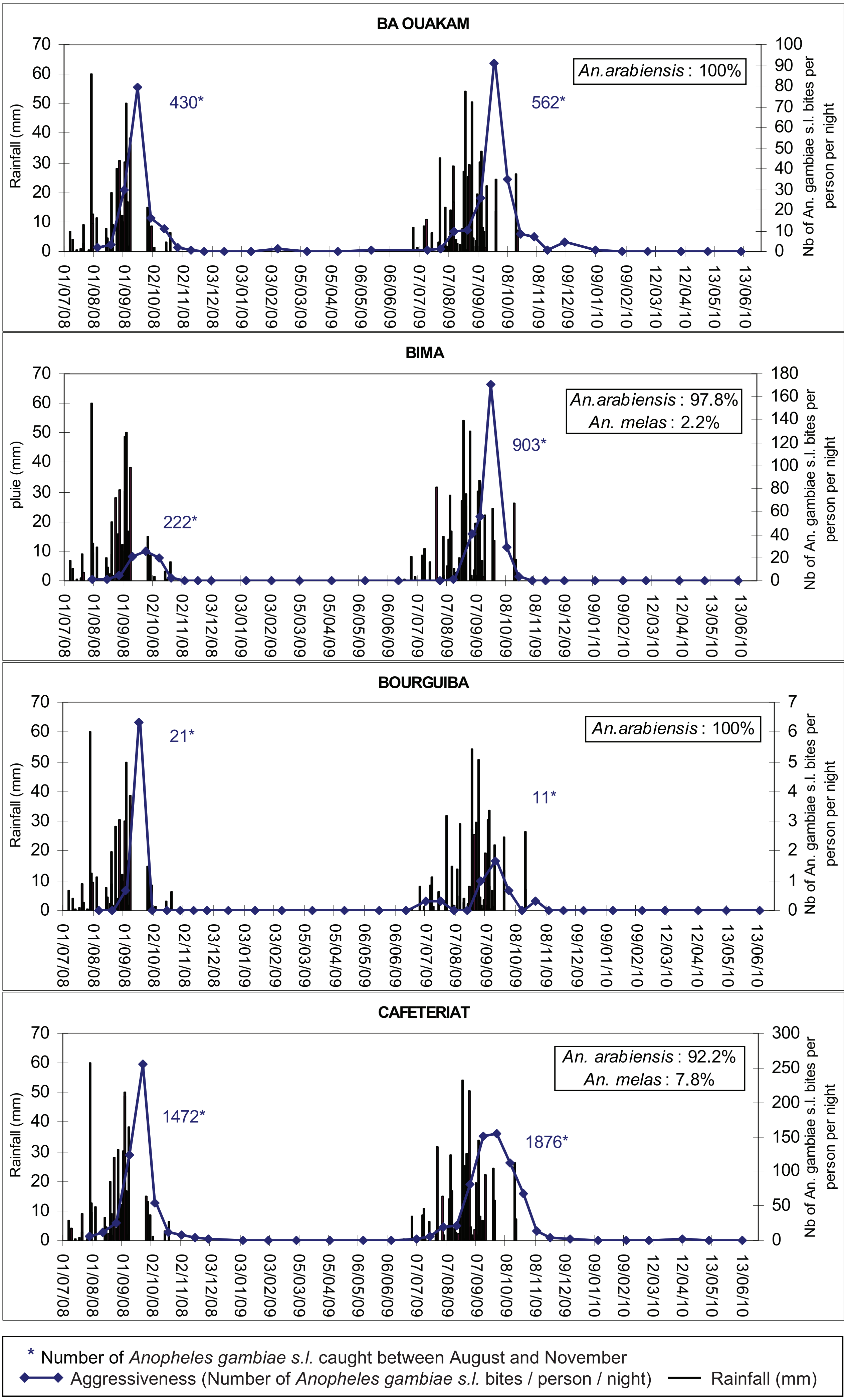
LIBERTE 5
Rainfall (mm)
Nb of An. gambiae s.l. bites per person per night
An. arabiensis : 100%
8*
30*
01/08/07 01/09/07 02/10/07 02/11/07 03/12/07 03/01/08 03/02/08 05/03/08 05/04/08 06/05/08 06/06/08 07/07/08 07/08/08 07/09/08 08/10/08 08/11/08 09/12/08 09/01/09 09/02/09 11/03/09 11/04/09 12/05/09 12/06/09
OUEST FOIRE
Rainfall (mm)
Nb of An. gambiae s.l. bites per person per night
An. arabiensis : 97%
An. melas: 3%
48*
1245*
01/08/07 01/09/07 02/10/07 02/11/07 03/12/07 03/01/08 03/02/08 05/03/08 05/04/08 06/05/08 06/06/08 07/07/08 07/08/08 07/09/08 08/10/08 08/11/08 09/12/08 09/01/09 09/02/09 11/03/09 11/04/09 12/05/09
PIKINE
Rainfall (mm)
Nb of An. gambiae s.l. bites per person per night
An. arabiensis : 75,6%
An. melas : 24,4%
228*
814*
01/08/07 01/09/07 02/10/07 01/11/07 02/12/07 02/01/08 02/02/08 04/03/08 04/04/08 05/05/08 05/06/08 06/07/08 06/08/08 06/09/08 07/10/08 07/11/08 08/12/08 08/01/09 08/02/09 10/03/09 10/04/09 11/05/09 11/06/09
UNIVERSITE
Rainfall (mm)
Nb of An. gambiae s.l. bites per person per night
An. arabiensis : 100%
134*
616*
01/08/07 01/09/07 02/10/07 02/11/07 03/12/07 03/01/08 03/02/08 05/03/08 05/04/08 06/05/08 06/06/08 07/07/08 07/08/08 07/09/08 08/10/08 08/11/08 09/12/08 09/01/09 09/02/09 11/03/09 11/04/09 12/05/09 12/06/09
* Number of Anopheles gambiae s.l. caught between August and November
Aggressiveness (Number of Anopheles gambiae s.l. bites / person / night)
Rainfall (mm)

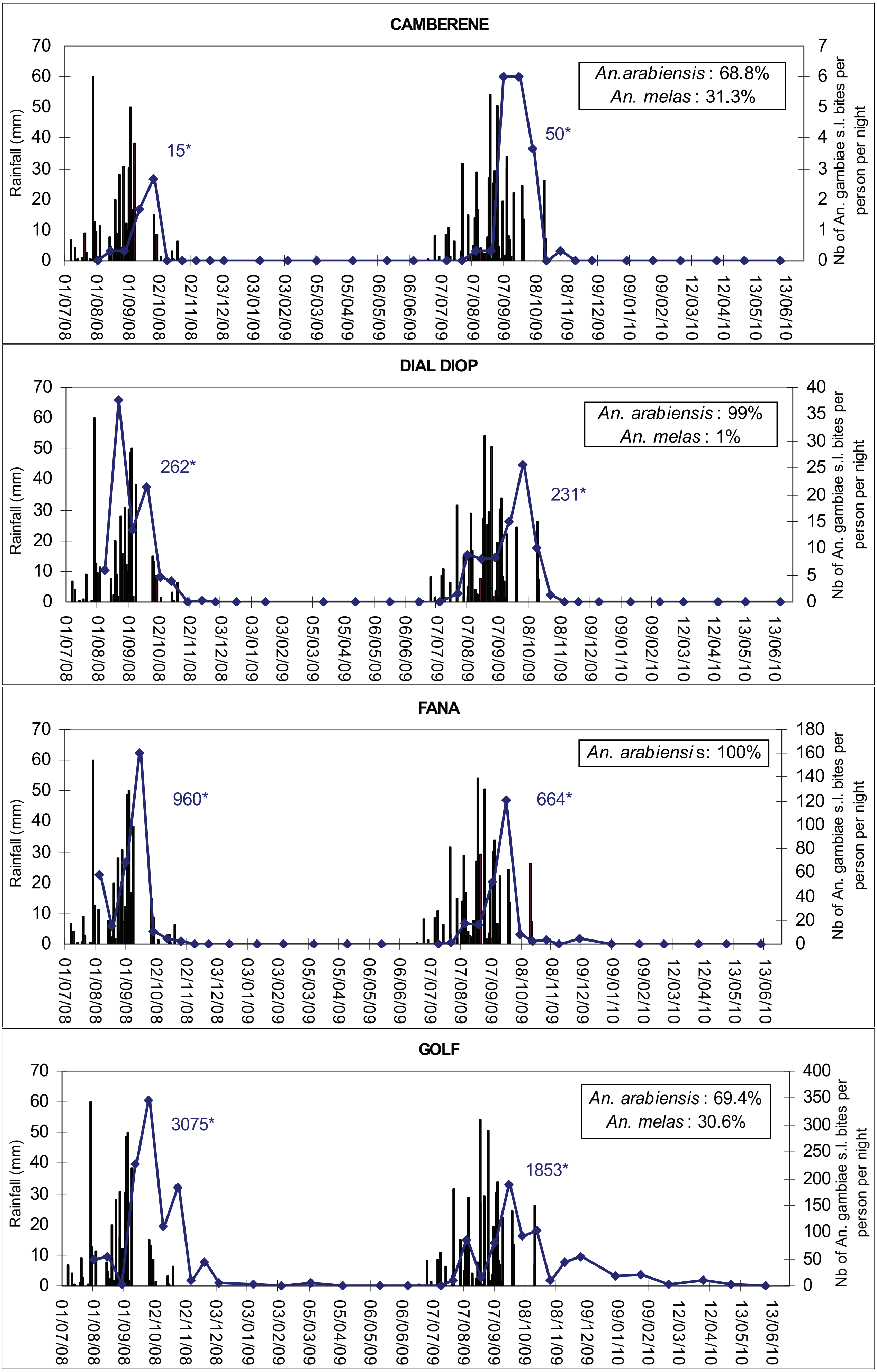


* Number of *Anopheles gambiae s.l.* caught between August and November

Aggressiveness (Number of *Anopheles gambiae s.l.* bites / person / night) — Rainfall (mm)

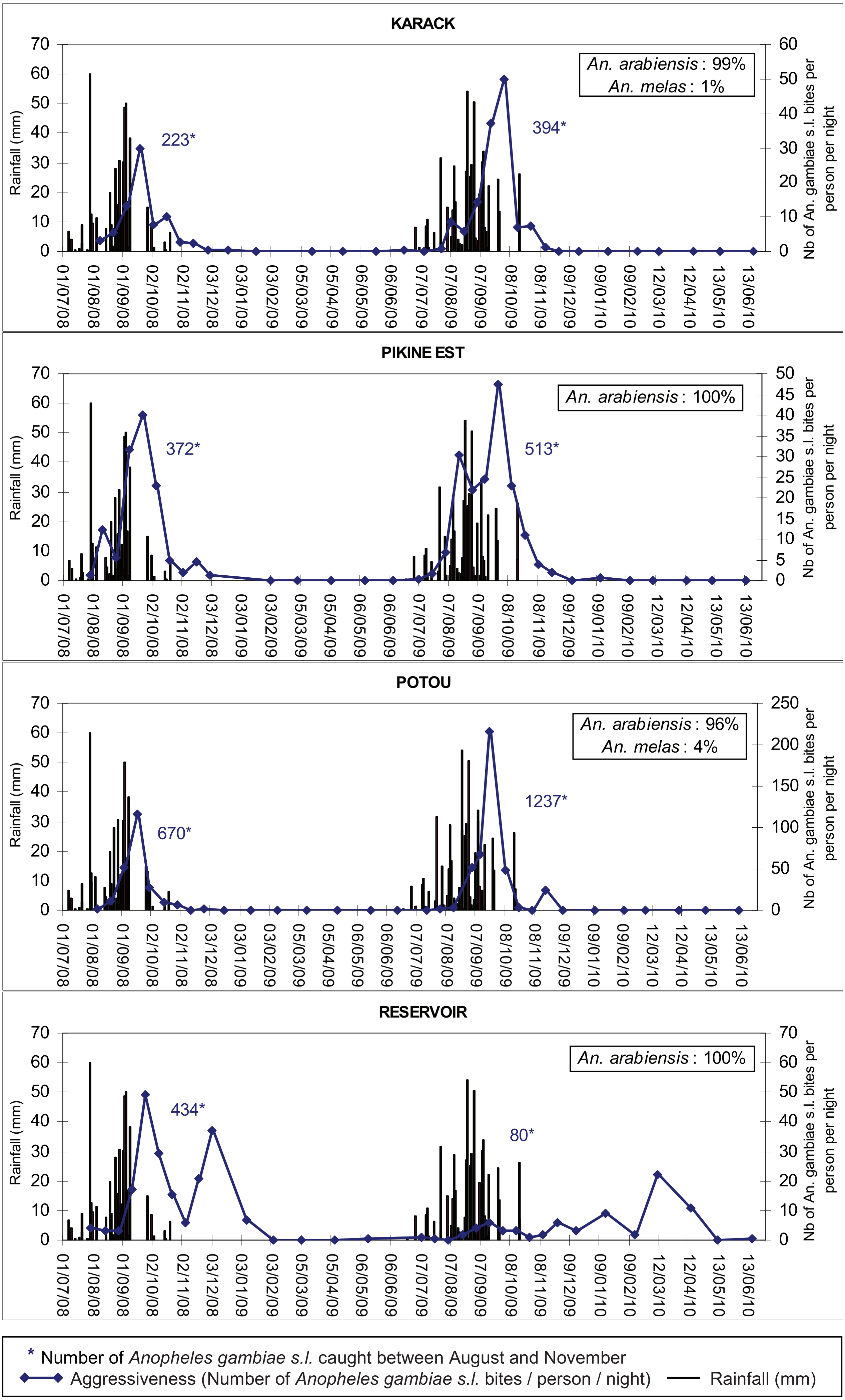

PARCELLES
An. arabiensis : 100%
2*
Rainfall (mm)
Nb of An. gambiae s.l. bites per person per night
01/07/08 16/07/08 31/07/08 15/08/08 30/08/08 14/09/08 29/09/08 14/10/08 29/10/08 13/11/08 28/11/08 13/12/08 28/12/08 12/01/09 27/01/09 11/02/09 26/02/09 12/03/09 27/03/09 11/04/09 26/04/09 11/05/09 26/05/09 10/06/09 25/06/09
PATTE D'OIE (1)
An. arabiensis : 80.9%
An. melas : 18.4%
An. gambiae M : 0.7%
956*
Rainfall (mm)
Nb of An. gambiae s.l. bites per person per night
ROI BAUDOUIN
An. arabiensis : 100%
177*
Rainfall (mm)
Nb of An. gambiae s.l. bites per person per night
BA 160
An.arabiensis : 100%
134*
250*
Rainfall (mm)
Nb of An. gambiae s.l. bites per person per night
01/07/08 01/08/08 01/09/08 02/10/08 02/11/08 03/12/08 03/01/09 03/02/09 05/03/09 05/04/09 06/05/09 06/06/09 07/07/09 07/08/09 07/09/09 08/10/09 08/11/09 09/12/09 09/01/10 09/02/10 12/03/10 12/04/10 13/05/10 13/06/10
* Number of Anopheles gambiae s.l. caught between August and November
Aggressiveness (Number of Anopheles gambiae s.l. bites / person / night)
Rainfall (mm)

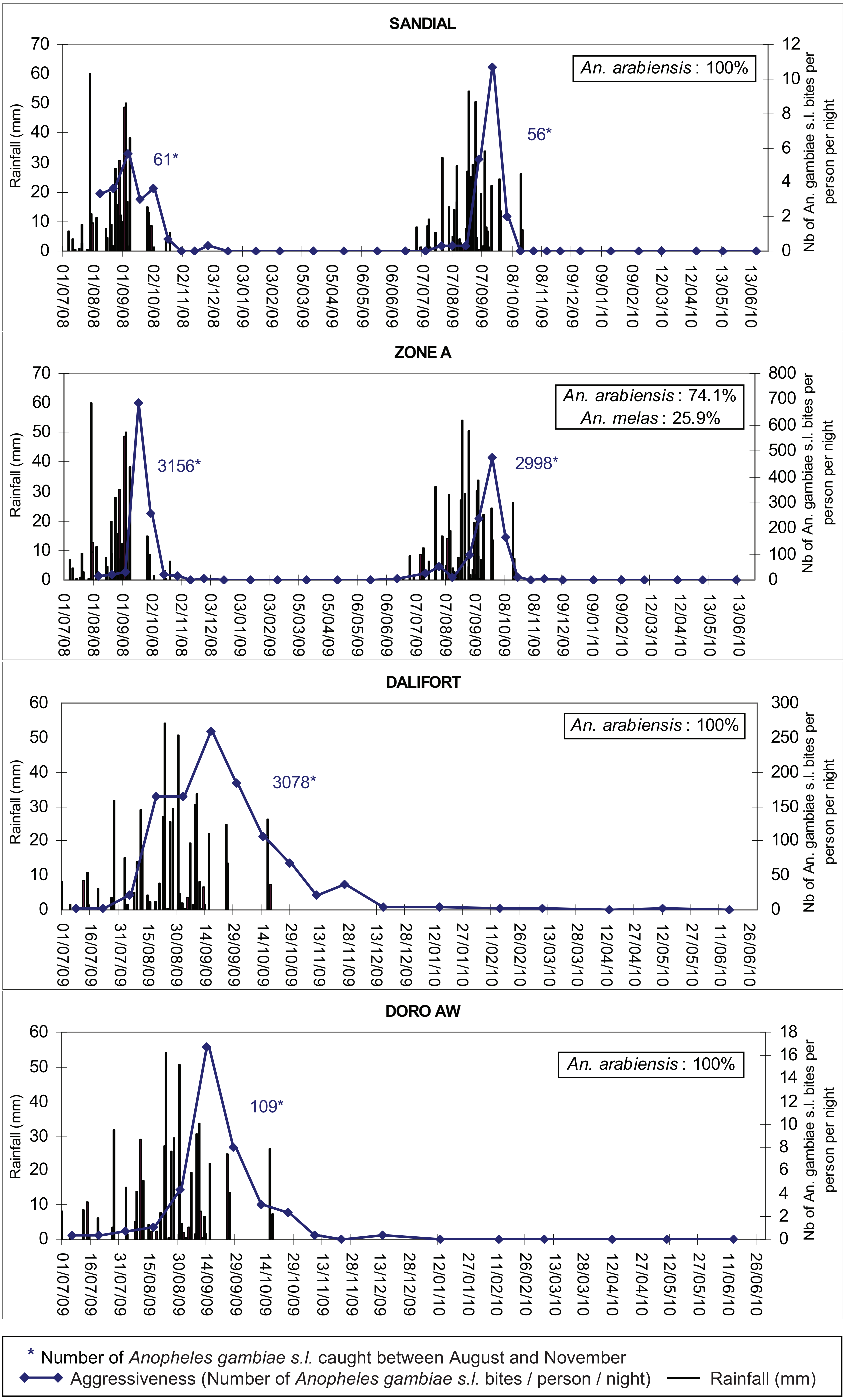

BA OUAKAM
Rainfall (mm)
Nb of An. gambiae s.l. bites per person per night
An.arabiensis : 100%
430*
562*
BIMA
pluie (mm)
Nb of An. gambiae s.l. bites per person per night
An.arabiensis : 97.8%
An. melas : 2.2%
222*
903*
BOURGUIBA
Rainfall (mm)
Nb of An. gambiae s.l. bites per person per night
An.arabiensis : 100%
21*
11*
CAFETERIAT
Rainfall (mm)
Nb of An. gambiae s.l. bites per person per night
An. arabiensis : 92.2%
An. melas : 7.8%
1472*
1876*
01/07/08
01/08/08
01/09/08
02/10/08
02/11/08
03/12/08
03/01/09
03/02/09
05/03/09
05/04/09
06/05/09
06/06/09
07/07/09
07/08/09
07/09/09
08/10/09
08/11/09
09/12/09
09/01/10
09/02/10
12/03/10
12/04/10
13/05/10
13/06/10
* Number of Anopheles gambiae s.l. caught between August and November
Aggressiveness (Number of Anopheles gambiae s.l. bites / person / night)
Rainfall (mm)

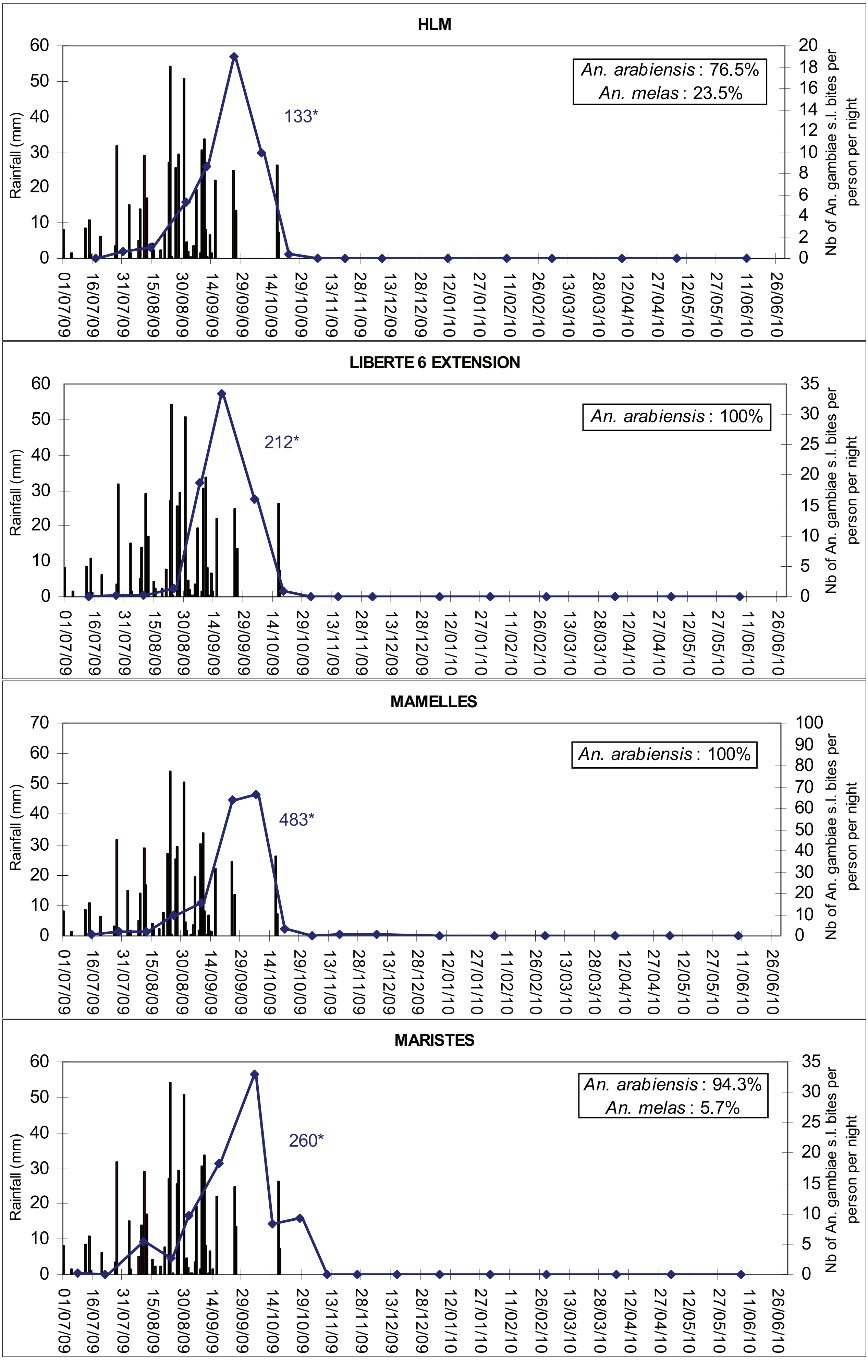


* Number of *Anopheles gambiae s.l.* caught between August and November

Aggressiveness (Number of *Anopheles gambiae s.l.* bites / person / night) — Rainfall (mm)

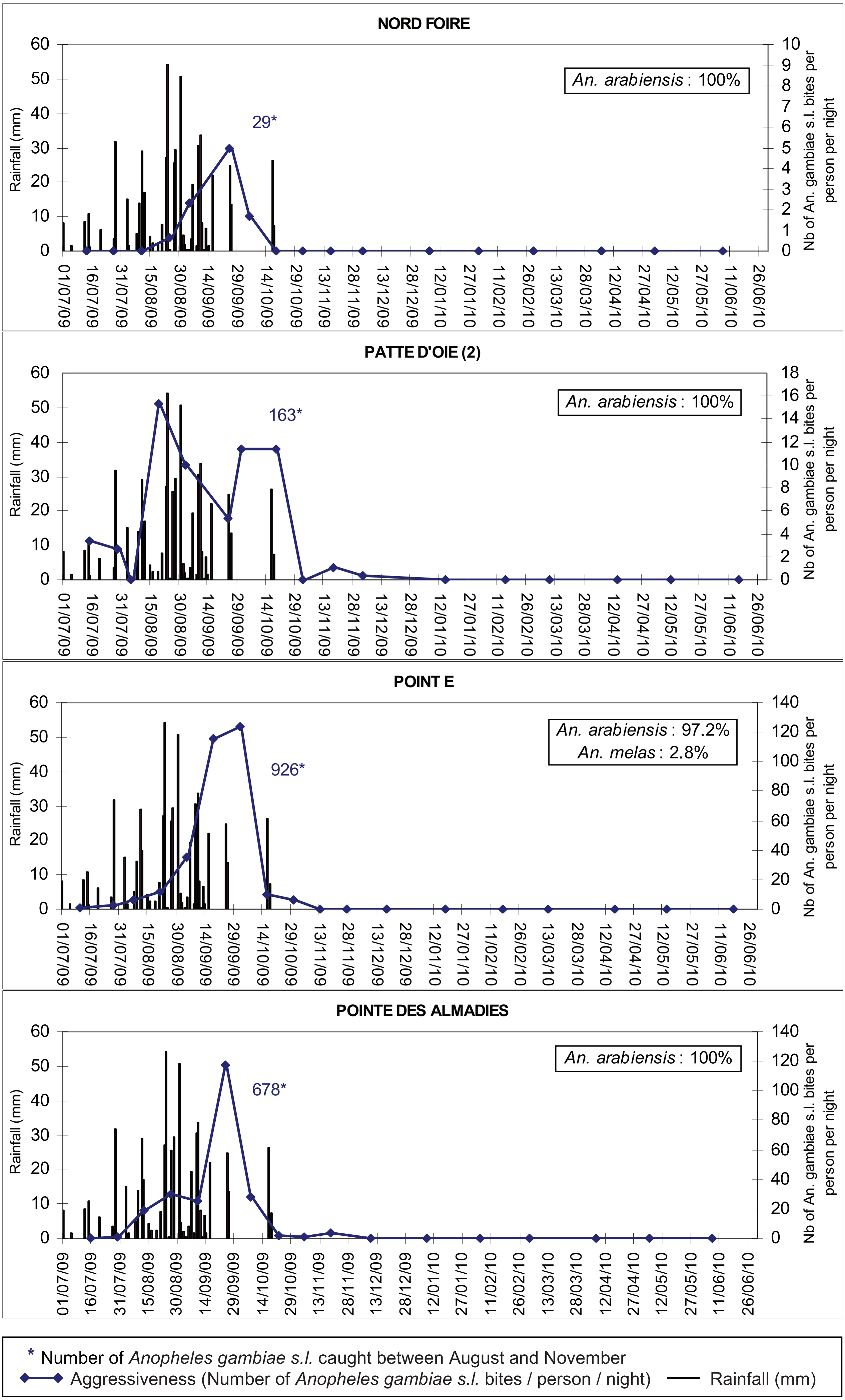

KARACK
An. arabiensis : 99%
An. melas : 1%
223*
394*
PIKINE EST
An. arabiensis : 100%
372*
513*
POTOU
An. arabiensis : 96%
An. melas : 4%
670*
1237*
RESERVOIR
An. arabiensis : 100%
434*
80*
Rainfall (mm)
Nb of An. gambiae s.l. bites per person per night
01/07/08
01/08/08
01/09/08
02/10/08
02/11/08
03/12/08
03/01/09
03/02/09
05/03/09
05/04/09
06/05/09
06/06/09
07/07/09
07/08/09
07/09/09
08/10/09
08/11/09
09/12/09
09/01/10
09/02/10
12/03/10
12/04/10
13/05/10
13/06/10
* Number of Anopheles gambiae s.l. caught between August and November
Aggressiveness (Number of Anopheles gambiae s.l. bites / person / night)
Rainfall (mm)

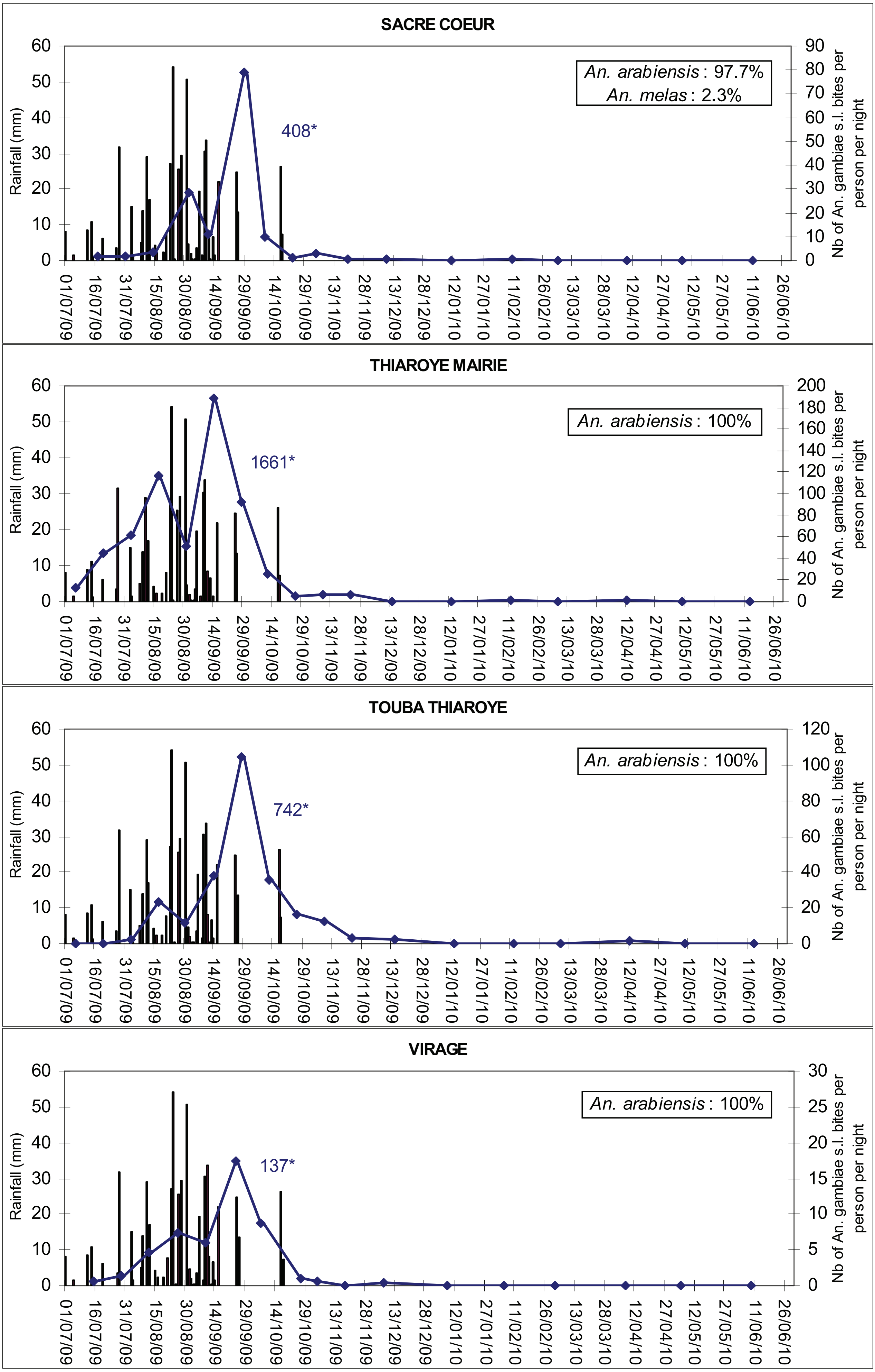

SANDIAL
Rainfall (mm)
Nb of An. gambiae s.l. bites per person per night
An. arabiensis : 100%
61*
56*
ZONE A
Rainfall (mm)
Nb of An. gambiae s.l. bites per person per night
An. arabiensis : 74.1%
An. melas : 25.9%
3156*
2998*
DALIFORT
Rainfall (mm)
Nb of An. gambiae s.l. bites per person per night
An. arabiensis : 100%
3078*
DORO AW
Rainfall (mm)
Nb of An. gambiae s.l. bites per person per night
An. arabiensis : 100%
109*
* Number of Anopheles gambiae s.l. caught between August and November
Aggressiveness (Number of Anopheles gambiae s.l. bites / person / night)
Rainfall (mm)



* Number of *Anopheles gambiae s.l.* caught between August and November

Aggressiveness (Number of *Anopheles gambiae s.l.* bites / person / night) — Rainfall (mm)


NORD FOIRE
Rainfall (mm)
Nb of An. gambiae s.l. bites per person per night
An. arabiensis : 100%
29*
PATTE D'OIE (2)
An. arabiensis : 100%
163*
POINT E
An. arabiensis : 97.2%
An. melas : 2.8%
926*
POINTE DES ALMADIES
An. arabiensis : 100%
678*
01/07/09
16/07/09
31/07/09
15/08/09
30/08/09
14/09/09
29/09/09
14/10/09
29/10/09
13/11/09
28/11/09
13/12/09
28/12/09
12/01/10
27/01/10
11/02/10
26/02/10
13/03/10
28/03/10
12/04/10
27/04/10
12/05/10
27/05/10
11/06/10
26/06/10
* Number of Anopheles gambiae s.l. caught between August and November
Aggressiveness (Number of Anopheles gambiae s.l. bites / person / night)
Rainfall (mm)



* Number of *Anopheles gambiae s.l.* caught between August and November

Aggressiveness (Number of *Anopheles gambiae s.l.* bites / person / night) — Rainfall (mm)

PARTIE III - Modélisation du risque de paludisme en milieu urbain

Les articles 3 et 4 ont démontré l'hétérogénéité spatiale et temporelle de la répartition des gîtes larvaires et des agressivités anophéliennes dans la ville de Dakar. Les données présentées dans ces deux articles constituent la base de travail entomologique pour cette partie III. A une échelle locale, le concept d'unité focale de transmission a été décrit comme un système dans lequel le gîte larvaire est le centre d'un foyer de transmission [20]. De plus, le management ciblé des gîtes les plus productifs permettrait une réduction de la population des moustiques mais aussi de l'incidence du paludisme [21]. Ainsi, le contrôle de la maladie dans la ville bénéficierait de la disponibilité de cartes de ces zones de plus forte transmission.

