MALARIA IN SURINAME: A NEW ERA

IMPACT OF MODIFIED INTERVENTION STRATEGIES ON *ANOPHELES DARLINGI* POPULATIONS AND MALARIA INCIDENCE

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THESIS

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ABSTRACT

Malaria is an infectious disease caused by *Plasmodium* blood parasites which live inside the human host and are spread by *Anopheles* mosquitoes. Every year an estimated 225 million new cases and near 800,000 malaria deaths are reported. Control of the disease is a formidable task involving all three living organisms (parasite, vector and host) and their environment. Human hosts are mobile and play an important role in the spread of the disease. *Anopheles* mosquitoes, also mobile, have a tendency to adapt to new environments and are able to counter-act control measures, for instance by developing insecticide resistance. *Plasmodium* parasites, highly adaptive, develop drug resistance, thus rendering existing malaria treatments useless. Finding new methods and strategies to control and eliminate malaria is a continuous struggle. Nowadays malaria control strategies contain a complex of measures complementing one another. New tools are being developed.

In 2005 a new 5-year malaria program was initiated in Suriname which combined a number of old, improved and new malaria control measures and strategies. This thesis describes the effect of these measures and strategies on malaria incidence and the *Anopheles darlingi* populations in Suriname and also evaluates some mosquito monitoring tools.

Suriname has been fighting malaria since the early 1900s. As in many other countries in Latin America, control efforts were successful in the 1950s and 1960s and Suriname succeeded in getting the coastal area free of malaria. In the interior, transmission continued and malaria incidence increased. An important role in malaria transmission is played by the mosquito *Anopheles darlingi*, the primary vector in Suriname. This vector species is important throughout its distribution area in South America. It has a preference for human blood and is highly effective in transmitting malaria even when population densities are low. It combines these capacities with diverse behavioral characteristics and an ability to adapt to new situations, which make it a difficult vector to control.

The density of host-seeking *Anopheles* mosquitoes is an important factor in determining malaria transmission risk. This density is traditionally assessed by collecting and counting the number of mosquitoes which land on a human collector in order to bite; the human landing collections. Most often the landing mosquitoes are collected before they bite, but obviously this method may hold a risk for the collectors when used in a malaria endemic area. Besides, it is a very labor-intensive and expensive method. Alternative tools are needed. The ability of the CDC Miniature light trap, the BG Sentinel™ trap and the Mosquito Magnet® Liberty Plus, with carbon dioxide or a protected human as bait, to act as an alternative to human landing collections in defining mosquito biting pressure was tested both for *An. darlingi* and *Anopheles aquasalis*. Even though in particular the BG Sentinel and the Mosquito Magnet Liberty Plus showed potential, none of the trapping methods proved as effective as the human landing collections. As it turns out carbon dioxide may not be a sufficient stand-alone bait for these *Anopheles* species. Alternative baits, especially human-derived stimuli, may improve the results, enabling a more cost-effective mosquito monitoring and surveillance tool, with less risks. The BG Sentinel trap, baited with CO_2, was very efficient in collecting *Culex* mosquitoes.

The most important changes in the malaria control strategy in Suriname as of 2005 were 1) the introduction of artemisinin-based combination therapy as a new first line treatment for malaria caused by *Plasmodium falciparum*, just prior to the onset of the new malaria program, and 2) the mass-distribution of free long-lasting insecticide-treated nets (LLINs) to the population at risk. Other measures included Indoor Residual Spraying, Active Case Detection and training of on-site Malaria Service Deliverers in remote areas. The effect of the control program on the mosquito populations was assessed in a 4-year longitudinal vector study in three sentinel sites, using human landing collections. *Anopheles darlingi* populations collapsed shortly after onset of the malaria control program and did not recover during the following years. The possible impact of the LLINs on the mosquito population is discussed. Our limited knowledge about the reasons for the vector populations' collapse and about *An. darlingi* ecology in general, prevent us from making predictions about future population dynamics. *An. darlingi* population densities remain low in the sentinel sites but a density increase could be triggered at any time. A continued monitoring of the vector and further studies with regard to its feeding behavior and ecology in general will therefore be necessary.

The new malaria control strategy led to a significant decrease of malaria in the villages in the interior of the country, enabling Suriname to reach the Millennium Development Goal for malaria in 2007. The malaria situation in Suriname changed to a state where the disease is almost completely controlled in the stabile populations of the villages in the interior where malaria incidence is down to near elimination levels. This is a significant success, but can Suriname hold on to it? Transmission still occurs in the mobile human populations in the forest, especially among the gold miners. Additionally, the border region with French Guiana is vulnerable due to cross-border movement of the people. The new challenge for Suriname is to further control and possibly (locally) eliminate malaria by establishing an integrated malaria control strategy with a strong malaria surveillance system and prompt interventions in areas of renewed outbreaks. This will require sufficient funds, dedication and regional collaboration.

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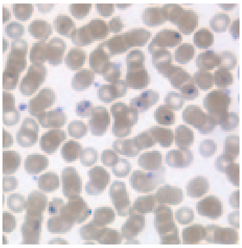
General Introduction

1

MALARIA IN SURINAME

Malaria has had a profound impact on the history of Suriname. It resulted in the death of numerous Amerindians after the import of the so-called malaria tropica, caused by *Plasmodium falciparum*, as a result of the introduction of African slaves in colonial times (16th and 17th century). Other malaria species in Suriname are *P. vivax* and *P. malariae*. Malaria forced the European colonists to make a treaty with runaway slaves in the forest of the interior in 1760, because malaria among the troops prevented them from getting these slaves back and return them to forced labor. It seriously hampered the development of gold, timber and balata industries and cost the lives of hundreds of contractors from overseas working on the railroad going from the capital to the interior in the early 1900s (Van der Kuyp, 1969). This mostly happened before the discovery by Charles Laveran in 1880 of the *Plasmodium* blood parasites which cause malaria and before Ronald Ross discovered the transmission of the parasite by *Anopheles* mosquitoes in 1897 (Fig. 1.1). Dr. Flu (1912) was the first to describe malaria transmission in Suriname, after which malaria control was initiated.

FIGURE 1.1: *Plasmodium falciparum (malaria parasite, inside red blood cells* ***(A)*** *(photo M. Zahniser), and Anopheles darlingi mosquito, most important malaria vector in Suriname* ***(B)*** *(photo S. Mitro).*



Almost one century after the first malaria control efforts in Suriname, malaria transmission still occurs in the country. Large vector control campaigns resulted in the elimination of the disease from the coastal area around 1968 (Rozendaal, 1990), but malaria continues to be an important health issue in the forested hinterland or so-called interior of the country. The ongoing problem of malaria transmission in this large area can be contributed to a number of factors, including the distribution of the vectors, the history of the malaria control programs, and the distribution and behavior of the human population. Current developments in malaria control efforts in Suriname may bring a change in this long-lasting status quo of malaria transmission in the Interior of the country.

GEOGRAPHY AND VECTOR DISTRIBUTION

Suriname is divided into three distinct ecological zones (Fig.1.2), which have a different species composition and distribution of *Anopheles* mosquitoes.

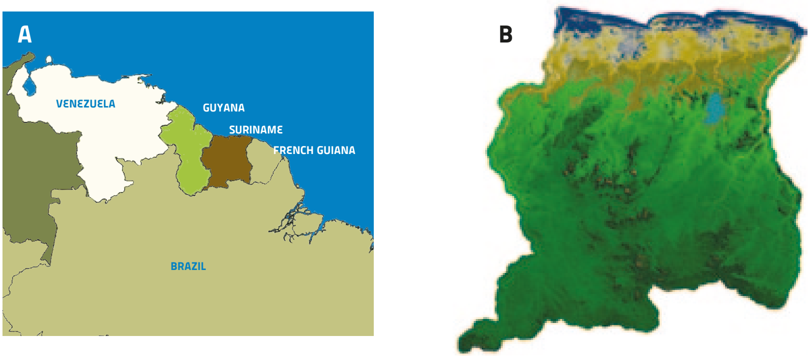


FIGURE 1.2: *Map showing Suriname* ***(A)*** *and satellite image of Suriname showing the three ecological zones; the northern coastal plain (light brown), the savannah belt (dark brown) and the tropical rainforest of the interior (green)* ***(B)****.*

- The majority of the human population lives in the northern coastal plain. This has diverse ecosystems ranging from agricultural lands to marshes and mangrove forests. The most common *Anopheles* species found along the coast is *An. aquasalis* Curry.
- The southern rainforest of the interior covers almost 80% of the country. The interior has several mountain ranges and isolated high peaks, the highest ones with an altitude of 1280 meters. This area is inhabited by Maroons (descendants of African slaves) and Amerindians who live in tribal communities along the major rivers.

  We also find significant communities of Brazilian goldminers (garimpeiros), living in the most remote areas (Peterson and Heemskerk, 2001; WWF, 2005). Common *Anopheles* mosquitoes are *Anopheles darlingi* Root, *Anopheles nuneztóvari* Gabaldon and *Anopheles oswaldoi* Peryassú.

- The northern coastal plain and southern interior of the country are separated by a so-called savannah belt, i.e. infertile white sand formations covered with shrubs. The very few communities living in this zone are mostly Amerindians. The savannah belt is an intermediate zone, which lies almost completely within the area of tidal influence. It has mosquito species from both the Interior and the coastal plain. At several occasions *An. darlingi* specimens were found in this zone, especially in temporary swamps (Rozendaal, 1987).

Anopheles darlingi is the primary malaria vector in Suriname. Rozendaal (1989, 1991) found a correlation between the presence of *An. darlingi* and the transmission of malaria in different localities in the interior of Suriname. Hudson (1984) was the first to study the effectiveness of vector control measures against this vector in Suriname. The distribution of *An. darlingi* in Suriname is thought to be related both to the tidal influence and to the physical characteristics of the major rivers and the resulting degree of flooding of forested river banks during the rainy season (Rozendaal, 1992). North of the tidal movement *An. darlingi* is virtually absent, except for sporadic occurrences in the savannah belt.

The second most common man-biting mosquito of the interior of Suriname is *An. nuneztovari*. During the construction of a hydropower reservoir in the interior in the 1960s, fear arose that this species would take the place of *An. darlingi* as a primary vector (Van Thiel, 1962). This did not happen, and the role of *An. nuneztovari* as a vector in malaria transmission in Suriname was never confirmed (Rozendaal, 1989). *Anopheles nuneztovari* is considered an important vector elsewhere in the Americas, with preference for outdoor biting (Tadei and Dutary-Thatcher, 2000; Galardo et al., 2007).

In the coastal plain of the country *An. aquasalis* is very common. It was suspected of playing a minor role in malaria transmission in Suriname in the past, due to malaria outbreaks in localities where this species was the only potential vector found (Rozendaal, 1990). *Anopheles aquasalis* has a history of replacing *An. darlingi* in coastal areas where it had been eradicated, and is known to bite and rest outdoors (Elliot, 1969). It has a preference for breeding in brackish water, and is a primary malaria vector in Venezuela and in parts of Brazil (Grillet, 2000; Povoa et al., 2003).

HUMAN POPULATION AND MALARIA TRANSMISSION

The interior of Suriname provides a challenge for malaria control due to a number of factors, including its lack of infrastructure. There are no roads beyond the Van Blommenstein power lake and travelling to and from the southern communities is done either by boat or by air, which in both cases is rather expensive. Another complicating factor is that the so-called "stabile" Maroon and Amerindian communities are in fact "semi-mobile", meaning that although the people live in a village, they will visit family in other villages or travel to their (slash-and-burn) agricultural grounds in the forest, and stay there for a longer period of time. This even involves crossing of the national borders to both French Guiana and Brazil, and occasionally Guyana. The communities of Brazilian garimpeiros (goldminers) and many of the young men of other ethnic backgrounds (Maroon, Amerindian, Chinese, Haitian etc) working in gold mines constitute yet another difficulty. They live and work in remote areas and are very hard to reach for health workers. They are mobile and interactive with the village communities in the surrounding area. Due to a lack of proper diagnosis and treatment in the remote mining areas, malaria transmission is ongoing, and the risk of transmission to the village communities is present. The mining operations themselves provide an added difficulty in malaria control as mining results in deforestation (Peterson and Heemskerk, 2001), which in turn may increase the vectorial capacity of the *Anopheles* mosquitoes (Vittor et al, 2006; Afrane et al., 2008).

AUTHORITY AND DATA FLOW

The public health working department of the Ministry of Health (MoH) in Suriname is the Bureau of Public Health (Bureau Openbare Gezondheidszorg; BOG). Since 1958 BOG has a special division dedicated tot malaria eradication, which is called the Anti Malaria Campaign (BOG/AMC). The BOG/AMC gathers malaria data from all health care providers in the country, and is responsible for monitoring malaria transmission, and detection of local epidemics.

For technical advice in the complex matters of malaria prevention, diagnosis, treatment and vector control, the MoH relies on the National Malaria Board, which is a sub-committee of the Country Coordinating Mechanism (CCM). The National Malaria Board has representatives from the MoH (including BOG/AMC), from the Regional Health Services, the armed forces, the Ministry of Regional Development, the University of Suriname, the Pan American Health Organization (PAHO), and the Medical Mission (MM). Important decisions on policy matters as well as on major ongoing activities are generally taken by this Board.

The MM is a partly government-funded NGO. It is the official public health care provider in the interior of Suriname, and has been in place since 1977. Currently the MM has 56 local health centres with trained health care assistants, and is still expanding this number with the help of international donors. As a result BOG/AMC is left with a formal controlling, evaluating and guiding role for malaria control in the interior of the country.

All data on malaria cases in the hospitals and the coastal health centres are compiled in a database by BOG/AMC. All data on malaria cases in the interior are compiled by the MM in a separate database and until 2006 were reported on a yearly basis to BOG. In 2005 a new Malaria Program (Medical Mission Malaria Program; MM-MP), supported by the Global Fund to fight Aids, Tuberculosis and Malaria (GFATM) and managed by the MM, came into being, which since 2006 combined the now weekly reported data of both the MM and BOG/AMC in one national database. Since 2009 this database is managed by the second GFATM-supported Malaria Program (MoH-MP), which is executed by the MoH. This national database will in time be incorporated within the structures of BOG.

MALARIA CONTROL

Both Schaapveld (1984) and Rozendaal (1990) have given a fairly detailed overview of the history of malaria control in Suriname until the 1980s (Table 1.1). The first intensive insect control program with insecticides was established in the country between 1949 and 1956, with an average of 30,000 houses sprayed each year with DDT (dichlorodiphenyltrichloroethane), Dieldrin or Gammahexane. Before 1949 malaria control consisted of case treatment and the use of prophylaxis. After 1957 the insect control program was converted in a malaria eradication program, which consisted of indoor spraying with DDT in the coastal area and savannah belt, and spraying with Dieldrin in the Interior. In 1961 spraying in the coastal area was stopped because of the complete disappearance of malaria from this area. The interior was sprayed with DDT twice a year, but coverage was below 40%, which was mostly attributed to a lack of cooperation of the local people. Reasons for lack of cooperation were found to be manifold, but much related to a lack of knowledge and awareness of local people, as well as to frictions between the local population and non-local spray men. The coastal area and savannah belt were free of malaria after 1967. In 1984, following many technical and organization problems in the malaria eradication campaign in the interior, the MoH decided to put the MM permanently in charge of the anti-malaria program in this area. This was done after a study by Schaapveld (1984) who predicted that this would lead to an increased efficiency and reduced costs. The result, however, was disappointing; long-term improved effectiveness did not occur, and indoor residual house spraying (IRS) was ultimately totally stopped. Malaria control activities were limited to case detection, treatment of malaria cases and incidental spraying.