Le paludisme, de part sa transmission vectorielle, est fortement dépendant de l'environnement, que cela soit au niveau entomologique ou épidémiologique. Des informations de télédétection pourraient donc apporter une aide précieuse à la cartographie du risque de paludisme.

L'objectif principal du travail de modélisation était d'obtenir, grâce à des données d'observation de la terre par satellite, 1) une carte des gîtes larvaires accompagné d'un indice de productivité dans la ville de Dakar, 2) une carte des densités d'agressivité des anophèles adultes, puis 3) de rendre ces cartes dynamiques, c'est-à-dire de prendre en compte les variations temporelles liées aux variations de leurs déterminants météorologiques. Les plans larvaires et adultes ont été considérés en cohérence avec un objectif opérationnel futur de cartographie utile à la fois dans la lutte antilarvaire et anti-imago. La démarche a donc été faite pas à pas, de la présence des collections d'eau de surface à leur utilisation comme gîte larvaire puis à la production et à la dispersion des imagos.

Pour obtenir une preuve de concept, un premier travail a utilisé les données d'agressivités anophéliennes dans des quartiers centraux de Dakar datant de 1994-1997, disponibles dans la littérature scientifique, et les données des travaux entomologiques que nous avions menés en septembre-octobre 2007 dans 10 quartiers de Dakar (présentés dans l'article 3). Une cartographie du milieu urbain a pu être extraite d'images satellites haute résolution puis associée aux niveaux des densités anophéliennes. La disponibilité de données de terrain et de données satellites à 11 années d'intervalle a permis, en appliquant des modèles de prédiction identiques, de mettre en évidence les évolutions des zones à risque de transmission et d'affirmer que le pourcentage de la population dakaroise à fort risque de transmission avait diminué entre 1996 et 2007. Pour ce travail, les niveaux d'urbanisation étaient les prédicteurs du risque de piqûres d'anophèles. La relation était recherchée directement entre l'environnement et les densités de moustiques adultes.

Au cours de l'étape suivante des travaux de thèse nous nous sommes attachés à mieux prendre en compte la connaissance de la biologie des vecteurs dans la modélisation de l'agressivité anophélienne. Trois niveaux de cartes ont ainsi été produits pour les années 2007, 2008 et 2009. Tout d'abord, plusieurs indicateurs extraits d'images satellite haute résolution ont été utilisés pour générer une carte des collections d'eau à Dakar. Ensuite, d'autres indicateurs satellites, ainsi que des données météorologiques de pluie (donnée de terrain) et de température (donnée de télédétection) ont pu être associés à la présence de larves d'anophèles mesurée sur le terrain, puis extrapolés à la carte des collections d'eau pour la transformer en carte de gîtes larvaires. Pour finir, les agressivités anophéliennes mesurées sur le terrain pendant ces trois années ont pu être ajustées par un modèle avec une précision satisfaisante grâce à la cartographie préalable des gîtes larvaires. Le calcul du risque d'agressivité a alors pu être étendu à tout Dakar pour les trois années. Les deux niveaux de cartographie - gîtes larvaires et densités d'adultes - pourraient être améliorés pour devenir des outils à part entière d'aide à l'orientation des actions de lutte sur le terrain.

Les deux sous parties suivantes présentent ces travaux de cartographie du risque. L'état actuel de préparation de l'article 6 est présenté. Quelques commentaires y sont associés en addendum.

ARTICLE 5

Machault V, Vignolles C, Pages F, Gadiaga L, Gaye A, Sokhna C, Trape JF, Lacaux JP, Rogier C: **Spatial heterogeneity and temporal evolution of malaria transmission risk in Dakar, Senegal, according to remotely sensed environmental data**. *Malar J* 2010, **9**:252.

ARTICLE 6

Machault V, Vignolles C, Pages F, Gadiaga L, Gaye A, Sokhna C, Trape JF, Lacaux JP., Rogier C: **Mapping of spatial and temporal distribution of *Anopheles* larvae and adults in Dakar.** *En préparation*

ARTICLE 5

Spatial heterogeneity and temporal evolution of malaria transmission risk in Dakar, Senegal, according to remotely sensed environmental data

Machault V, Vignolles C, Pages F, Gadiaga L, Gaye A, Sokhna C, Trape JF, Lacaux JP, Rogier C

Malar J 2010, **9**:252.

Machault et al. Malaria Journal 2010, 9:252
http://www.malariajournal.com/content/9/1/252

RESEARCH Open Access

Spatial heterogeneity and temporal evolution of malaria transmission risk in Dakar, Senegal, according to remotely sensed environmental data

Vanessa Machault[1,2,3,4*], Cécile Vignolles[3], Frédéric Pagès[2], Libasse Gadiaga[5], Abdoulaye Gaye[5], Cheikh Sokhna[5], Jean-François Trape[5], Jean-Pierre Lacaux[4], Christophe Rogier[1]

Abstract

Background: The United Nations forecasts that by 2050, more than 60% of the African population will live in cities. Thus, urban malaria is considered an important emerging health problem in that continent. Remote sensing (RS) and geographic information systems (GIS) are useful tools for addressing the challenge of assessing, understanding and spatially focusing malaria control activities. The objectives of the present study were to use high spatial resolution SPOT (*Satellite Pour l'Observation de la Terre*) satellite images to identify some urban environmental factors in Dakar associated with *Anopheles arabiensis* densities, to assess the persistence of these associations and to describe spatial changes in at-risk environments using a decadal time scale.

Methods: Two SPOT images from the 1996 and 2007 rainy seasons in Dakar were processed to extract environmental factors, using supervised classification of land use and land cover, and a calculation of NDVI (Normalized Difference Vegetation Index) and distance to vegetation. Linear regressions were fitted to identify the ecological factors associated with *An. arabiensis* aggressiveness measured in 1994-97 in the South and centre districts of Dakar. Risk maps for populated areas were computed and compared for 1996 and 2007 using the results of the statistical models.

Results: Almost 60% of the variability in anopheline aggressiveness measured in 1994-97 was explained with only one variable: the built-up area in a 300-m radius buffer around the catching points. This association remained stable between 1996 and 2007. Risk maps were drawn by inverting the statistical association. The total increase of the built-up areas in Dakar was about 30% between 1996 and 2007. In proportion to the total population of the city, the population at high risk for malaria fell from 32% to 20%, whereas the low-risk population rose from 29 to 41%.

Conclusions: Environmental data retrieved from high spatial resolution SPOT satellite images were associated with *An. arabiensis* densities in Dakar urban setting, which allowed to generate malaria transmission risk maps. The evolution of the risk was quantified, and the results indicated there are benefits of urbanization in Dakar, since the proportion of the low risk population increased while urbanization progressed.

Background

Malaria and urbanization

Urbanization is occurring at a rapid pace in Africa, and the United Nations forecasts that by 2050, more than 60% of the African population will live in cities [1]. Inescapably, those changes will have consequences on the health of local populations. Regarding malaria, many papers and reviews have reported the existence of transmission in urban areas, even if levels are usually lower than in peri-urban and rural places [2,3]. The epidemiology of malaria in cities is specific, and the urban form of the disease is considered to be an emerging health problem of major importance in Africa [4].

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Full list of author information is available at the end of the article

The urban malaria burden, as well as its spatial and temporal distribution, is closely related to a wide range of factors, such as the degree and type of urbanization, the density of the human population, vector control measures, access to health care [2,5] and adaptation of the vector to new or polluted breeding sites [6-9]. Urbanization has a great impact on the composition of the vector system and malaria transmission dynamics [10]. Moreover, in urban settings, blood meal sources are abundant, dispersion of the vectors is low and malaria transmission is highly driven by the proximity of breeding sites [11,12]. Malaria risk is heterogeneous over small distances, and transmission can vary among different districts of the same city, as shown in Brazzaville [13] and Dakar [14].

Malaria and remote sensing

Risk maps can be useful for decision-makers who are seeking to address the challenge of assessing, understanding and spatially focusing malaria control activities [15]. Over the last several decades, remote sensing (RS) and geographic information systems (GIS) have become popular tools for evaluating the environmental, meteorological and climatic factors leading to the geographical and temporal distribution of malaria risk [16-20]. The majority of studies have been conducted in rural settings, but some research has focused on cities. For example, previous studies have conducted direct and indirect identification of *Anopheles* breeding sites, sought to predict malariometric indices and assessed impact of environmental changes on malaria risk [21-24].

Malaria in Dakar

In Dakar, the capital city of Senegal, parasite rates and incidences of clinical malaria attacks in the city and its periphery have been at low levels relative to continent-wide rates [2,11,25,26]. Nevertheless, some malaria cases have been recognized as autochthonous [26], and severe cases have been reported [27-29]. Additionally, placental malaria infections have been associated with preeclampsia in pregnant women with poor malaria immunity [30]. In this clinical context, local malaria transmission has been studied for several decades and has been assessed in Dakar [14,31] and its close suburb Pikine [11,32].

Objectives

The objectives of the present study were to use high spatial resolution SPOT (*Satellite Pour l'Observation de la Terre*) satellite images to identify some urban environmental factors associated with *Anopheles arabiensis* densities in Dakar, to assess the persistence of these associations and to describe spatial changes in at-risk environments at a decadal time scale.

Methods

Study site

Dakar (14°40'20" North, 17°25'22" West), the capital city of Senegal, is located in the Cap-Vert peninsula at the westernmost point of Africa. The altitude peaks at 104 m above sea level (Mamelles). The climate is mild sahelian. The hot and wet season lasts from June to November with average temperatures between 24 and 30°C. The cool and dry season lasts from December to May with average temperatures between 19 and 25°C. The first rains generally occur at the end of June or the beginning of July, and the last ones at the beginning of October. In 1994, 1996 and 2007, the annual rainfalls were 252, 350 [25,26] and 178 mm (data from the national weather forecast), respectively. The estimated population of the Dakar urban area was close to 1 million inhabitants in 2005, accounting for about 20% of the country's population. The population density was about 12,000 inhabitants per km^2.

Entomological data from the literature

Part of the entomological data used in the present study were from two studies of Diallo *et al*, which were conducted in both the south [25] and centre [26] sanitary districts of Dakar, and published in 1998 and 2000, respectively.

The first paper described entomological fieldwork from June 1994 to May 1995 in 13 sites, and the second paper reported mosquito prospecting from March 1996 to February 1997 in 12 sites. In both studies, each site was 1.5 km (North-South) and 1 km (East-West) distant from the adjacent ones. Adult mosquito sampling was carried out once every month during the studied period by human landing catch, both indoors (one catching point) and outdoors (one catching point).

In the southern district, a total of 308 person/nights of adult mosquito captures were undertaken. Among the 16 637 females collected, 83 (0.5%) were *Anopheles*, 81 were *An. arabiensis* and two were *Anopheles pharoensis. Anopheles arabiensis* densities were low, peaking at 1.76 bites per person per night in October 1994 (mean value for all sites). In the centre district, a total of 308 person/nights of capture were also conducted. Among 6 157 collected female mosquitoes, 92 (1.5%) were *Anopheles*, and all of them were *An. arabiensis*. Maximum aggressiveness was 2.25 bites per person per night in September 1996 (mean value for all sites). In both districts, none of the *An. arabiensis* were CSP antigen positive.

For the purpose of the present study, the mean number of *An. arabiensis* bites per person per night for both of the 12-month study periods was considered and hereafter is called the "1994-97 aggressiveness". Each site was surveyed between 22 and 24 nights so the full transmission season (September-October) was covered. Thus, even if the study periods for the two sanitary districts were a little bit staggered, it was considered that the figures from the two studies were comparable.

Field entomological data collection

The field work in the present study has been described elsewhere [14]. Briefly, it was conducted in Dakar and Pikine, a nearby suburb. The studied sites were sampled in order to cover as many diverse environments as possible in terms of type of urbanization and vegetation. The results for nine studied areas are reported in the present paper. Adult mosquito sampling was carried out once every two weeks during September and October 2007. Human landing catch of adult mosquitoes was conducted both indoors (one catching point) and outdoors (two catching points) for a total of four nights of capture in each of the studied areas. Published *An. arabiensis* densities were calculated as the mean aggressiveness for two months of mosquito collection [14]. In order to compare those data with the data from the centre and south districts, aggressiveness values were averaged over a 12-month period for the present study, assuming that most of the annual *Anopheles* density was caught during the September-October period.

Geolocation of study sites

The geolocations of the catching points of the south and centre districts were not reported in the published articles. Therefore, a member of the team who had participated in the fieldwork in 1994-95 and 1996-97 went back to the capture houses with a GPS (global positioning system) receiver to record appropriate geographic coordinates. Between both districts, 22 sites out of 25 were successfully geolocated on the ground, and three points were approximated. The "Hann - Village" and "Caserne Gendarmerie de Potou" catching houses could not be relocated, so the point was located in the neighbourhood. In "Bop-CerfVolant", the slum existing in 1996-97 was destroyed, so the point was set in the formerly built-up area identified on available aerial photographs from 1997. For the 2007 sites, precise geographic coordinates were available for every capture location. The geographic centre of the three catching points was used for each of the nine sites as the unique location. A description of all the sampled areas is available in the three original articles.

Satellite images and aerial photographs

SPOT View imagery products were acquired for the following dates: 30 October 1996 and 26 September 2007. Images were "Level 3" pre-processed (orthoimages), so they were already georeferenced, and their map projection (UTM zone 28, WGS 84 datum) was based on ground control points and a digital elevation model (DEM). The location accuracy of the images was less than 10 m [33]. For 1996, the SPOT-4 image had a 20-m spatial resolution and three spectral bands: two in the visible (green and red) and one in the near infrared (NIR). For 2007, one SPOT-5 image had a 2.5-m spatial resolution and the same three spectral bands than the 1996 image. A second SPOT-5 image had one band at a 10-m spatial resolution for short wave infrared (SWIR). These SPOT images covered a large area of the Cap-Vert Peninsula, so views were resized to cover Dakar city and its suburbs until Pikine (lower left corner: 17° 31'44"W 14°38'44"N; upper right corner: 17°23'21,95"W 14°47'26,23"N).

A panchromatic QuickBird image (0.61 m resolution, projection UTM zone 28, WGS 84 datum) from 2005 was also available for the studied urban area. Finally, aerial photographs from 1997 were scanned from paper at the "*IGN France International - Bureau de Coordination Projet Cartographie du Sénégal 1/200 000*" in Dakar. Quickbird image and aerial photographs were georeferenced on the SPOT images using control points in ENVI 4.3 (ITTVIS software). QuickBird image and aerial photographs were used for visual support only, whereas SPOT images were processed for the analysis.

Pre-processing of SPOT images

All image processing was conducted with ENVI 4.3. A common mask of the sea was digitized and applied to all the images, whereas specific cloud masks were digitized for each date. Clouds and their shadows covered 379 Ha on the 2007 image but did not hide any of the entomological sampling zones. No clouds were observed in the studied zone on the 1996 image. To reach the objective of comparing images of different spatial resolutions, the 2007 views were resampled at 20 m, averaging values of the pixels contributing to the output pixel. Both resulting images were stacked to produce a new multiband image encompassing the full spectral resolution, hereafter called the 2007 image. Because comparisons of multi-date images could be impeded by differences in atmospheric conditions from one date to another, internal average relative reflectance (IARR) calibration was undertaken to normalize images to a scene average spectrum [34,35]. All further image processing was based on these calibrated images.

NDVI and distance to vegetation

The NDVI is the ratio of two spectral bands available in SPOT imagery and is calculated as follow: NDVI = (NIR - Red)/(NIR + Red). The result can range from -1 to +1, where high values correspond to a dense and active vegetation cover. The NDVI images were calculated from both the 1996 and 2007 images. A threshold of 0.1 was used to best separate vegetated from non-vegetated pixels and to produce binary images for 1996 and 2007. This step was conducted by an operator with good knowledge of Dakar city, who was aided by the examination of the QuickBird image and the aerial photographs. To eliminate isolated pixels, a majority filter was applied to the resulting binary images. All pixels were replaced by the majority class in a passing window (62.5 × 62.5 m).

Land use and land cover classification

Various classification techniques were investigated. Unsupervised classification (ISODATA) was ignored as many confusions occurred, e.g., between water and dark asphalt. Supervised maximum likelihood classification was chosen to generate maps of land use and land cover. Each pixel was assigned to the class having the highest probability to be the correct one based on a set of training areas. No exclusion threshold was defined, so every pixel of the studied zone was classified.

Training polygons were digitized by an operator with good knowledge of the town who photo-interpreted and examined the SPOT and QuickBird images and aerial photographs. Separability of the different classes was regularly computed to assist the definition of the polygons. Training sets were chosen exclusively where no visible land changes occurred between 1996 and 2007. Thus classifications were done in 1996 and 2007 with the same training polygons, which should maximize the comparability of the results. Three-hundred forty-nine training polygons were digitized in the 1996 and 2007 images. They covered 127 ha, representing about 1% of the total zone (excluding the sea). Thirteen land cover classes were defined, which were distributed as five urban classes (depending of the type of buildings and soils), one vegetation class, one water class and six bare soil classes (asphalt, sand, other types of soils, mixed or not with vegetation) (Additional File 1).

The quality of the resulting supervised classified images was assessed by calculating the kappa statistics, which provide a measurement of the agreement between the classes issued from the classification and the training polygons. The majority filter (62.5 × 62.5 m) was also applied to the resulting images to eliminate isolated pixels. The sea coast was masked as the classification quality was low for this particular land cover.

Geographic information system (GIS)

A GIS was built in ArcGIS 9.2 (Environmental Research Systems Institute, Redlands, CA). The layers were added as follows: map of vegetation (corresponding to the filtered map of NDVI > 0.1) for 1996 and 2007, results of the filtered supervised classifications for 1996 and 2007, 23 points corresponding to the 1994-97 sampling locations with related aggressiveness values and nine points for 2007 also with aggressiveness values. For every catching point, the Euclidian distance to the first vegetated pixel was computed, and the number of pixels of each of the 13 land use and land cover classes at several radius buffers (from 100 m to 500 m) was calculated. Spatial autocorrelations between aggressivenesses were investigated using the Moran's I index.

Statistical analysis

Statistical associations between the 1994-97 aggressiveness and the data issued from the 1996 image were first investigated to identify which environmental factors were associated with the *An. arabiensis* densities (Step 1). Then, associations between the 1994-97 aggressiveness and the data issued from the 2007 image were examined in order to assess the persistence of the associations over time (Step 2). Finally, external validation (Step 3) was undertaken by researching the associations between the 2007 aggressiveness and the 2007 image, using the variables found to be significantly associated in Step 1. Thus, the quality of the predictions of *An. arabiensis* densities from Step 1 was assessed. The dependent variables were square root transformed, and linear regression models were fitted. All combinations of classes in the 100-m to 500-m radius buffers were tested as independent variables in order to obtain the best association. All statistical analyses were performed using STATA 9.0 (Stata-Corp LP). Spatial autocorrelation was researched among the residuals of the fitted regressions using the Moran's I index in ArcGIS 9.2.

Risk maps

Following the results of the statistical analysis, risk maps were drawn for 1996 and 2007 by computing for every pixel of the studied zone the environmental factors found to be statistically associated with aggressiveness. This calculation was done for populated areas only. Masks were applied specifically on the 1996 and 2007 images to hide any non-urban pixels, such as vegetation, water, swamp areas and bare soils. The masks were issued from the results of the supervised classification and assisted by a manual digitisation. The areas that were not masked depicted the built-up areas and allowed the urban evolution in the 11 years to be described and quantified.

Results

Image processing

All satellite image pre-processing was successfully conducted and enabled the generation of vegetation images as well as land use and land cover maps for 1996 and 2007. The quality of the supervised classification was validated thanks to the high kappa coefficient (0.85 for the 1996 image and 0.95 for the 2007 image). The built-up area was defined as the total surface of all urban classes plus the asphalt class.

GIS and statistical analysis

All entomological data are presented in the five first columns of Tables 1 and 2 for the 1994-97 and 2007 studies respectively.

Step 1. Processing of ecological and entomological data in the GIS allowed the environmental variables to be extracted from the 1996 image for the 23 *Anopheles* catching points from the 1994-97 studies. Results are presented in Table 1. The Moran's I index was -0.01, indicating that the pattern where neither clustered nor dispersed. The z-score was equal to 0.9 standard deviations so it was associated with a high p-value. Those results were not statistically significant, possibly due to the small number of observations. In the absence of evidence of spatial autocorrelation between the 23 aggressiveness values from 1994-97, no cluster parameter was taken into account in the statistical analyses. In the univariate analyses, the anopheline aggressiveness measured in 1994-97 was significantly negatively associated with the distance to the vegetation (R^2 = 0.38) and the built-up area in a 300-m radius buffer (R^2 = 0.42) in the 1996 images (Table 3). When excluding one by one the observations for which the geographical locations were approximated, the parameters did not differ from the ones estimated in models including all the observations. Thus, all the observations where kept in the models.

Step 2. The GIS also allowed the extraction of the environmental variables from the 2007 image for the 23 *Anopheles* catching points from the 1994-97 studies (Table 1). The R^2 was 0.45 for the linear regression including the distance to vegetation and the R^2 was 0.57 for the linear regression including the built-up area in a 300-m radius buffer (Table 3). The statistical associations found in Step 1 persisted over time. Table 4 contains the statistical parameters for buffers of different sizes and indicates that the 300-m buffer fit the best.

Step 3. The GIS was used to extract the environmental values from the 2007 image at the 2007 catching point locations (Table 2). The Moran's I index was -0.07 with a z-score equal to 0.84 standard deviations. Those results were not statistically significant but in the absence of evidence of spatial autocorrelation between the 9 aggressiveness values from 2007, no cluster parameter was taken into account in the statistical analyses. Table 5 provides the parameters of the linear regression fitted for validation using the independent set of data (R^2 = 0.68).

Following the results of the Moran's I statistics at each of those 3 steps, no significant spatial autocorrelations could have been showed among the residuals of the fitted regressions.

Risk maps

As the built-up area was found to be the factor most strongly associated with *Anopheles* density in the statistical analysis, risk maps were derived from the built-up area in a 300-m radius buffer for every pixel of the populated areas, using the results of the fitted regression model. Continuous values of the computed risk map were discretized to generate three classes, which were based on a calculation of the terciles of the built-up surface in the 300-m radius buffer, followed by a manual adjustment. Breaking values were chosen at 20 and 26 Ha around every pixel. Figures 1a and 1b show the 1996 and 2007 risk maps overlaid with the measured 1994-97 and 2007 *An. arabiensis* densities. A comparison of the maps indicated an increase in built-up surfaces in 11 years (about 1 300 ha), and also depicted the evolution of the areas of each risk class in Dakar and its suburb. Table 6 gives a summary of the information provided by the risk maps. The built-up area, the related anopheline aggressiveness predicted by inverting the best statistical association (R^2 = 0.57), the total surface of the risk class and the proportional surface of the risk class with respect to the total built-up area is given for each class. Table 1 and Table 2 provide the risk class at each catching point for the 1994-97 and 2007 studies, respectively.

Discussion

Association between remotely sensed environmental data and *An. arabiensis* densities

Statistical associations were found between 1994-97 *Anopheles* aggressiveness [25,26] and 1996 and 2007 SPOT images. Even with the relatively small number of observations, aggressiveness values were statistically associated with the distance between the vegetation and the catching points and the built-up area in a 300-m radius buffer around the catching points. No multivariate model could be implemented because both ecological variables were highly correlated. Indeed, the presence of built-up areas was mainly colinear with the absence of vegetation. The validity of the model was assessed by fitting the model with an independent set of data. The aggressiveness recorded in 2007 was significantly associated with environmental data extracted from the 2007 image. The resulting agreement (R^2 = 0.68) improves our confidence in the statistical results from the present study.

The results of the present study provide evidence that environmental data retrieved from high spatial resolution SPOT satellite images (acquired for the rainy season) can be associated with *An. arabiensis* densities in

Table 1 Description of the 1994-97 entomological data and the environmental variables evaluated from 1996 and 2007 SPOT images.

Catching point	N° of catching point	District	Geographic coordinates (Decimal degrees)	*An. arabiensis* aggressiveness (number of bites /person/night,, averaged for 12 months)	Year*	Built-up area in 300-m radius buffer (Ha)**	Distance to vegetation (NDVI > 0.1) (m)	Risk map class from the final model
Usine Niari Talli	1	Centre	14.7105; -17.4519	0				
					1996	28.1	447	Low
					2007	28.2	449	Low
Point E (Zone B)	2	Centre	14.6976; -17.4558	0				
					1996	26.0	149	Medium
					2007	26.8	189	Low
SICAP Liberte I	3	Centre	14.7083; -17.4605	0				
					1996	25.3	286	Medium
					2007	28.0	409	Low
HLM III	4	Centre	14.7107; -17.4447	0				
					1996	26.4	404	Low
					2007	27.5	449	Low
Derklé- Castor	5	Centre	14.7238; -17.4497	0				
					1996	24.3	351	Medium
					2007	26.5	438	Low
SICAP Liberte VI	6	Centre	14.7244; -17.4630	0				
					1996	16.2	55	High
					2007	26.0	181	Low
Hann Village	7	Centre	14.7177; -17.4365	0.15				
					1996	21.0	167	Medium
					2007	21.5	162	Medium
Cite des eaux	8	Centre	14.7246; -17.4431	0.19				
					1996	20.3	0	Medium
					2007	22.0	98	Medium
Bop - Cerf Volant	9	Centre	14.6992; -17.4498	0.38				
					1996	18.0	117	High
					2007	18.5	25	High
Hann - Pêcheurs B	10	Centre	14.7286; -17.4234	0.46				
					1996	14.5	343	Low
					2007	14.7	316	Low
Hann - Pêcheurs A	11	Centre	14.7192; -17.4320	0.85				
					1996	19.1	55	Medium
					2007	21.0	65	Medium
Zone des hydrocarbures	12	Centre	14.7144; -17.4385	1.5				
					1996	15.4	0	High
					2007	18.8	50	High
Mboth (Plateau)	13	South	14.6701; -17.4357	0				
					1996	28.3	338	Low
					2007	28.0	317	Low
Gueule - Tapée	14	South	14.6802; -17.4572	0				
					1996	28.2	487	Low
					2007	27.6	398	Low
Diecko Nord (Médina)	15	South	14.6752; 17,43237	0				
					1996	28.3	424	Low

Table 1: Description of the 1994-97 entomological data and the environmental variables evaluated from 1996 and 2007 SPOT images. *(Continued)*

					2007	28.2	392	Low
Niayes Thioker	16	South	14.6716; -17.4413	0.04				
					1996	28.2	351	Low
					2007	27.6	251	Low
Fann - Hock	17	South	14.6817; -17.4614	0.04				
					1996	21.0	135	Medium
					2007	21.2	125	Medium
Cite du port autonome (Plateau)	18	South	14.6752; -17.4323	0.04				
					1996	23.4	151	Low
					2007	24.1	295	Low
Camp Dial Diop	19	South	14.6587; -17.4371	0.18				
					1996	13.2	28	High
					2007	18.5	90	High
Caserne des sapeurs pompiers (av Malick Sy)	20	South	14.6816; -17.4381	0.21				
					1996	27.6	476	Low
					2007	27.1	343	Low
HLM Fass	21	South	14.6952; -17.4507	0.21				
					1996	23.4	143	Medium
					2007	19.0	147	High
Caserne gendarmerie de Potou	22	South	14.6947; -17.4346	0.25				
					1996	26.8	120	Low
					2007	26.8	491	Low
Fann hôpital	23	South	14.6900; -17.4663	0.79				
					1996	7.2	7	High
					2007	12.3	0	High

*1996: data extracted from the 1996 SPOT-4 image (20 m) and 2007: data extracted from the 2007 SPOT-5 image (2.5 m degraded to 20 m)

** Total surface in a 300-m radius buffer is 28.3 Ha

The aggressivenesses are given for 23 adult *Anopheles* catching points in the south and centre districts of Dakar. All values are rounded.

the urban setting of Dakar. Almost 60% of the anopheline aggressiveness variability was explained with only one variable, the built-up area in a 300-m radius buffer, in a linear regression model.

Risk maps

Comparable risk maps were drawn for 1996 and 2007 because the same ecological information could be extracted for both dates. Because the amount of built-up area in a 300-m radius buffer gave the best R^2 in the linear regressions, maps were computed based on this predictor. The built-up area in a 300-m buffer was calculated for every populated pixel of both images. Indeed, it is known that the peak of anopheline aggressiveness occurs in the middle of the night [14]. Because the evening and night activities are expected to take place mainly in or around dwellings, the non built-up areas were excluded from predictions.

Evolution of urbanization

According to the report of the United Nations on population prospects' [1], 3 600 000 people lived in urban settings in Senegal in 1995, which grew to 4 890 000 in 2005, an increase of 36%. The results of the present study showed that urbanized areas in Dakar rose from 4510 Ha to 5847 Ha (+30%) between 1996 and 2007. Assuming that urban surfaces are proportional with the population figures and that the population increase in Dakar is proportional with the increase in the Senegalese population as a whole; the results are consistent with the increase in population. Most of the newly built-up areas were located around the airport located in the North-West of the city (in Almadies, north and west of CICES, Ouakam, Mermoz), near the Corniche (the sea coast west of the city), south of the Grande Niaye (in Hann Maristes) and around Yoff Plage (the long beach north of the city). The examination of the 1996 and 2007 maps confirmed that the city centre did not experience significant changes. Instead, the city is growing at its periphery where empty spaces still exist.

In Dakar, there was relatively little conversion from built-up zones to non-urban areas, which contrasts with Malindi and Kisumu, Kenya, where comparison of

Table 2 Description of the 2007 entomological data and the environmental variables evaluated from 2007 SPOT image.

Study area	N° of catching point	Geographic coordinates (Decimal degrees)	Total number of *An. arabiensis* (12 person/nights of capture in each study area)	*An. arabiensis* aggressiveness (number of bites/ person/night, averaged for 12 months)	Built-up area in 300-m radius buffer (Ha)*	Risk map class from the final model
Pikine	1	-17.4634; 14.6892	180	2.50	15.2	High
Universite	2	-17.4322; 14.7321	138	1.92	10.6	High
Hann Maristes	3	-17.4711; 14.7453	60	0.84	15.0	High
Ouest Foire	4	-17.4443; 14.6849	49	0.68	18.2	High
Gibraltar	5	-17.4353; 14.7159	43	0.60	27.4	Low
Yarakh	6	-17.4600; 14.7238	42	0.58	21.9	Medium
Liberte 5	7	-17.4448; 14.7496	8	0.11	27.8	Low
Grand Medine	8	-17.4803; 14.7609	3	0.04	26.4	Low
Yoff	9	-17.3987; 14.7584	1	0.01	27.1	Low

* Total surface in a 300-m radius buffer is 28.3 Ha
The aggressivenesses are given for nine adult *Anopheles* catching points in Dakar and Pikine. All values are rounded.

multi-date images showed important changes from urban to non-urban areas [23]. In Dakar, no major climatic or political events occurred, and desapearance of buildings can be related to destruction of slums or minor changes due to the 2006 flood.

Malaria transmission risk evolution

The distribution of the risk classes evolved over time. The high-risk surface slightly decreased over 11 years (-262 Ha). Consequently, the raw number of at-high risk persons also slightly decreased. The geographical distribution of this class did not notably change. Indeed, the majority of the high-risk areas are located around the airport, the "Grande Niaye" (big marshland) and the University, which are places that have been quite stable over time. However, relative to the amount of built-up area in Dakar, the high-risk surface decreased significantly from 32 to 20%, and consequently, the proportion of the population at risk diminished between 1996 and 2007.

In contrast, the raw surface of the low-risk class increased greatly (+1066 Ha). In proportion of the total population also, the low-risk area rose significantly from 29 to 41%, meaning that both the raw number and the percentage of the urban population that is less exposed to malaria risk were greater in 2007 than 11 years before.

Finally, the raw surface of the medium-risk class increased moderately (+532 Ha), but the proportion of the population exposed to this medium transmission risk remained stable over the 11 years (39% of the total population).

Table 3 Environmental factors evaluated from 1996 (Step 1) and 2007 (Step 2) SPOT satellite images and associated with the 1994-97 anopheline aggressiveness.

	1996 SPOT-4 image (20 m) (Step 1)				2007 SPOT-5 image (2.5 m degraded to 20 m) (Step 2)			
	Coefficient	95% IC	p-value	Adjusted R^2	Coefficient	95% IC	p-value	Adjusted R^2
Distance to vegetation (per 100 m and square root transformed)	-0.33	-0.51; -0.15	0.0010	0.38	-0.41	-0.60; -0.21	0.0003	0.45
Built-up area in 300-m radius buffer (per Ha)	-0.04	-0.06; -0.02	0.0005	0.42	-0.06	-0.07; -0.04	< 0.0001	0.57

Univariate linear regression. Twenty-three observations. Independent variables are not rounded. Anopheline aggressiveness is quare root transformed.

Table 4 Built-up area in 100- to 500-m radius buffers evaluated from 2007 SPOT image and associated with the 1994-97 anopheline aggressiveness.

	2007 SPOT-5 image (2.5 m degraded to 20 m)			
	Coefficient	95% IC	p-value	Adjusted R^2
Built-up area in 100-m radius buffer (per Ha)	-0.51	-0.73; -0.29	0.0001	0.49
Built-up area in 200-m radius buffer (per Ha)	-0.13	-0.18; -0.07	0.0001	0.51
Built-up area in 300-m radius buffer (per Ha)	-0.06	-0.07; -0.04	< 0.0001	0.57
Built-up area in 400-m radius buffer (per Ha)	-0.03	-0.04; -0.02	0.0001	0.53
Built-up area in 500-m radius buffer (per Ha)	-0.02	-0.02; -0.01	0.0004	0.44

Univariate linear regression. Twenty-three observations. Anopheline aggressiveness is quare root transformed.

These results highlight the benefits of urbanization in Dakar where the total population increased but the proportion of the population at higher risk for malaria transmission greatly decreased.

Persistence of the associations

The persistence of the associations between *An. arabiensis* densities and ecological data was shown in the Dakar urban centre. Statistical results (*i.e.* estimated parameters) were similar when analysing the association between the 1994-97 agressiveness and the 1996 image in one hand, and analysing the association between the 2007 aggressiveness and the 2007 image in the other hand. Thus the relationship between the environment and the anopheles densities remained unchanged at a decadal time scale. No other parameters, such as a, evolution of antivectorial methods, were introduced in Dakar to modify this relationship. In addition, statistical associations remained significant when fitting the linear regression between the 1994-97 aggressiveness and the data issued from the 2007 image. Although one site changed (Bop - Cerf Volant, where slums were destroyed), the land cover of central and southern Dakar did not change significantly. This is consistent with the fact that Dakar is now evolving outside of its "historical" city centre. Thus, in city centres or places that are remaining stable, remotely sensed data could be used to predict vectorial risk even if only former and no contemporary data from the ground are available.

Urbanization

Sparsely built-up areas are known to be risk factors for malaria in cities [24]. Furthermore, it is known that malaria transmission is reduced in urban centres compared to peri-urban and rural areas [2,3]. The present results confirm these patterns, as a highly built-up area around a catching point was a protective factor in the statistical model and was associated with lower *An. arabiensis* densities. Regarding scales of associations, aggressiveness was found to be associated with a 300-m radius buffer, which is consistent with previous findings in Pikine that found that most *An. arabiensis* were caught less than 285 m from the marshland, *i.e.* the breeding sites [11].

Vegetation

In the present work, the NDVI has been used for vegetation mapping. It is a common index that quantifies coverage by green leaf vegetation [36] and captures some combined effects of temperature, humidity, rainfall, sunlight, altitude, land-use and land-cover in one value. The NDVI threshold was defined specifically for both the 1996 and 2007 satellite images to delineate the vegetation in the Dakar urban setting. Distance to vegetation was associated with *An. arabiensis* densities, which is consistent with several previous studies that suggested that vegetation, as measured with the NDVI, is a factor associated with the risk for malaria [37-40]. In the present study the NDVI has been used for the definition of the "vegetation areas"

Table 5 Validation of 2007 risk map with 2007 entomological figures.

	2007 SPOT-5 image (2.5 m degraded to 20 m) (Step 3)			
	Coefficient	95% IC	p-value	Adjusted R^2
Built-up area in 300-m radius buffer (per Ha)	-0.07	-0.10; 0.03	0.0037	0.68

Univariate linear regression for nine observations.

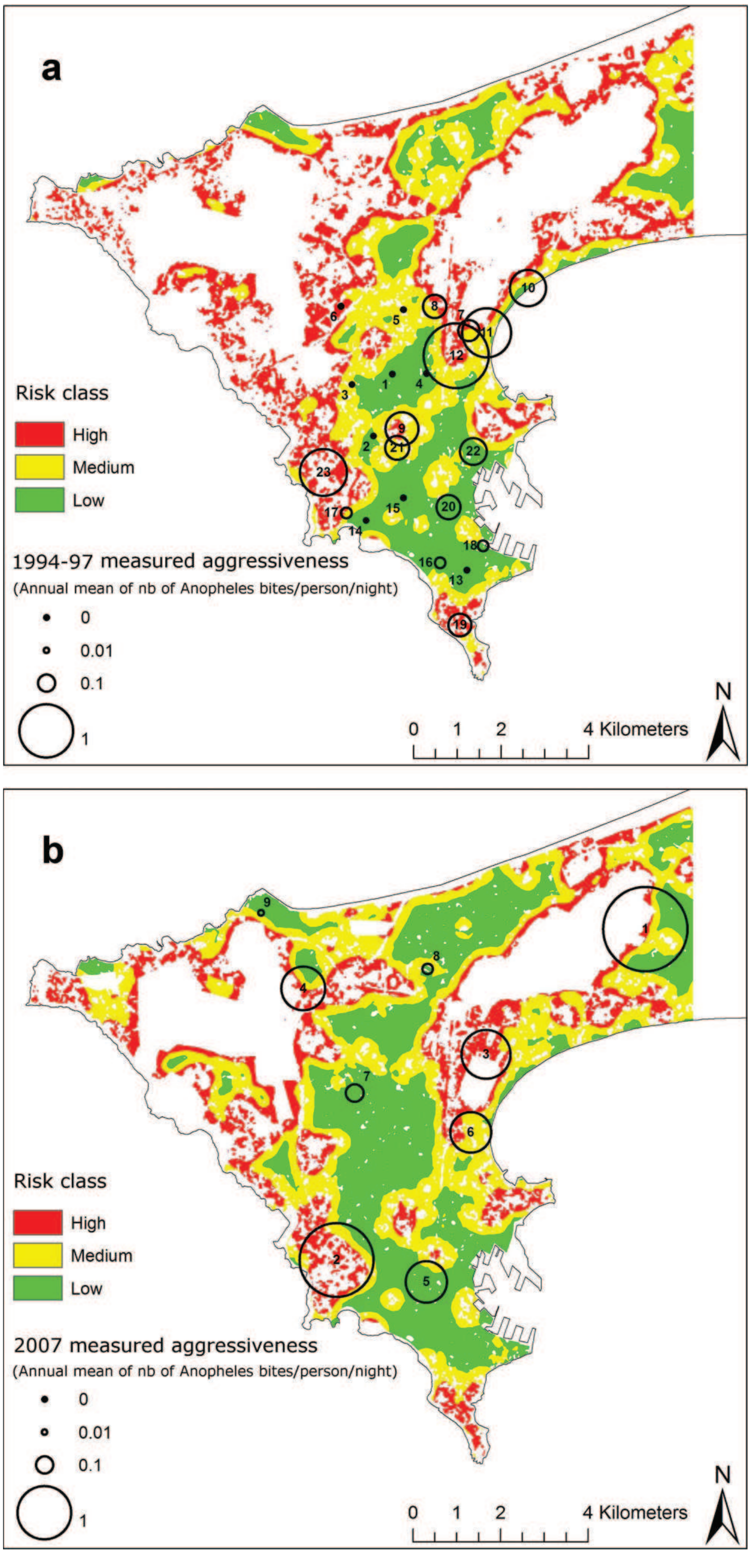

Figure 1 Measured anopheline aggressiveness overlaid on risk maps computed using the built-up surface in a 300-m radius buffer for every pixel of the populated areas. 1a. Anopheline data from 1994-97 and risk map computed with the 1996 SPOT satellite image. Refer to Table 1 for names of the study sites. 1b. Anopheline data from 2007 and risk map computed with the the 2007 SPOT satellite image. Refer to Table 2 for names of the study sites.

Table 6 Description of the risk classes in 1996 and 2007.

Class	Risk	Built-up area in 300-m radius buffer	*An. arabiensis* aggressiveness evaluated from the model (number of bites/ person/night)	Area of the risk class in 1996 (Ha)	Area of the risk class in 2007 (Ha)	Difference in risk class areas between 1996 and 2007	% difference in risk class areas between 1996 and 2007	% of the risk class area in 1996	% of the risk class area in 2007
1	High	< 20 ha	[2.82 - 0.28[	1457	1195	-262	-18%	32%	20%
2	Medium	[20-26[ha	[0.28 - 0.03[	1735	2267	+532	+31%	39%	39%
3	Low	> = 26 ha	< = 0.03	1319	2385	+1066	+81%	29%	41%
Total				4511	5847	+1336	+30%	100%	100%

All values are extracted from Figure 1.

(*i.e.* presence/absence of vegetation) instead of the results of the supervised classification that distinguished several types of vegetations and associated bare soils (*i.e.* characteristics of vegetation). The small number of entomological observations did not allow any powered analysis of the association between the aggressiveness and the different classes of vegetation. Indeed, vegetation can play various roles in malaria transmission, depending on its characteristics. It can provide resting or feeding sites for mosquitoes or can be a proxy for the presence of breeding sites. For example, in Dakar, the "Grande Niaye" is a large, vegetated marshland known to provide habitat for mosquito breeding activity [11]. The presence of vegetation can also be an indicator of the presence of urban agriculture, which was reported to be associated with malaria in several African cities, such as in Côte d'Ivoire [41] and Ghana where irrigation led to the emergence of larval habitats [42,43] and a higher malaria prevalence [44,45]. In Dakar, non-cemented wells, locally called "ceanes", are used for market-garden activity and are known to be *Anopheles* breeding sites [14,46]. In addition, urban agriculture may provide potential resting sites for vectors [47]. It is also recognized that modifications in the vegetation cover, such as deforestation, are associated with changes in malaria transmission level [48]. Finally, vegetation type can be a determinant of mosquito density [49].

Remote sensing in cities

In cities, there are obstacles to the use of remotely sensed data. Urban cover is spatially highly heterogeneous, the number of different building materials is high and the occurrence of mixed pixels is important [50]. Thus, even with high or very high spatial resolution images, distinguishing urban land uses and land covers could be difficult. Despite these difficulties, satellite images have been used in several ways in cities for a few years, and studies have attempted to describe vector presence and density or other malariometric indices.

The results of the present study are consistent with other findings in urban settings where malaria risk has been studied using environmental proxies of the presence of breeding sites and the distance to known breeding sites. In the cities of Malindi and Kisumu, Kenya, the NDVI was associated with a low housing density and thus with a higher probability of *Anopheles* breeding sites. The scale of the study was 270*270 m [22]. Using 15-m to 30-m resolution ASTER (Advanced Spaceborne Thermal Emission and Reflection Radiometer) images in New Haven, United States, the amount of vegetation in 50-m buffers around Aedes and Culex capture point, as well as the distance to water bodies, were related to mosquito densities [51].

As it has been done in this study, environmental changes and their impact on malaria risk have been studied with remotely sensed data. In two cities in Kenya, MTI (Multi-spectral Thermal Imager) satellite images acquired at 14-year intervals were used to detect changes in land use and land cover and showed that the presence, abundance and spatial distribution of breeding sites were driven by the evolution in the urbanization. Larval-positive water collections were primarily found in changing environments [23]. In Brazil, environmental changes due to the creation of a dam, as mapped from 1996 and 2001 Landsat-5 images were related to malaria incidence [52].

Other works have used different approaches, such as the study of other malariometric indices or the direct mapping of breeding sites. In Ouagadougou, Burkina Faso, overall prevalence of anti-CSP (circumsporozoite) antibodies and *P. falciparum* infections among children were associated with specific urban environments that were partly defined on a SPOT-5 satellite image [24]. In Malindi and Kisumu, Kenya, researchers attempted to directly identify *An. gambiae s.l.*, *An. funestus* and *Anopheles merus* breeding sites using 5- to 20-m spatial resolution MTI images, but only 6% of the sites were detected [21]. On the contrary, in Dar-Es-Salaam, Tanzania, aerial photographs were visually interpreted to identify breeding sites and thus helped guide an integrated fight against malaria in the city [53].

Finally, radar images have been useful in the study of malaria in tropical areas [54,55], even in urban settings [56]. Indeed, they have a powerful capacity for detecting water and do not have cloud cover acquisition problems.

Validity of image classification

The kappa coefficient calculated following the supervised classification step was 0.85 in 1996 and 0.95 in 2007, indicating a strong agreement between ground-truthed data and the classes from the supervised classification. Most of the confusions were not of major importance because they occurred mainly between different urban types, which were further aggregated in the built-up class. However, other confusions could not be totally erased, such as water and some types of asphalt.

Comparison of multi-date images

Comparing images can be difficult because some differences are not due to actual ecological differences; instead, they are related to the specificity of the images, such as differences in atmospheric conditions, solar angle, sensor calibration, period of the view or the registration of images [57]. The method used in the present study improved the validity of the comparison in several ways. First, comparisons between images were not done directly on the images but rather on the results of classifications and calculations of the vegetation index, thus taking into account intrinsic parameters for each image. Furthermore, images were acquired in level 3 pre-processing, thereby avoiding all problems of spatial misregistration. Finally, differences in atmospheric conditions were taken into account thanks to the IARR pre-processing. This was of particular importance because it allowed an NDVI common threshold to be chosen for the 1996 and 2007 images, which was not feasible without this pre-processing step.

Comparison of environmental data from multi-date images was also made possible because views were acquired from the same period of the year (during the wet season). This was of major importance, especially for vegetation measurement. Rainfall was 350 mm in 1996 and 178 mm in 2007, but images were both taken just a few days after the last rain (13 days in 1996 and 10 days in 2007). At that time, the vegetation should have been at its maximum growth so vegetation developments were considered comparable.

The validity of the comparison of the supervised classifications in 1996 and 2007 was inescapably related to differences in spatial and spectral resolutions. The 1996 SPOT-4 image has three bands at 20 m whereas the 2007 SPOT-5 image had three bands at 2.5 m and one band at 10 m. Even if the 2007 image was resampled to 20 m to allow comparison with the 1996 image, every averaged 20-m pixel may contain more information than pixels initially aqcuired at 20-m spatial resolution. In Korea, it was shown that about 20% of a scene could have been classified into different classes based on two images at different spatial resolutions (Ikonos and Landsat) [58]. Whereas this could impede the validity of multi-date comparisons, choosing common training polygons for supervised classification of both images should have improved this quality.

Conclusion

Remotely sensed environmental data were statistically associated with the *An. arabiensis* densities in Dakar city. Accordingly, risks maps were drawn for the years 1996 and 2007. Based on these maps, urbanization led to an increase in the proportion of the population at low risk for malaria transmission, *i.e.*, when urbanization increased, malaria risk was reduced. These maps should be seen as a first step towards creating operational risk maps that could drive antivectorial control in the city.

Additional material

Additional file 1: Spatial repartition of the training polygons digitized for supervised classification process.

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Authors' contributions

VM was responsible for study design, analysis, remotely sensed images processing, interpretation, production of the final manuscript and revisions. CV contributed to the study design, remotely sensed images processing, analysis, interpretation and production of the final manuscript and revisions. LG was co-responsible for field data collection. FP was responsible for overall scientific management, study design, analysis, interpretation, preparation of the final manuscript and revisions. CS contributed to overall scientific management. JFT contributed to overall scientific management. JPL was responsible for overall scientific management, study design, analysis, interpretation, preparation of the final manuscript and revisions. CR was responsible for overall scientific management, study design, analysis, interpretation, preparation of the final manuscript and revisions. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

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ARTICLE 6

Mapping of spatial and temporal distribution of Anopheles larvae and adults in Dakar

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En préparation

Mapping of spatial and temporal distribution of *Anopheles* larvae and adults in Dakar

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Abstract

As a consequence of the vectorial transmission of malaria, the environment has been clearly identified as a determinant of the biodiversity of the disease and a key factor for vector species or sub-species distribution. Climate seasonality, rainfall pattern, air temperature and humidity or presence of vegetation or surface water, as well as anthropogenic activities, can be related to every step of the malaria transmission cycle. Regarding the ability of numerous orbiting satellites to provide ecological, meteorological and climatic information, the study of malaria took advantage of the growing availability and precision of remotely sensed data. As malaria transmission is spatially and temporally heterogeneous, entomological maps of larval or adult mosquito spatial and temporal distribution, drawn at appropriate scales can provide valuable information for targeted malaria control and selective allocation of resources. Regarding the rapid increase of African urban populations, urban malaria is considered an important emerging health problem in that continent. In Dakar, malaria transmission has been showed to be highly heterogenic so the study and control of malaria in the city could benefit from the availability of risk maps. The objective of the present study was to use ground data and remotely-sensed data to obtain malaria risk maps in Dakar at two levels: risk maps of the *Anopheles* breeding sites with their larval production level and risk maps of the *Anopheles* adult density levels for years 2007 to 2010.

The present study showed that remotely sensed environmental and meteorological data were successfully used to draw high resolution *Anopheles* larval and adult risk maps in Dakar, the capital city of Senegal. The results first showed that high spatial resolution SPOT (*Satellite Pour l'Observation de la Terre*) satellite images provided some indicators related to the presence of water in the city. Second, some remotely sensed environmental factors as well as ground and satellite meteorological information, were successfully associated with the presence of *Anopheles* larvae in water collections studied on the ground. Third, the *Anopheles* adult densities field measurements could have been predicted by some remotely sensed environmental factors as well as with an *Anopheles* larval productivity surrogate extracted from the breeding sites mapping.

In conclusion, remotely-sensed environmental and meteorological data allowed drawing different levels of maps that could be of interest for focusing malaria control in urban settings. In addition to research interests, one key issue of the present work is to be a preliminary step for the implementation of an operational system to facilitate real-time monitoring of human health.

Introduction

Malaria is caused by a *Plasmodium* parasite which is transmitted among humans by the bites of female mosquitoes of the genus *Anopheles.* As a consequence of this vectorial transmission, the environment has been clearly identified as a determinant of malaria biodiversity [1, 2] and a key factor for vector species or sub-species distribution [3-6].

Climate seasonality, rainfall pattern, air temperature and humidity or presence of vegetation or surface water, as well as anthropogenic activities, can be related to every step of the malaria transmission cycle. Indeed, environmental factors impact on a wide range of malariometric indices *i.e.* the presence and persistence of *Anopheles* breeding sites, the larval densities, the aggressiveness or Human Biting Rate (HBR - number of *Anopheles* bites per person per unit of time), the percentage of *Anopheles* that are infected with a *Plasmodium*, the Entomological Inoculation rate (EIR - number of infected *Anopheles* bites per person per unit of time), the parasite prevalence (percentage of persons infected with a *Plasmodium*), the morbidity and the mortality in human population.

Regarding the ability of numerous orbiting satellites to provide ecological, meteorological and climatic information, the study of many infectious diseases took advantage of the growing availability and precision of remotely sensed data [7-12]. About mapping malaria, there are many examples of the use of remotely sensed environmental data that allowed drawing risk maps or setup risk models at every level of the malaria transmission cycle [13].

Urban malaria

Urbanization is occurring at a rapid pace and the United Nations forecasts that by 2030, nearly 60% of the world population will live in cities [14]. Regarding malaria, many studies have reported evidences of transmission in urban areas, even if levels are usually lower than in peri-urban and rural places [15, 16]. The epidemiology of malaria in cities is specific, and urban malaria is considered to be an emerging health problem of major importance in Africa [17]. Urban malaria specificity is led by the degree and type of urbanization, the density of the human population, the vector control measures or the access to health care. In addition, urban malaria risk is heterogeneous over small distances, and transmission can vary among different districts of the same city, as shown in Brazzaville [18] and Dakar [19, 20]. In cities, blood meal sources are abundant, dispersion of the vectors is low and malaria transmission is highly driven by the proximity of breeding sites [20, 21]. At local scales, the concept of focal units of transmission has been described as a system where the breeding site of malaria vectors is the centre of a focus of transmission [22].

Malaria control

The objective of malaria control can be to reduce the amount of parasites in the population by the use of appropriate antimalarial drugs or to decrease the human-vector contact tanks to the use of insecticide-treated bednets for example. At the entomological level, control aims at reducing larval or adult *Anopheles* densities, implementing environmental management by the use of larvicides or by insecticides spraying.

Larval control has been showed to be a powerful tool for malaria control and even elimination in many historical examples [23-27]. Modelling also highlighted that targeted management of the most productive breeding sites can lead to significant reductions in adult mosquito productivity but also in incidence and prevalence of malaria [28]. Several examples have also documented the efficacy of adult mosquito control by the use of insecticides [29, 30].

In consequence, entomological maps of larval or adult mosquito spatial and temporal distribution, drawn at appropriate scales can provide valuable information for targeted malaria control and selective allocation of resources. In Dakar, a preliminary step towards malaria risk mapping has been achieved by setting up two risk maps for years 1996 and 2007 [31].

Malaria risk mapping

To attain the objective of accurate risk mapping, collection of appropriate field data is the basic requirement. Next, an informed choice of remote sensing products, geographic data and meteorological or climatic information, should be made in light of all available spatial, temporal and spectral resolutions. The analysis methods must be chosen based on the type of data to avoid as much bias as possible. It is also of major importance to carefully understand the mechanisms of the relationships between environmental data and each step of the transmission cycle.

Objectives

In this context, the global objective of the present study was to obtain malaria risk maps in Dakar at two levels: risk maps of the *Anopheles* breeding sites with their larval production level and risk maps of the *Anopheles* adult density levels. The methodology for obtaining those risk maps for years 2007, 2008-2009 and 2009-2010 was divided in three sub-objectives described as follow:

a) Using high spatial resolution SPOT (*Satellite Pour l'Observation de la Terre*) satellite images to identify the indicators related to the presence of surface water collections in Dakar, and extrapolating the results to build water collection maps.

b) Identifying the remotely sensed environmental factors associated with the presence of *Anopheles* larvae in water collections studied on the ground, and extrapolating the results to build breeding sites maps, with their daily probability for the presence of *Anopheles* larvae, as well as the derived yearly maps.

c) Predicting the *Anopheles* adult densities measured on the ground using the *Anopheles* larval productivity surrogate extracted from the preceding step and remotely sensed environmental factors, in order to draw high resolution adult *Anopheles* risk maps.

Methods

Study site

Dakar (14°40'20" North, 17°25'22" West), the capital city of Senegal, is located in the Cap-Vert peninsula at the westernmost point of Africa. The estimated population of the Dakar urban area was close to 1 million inhabitants in 2005, accounting for about 20% of the country's population. The population density was about 12,000 inhabitants per km². The altitude peaks at 104 m above sea level (Mamelles). The climate is mild sahelian. The hot and wet season lasts from June to November with average temperatures between 24 and 30°C. The cool and dry season lasts from December to May with average temperatures between 19 and 25°C. The first rains generally occur at the end of June or the beginning of July, and the last ones at the beginning of October. In 2007, 2008 and 2009, the annual rainfall was respectively 178 mm, 510 mm and 565 mm (data from the national weather forecast).

Entomological data

Entomological field study have been undertook in September-October 2007 and from July 2008 to June 2010 and they are described elsewhere [19, 32]. Briefly, the study was conducted in Dakar, as well as in Pikine, Thiaroye and Guediawaye, three of its satellite cities, in a total of 45 areas. The choice of the studied zones was done to encompass as wide a variety of environments as possible in terms of type of urbanization, road network, vegetation and socio-economic level. Each site was delimited on the ground to cover an area of about 200 x 200 m, depending on the technical and logistical limitations presented by the landscape.

Ten zones were studied in September-October 2007 (further named year 2007), 30 zones in July 2008-June 2009 (further named year 2008) and 30 zones in July 2009-June 2010 (further named year 2009). Each zone was followed for one or two years for a total of 70 zones-year. The studied zones and the periods under study are presented in [32].

Adult mosquito sampling was carried out once every two weeks in every studied zone. Human landing catch of adult mosquitoes was conducted both indoors (one catching point) and outdoors (two catching points) for a total of 3 120 person-nights of capture. Most of the caught *Anopheles* mosquitoes were *An. arabiensis*.

Larval investigations were undertaken every 10 days and each yard within every studied area was searched for open water collection sites and all the found water bodies were examined for larvae. Larvae and pupae were sampled using a standard dipping method. When anopheline specimens were found, larval density was calculated as the number of larvae (all instars) and pupae (further emerged and identified at the laboratory) per dip and recorded for each water collection site.

Satellite images

SPOT View imagery products were programmed and acquired during rainy seasons at the following dates: 26 September 2007, 24 September 2008, 28 September 2009. One image was acquired the 11 May 2009 during the dry season. For each date, one SPOT-5 image had a 2.5-m spatial resolution and three spectral bands: two in the visible (green and red) and one in the near infrared (NIR); and one 4-bands SPOT-5 image at a 10-m spatial included one band for short wave infrared (SWIR). All images were "Level 3" pre-processed (orthoimages), so they were already georeferenced, and their map projection (UTM zone 28, WGS 84 datum) was based on ground control points and a digital elevation model (DEM). The location accuracy of the images was less than 10 m [33]. These images covered a large area of the Cap-Vert Peninsula, so views were resized to cover Dakar city and its suburbs until Pikine (12 439 Ha of land, lower left corner : 17°31'44"W 14°38'44"N; upper right corner : 17°20'54.005"W 14°48'14.219"N).

A DEM at 90-m resolution (version 4.1) was downloaded from the Shuttle Radar Topography Mission [34, 35].

Day and night Land Surface Temperatures (LST) were extracted from decadal MODIS images for the full duration of the field work.

Pre-processing of images

All pre-processing and processing were done using ENVI 4.3. A common mask of the sea was digitized and applied to all the images, whereas specific cloud masks were digitized for each date. Clouds and their shadows covered 3.8 km² on the 2007 image and did not hide any of the entomological sampling zones. In 2008, clouds and their shadow covered 19.5 km² and hide most part of five zones out of 30 under study in 2008. One zone was totally under the clouds. Finally, clouds covered 9.8 km² of the September 2009 image, hiding most part of four areas out of 30 having being investigated this year. No clouds were detected in the May 2009 SPOT image.

For every pair of images at a given date, the 10m-SWIR band was resampled at 2.5m in order to be stacked with the 3-bands image. Then, because comparisons of multi-date images could be impeded by differences in atmospheric conditions from one date to another, internal average relative reflectance (IARR) calibration was undertaken to normalize images to a scene average spectrum [36, 37]. All further image processing were based on these calibrated images.

The DEM was reprojected in UTM zone 28, WGS 84 datum.

The freely available MODIS Reprojection Tool was used to extract LST values from MODIS images and to reproject the resulting images in UTM zone 28, WGS 84 datum. The LST was averaged for the Cap-Vert peninsula. Then, the LST values were smoothed by calculating for each decade the averaged value among 3 decades (from the previous one to the following one). The daily values were deducted as linear values between each decadal value.

Calculation of indicators

For each 4-bands SPOT image, several indicators were calculated using mathematical combinations of spectral bands:

- Vegetation indicators:
  - NDVI (Normalized Difference Vegetation Index) [38, 39] was calculated as (NIR - Red)/(NIR + Red). It is the most commonly used in the field of disease mapping. Its value ranges from -1 to +1. Even if no strict threshold can be depicted, a value superior to 0.2 usually corresponds to a vegetated area, which gets denser when this value rises. A negative value indicates non-vegetated features such as barren surfaces (rocks and soils), water, built-up areas or asphalt.
  - SAVI (Soil Adjusted Vegetation Index) [40] was calculated as ((NIR - Red)/(NIR + Red + L))*(1 + L), where L, the adjustment factor, is typically chosen at 0.5. The SAVI minimizes the effect of soil background conditions and it also ranges from -1 to +1.

- Humidity indicators that can highlight the presence of water or evaluate vegetation water content, ranging from -1 to +1:
  - The SWIR band was used such as, without any additional processing. The SWIR is absorbed by water, being it free water bodies or the water contained in plant cells.

- NDWI (Normalized Difference Water Index) Gao [41] was calculated as (NIR - SWIR)/(NIR + SWIR). It increases with vegetation water content or from dry soil to free water.
- NDWI Mac Feeters [42] was calculated as (Green - NIR)\(Green + NIR). It has been developed to delineate open water features while eliminating the presence of soil and terrestrial vegetation features. It is also suggested that the NDWI may provide turbidity estimations of water bodies. Its value increases with the presence of water and decreases with the presence of vegetation.
- MNDWI (Modified NDWI Mac Feeters) [43] was calculated as (Green - SWIR)/(Green + SWIR). It can enhance open water features detection while efficiently suppressing and even removing built-up land noise as well as vegetation and soil noise. It can be noted that this indicator is calculated with the same spectral bands than the NDPI (Normalized Difference Pond Index) used to detect ponds in the North of Senegal [44].