TABLE 1.1: *Overview of malaria control activities in Suriname (Schaapveld (1984), Rozendaal (1990)).*

YEAR	ACTIVITIES
1930	Mass active case detection by all district medical officers
1950	Introduction of bed nets (coastal plain and interior)
1949-1955	Insect control program with DDT in the coastal plain
1957	Establishment of Anti-Malaria Campaign by the government
1958-1960	Eradication program with DDT (coastal plain)
1958-1960, 1963-1974	Eradication program with Dieldrin and DDT (interior)
1968	Maintenance phase - middle and west coast
1967-1973	Distribution of medicated salt, with Amodiaquine (interior)
1981	Maintenance phase – east coast (excluding Albina)
1984	Vector control in the interior responsibility of Medical Mission

Since 1965 the number of malaria cases reported annually varied between ca. 500 and 4000 cases, and originated mostly from the interior. In 1987/1988, following the internal war, the number of malaria cases in the interior exceeded 7500 (data MM). From 1993/1994 onwards the number of cases again started to increase (Fig. 1.3). The peak in number of malaria cases between 1995 -1996 is thought to be partly related to improved health care services, including improved capacity of diagnosis. This means that during the preceding years malaria may have been underreported.

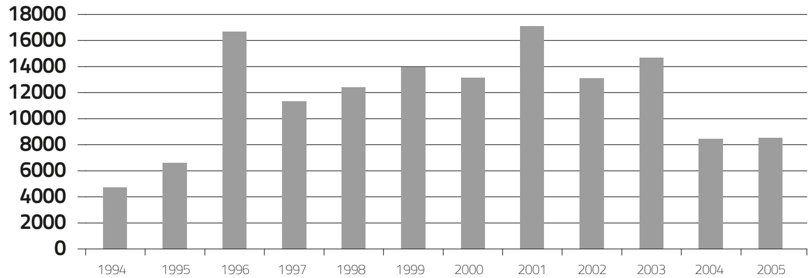


FIGURE 1.3: *Number of malaria cases in Suriname between 1994-2005 (WHO, 2005; data MM).*

Following the increased resistance of *Plasmodium falciparum* to chloroquine (and other drugs) and the ongoing developments on the drug market, the National Malaria Board decided on a new protocol for malaria chemoprophylaxis and treatment in December 2003 (National Malaria Board, 2003). Mefloquine (Lariam®) became the first line prophylaxis for both adults and children, replacing the combined use of paludrine and chloroquine. The protocol for treatment of *P. vivax* infections is a combination of chloroquine (3 days) and primaquine (14 days). *Plasmodium malariae* infections are treated with chloroquine only. A 3-day artemisinin-based combination therapy (Coartem®) (lumefantrine and artemeter; 3 days) was introduced as the first choice therapy for non-complicated *P. falciparum* infections. Complicated *P. falciparum* infections are treated with quinine. A separate protocol for treatment and prophylaxis for pregnant women was established.

In 2005 Suriname entered a new phase in malaria control with the onset of the MM-MP. The main objectives of the MM-MP were;

i) expansion of the prompt access to diagnosis and treatment
ii) promotion of the use of long-lasting Insecticide Treated bed Nets (LLINs) in the interior, and
iii) strengthening of the intelligence of the Malaria control program.

The most important control activities within the program are improved case detection and treatment, especially in high malaria risk areas, free distribution of LLINs and (re-)impregnation tablets to the communities of the interior, and Indoor Residual House Spraying (IRS) in high risk areas.

PROBLEM DEFINITION AND RESEARCH OBJECTIVES

With funding of the MM-MP a significant amount of money became available for malaria control, including vector control, in Suriname. The control measures were expected to have an effect on the number of malaria cases in the country and on the vector populations. The aim of distributing LLINs is to reduce the vector-host contact possibility, which is thought of as one of the most successful control measures (Lindsay et al., 2002) in a world with increasing drug resistance of the malaria parasite. Aim of the research discussed in the following chapters is to study to what extent *An. darlingi* in Suriname is affected by the vector control strategies executed within the MM-MP. What is the effect on the population densities, dynamics and behavior of the *An. darlingi* mosquitoes, and in turn on malaria incidence? It may be especially difficult to assess the impact on mosquito behavior, because even if *An. darlingi* is an important vector in South America, little information is available on its behavioral patterns, especially its biting behavior and preferences. This is an important knowledge gap because changes in the behavioral or biting preference of this vector, possibly induced by control measures, may in time render these measures non-effective and will need to be detected as soon as possible. Part of the research described here will, therefore, focus on the vector's biting behavior and preferences. Monitoring of the effectiveness of vector control strategies is necessary since vector control worldwide is threatened by the increased development of insecticide-resistant mosquitoes (N' Guessan et al., 2007; Ramphul et al., 2009).

The ethical issues related to the human landing collections of mosquitoes, a method conventionally used in vector monitoring programs, force us to look for alternative methods which provide a representative picture of the population dynamics and behavior of the malaria vector. With an eye on future monitoring needs in Suriname a study was made to determine which mosquito surveillance method may be the most representative alternative for human landing catches to monitor densities and biting characteristics of *An. darlingi*, the main malaria vector in this country.

In short; the main objectives of this study are;

1) To obtain baseline data on behavioral characteristics, especially biting and host-seeking behavior, of *An. darlingi*, from selected sites in high-malaria risk areas in Suriname.

2) To assess which mosquito surveillance method may be the most representative alternative for human landing catches to monitor densities and biting characteristics of the main malaria vector in Suriname.

3) To study the effect of vector control measures on the *An. darlingi* populations and malaria incidence over time in Suriname

The study objectives and results are discussed in the following chapters. **Chapter 2** gives a review of vector studies on *An. darlingi* and provides insight in the importance of this vector for malaria transmission in South America. It addresses the ecology of the species. In **Chapter 3** the relation between the biting preferences of *An. darlingi* and the usefulness and efficiency of some alternative trapping methods is addressed. **Chapter 4** provides a parallel by describing the testing of alternative trapping methods for *An. aquasalis*, a (potential) malaria vector species in the coastal plain of Suriname. In **Chapter 5** vector data are presented which serve as a baseline for the longitudinal study on population dynamics and vector behavior since the onset of the MM-MP. These longitudinal data are presented in **Chapter 6** and are related to the interventions executed by the MM-MP. **Chapter 7** describes the impact of the combined interventions on malaria within the MM-MP on a broad scale and presents the resulting changes in the malaria situation in Suriname. **Chapter 8** summarizes the results in light of the objectives and discusses the future of malaria control (and elimination) in Suriname in relation to global developments in malaria control.

Ecology of *Anopheles darlingi* Root with respect to vector importance: a review

2

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ABSTRACT

Anopheles darlingi is one of the most important malaria vectors in the Americas. In this era of new tools and strategies for malaria and vector control it is essential to have knowledge on the ecology and behavior of vectors in order to evaluate appropriateness and impact of control measures. This paper aims to provide information on the importance, ecology and behavior of *An. darlingi*. Publications addressing ecological and behavioral aspects which are important to understand the role and importance of *An. darlingi* in the transmission of malaria throughout its area of distribution are reviewed.

Anopheles darlingi is especially important for malaria transmission in the Amazon region. Although numerous studies exist, many aspects determining the vectorial capacity of *An. darlingi*, i.e. its relation to seasons and environmental conditions, its gonotrophic cycle and longevity, and its feeding behavior and biting preferences, are still unknown. The vector shows a high degree of variability in behavioral traits. This makes it difficult to predict the impact of ongoing changes in the environment on the mosquito populations. Recent studies indicate a good ability of *An. darlingi* to adapt to environments modified by human development. This allows the vector to establish populations in areas where it previously did not exist or had been controlled to date.

The behavioral variability of the vector, its adaptability, and our limited knowledge of these impede the establishment of effective control strategies. Increasing our knowledge of *An. darlingi* is necessary.

INTRODUCTION

The malaria vector *Anopheles darlingi* was first described in 1926 by Root and named after Dr. Samuel Taylor Darling (1872-1925), a leading expert in tropical diseases in the early twentieth century. This mosquito species has a wide geographic distribution in South and Central America, stretching from South Mexico to North Argentina, and from the East of the Andes chain to the coast of the Atlantic Ocean (Komp, 1941; Deane et al., 1948; Rachou, 1958; Forattini, 1987). In coastal areas its distribution is restricted by the salinity of water (Deane et al., 1948) and the species is absent from regions with long dry seasons. *Anopheles darlingi* has an elaborate history of vector incrimination in South America (Deane et al., 1948; Giglioli, 1956; Rachou, 1958; Tadei et al., 1988; Sinka et al., 2010) and its presence has been associated with malaria epidemics, like the one in the Paranápanema River, Brazil, in 1950 (Falavigna-Guilherme et al., 2005).

Studies on the biology and behavior of this mosquito have been relatively few, especially when considering its important role in malaria transmission and its association with severe endemic or epidemic malaria (Deane et al., 1948; Rachoe, 1958; Forattini, 1962; Forattini, 1987; Arumburú et al., 1999; Flores-Mendoza et al., 2004; Moreno et al., 2007).

The malaria situation in the world is changing. Not only is there an increased international effort to control and where possible eliminate malaria (Roll Back Malaria, Millennium Development Goals (United Nations, 2010)), supported by a changed funding environment, but also ongoing developments have resulted in new tools for malaria and vector control. Current strategies of integrated vector management, including the use of insecticide treated bed nets and indoor residual spraying, may not be sufficient to eliminate malaria transmission in all endemic areas. Considering the long-term challenge of eradication of malaria it is essential to increase knowledge on the ecology and behavior of malaria vectors like *An. darlingi*, in order to evaluate appropriateness and impact of these strategies (Ferguson et al., 2010; MalERA, 2011).

VECTOR IMPORTANCE OF *ANOPHELES DARLINGI* IN THE COUNTRIES OF DISTRIBUTION

Intensified malaria control activities have led to a decrease in the number of malaria cases in many countries in Latin America. According to the WHO (World Health Organization, 2011), Mexico, El Salvador, Paraguay and Argentina have entered the elimination or pre-elimination phase and only four countries in the Americas, namely Brazil, Colombia, Haiti and Peru, are responsible for 90% of the malaria in this region in 2009. *Anopheles darlingi* is among the most efficient malaria vectors in the Neotropical Region (Manguin et al., 2008). The exact extent of its distribution is subject to discussion and changes continuously due to ecological changes and adaptations of this mosquito. A predicted distribution based on published records and expert opinions was recently produced by Sinka et al. (2010) (Fig 2.1). The vector importance of *An. darlingi* varies throughout its area of distribution. In **Mexico** the main vectors are *Anopheles pseudopunctipennis* Theobald (inland) and *Anopheles albimanus* Wiedemann (coastline and marshland). *Anopheles darlingi* plays a minor role in the south-eastern region of the country (Loyola et al., 1991; Manguin et al., 2008), especially in the state of Oaxaca. Overall malaria in Mexico is down since a change of control strategy towards ecological measures (clearing of vegetation around houses and in waterways) in 1998 (Shah, 2010). In **Guatamala** the main vectors are *An. albimanus, An. pseudopunctipennis* and *An. vestitipennis*, Dyar & Kanb, but *An. darlingi* has been collected along the various river systems (Komp, 1941; Manguin et al., 2008; Mirabello and Conn, 2006) and is considered to play a role in the malaria transmission (Hernandez et al., 2009). The same four vectors play an important role in malaria transmission in Belize and Honduras (Manguin et al., 2008).

In **Belize** *An. darlingi* is uncommon in the northern part of the country. In the southern regions it is mostly endophagic, more so than *An. albimanus* (Robert et al., 2002). An interesting study by Roberts et al. (1993) shows that in the Toledo District, where *An. darlingi* was the most common species in the 1940s (based on Kumm & Ram, 1941), no *An. darlingi* were found in 1992. Instead, the most abundant mosquito was *An. vestitipennis*. Malaria transmission in Belize decreased after 1995 as result of a vector control programme with DDT (Manguin et al., 2008). In **Honduras**, *An. darlingi* was a suspected vector in severe malaria transmission in the early 20th century (Komp, 1941). Sugar-cane and cotton farming had dried up the southern part of the country, which led to a significant decrease of malaria cases and a move of the human population to the north. The resulting forest clearing in the north, however, has led to an increase of malaria in that area due to *An. albimanus* (Manguin et al., 2008; Almenderes et al., 1993).

The incidence of malaria in **El Salvador** is among the lowest in the Central American countries. *Anopheles darlingi* specimens have been found (Manguin et al., 1996), but transmission is considered due mainly to *An.*

albimanus and *An. pseudopunctipennis*, as is the case in **Nicaragua** where malaria transmission was high but is decreasing (Garfield, 1999; PATH, 2010). The northern and eastern regions still have a high transmission risk (Centers for Disease Control and Prevention, 2010). Nicaragua and **Costa Rica** have no collection data of *An. darlingi* despite numerous and comprehensive surveys in the area trying to locate it. Official reporting of *An. darlingi* from **Panama** did not exist for a long time (although it was one of the first countries worldwide from where successful malaria control was reported (Dehné, 1955)). Malaria transmission is considered due to *An. albimanus* and *Anopheles punctimacula*, Dyar & Kanb which are the most abundant species with the highest human biting rate. *Anopheles aquasalis* is suspected of playing a role in transmission on the eastern Atlantic coast.

Recent genetic studies suggest a long and stable population of *An. darlingi* in (eastern) Panama, possibly originating from Colombia (Loaiza et al., 2008; Loaiza et al., 2009). In **Colombia**, *An. darlingi* is the principal malaria vector in the Llanos Orientale, The Amazonia, The Orinoquia, Urubai, Bajo Cauca and Magdalena Media (Olana et al., 2001). The most important vector in Colombia is *An. albimanus*, representing 99% of the *Anopheles* population along the Pacific coast and 61% of the population along the Atlantic coast (Guttierez et al., 2008). **Venezuela** has a very diverse *Anopheles* population. *Anopheles darlingi* is especially important in the Amazonian south along the rivers (Giglioni, 1956; Charlwood, 1996; Magris et al., 2007a). Studies by Moreno et al. (2002, 2007) showed that *An. darlingi* represented over 30% of the *Anopheles* populations in locations in the Amazonias and Bolivar state. Other important vectors are *An. aquasalis* (north east coast), *An. albimanus* (coast and subcoastal areas), *An. pseudopunctipennis* (Andes foothills) and *An. nuneztovari* (northwest) (Manguin et al., 2008). Vector control with DDT between the 1940s and 1960s proved very efficient in reducing malaria in the **Guyanas** (Guyana, Suriname, French Guiana), eliminating it from the coastal areas of Guyana (1951) and Suriname (1968) (Charlwood, 1996; Rambajan, 1984; Rozendaal, 1987). *Anopheles darlingi* is considered the most important and often only malaria vector in the sparsely populated interiors of the three countries which are covered with rainforest (Giglioni, 1956; Pajot et al., 1977; Juminer et al., 1981; Rozendaal, 1987; Moreno et al., 2002; Girod et al., 2008; Hiwat et al., 2009). Hudson (1984), for instance, found that females of *An. darlingi* were the commonest (98.6%) of the 5,464 anophelines he collected in the rainforest of Suriname during 1979-1981. In 1980 malaria re-appeared in the coastal area of Guyana and transmission continues among the inland Amerindians (Manguin et al., 2008; Rambajan, 1984). Despite the resurgence of malaria in the early 1990s and the continued high transmission among Amerindian and Maroon populations of the interior, the coast of Suriname is currently still free of malaria. Since 2005 a sharp decline in number of malaria cases is reported following a scale-up of intervention activities (World Health Organization, 2011). In French Guiana malaria continues to be a problem in the Amerindian populations along the Oyapock and Lawa Rivers and the Maroon population along the Marowijne River. Coastal malaria, transmitted by *An. darlingi*, has disappeared except from import cases due to Haitian and Brazilian immigrants (Claustre et al., 2001; Manguin et al., 2008). Almost all cases in **Brazil** originate from the Brazilian Amazon, with 74% of the cases coming from the three states Rondonia, Pará and Mato Grosso (Manguin et al., 2008). *Anopheles darlingi* is one of the most important vectors, with a natural infection rate varying between 2.7% and 4.2% in the state of Pará (De Arruda et al., 1986). Other vectors include *An. nuneztovari*, *Anopheles triannulatus*, Neivo & Pinto *An. oswaldoi*, *Anopheles albitarsis* Linthicum and *Anopheles intermedius* Peryassú. Povoa et al. (2006) reconfirmed the importance of *An. darlingi* in malaria transmission in the savannah eco-region of northern Amazonian Brazil. Here, the species was named an important vector in peri-urban environments.