- Soil indicator:
  - A Soil BI (Brightness Index) was calculated using the four bands, as $\sqrt{((Red^2)+(Green^2)+(NIR^2)+(SWIR^2))/4}$ [45]. It characterizes soil physical properties, roughness, compactness or moisture content. High values correspond to natural or anthropic bar soils, without vegetation.

Every indicator was calculated in ENVI 4.3.

Land use and land cover (LULC) classification

Unsupervised ISODATA classification allowed generating maps of land use and land cover from each SPOT image. The ISODATA parameters were chosen in order to obtain a maximum of 20 classes with 50 iterations. The classification results were examined by an operator with good knowledge of the town. Similar resulting classes were merged and the final classification included six land cover classes distributed as building, asphalt, sand, water, vegetation, bare soils (different types, mixed or not with vegetation). The sea coast was masked as the classification quality was low for this particular land cover.

Geographic information system (GIS)

A GIS was built in ArcGIS 9.2 (Environmental Research Systems Institute, Redlands, CA, USA). For each year (2007, 2008-2009 and 2009-2010), the layers were added as follows:

1) Raster images containing every calculated indicator.

2) Maps of land use and land cover issued from the classification process.

3) Grid of 10m-squares covering all the studied area (about 1 million squares). Subsamples of this grid were extracted to cover all the studied zones for each year, excluding places covered with clouds (18 479 squares in 2009, 25 447 in 2008 and 6 313 in 2007).

4) For each year, shape of every single water collection visited on the ground at each date of the field visits.

5) For each year, shape of every single water collection visited on the ground at the date the closest to the date of the acquisition of the SPOT image.

6) Points corresponding to the three adult mosquito catching points (two indoors and one outdoors) for every studied zone. The centroide of the three catching points was extracted for every zone.

Ground meteorological data

Ground measures of daily rainfall event were available from the Senegalese national meteorological agency for year 2007, 2008 and 2009. They were recorded at the International Airport.

Water collections mapping (step 1)

SIG

In the SIG, the value 0 or 1 was affected to every 10m-square of the studied zones, depending on the status concerning the presence of surface water. Indeed, if a square was overlaying a water collection, even for a very small area, its value was chosen at 1. The water collections were the ones recorded at any date during each yearly ground study. Then, the mean, maximum and minimum values of every indicator, as well as the surface of every LULC class and the altitude (from the DEM image) were calculated for every 10-m square, fore each year separately.

Statistical analysis and validation of the models

All the variables calculated in the SIG at the 10m-square level, were exported to Stata 11 (Stata Corporation, College Station, Texas) to perform the statistical analysis.

Exploratory analysis allowed detecting the correlated variables thanks to the examination of the correlation matrix. In order to respect the assumption of independence of observations required to undertake classical statistics, the variables which were correlated at more than 0.5 (Spearman rho or Pearson correlation coefficient) were not included in a same statistical model.

It was assumed that the outcome (*i.e.* presence/absence of water in the 10m-squares) followed a Bernoulli distribution so logistic regressions were fitted to identify the environmental variables significantly associated with the presence of water in the 10m-squares. The sampling scheme in this study implied that some correlations could exist between observations done in a same studied zone, as nearby observations could be influenced by the same environmental factors. Thus, a random effect that could account for this spatial correlation was added to the model (xtlogit function in Stata). Models were fitted with data for the three studied years together, or for each year separately.

The variables associated with the presence of water with a p-value <0.25 in univariate analysis were retained for multivariate analysis. A backward stepwise selection procedure was applied in the final model to keep variables with a p-value <0.05.

For validation purpose, models were fitted with a random sample of 80% of the total number of observations. The remaining 20% observations were used to assess the predictive ability of the model by the calculation of the area under the ROC (Receiving Operating Characteristics) Curve. The predictions of the probability of presence of water were calculated for the validation dataset by inverting the fitted model. Then, the predictions were compared with the actual presence of water in the 10m-squares in order to measure accuracy of the predictions. The higher is the area under the ROC curve (between 0 and 1), the best the model fitted to the data. The examination of the ROC curve allowed choosing a cut-off in order to transform the predicted probabilities in a dichotomous presence/absence outcome. This threshold was chosen to maximize sensitivity (Se) first, and then specificity (Sp). Validation was also undertaken on the yearly subsamples to assess reproducibility of the global model along years. In addition, sub-models were fitted for each year and the regression coefficients were compared in order to assess that the variables are similarly implied each year.

Maps of the water collections

In the GIS, the predictions of the probability of presence of surface water were calculated for each 10m-square for the full 10m-grids of each year, by inverting the regression formula of the best fitted multivariate model. Grids of the probabilities of presence of water were transformed in grids of the presence of water using the cut-off value.

A closing morphology filter (3x3 10m-squares) was applied to the water presence image. Closing filters smooth the contours, fuse narrow breaks and long thin gulfs, eliminate small holes, and fill gaps in the contours of an image. The closing of an image is defined as the dilation of the image followed by subsequent erosion using the same structural element, with the advantage that it does not erase small water areas.

The resulting smoothed map of presence of water was vectorized, meaning that every water collection was created as a single object, with a unique identifier. An operator having a good knowledge of the city validated the map and corrected any obvious misclassification. The final result was one map of the water collections for each year.

Anopheles larvae mapping (step 2)

SIG

In the SIG, the mean, maximum and minimum values of every indicator, as well as the surface of every LULC class, were calculated in the water collections and in 10m-rings around them for each water body recorded on the ground at the dates the closest to the acquisition of the SPOT images. Water collections that were not flooded at that time were not included in the analysis.

Meteorological data

The rainfall data was combined into several variables: cumulative number of rainfall events and cumulative rainfall amount in several lag times (from 1 to 30 days before the recording of the ground information). The LST was included as a daily value.

Statistical analysis and validation of the models

All the variables extracted from the SIG were exported to Stata 11 (Stata Corporation, College Station, Texas) to perform the statistical analysis.

The statistical analyse aimed to identify the remotely-sensed and meteorological determinants of the presence/absence of anopheline larvae in the water collections for each observation recorded on the ground at the dates the closest to the dates of acquisition of the SPOT images. This analysis attempted to explain the presence of larvae in the water bodies under study on the ground and did not take into account the water collections predicted at step 1.

The presence/absence of larvae was analysed using a logistic regression model with a water collection random effect accounting for any autocorrelation among the observations.

The variables associated with the presence of larvae with a p-value <0.25 in univariate analysis were retained for multivariate analysis. A backward stepwise selection procedure was applied in the final model to keep variables with a p-value <0.05.

For validation purpose, models were fitted with a random sample accounting for 80% of the observed water collections. The remaining 20% water collections were used to assess the predictive ability of the model by the calculation of the area under the ROC (Receiving Operating Characteristics) Curve. The sensitivity and specificity were deducted from the choice of a cut-off probability value defined thanks to the examination of the ROC curve. Validation was also undertaken on the yearly subsamples to assess reproducibility of the global model along years. In addition, sub-models were fitted for each year in order to verify that the coefficients of the associated variables were comparable.

Maps of the Anopheles breeding sites

In the GIS, the indicators found to be statistically associated with the presence of *Anopheles* larvae were computed in and around every water collection predicted at step 1 of the present study, for each year. Then, the predictions of the probability of presence of *Anopheles* larvae were calculated for those predicted water collections for every single day of the full duration of the study (for the three years), by inverting the regression formula of the best fitted model. Indeed, a temporal aspect was introduced in the model fitted at this step 2, by including daily rainfall amount and daily LST, allowing calculating a probability of presence of *Anopheles* larvae day by day. Thus, daily maps of the probabilities of the presence of *Anopheles* larvae were generated.

The daily probabilities of presence of *Anopheles* larvae were also combined yearly in order to obtain a map of the probability for the water collections to harbour larvae during the year.

Anopheles adult mapping (step 3)

SIG

Different buffers (from 100 to 400m) were drawn around the centroids of the adult *Anopheles* catching points in order to summarize environmental variables around the places for which ground mosquitoes measurements were available. On one hand, the mean, maximum and minimum values of every indicator, as well as the surface of every LULC class, were calculated in the buffers. On the other hand, the daily maps of the probabilities of presence of *Anopheles* larvae were used as the basis for evaluating a surrogate variable of the adult *Anopheles* daily productivity calculated as follow.

Calculation of a larval productivity surrogate weighted by water surface

The adult *Anopheles* survival rate has been evaluated at 82% in Pikine in 1985, meaning that from one day to another, 82% of the adult mosquitoes survived [46]. In consequence, after 35 days, an initial population would be close to extinction. In addition, a new emerged adult mosquito needs one or two days to reinforce its cuticle, rest and mate before researching its first blood meal. In consequence of those figures, a total probability of larval presence was calculated in the buffers as the product of the surface of each water collection included in the buffer, and the probability of presence of *Anopheles* larvae in each water collection. The resulting values were summed for days “minus 2” to “minus 35” and weighted by the 82% daily survival rate in order to generate the larval productivity surrogate. This surrogate could be calculated at any date.

In parallel, a non-linear statistical model was fitted in Stata to model the surface fraction of the maximum superficies of the water collections measured on the ground, using the rainfall amount in the preceding days and the environment of the water collections. As water collection surface variations can have very diverse patterns depending on their characteristics, a global surface has been taken into account at the scale of a studied zone. The water content of the zones was first modelled as permanent (no or low surface variations) or temporary (important surface variations). Then, for temporary patterns, the total rainfall amount in the seven preceding days allowed predicting the fraction (less than one) of the maximal water surface in a studied zone. This model was inverted in order to weight the *Anopheles* larval productivity surrogate by the daily changes of surface water along the year.

Statistical analysis and validation of the models

The variables calculated in the buffers were transferred in Stata to perform the statistical analysis aiming at describing the relationship between *Anopheles* larval productivity surrogate coming from modelling, remotely sensed environmental factors and *Anopheles* adult densities measured on the ground.

As the outcome was a count (number of adult *Anopheles* caught), a binomial negative regression was fitted. The validity of the model was assessed with the Spearman rank correlation coefficient computed between the adult *Anopheles* count predictions and field measurements, weighted by the total number of catching nights per zone, summed at the zone-year level. Validation was undertaken at the global level as well as at the yearly level. In addition, yearly sub-models were fitted and their estimated coefficients compared.

Maps of the adult Anopheles

In the GIS, the variables significantly associated with the adult *Anopheles* densities were mapped at appropriate scales. The fitted statistical model was then reverted in order to generate an adult *Anopheles* densities level risk map in Dakar for year 2007, 2008 and 2009.

Results

Water collections mapping (step 1)

Statistical analysis and validation of the models

The layers were successfully overlaid in the GIS and all the environmental information was extracted from the 10m-squares and transferred into Stata for classical statistics modelling.

Significant results of the univariate and multivariate logistic regression with the random effect accounting for any zone effect are presented in Table 1 (global model for all years). The results of the univariate analysis are presented only for variables that remained significant in the multivariate analysis. Those variables were the mean of the Modified NDWI of the rainy seasons (extracted from 2007, 2008 and 2009 wet seasons SPOT images), the mean of the NDVI of the dry season (extracted from the 2009 dry season SPOT image), the built-up surface (extracted from the unsupervised classification of the 2009 dry season SOT image) and the elevation (extracted from the DEM) in the 10m-squares. The MNDWI and NDVI were risk factors whereas the built-up surface and the altitude were protective factors. No large differences were noted between the coefficients of the univariate and multivariate analysis, meaning that there was no confusion factor among the variables. Analysis was performed on 80% of the total 10m-squares of the three years (40 191 observations).

Table 2 provides the results for the sub-models for each year, fitted with yearly subsamples of the 80% of the total observations. For each year, each studied zone was included, except in 2008 were one studied zone was completely covered by clouds. The four variables remained significant in the yearly multivariates models. Even if the 95% confidence intervals obtained in the four models were not all spanning, the directions and strength of the associations remained similar.

Table 1. Environmental factors associated significantly with the presence of water in the 10m-squares at any date, including all the observations for years 2007, 2008 and 2009. Logistic regression with zone random effect.

All years	Logistic regression with zone random effect					
	Univariate			Multivariate		
80% of obs. = 40 191 Nb zones = 45	Coef	95% IC*	p-value	Coef	95% IC*	p-value
MNDWI rainy season (mean)						
Per 1 unit increase	11.00	10.50 - 11.49	<0.001	13.09	12.45 - 13.72	<0.001
NDVI dry season (mean)						
Per 1 unit increase	7.61	7.12 - 8.09	<0.001	10.59	9.95 - 11.23	<0.001
Built-up surface						
Per pixel (6.25m²) increase	-0.17	-0.18 - -0.16	<0.001	-0.10	-0.11 - -0.09	<0.001
Elevation						
Per meter increase	-0.34	-0.36 - -0.32	<0.001	-0.26	-0.28 - -0.24	<0.001

*95% confidence interval

Table 2. Environmental factors associated significantly with the presence of water in the 10m-squares at any date, including observations for years 2007, 2008-2009 and 2009-2010 separately. Logistic regression with zone random effect.

	Multivariate logistic regression with zone random effect								
	2007 (5 062 obs. in 10 zones)			2008-2009 (20 328 in 29 zones)			2009-2010 (14 801 in 30 zones)		
	Coef	95% IC*	p-value	Coef	95% IC*	p-value	Coef	95% IC*	p-value
MNDWI rainy season (mean)									
Per 1 unit increase	29.34	26.09 - 32.60	<0.001	9.64	8.78 - 10.50	<0.001	24.98	23.39 - 26.57	<0.001
NDVI dry season (mean)									
Per 1 unit increase	15.13	12.61 - 17.65	<0.001	12.62	11.50 - 13.73	<0.001	9.57	8.51 - 10.64	<0.001
Built-up surface									
Per pixel (6.25m²) increase	-0.10	-0.14 - -0.05	<0.001	-0.10	-0.11 - -0.08	<0.001	-0.11	-0.13 - -0.09	<0.001
Elevation									
Per meter increase	-0.08	-0.15 - 0.01	0.019	-0.34	-0.37 - -0.31	<0.001	-0.17	-0.21 - -0.13	<0.001

Concerning the model including all years, the results of the likelihood ratio test ($p<0.001$) showed that the random effect model was significantly different than a model fitted without accounting for the zone effect. Predictions of the probability for the presence of water at any date during the field follow-up for the validation sample (10 048 observations) allowed calculating the area under the ROC curve at 0.86 (95% confidence interval: 0.84 - 0.87).

The area under the ROC curve calculated for the presence of water at the date of the image was 0.93 (0.92 - 0.94) with Se: 90%, Sp: 84% and correctly classified: 84%.

The areas under the ROC curve calculated after the fitting of the global model on the 2007, 2008 and 2009 validation subsamples were respectively 0.95 (0.93 - 0.97), 0.88 (0.86 - 0.91) and 0.95 (0.94 - 0.96). The respective Se/Sp couples were: 93%/83%, 84%/85% and 93%/82%.

Maps of the water collections

The extrapolation of the global model to the full sets of 10m-squares of the 2007, 2008 and 2009 images allowed to draw the maps of the probability of presence of surface water for the whole studied area of Dakar and its suburb for those three years. The chosen cut-off value allowed transforming those maps in the maps of presence of water in Dakar.

Visual examination of the results allowed identifying areas in which prediction was incorrect. In the port, the docks were largely detected as water, probably due to the very "dark" asphalt. The golf course was also mainly detected as water, probably because of the important watering. As those large zones could not reasonably be considered as actual potential breeding sites, they were hidden with manually digitized masks. The masked water area was 0.02 km². In addition, the sea coast (beaches and falls close to the sea) was masked as the misclassifications could be important in this particular land cover and their bio-ecological conditions were not favourable for *A. arabiensis* breeding sites.

Then, the filtering (closing filter) followed by the vectorization process transformed the map of the presence of water in the 10m-squares in a map of water collections objects. After masking, in 2007, 2008 and 2009, a total of 1 119 (7.9 km², 0.070% of the total surface outside cloud cover), 1 002 (7.0 km², 0.073% of the total surface outside cloud cover) and 890 (8.5 km², 0.080% of the total surface outside cloud cover) water collections were respectively detected in the areas that were not masked by clouds. The correlation between the annual rainfall amount and the percentage of the total water surface mapped outside of cloud cover was 0.87.

Water collections size ranged from 68 m² to 3.7 km² (a big marshy area in Dakar and Pikine). It can be noted that the vectorization process done in ArcGIS, with the option to simplify the polygons, implied a reduction of the minimum size of a water collection from 100 m² (corresponding to one 10m-square) to 68 m². The figure 1 shows the results of the water collections mapping for 2009.

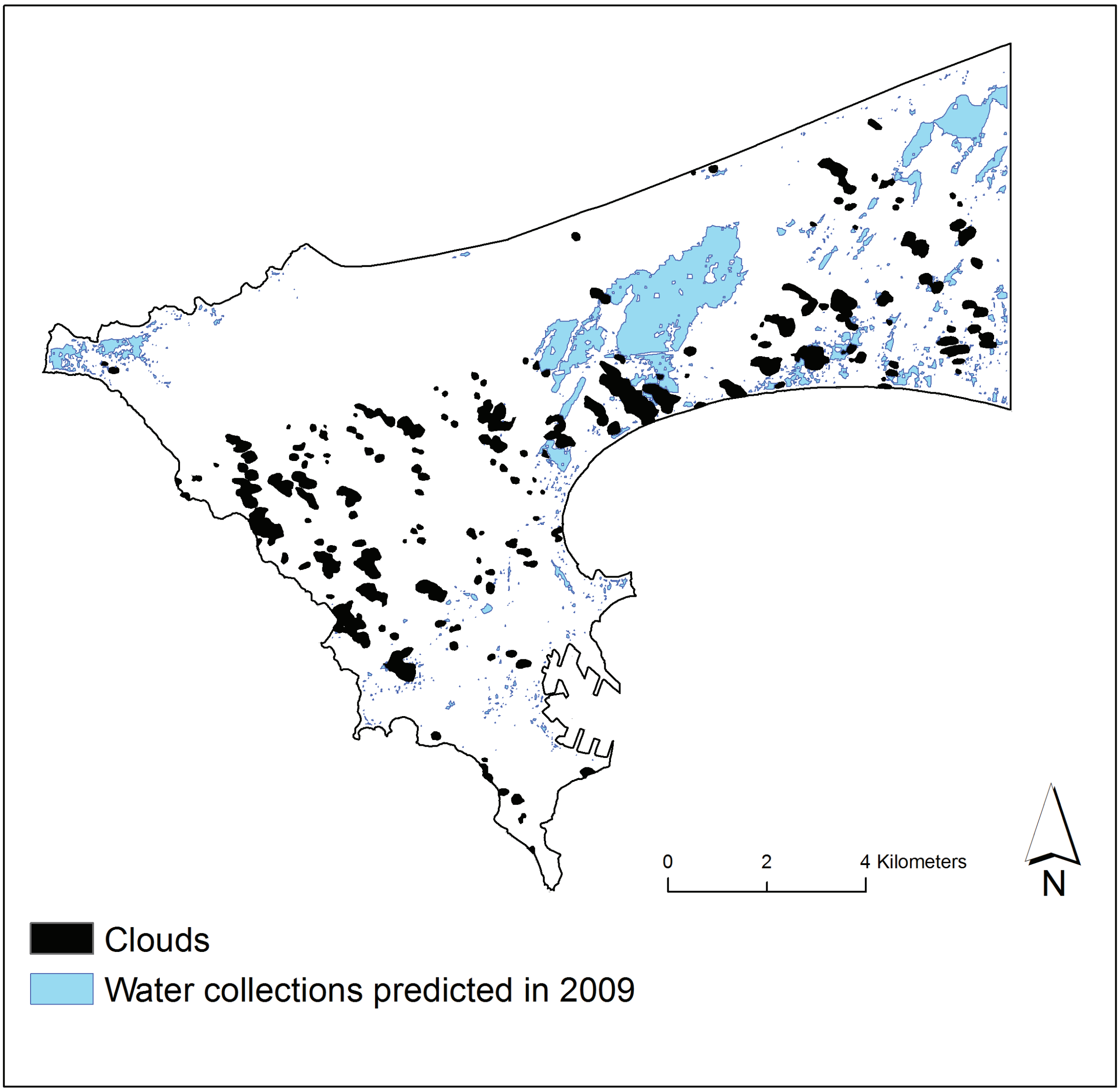

Figure 1. Results of the water collections mapping for 2009.

Anopheles larvae mapping (step 2)

Statistical analysis and validation of the models

At the date of acquisition of the three rainy seasons SPOT images, 170 water collections were flooded and outside of cloud cover. All the observations associated with those 170 collections were included in the analysis, for a total of 2 051 observations. The results of the univariate and multivariate logistic regressions are presented in Table 3 (global three years model).

Table 3. Meteorological factors and environmental factors extracted from SPOT images and associated significantly with the presence of *Anopheles* larvae in the water collections followed on the ground, including all the observations for years 2007, 2008 and 2009. Logistic regression with water collection random effect.

	Logistic regression with water collection random effects					
	Univariate			Multivariate		
80% of water collections = 140 Number of obs. = 1 638	Coef.	95% IC	p-value	Coef.	95% IC	p-value
NDWI Mc Feeters dry season (mean in the collection and a 10m-buffer around)						
Per 1 unit increase	9.85	4.01 - 15.68	0.001	6.79	0.91 - 12.67	0.024
Soil BI dry season (mean in the collections and a 10m-buffer around)						
Per 1 unit increase	3.96	1.58 - 6.34	0.001	2.79	0.42 - 5.15	0.021
Current night temperature						
Per °C increase	0.28	0.21 - 0.35	<0.001	0.13	0.05 - 0.21	0.002
Rainfall amount in the preceding 30 days						
Per 10 mm increase	0.08	0.07 - 0.09	<0.001	0.05	0.03 - 0.07	<0.001

The results of the univariate analysis are presented only for variables that remained significant in the multivariate analysis. Those variables were the mean of the NDWI Mc Feeters of the dry season (extracted from the May 2009 SPOT image in the collection and a 10m-buffer around), the soil BI of the dry season (extracted from the May 2009 SPOT image in the collection and a 10m-buffer around), the current night temperature (extracted from MODIS images) and the rainfall amount in the preceding 30 days (ground measurements). No large differences were noted between the coefficients of the univariate and multivariate analysis, meaning that there was no confusion factor among the variables. Analysis was performed on 80% of the total number of water collections of the three years (1 638 observations in 140 water collections). The four variables were positively associated with the presence of *Anopheles* larvae in the water collections.

Table 4 provides the results for the sub-models for each year, fitted with yearly subsamples of the 80% of the water collections. Several variables were not anymore significantly associated with the presence of Anopheles larvae in the water collection, probably because of the reduced number of water collections in those sub-models, especially for 2007 where the ground measurement effort was inferior than in 2008 and 2009. For the other years, even if the 95% confidence intervals obtained in the four models were not all spanning, the directions and strength of the associations remained similar.

Table 4. Meteorological factors and environmental factors extracted from SPOT images and associated significantly with the presence of *Anopheles* larvae in the water collections followed on the ground, including observations for years 2007, 2008 and 2009 separately. Logistic regression with water collection random effect.

	Multivariate logistic regression with water collection random effect								
	2007 (237 obs. in 37 water collections)			2008-2009 (651 obs. in 59 water collections)			2009-2010 (750 obs. in 75 water collections)		
	Coef	95% IC	p-value	Coef	95% IC	p-value	Coef	95% IC	p-value
NDWI Mc Feeters dry season (mean in the collection and a 10m-buffer around)									
Per 1 unit increase	6.01	-9.10 - 21.12	0.436	11.13	3.12 - 19.15	0.006	9.52	0.42 - 18.63	0.040
Soil BI dry season (mean in the collections and a 10m-buffer around)									
Per 1 unit increase	2.34	-3.62 - 8.30	0.441	3.85	-0.26 - 7.96	0.066	2.19	-1.08 - 5.46	0.190
Current night temperature									
Per °C increase	-0.15	-0.62 - 0.32	0.527	0.39	0.18 - 0.61	<0.001	0.11	0.003 - 0.22	0.045
Rainfall amount in the preceding 30 days									
Per 10 mm increase	-0.11	-0.26 - 0.04	0.158	0.004	0.01 - 0.06	0.009	0.05	0.03 - 0.07	<0.001

Concerning the global model, the results of the likelihood ratio test ($p<0.001$) showed that the random effect model was significantly different than a model fitted without accounting for the water collection effect. Predictions of the probability for the presence of larvae from the validation sample (413 observations in 30 water collections) allowed calculating the area under the ROC curve at 0.81 (95% confidence interval: 0.77 - 0.85). The Se was 75% and the Sp was 70% (72% of correctly classified).

The areas under the ROC curve calculated after the fitting of this global model on the 2007, 2008 and 2009 validation subsamples were respectively 0.91 (0.80 - 1.00), 0.85 (0.80 - 0.91) and 0.77 (0.70 - 0.84). The Se/Sp couples were respectively 87%/57%, 73%/82% and 73%/62%.

The global model was also evaluated for its capacity to predict that a water collection was breeding site at least once during a year. The area under the ROC curve was calculated using a composite variable for the presence of *Anopheles* larvae at least once during the year and the maximum probability of presence of larvae in a water collection during its full follow-up. Results were 0.69 (95% confidence interval: 0.64 - 0.74) on the validation subsample.

Maps of the Anopheles breeding sites

Daily maps were drawn by inverting the global model predicting the presence of *Anopheles* larvae for each water collection detected at step 1. For the full duration of the follow-up, the daily probability of presence of *Anopheles* larvae was calculated for each predicted water collection. Those maps were the basis of the study undertaken at step 3.

A map presenting the total probabilities of presence of Anopheles *larvae* for the whole year 2009 is provided in Figure 2. It was drawn by summing the full set of daily maps from July 2009 to June 2010. This map allowed providing an estimate of the annual risk level of the water collections detected in the city.

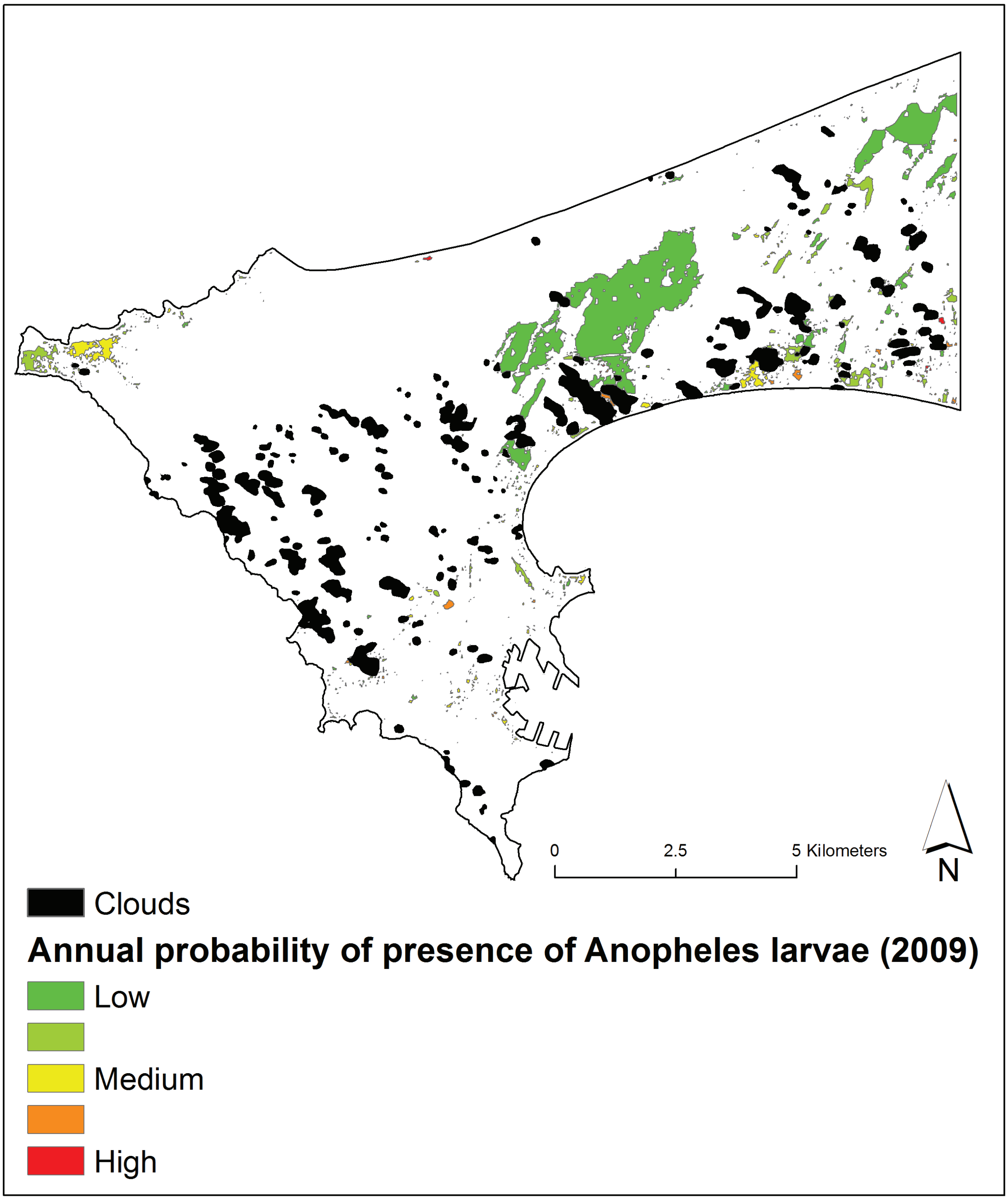

Figure 2. Total probabilities of presence of Anopheles *larvae* in 2009.

Anopheles adult mapping (step 3)

Statistical analysis and validation of the models

Results of the statistical analysis aiming at modelling the number of adult *Anopheles* mosquitoes caught on three human baits at a given date are presented in Table 5 (global model) and Table 6 (yearly models). The results of the univariate analysis are presented only for variables that remained significant in the multivariate analysis. The first significant explanatory variable was the *Anopheles* productivity surrogate in a 300m-buffer around the centroid of the three catching points (*i.e.* the product of the surface of each water collection included in the buffer, and the probability of presence of *Anopheles* larvae in each water collection, summed for "day minus 2" to "day minus 35" and weighted by the *Anopheles* adult survival rate and the surface water fraction). The second significant variable was the proportion of building and asphalt in a 200m-buffer around the centroid.

Among the studied 70 zones-years, 61 were outside of cloud cover. Two variables were significantly associated with the number of *Anopheles* adult mosquitoes caught on human bait. The *Anopheles* larval productivity surrogate in a 300m-buffer, weighted by the water surface was a risk factor whereas the built-up and asphalt surface percentage in a 200m-buffer was a protective factor.

The yearly coefficients were not all significant but the directions and strength of the associations remained similar with the coefficients of the global model.

Table 5 *Anopheles* larval productivity surrogate extracted from modelling weighted by the water surface and environmental factors extracted from SPOT images and associated significantly with the number of *Anopheles* adult mosquitoes caught in the 45 studied zones, including all the observations for years 2007, 2008 and 2009. Negative binomial regression.

	Negative binomial regression					
	Univariate			Multivariate		
100% of adult catching points (61 zones/years) Nb obs. = 858	Coef.	95% IC	p-value	Coef.	95% IC	p-value
***Anopheles* larval productivity surrogate in a 300m-buffer weighted by the water surface**						
Per unit increase	3.28	2.68 - 3.87	<0.001	3.14	2.54 - 3.75	<0.001
Built-up and asphalt percentage in a 200m-buffer						
Per % increase	-3.07	-4.26 - -1.88	<0.001	-1.16	-2.23 - -0.09	0.033

Table 6. *Anopheles* larval productivity surrogate extracted from modelling weighted by the water surface and environmental factors extracted from SPOT images and associated significantly with the number of *Anopheles* adult mosquitoes caught in the 45 studied zones, including observations for years 2007, 2008 and 2009 separately. Negative binomial regression.