In **Equador** fifty percent of the human population lives in malaria endemic areas. Especially in the Amazonian plains, east of the Andes, *An. darlingi* plays an important role. By contrast, *An. albimanus* is the main vector along the Pacific coast and *An. pseudopunctipennis* transmits malaria along the slopes and southern valleys of the Andes (Manguin et al., 2008). The resurgence of malaria in **Peru** after 1991 was thought to be associated with the spread of *An. darlingi* into new areas of the Amazon Basin (Aramburú et al., 1999; Schoeler et al., 2003; Pinedo-Cancino et al., 2006). A study by Schoeler et al. (2003) shows that in the departments of Loreto and Ucayali, where over 60,000 mosquitoes were collected, 71% of the mosquitoes were *Anopheles benarrochi* Galabadon, Cova-Garcia & Lopez and 24% were *An. darlingi*. The latter species was found in almost 50% of the study areas including areas where the species had not been reported before. Flores-Mendoza et al. (2004) found positive specimens of both *An. benarrochi* and *An. darlingi* at rates of 0.14% and 0.98%, respectively. In **Bolivia**, *An. darlingi* is thought to have played an important role in malaria epidemics in the first half of the 20th century (Manguin et al., 2008). The species is found in the plains of northeastern Bolivia in the Departments of Beni, Panel and Santa Cruz (Walter Reed Army Medical Center, 1998). *Anopheles pseudopunctipennis* is an important vector in areas above 500 m. asl. Other vectors include *An.*

nuneztovari, *An. triannulatus, Anopheles marajoara* Galvao & Damasceno and *Anopheles braziliensis* Chagas. **Paraquay** is a transition area between the Andean countries and Brazil. Areas of moderate malaria risk are found in the Alto Paraná, Caaguazú and Canendiyú, where *P. vivax* is transmitted (Centers for Disease Control and Prevention, 2010). Negligible risk exists in the remaining areas. *Anopheles darlingi* is the only malaria vector reported (Manguin et al., 2008) and its reappearance along the Paraguay border with **Argentina** is assumed to have been the reason for an increase in malaria on the Argentinean side (De Casa and Isabel, 1992), together with increased border traffic along the Bolivian and Paraguayan border and the ecological changes due to the construction of dams in the Paraná basin. Malaria in the north-western area of Argentina (Satla and Jujuy provinces) is mostly due to *An. pseudopunctipennis* (Davis, 1927; Juri et al., 2005; Juri et al., 2009). In the northeastern part (Missiones) *An. darlingi* is a vector (Manguin et al., 2008).

From the combined studies reviewed above, it is apparent that *An. darlingi* has a very wide distribution and acts as a malaria vector in almost all South American countries, being the principle vector in the Amazon basin.

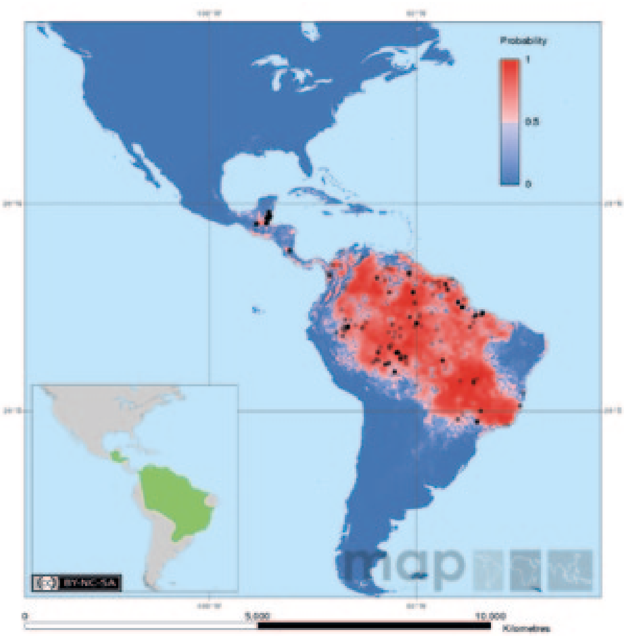

FIGURE 2.1: *Map of the predicted distribution of Anopheles darlingi. Map by Sinka et al. (2010) based on hybrid data (318 occurrence data plus 500 pseudo-presences weighted at half that of the occurrence data and randomly selected from within the Expert Opinion range).*

SEASONALITY

Seasonality patterns of *An. darlingi* are closely related to the annual cycle of rainfall, although the relation of the occurrence of peak abundances to rainfall patterns seems to differ at different localities or regions. Deane et al. (1948) discusses the species' sensitivity to dry season conditions. Rains are thought to increase availability of breeding sites (Giglioli, 1938) and peak abundances of *An. darlingi* in the rainy season have been reported (Forattini, 1962; Elliot, 1968; Tineo et al., 2003; Léon et al., 2003). The local distribution area of *An. darlingi* may expand during the rainy season, as was observed by Roberts et al. (2002) in Belize who found adult females within the range of rivers all year round, but further away from rivers only in the wet season. Forattini (1987) found a greater density of *An. darlingi* during the hot months in southern Brazil and considered that the annual cycle of activity of this species may depend on exogenic factors, including those which can affect the productivity of breeding sites. He considered that the occurrence of heavy rains could possibly flood breeding places and create flood currents that carry away immature forms. This was also found by Pajot et al. (1977) in French Guiana who discovered that heavy rains are followed by a decrease in and sometimes a total absence of *An. darlingi*. This mechanism is thought to have some influence on the increase in the density of *An. darlingi* in the dry season or in the transitional period between the dry and the wet season (Forattini, 1987; Charlwood, 1980).

Following a study in Rondônia (Brazil), Gil et al. (2007) make a distinction between malaria of riverine areas and dry land malaria, explaining that differences in the nature and timing of establishment of breeding sites favorable to *An. darlingi* may result in different ecological models for malaria transmission. In dry land conditions, rivers can flood the margins during the rainy season, but the strong water flow will prevent successful breeding. Only after rains have ended, when rivers retreat to their original size, breeding sites originate as large stable water collections left behind in previously inundated areas. This results in high mosquito densities at the beginning of the dry season and malaria outbreaks in the dry season. By contrast, in riverine areas water levels of the major rivers rise significantly during the rainy season, due to draining from the tributaries. Not only the river margins, but complete forest areas can be flooded, creating inundated forest floors with low water flow which serve as excellent breeding sites for *An. darlingi*. Peak occurrences will be found in the rainy season, decreasing at the beginning of the dry season, when the flooded forest floors dry up. Such differences in ecological settings may explain a study in Suriname, where Hudson (1984) discovered two different seasonal patterns (rainy season peak density and dry season peak density) in areas only 40 km. distant. Rozendaal (1992) adds to the evident diversity of seasonal patterns when he finds that peaks in biting densities in a focus of malaria along the Marowijne River in Suriname correlated well with periods of (i) high water level in the long rainy season, (ii) low water level in the long dry season, and (iii) abundant rainfall in the short rainy season. Critical levels of river height and rainfall were defined, which could explain most of the monthly fluctuations in malaria parasite incidence observed in this area.

Knowledge about the relationship of *An. darlingi* with environmental conditions and the impact of seasonal cycles on the mosquito population densities is required to predict areas and seasons of high malaria risk. Considering the large variety of larval habitats of *An. darlingi*, depending on the (changes in) ecological environments, and considering the adaptability of the vector, there is no way of assessing seasonality of the population densities than through the study of local settings.

LARVAL HABITATS

Larvae of *An. darlingi* are thought to require a stable chemical and physical condition in the breeding sites, which is generally not found in small water bodies (Deane, 1948). This species breeds preferentially in large, deep and clear water collections like lakes, swamps or large rivers (Deane, 1948; Rachou, 1958; Forattini, 1962; Rozendaal, 1992). Adequate larval conditions depend on depth of the water, temperature, pH, chemical stability and light/shade proportions (Rachou, 1958). A combination of shade and direct sunlight, with a certain amount of cover in the form of vertical vegetation is preferred, with a temperature of 20-28°C, and a pH of 6.5-7.3 (Giglioli, 1938; Rachou, 1958). Singer and Castro (2001) considered the forest margins to be the principal breeding sites for *An. darlingi* in the Amazon. Undisturbed forests rarely provide ideal breeding sites due to a high acidity of the water and an absence of partly shaded water bodies.

Black rivers with a pH below 5 and with little vegetation or plankton are unsuitable breeding sites, but Giglioli (1938) found how intense rainfall created adequate breeding conditions by diminishing the acidity of black water rivers and by creating clear water swamps in areas where the water bodies had a low pH or high salinity. Rozendaal (1987) collected *An. darlingi* larvae in water from at a temperature of 40°C, which contradicts the consideration that the breeding sites are always in cool waters (Deane, 1948). This has implications for the range of possible breeding sites for this species, especially when considering ecological changes due to for instance deforestation, dam construction or mining. Vittor et al. (2009) found that mosquito breeding sites with *An. darlingi* larvae have an average of 24% forest cover, compared with 41% for sites without *An. darlingi*. Further analysis of breeding-site characteristics identified seasonality, presence of algae, size of water body, presence of human populations, and the amount of forest and secondary growth as significant determinants of *An. darlingi* presence. Larvae are generally found around trunks, emergent plants and floating debris (Tadei et al., 1988; Hudson, 1984; Rozendaal, 1992; Achee et al., 2006), which seems to provide them with some amount of shadow and a stability of water condition in terms of water flow in this particular microhabitat.

Table 2.1 provides an overview of the various breeding sites of *An. darlingi*. When breeding in the large sites, *An. darlingi* larvae seem to prefer deeper areas, away from the edges. This is an important consideration as it causes difficulty for larval surveillance, which may lead to biased information on larval presence and population densities.

TABLE 2.1: *Categorical overview of breeding sites of An. darlingi*

BREEDING SITE CATEGORY	BREEDING SITE CHARACTERISTICS	REFERENCES
Lakes and Lagoons	in lake side vegetation and floating debris, in more exposed deeper parts of the lakes	Giglioli, 1938; Galvao et al., 1942; Deane et al., 1948, Rachoe, 1958; Forattini, 1962, Tadei et al. 1988, Brochero et al. 2005, Nagm et al. 2007
Large rivers	Semi-shaded, along the river edges, between floating debris and superficial vegetation, sections with slow water flow	Deane et al., 1948, Rachoe, 1958, Hudson, 1984; Forattini, 1962; Rozendaal, 1992, Manguin et al. 1996, Achee et al. 2006,
Small rivers	Slow flow rivers, creeks, residual pools in riverbeds during the dry season, irrigation canals	Giglioli, 1938; Hudson, 1984, Hayes and Charlwood, 1979; Tadei, 1988, Tadei et al. 1998
Flooded forest	Flooded forested riversides in the rainy season, swamps	Davis, 1931; Giglioli 1938; Galvao et al 1942; Deane et al. 1948; Tadei, 1988; Rozendaal, 1992
Small water collections	Ditches, drains, wells, rain pools, old, abandoned (mining) pits	Gigliolo 1938; Deane et al. 1948; Rachoe, 1948; Forattini 1962, Moreno et al. 2000
Rare breeding sites	Fully shaded water bodies, very small water collections, brackish or acid water, polluted water	Deane et al., 1948; Rachoe, 1958, Manguin et al. 1996, Singer and Castro 2001, Da Silva-Vasconcelos et al. 2002

VECTORIAL CAPACITY

According to Dean (1948) *An. darlingi* is capable of maintaining a relatively high transmission of malaria even when found in low densities. The mosquito species is considered a good vector, despite its infection rates tending to be low, even in high risk malaria areas. The re-emergence of malaria in eastern Peru is for instance attributed to the spread of *An. darlingi* into new areas, but the vector infection rate found was less than one percent (Schoeler et al., 2003; Flores-Mendoza et al., 2004). In studies in the high-malaria risk area Rondônia in Brazil by Tadei et al. (1988) and De Oliveira-Ferreira et al. (1990), an infection rate in *An. darlingi* of 0.48% (one positive out of 210 and 47 positive out of 9,838, respectively) was found. Gil et al. (2007) also found a low infection rate and considered that *An. darlingi*, which is the primary vector in that area, maintains malaria transmission by its high human biting rate and that transmission is supported through the high number of asymptomatic malaria cases in the human population. *Anopheles darlingi* has a relatively high susceptibility to *Plasmodium* infection when compared to other Amazonian species (Klein et al., 1991), and asymptomatic cases with very low parasitemias can be infective to *An. darlingi*, even if it is at a much lower rate than symptomatic cases (Alves et al., 2005). The recent discovery of sub-microcopic, but highly infectious, low-density gametocytes of *Plasmodium falciparum* may be an additional explanation for the relatively continuous infections in the Amazon (Schneider et al., 2007). Mosquito infection rate can be relatively high at times (De Arruda et al., 1986). Da Silva Vasconcelos et al. (2002) found 8.5% of over 700 *An. darlingi* collected in Roirama (Brazil) to be infected with a *Plasmodium* parasite. This rate would be comparable to infection rates found for instance for *An. gambiae* and *An. funestus* in Tanzania (11.1% and 6.2% respectively (Taylor, 1999)).

Gonotrophic cycle, longevity and age composition are considered important vector characteristics that are essential in determining the ability to transmit malaria. Based on the view that the gonotrophic cycle in other tropical anophelines lasts approximately three days (Gillies and Wilkes, 1965) Charlwood (1980) calculated a daily mortality of *An. darlingi* at Arapuaña, Brazil, of approximately 38%. He considered that only those females that had completed four or more cycles would be old enough to contain malaria sporozoites (Charlwood and Wilkes, 1979). Of 1,596 dissected females in his study, only seven had laid eggs four times or more, resulting in a proportion of females that could be potentially infective with malaria sporozoites of 0.4%. In reality the gonotrophic cycle of *An. darlingi* may be two rather than three days (Roberts et al., 1983), which means that the daily mortality at Arapuaña would actually be higher than estimated by Charlwood (approximately 51%).

Terzian and Stahler (1949) considered that the male-female composition of a mosquito population may influence feeding behavior, after a laboratory study with *Anopheles quadriannulatus* Theobald in which the virgin females never took a blood meal. This supports the hypothesis that take-off or host-seeking behavior is inhibited until a substance is transmitted to the female during mating. Recent studies on the behavior of several other anopheline species show that pre-gravid biting is common and sometimes needed before mating can occur (Charlwood et al., 2003; Takken et al., 2005a). Multiple blood meals within a single gonotrophic cycle appear to be less important in the life histories of Neotropical Nyssorhynchus species, including *An. darlingi*, compared to Afrotropical malaria vectors. One blood meal is usually enough for egg maturation (Lounibos et al., 1998; Monteiro de Barros and Honório, 2007).

Age of female mosquitoes is estimated from the parity rate of a population, i.e. the rate of females which have had a blood meal and have laid eggs (as determined using the ovarian tracheole dissection by Detinova (1962). The reliability of this method, especially in older females is subject to discussion (Hoc and Charlwood, 1990). Age composition of female *An. darlingi* collected in the field differs over collection time, collection seasons and locality. Higher variability in age composition may be found in the dry season, possibly related to availability of breeding sites and more stable climatic conditions (Monteiro de Barros et al., 2007). Hudson (1984) found that the parous rates of females he caught in Suriname from 19.00-22.00 h were 10-20% higher than those of females caught at other times of the night. The differences in age composition over season and time of day or night will need to be taken into account when collecting the mosquitoes for determination of infection rate (Tadei and Dutary-Thatcher, 2000). Age composition may also depend on the distance of breeding sites to nearby human settlements. More gravid females and less nulliparous females are found in villages close to breeding sites than in villages away from breeding sites (Ulloa et al., 1997).

Flight range of malaria vectors is an important determinant for their success in transmission over distance. Deane et al. (1948) found breeding sites as far as 1.5 to 2 km. from the sites of adult captures in the Amazon. In a study in Jardim das Copaíbas (Roirama, Brazil) Monteiro de Barros et al. (2007) found that 20.3% of *An. darlingi* would fly over 500 m., 4.6% would fly over 1000 m., and less than 1% further than 1200 m. In comparison, Achee et al. (2005) found recovery rates of 29%, 11.6%, 5.8% at distances of 0, 400 and 800 m., respectively, in Belize. Tadei (1988) estimates a possible flight range of 5 km. when going downwind, and Charlwood and Alecrim (1989) in a capture-recapture study in Brazil found two *An. darlingi* females at 7.2 km. from their release site, nine days after release. Recapture rate at the release site was 12-19%. This dispersal behavior is similar to what is found in African anopheline species (Takken et al., 1998).

Due to the variability of the vectorial capacity determinants and their dependence on external factors vectors, obtaining insight into local *An. darlingi* vectorial capacity is difficult and requires a sound methodology and understanding of the variabilities.

RELATIVE ABUNDANCE AND FEEDING BEHAVIOR

Anopheles darlingi is attracted to the human host (Gigliolo, 1938; Charlwood and Alecrim, 1989; Zimmerman et al., 2006). Deane et al. (1948) and Rachou (1958) compared the attraction of the mosquitoes to humans and other mammals and found that especially humans and large mammals, like horses, are preferred. Zimmerman et al. (2006) considered that host selection may in fact vary much depend on host availability and can differ significantly in different sites within one region. The tendency of *An. darlingi* to go indoors for biting was confirmed very early (Shannon, 1933; Galvao et al., 1942; Deane et al., 1948). A study in Belize showed an indoor-outdoor ratio of 1:0.6 for *An. darlingi* (Roberts et al., 2002). In Brazil a larger degree of variation in behavior was found by Deane et al. (1948), who discovered a stronger exophilic tendency in the interior of Brazil. Charlwood (1996) confirmed this with his study in Manaus, where he found *An. darlingi* to be primarily exophagic and exophilic, while in northern Brazil (towards the border with Guyana and Venezuela) it was more endophagic. Rozendaal (1989) captured 73% of the *An. darlingi* in the interior of Suriname outside of the houses, in the peridomestic areas. He discovered that, even when *An. darlingi* is anthropophilic and prospers in the presence of human blood, it is able to survive as a 'wild' population in much lower numbers on animal blood. This was also found in French Guiana by Pajot et al. (1978), and in Brazil by Deane et al. (1948) where *An. darlingi* was collected in uninhabited areas.

Elliot (1968) found that in periods of increased mosquito density the relative importance of outdoor biting (during times of human activity) declines. He offers two possible explanations, the first one being that in periods of highest density the females may have a 48 hr. gonotrophic cycle, causing them to lay eggs in the early evening and start feeding in the late night of the same night. The second explanation would be that high density of mosquitoes often coincides with periods of heavier rainfall and higher relative humidity. Relative humidity inside houses, which may well inhibit entry when it is low in the first hours after sunset, would rise sooner in the wet season as the house cools, which may result in higher numbers of entry. Either or both hypotheses could be true.

Hudson (1984) studied the resting time of mosquitoes before and after biting in Suriname and found at Aselikamp in June 1979 that the mean resting periods observed were 7.7 min. (range 1-35) for 52 unfed females, and 17.1 min. (range 2-41) for 10 blood fed females.

Biting cycles of *An. darlingi* seem to differ significantly between various regions of its distribution area, and even in localities not far apart. Some studies record unimodal (Elliot, 1972) and other bimodal biting rythms (Forattini, 1987; Tadei et al., 1988; Pajot et al., 1977; Léon et al., 2003). Da Silva-Vasconcelos et al. (2002) found no defined biting peak for *An. darlingi* in Boa Vista (Roraima, Brazil) where over 10% of the anopheline population was found to be *An. darlingi* and where *An. darlingi* had the highest *Plasmodium* infection rate and together with *An. albitarsis* was considered a primary vector for this area. Forattini (1987) found that the distribution of the 9,523 *An. darlingi* caught in southern Brazil at 1500 feet displayed a distinct bimodal distribution in the daily blood feeding periodicity with peaks at both dusk and dawn. Hudson (1984) discovered that biting cycles of *An. darlingi* at his two study sites (Aselikamp and Apoma Tapoe, Suriname) showed a single main peak, but the peak would occur one hour later at Aselikamp (22.00-23.00 hr.) than at Apoma Tapoe (21.00-22.00 hr.), with smaller secondary peaks at 18.00-19.00 and 05.00-06.00 hr. Pajot et al. (1977) found a trimodal cycle in nearby French Guiana, including both twilight periods and a clear nocturnal peak between 01.00 - 02.00 hr.. He also found that cycles of biting activity of parous and nulliparous females are similar, both inside the house and on the veranda. That different biting cycles of *An. darlingi* can be found in a single locality over the seasons was discovered by Léon et al. (2003) in St. Clara (Peru) where a unimodal cycle was found from August to December and a bimodal cycle from January to June. Vector control measures like IRS or the use of insecticide treaded nets (ITNs) can result in a change in biting behavior (Lourenço-de-Oliveira et al., 1989; Bustamante, 1959; Takken, 2002).

Lunar cycles do not appear to influence daily biting rhythms of most mosquito species, but larger numbers of mosquitoes can be collected during new moon (Guimarães et al., 2000). Voorham (2002) discarded the likelihood of mosquito density interfering with biting behavior after his study in the State of Amapa (Brazil). This is consistent with studies in for instance French Guiana (Pajot et al., 1977), but is not in line with results obtained in studies in Colombia and Brazil (Tadei et al., 1998). Voorham acknowledges that intra-population variation of biting activity can be as significant as inter-population variation, and states that plasticity in biting activity patterns can result in increased vectorial potential of mosquitoes and control strategies may have to be adjusted to account for differences in human-vector contact over time.

Mosquitoes may very well display a preference in their biting sites on their available or preferred host. Observations by De Jong and Knols (1996) on mosquito biting on humans revealed that many species have preferred biting sites, and that not all species share the same preferences. Selection of these sites may be related to several factors, depending on the mosquito species, including visual and chemical properties of the host. This was confirmed by the shown differential attractiveness of Kenyan men to the African malaria vector *Anopheles gambiae* Giles (Mukabana et al., 2002) and by another study that revealed that allomonal breath contributes to differential attractiveness of humans to the African malaria vector *An. gambiae* (Mukabana et al., 2004). In a study on the South American malaria vector *An. albimanus*, biting sites were recorded mostly from the head region, suggesting that this species responds mostly to human breath (Knols et al., 1994). Studies on biting site preferences of *An. darlingi* are necessary to provide information on cues that are important in the finding and selection of a host. This information could be applied in trapping and control activities (Hiwat et al., in press).

BIOLOGICAL VARIATION

The possibility that the mosquito species *An. darlingi* may consist of a species complex is subject of continued research. If true, it could have important implications for future malaria control schemes in Latin America. Charlwood (1996) found that *An. darlingi* mosquitoes from the Manaus area are more chromosomically diverse than mosquitoes towards the northern edge of the distribution area (Venezuela and Guyana). He also found that female wing size can vary between populations. Similarly, a study by Harbach et al. (1993) showed that the *An. darlingi* specimens found in Belize show variation in their hind tarsal markings at a more than incidental rate.

Rosa-Freitas et al. (1998) related iso-enzymatic, behavioral and mitochondrial DNA studies on Brazilian and other Latin-American populations and deducted that *An. darlingi* is a monotypic species. Mirabello and Conn (2006) studied the genetics of *An. darlingi* mosquitoes to determine whether there is a division in the gene pool between Central and South America and found no significant evidence for this. Conn et al. (2006) continued this research in an attempt to find a population bottleneck in *An. darlingi* due to possible pressure as a result of insecticide use. The bottleneck was not found but significant differentiation between locations north and south of the Amazon River were discovered, suggesting a degree of genetic isolation between them, which was attributed to isolation by distance. Continued studies by Mirabello et al. (2008) result in the conclusion that "all of the data confirm a deep divergence between Amazonia and southern Brazil (genotype 1), and Central America, Colombia, and Venezuela (genotype 2).", which indicate incipient speciation. Recent studies in Brazil and Colombia show that on a more local level speciation is less likely due to high levels of gene flow, although even on that level evidence for isolation by distance exists (Scarpassa and Conn, 2007; Gutiérrez et al., 2010).

ECOLOGICAL CHANGE

In the whole of South America ongoing developments result in changing environments: agriculture and industries, colonization of uninhabited areas by humans, construction of hydropower dams, and forestry and mining activities are some of the causes. The globally changing climate is another. Change of ecosystems can result in a change in availability of breeding sites for mosquitoes or a change of survival rate and reproduction (Giglioli, 1963; Takken et al., 2005b). This may affect the malaria transmission risk. According to Patz & Olson (2006), changing temperature trends, due to influences from global climate change and local land use practices, may alter malaria risk, due to 1) a shift in time needed for parasite development, 2) changing mosquito abundance and survivorship, 3) a change in gonotrophic cycle, and 4) a change in larval development and pupation rates. Non-sustainable forestry, resulting in large-scale deforestation, will have an effect on local temperature, and possibly on the availability of breeding sites. Vittor (2003) found that the biting rate of *An. darlingi* is positively related to the amount of deforested land, and further found that deforested sites had an *An. darlingi* biting rate that was more than 278 times higher than the rate determined for areas predominantly forested (Vittor et al., 2006). In accordance, Harris et al. (2006) considered that the growing malaria problem in the Bolivian Amazon (a four-fold increase between 1991 and 1998) was largely due to forest clearance, bringing human and vector populations into closer contact. Malaria outbreaks were predicted for Belem (Brazil) as a result of the continued expansion of the city into the surrounding forest in the 1990s, and the observed increase in the population sizes of *An. darlingi* in these locations (Povoa et al., 2003).

The construction of hydropower dams often came with special awareness of a possible increase of the malaria risk, which resulted in related studies. De Carvallo (1953) found a decline in *An. darlingi* densities and malaria transmission after the construction of the Lages dam in Brazil. This was attributed to the variations in water level, which destroyed the preferential breeding places of *An. darlingi*. Rozendaal (1992) found that, in contrast to the prediction by Van Thiel (1962), the hydropower scheme which created the Van Blommenstein Lake in 1971 in Suriname, did not cause a malaria problem. No *An. darlingi* were found in that area. He assumed that the non-shaded shores of the lake are unsuitable breeding habitats. With the completion of the (binational) Itaipu dam between Brazil and Paraguay and the maintenance of the breeding places for *An. darlingi* an increasing number of malaria cases, especially in the Upper Parana River, was expected. Falavigna-Guilherme et al. (2005) describe the occurrence of some *P. vivax* outbreaks after the completion of the dam, but believe that with the adoption of satisfactory preventive measures, including health educational and social actions, malaria can be controlled. Zeilhofer et al. (2007) found a positive relationship between the *An. darlingi* presence and increased proximity to forested areas near reservoirs, especially in bays protected from wind and wave action. Breeding site classification with satellite imaging together with entomological studies are proposed as a valuable tool for spatial modeling of *An. darlingi* habitats in hydropower reservoir areas (Zeilhofer et al., 2007).

One increasingly common human activity in South America is gold mining, especially in the forested areas of the Amazon. The relation between gold mines and malaria has been discussed often (Silbergeld et al., 2002). In fact, the re-emergence of *P. falciparum* malaria in Cuyuni-Mazaruni-Potaro in Guyana, after 28 years of absence of cases, was considered due to the 'gold rush' (Rambajan, 1988). In Mato Gross (Brazil) a positive correlation existed between amount of gold extraction and malaria incidence rate (Duarte and Fontes, 2002). Very early on it was recognized that in mining areas, old abandoned pits could be suitable as *Anopheles* breeding sites

(Giglioli, 1938) and this was recently re-established (Moreno et al., 2007). Clear and deep water bodies remain after sand and debris (resulting from the mining activity) are deposited on the bottom of the mining pits over time. Destruction of the surrounding forest may result in a less than optimal amount of shadow, but *An. darlingi* has shown tolerance to high water temperatures (Rozendaal, 1987) and an ability to adapt. Following a successful malaria intervention program in the interior of Suriname between 2005 and 2010, the only persistent malaria areas are associated with gold mines (H. Hiwat, unpublished results).

SURVEILLANCE AND CONTROL

Capturing *An. darlingi* is not easy. So far, human landing catches seem the 'golden standard' for collecting this vector. The generally disappointing results of various trapping devises when compared to human landing collections (see for instance Moreno et al. (2002), Brochero et al. (2005), Turrel et al.(2008), Dusfour et al.(2010), Hiwat et al. (in press)) may lie in the high degree of anthropophily which is often found in this species. This high degree of anthropophily could indicate that *An. darlingi* is attracted by very specific human-related cues, and is therefore less inclined to enter traps which fail to present these cues. Since human landing catches are costly and labor-intensive and may present a risk to the collector, alternative methods are needed. Further studies into the biting behavior and preferences of *An. darlingi* may be instrumental in the development of an efficient alternative collecting method which can be used in vector surveillance. Identifying the host-related cues which attract the vector can ultimately result in more target-specific control measures. This line of study and tool development is currently employed for *An. gambiae* s.s. surveillance and control (Verhulst et al., 2011).

Evidence exists that vector control measures can result in changing characteristics of the targeted vector (Rozendaal et al., 1989). Long-term DDT use resulted in a changed susceptibility of *An. darlingi* populations to the insecticide over time (Suarez et al., 1990). IRS and ITNs reduced intra-domiciliary vector densities in several species and variation in biting time after their introduction has been recorded (Santos et al., 1999; Pates and Curtis, 2005). Which vector control method to use at a certain location, depends very much on the characteristics of the vector and requires adequate baseline information and continuous monitoring to detect changes (Ferguson et al., 2010). ITNs for instance proved very successful against *An. darlingi* in southern Venezuela, where a reduction of 56% of malaria cases was recorded in local indigenous populations after the introduction of lambdacyhalotrin-treated hammock nets (Magris et al., 2007b). The use of the same ITNs in the Bolivian Amazon might be less successful because, as Harris et al. (2006) found, 48% of mosquito biting takes place between 19.00 and 21.00 h., when most people are not yet in the protective area of the net.

The mobility of larval mosquitoes is low compared to that of the adult forms, which is why larval control of vectors can be a powerful tool in malaria control (Killeen et al., 2002). Possible options are biological control with larvivorous fish or bacteria, or chemical control, for instance with oil. Whether larval control can be successful depends on the characteristics, especially the size, of breeding habitats (Curtis, 2006). *Anopheles darlingi* breeds in various habitats, smaller and larger, often covering wide areas (see above). In clearly defined breeding sites, like the stagnant water bodies remaining after the rainy season, limited areas of swamps or inundated forest floors, and possibly mining pits or other limited breeding sites derived from human activity, larval control with microbial larvicides could be effective. Bacterial control has been successful in Africa when directed at *An. gambiae* (Fillinger et al., 2003), but also in breeding sites in Peru and Equador (Kroeger et al., 1995). The difficulty of localizing the breeding sites where larval control of *An. darlingi* could be successful to reduce malaria incidence is yet another issue. The Amazon rainforest is still a sparsely inhabited area, and the logistics of locating and treating individual breeding sites may preclude control directed at the larval stage. Satellite imaging may prove a useful tool in this endeavor. Studies on this subject are currently undertaken (Zeilhofer et al., 2007).