	Negative binomial regression								
	2007 (40 obs. in 10 zones)			2008-2009 (391 obs. in 25 zones)			2009-2010 (427 obs. in 26 zones)		
	Coef	95% IC	p-value	Coef	95% IC	p-value	Coef	95% IC	p-value
***Anopheles* larval productivity surrogate in a 300m-buffer weighted by the water surface**									
Per unit increase	3.63	2.25 - 5.00	<0.001	3.26	2.20 - 4.31	<0.001	3.15	2.36 - 3.93	<0.001
Built-up and asphalt percentage in a 200m-buffer									
Per % increase	-4.66	-7.63 - -1.62	0.003	-1.33	-3.01 - 0.34	0.120	-0.74	-2.28 - 0.80	0.347

Concerning the global model, the Spearman correlation coefficient was 0.72 (p<0.001) between the total adult *Anopheles* count predictions and field measurements in each zone, weighted by the total number of catching nights per zone. The Spearman coefficients calculated for each year separately were 0.90 (p<0.001), 0.67 (p<0.001) and 0.75 (p<0.001) for 2007, 2008 and 2009.

The Figure 3 shows the scatter plot of the predicted *Anopheles* adult number for each 61 zones/year, on a logarithmic scale.

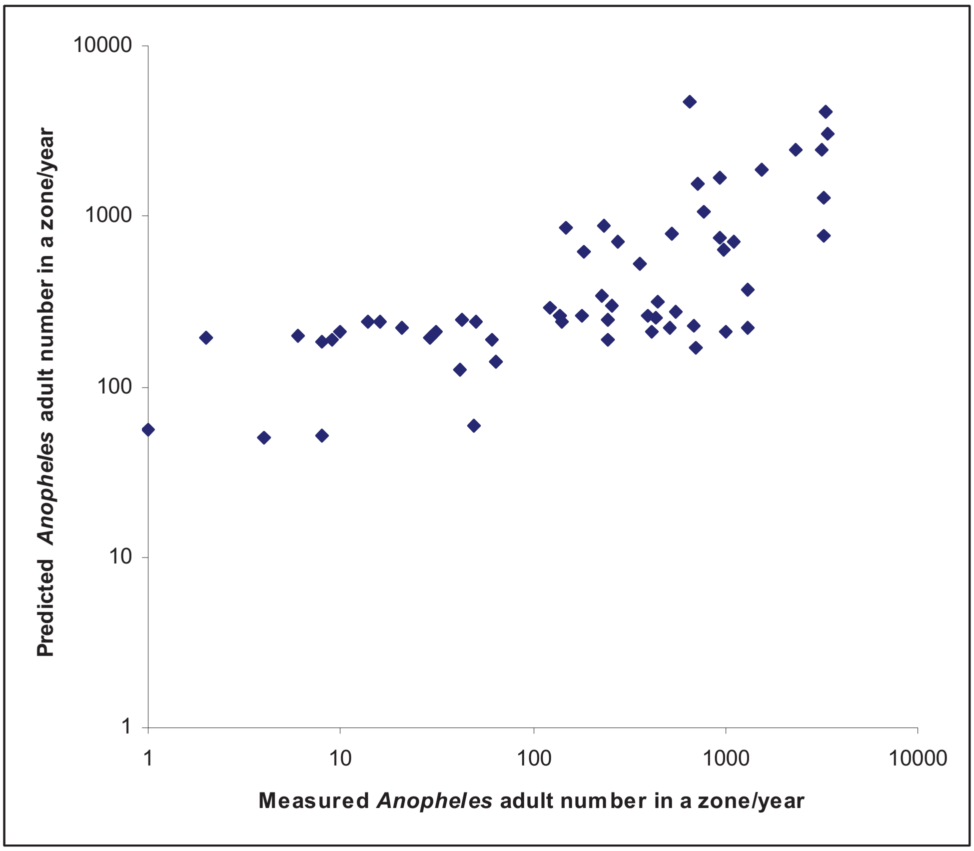

Figure 3. Scatter plot of the predicted *Anopheles* adult number for 61 zones/year, against observed values. Logarithmic scale.

Maps of the adult Anopheles densities

The inversion of the negative binomial statistical model, using the breeding sites map and productivity surrogates obtained in step 2, can lead to the prediction of the number of adult *Anopheles* in any location of Dakar (Figure 4, example of predicted number of *Anopheles* bites per person per night for the 20 September 2009). Figures 5 presents the same prediction, with the aggressivenesses measured on the ground at the same period (between 15 and 30 September 2009 depending on the studied areas).

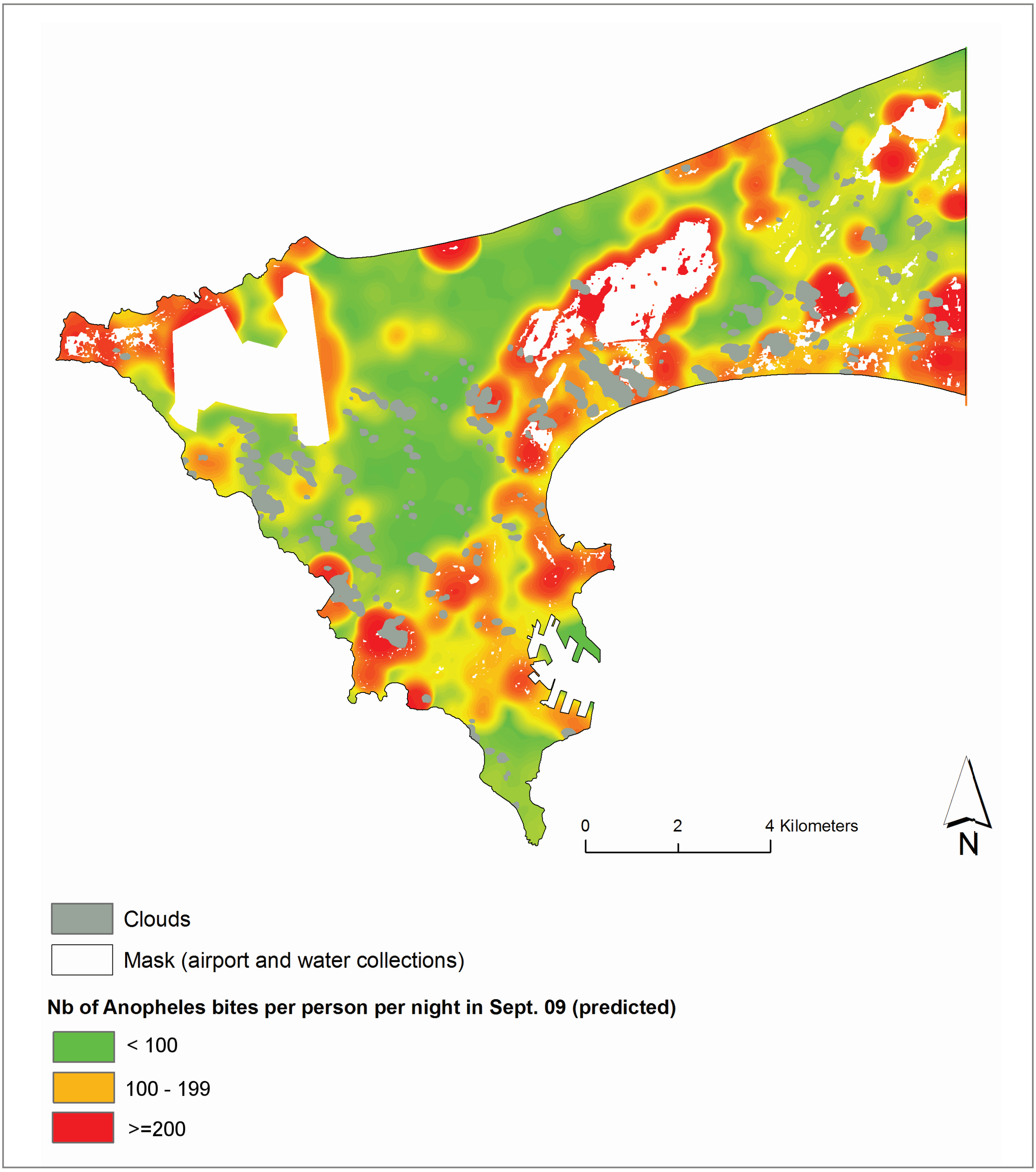

Figure 4. Predicted number of *Anopheles* bites per person per night in the second half of September 2009 (20 September 2009).

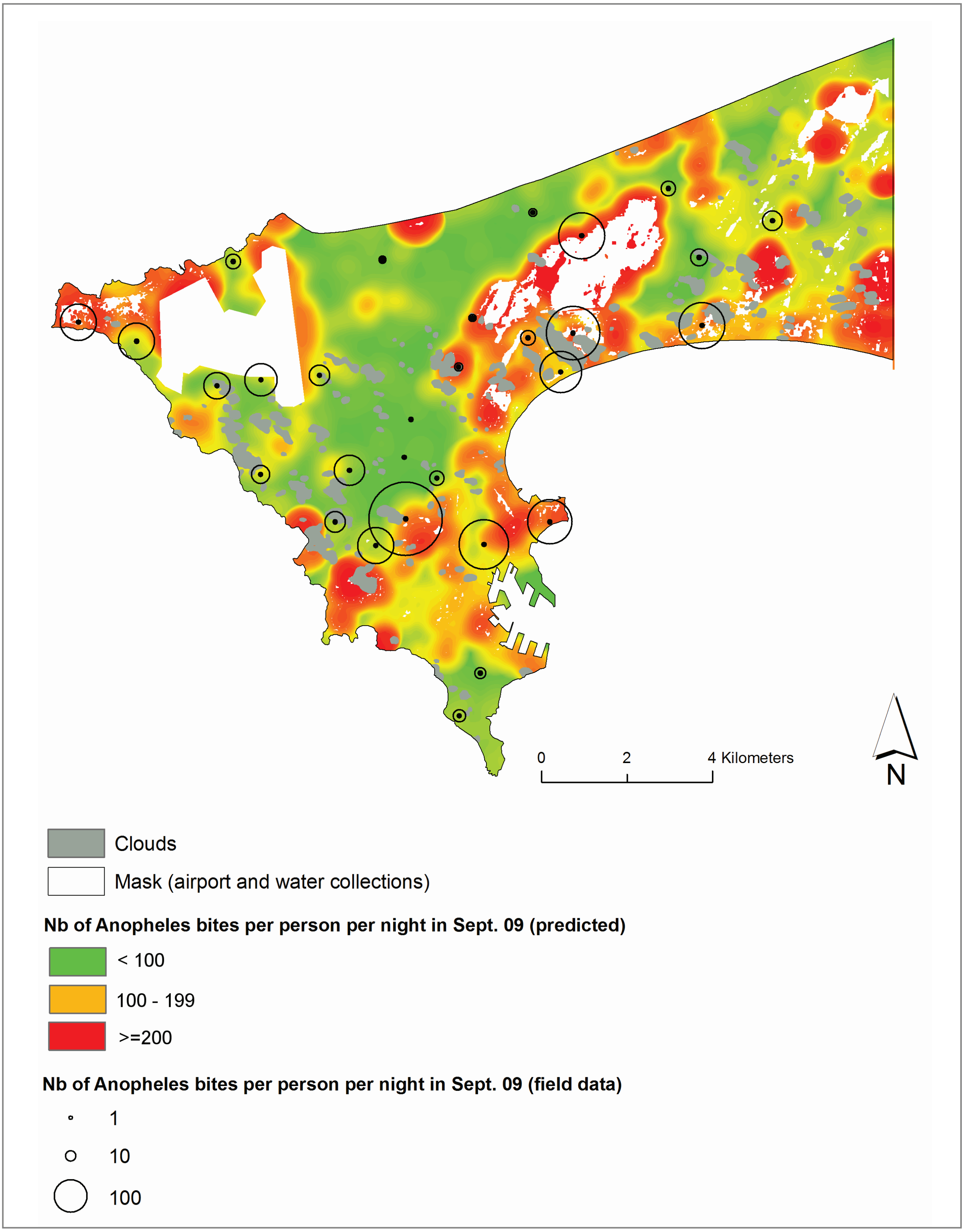

Figure 5. Predicted number of *Anopheles* bites per person per night in the second half of September 2009 (20 September 2009) and observed aggressivenesses between 15 and 30 September 2009.

Discussion

In the present study, remotely sensed environmental and meteorological data were successfully used to draw high resolution *Anopheles* larval and adult risk maps in Dakar, the capital city of Senegal. The results first showed that high spatial resolution SPOT (*Satellite Pour l'Observation de la Terre*) satellite images provided some indicators related to the presence of water in the city. Second, some remotely sensed environmental factors as well as ground and satellite meteorological information, were successfully associated with the presence of *Anopheles* larvae in water collections studied on the ground and allowed drawing breeding sites maps. Third, the *Anopheles* adult densities field measurements could have been predicted by some remotely sensed environmental factors as well as with an *Anopheles* larval productivity surrogate extracted from the breeding sites mapping.

<u>Water collections mapping (step 1)</u>

The water collections mapping was achieved using three variables extracted from the SPOT images of the wet and dry seasons, as well as data from a Digital Elevation Model.

First, the Modified NDWI is suitable for enhancing and extracting water information [43] so it was logically positively associated with the presence of water in the present study.

Second, the NDVI of the dry season allowed mapping locations with permanent vegetation and this was a proxy for the presence of persistent water. In Dakar, there is almost no rain during the dry season so any remaining vegetation outside of the wet season is associated with the presence of permanent water, such as the swamps created by the rise of the water table, locally known as "niaye" [20].

Third, the increase of the built-up surface was negatively associated with the presence of water in the study unit (10m-squares). This was expected that the presence of water is not favoured by high urbanization. In addition, this result was in adequacy with a longitudinal field study undertaken in Dakar in September 2009 in 355 private properties which showed that the majority of the gardens, flat roofs or balconies did not harbour large surfaces of persistent water (personal observation and [32]).

Fourth, the presence of water decreased with altitude. In Dakar, this was coherent as the water table come up to ground level in low altitude areas that can even be under the sea level. Those results confirm that the relatively low spatial resolution (90m, compared to the 2.5m SPOT images) and the intrinsic lack of precision (+/-15m-horizontal and +/- 6m-vertical) of the DEM could bring satisfactory information for the detection of water. Even in Dakar which is a rather flat city, the DEM allowed catching some contrasts. Elevation had already been described as a risk factor for the presence of aquatic habitats, such as in Kenya [47]. Elevation-derived indicators have also been used as predictors for the presence of water [48, 49]. In the present study, the curvature was associated with the presence of water in univariate analysis but not in the multivariate model.

The scale was chosen at 10m-spatial resolution and each indicator was averaged in the 10m-squares. This constituted a reasonable degradation of the 2.5m-spatial resolution of the 3-bands SPOT images, (the SWIR band already being at 10m-spatial resolution) which allowed taking into account the imprecision of the ground measurements of the water collection shapes, without loosing the capacity to capture the very high urban environmental heterogeneity.

The validation of the model using a random subsample of 20% of the observations allowed improving confidence in the results and the high area under the ROC curve (0.93) showed the high capacity of the model to detect water in this heterogeneous environment. The reproducibility of the model on subsequent years also improved confidence, especially because annual differences in the rainfall patterns were high in the present study with 178 mm, 510 mm and 565 mm for 2007, 2008 and 2009 respectively. In consequence, the use of the model could be considered for detection of water on future wet season images.
The validity of the model (Se: 90%, Sp: 84%, correctly classified: 84%) was comparable to some results obtained in non exclusively urban areas, such as in the Kakamega District in Kenya were computer models based on topographic features and land-cover information obtained from the Ikonos image yielded a misclassification rate of 20.3–22.7% for aquatic habitats [47].

In the present study, it has been preferred maximize sensitivity, implying the known risk to detect too many water collections than too less. Indeed, it was expected that the false positives will further be predicted with very low probability to harbour larvae. In the case of the use of such model in an operational system, this threshold could be adapted depending on the action plan for ground control. For example in conditions of important resources, it could be preferred to increase the false negatives in order to be sure not to miss any positive.

The size of the water collections in the present study could have been overestimated. Indeed, when fitting the model, it was decided to consider a 10m-square as water, even if it was only partly covering a water body registered on the ground. In an operation situation, this overestimation would not have any impact on the planning of the field control work.

In Dakar, the diversity of the water bodies is high, including puddles, ditches, wells, holes or marshy areas [19, 32]. A validation could be undertaken for each type of water bodies in order to detect any weakness in the detection of some types of aquatic habitats and guide any future work for improvement of the methodology. Nevertheless, in the objective of testing the model in other cities, very specific characteristics of Dakar water collections should not strongly impact the model validity.

Anopheles larvae mapping (step 2)

The prediction of the presence of *Anopheles* larvae in the water collections studied on the ground was achieved using two variables extracted from the SPOT images of the dry seasons, as well as ground and satellites meteorological data. Even if ground physical and environmental characteristics in and around water bodies were available in the scope of the present work, it was an informed choice to focus the analysis on environmental remotely-sensed variables, at the difference of other works that attempted to include both remotely-sensed and ground variables in their work [50].

The mean NDWI Mc Feeters (dry season) measured in the water collection and their 10m surrounding was positively associated with the presence of larvae. This indicator decreases with vegetation. It could have been related to the permanent/temporary characteristic of the water collections as permanent collections could be related with the presence of vegetation in the dry season. In the analysis of the ground factors associated with the presence/absence of *Anopheles* larvae presented in [32], it has been clearly showed that temporary water collections were more likely to harbour *Anopheles* larvae (OR=10.25, p<0.0001). Examination of the boxplot showed higher NDWI Mc Feeters values in temporary collections. In addition, muddy bottom for a water collection was a risk factor for the presence of larvae (OR=2.57, p=0.0006) [32]. Boxplot highlighted higher NDWI Mc Feeters values for collection with muddy bottom. The second significant variable was the mean soil BI and the same associations were found between the mean soil BI of the dry season and the presence of larvae. The BI was also positively related to temporary collections and muddy bottom. The analysis of the ground measurements also showed that a water collection located in a market-garden was less likely to harbour larvae (OR=0.09, p<0.0001) [32]. Boxplot showed higher NDWI Mc Feeters for collections outside of market-gardens.

Both indicators, NDWI Mc Feeters and soil BI have been found to be proxies for several variables measures on the ground. Nevertheless, they are low correlated (<0.20) so they each of them bring different environmental information. Their biological and physical meaning have been interpreted but could be deeper understood.

The LST night temperature of the ground prospecting day was also significantly associated with the presence of *Anopheles* larvae in the water collections. In breeding sites, the entire development cycle from egg to emerging adult, which passes through four larval stages or instars, can be completed under favorable conditions. This cycle can range between one and three weeks, depending on the water and air temperatures, assuming sufficient food availability. Thus, it was consistent that air temperature was significantly positively associated with the presence of larvae. Night temperature was better associated than day temperature, probably because it was less impacted by solar radiation and more representative of the averaged atmospherical conditions. In addition, comparability along time of night temperatures was likely more accurate than for day temperatures.

The total rainfall amount in the 30 days preceding the ground prospecting day was positively associated with the presence of larvae. On one hand, rainfall pattern drives the availability of surface water for mosquito breeding. In this part of the study, only flooded water collections were included so rainfall was already implicitly included. Nevertheless, the rainfall amount could be a proxy for the persistence of water bodies. Indeed, in the analysis of the ground data, the persistence of water bodies assessed from one field visit to another was significantly associated with the presence of *Anopheles* larvae (OR=1.21, p<0.001) [32]. On the other hand, rainfall was a surrogate variable for the global larval productivity in the city. The temporal graphics of the rainfall amounts and the total larval production in all the studied zones were closely superimposed [32]. Thus, in a high productivity period, *e.g.* the middle of the rainy season, every water collection will have increased probability to harbour larvae so the inclusion of rainfall in the model could have accounted for this temporal heterogeneity. Finally, it is known that too much water can flush away larval habitats but this threshold from which the rainfall amount becomes an unfavourable factor has not been taken into account.

The results of the present study are consistent with the literature as meteorological data have already been included in remotely-sensed models for the prediction of larval risk. In Indonesia, modelling of *An. subpictus* larval densities achieved using the season and the distance from the coast to larval habitats [51]. In Mali, Bayesian modeling allowed mapping of spatial distribution of *Anopheles gambiae s.s*. and *Anopheles arabiensis* [5] or of chromosomal forms of *Anopheles gambiae* [4] to be drawn, based on environmental and climatic (rainfall, temperature) factors.

The validation of the model using a random subsample of 20% of the water collections allowed improving confidence in the results and the area under the ROC (0.81) curve showed the satisfactory capacity of the model to detect larvae in the water bodies. The reproducibility of the model on subsequent years also improved confidence. The validity of the present results (Se: 75%, Sp: 70%, correctly classified: 72%) was in agreement with previous results in non urban setting obtained with remotely-sensed data, in Kenya (<25% of misclassifications) [47] or France (Se:0.76, Sp:0.78) [52].

Modelling of the larval densities using remotely sensed data has been tested in the present study. Some remotely-sensed variables have been significantly associated with the larval densities but the predictive ability of the model was low. This could have been due to the lack of informative satellite variables but also on the imprecision of the ground measurement. Indeed, even if the dipping was carefully undertaken on the ground, this sampling method is prone to numerous sources of imprecision. For example, the efficacy of dipping can be different depending on the larval instars or the capacity of the larvae to escape when disturbed can be varied [53].

In the present study, the detected water collections were built as objects, with their own environmental characteristics as it has been done in the North of Senegal in the study of the ponds harbouring larvae for the vector of Rift Valley Fever [44, 54]. Indeed, important predictors for the presence of larvae, such as shade around the collection [32], were water body-related. In consequence, it appeared to be accurate to map the environmental data at the level of the water collection and not at the pixel-level which could be relevant for large rural breeding sites [55] but which has less biological meaning in urban settings. Predictions of the probability of presence of *Anopheles* larvae could have done in each predicted water body. As the model was dynamic, a daily probability could have been predicted for every single water collection.

In an objective of using such map for guiding the larval control in the city, daily maps would not be informative and should be summed in weekly, monthly or yearly maps in order to classify the urban areas in different risk levels of presence of *Anopheles* larvae.

Anopheles adult mapping (step 3)

The calculation of the larval productivity surrogate took into account the biology of the *Anopheles* vector in urban settings. The daily survival rate had been evaluated in Pikine at 82% [46], meaning that from one day to another, 82% of the adult mosquitoes survived. After 35 days, an initial population would be close to extinction. In addition, a new emerged adult mosquito needs one or two days to reinforce its cuticle, rest and mate before researching its first blood meal. From those numbers was deducted the calculation of the probability of presence of larvae from “day minus 2” to “day minus 35”, with a daily survival rate at 82%.

The step 1 of the present study allowed drawing a unique water collection map each year, directly depending on the characteristics of the wet season SPOT image. As the images of 2007, 2008 and

2009 wet seasons were acquired close to the peak of rainfall, the water collections were in most cases at their maximum size. The size of the breeding sites impacts on the production of adult *Anopheles* from a same larval density so it is a major determinant of the adult densities. To account for the variations of water surface, the non linear model first allowed to differentiate temporary from permanent collections and to calculate, for temporary collections, the water surface fraction (from the maximum size) depending on the rainfall amount in the preceding 7 days. This fraction allowed weighting the larval productivity surrogate. It was assumed that permanent collections had a fixed size, even if ground data showed that some of them moderately increase in the rainy season. The model including the weighted larval productivity surrogate had a better predictive capacity than the model with the non-weighted variable.

The prediction of the presence of adult *Anopheles* measured on the ground was done using: i) the larval productivity surrogate, weighted by the water surface, summed for all the water collections found in a 300m-buffer around the catching points and ii) the percentage of built-up and asphalt area in a 200m-buffer. A similar result has been found in Camargue, a marshy area of France, where a larval index (*i.e*. probability of the presence of larvae) modeled at each pixel using remotely-sensed data were a very accurate predictor for the *An. hyrcanus* adult abundance caught at 300m distance [52].

Regarding the scales of associations, the findings are consistent with previous works in Pikine that found that most *An. arabiensis* were caught less than 285 m from the marshland, *i.e.* the breeding sites [20]. Dispersal of *Anopheles* is generally <300m in highly-populated urban settings [20, 56-59].

The built-up and asphalt surface was a protective factor and, in adequation with the fact that sparsely built-up areas are known to be risk factors for malaria in cities [57]. First, the urban environment is unfavorable for the apparition of breeding sites. Thus, if it is assumed that some breeding sites have been omitted in the mapping, the variable could partly correct this weakness. Second, urbanization decreases the life span of the adult mosquitoes as they do not provide appropriate resting and feeding sites. Third, the high presence of buildings can be a proxy for a high human population density, leading to the dilution of the *Anopheles* bites.

In cities, the urbanization has already been associated with entomological factors. In Dakar, a preliminary study to the present work showed that almost 60% of the variability in anopheline aggressiveness measured in 1994-97 was explained with only one variable: the built-up area in a 300m-radius buffer around the catching points. This association remained stable between 1996 and 2007 [31]. In Malindi and Kisumu, Kenya, the NDVI was associated with a low housing density and thus with a higher probability of *Anopheles* breeding sites, at the scale of 270*270 m [60]. In New

Haven, United States, the use of 15m to 30m resolution ASTER (Advanced Spaceborne Thermal Emission and Reflection Radiometer) images to map the amount of vegetation in 50m-buffers around *Aedes* and *Culex* capture point, as well as the distance to water bodies, were related to mosquito densities [61].

The validity of the model was good, with a Spearman correlation coefficient at 0.72 ($p<0.001$) between the yearly total adult *Anopheles* count predictions and the field measurements in each zone.

Ground data

The present study relied on a very large entomological dataset, collected at different years and different seasons from an extensive study conducted in 45 zones in Dakar and its suburbs from 2005 to 2010 [19, 32, 62]. The high temporal frequency of the field prospecting allowed including the temporal and seasonal evolution of the presence of *Anopheles* larvae in the models and they showed their capacity to catch the contrasts between years and seasons.

Rainfall data used in the present study were issued from ground measurements done at one point in Dakar. In sahelian climate, rainfall can be very focused and daily rainfall amounts and patterns are not always the same in each part of a same city. More accurate ground data, if available, could be tested for model improvement. Remotely-sensed rainfall data, such as the ones provided by the near-real-time TRMM (Tropical Rainfall Measuring Mission) satellite [63] could also be tested and will be useful in places where ground data is not available.

The LST correlates highly with air temperature, but the relationship can differ depending on land cover and humidity, atmospheric conditions, the study area and the period of the day that the image is recorded [64-67]. Nevertheless, no absolute values were needed in the present study and relative differences were used. In addition, the use of 8-days MODIS composite images should have smoothed any daily measurement discrepancies.

Remotely-sensed data

Comparing images acquired at different dates can be difficult because some differences are not due to actual ecological differences; instead, they are related to the specificity of the images, such as differences in atmospheric conditions, solar angle, sensor calibration, period of the view or the registration of images [68]. The method used in the present study improved the validity of the comparison in several ways. First, comparisons between images were not done directly on the images but rather on the results of classifications and calculations of indices, thus taking into account intrinsic parameters for each image. Furthermore, images were acquired in level 3 pre-processing, thereby avoiding all problems of spatial misregistration. Finally, differences in atmospheric conditions were taken into account thanks to the IARR pre-processing.

The SPOT images were fully contemporary to the field work and this largely improved the comparison validity between ground data and remotely-sensed data.

On the contrary, for the dry season, only one image was acquired. This was probably not a problem as no important changes of land use and land cover was recorded on the ground in the studied zones. In addition, the dry season is not submitted to annual variations such as in the wet seasons were rainfall patterns can be very different.

Despite all the precautions taken to use and process satellite images in a right way, some misclassifications occurred. Some large areas were manually masked but the authors are aware that other misclassifications occurred, mainly between water and dark asphalt. Whatever the indicator calculated or the classification process undertaken, black soils (*e.g.* parking areas) were sometimes recognized as water. This point will be particularly emphasized in future works.

Technical perspectives

In future works, the detection of small collections and the accuracy of the maps of indicators and of the classifications could be improved by the use of higher spatial resolution images, such as Quickbird, Worldview-2 or Pleiades images. In cities, the cover is spatially highly heterogeneous, the number of different building materials is high and the occurrence of mixed pixels is important [69]. Thus, even if some very high resolution images do not have any SWIR band, it can be expected that they will bring useful information. Focused should also be done on new forms of classification based on object-oriented techniques that are particularly well adapted for very high spatial resolution image analysis. The benefit of the object-based approach, moving from a pixel to object representation, is that it allows the incorporation of information of spatial neighborhood and not only information from a single pixel at once [70, 71].

Radar imagery could also be of interest as it has an enhanced capacity to detect water and humidity and it can overcome the acquisition problems caused by cloud cover. A sub-study has been undertaken in the scope of the present study using TerraSAR-X images at 1m-theoretical resolution acquired in the 2007 wet season processed by interferometry techniques to detect free water in Dakar but first results could not improve water detection and showed important misclassifications of water. Nevertheless, it has already been useful in the study of malaria in tropical areas [72, 73], even in urban settings [74].

In addition photointerpretation done by operators having a good knowledge of the possible water collections in Dakar could assist the automatic detection, at the level of the detection of water bodies or the detection of risk factors for the presence of larvae. Synergy between both methods could be of interest. In the present study it was an informed choice to totally automate the mapping process and to work exclusively with models, except for masking the two large areas wrongly detected as water. Indeed, in the objective of an operational tool, a fully automatic system could be of interest. Nevertheless, photointerpretation come as a support of an automated process. In the present study, a sub-sample of the water collections recorded on the ground has been characterized by experimented photointerpretators. The detected market-garden could have been significantly associated with the presence of larvae in the water collection. The availability of very high spatial resolution images, at less than 1m, should improve the possibilities of human detection of objects or characteristics. In Kenya, photointerpreation of 1m-spatial resolution Ikonos images allowed detecting 41% of the aquatic habitats [47]. In Malindi and Kisumu, Kenya, researchers attempted to directly identify *An. gambiae s.l.*, *An. funestus* and *An. merus* breeding sites using 5- to 20-m spatial resolution MTI (Multi-spectral Thermal Imager) images, but only 6% of the sites were detected [75]. On the contrary, in Dar-Es-Salaam, Tanzania, aerial photographs were visually interpreted to identify breeding sites and thus helped guide an integrated fight against malaria in the city [76].

Focus could also be put on the study of the evolution of the environmental changes that could lead to an increased entomological risk. In two cities in Kenya, MTI satellite images acquired at 14-year intervals were used to detect changes in land use and land cover and showed that the presence, abundance and spatial distribution of breeding sites were driven by the evolution in the urbanisation. Larval-positive water collections were primarily found in changing environments [77].

An enhanced classification of vegetation could also bring new information in the modelling process. Indeed, the different types of vegetation, such as trees or grass, may have different impact on the presence of larvae in water collections (providing shade or not) or the *Anopheles* adult survival (providing resting sites or not).

Finally, a validation of the maps should be undertaken in other cities of the sub-Saharan region, such as N'Djamena or Bamako in order to test the mapping capacity of the model in other context. It could be envisaged that a limited field work in new cities would allow fine-tuning the model.

Health perspectives

In addition to any entomological use of the risk maps, it could be public health interest to map the spatial distribution of malaria in cities using remotely-sensed data. In Ouagadougou, Burkina Faso, the overall prevalence of anti-CSP (circumsporozoite) antibodies and *P. falciparum* infections among children were associated with sparsely built-up areas and irregularly built areas that were partly mapped using a SPOT-5 satellite image [57]. In a village of Cambodia, an increased distance to the forest was a significant protective factor for *Plasmodium* infection [78]. In Antananarivo, the capital city of Madagascar, the rice field surface area, together with altitude, temperature, rainfall and population density, were investigated as potential risk factors for confirmed malaria cases [79]. At larger scales, numerous works have been done to draw prevalence maps [80-84]. Nevertheless, following the principle of modelling on the basis of a deep understanding of the subjacent biological mechanisms, a third level of risk map for malaria parasite prevalence in urban settings could be derived for entomological risk maps drawn in the present study. To achieve such goal, powerful set of epidemiological data should be collected on the ground and compared with entomological ground data and models. In addition, malaria occurs only where and when a competent vector meets a human sensitive population and a *Plasmodium* rservoir. Urban population mapping should also be a challenging objective when no population census report is available. Indeed, risk areas should be defined as the areas where hazard and vulnerability overlap. Hazard represents the “potential risk”, e.g., the vector distribution, and vulnerability relates to the distribution, sensitivity and exposure of human populations.