CONCLUSIONS

Anopheles darlingi is widely distributed across South America, but the species is especially important as malaria vector in the Amazonian countries. Even though the natural infection rate and population densities of this vector are often low, its efficiency in malaria transmission, through high biting rates and a good susceptibility to *Plasmodium* infection, is high. *Anopheles darlingi* is mostly anthropophilic and shows a capacity to adapt to changing environmental situations. Local variability in determinants for the vectorial capacity of *An. darlingi* is high and many aspects determining this capacity, i.e. its relation to seasons and environmental conditions, its gonotrophic cycle and longevity, and its feeding behavior and biting preferences, are still unknown. This means that the establishment of an effective control strategy will require elaborate studies on the (local) vector situation. Also this behavioral plasticity makes it difficult to predict the impact of changes in ecological environment and in (macro) climate on the vector populations. Adaptation through natural selection is to be expected. This allows the vector to establish populations in areas where it previously did not exist or had been controlled. The behavioral variability of the vector, its adaptability, and our limited knowledge of it impede the establishment of effective control strategies. Increasing our knowledge of *An. darlingi*, therefore, is necessary.

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3

Evaluation of methods for sampling the malaria vector *Anopheles darlingi* in Suriname and the relation with its biting behavior

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ABSTRACT

The effectiveness of CO_2-baited and human-baited mosquito traps for the sampling of *Anopheles darlingi* was evaluated and compared with human landing collections (HLC) in Suriname. Biting preferences of this mosquito on a human host were studied and related to trapping data. Traps used were the CDC Miniature light trap, the BG Sentinel™ Mosquito Trap, the Mosquito Magnet ® Liberty Plus Mosquito Trap (MM-Plus), and a custom-designed trap. Carbon dioxide and humans protected by a bed net were used as bait in the studies. The number of *An. darlingi* collected was greater with HLC than with all other collection methods. *Anopheles darlingi* did not show a preference for protected humans over CO_2 bait. The BG Sentinel™ Mosquito Trap with CO_2 or human odor as bait and the MM-Plus proved the best alternative sampling tools for *An. darlingi*. The BG Sentinel™ Mosquito Trap with CO_2 or human odor as bait was also very efficient at collecting *Culex* spp.. In a field study on biting preferences of wild *An. darlingi*, the females showed directional biting behavior ($p<0.001$) with a majority of females (93.3%) biting the lower legs and feet when approaching a seated human host. Higher efficiency of the closer-to-the-ground collecting MM-Plus and BG Sentinel™ Mosquito Trap when compared to the other trapping methods may be a result of a possible preference of this mosquito species for low-level biting. It is concluded that odor-baited sampling systems can reliably collect *An. darlingi* but the odor bait needs to be improved, for instance by including host-specific volatiles, to match live human baits.

INTRODUCTION

In South America, *Anopheles darlingi* has been incriminated as a malaria vector since 1931 (Davis 1931, Deane 1948, Rachou 1958, Tadei et al. 1988) and is associated with severe malaria epidemics (Falavigna-Guilherme et al. 2005). Hudson (1984) and Rozendaal (1987) found *An. darlingi* to be the primary malaria vector in the interior of Suriname. Following the free distribution of long lasting impregnated bed nets throughout the interior of the country in 2006, Suriname experienced a significant decrease in the annual number of malaria cases. However, malaria still occurs in specific parts of the interior, especially in an increasing number of remote gold mining areas (data Ministry of Health Malaria Program Suriname). These mining areas have hardly been explored concerning key characteristics of malaria transmission such as mosquito population diversity and densities or human biting rates, and vector studies are urgently needed.

Sampling *An. darlingi* for epidemiological malaria studies is traditionally conducted by the use of human landing collections (HLC) (Turell et al., 2008; Rubio-Palis, 1996) because existing traps proved inefficient. The HLC has risks for the collectors due to exposure to potentially infected vectors and is thus less accepted on ethical grounds (Rubio-Palis and Curtis, 1992a). HLC is the most widely used method to estimate the human biting rate of malaria vectors, even though its reliability depends on dedication and personal attractiveness (Knols et al., 1995; Olanga et al., 2010) of the individual collectors. In addition, the method is costly and labor-intensive (Rubio-Palis and Curtis, 1992a). As the degree of anthropophily of *An. darlingi* varies considerably (De Oliveira-Ferreira et al., 1992; Charlwood, 1996; Zimmerman et al., 2006), the efficacy of HLC for this vector is unknown and adds a factor of uncertainty to the reliability of data on intensities of malaria transmission in South America. Finding an alternative method representing mosquito population structure and dynamics in a reliable and comparable way is a necessary but time-consuming and difficult enterprise, particularly when the target mosquitoes exhibit variation in biting habits. The ideal alternative method holds no transmission risks for the collector and is not influenced by the collector's ability or interaction. It is cost-effective and easy to use.

Studies have been conducted worldwide to compare different mosquito sampling methods and find alternatives for HLC to obtain accurate estimates of the population density of malaria vectors. Among these we find a variety of light traps (Faye et al., 1992; Rubio-Palis and Curtis, 1992a; Davis et al., 1995; Rubio-Palis, 1996; Mboera et al., 2000; Burkett et al., 2001);

and odor-baited entry traps (Duchemin et al., 2001; Schmied et al., 2008). Kline (2006) provides an overview of evaluations of mosquito traps, but few of these comparative studies have been conducted with *An. darlingi* and the efficacy of the evaluated sampling methods varies greatly depending on local malaria vectors and local situations. The CDC Miniature light trap (hereafter termed CDC trap) proved effective in the collection of indoor biting African anopheline mosquitoes (Garrett-Jones and Magayuka, 1975; Lines et al., 1991) and became the standard sampling tool for the collection of malaria mosquitoes in many areas of the world. The CDC trap is often used in combination with protected human bait (Costantini et al., 1998a), but the efficacy of this set-up depends on the feeding preference of local vectors, which may vary from zoophilic to anthropophilic and from exophilic to endophilic.

Kline (2002) demonstrated that propane-powered traps, using CO_2 as bait, collect large numbers of mosquitoes in Florida and are easy in use. The MM-Plus for instance, is a propane powered trap that has been evaluated under a variety of conditions and compared with light traps and HLC. Sithiprasasna et al. (2004) compared a number of traps to HLC. They found the performance of the MM-Plus in collecting large numbers of Thai *Anopheles* mosquitoes less effective than HLC, but among the best of the alternative methods tested.

The principle behind many mosquito traps is to combine a mosquito attractant (CO_2, human bait, or (human-derived) odors) with a suction mechanism that will trap the approaching mosquitoes in a holding chamber. Studies on biting behavior of antropophilic anophelines show that they are attracted to skin temperature, exhaled CO_2, skin humidity, body odors or a combination of these factors (De Jong and Knols, 1995; Costantini et al., 1996; Takken et al., 1997; Dekker et al., 1998; Mukabana et al., 2004). Knowledge about the biting preferences of the target mosquitoes could be used to fine-tune sampling methods for the vector. The biting preferences of *An. darlingi* females were never determined and may vary locally (Charlwood, 1996). *Anopheles darlingi*, like *An. gambiae* s.s., may have preferential biting locations on the human body guided by odors or other factors (Dekker et al., 1998; Braks and Takken, 1999; Olanga et al., 2010).

The first goal of the present study was to evaluate different sampling methods for their efficacy in collecting *An. darlingi* and to assess their ability to replace the conventional HLC as a tool to determine the malaria transmission risk in endemic areas of Suriname. Different trap models were selected, including the CDC trap, the BG Sentinel™ Mosquito trap, the MM-Plus trap and a custom-designed mosquito trap. Traps were baited with CO_2 or with the emanations of a human protected by a bed net. The second goal was to investigate whether *An. darlingi* females, under field conditions, show non-random biting behavior on human hosts. Directional biting may indicate certain preferences, which could explain or influence the efficiency of the evaluated trapping methods.

MATERIALS AND METHODS

STUDY SITE The study was conducted between April and June 2009 in Palumeu (N 03°21' W 55° 26'), an Amerindian village along the Tapanahony River in the Interior of Suriname (Figure 3.1). The villagers spent their time, when not away from their homes, mostly underneath their houses, which are built on stilts. The village has a relatively high density of *An. darlingi* mosquitoes (compared to other sites in Suriname), with peak biting between 01:00 and 02:00 hr. (H.Hiwat, unpublished data). Domestic animals in the village consist of dogs and chickens. Occasionally a monkey or forest bird is kept as pet.

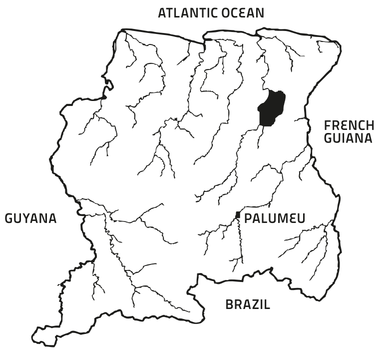

FIGURE 3.1: *Map of Suriname with study location (Palumeu village).*

MOSQUITO TRAPS During a first round of evaluation (April 14-26, 2009) five trapping methods and HLC were compared in a 6x6 Latin square which was performed twice. During a second round (May 26 - June 7, 2009) one method tested in the previous round was excluded from further testing and replaced by another collection method. This new method and the remaining methods (some in adapted form, see trap details below) were tested in a 6x6 Latin square, again performed twice. Testing of the traps was done between 21:00 and 03:00 hr. The trap evaluations were done outdoors, mostly underneath the houses in which the local people were sleeping. Locations of houses with traps were at least 50 m apart to prevent interference. Outdoor temperature and relative humidity were recorded for each hour of collection with a digital temperature and humidity measuring device.

The day after each nightly collection, the catches of the night before were, for each collection method, sorted and the mosquitoes were counted. *Culex* spp. and *Aedes* spp. mosquitoes were identified to genus level. *Anopheles* mosquitoes were sexed and identified to species level. Identification was done using the keys of Faran and Linthicum (1981), Linthicum (1988), and Gorham et al. (1967),

The trapping methods used were:

- *CDC Miniature light trap* (CDC trap) baited with carbon dioxide (500 cc./min.) provided from a gas cylinder (see below). Carbon dioxide was led to the trap by a polyethylene hose of 5 mm. diameter, and released at 1 cm. from the trap entrance. The CDC trap was placed with the trap entrance at a height of approximately 150 cm.. During the first evaluation round, the CDC trap was used with light. During the second round the trap was operated without light because of the large number of insects of no interest in the trap. By removing the light most of this bulk of non-target insects will not enter, which makes sorting and counting of the mosquitoes easier. Leaving the light off could actually improve the collection by the CDC trap (Carestia and Savage 1967, Takken and Kline, 1989).
- *CDC Miniature light trap* (CDC trap) baited with a protected person. The CDC trap was placed at approximately 150 cm. height next to a mosquito net baited with a person (local male, age 21) sleeping in a hammock. During the first round of evaluation the light bulb of the CDC trap was switched on; during the second round the trap was operated without light, for reasons explained for the CDC trap baited with carbon dioxide.
- *BG Sentinel™ Mosquito Trap* (BG Sentinel), baited with carbon dioxide (500 cc./min.) from a gas cylinder, released near the trap entrance from a 5 mm. polyethylene hose. The BG Sentinel is normally provided with BG Lure, containing components of human skin odor, as a mosquito attractant. This bait was not used in the current setup, in order to make the BG Sentinel comparable to the other trapping methods which were used in combination with only carbon dioxide or a protected human as attractant.

- *BG Sentinel™ Mosquito Trap* (BG Sentinel) baited with a protected person (as with CDC trap). In this experiment, the BG lure was also not used as bait, as natural human odor from a person sleeping under a bed net served as bait. Two BG Sentinel traps were placed at ground level next to a mosquito net with a person sleeping under it: one near the head and one near the feet (local male, age 29) sleeping in a hammock. The mosquitoes collected with both BG Sentinels were summed and a mean per trap was calculated to account for differential attractiveness of different body parts. This collection method was tested during the second round only.
- *Mosquito Magnet® Liberty Plus* (MM-Plus). The MM-Plus converts propane into CO_2 (flow 500 cc./min.), which is used as bait (Kline, 2002). The MM-Plus is generally provided with 1-octen-3-ol (octenol) as bait, but in this experiment the octenol was not used and CO_2 was the only chemical stimulus. For technical reasons (connecting parts for the cylinder damaged) the MM-Plus could not be tested in the first round of evaluation and was only tested during the second round.
- *Mosquito net trap* (MNT). A MNT designed by the authors and made of cotton cloth was used. The trap section at the bottom of the mosquito net was made of gauze. The MNT was baited with a person (local male, age 29) sleeping in a hammock. This method proved very inefficient in the first round of evaluation and was replaced by the BG Sentinel baited with a protected person in the second round.
- *Human Landing Collection* (HLC): The HLC to collect *An. darlingi* was performed by eight persons, working as a pair in different combinations. Mosquitoes landing on their exposed lower legs were aspirated. The number of mosquitoes that was obtained with HLC was divided by two to obtain the number of biting female mosquitoes per person, thereby decreasing the influence of differences in personal attractiveness.

The CO_2 used in combination with the CDC trap and the BG sentinel was obtained from a gas cylinder. Using a pressure regulator (Concoa, model CGA 320) and a flow meter (Brooks, model 1355) a constant flow of approximately 500 cc/min CO_2 was obtained. This was similar to the CO_2 production of the propane driven MM-Plus trap, which is calibrated to produce a 500 cc/min flow.

BITING LOCATION The experimental design used to establish biting preferences was an 'in-the-field' design similar to the method used by Self et al. (1969). A human bait (one of the researchers) sat outdoors on a stool wearing only shorts while a second person walked around him with a headlight to check exposed body surfaces for probing or biting *Anopheles* females. At regular intervals the human bait would turn 180 degrees to account for position effects. For each *Anopheles* female discovered on the bait, the biting location was determined and the mosquito was collected and stored for identification. The biting tests took place between 21:15 and 22:00 hr. and were repeated on 8 nights between 29 May and June 7 2009. Temperature and relative humidity were recorded at onset of the collections with a handheld temperature and humidity meter, and considered stabile for the 45 min. test period.

STATISTICAL ANALYSIS Numbers of *An. darlingi* were Ln(x+1) transformed and imported in a General Linear Model (SPSS version 17) to locate differences in the total number of *An. darlingi* mosquitoes taken between collection methods. Tukey's HSD (post-hoc) tests were used to find differences per collection method. A comparison was made between the two Latin square designs within each period to determine if the data per period could be summed, and a comparison was made between the two evaluation periods. Tukey's HSD (post-hoc) tests were also used to determine the influence of temperature, relative humidity, and locations. Ranking of the data and Spearman's rho tests were used to determine the correlation between alternative collection methods and the conventional HLC over time.

To determine biting preferences in the biting study we compared the observed and expected number of bites per body part in relation to their relative skin surface areas (Mitchell and Wyndham 1969) with a Chi-square test and based on the null hypothesis that mosquito bites are distributed in proportion to the exposed skin surface areas per body part. Average temperature and humidity over the testing days were determined.

RESULTS

TRAP EVALUATION Combined over the two trap evaluation periods a total of 4,027 mosquitoes were collected, consisting of 790 *An. darlingi*, 10 unidentified *Anopheles* spp., 3,222 *Culex* spp., and 5 *Aedes* spp. All anophelines were females, except for one. No significant differences in the total number of collected *An. darlingi* mosquitoes were found per collection method within the evaluation rounds (all $P>0.05$), which allowed for pooling of the results within the evaluation rounds.

TRAP EVALUATION ROUND 1 Table 3.1 shows the number of *An. darlingi* collected and the number of collection days per method. The average temperature and average relative humidity were 24.8°C and 74.5% for the first Latin square and 25.3°C and 69.8% for the second Latin square respectively. Significant differences in total number of collected *An. darlingi* mosquitoes were found only between the HLC and the three other collecting methods (for all three combinations: $P<0.001$). Between CDC trap + protected person, CDC trap + CO_2 and BG Sentinel + CO_2 no significant differences in the number of *An. darlingi* collected were found (all combinations: $P>0.05$).

TABLE 3.1: *Mean number +SEM of An. darlingi and Culex spp. mosquitoes and collection days per collection method in evaluation round 1.*

COLLECTION METHOD	NO. OF DAYS	MEAN NO. OF AN. DARLINGI	MEAN NO. OF CULEX SPP.
HLC (per person)	11	7.82 ± 8.42a	46.59 ± 23.14a
CDC trap* + protected person	10	0.90 ± 1.26b	6.70 ± 9.36b
CDC trap* + CO_2	9	1.11 ± 1.36b	5.44 ± 5.43b
MM Plus	-	-	-
BG Sentinel + CO_2	9	2.56 ± 4.00b	46.00 ± 35.26a
MNT	11	0.00b	0.00c

*Means within a column followed by the same letter are not significantly different (P>0.05); * , with light*

TRAP EVALUATION ROUND 2 The mean number of female *An. darlingi* and *Culex* spp. collected and the number of collection days per method is shown in Table 3.2. The average temperature and average relative humidity were 24.5°C and 88.3% for the first Latin square and 24.3°C and 91.8% for the second Latin square respectively. Significant differences in total number of collected *An. darlingi* mosquitoes were found between the HLC and all other collection methods (all combinations $p<0.05$). The BG Sentinel + CO_2 and the BG Sentinel (2) + protected person each collected significantly more *An. darlingi* than either CDC traps ($P<0.05$). The number of *An. darlingi* collected in the two BG Sentinel trap setups (with CO_2 or a protected person) was not significantly different ($p>0.05$). The number of *An. darlingi* collected in the two CDC trap setups was not significantly different ($p>0.05$). By ranking the data and using Spearman's rho test, we found that the total number of *An. darlingi* mosquitoes collected per day with the alternative methods showed no correlation with the total number of *An. darlingi* mosquitoes collected per day using the HLC. This was found for the CDC + CO_2 traps (both periods), the CDC trap + protected person (both periods), the BG Sentinel + CO_2 (both periods), the BG Sentinel (2) + protected person as well as the MM-Plus. In other words, none of the traps compared to HLC when monitoring population dynamics over time. Temperature, relative humidity and location were not influencing the results obtained in either of the study periods (all P>0.05).

Of the mosquitoes found besides the anophelines, some were *Aedes* spp. but most turned out to be *Culex* spp. (Tables 3.1 and 3.2). The BG Sentinel traps, both with CO_2 and with a protected person as bait, collected large numbers of *Culex* spp. mosquitoes, comparable to the numbers collected by HLC.

TABLE 3.2: *Mean number +SEM of An. darlingi and Culex spp. mosquitoes and collection days per collection method in evaluation round 2.*

COLLECTION METHOD	NO. OF DAYS	MEAN NO. OF AN. DARLINGI	MEAN NO. OF CULEX SPP.
HLC (per person)	12	15.08 ± 10.60a	22.71 ± 15.01a
CDC trap* + protected person	12	1.33 ± 2.06b	8.75 ± 4.83ab
CDC trap* + CO_2	11	0.73 ± 0.65b	10.82 ± 8.04ab
MM Plus	12	3.42 ± 4.85bc	3.58 ± 3.23b
BG Sentinel + CO_2	12	6.50 ± 8.27c	39.33 ± 28.33a
BG Sentinel(2) + protected person	10	6.00 ± 6.09c	38.50 ± 42.92a

*Means within a column followed by the same letter are not significantly different (P>0.05); * , without light*

BITING STUDY In the biting behavior study the biting location of 105 *An. darlingi* females was determined. The expected and observed distributions of the biting sites in relation to skin surface areas are shown in Table 3.3. Figure 3.2 shows the location of the individual bites. The mean temperature during the tests for the eight test days was 24.3°C and the mean relative humidity 81%.

The observed distribution of biting sites differed significantly (p<0.001) from the expected distribution in relation to the skin surface area. A total of 93.3% (98 out of 105) of the bites were located on the legs and feet. More than half of the total number of bites were located on the feet (56 out of 105).

DISCUSSION

None of the alternative sampling methods tested in this study collected as many *An. darlingi* mosquitoes as the HLC, but some showed potential as a surveillance trap for this malaria vector species. Data from the second

TABLE 3.3: *Expected and observed distribution of Anopheles darlingi bites (N=105) on the various body parts of the human host in relation to the skin surface area.*

	HEAD[A]	TRUNK	ARMS	LOWER BODY			
				TOTAL	UPPER LEGS	LOWER LEGS	FEET
Skin surface (% of total)[B]	7	35	19	39	19	13	7
Expected nr of bites	7	37	20	41	20	14	7
Received nr of bites	0	6	1	98	11	31	56

[A] *head includes neck region;* [B] *Mitchell and Wyndham (1969)*

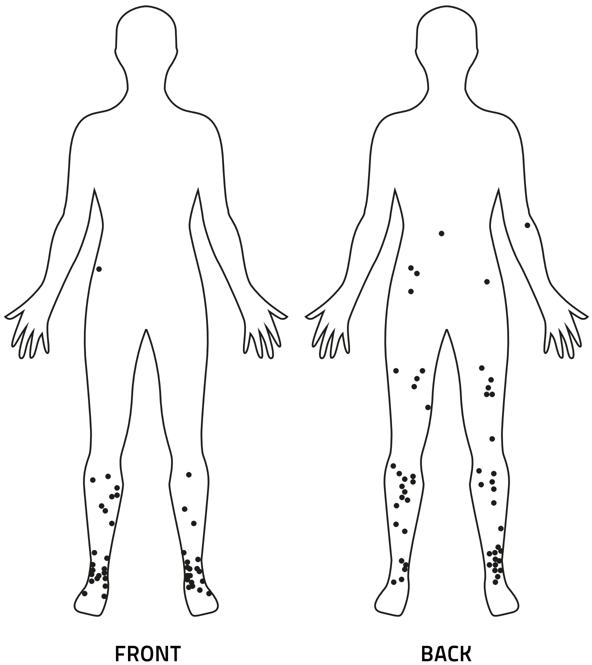

FIGURE 3.2: *Distribution of observed biting sites of Anopheles darlingi (N=105) on the human body.*

evaluation period suggested that when the objective of collection would be to collect as many *An. darlingi* mosquitoes as possible the BG Sentinel baited with CO_2 or a protected person is a better alternative method than the CDC trap baited with CO_2 or a protected person and was comparable to the MM-Plus. Both the BG Sentinel and the MM-Plus need much less manpower, and are therefore less expensive methods, than the HLC. As CO_2 can be difficult to obtain and transport in the field, the BG Sentinel baited with a protected person or the MM-Plus (if propane is available) may be the most workable alternative. Recently, a cheap and effective method for the production of CO_2 has been developed, which does not rely on gas cylinders, but produces CO_2 locally from a sugar-yeast solution (Smallegange et al. 2010). Using this method in combination with the BG Sentinel may be preferable. It allows the traps to be operated in the absence of a human host. Addition of human odor components to the CO_2 –bait could possibly increase the catch, depending on the mosquito preferences.

For both the CDC trap and the BG Sentinel trap, we found no significant difference in *An. darlingi* numbers between CO_2-baited or human-baited traps. This suggests that a person (with natural host emanations) does not provide an added attractive effect over CO_2 alone. This implies that CO_2 is the main host attractant for *An. darlingi* in Palumeu and that other hosts, exhaling equal amounts of CO_2, could be as attractive as humans to the mosquitoes. The degree of anthropophily of *An. darlingi* may thus be low, but a thorough evaluation with other host species remains to be executed. Given the recent discovery of alternative odor baits for *An. gambiae* (Okumu et al., 2010; Olanga et al., 2010), it is possible that simular studies on odor baits for *An. darlingi* can significantly improve the catch of *An. darlingi*, compared to trapping with CO_2 alone.

The traps with CO_2 or with a protected person as bait yielded equal amounts of *Culex* spp. mosquitoes, but contrary to *An. darlingi* collections, the yield of the traps (except for the MM-Plus) was comparable to the HLC. In other words, both the CDC traps and the BG Sentinel traps can collect as many *Culex* spp. as HLC when CO_2 or a protected person is used as bait. The BG sentinel trap collected significantly more than the MM-Plus, which makes it a better option as sampling tool for these mosquito species.

None of the tested methods proved a good alternative to the HLC to monitor the population dynamics (especially density over time and feeding behavior) of *An. darlingi*. Studies conducted elsewhere with other *Anopheles* species indicate that CDC traps baited with a protected person may have the potential to provide population dynamics data which are comparable to HLC (Davis et al., 1995; Mathenge et al., 2004; Sithiprasasna et al., 2004; Sadanadane et al., 2004).

The sampling methods evaluated in the second period of the present studies showed differences in efficiency. The BG Sentinel baited with CO_2 or with a protected person collected significantly more *An. darlingi* mosquitoes than the two CDC traps (without light) and as many as the MM-Plus. This trend in mosquito yield was also observed during the first evaluation round, but the differences in number of *An. darlingi* mosquitoes collected proved not significant. This may have been due to the lower number of trapping nights for the BG Sentinel trap due to technical difficulties.

Removal of the light from the CDC light trap eliminated most of the unwanted insects from the collections and at the same time made the method more comparable to the other methods with CO_2 or a protected person as bait (Carestia and Savage 1967, Takken and Kline 1989). Whether or not the light would have had an effect on *An. darlingi* catches is questionable. A similar study with the mostly anthropophilic *An. gambiae* s.l. in West Africa for instance showed that light increased collections indoors but not outdoors (Costantini et al. 1998a). *Anopheles darlingi* is in fact more attracted to black light (UV-light) than to white light (Laubach et al. 2001). Using the baited CDC trap in combination with a black light may improve its performance.

An advantage of the BG Sentinel over the MM-Plus is its compactness. A disadvantage of the BG Sentinel is its constant need of an energy source (batteries or electric grid). This applies also for the CDC trap. The MM-Plus is difficult to transport, but can last up to three weeks without renewal of its propane supply or batteries.

Both the BG Sentinel trap and the MM-Plus trap collect mosquitoes closer to the ground than the CDC light trap. They thus select for mosquitoes flying at ground-level at the time of capture. In the biting preference study, a significant deviation from the expected distribution of bites was found, with almost all of the bites occurring on the leg and foot regions. This was thus, since the feet were down, at ground-level. Whether the higher number of *An. darlingi* mosquitoes collected by the BG Sentinel traps when compared to the CDC traps is related to this biting behavior needs further study.

The biting behavior of *An. darlingi* compares to that of *An. gambiae* s.s. in preference for biting the foot regions. De Jong and Knols (1995) studied the biting preferences of *Anopheles atroparvus* Van Thiel and *An. gambiae* s.s. and found that the anthropophilic *An. gambiae* s.s. preferred to bite the foot regions and the more opportunistic *An. atroparvus* preferred biting the head region. The selection of biting sites of these two mosquito species correlated with particular combinations of skin temperature and eccrine sweat gland densities on the human skin. Other factors influencing their biting site selection were the presence of CO_2 for *An. atroparvus* and the presence of skin bacteria for *An. gambiae* s.s.. Similar to *An. atroparvus*, the Central American malaria vector *An. albimanus* prefers the head region above other parts of the human body (Knols et al., 1994). Dekker et al. (1998) compared the preferences of the anthropophilic *An. gambiae* s.s. with those of *Anopheles arabiensis* Patton (an opportunistic species), and *An. quadriannulatus*, a zoophilic species. The results showed that *An. gambiae* s.s is attracted to the feet, but that the females may also base their preference on factors like the position of body parts relative to the ground (preference for ground-level biting), or the convection currents created by body heat, which lead them towards these body parts. Both *An. darlingi* and *An. gambiae* s.s. bite at the lower extremities of a human host, and both seem to be attracted to foot odors and humidity, or find their way across the host along convection currents. They may even use a combination of these traits to reach the preferred biting site. Considering our previous discussion on the degree of anthropophily of *An. darlingi*, biting of lower legs and feet appears to be a short-range preference of the vector or may be guided by a preference for ground level biting. For instance, Braack et al. (1994) found, in a comparable set-up, that ground-level biting *An. arabiensis* would not shift to higher body parts if the feet were covered. For this species ground-level biting seems a behavioral trait which is independent of the availability of host body parts. Additional testing using different body positions and different persons (to eliminate individual differences in attractiveness (Khan et al., 1965; Braks and Takken, 1999; Dekker et al., 1998) or using a dual-port olfactometer will provide additional information on the host-related cues that attract *An. darlingi*. Knowing what drives its preference makes it possible to ultimately develop specialized traps needed for entomological surveillance and evaluation systems. Also, evaluating the traps at different heights may reveal the impact of height on trap efficiency.

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4

Studies with carbon dioxide baited traps as alternative for human landing collections of *Anopheles aquasalis* in Suriname

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ABSTRACT

Three types of mosquito traps, i.e. CDC Miniature Light Trap (without light), the BG Sentinel™ Mosquito Trap and the Mosquito Magnet ® Liberty Plus Mosquito Trap, all baited with carbon dioxide only, were compared to human landing collections in their efficiency in collecting *Anopheles (Nyssorhynchus) aquasalis* mosquitoes. From a total of 13,549 mosquitoes collected 1019 (7.52%) were *An. aquasalis*. The majority of *An. aquasalis* (83.8%) were collected by the human landing collections. None of the traps correlated with human landing collection in the number of *An. aquasalis* captured over time. The high efficiency of human landing collections suggests (local) anthropophily of this malaria vector. Carbon dioxide was insufficiently attractive as stand-alone bait. Alternative traps using carbon dioxide in combination with host specific cues may provide better results. The BG Sentinel™ Mosquito Trap proved very efficient in collecting *Culex* mosquitoes.

INTRODUCTION

Anopheles (Nyssorhynchus) aquasalis is a Neotropical coastal mosquito. It is a very common mosquito in Suriname, often found in high densities in the coastal area. It is a suspected vector in some coastal malaria outbreaks of the past, for instance in the village of Galibi in 1972 (Rozendaal 1990). *Anopheles aquasalis* is an important malaria vector in Brazil (Deane, 1948; Flores-Mendoza & Lourenço-de-Oliveira, 1996a; Povoa et al., 2003) and a proven or potential vector in neighbouring countries (Rozendaal, 1987; Laubach et al., 2001). The species shows diverse biting behaviour with local anthropophilic and endophilic biting patterns (Deane et al., 1948; Ferreira, 1964; Flores-Mendoza et al., 1996). It is thought to be an opportunistic and exophilic species in Suriname, biting both domestic animals and humans. *Anopheles aquasalis* can be collected using human landing collections (HLC), but this method leaves the collector exposed to potentially malaria-infected mosquitoes and is therefore becoming less accepted on ethical grounds. In addition, collecting *An. aquasalis* with HLC in an environment with high densities of other (*Culex*) mosquito species, as is often the case in Suriname, is very uncomfortable.