Methodology

The methodology of the present study was chosen as a step by step approach in order to highly relate modelling to the biological mechanisms of the larval development and adult densities. Each indicator having found to be associated with the presence of larvae in pools or with an increased biting risk has been put in relation with ground measurements and biological aspects. Nevertheless, several studies pulled from the environment and climate to the adult densities or even to the parasite prevalence, morbidity or mortality and they reached satisfactory results. The step by step approach could have the disadvantage to multiply errors due to successive modelling. The choice of the methodology probably could lie in between the two approaches on order to introduce enough comprehension of the subjacent biological mechanisms but not to multiply the steps that could be shunted.

A large panel of remotely-sensed indicators have been tested in order to maximize the possibilities of highlighting environmental information associated with the presence of water and the presence of larvae in the water collections. It has been an informed choice to include numerous indicators as they could all bring some ecological information linked to biological and physical mechanisms.

Conclusion

Remotely-sensed environmental and meteorological data allowed drawing different levels of maps that could be of interest for focusing malaria control in urban settings. The first map is a map of the potential *Anopheles* breeding sites in Dakar that could guide larval management. The second map is a map of the adult *Anopheles* densities that are driven by the proximity, surfaces and potential productivity of breeding sites as well as urbanization levels, which could guide adult mosquito control activities. In addition to research interests, one key issue of the present work is to be a preliminary step for the implementation of an operational system to facilitate real-time monitoring of human health.

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Addendum à l'article 6

Dans l'article 6, il a été évoqué que le plan d'échantillonnage mis en place pouvait introduire des autocorrélations spatiales entre les observations. Au niveau de la détection de l'eau, des pixels proches les uns des autres avaient plus de chance de se ressembler que des pixels éloignés. De la même façon, deux collections d'eau d'une même zone d'étude (*i.e.* qui partageaient des caractéristiques environnementales similaires) étaient plus susceptibles de comporter toutes deux des larves. Enfin, les mesures des densités de moustiques adultes pouvaient aussi être soumises à des autocorrélations spatiales.

Négliger des autocorrélations peut conduire à surestimer la force des associations entre les variables explicatives et la variable à expliquer car elles tendent à sous-estimer l'erreur standard [22]. Pour palier à ce problème, des modèles multi niveaux ont souvent été utilisés pour analyser la corrélation des indicateurs paludométriques imbriqués dans un niveau hiérarchique supérieur, tel que des individus dans des maisons [14, 23, 24]. Des modèles de régression logistique avec effets aléatoires (effet zone ou effet collection d'eau) ont ainsi été utilisés dans l'article 6 pour modéliser la présence de collections d'eau (par zone) et la présence de larves (par collection d'eau). Dans ces modèles, les effets zones et collection d'eau étaient significatifs.

Une autre façon de prendre en compte l'autocorrélation spatiale est d'utiliser des modèles spatiaux qui permettent d'incorporer explicitement la corrélation spatiale comme une fonction de la distance ou du voisinage des observations [25]. Etant donné le grand nombre de paramètres à estimer dans ces modèles (il y a un effet aléatoire par unité d'espace, tel que le pixel ou le département par exemple), ils peuvent être plus facilement ajustés dans le cadre des statistiques bayésiennes grâce à l'utilisation des simulations des chaîne de Markov Monte Carlo (MCMC) [26, 27]. Les prédictions pour un lieu donné sont faites en tenant compte de la distance ou du niveau de voisinage entre le lieu et tous les lieux qui ont servi à ajuster le modèle. Ces modèles sont de plus en plus utilisés pour la cartographie du risque de paludisme [26, 28-34] et ils sont les mieux adaptés à l'analyse des données géolocalisées.

Dans le cadre de la cartographie du risque entomologique à Dakar, nous allons utiliser l'approche Bayesienne. Un modèle bayésien a déjà été ajusté pour la détection de l'eau sur un sous échantillon des carrés de 10 m de l'année 2009 (analyse préliminaire non présentée). En effet, le calcul des modèles dans le logiciel Open BUGS est soumis aux limites de calcul de l'outil. D'autre part, la prise en compte des coordonnées géographiques au niveau de chaque carré de 10 m est techniquement difficilement réalisable (augmentation importante du temps de calcul pour l'ajustement des modèles) et n'est probablement pas nécessaire. En conséquence, pour cette analyse préliminaire une grille de 40 m a été superposée aux grilles de 10 m. L'effet lieu a été pris en compte au niveau des carrés de 40 m auxquels étaient affiliés 4 carrés de 10 m et il a alors été possible d'ajuster des modèles spatiaux bayesiens. Cette analyse préliminaire sur un sous-échantillon des données 2009 a montré que les estimations des coefficients de régression obtenus par les modèles bayésiens etaient très proche de ceux obtenus avec les modèles mixtes classiques (données non présentées). Or les résultats des modèles bayésiens montrent que la distance à partir de laquelle la corrélation est inférieure à 5% est de presque 800m. Ainsi, un effet zone pourrait suffire à prendre en compte la corrélation spatiale. Quand à la prédiction, un programme a été écrit en langage R qui permettra de générer les cartes de risque.

Ces analyses seront poursuivies et étendues à l'ensemble des données des 3 années. Des modèles bayésiens seront aussi ajustés pour la prédiction de la présence de larves et la prédiction des densités agressives d'adultes. Les résultats seront inclus dans l'article 6 pour être soumis pour publication.

DISCUSSION - CONCLUSION

Les résultats des travaux de thèse ont montré que des données de télédétection associées à une grande quantité de données de terrain peuvent permettre l'ajustement de modèles prédictifs et la construction de cartes de risque entomologique. D'une part, pour des populations mobiles, les résultats obtenus ont indiqué que la télédétection peut être utilisée pour évaluer la part de l'environnement dans le risque de paludisme rencontré chez des voyageurs. D'autre part, dans les villes, malgré les échelles fines d'hétérogénéité du risque de paludisme, des images satellites à haute résolution spatiale, associées à des données météorologiques, ont apporté l'information nécessaire pour cartographier les collections d'eau, les gîtes larvaires productifs et les densités d'anophèles adultes. L'originalité de ce travail réside dans l'importance des données de terrain qui ont été récoltées spécifiquement pour ce travail (en termes de nombre de sites et de fréquence des investigations) et dans la construction de modèles reposant étroitement sur la connaissance de la bio-écologie de la transmission du paludisme plutôt que sur la recherche aveugle de corrélations spatiales et temporelles.

La revue de la bibliographie a montré que de nombreux résultats ont été publiés quant à la modélisation du risque de paludisme (au niveau entomologique, parasitologique ou épidémiologique) s'appuyant sur des données de télédétection. Nos travaux de thèse se sont appuyés sur les méthodologies existantes (*e.g.* classification des images, utilisation d'indicateurs classiques tels que le NDVI, utilisation de zones tampons…) tout en s'en détachant pour certains aspects novateurs. Ainsi, certains pans de la méthodologie mise en œuvre pendant la thèse sont originaux, tels que le plan d'échantillonnage de terrain, l'utilisation d'indicateurs peu communément employés, la cartographie par étape (eau - larves -adultes).

A notre connaissance, aucun travail n'avait été mené pour étudier le risque environnemental de paludisme, mesuré par télédétection, pour des populations mobiles. Dans un contexte de déplacements de militaires dans différents pays, et au sein de chaque pays, dans différentes régions, l'exposition environnementale a pu être fortement associée au risque d'accès de paludisme clinique. Les images de NDVI MODIS avaient l'avantage d'être disponibles pour toutes les zones d'étude et leur résolution kilométrique était appropriée pour une étude sous continentale contenant des points d'études éloignés. Ainsi, la preuve de concept que les données de télédétection peuvent être utilisées pour évaluer le risque palustre chez des voyageurs et pour standardiser des études d'efficacité des mesures de protection contre la maladie, a été apportée. A cette étape du travail, une grande quantité de données de terrain était déjà à notre disposition et la numérisation des « carnets de déplacement » a permis de reconstituer les itinéraires individuels qui ont ensuite été introduits dans un Système d'Information Géographique (SIG). Le traitement des images MODIS a été simple car il s'agissait de convertir le format des images, de les reprojeter et de les inclure dans le SIG. Au niveau des analyses statistiques, le plan d'échantillonnage a permis de mettre au point l'utilisation de modèles a effets aléatoires. Cette partie de la thèse a constitué un premier contact avec les SIG, la télédétection et les analyses statistiques permettant de mettre en évidence des facteurs de risque de paludisme. Même si ces premiers résultats sont encore éloignés d'une éventuelle application opérationnelle, il n'en reste pas moins que ces travaux ont permis d'apporter une connaissance nouvelle dans l'utilisation des données de télédétection dans les questions de santé humaine.

Pour notre étude en milieu urbain, l'approche méthodologique a reposé sur les étapes suivantes. Tout d'abord, un plan de collecte de données entomologiques a été suivi rigoureusement pendant plusieurs années dans la ville de Dakar. Des données de prospection larvaire et de capture d'adultes se posant sur appât humain ont été collectées dans 45 zones, à une fréquence élevée. Les résultats de ces collectes ont permis, 1) un état des lieux du risque de paludisme dans la capitale du Sénégal, 2) une mise en évidence de l'hétérogénéité spatiale et temporelle du risque entomologique de paludisme dans la ville, 3) la construction d'une base de données de terrain géoréférencées et 4) l'identification d'indicateurs environnementaux et météorologiques obtenus par télédétection (*i.e.* par traitement le plus souvent automatisé d'images satellites) associés à la présence de gîtes larvaires et/ou à l'agressivité des anophèles.

La méthodologie et les résultats des collectes de données entomologiques effectuées dans la ville de Dakar ont été présentés dans trois articles. Le jeu de données collecté pendant cinq ans dans la capitale du Sénégal est important et il a permis de donner une vision actualisée de la situation entomologique dans la ville, du point de vue des espèces d'*Anopheles*, de la description des gîtes larvaires et de l'hétérogénéité de l'agressivité anophélienne selon les quartiers. La base de données de terrain constituée pendant cette période de suivie a fourni une base solide aux étapes de modélisation du risque urbain de paludisme. Elle semble représenter l'une des bases d'information les plus importantes utilisées pour la cartographie du risque basée sur l'utilisation de données de télédétection.

D'une part, l'acquisition et le traitement d'images satellite SPOT-4 (20 m) et SPOT-5 (2.5m) à 11 années d'intervalle et la mise en relation avec des données de terrain issues de ce projet et de la littérature, a permis de montrer que le pourcentage de la population à fort risque de paludisme avait baissé à Dakar entre 1996 et 2007. Ainsi, un indicateur simple du niveau d'urbanisation a pu prédire avec une précision satisfaisant les niveaux de densités d'agressivité d'anophèles. Ces résultats étaient cohérents avec le fait que les zones urbaines partiellement construites sont connues pour être des zones à risque de paludisme [14]. En effet, elles représentent une combinaison de facteurs de risque tels que la présence de gîtes larvaires, la survie des adultes et la concentration des piqûres d'*Anopheles* sur une faible population. A cette étape, les phénomènes biologiques en jeu ont été pris en compte de façon globale et non détaillée et l'enjeu du travail a plutôt porté sur le traitement approprié d'images satellites à des résolutions spatiales différentes et acquises à des dates différentes dans l'objectif de mettre en évidence l'évolution temporelle du risque. Les résultats étaient en adéquation avec des études menées en milieu urbain, tel qu'à Malindi et Kisumu, au Kenya, où la faible densité des maisons dans une grille de 270x270 mètres était associées à une probabilité accrue de présence de gîtes larvaires d'anophèles [13]. Les cartes de risques dressées n'avaient pas pour objectif d'être opérationnelles mais elles ont permis de bien documenter les évolutions du risque de paludisme pour la population dakaroise.

D'autre part, des modèles ont été construits à partir des conditions environnementales de présence de collections d'eau et des déterminants connus de la biologie des vecteurs et des cartes de risque en ont été déduites. L'acquisition et le traitement d'images SPOT-5 à 2.5m de résolution spatiale, contemporaines aux collectes de données de terrain, a ainsi permis d'identifier et de valider des indicateurs utiles et pertinents pour une cartographie raisonnée des collections d'eau, des gîtes larvaires productifs et des densités agressives d'anophèles. Cette identification a été possible car elle s'est appuyée à chaque étape sur les données de terrain et sur la connaissance de la biologie des vecteurs. C'est cette étape qui a constitué réellement la partie innovante des travaux de thèse. Tout d'abord, le travail de modélisation a reposé sur une grande quantité de données de terrain, collectées sur plusieurs années, et sur plusieurs images satellites contemporaines à ces informations in situ. Dans la bibliographie, les travaux se heurtent souvent à la disponibilité décalée de données de télédétection et de données de terrain. Pour cette thèse, les passages de SPOT-5 ont été guettés pendant les prospections larvaires. De plus, les données entomologiques étaient collectées à un nombre de sites élevé (45 sites) permettant la mise en évidence de l'hétérogénéité spatiale, et une fréquence temporelle très élevée (tous les 10 jours pour les prospections larvaires et tous les 15 jours pour les collectes de moustiques adultes) permettant l'inclusion d'un aspect dynamique dans les modèles, fondamental pour une mise en place opérationnelle de la cartographie du risque.

Les images SPOT-5 ont fourni la base de l'information environnementale utilisée dans ces travaux. Le traitement adapté des quatre images, le choix des classifications et des indicateurs a permis de fournir des données de substitution à des facteurs mesurés sur le terrain. Le souci constant de comprendre les effets de ces variables « proxies » sur les processus physiques (*e.g.* présence d'eau) et biologiques (*e.g.* environnement des gîtes larvaires) a permis de rester en adéquation avec les connaissances du terrain. Les données de télédétection ont eu un rôle central dans la modélisation du risque car elles ont substitué ces données de terrain, une fois les mécanismes compris.

La cartographie de la présence d'eau dans la ville de Dakar a été guidée par la présence de collections d'eau pouvant potentiellement contenir des larves d'*Anopheles (e.g.* flaques d'eau recouverte de végétation de surface). Ainsi, la cartographie de l'eau a été adaptée à la problématique du paludisme à Dakar en prenant en compte les multiples caractéristiques que peuvent revêtir les gîtes larvaires potentiels.

Concernant la cartographie des gîtes larvaires, l'originalité du travail a été de prendre les gîtes larvaires en tant qu'objets. Souvent, les articles décrivent une probabilité de présence de larves au niveau du pixel. Or, les études entomologiques ne décrivent pas des facteurs de risque de présence de larves à un autre niveau que celui de la collection d'eau. Dans notre volonté de rester en adéquation avec les observations de terrain, le risque de présence de larves a donc été recherché au niveau de l'objet collection d'eau.

Pour la cartographie des agressivités d'*Anopheles* adultes, la force de ce travail a été de mettre en place une variable de substitution de la productivité larvaire (somme des probabilités de présence de larves autour d'un point de capture, sommées sur environ un mois, avec application d'un facteur de survie quotidienne des *Anopheles* adultes en milieu urbain) qui a été associée avec succès aux densités de moustiques capturés sur le terrain.

Un atout majeur des résultats aux trois niveaux de cartographie a été la reproductibilité des modèles sur les années 2007, 2008 et 2009. Dans l'objectif de la mise en place d'un système opérationnel, il n'est pas envisageable de ré-ajuster des modèles statistiques chaque année. En

utilisant des données environnementales et météorologiques sur plusieurs années, il a été possible de mettre en place un seul et unique modèle à chacun des trois niveaux de cartographie de l'eau, des larves et des adultes, qui a pu s'adapter sur ces trois années. Ceci est d'autant plus intéressant que 2007 a été une année sèche en comparaison à 2008 et 2009. Les modèles, grâce à l'utilisation des données de pluviométrie et de température ont donc été capables de capter les variations géo-climatiques saisonnières et annuelles.
Le travail de cartographie effectué à Dakar constitue donc un apport original dans la méthodologie de cartographie du risque de paludisme par rapport aux travaux identifiés dans la revue de la bibliographie.

Les limites rencontrées dans ces travaux de thèse se situent à différents niveaux. Tout d'abord, la transportabilité des modèles doit être discutée si l'on souhaite les appliquer à d'autres villes d'Afrique sub-saharienne. Un effort particulier a été porté sur l'automatisation des processus de génération des cartes de risque afin que les modèles ne souffrent pas d'une subjectivité liée à la bonne connaissance de la ville, ce qui pourrait ne plus être le cas dans d'autres cités. Le calcul automatique des indicateurs de télédétection faciliterait la transposition de ces modèles alors que la forte implication humaine dans les processus de classifications serait plutôt un facteur défavorable. Dans le cas présent, la mise en place et la validation des classifications dépendaient en effet de la connaissance du terrain et de la photo-interprétation. Cependant, c'est majoritairement la classe de bâti qui a été utile dans la modélisation et la reconnaissance du bâti sur les images à haute ou très haute résolution est généralement aisée. Il est donc très probable que la photointerprétation de ce type d'image, même sans « vérité terrain », suffise à valider la qualité des classifications. Par ailleurs, dans la première étape de notre travail (évolution du risque en 11 années), la validation a été effectuée sur les mêmes polygones que ceux utilisés pour la classification (*i.e.* polygones non indépendants). Cela peut avoir entraîné une surestimation de la qualité de la classification. Dans l'avenir (*i.e.* pour d'autres villes), des visites de reconnaissance du terrain éviteraient que le traitement des images soit fait « en aveugle » et il serait plus approprié d'utiliser pour la validation des jeux de données indépendants des polygones d'apprentissage.
Au niveau des relevés entomologiques de terrain, il ne peut être exclu, malgré l'intensité des efforts de collecte, que l'imprécision des mesures ait pu limiter la qualité de l'ajustement des modèles statistiques. L'évaluation des densités larvaires est approximative à cause de la capacité des larves à s'échapper lorsqu'elles sont dérangées, capacité qui peut être différente selon les stades larvaires [35] et la nature des gîtes prospectés. De même, l'évaluation des

densités d'adultes sur trois postes de captures était imprécise même si les captureurs se relayaient au sein de chaque poste. L'estimation de l'agressivité anophélienne dépend de l'attractivité propre à chaque individu et du microenvironnement (*e.g.* elle peut différer entre deux pièces d'une même maison, entre deux maisons ou entre deux jardins). De plus, les conditions météorologiques étaient variées (vent, pluie, température) et elles on pu jouer sur la comparabilité des résultats des captures dans un même site, d'une séance à une autre. Cette imprécision de l'estimation des variables dépendantes (*i.e.* variables entomologiques « à expliquer ») pouvait biaiser vers zéro l'estimation de l'effet des facteurs prédictifs (*i.e.* coefficients de régression) et donc sous-estimer leur valeur prédictive. De ce point de vue, il est possible que la validité de nos cartes de prédiction ait été sous-estimée.

Pour l'aspect technique du traitement des images satellites, plusieurs limites ont pu exister. La comparaison d'images prises à plusieurs dates doit être faite avec précaution. Même si une standardisation préalable aux traitements des images a été réalisée, il ne peut être totalement exclu que des différences entre des valeurs de pixels soit liées à des différences propres aux conditions d'acquisition des images (angle de vue, conditions atmosphériques) plutôt qu'à de réelles différences écologiques. D'autre part, des confusions ont clairement été identifiées dans les résultats des classifications ou des calculs d'indicateurs. En particulier, l'eau et certaines zones d'asphalte ont une signature radiométrique similaire dans certaines zones.

Même si les validités des modèles de détection de l'eau, de prédiction des gîtes larvaires et des densités d'*Anopheles* adultes étaient bonnes, elles pourraient probablement être améliorées, peut-être par l'utilisation d'autres images (radar, ou optique très haute résolution), d'autres indicateurs ou combinaisons d'indicateurs ou d'autres classifications (*e.g.* classification orientée-objet possible sur les images à très haute résolution spatiale).

Pour finir, nous souhaiterions préciser les limites de la signification des résultats de cette thèse. Des cartes de risque entomologique ont été dressées pour cibler la lutte anti-larvaire d'une part et anti-imago d'autre part. Il doit être clairement précisé que les cartes d'agressivité anophélienne ne sont pas des cartes de transmission des plasmodiums ni des cartes épidémiologiques du poids du paludisme. L'utilisation des cartographies mises en place dans le cadre de la thèse est destinée à lutter contre le moustique, quel que soit son statut vis-à-vis de l'infection par les plasmodiums. Des cartes de transmission devraient prendre en compte, en plus de la densité vectorielle, les déterminants de la durée du cycle sporogonique et l'importance du réservoir humain de plasmodiums. Ce dernier dépend de l'immunité antipaludique de la population humaine (phénomène encore mal connu et mal évalué), de l'accès aux traitements antipaludiques et de la sensibilité des plasmodiums à ces médicaments. Ces paramètres sont difficiles ou impossibles à évaluer par télédétection. La mise en place de cartes de risque de la maladie devrait prendre en compte, en plus des paramètres cités ci-dessus, l'utilisation de mesures de protection anti-vectorielle et la mobilité des populations qui permet l'importation dans les villes de cas contractés en milieu rural. Il est donc complexe de passer des cartes de risque entomologique à des cartes de « santé humaine ».

Les échelles de cartographie obtenues paraissent appropriées pour la mise en place, à terme, d'un système opérationnel. L'eau est détectée à une résolution spatiale de 10 m, ce qui paraît tout à fait suffisant pour l'envoi d'équipes de terrain au niveau des collections d'eau. L'utilisation future d'images à très haute résolution (<1 m) permettra peut-être d'améliorer la validité des modèles et d'augmenter la détection des petits gîtes mais le but de leur utilisation ne sera pas l'obtention d'une carte finale à 1 m de résolution spatiale. La résolution temporelle des cartes de risque est pseudo quotidienne (pluviométrie quotidienne mais température et données de terrain décadales ou bi-mensuelles). Une carte quotidienne n'aurait pas de sens biologique, entomologique ou épidémiologique alors que la possibilité de générer des cartes hebdomadaires, mensuelles ou annuelles pourrait avoir un intérêt dans la mise en place de la lutte contre le paludisme variable selon les contextes épidémiologiques. Dans les zones de paludisme stable et pérenne, une cartographie annuelle serait probablement suffisante. Dans les zones où le paludisme est strictement saisonnier voire épidémique, des cartes mensuelles voire hebdomadaires pourraient être plus utiles.

En conclusion, les populations urbaines, ainsi que les voyageurs ou les militaires, qui ont peu ou pas d'immunité acquise contre les *Plasmodium* et pour qui le risque de paludisme est fortement lié au risque entomologique, pourraient bénéficier à l'avenir de l'apport de cartes de risque à des stratégies de lutte antivectorielle plus ciblées ou au choix des lieux d'implantation et de séjour les moins à risque de paludisme. Notre travail a permis des avancées significatives dans ce sens.

PERSPECTIVES

Plusieurs pistes d'amélioration ou de développement des modèles construits dans le cadre de cette thèse sont en cours ou seront prochainement explorées.

Premièrement, le Programme d'Accompagnement ORFEO-Santé a été mis en place afin de préparer, accompagner et promouvoir l'utilisation et l'exploitation des images issues de satellites Pléiades (PHR) et Cosmo-Skymed (CSK). Dans ce cadre, des images de Dakar à très haute résolution spatiale (<1m) ont été récemment mises à disposition des équipes du CNES et du SERTIT (Service Régional de Traitement d'Image et de Télédétection, Strasbourg). Elles seront traitées afin de vérifier si elles permettent d'améliorer la précision et la qualité d'ajustement des modèles construits dans le cadre de la thèse.

Deuxièmement, un projet de validation des modèles construits dans le cadre de la thèse est en cours d'élaboration. Des données satellites et des données entomologiques de terrain (pour la validation des modèles) seront collectées à Ndjamena, Tchad et à Bamako, Mali, deux autres villes sub-sahariennes. Les modèles construits dans le cadre de la thèse seront appliqués aux données obtenues par télédétection sur ces deux villes. Leurs prédictions seront comparées aux données obtenues sur le terrain afin de vérifier la transposabilité de ces modèles. Des ajustements seront probablement nécessaires pour prendre en compte les spécificités de chaque lieu. Dans cette perspective, des études de terrain seront lancées en 2011, en collaboration avec un projet de recherche mené par le MRTC (Malaria Research and Training Center) à Bamako.

Troisièmement, le projet EOS-Malaria (Epidemiology Earth Observation Services-Malaria) a débuté en septembre 2010, en collaboration avec le CNES, le SIRS (Système d'Information à Référence Spatiale, Lille), le SERTIT (Service Régional de Traitement d'Image et de Télédétection, Strasbourg), l'IRBA (Institut de Recherche Biomédicale des Armées) et le laboratoire d'Aérologie de l'OMP (Observatoire Midi Pyrénées). Il vise à développer un démonstrateur de service de cartographie de risques sanitaires s'appuyant sur les cas de Dakar et N'Djamena. Ce projet se basera sur les modèles développés dans le cadre de la thèse pour concevoir des outils automatisés.

Quatrièmement, si les travaux de thèse se sont tournés vers l'étude des milieux urbains, une étude prend aussi place en milieu rural, à Nouna, Burkina-Faso, en collaboration avec l'Université de Heidelberg en Allemagne. Leurs travaux préliminaires sont présentés en annexe de cette thèse. Des campagnes de terrains seront lancées en 2011 afin de collecter des données entomologiques en vue d'adapter la méthodologie de cartographie du risque pour les milieux ruraux.

Cinquièmement, à partir des cartographies entomologiques, une cartographie épidémiologique pourrait être envisagée. Pour cela, des données de prévalences parasitaires et éventuellement d'incidence des accès palustres doivent être collectés afin d'être mis en relation, à des échelles appropriées, avec les cartes de risque entomologique (projet Actu-Palu : R. Lalou, J.Y. Le Hesran, F. Remoué IRD). Une rapide mise en relation des données entomologiques à Dakar, avec les données épidémiologiques fournies par le PNLP (Plan National de Lutte contre le Paludisme) du Sénégal n'a pas permis de mettre en évidence d'association spatiale. Cela a pu être dû à un manque de précision géographique des lieux d'inoculation plasmodiale des cas cliniques diagnostiqués (dans Dakar et sa banlieue), de l'absence de prise en compte de facteurs comme l'accès aux soins, à la prophylaxie antipaludique ou l'immunité antipaludique, ou à l'importation en ville de cas de paludisme clinique depuis le milieu rural. Des études suggèrent que prés de 50% des cas de paludisme clinique observés dans Dakar surviennent chez des individus ayant voyagé dans d'autres zones du pays au cours du mois précédent.

Au delà de la présente thèse, les travaux continuent dans l'objectif d'obtenir à moyen terme un système opérationnel qui pourra être utilisé à plusieurs niveaux, selon les besoins et les contextes nationaux : service d'alerte précoce du risque entomologique basé sur des prévisions météorologiques, ciblage des épandages d'insecticides dans les collections d'eau prédites comme étant à fort risque de production de larves d'anophèles, contrôle dirigé des populations d'anophèles adultes dans les zones les plus à risque, évaluation des quartiers exposés à de forts niveaux de piqûres afin d'y renforcer les actions de préventions…

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ANNEXES

Modélisation du risque de paludisme en milieu rural

A Nouna (Burkina Faso), l'occupation du sol a été étudiée pour estimer le risque de paludisme associé à l'environnement autour des villages de la région. A partir d'une étude bibliographique sur la présence d'anophèles dans différents environnements, des types d'occupation du sol ont été associés au risque de paludisme. Pour cela, chaque type d'occupation du sol a été classé en fonction du risque de paludisme qui lui était attaché : habitat potentiel (gîtes larvaires) et zone productrice d'anophèles. Ainsi, une carte d'occupation du sol de la zone d'étude, établie à partir de la classification supervisée d'image à haute résolution spatiale (de type SPOT-5) a permis d'associer différents types d'occupation du sol à un risque environnemental palustre. L'analyse des différents types d'occupation du sol dans une zone tampon autour des villages, correspondant à la distance de vol moyenne des anophèles, a permis de classer les villages en deux catégories : "village à faible risque palustre" et "village à fort risque palustre". Ainsi, pour chaque village, le risque de prévalence du vecteur a pu être obtenu en fonction des types d'occupation du sol présents dans la zone tampon.

Ces travaux constituent une étape préliminaire à l'élaboration d'un système comparable à celui développé à Dakar, à savoir, un échantillonnage important de données entomologiques larvaires et adultes, une acquisition de produits spatiaux adaptés contemporains aux prospections de terrain, et une modélisation statistique adéquate permettant de dresser les cartes de risque.

L'article décrivant le travail effectué est présenté ci-dessous.

Using high spatial resolution remote sensing for risk mapping of malaria occurrence in the Nouna district, Burkina Faso

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Introduction: Malaria control measures such as early diagnosis and treatment, intermittent treatment of pregnant women, impregnated bed nets, indoor spraying and larval control measures are difficult to target specifically because of imprecise estimates of risk at a small-scale level. Ways of estimating local risks for malaria are therefore important.

Methods: A high-resolution satellite view from the SPOT 5 satellite during 2008 was used to generate a land cover classification in the malaria endemic lowland of North-Western Burkina Faso. For the area of a complete satellite view of 60×60 km, a supervised land cover classification was carried out. Ten classes were built and correlated to land cover types known for acting as Anopheles mosquito breeding sites.

Results: According to known correlations of Anopheles larvae presence and surface water-related land cover, cultivated areas in the riverine vicinity of Kossi River were shown to be one of the most favourable sites for Anopheles production. Similar conditions prevail in the South of the study region, where clayey soils and higher precipitations benefit the occurrence of surface water. Besides pools, which are often directly detectable, rice fields and occasionally flooded crops represent most appropriate habitats. On the other hand, forests, elevated regions on porous soils, grasslands and the dryer, sandy soils in the north-western part turned out to deliver fewer mosquito breeding opportunities.

Conclusions: Potential high and low risks for malaria at the village level can be differentiated from satellite data. While much remains to be done in terms of establishing correlations between remotely sensed risks and malaria disease patterns, this is a potentially useful approach which could lead to more focused disease control programmes.

Keywords: *high spatial resolution*; *remote sensing*; *malaria*; *West Africa*; *Burkina Faso*; *Anopheles*; *risk mapping*; *SPOT 5 satellite*

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There is a widespread consensus on malaria control measures, including early diagnosis and treatment, intermittent treatment of pregnant women, impregnated bed nets, in-door spraying and larval control measures. The policy conundrum, however, is the low and often unfocused coverage with these measures. In the study district of Nouna, Burkina Faso, only 8% of children actually sleep under insecticide treated bed nets (and about 20% of children with fever present at health centres get appropriate treatment in case of malaria) (1, 2). Neither larval control measures nor indoor spraying is practised.

There are two different policy options to respond to this unfortunate situation in a holo-endemic area. One would propose an overall improvement of the effectiveness of health systems to deliver the control measures to the entire population. This is based on good public health practice and theory which stipulates that when conditions or risk factors are highly prevalent in a population, it is best to offer control

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measures to everyone irrespective of the level of risk exposure.

The alternative – unorthodox – approach proposed here is based on the assumption that the financial and logistical constraints of health systems in districts such as the one under study are so formidable that focusing measures on populations at high risk of transmission is justified. No one would challenge a *temporal* focus in an area of highly seasonal transmission. Following this rationale, the recent WHO malaria report (3) suggests distributing bed nets and drugs before the rainy season so that populations have better access during the peak transmission season.

In this paper, we argue that an additional *spatial* focus should be considered. This is based on consistent findings in the study area of very varied malaria incidence rates between even adjacent villages (4). Regions in and close to Sahel are known for very focal and seasonal transmission (5–8). The combination of the advent of low-cost high-resolution remote sensing and reports of different malaria transmission risks based on different surface water quality, size and land cover led us to carry out the current study. The main objective of this paper is to answer the question to which extent remote sensing can validly identify different larval habitats producing different malaria transmission risks. The spatial resolution of sensors is still limited to habitats at least several metres in diameter, and the revisit rate of high-resolution satellites is too low to map dynamic changes.

We are of course aware that, having answered this question, further studies would be needed on

1) the statistical associations between remotely sensed risk zones and actual entomological data within them;
2) the relationship between both entomological and remotely sensed data, and incident malaria cases and their severity, together with malaria mortality; and
3) the evaluation of cost effectiveness of interventions in a target area. These could be raising bed net coverage in high-risk areas, coupled with larval control and indoor spraying. This should be carried out through cluster-randomised and controlled intervention studies.

The next generation of satellites will deliver new dimensions of spatial resolution within the sub-metre range and hence allow detection of even smaller habitats. The more limiting factor will still be the flyover frequency, so even usage of a higher spatial resolution will require temporal modelling of habitat dynamics. New and original approaches on dynamics have been set up for others diseases such as Rift Valley fever in Senegal (9). The predominant percentage of prevalent surface water and related land cover is already detectable with current technology. Risks emerging from small-scale water agglomerations, e.g. puddles, skid marks, etc. that often do not evaporate completely for periods of several weeks has to be modelled from the implications of their characteristic larvae production, occurrence and duration since they cannot be detected directly via remote sensing.

Materials and methods

The study site lies in the north-western part of Burkina Faso in the Kossi district and correlates to the satellite view of the SPOT 5 satellite from 2008. In the centre of this area, which is 60×60 km, the village of Nouna is located at 12° 44′ N; 3° 51′ W. Most areas in this region lie on an altitude of 150–250 m above the sea level and belong to a Precambrian peneplain. Mean precipitation for Nouna during the last 10 years has been 817 mm per year. The monthly maxima during the rainy season between May and September can reach up to 350 mm. The yearly average temperature of Nouna is 27.8°C.

While some studies (10) have dealt with an extensive collection of ground data for a relatively small study site of few square kilometres, for this study wide parts of a 3,600 km^2 satellite view were used to map habitats which are known to be appropriate for *Anopheles gambiae* breeding from other studies. A SPOT 5 (Satellite Pour l'Observation de la Terre) satellite image was utilised for this study. Since the study area was visited during late rainy season and the collection of ground truth points had to be close in time to the flyover, a satellite view of 1 September 2008 was programmed. The multispectral image used consists of three bands (red, green and near infrared) and resolution is 2.5 m per pixel. Images were received orthorectified and georeferenced (level 3) in UTM system (zone 30P).

Training zones

During the six-week field phase from August to October 2008, an overall number of 45 ground truth points were taken in different geographic regions within the satellite view in order to produce a classification scheme (Fig. 1).

Fig. 1. Characteristic pool in the vicinity of Nouna. In some parts it is used as brickyard while other parts show lateritic substratum on the ground. (Location: see Fig. 5).

These ground truth points are objects in the terrain that are needed for recognising different land cover in the satellite image. Knowing the location and land cover type of ground truth points in the terrain allows the determination of similar zones in the satellite image. Ground truth points contained rice fields, sorghum, water pools, bare soil, buildings, bush, etc. Most locations were visited contemporaneously with satellite overflight; positions being recorded using GPS handheld receivers (Garmin GPS Map 76s). All ground truth objects were saved as waypoints, and some additionally as polylines using the track recording function of the GPS.

Supervised classification

For analysis of the SPOT image ITTVIS, ENVI image processing software was used. The image was classified by using validation data collected during a six-week field study in 2008. Using the Region of Interest-Tool (ROI) in ENVI, 45 ground truthing points were used for spectral reference. These training signatures were distributed in 15 classes, which were merged later into 10 classes to run the classification. Using three bands (red, green and near infrared), the image was processed using the maximum likelihood calculation for supervised classifications. The maximum likelihood classification assumes that the statistics for each class in each band are normally distributed and calculates the probability that a given pixel belongs to a specific class. During the process each pixel is assigned to the class that has the highest probability; if the highest probability is smaller than a specified threshold, the pixel remains unclassified.

Data analysis

The classified satellite image was saved in ENVI as an ASCII file. The ASCII format was then transformed into a raster file using ArcMaps integrated conversion tools. This procedure allowed keeping the calculated classes in ArcMap in a selectable raster dataset. Classes were renamed and fitted to original colour set. For 30 villages, buffers of 500 m radius were constructed around the centre using ArcMaps buffer wizard. These buffer zones represent the assumed Anopheles mosquito flying range (11, 12). The surface of each class within the radius around each village was calculated. This was performed using the 'zonal histogram' tool in the 'spatial analyst' extension in ArcMap. According to data from the 2008 study, as well as to typical Anopheles presence in different land cover types known from literature, the land cover types have been evaluated (10, 13–23). Since for this region there are no existing studies that deal with absolute numbers of mosquito larvae per habitat per time, a relative risk classification was constructed. Four classes of relative mosquito larvae presence in environmental habitats from low to very high were incremented (see Fig. 2).

No.	Land cover class	Risk level
1	Sandy soil	Low
2	Bare soil	Low
3	Dry vegetation	Low
4	Housing	Medium
5	Forest and bush	Medium
6	Field crops	High
7	Turbid water	High
8	Rice field (submerged/irrigated)	Very high
9	Submerged vegetation	Very high
10	Water covered with vegetation	Very high

Fig. 2. Land cover classes and risk levels according to various literature (10, 13–23).

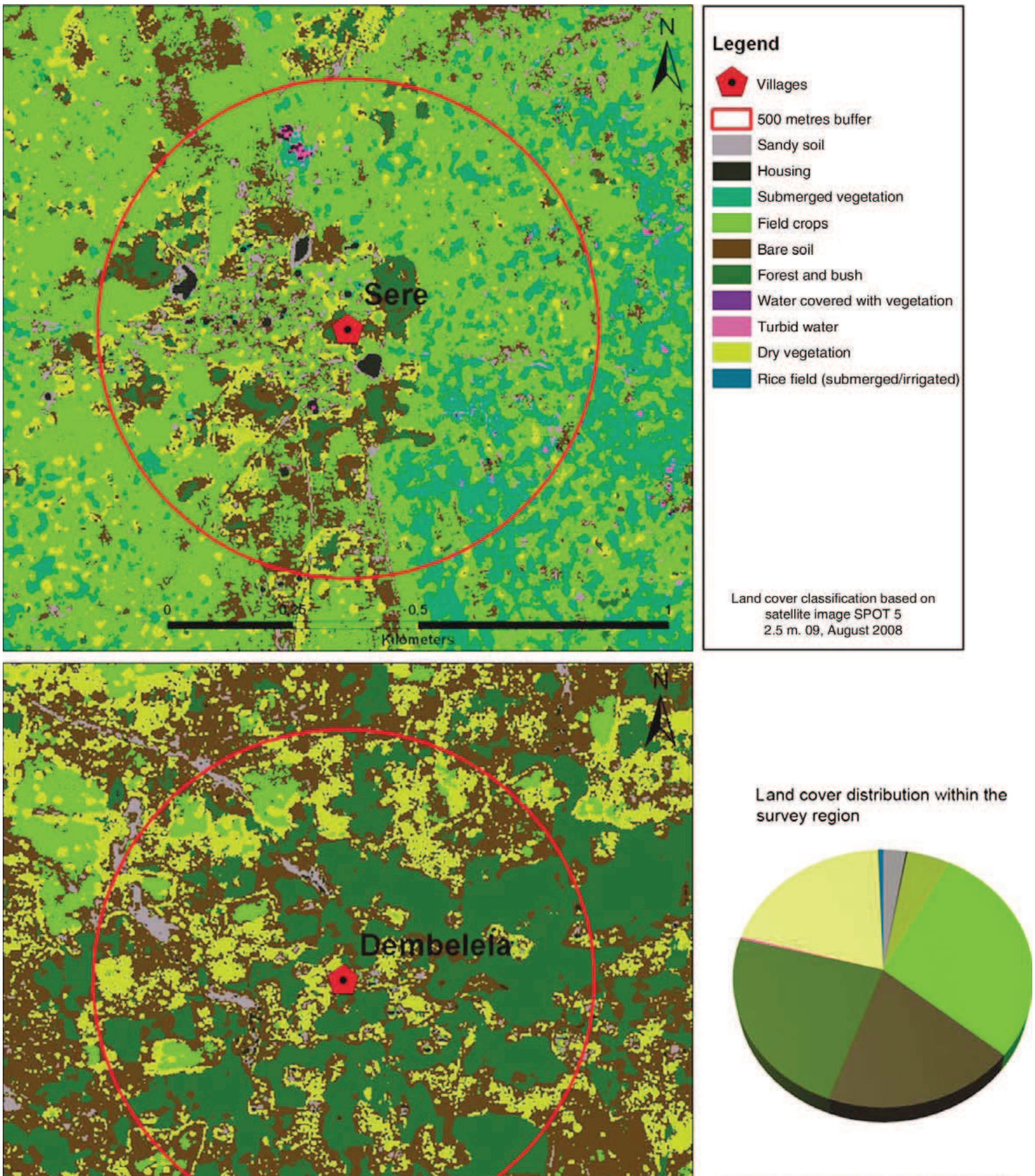
The percentage of very high and high-risk land cover within the 500 m buffer zone of all villages was compared in a diagram and sorted by percentage (see Fig. 4). On the base of this graduation, two groups of villages were featured, one with a percentage of risk-related land cover lower than 25% of area with high and very high risk, another with more than 25%. This threshold marks at the same time a significant increase in very high risk land cover per village. In ArcMap, villages were redrawn on the satellite image indicating their calculated risk (see Fig. 5).

Results

For the 30 villages included in the local demographic surveillance system, the area of potential habitats with very high risk (submerged and irrigated rice fields, water covered with vegetation and submerged vegetation) and high risk (field crops with clayey soil and turbid water) was calculated for the 500 m buffer zone. The share of total surface accounted for by very high and high-risk habitats within the buffers showed a difference between the lowest and highest by nearly a factor of 20. Some villages (Dembelela) had around 3% of surface within the 500 m buffer covered with very high and high-risk habitats, while it reached up to 60% in the vicinity of other villages (Sere, Tissi). This is shown in Fig. 3.

Villages that already showed a high percentage of high-risk land cover within their 500 m buffer zone also had a higher percentage of very high-risk land cover types (see Fig. 4). Since risk is defined as appropriateness for larvae breeding, which is bound to surface water, the results show that an underlying factor exists that influences the presence or absence of both risk types at the same time.

Villages with similar risks turned out not to be randomly distributed over the survey area but lay together in certain regions. Two zones around villages with elevated risk (risk-related land cover share higher than 25%) and three zones containing villages at lower risk could be separated (see Fig. 5). These zones alternated from South-West to North-East. This remarkable difference in distribution of natural and anthropogenic land cover between regions seems to



Relative distribution of land cover risk in 500 m buffer		
Risk	Sere	Dembelela
Low	31,41	58,10
Medium	6,65	38,76
High	51,18	3,14
Very high	10,76	0,00
Sum total	100	100

Fig. 3. Land cover distribution in 500 m buffer zone around the villages of Sere (highest risk) and Dembelela (lowest risk). Land cover risk within the survey region (pie diagram, same legend).

have its origin in the natural distribution of geographic and geologic factors. Suitable factors could be the prevailing type of soil and or as additional effect depressions in topography. Those depressions played a role in regional water distribution although only showing height differences of 15 m or less. Since the survey region was relatively small and risk zones alternated within it, climatic differences do not seem capable of explaining those distributions. Soil types in this region often vary within small areas and show considerable differences in infiltration behaviour and water retention capacity. Some areas have mostly sandy soils, which leads to less environmental water

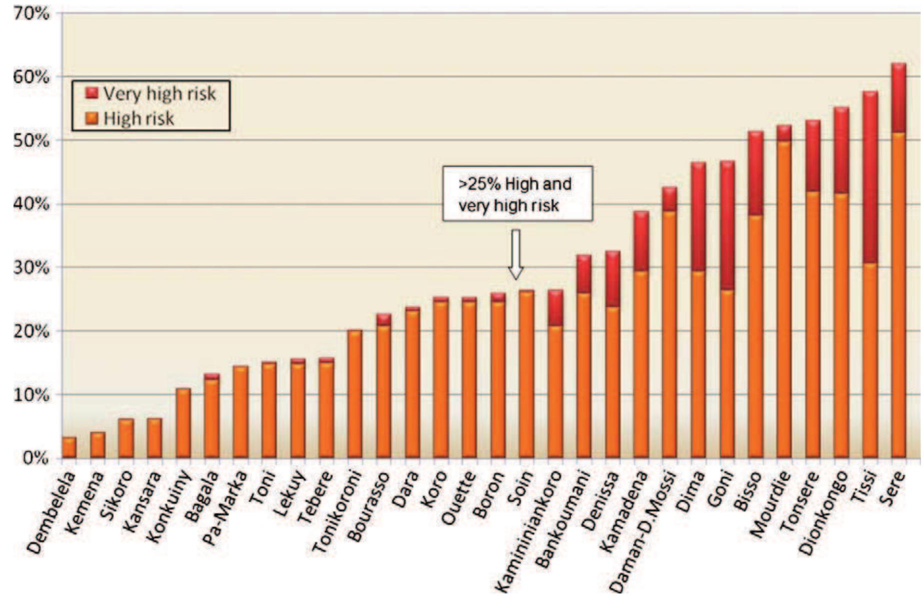


Fig. 4. Percentage of land cover areas with elevated risk for Anopheles larvae breeding of total surface in 500 m buffer zones around 30 villages.

reservoirs. Regions with clayey soils often show swampy characteristics during the rainy season and keep water for several days or weeks. Lateritic crusts at the surface are a prevalent type of substratum as well and allow nearly no infiltration but high runoff rates (see Fig. 5).

Discussion

We showed and validated with ground data that high-resolution satellite images can indeed identify small-scale habitats with sizes of only few metres diameter conducive to Anopheles larvae development. Micro habitats in the sub-metre scale are not directly detectable at the current state of technology, but need imputation via modelling in further studies. Those micro-habitats mainly play a role within villages where they are close to the population and this mostly during the peak of the rainy season. After more than one week without precipitation they mostly evaporate or are infiltrated and cannot act as productive habitats. Being not directly detectable, these micro-habitats need to be estimated by their average occurrence in typical villages and their occurrence attached to different land cover types. The extremely varied micro-distribution of risks between villages is compatible with the findings of Yé et al. (24), who reported considerable differences in malaria incidence between villages in the region.

The most extensive work on geographical variation of malaria risk in Africa has been made at the continental scale, based on meteorological data and historical ground data from various sites across the continent (25, 26), but using a much coarser resolution and were not useful for malaria control at the district level. At the time those studies were carried out, the current resolution was not available. Studies mapping Anopheles mosquito breeding habitats, transmission or disease, partly with higher resolution, have been made in Africa (8, 22, 25, 27–31) and South and Central America (32–35). Reliable information about vector density and malaria transmission risk is essential for understanding variations in local disease epidemiology and to stratify intervention programmes. The next step is to correlate malaria case data from the demographic surveillance system with the risk modelled by using high-resolution satellite imagery.

We are aware that there is a long and non-linear causal pathway between the number of larvae in a given habitat and the incidence, severity and cause-specific mortality of malaria so we urge for prudence in interpreting our data. Our mapping of villages into two risk categories for malaria transmission is a first step towards exploring the usefulness of targeted control measures. As pointed out in the introduction, our findings need to be connected with entomological and clinical data. On the basis of further results the application of counter measures can be considered. Since risk seems to be focused on certain zones, interventions like bed net distribution and indoor-spraying, but also the use of bacteria produced toxins that selectively kill larvae of certain mosquito species (36) seem to be putative approaches. It will only be after carefully designed intervention studies that any policy implications can be considered.

Conflict of interest and funding

The authors have not received any funding or benefits from industry to conduct this study.

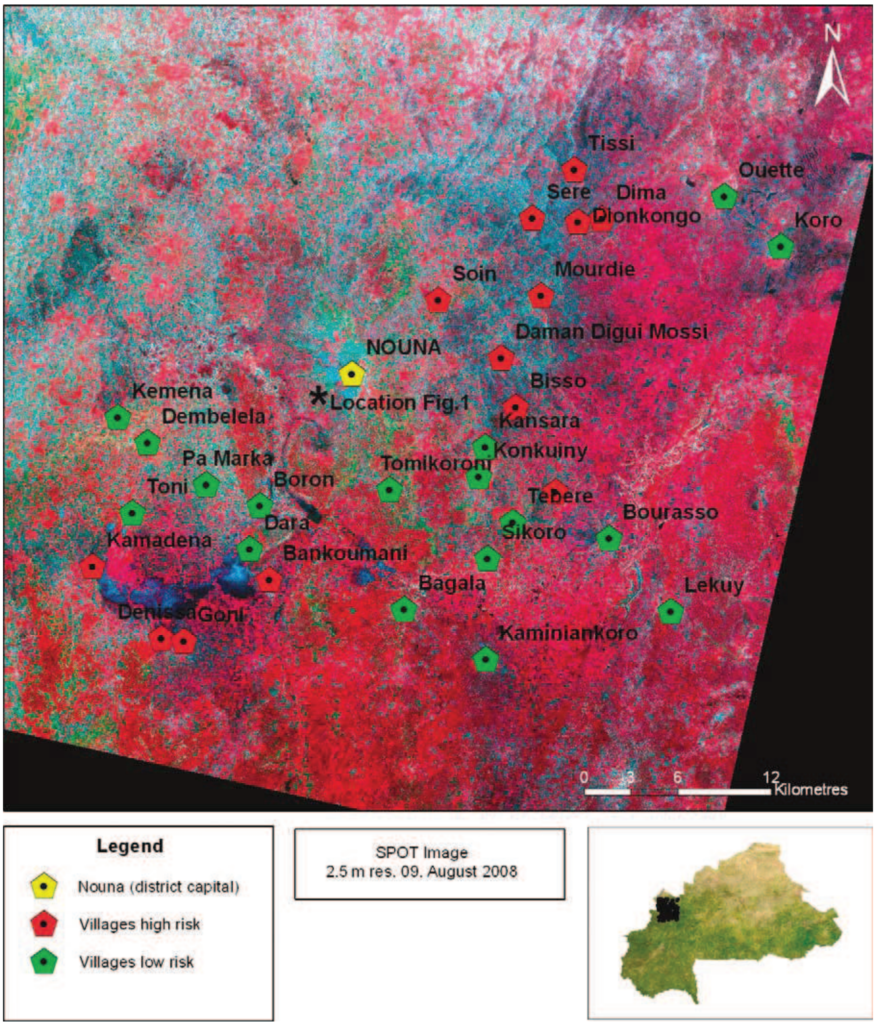


Fig. 5. Villages with similar land cover risk in their 500 m buffer zones. Similar risks show spatial agglomeration in certain zones. Villages with high-risk habitats exceeding the 25% threshold in Fig. 4 have significantly higher land cover with very high risk (red columns in Fig. 4). The asterisk marks the position where Fig. 1 was photographed.

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Apport de la télédétection à la lutte contre le paludisme

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Une première revue de la littérature, écrite en français, a été publiée en 2009 dans la revue Médecine Tropicale. Cette revue est présentée en annexe car elle constitue une version préliminaire à la revue en anglais qui constitue l'introduction de cette thèse.

Cette revue en français est présentée ci-dessous.

Apport de la télédétection à la lutte contre le paludisme

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RÉSUMÉ • Malgré les efforts nationaux et internationaux, le paludisme reste un problème de santé publique majeur et les avancées dans la lutte contre la maladie se heurtent à de nombreux obstacles. L'importance du paludisme doit pouvoir être évaluée, au niveau spatial et temporel, afin de renseigner les décideurs et de prioriser les interventions, y compris dans les zones où les informations de terrains sont insuffisantes et où les systèmes d'information sanitaire sont défaillants. L'évaluation du risque palustre doit aussi permettre d'anticiper la survenue d'épidémies par des systèmes d'alerte précoce. Des satellites civils d'observation de la terre survolent la planète depuis les années 1960-70 et apportent des informations pour la mesure ou l'évaluation de certains des facteurs géo-climatiques et anthropogéniques dont dépendent la transmission et l'importance du paludisme. De nombreux travaux civils ou militaires, en milieu rural ou urbain, ont permis la mise en place de modèles et de cartes de risques entomologique, parasitologique ou épidémiologique du paludisme à partir de données de télédétection. La cartographie des populations humaines à risque a aussi bénéficié des apports de la télédétection. Les résultats des travaux publiés montrent que la télédétection est un outil adapté pour améliorer la planification, l'efficacité et l'efficience de la lutte antipaludique.

MOTS-CLÉS • Lutte antipaludique. Lutte antivectorielle. Télédétection. Satellites. Epidémiologie. Paludisme.

CONTRIBUTION OF REMOTE SENSING TO MALARIA CONTROL

ABSTRACT • Despite national and international efforts, malaria remains a major public health problem and the fight to control the disease is confronted by numerous hurdles. Study of space and time dynamics of malaria is necessary as a basis for making appropriate decision and prioritizing intervention including in areas where field data are rare and sanitary information systems are inadequate. Evaluation of malarial risk should also help anticipate the risk of epidemics as a basis for early warning systems. Since 1960-70 civilian satellites launched for earth observation have been providing information for the measuring or evaluating geo-climatic and anthropogenic factors related to malaria transmission and burden. Remotely sensed data gathered for several civilian or military studies have allowed setup of entomological, parasitological, and epidemiological risk models and maps for rural and urban areas. Mapping of human populations at risk has also benefited from remotely sensing. The results of the published studies show that remote sensing is a suitable tool for optimizing planning, efficacy and efficiency of malaria control.

KEY WORDS • Malaria control. Vector control. Remote sensing. Satellites. Epidemiology. Malaria.

Malgré les efforts nationaux et internationaux, le paludisme reste un problème de santé publique majeur et les avancées dans la lutte contre la maladie se heurtent à différents types d'obstacles. Le risque de paludisme doit pouvoir être évalué, au niveau spatial et temporel, afin de renseigner les décideurs et de prioriser les interventions. La variabilité de l'importance du paludisme peut être importante, parfois à des échelles réduites, et il est primordial de pouvoir concentrer et cibler les moyens de lutte vers les zones et les périodes où cette charge est la plus lourde.

Bien que des efforts soient faits pour rassembler les informations entomologiques, parasitologiques et épidémiologiques existantes (1-3), la disponibilité des données de terrain est souvent ponctuelle, parcellaire et reste une entrave à l'évaluation de l'importance du paludisme. Dans certaines régions, les informations épidémiologiques peuvent être insuffisantes, voire inexistantes, lorsque les systèmes de santé et d'information sanitaire sont déficients. Si des recherches ponctuelles sont menées, les méthodes de collecte de données de terrain sont coûteuses en temps et en investissement financier et les résultats donnent une image localisée et généralement instantanée de la situation. Il en est de même pour les données entomologiques de terrain. La lutte antipaludique souffre de l'absence d'informations globales et continues pouvant guider les interventions de lutte antivectorielle.

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Par ailleurs, l'anticipation du risque est un enjeu majeur dans la lutte contre le paludisme et particulièrement contre ses épidémies. L'histoire des systèmes d'alerte précoce de paludisme remonte à 1921 en Inde où l'intensité et la distribution d'une épidémie a été prévue par la combinaison de l'absence de paludisme dans les 5 années précédentes, des anomalies de pluie de juillet à août et du prix local du blé qui représentait l'état nutritionnel de la population (4). L'introduction des insecticides dans les années 1950-60 et le lancement du programme mondial d'éradication du paludisme ont laissé croire que la prédiction des épidémies n'était plus nécessaire. Depuis l'apparition des résistances aux insecticides mais aussi aux antipaludiques, l'intérêt pour ces systèmes d'alerte renaît.

En matière de lutte contre le paludisme, il est nécessaire d'améliorer la planification, l'efficacité et l'efficience des méthodes de lutte, de surveillance et de contrôle. Mieux prédire et mieux évaluer l'importance du paludisme passe nécessairement par une meilleure compréhension des déterminants de sa transmission et de son impact.

Déterminants de la transmission du paludisme

La biodiversité et l'importance du paludisme dépendent des combinaisons et interactions entre des facteurs abiotiques (environnement, climat, facteurs écologiques, météorologiques et anthropogéniques) et des facteurs biotiques (espèces vectrices endémiques, sensibilité de la population humaine, résistance des parasites aux antipaludiques, résistance des vecteurs aux insecticides) (5).

Les facteurs géo-climatiques, tels que la saisonnalité du climat, le régime des pluies, la température ambiante, l'humidité, la présence d'eaux de surface et de végétation, conditionnent l'apparition et la persistance des gîtes larvaires d'anophèles, la vitesse de développement des larves, l'abondance des vecteurs, leur taux de survie et leur dispersion, et la durée du cycle extrinsèque du Plasmodium. Des facteurs anthropogéniques, tel que les activités agricoles, l'irrigation, la déforestation, l'urbanisation, les mouvements de population, les changements économiques, sont aussi susceptibles de peser sur les niveaux de transmission et l'épidémiologie des infections plasmodiales (6-8).

La biodiversité du paludisme est importante car, « comme aux échecs, il y a peu de pièces mais la variété des situations est infinie ». Pour saisir le jeu des déterminants et prédire l'importance du paludisme, il est nécessaire de comprendre l'interrelation entre ces facteurs et à quelles échelles ils doivent être pris en compte (6). L'évaluation des facteurs géo-climatiques a bénéficié, au cours des quarante dernières années, de l'apport de la conquête spatiale et des images de la terre obtenues par satellite.

Télédétection : méthodes

Des satellites civils d'observation de la terre survolent la planète depuis les années 1960-70 et fournissent depuis des images de télédétection. La télédétection correspond à l'ensemble des connaissances et techniques utilisées pour déterminer des caractéristiques physiques et biologiques par des mesures effectuées à distance, sans contact matériel (9). Le principe de la télédétection repose sur l'enregistrement par les capteurs embarqués à bord des satellites, de l'énergie réfléchie ou émise par la surface de la terre et transportée par un rayonnement électromagnétique. L'énergie radiative enregistrée par le capteur est convertie en un signal électrique mesurable. La détection des différents écosystèmes repose sur les différences de réflexion et d'émission des objets au sol. Les capteurs passifs réceptionnent certaines longueurs d'onde du rayonnement solaire et les capteurs actifs, tels que les radars, enregistrent l'écho terrestre du rayonnement artificiel qu'ils ont eux-mêmes émis. Les capteurs peuvent enregistrer l'énergie radiative dans une ou plusieurs longueurs d'onde (ou bande spectrale). Une image panchromatique est constituée d'une seule bande spectrale et une image multispectrale de plusieurs bandes. Une image satellite est ainsi constituée de pixels, chacun contenant une ou plusieurs valeurs numériques selon le nombre de bandes spectrales enregistrées par le capteur. La mesure des indicateurs environnementaux, climatiques ou météorologiques, utilisables en épidémiologie dépend des bandes spectrales disponibles et donc de capteurs spécifiques.

Les capteurs des satellites sont caractérisés par leurs résolutions spatiale et temporelle, c'est-à-dire respectivement la surface réelle que représentent les pixels de l'image et la fréquence des prises de vue. Pour les capteurs à haute ou très haute résolution spatiale (jusqu'à 0,61 m), tels que ceux embarqués sur les satellites commerciaux SPOT (Satellites Pour l'Observation de la Terre), Ikonos, Quickbird, Landsat ou les radars RADARSAT ou TerraSAR-X, la résolution temporelle est faible et plusieurs semaines peuvent s'écouler entre deux prises de vue. Pour les satellites à faible résolution spatiale (à partir de 1 km), tels que Meteosat ou NOAA (National Oceanographic and Atmospheric Administration), la résolution temporelle peut en revanche atteindre plusieurs passages par jour sur la même zone. Ainsi, les indicateurs environnementaux pour lesquels sont requises des mesures à une précision de quelques mètres, pour la cartographie par exemple, seront dérivés du premier type de capteurs. Les applications associées seront locales ou régionales. Les indicateurs qui doivent faire l'objet d'un suivi temporel, telles que des mesures de végétation ou de pluviométrie, pourront être évalués par le second type de satellites. L'étendue des zones étudiées pourra atteindre un pays ou même un continent (7). Souvent, les images à faible résolution spatiale sont gratuites ou peu onéreuses, à l'inverse des images à haute ou très haute résolution.

Après acquisition, une image satellite doit généralement subir différents types de traitements informatiques pour être exploitée, tels que le filtrage, l'amélioration des contrastes, la classification ou le calcul d'indices (voir encadré « Images »). La classification permet de regrouper des pixels similaires et ainsi de caractériser des écosystèmes. Le calcul d'indices est réalisable pour les images multispectrales. Il consiste à combiner les valeurs de différentes bandes spectrales de chaque pixel dans des formules mathématiques plus ou moins complexes. Les indices les plus communément utilisés pour l'étude de l'épidémiologie du paludisme et des maladies à transmission vectorielle sont des indicateurs de végétation (Normalized Difference Vegetation Index [NDVI, voir encadré « NDVI »]), de pluviométrie (CCD [Cold Cloud Duration]) ou de température à la surface de la terre.

La technologie GPS (Global Positionning System) permet la géolocalisation des données entomologiques ou épidémiologiques recueillies sur le terrain. Il est alors possible, grâce aux Systèmes d'Information Géographique (SIG) d'intégrer les informations spatialisées (appelées couches) issues des images satellites et celles obtenues sur le terrain, pour dresser des cartes de risque. Des couches spatialisées ne provenant pas de la télédétection peuvent aussi être incluses, telles que des cartes topographiques (altitude, types de sol), démographiques, socio-économiques ou sanitaires (implantations des structures de santé).

Des associations statistiques peuvent alors être recherchées par régression entre les variables paludométriques, les indicateurs environnementaux et les autres variables incluses dans les SIG. L'inversion des relations (*i.e.* de la formule du modèle de régression) permet de générer des modèles et des cartes de risque. Alors que l'hypothèse principale conditionnant l'utilisation des statistiques classiques est l'indépendance des données, les observations spatialement proches peuvent être plus semblables que des observations éloignées. Négliger cette autocorrélation spatiale des données dans l'analyse statistique tend à surestimer la signification des effets des associations et peut conduire à conclure à des associations qui n'existent pas (10). Par exemple au Kenya, 87% de la variance de la densité de mouches tsé-tsé étaient expliquées grâce à des données de télédétection mais la prise en compte des autocorrélations spatiales ramenait les résultats à une association non significative (11). Il est donc important d'utiliser des méthodes statistiques adaptées lorsque l'on analyse des données spatialisées. Ces méthodes statistiques sont le plus souvent classiques et conventionnelles. Des méthodes d'analyse et de prédiction non conventionnelles telle que la logique floue («fuzzy logic») ont aussi été utilisées pour dresser des cartes de risque.

Télédétection et lutte antipaludique

Les capteurs des satellites ne sont pas spécifiquement dédiés à l'enregistrement de données pour l'épidémiologie. Les données obtenues par télédétection peuvent cependant être utilisées pour l'étude des déterminants de santé des populations. Les satellites apportent des renseignements utiles pour la mesure ou l'évaluation de certains des facteurs géo-climatiques et anthropogéniques dont dépendent des pathologies humaines et animales. Depuis 1970 (12), des travaux dans le domaine des maladies infectieuses, en particulier à transmission vectorielle, ont utilisé des données obtenues par télédétection pour élaborer des modèles et dresser des cartes de risque de paludisme (6, 13-17). Les résultats montrent que les informations ainsi obtenues peuvent être utiles en matière de lutte antipaludique, que cela soit pour stratifier le risque, évaluer sa variabilité, pallier au manque de données de terrain, cartographier les populations à risque, extrapoler un niveau de risque à des échelles spatiales plus large ou pour anticiper des épidémies.