Many alternative methods for collecting mosquitoes have been evaluated in order to find a suitable alternative for the HLC (Rubio-Palis, 1996; Kline, 2002; Mboera et al., 2000; Laubach et al., 2001). Very few of these studies were conducted with *An. aquasalis*. Light traps are promising candidates to replace HLC in epidemiological monitoring of malaria vectors, especially when used in combination with human bait (Davis et al., 1995; Mbogo et al., 1993; Costantini et al., 1998a). The limitation of light traps found for afrotropical vectors is that they do not represent the true man-biting mosquito population, which reduces their reliability in the estimation of the entomological inoculation rate (Mboera, 2005). Rubio-Palis (1996) established that *An. aquasalis* is attracted to UV light traps used in combination with CO_2, but far less then to human bait. An alternative for mosquito collections with HLC are carbon dioxide baited traps. The attractiveness of CO_2 to mosquitoes was discovered by Reeves (1953) and was later confirmed for several anopheline species (Costantini et al., 1996; Gillies, 1980; Service, 1993). Healy and Copland (1995) for instance found that a carbon dioxide concentration level of 0.01% above background activated *Anopheles gambiae* females. Mosquitoes are collected in higher numbers with increasing levels of carbon dioxide (either from cylinders or from dry ice), until a level of about 500 ml./min.. Generally no further increase of captures is found above this level (Service, 1993).

The goal of this study was to evaluate the efficiency of different trapping devices baited only with carbon dioxide in collecting *An. aquasalis* mosquitoes and to assess the potency of these traps to replace HLC in entomological surveys or long-term monitoring. We used three types of traps to compare with the HLC; the CDC Miniature light trap (without light) at a level of one meter above the ground, the BG Sentinel™ Mosquito Trap (BG Sentinel) at ground level and the Mosquito Magnet ® Liberty Plus also at ground level.

MATERIAL AND METHODS

Study design - Between May 5 and May 21, 2009, three carbon dioxide baited mosquito traps were tested for their efficiency in collecting *An. aquasalis* mosquitoes and compared to the conventional HLC. The collections took place along the roadside in a residential area in northern Paramaribo, close to the Atlantic coast. Nearby ecosystems consisted mostly of coastal marshes. *Anopheles aquasalis* has a biting peak in northern Paramaribo between 19:00 and 19:30 hr.. Before and after this biting peak almost no *An. aquasalis* can be found. Therefore, we collected mosquitoes between 18:45 and 19:45 hr. at four locations, separated by at least 50 meter to prevent interference, and according to a rotation scheme based on a 4x4 Latin square design. This was repeated three times (12 days) separated by a three day time interval. Temperature and humidity levels were measured during the collecting times. Every next day the collections of the night before were sorted and counted per collection method. *Anopheles* mosquitoes were sexed and identified to species level, *Culex* spp. and *Aedes* spp. mosquitoes were also collected and identified to genus level.

Traps - The three carbon dioxide baited traps were:

- *Mosquito Magnet ® Liberty Plus* (MM-Plus); the MM-Plus Plus converts propane into CO_2 (flow 500 cc./min.), which is used as bait. The MM-Plus is generally provided with 1-octen-3-ol (octenol) as bait. In this experiment the octenol was not used, because it was the objective of the study to compare the efficiency of the collection methods baited only with CO_2.
- *CDC Miniature light trap* (CDC trap); the CDC trap was placed at approximately 1.0 meter height. Because of the high amount of insects of no importance attracted by the light of this trap, this light was removed. The CO_2 was obtained from a gas cylinder. Using a pressure regulator (Concoa, model CGA 320) and a flow meter (Brooks, model 1355) a constant flow of approximately 500 cc./min. CO_2 (same as MM-

Plus) was obtained. The CO_2 was released through a tube near the trap opening.
- *BG Sentinel™ Mosquito Trap* (BG Sentinel); the BG Sentinel was baited with a flow of CO_2 of approximately 500 cc./min., obtained and released as described for the CDC trap. BG Lure, a mosquito attractant generally provided with these traps, was not used.

The trap collections were compared with HLC. The HLC were done by four collectors, working in alternating shifts of 2 persons. When many more mosquitoes landed than the collectors were able to catch, the amount of exposed skin was reduced. From the resulting number of mosquitoes a mean number of mosquitoes collected per person was calculated and compared with the traps.

Statistical analysis - Anopheles aquasalis numbers were Ln(x+1) transformed and imported in a General Linear Model (SPSS version 17) to locate differences in total collected *An. aquasalis* mosquitoes between collection methods. Tukey's HSD (post-hoc) tests were used to find differences between and within periods per collection method. Temperature and relative humidity were taken into account as explanatory factors for differences in mosquito collections. Pearson's correlations were calculated to define possible dependence of the alternative methods in relation to HLC in the number of mosquitoes collected over time.

RESULTS

A total of 13,549 mosquitoes (all females) were collected, of which 7.52% were *An. aquasalis*, 91.11% *Culex* spp. (the coastal *Culex* population consists for a large part of *Culex quinquefasciatus* Say; H. Hiwat unpublished observations) and 0.03% *Aedes* spp.. For five collections the data of different collection methods were not usable due to technical or human related problems (nights 3, 6 and 11 for the MM-Plus and nights 4 and 10 for the BG Sentinel). No significant differences in total number of *An. aquasalis* mosquitoes were found per collection method between periods of collection ($P>0.05$), which allowed for summing of the results for all periods per collection method.

Overall (adjusting for missing data) the BG Sentinel collected 52.7% of all mosquitoes, the CDC light trap 26.7%, the MM-Plus 7.4% and the HLC (per person) 13.2%. Of the *An. aquasalis* mosquitoes the BG Sentinel collected 5.9%, the CDC light trap 6.4%, the MM-Plus 15.5% and the HLC (per person)

72.1% (note: the overall HLC percentage of *An. aquasalis*, not corrected with a mean-per-person calculation, was 83.8%). In Table 4.1 the mean number of collected mosquitoes per collection method and per day is shown. The HLC collected many more *An. aquasalis* per day then any of the other methods (P<0.05). Both the CDC trap and the BG Sentinel were effective in collecting large numbers of *Culex* mosquitoes.

TABLE 4.1: *Mean number of An. aquasalis and Culex spp. mosquitoes per collection method per day.*

	COLLECTION DAYS	NR. OF AN. AQUASALIS	NR. OF CULEX MOSQUITOES
HLC (per person)	12	35.58±19.84a	88.71±29.17a
BG Sentinel	10	3.50±3.63b	626.40±287.72b
CDC Trap	12	3.17±4.63b	263.00±170.28a
MM-Plus	9	10.22±14.48b	88.44±45.04a

[ab] results with the same letter do not differ significantly (p>0.05)

Significant differences in total collected *An. aquasalis* mosquitoes were found between the HLC and all three other collection methods (for all three combinations: P<0.001). Between BG Sentinel, CDC trap and MM-Plus no significant differences in total number of collected *An. aquasalis* mosquitoes were found (for all combinations: P>0.05).

Temperature, relative humidity and location were not influencing the obtained results (P>0.05). The number of *An. aquasalis* mosquitoes collected per day with the BG Sentinel, the CDC light trap as well as with the MM-Plus is not correlated with the number of *An. aquasalis* mosquitoes collected per day by the HLC (Pearson's correlation coefficient: BG Sentinel 0.27, MM-Plus 0.30, CDC trap 0.64; Figure 4.1).

DISCUSSION

From the alternative trapping devices tested in this study, no method collected as many *An. aquasalis* mosquitoes as HLC. The human collectors, although collecting all species, may have had (an unconscious) preference for *An. aquasalis* knowing its importance as a malaria vector. Also the differential attractiveness of carbon dioxide for the different mosquito species collected in the traps could be an explaining factor, but neither would account for the large differences found.

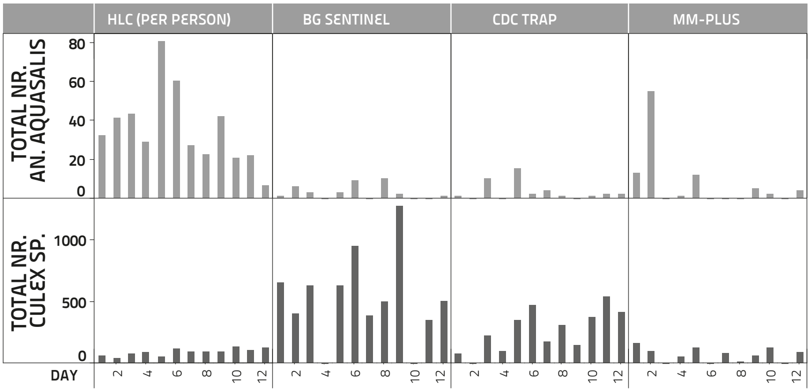

FIGURE 4.1: *Total number of mosquitoes, total number of An. aquasalis and total number of Culex sp. per collection method per day.*

The difference between HLC and the other three collecting methods, and the lack of difference between the alternatives indicate that, rather than a difference in efficiency of traps (or an effect of the height level of the trap), CO_2 is not a sufficiently attractive trapping bait for *An. aquasalis*. At least it is not in northern Paramaribo, where *An. aquasalis* seems more anthropophilic than expected and guided by more complex host specific cues than CO_2 alone.

Anopheles aquasalis is known for its local variations in blood-host preferences, and is zoophilic or opportunistic in large parts of its distribution area. In north-eastern Brazil it is thought to be anthropophilic (Flores-Mendoza et al., 1996) but its role as malaria vector in the Guyanas is as yet unclear. Malaria infected specimens were found in Surinames neighbouring country Guyana (Laubach et al., 2001).

What attracts *An. aquasalis* if it is not carbon dioxide? If the local population of the species is indeed anthropophilic, human emanations may play an important role. Studies on the role of human odours in attracting anthropophilic malaria vectors are ongoing, especially with African malaria vectors (Costantini et al., 1998b; Knols, 1997; Schmied et al., 2008; Jawara et al., 2009). The results show that host specific odours play an important role in the host finding behaviour of the vectors and that odour baited traps, especially in combination with CO_2 may have a high potential as effective methods for entomological surveillance (Jawara et al., 2009). This is something worth considering for Latin American vectors.

Collecting as many mosquitoes as possible may not always be the purpose of mosquito collecting. When the purpose of a study is to determine the species composition and feeding behaviour over time between indoor, peridomestic and outdoor areas, like Rodriguez et al. (2009) did in Colombia, the used collection methods should have a correlation with the HLC with respect to the relative amount and composition of collected mosquito species over time. None of the tested alternative collection methods in the current study showed a correlation with the HLC with respect to the amount of collected *An. aquasalis* mosquitoes over time. This means that none of the alternative methods proved a reliable tool in determining malaria vector potential of *An. aquasalis*.

When taking into consideration the collected numbers of *An. aquasalis* per trap and considering the missing data, the MM-Plus shows some potential as an alternative for collecting this vector. Studies on propane-powered traps, like the MM-Plus, showed them to provide relatively good results and to be easy in use (Kline, 2002). The MM-Plus is generally used with 1-octen-3-ol (octenol), which is thought to act as a synergist in attracting mosquitoes when combined with carbon dioxide (Takken & Kline, 1989; Kline et al., 1991). Here we tested the trapping device with only carbon dioxide as bait, for fair comparison between traps, but using it with octenol or host-specific attractants may significantly increase the yield of *An. aquasalis*. The advantage of the MM-Plus over the CDC light trap and BG Sentinel is its endurance. It can function for weeks without needing new batteries or additional propane, and may in time even be used as a tool in the integrated mosquito control rather than for mosquito monitoring (Kitau et al., 2009).

The CDC trap and BG Sentinel showed promising results in previous studies when collecting anopheline mosquitoes in a number of set-ups with different baits (Faye et al., 1992; Davis et al., 1995; Schmied et al., 2008). Using the CDC trap without light decreases the vast amount of insects of no-interest in the collections and may even increase the yield of insects of interest (Carestia and Savage, 1967), which is why, when using the CDC trap with alternative attractants it is often decided to eliminate the light source as bait. In our study the CDC trap showed potential as a tool for collecting *Culex* mosquitoes, but failed to be effective in the collection of *An. aquasalis*.

The advantage of the BG Sentinel is that it is very compact and easy to take along. The BG Sentinel is generally provided with BG Lure, a mosquito attractant containing ammonia, lactic acids and fatty acids, and developed to mimic human skin odour. Studies with different vector mosquito species show it to be a fairly good trapping method (collecting high numbers of females) when compared to for instance the CDC light trap, especially when used in combination with carbon dioxide (Maciel-De-Freitas et al., 2006, Bhalala & Aria, 2009). Using these traps in combination with BG Lure or other (human) host specific baits, for instance a worn sock with emanating foot odour, may increase its efficiency in collecting (anthropophilic) *An. aquasalis*.

The benefit of using alternative collection methods for the HLC, besides the reduction of infection risk, may be the lower costs and the possibility for long-term, more constant collections. Furthermore, the element of differences in personal attractiveness, an aspect of HLC (Kahn et al., 1965, Knols et al., 1995), is eliminated. Using CO_2 as bait has some disadvantages as CO_2 is generally not easy to transport and use in remote areas. The use of propane powered traps overcomes this disadvantage. Recent findings show that sugar-fermenting yeast can serve as a reliable alternative source for the production of CO_2 in mosquito traps (Smallegange et al., 2010). This can help solve the problem of logistics and allow for a wider use of this attractant in mosquito monitoring tools.

This study indicates that CO_2 as a single attractant is not sufficient to trap *An. aquasalis*. Using it as bait did not help to provide a good alternative collecting method to replace HLC, even though some of the collecting methods used in the study showed potential in collecting large numbers of mosquitoes. Since the HLC are very efficient, *An. aquasalis* obviously needs more host-specific cues. Knowing what attracts the mosquito will lead to added opportunities for the development of alternative collection methods.

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Behavioral heterogeneity of *Anopheles darlingi* and malaria transmission dynamics along the Maroni River (Suriname, French Guiana)

5

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ABSTRACT

The border area between Suriname and French Guiana, is considered the most affected malaria area in South America. A one-year cooperative malaria vector study was performed by the two countries, from March 2004 until February 2005, in four villages. *Anopheles darlingi* proved the most abundant anopheline species. Human biting rates differed between villages. The differential effect of high rainfall on mosquito densities in the villages suggests a variety in breeding sites. Overall parity rates were low, with means varying from 0.31 to 0.56 per study site. Of the 2,045 *An. darlingi* mosquitoes collected 13 were found *Plasmodium*-infected; 10 *P. falciparum*, 2 *P. malariae* and 1 mixed *P. malariae/P.vivax*. The overall annual Entomological Inoculation Rates in the villages ranged from 8.7 to 66.4. The seemingly non-relation of number of malaria cases with periods of high mosquito densities is discussed. *An. darlingi's* tendency to bite during sleeping hours provides opportunity for malaria control with impregnated bed nets; a strategy just introduced in Suriname, which may also find its way into French Guiana.

INTRODUCTION

A majority of the number of malaria cases in Suriname and French Guiana originates from the Maroni River[1], the border river between the two countries (unpublished report Medical Mission; Chaud et al., 2005). Between 1999 and 2003 this amounted to 77.2% of all cases in French Guiana with the highest incidence reported from the health post of the village of Gran Santi (28.9% of the overall). In Suriname, the 8 health clinics of the Maroni River accounted for 37.5% of the national total, with the highest incidence reported from the health post of Stoelmans-island (11.3% of the overall).

The human population in this area consists mostly of Maroons or so-called Bush Negroes, living in small villages and camps along the river. Other ethnicities in the area include Brazilians, often involved in small-scale gold-mining, and Amerindians. Due to a genetically defined immunity of the Maroon population to *Plasmodium vivax* infection, the majority of malaria cases reported is caused by *P. falciparum*.

The most important malaria vector recognized is *An. darlingi*, one of the most common and efficient malaria vectors in the Americas. Other potential vectors such as *An. nuneztovari* or *An. oswaldoi* are also present but their role in local malaria transmission has never been proven (Mouchet et al., 1989; Rozendaal, 1990).