Exemples d'application

Les données de télédétection ont été associées à divers indicateurs paludométriques, tels que les densités larvaires, les densités vectorielles, l'agressivité (nombre de piqûres reçues par individu et par unité de temps), le taux d'inoculation entomologique (TIE, nombre de piqûres infectées reçues par individu et par unité de temps), la prévalence parasitaire et l'incidence du paludisme.

Données de télédétection et gîtes larvaires

A partir du lancement en 1972 du satellite Landsat-1 (résolution spatiale de 30 m), la recherche s'est intéressée à la cartographie spatiale du risque de paludisme par l'identification, la caractérisation et la cartographie des environnements propices à la présence de gîtes larvaires, que cela soit par une classification de l'image satellite ou par le calcul d'indices tel que le NDVI. La détection de ces environnements repose soit sur les connaissances a priori des écosystèmes pouvant contenir des gîtes larvaires soit sur les résultats de modèles statistiques plus ou moins complexes. Ces modèles sont élaborés par la mise en relation de données de terrain issues de prospections entomologiques et des données environnementales extraites de l'image obtenue par télédétection sur des zones limitées. L'extrapolation spatiale permet de définir les niveaux de risque de présence de gîtes larvaires pour des zones plus étendues.

En Thaïlande, il a été montré que les données de télédétection pouvaient apporter une information aussi fiable que celle collectée sur le terrain. La présence de larves de *An. minimus, An. maculatus* et *An. barbirostris* dans des habitats a en effet été prédite de façon similaire par les modèles de régression incluant des variables environnementales issues soit d'une image Landsat à 30 m de résolution soit d'investigations de terrain (18). Dans plusieurs pays, des associations ont été recherchées entre la présence de gîtes larvaires sur le terrain et les environnements issus de la classification d'images à différentes résolutions spatiales (de QuickBird à 0,6 m de résolution à Landsat à 30 m de résolution). Les écosystèmes propices aux gîtes larvaires de différentes espèces d'anophèles ont ainsi pu être cartographiés (19-21). La précision de la cartographie est allée jusqu'à distinguer les aires de répartitions des formes moléculaires d'une même espèce d'anophèle. Le caryotype 2Rbc de la forme chromosomique Mopti de *An. gambiae s.s.* est présent en saison sèche dans des zones arides irriguées. Au Mali, la présence de cette forme a pu être corrélée négativement aux quantités de pluie du mois de collecte ou du mois précédent (22). Les mêmes données de terrain ont aussi été associées au NDVI, avec une présence accrue du caryotype 2Rbc dans les zones de plus faible NDVI (17).

Une méthodologie différente consiste, non plus à cartographier un environnement propice à la présence de gîtes larvaires, mais à détecter directement des collections d'eau sur des images satellites. L'identification des gîtes est alors visuelle et directe, la collection d'eau étant prise en compte en tant qu'objet.

Dans un environnement aussi hétérogène que celui des hautes terres du Kenya, les erreurs de classification des écosystèmes sur une image ont pu être importantes. La photointerprétation a alors été choisie pour détecter les gîtes larvaires. Seules les images Ikonos de résolution 1 m ont permis une identification partiellement satisfaisante des collections d'eau (environ 41% d'entre elles), sans tenir compte de la présence de larves. Les images Landsat 7, dont la résolution de 30 m était supérieure à la taille de la majorité des collections d'eau pouvant servir de gîte larvaire, n'apportaient aucune information. Il était suggéré que la détection d'objets sur une image satellite nécessite qu'ils aient une taille au moins 1,5 fois supérieure à la taille du pixel (23). Le repérage direct des gîtes souffre donc de limitations dues aux résolutions spatiales des images satellites mais aussi au couvert végétal pouvant masquer les gîtes. Dans l'exemple kenyan, la prédiction visuelle a été améliorée par les résultats d'une régression logistique, les variables indépendantes étant issues de l'image classée et d'un Modèle Numérique de Terrain (MNT, image des courbes de niveaux) (23).

En plus des images satellites optiques, des images radar ont aussi été utilisées pour la détection des gîtes larvaires, mettant à profit leurs capacités à détecter l'eau et l'humidité et à acquérir des images du sol malgré le couvert nuageux. Au Mali, une équipe a mis en évidence l'association entre les données radar et le stade de croissance des rizières, lui-même associé aux densités larvaires d'anophèles (*An. gambiae s.l, An. pharoensis, An. rufipes* et *An. funestus*). Neuf images ERS-2 SAR de résolution 12,5 m avaient été acquises, trois d'entre elles étant contemporaines des prospections larvaires sur le terrain. Grâce à la multiplicité des images, des différences de profils temporels des rizières ont été détectées et associées à différents niveaux de densité larvaire (24).

Assez vite, l'aspect temporel de la prévision du risque a aussi fait l'objet de travaux. En Californie, les rizières les plus productives en *An. freeboni* ont été cartographiées 2 mois avant le pic de densité larvaire. Les indicateurs significativement associés aux densités larvaires étaient la présence d'une végétation développée en début de saison, mesurée sur une image Landsat de résolution spatiale 30 m, et la proximité du bétail qui fournit les repas sanguins, dont les pâturages étaient détectés sur des photographies aériennes infrarouges (25).

Donnés de télédétection et densités vectorielles

En se reposant sur la corrélation existante entre les densités larvaires et les densités vectorielles, *i.e.* d'adultes, des études exploitant les données de télédétection pour l'évaluation des densités des vecteurs du paludisme ont été initiées en 1992. Au Mexique, la classification d'une image Landsat 30 m a permis d'identifier les écosystèmes favorables aux densités élevées d'*An. albimanus* adultes pendant la saison des pluies autour de 40 villages (26). Dans cet exemple, la modélisation reposait sur la mesure des densités de vecteurs adultes pour déterminer les écosystèmes susceptibles de fournir les gîtes larvaires.

Une approche inverse consiste à utiliser la cartographie des gîtes larvaires connus pour évaluer ou prédire les niveaux de densités de vecteurs. La modélisation repose alors sur les données entomologiques larvaires et les variables de prédiction sont issues des images satellites ou d'autres informations spatialisées, comme la distance aux gîtes larvaires ou la distance de vol connue des anophèles. Les prévisions du modèle peuvent être confirmées a posteriori par des mesures de terrain des densités vectorielles (captures sur appâts humain, pièges, faune matinale résiduelle).

Au Belize, la densité d'*An. albimanus* a pu être prédite en deux classes pour des habitations ou des villages en fonction de leur distance aux principaux gîtes larvaires de la région. Ces gîtes, à savoir les zones frontières entre des surfaces en eau et de la végétation éparse, étaient cartographiés grâce à la classification d'une image SPOT de résolution 20 m (27). Le même principe a été appliqué, toujours au Belize, pour la prédiction de la présence d'*An. pseudopunctipennis* adultes dans des maisons. Une image SPOT de résolution 20 m et des cartes topographiques ont permis l'extraction des variables indépendantes prédictives : les rivières visibles sur l'image, étant identifiées comme gîtes larvaires principaux, la distance, la différence d'altitude et l'éventuelle présence d'un couvert forestier entre les maisons et ces rivières. Dans cette étude, les images n'étaient pas contemporaines des prospections de terrain mais la persistance de leur validité a été vérifiée sur le terrain (28). Dans les deux études précédentes, l'absence ou les faibles densités d'anophèles ont été prédites avec plus d'exactitude que la présence ou les fortes densités.

En Camargue, une zone marécageuse du sud de la France, les données d'une image Landsat 30 m'ont permis de cartographier la présence d'*An. hyrcanus* aux stades larvaires et adultes dans le cadre d'une étude du risque de réémergence du paludisme. La probabilité de présence des larves d'*An. hyrcanus* (appelé indice larvaire) était calculée pour chaque pixel de l'image, selon le biotope, la distance à la rizière la plus proche et l'application éventuelle de traitements larvicides. Un indice d'anophèles adultes était alors calculé comme la moyenne des indices larvaires dans des zones tampons de différentes surfaces. La surface retenue était celle qui permettait d'obtenir la meilleure corrélation entre l'indice d'anophèles adultes et les densités d'adultes capturés sur le terrain. (29).

Données de télédétection et taux d'inoculation entomologique des Plasmodiums

Selon les mêmes principes de corrélation entre les densités larvaires et adultes, certaines études ont eu pour objectif de rechercher des associations entre les écosystèmes et le taux d'inoculation entomologique (TIE).

Au Kenya, les agressivités et les TIE de *An. gambiae* et *An. funestus* ont été prédits avec plusieurs semaines d'avance par un modèle hydrologique d'humidité du sol basé sur les pluies, le type de sol, l'écoulement de l'eau et l'évapotranspiration. Les prédictions de ce modèle étaient meilleures que celles d'un modèle construit à partir des précipitations et de la température. Le NDVI issu du capteur AVHRR (Advanced Very High Resolution Radiometer) permettait la prédiction de l'agressivité de *An. gambiae* avec une exactitude similaire mais avec le désavantage d'être disponible à une résolution temporelle un peu moins élevée (30). En Gambie, les TIE de *An. gambiae s.l.* ont pu être estimés dans des villages, en tenant compte de la distance aux gîtes larvaires cartographiés sur des images à haute résolution (31). En Gambie toujours, une étude a permis la cartographie des écosystèmes propices à la présence de gîtes larvaires autour de 10 villages, par la classification d'une image SPOT de résolution 20 m. Dans ces villages, le TIE était connu. La relation entre le TIE d'une part et la présence et la distance aux gîtes larvaires d'autre part a été extrapolée à d'autres villages afin de prédire le risque de recevoir des piqûres infectées. Ce TIE estimé était alors comparé à des données de prévalence parasitaire. La prévalence parasitaire était positivement corrélée avec le TIE prédit jusqu'à un certain niveau, puis la relation s'inversait, probablement à cause de l'immunité antipalustre des populations humaines fortement exposées à la transmission du paludisme (32).

Donnés de télédétection, transmission et prévalence parasitaire

Alors que le TIE peut être assez directement associé à la densité de larves et de vecteurs adultes, la relation entre les populations de vecteurs et l'incidence ou la prévalence des infections plasmodiales ou du paludisme maladie est plus complexe. Elle dépend des niveaux de transmission mais aussi de nombreux facteurs comme les moyens de protection antivectorielle, l'immunité acquise, l'accès aux soins et aux traitements antipaludiques (5). Peu de travaux ont eu pour intention de définir les niveaux de prévalence parasitaire à partir de la présence de gîtes larvaires ou des densités vectorielles. En Thaïlande, aucune association n'a pu être trouvée entre le nombre de cas de paludisme dans des maisons et la distance aux écosystèmes favorables aux gîtes larvaires, cartographiés grâce à une image Ikonos de résolution 1 m (33).

La plupart des modèles de prédiction de la prévalence ou de l'incidence du paludisme rapportés dans la littérature ont été construits à partir d'indicateurs climatiques, tels que la pluviométrie, la présence de végétation ou la température, issus d'images à faible résolution spatiale. En Gambie, un modèle de prédiction de la prévalence parasitaire chez des enfants de 65 villages a été construit à partir de variables telles que l'âge, l'utilisation de moustiquaires imprégnées d'insecticides et du NDVI (estimé par la valeur de l'aire sous la courbe d'une série temporelle), qui était utilisé comme une variable de substitution (*i.e.* un «proxy») de la longueur de la saison de transmission. Cette modélisation permettait de prédire l'effet des changements des niveaux d'utilisation des moustiquaires sur les niveaux de transmission (34).

Assez tôt, la nécessité de dresser des cartes de niveau de transmission du paludisme à l'échelle du continent Africain a été soulignée (35). Le projet MARA/ARMA (Mapping Malaria Risk in Africa/Atlas du Risque de Malaria en Afrique) a été un travail collaboratif visant à produire des cartes de risques de paludisme à l'échelle du continent africain. Des données paludométriques de plusieurs milliers d'études de terrain ont été collectées dans la littérature ou auprès d'acteurs du terrain. Elles concernaient la transmission, la prévalence parasitaire et l'incidence de la maladie. Une modélisation des facteurs géo-climatiques associés au paludisme a permis de suppléer cette collecte de données dans les zones où elles n'étaient pas disponibles (35, 2). Les limites d'une transmission stable du paludisme ont ainsi pu être déterminées en définissant les limites d'un climat approprié (36).

Plusieurs travaux ont reposé sur cette base de données. En Afrique de l'Ouest et Centrale, des cartes de prévalence parasitaire de *Plasmodium falciparum* ont été dressées à partir des données empiriques de prévalence parasitaires issues du projet

MARA/ARMA et d'un modèle de saisonnalité basé sur le NDVI, la température et les pluies (37). Au Mali, une carte de risque de prévalence du paludisme a été calculée à partir de la distance à l'eau, la moyenne de NDVI pendant la saison des pluies, la température maximale dans les 3 mois précédant le début de la saison des pluies et le nombre de mois avec une pluviométrie supérieure à 60 mm (38). Au Kenya, en Ouganda et en Tanzanie, les prédictions des niveaux de prévalence parasitaire reposant sur la température, la pluviométrie, l'humidité, le NDVI et l'altitude étaient concordantes avec des cartes historiques de transmission dérivées d'expertises des données paludométriques et climatiques (39). Dans les mêmes pays, d'autres cartes ont été calculées à partir des prédicteurs précédents et de données de densité de population humaine, d'urbanisation, de présence de collections d'eau et de grandes classes écologiques (40).

Plus récemment, le projet MMP (Mapping Malaria Project) a vu le jour dans l'objectif de rassembler les données de prévalence parasitaire disponibles à l'échelle mondiale et de les mettre librement à disposition (41). La création d'un modèle détaillé des limites spatiales et du degré d'endémicité du paludisme à *P. falciparum* et *P. vivax* repose sur ces données, ainsi que sur des connaissances d'experts et sur la température et l'aridité déterminant l'exclusion de la transmission, selon les espèces anophéliennes en cause (42).

Données de télédétection et morbidité

Les mêmes types d'indicateurs environnementaux et climatiques ont été exploités pour l'évaluation des niveaux de morbidité palustre.

Au Bangladesh, le réseau national de suivi météorologique n'était pas suffisamment dense pour fournir une information utile en épidémiologie. Des indices de végétation et de température, issus de la télédétection, ont pu être corrélés au nombre de cas admis dans les hôpitaux, principalement pendant la saison des pluies. Les données étaient disponibles pour 12 années de suivi et les indices utilisés étaient calculés à partir du NDVI et d'un indice de température de façon à tenir compte des fluctuations météorologiques, sans tenir compte des effets climatiques à long terme (43). Au Kenya, un NDVI supérieur à 0,35-0,40 pour un mois donné a permis de prédire pour le mois suivant que le taux d'admission hospitalier de cas de paludisme pédiatrique grave dépasserait 5% des admissions annuelles totales (44). En Asie, des cartes de NDVI supérieur à 0,4 pendant au moins 6 mois correspondaient à la distribution de l'incidence des cas paludisme à *P. falciparum* de cartes historiques (45). Au Burundi dans une zone de paludisme instable, un modèle incluant le NDVI calculé à partir d'images AVHRR, la température et la pluviométrie mesurées dans les stations météorologiques, et le nombre de cas de paludisme pour un mois donné permettait de prédire l'incidence du paludisme pour le mois suivant (46).

Dans les zones de paludisme instable, l'émergence d'épidémies dépend des variations à long terme du climat et de variations météorologiques à court terme. L'application de modèles reposant sur l'environnement peut permettre de prévoir le risque d'épidémies (47). A l'échelle du continent africain, un système d'alerte précoce des épidémies de paludisme a été mis en place sous l'égide du programme Roll Back Malaria. Il repose sur l'association entre les épidémies et les anomalies de pluie observées sur dix jours. L'accès à ses prédictions est libre sur Internet (48).

Applications pour des populations de militaires

En plus des populations civiles ciblées dans les études décrites précédemment, les militaires peuvent bénéficier des apports de la télédétection et des travaux ont été menés dans cet objectif.

Une étude a récemment montré que le NDVI et des anomalies de NDVI par rapport aux moyennes calculées sur les années précédentes pouvaient être associés à des changements dans des populations de vecteurs près des installations militaires américaines. Ces travaux sont présentés comme préliminaires à l'élaboration d'un système automatisé d'alerte précoce du risque de maladie à transmission vectorielle aux Etats-Unis, reposant sur le climat (49).

Des travaux menés par les militaires américains en République de Corée ont apporté une preuve d'application concrète de la modélisation basée sur la télédétection dans la lutte contre le paludisme. Dans deux camps militaires, un système d'aide à la décision relatif au choix de la méthode de lutte à mettre en place a été testé. Des images de télédétection ont permis de connaître la surface des gîtes larvaires à traiter autour des camps pour évaluer le coût d'une stratégie reposant sur l'utilisation de larvicides. Ce coût a été comparé à celui d'une chimioprophylaxie pour les personnels des camps (50).

Chez les militaires français en mission de courte durée en Afrique intertropicale, le NDVI moyen calculé à partir des données du capteur MODIS (Moderate Resolution Imaging Spectroradiometer) - Terra pour les environnements auxquels étaient exposées les troupes, était significativement et fortement associé au risque d'accès palustre. Ce facteur expliquait une plus grande part de l'incidence du paludisme que l'observance de la chimioprophylaxie (51).

Notion de risque et de densité de population humaine

Le risque de paludisme dépend des populations humaines exposées aux piqûres d'anophèles et du réservoir de parasites. En plus de son apport pour la cartographie des zones propices aux anophèles et l'obtention de données géo-climatiques, la télédétection peut aussi fournir des données relatives aux populations humaines.

Au Kenya, le traitement d'images du capteur radar RADARSAT-1 a permis de cartographier les zones propices à la présence de gîtes larvaires mais aussi les zones peuplées. La zone de risque était définie comme la superposition des aires peuplées et des environnements propices aux gîtes larvaires, augmentés d'un contour de 2 km représentant la distance de vol des anophèles dans cet écosystème (52).

Pour les objectifs de cartographie du risque de paludisme à des échelles plus larges, des bases de données démographiques spatialisées existent, tel que GRUMP (Global Rural-Urban Mapping Project) ou GPW3 (Gridded Population of the World, version 3) (http://beta.sedac.ciesin.columbia.edu/gpw/index.jsp, accédé le 11 décembre 2008). Cependant, il a été récemment souligné que ces sources ne sont pas toujours suffisamment précises pour pouvoir les relier au risque de paludisme, en particulier pour les zones de faible densité de population (53).

Paludisme urbain

La population urbaine croît rapidement et les estimations prévoient que plus de 70% de la population mondiale et plus de 60%

de la population africaine vivront dans les villes d'ici 2050 (54). En milieu urbain, la population est dense, l'accès aux soins est généralement aisé et l'hétérogénéité des écosystèmes peut être importante. La transmission du paludisme peut y être très focalisée. L'urbanisation a un impact sur l'importance et la répartition de la maladie. Le paludisme urbain devrait donc être étudié de façon spécifique. Dans cette optique, les données de télédétection ont été utilisées pour rechercher les gîtes larvaires et mesurer les densités de populations humaines.

A Malindi et Kisumu, au Kenya, l'identification directe des gîtes larvaires de *An. gambiae s.l.*, *An. funestus* et *An. merus* a été tentée à partir d'images du satellite Multi-spectral Thermal Imager (MTI) d'une résolution spatiale de 5 à 20 m en fonction des bandes spectrales. Les résultats ont montré que, même en incluant des examinateurs ayant une grande expérience de photointerprétation, seuls 6% des gîtes larvaires étaient identifiés et les gîtes les plus grands (zones marécageuses, mares, fossés) n'étaient paradoxalement pas mieux détectés que les plus petits (réservoirs d'eau, canaux, flaques, trous d'arbres...) (55). Ces résultats soulignent de nouveau les difficultés de la détection directe des gîtes, surtout dans les villes où ils sont généralement petits. L'utilisation d'images à très haute résolution peut apporter une aide précieuse. A Dar-Es-Salaam en Tanzanie, l'interprétation visuelle de photographies aériennes a permis d'aider à l'identification des gîtes larvaires d'anophèles. La lutte intégrée mise en œuvre dans la ville a pu reposer en partie sur une lutte larvicide guidée par les résultats obtenus par télédétection (56).

A des échelles moins fines et sans détection directe des gîtes larvaires, la télédétection a aussi permis le calcul de variables environnementales de substitution (« proxys » environnementaux) en milieu urbain. Dans deux villes du Kenya, le NDVI calculé sur des pixels de 270 m x 270 m a été associé à la densité de maisons et donc à la présence de gîtes larvaires d'anophèles. Les fortes valeurs de NDVI indiquaient une densité de maison moindre et donc une plus grande probabilité de présence de gîtes larvaires (57).

Dans le domaine de l'épidémiologie du paludisme urbain, la télédétection a aussi été utilisée pour cartographier et modéliser les populations humaines urbaines (58). Par exemple, des images des lumières de nuit ont pu donner des indications sur les zones habitées et ont été utilisées pour estimer les populations urbaines touchées par le paludisme (59) mais leur interprétation quantitative doit être faite avec précaution à cause de la diffusion de la lumière des villes sur leurs environs (60). Les informations de GRUMP sont en partie obtenues par la technique des lumières de nuit. A l'échelle mondiale, il a été montré que les zones urbaines de GRUMP correspondaient bien aux descriptions plus ou moins détaillées dans les articles traitant du paludisme (53). Pourtant, la difficulté d'obtenir une définition quantitative des environnements urbains a encore récemment été soulignée, pouvant gêner l'estimation de l'importance du paludisme et l'extrapolation des résultats à d'autres villes ou quartiers (61).

Précautions

Certaines précautions sont à prendre dans l'utilisation des modèles et cartes de risque calculés à partir de données de télédétection.

L'un des objectifs des modèles est de suppléer les données de terrain indisponibles. Pour cela, ils doivent être construits à partir de jeux de données de qualité et de quantité suffisante pour pouvoir conclure à des associations. Ils doivent aussi être validés sur des jeux de données indépendants et de qualité. L'interprétation des relations statistiques doit être prudente et tenir compte des mécanismes sous jacents à toute corrélation identifiée entre des données de télédétection et des variables paludométriques.

L'extrapolation spatiale est une des propriétés les plus intéressantes des modèles et des cartes mais l'étendue des prédictions ne doit pas dépasser leurs possibilités intrinsèques, en particulier l'étendue des valeurs des données utilisées pour la construction des modèles, les caractéristiques des populations de vecteurs, des populations humaines et des environnements concernés. Ainsi, l'étendue des extrapolations possibles doit être discutée spécifiquement pour chaque situation.

La disponibilité des données de télédétection et des données de terrain est souvent contrainte par des considérations logistiques. Pourtant, les images doivent être choisies de façon spécifique et leur échelle doit être définie en fonction des besoins, en particulier en fonction de l'échelle des mécanismes biologiques étudiés. La cohérence des échelles des différents types de données doit être vérifiée.

La disponibilité d'images contemporaines aux prospections de terrain n'est pas systématique. Les conséquences d'une discordance temporelle entre la prise de l'image et la collection des données doivent être évaluées et discutées. Une étude a montré que la présence de gîtes larvaires d'*An. arabiensis* était associée à des changements écologiques dans le temps dans un village du Kenya (62). Il est donc impératif de prendre en compte ces éventuels changements et de vérifier dans quelle mesure ils peuvent modifier les prédictions des modèles.

Conclusion

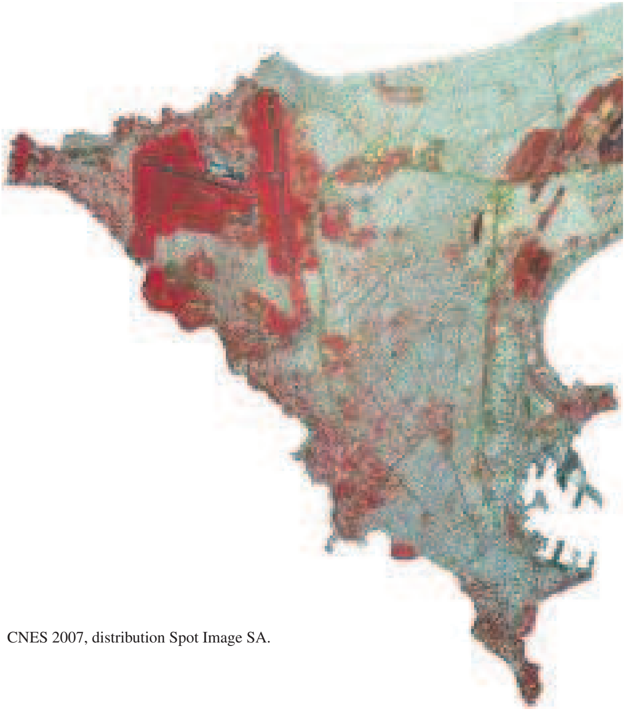
La télédétection peut améliorer la planification, l'efficacité et l'efficience de la lutte antipaludique.

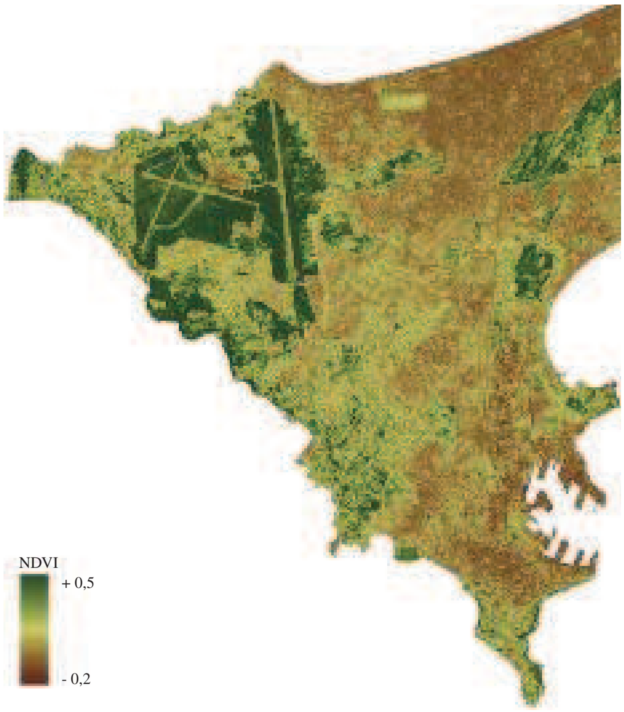
Depuis plusieurs années, les résultats de nombreux travaux montrent que la télédétection peut constituer une source de données importante pour la mise en place de modèles et cartes de risques entomologique, parasitologique et épidémiologique du paludisme. Les images enregistrées par les satellites fournissent des indicateurs d'environnement, de végétation, de climat, de couvert et utilisation du sol, qui peuvent tous jouer un rôle dans l'épidémiologie du paludisme. La disponibilité de ces informations au sol est souvent limitée et les images satellite pallient à ce déficit.

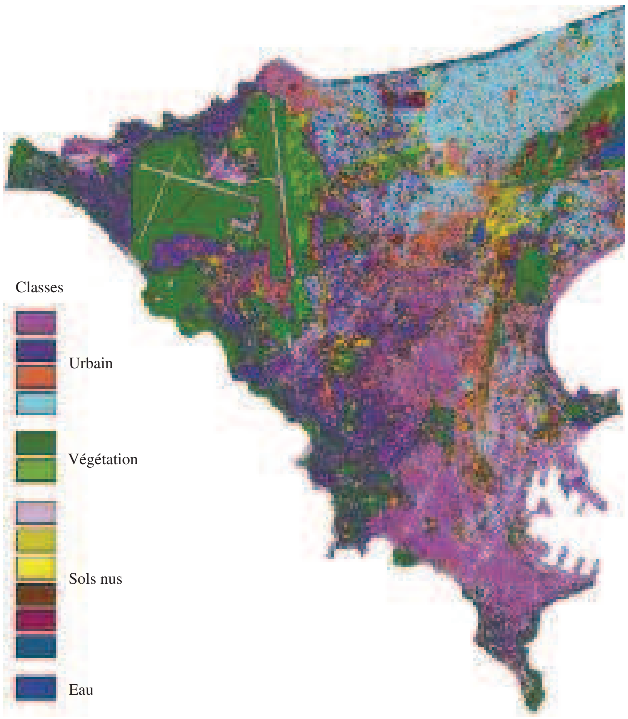
Les données paludométriques ne sont généralement disponibles que de façon ponctuelle, en particulier dans les régions où le système de soin et d'information sanitaire est défaillant. Les cartes et modèles de risque calculés à partir de jeux de données restreints peuvent, dans une certaine mesure qui doit être discutée, être extrapolés dans l'espace et le temps. L'importance du paludisme peut ainsi être évaluée et stratifiée dans des zones et des périodes plus étendues.

Des systèmes d'alerte précoce reposant sur des données géo-climatiques issues de la télédétection permettent de prédire les épidémies de paludisme et de préparer à temps la riposte dans des pays généralement démunis.

Enfin, la connaissance de la répartition et des densités des populations humaines doit être prise en compte pour l'évaluation de l'importance du paludisme. La télédétection permet d'apporter certaines réponses dans ce domaine en contribuant à la cartographie des populations.







NDVI

Le Normalized Difference Vegetation Index (NDVI) a été défini dans les années 1970 (63, 64). Il est encore l'indice de végétation le plus communément utilisé pour les applications en santé humaine. Il capture en un seul indice, des effets combinés des température, humidité, pluviométrie, ensoleillement, altitude, type et utilisation des sols.

Le couvert végétal réfléchit d'autant plus le rayonnement proche infrarouge (PIR) et absorbe d'autant plus le rayonnement rouge (R) que son activité chlorophyllienne est élevée. Ainsi le calcul du NDVI repose sur la différence d'absorption par les plantes des lumières rouge et proche infrarouge et il permet la mesure de l'abondance et la caractérisation de la couverture végétale. Sa formule est un rapport normalisé : $NDVI = (PIR - R) / (PIR + R)$ et sa valeur est comprise entre –1 et +1. En pratique, bien que des seuils fixes ne puissent être définis, une valeur de NDVI comprise entre 0 et 0,2 correspond à des sols secs et dégagés et des valeurs de NDVI supérieures à 0,2 correspondent à de la végétation d'autant plus dense et active que ces valeurs augmentent. Une valeur négative ou proche de zéro peut correspondre à des zones aquatiques, à certains types de bâti ou à des zones asphaltées (routes).

Le calcul du NDVI à partir d'une image multispectrale permet la création d'une nouvelle image dont chaque pixel contient une valeur de NDVI.

Images : Image SPOT / image du NDVI / image classifiée de la Péninsule du Cap Vert (Dakar et ses environs), Sénégal (voir Figure).

Image SPOT : Image SPOT multispectrale à 2,5 m de résolution spatiale, acquise le 26 septembre 2007. Les bandes spectrales de l'image sont converties en rouge, vert et bleu pour l'affichage d'une composition colorée. La végétation apparaît en rouge.

Image de NDVI : Image issue du calcul du NDVI effectué à partir de l'image SPOT. Chaque pixel de 2,5 m contient une valeur de NDVI. Les couleurs vertes correspondent à de la végétation d'autant plus dense que le vert est foncé. Les couleurs brunes correspondent à des sols nus et du bâti.

Image classifiée : Image issue d'une classification ayant permis de regrouper les pixels similaires en 4 classes d'urbains (selon le type d'urbanisation, ex : centre ville, urbain non planifié, quartiers de maisons avec jardins), 2 classes de végétation (selon la densité et le type de couvert végétal, ex : herbe, arbres), 6 classes de sols nus (ex : sable, asphalte, différents types de terre) et 1 classe d'eau (ex : mare, réservoir d'eau).

La disponibilité d'images de télédétection est croissante, leurs résolutions augmentent et les possibilités techniques de traitement s'améliorent. Ainsi, de nouveaux travaux verront le jour pour construire des modèles et cartes de risque de plus en plus précis, en particulier dans des milieux pour lesquels beaucoup de réponses restent à apporter, tel que les zones urbaines. Les applications de la télédétection à la lutte antipaludique devraient donc se développer dans le futur.

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