An. darlingi breeds in creeks, along river edges, in flooded river banks, and in pools remaining after the rainy season. Seasonal fluctuations in biting densities were studied in Suriname and differed between collecting sites. This is believed to be due to differences in availability of breeding sites (Hudson, 1984; Rozendaal, 1992). Nightly biting activity of the vector also shows variability. Rozendaal (1990) reported a single peak biting time of *An. darlingi* at 23:00 hr. along the Maroni River, and Hudson (1984) reported a peak between 21.00 and 23.00 hr. Pajot et al.(1977) reported peaks at dusk, midnight and dawn in the coastal area of French Guiana. Rozendaal (1989) also reported a relatively short indoor resting period of the mosquitoes, but nevertheless believed that transmission takes place indoors, based on the late peak biting time.

Large vector control programs with DDT and/or Dieldrin Indoor Residual Spraying (IRS) performed in the 1950s and 1960s resulted in the coastal area of both countries being free of malaria in the 1970's. This was never achieved for the interior. More recent malaria control activities in the Maroni area were not coordinated between Suriname and French Guiana. IRS and Active Case Detection (ACD) were the most important activities in malaria

[1] *In Suriname this river is called Marowijne River.*

control in French Guiana (Chaud et al., 2005). Vector control in this country is the mission of the Service Départemental de Désinfection (SDD). In Suriname the Bureau of Public Health (BOG, Ministry of Health) is responsible for malaria control, but health clinics in the interior are run by Medical Mission (MM), a partly government funded NGO. Recent control activities along the Surinamese side of the Maroni River included case treatment, and small-scale projects with non-impregnated and impregnated bed nets.

In spite of continuing vector control activities, malaria transmission still exists in the Maroni border area. In fact, it was reported the area with the highest Parasitic Index in South America (WHO, 2005). One important obstacle in control programs is that little information is available on the malaria vector, especially its behavior and characteristics. This makes it difficult to identify routes of transmission, and so define and evaluate vector control measures.

Authorities in French Guiana and Suriname decided to make a cooperative effort to decrease malaria in their countries, and especially in the border area. As part of this joint French-Surinamese Project (FSP), entomological surveys were performed along the Maroni River by teams of the Institute Pasteur (French Guiana) and the Bureau of Public Health (Suriname) in 2004-2005. The results of these surveys are reported upon in this paper.

MATERIALS AND METHODS

STUDY SITES

This study was implemented in four Maroon villages; Jamaica and Flavien Campou located on the Upper Maroni River (southern part of the border), and Langatabiki and Midenangalanti located on the Lower Maroni River (northern part of the border) (Fig. 5.1). The villages Jamaica and Flavian Campou are situated in the heart of the high malaria risk area along the Maroni River. Langatabiki and Midenangalanti were chosen for their easy accessibility. Within each village the specific study site was selected based on availability and in agreement with the local population.

Jamaica (N 4°20', W 54°23') is a Surinamese settlement on a small island in the middle of the Maroni River, situated just south of the larger Stoelmans-island. The island is partly vegetated and has a small swampy area on the southwestern side, a presumable ideal breeding site for *An. darlingi*. Jamaica is a location where a high number of malaria cases originated from. The nearest health post is at Stoelmans-island.

Flavien campou (N4°19', W 54°22') is a small settlement situated close to a French touristic camp, located about 5 km. upstream from Jamaica.

The camp itself is cleared from high vegetation, which allowed the owner to keep sheep. The surroundings are covered with rainforest. Flavien campou is situated on a high river bank on the French Guianese side of the Maroni River. It has no swampy areas or obvious breeding sites near the habitations. Malaria diagnosis and treatment are essentially made in the Gran Santi health center.

Langatabiki (N 4°59', W 54°26') is a Surinamese island in the Maroni River of about 2 kilometers long, at about 20 kilometers upstream from the French Guianese village of Apatou. The village of Langatabiki, located at the south of the island, is inhabited by 570 people (data Medical Mission). There is a school, some shops and a Medical Mission health center where malaria cases are regularly diagnosed and treated. The island is covered by rainforest but the village is surrounded by swampy areas in the rainy season.

Midenangalanti (N 5°02', W 54°25') is located on the French side of the Maroni River at about 15 kilometers upstream from Apatou. The village has about 70 people living in about 15 traditional houses. There is no health center and malaria diagnosis is made in the Apatou health center. The village is located on the bank of the river below a hill where cassava (manioc) is cultivated. It is surrounded by Amazonian rainforest.

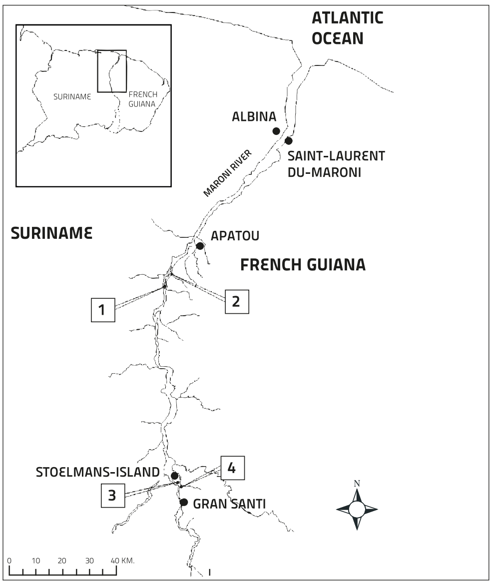

FIGURE 5.1: *Map of the Maroni River, border area between Suriname and French Guiana, showing the localisation of the villages of Midenangalanti (1), Langatabiki (2), Jamaica (3) and Flavien Campou (4).*

CLIMATOLOGY

The study sites are situated in the tropical rainforest. Climate in this area is hot and humid with average temperature of 27°C and average annual relative humidity of around 80%. Average monthly rainfall during the year of study was 182.5 mm. in Apatou and 205.2 mm. in Gran Santi, with a maximum from April to June in the two towns. Four seasons are identified, the major rainy season from Mid-April to Mid-August, the major dry season from Mid-August to November, the minor rainy season from December to January and the minor dry season from February to Mid-April.

COLLECTING AND PROCESSING OF MOSQUITOES

The outdoor collecting in Langatabiki and Midenangalanti was done by a team from the Institute Pasteur (French Guiana), and in Jamaica and Flavien Campou by a team from the BOG (Suriname), in all sites with assistance from local residents. Peri-domestic *Anopheles* mosquitoes were collected using human landing collections. During monthly fieldtrips of four nights, each team would stay two nights in one study site and two nights in the other. Every night 4 persons would work, in rotating shifts of 2 persons, from 18.30 until 06.30 hr. Collectors (males and females of variable age and body mass) were rotated based on availability. Mosquitoes landing on the lower leg parts of the collectors were collected with aspirators. The collected mosquitoes were counted, identified and stored per hour of collection. Identification was done using the keys of Faran & Linthicum (1981), Linthicum (1988), and Gorham et al. (1967). Ovaries of female *Anopheles* mosquitoes were dissected to determine parity rate (Detinova, 1962). Head and thorax were then stored in tubes with silica gel for further processing in the laboratory.

In the laboratory of the Institute Pasteur in Cayenne (French Guiana) heads and thorax of anopheline females were tested individually on the presence of *Plasmodium falciparum*, *P. vivax* 210, *P. vivax* 247, and/or *P. malariae*, using the sandwich ELISA method for mosquito analyses according to Wirtz et al. (1987).

DATA ANALYSIS

All data of the four study sites on number and species of mosquitoes per location per hour of the night were processed in an Excel database. Parous rate (percentage of dissected mosquitoes with positive parity), Human Biting Rate (number of bites per person per hour, and per night), Circumsporozoite (CSP) Index (percentage of *Plasmodium* positive mosqui-toes) and Entomological Inoculation Rate (number of infective bites per person per night, and per year) were calculated. The correlation between monthly rainfall and human biting rate per night in each month was studied, using Pearsons correlation test, one-tailed, $\alpha<0.05$.

RESULTS

From March 2004 to February 2005, 182 human-nights outdoor captures in the four villages yielded an overall of 2,274 *Anopheles* mosquitoes, of which about 90% were *An. darlingi* (2045). Of the other *Anopheles* species 85.6% were *An. nuneztovari*. The remaining few belonged to the species *An. oswaldoi*, *An. intermedius*, or *An. triannulatus*. A few specimens remained unidentified because legs and/or wings were lost (Table 5.1).

TABLE 5.1: *Summary of Anopheles mosquitoes collected in Langatabiki, Midenangalanti, Flavien Campou and Jamaica between March 2004 and February 2005.*

	MIDENANGALANTI	LANGATABIKI	JAMAICA	FLAVIEN CAMPOU
Human nights	48	42	44	48
An. darlingi	911 (99.8%)	145 (99.3%)	783 (93.2%)	206 (54.9%)
An. nuneztovari	2	1	55	138
An. triannulatus	0	0	1	5
An. oswaldoi	0	0	1	16
An. intermedius	0	0	0	9
***An. darlingi* mean nightly HBR**	190	35	185	43

An. darlingi mean nightly Human Biting Rate (HBR) over the study year were, 19.0 ± 15.23, 3.5 ± 2.87, 18.5 ± 16.89 and 4.3 ± 4.06 bites/man/night respectively in Midenangalanti, Langatabiki, Jamaica and Flavien Campou (Table 5.1). The percentage of *An. darlingi* in relation to all anophelines collected was lowest in Flavien Campou, were the majority of other anophelines consisted of *An. nuneztovari.* These *An. nuneztovari* were, except for 3% of the specimens, collected before 23:30 hr. in the evening.

Figure 5.2 displays population fluctuations of the *An. darlingi* nightly HBR in the four villages along the different seasons, and shows the relation with rainfall (data from Gran santi / Apatou). A positive correlation exists between monthly rainfall in Gran Santi and HBRs in Jamaica and Flavian Campou, and a near positive correlation between monthly rainfall in Apatou and HBR in Langatabiki. In Midenangalanti and Jamaica, a very high number of mosquitoes were collected in May and June 2004, during the main rainy season. Unfortunately no collections were made in Langatabiki during that same period due to logistic limitations. The human biting rate of *An. darlingi* did not differ much among the four locations during the remainder of the year.

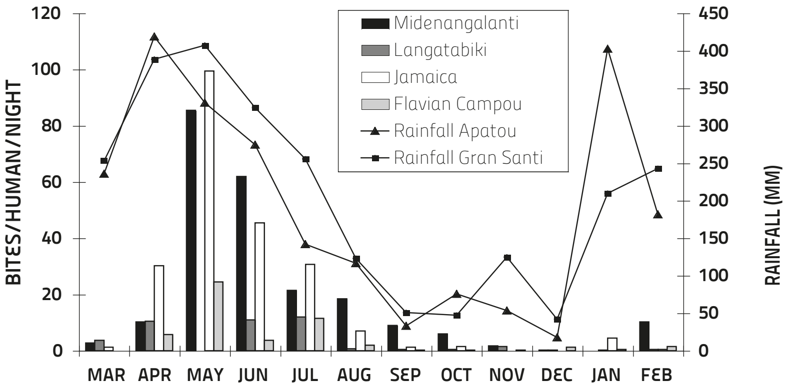

FIGURE 5.2: *Human biting rates (bites/man/night) of An. darlingi from March 2004 to February 2005 in Langatabiki, Midenangalanti, Jamaica and Flavien Campou, and their relation to the amount of rainfall measured in Apatou and Gran Santi. (data from May 2004 in Langatabiki not available).*

Figures 5.3A, 5.3B, 5.3C and 5.3D show *An. darlingi* HBR in the four villages in relation to the number of monthly confirmed malaria cases from the four health clinics of the study area during the study period. A peak incidence of malaria was reported in the period Oct-Dec 2004, in Langatabiki, with increasing numbers in January/February 2005 in Langatabiki and Gran Santi. This did not coincide with high biting rates.

The hourly biting activity of *An. darlingi* in the four study sites differs per location. It is difficult to identify clear peaks in biting activity, although the mosquitoes seem to have a preference to bite after 21:30 hr..

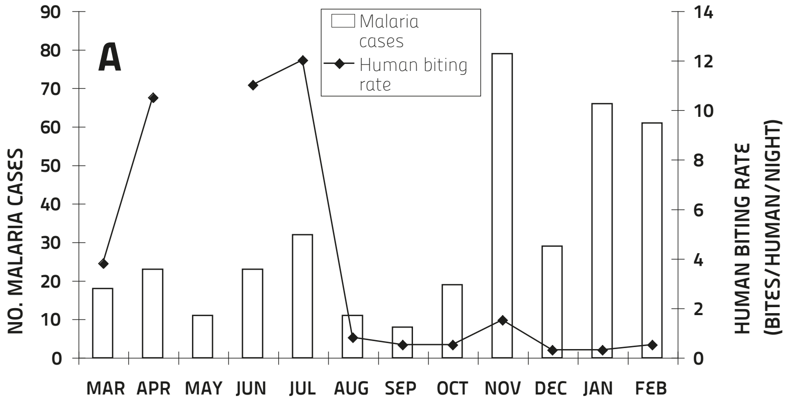

FIGURE 5.3A: *Number of confirmed malaria cases in the health post of Langatabiki, in relation to the An. darlingi HBR measured in Langatabiki.*

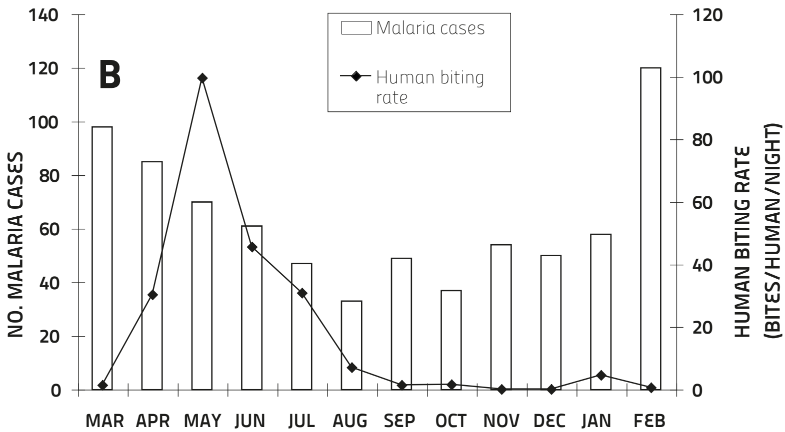

FIGURE 5.3B: *Number of confirmed malaria cases in the health post of Stoelmans-island (B) in relation to the An. darlingi HBR measured in Jamaica.*

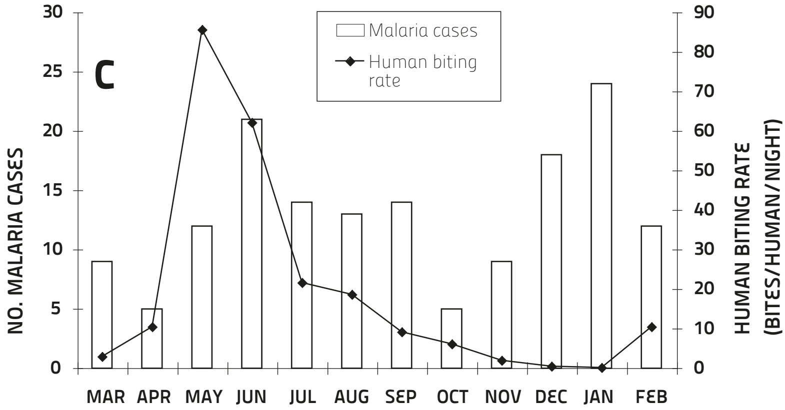

FIGURE 5.3C: *Number of confirmed malaria cases in the health post of Apatou, in relation to the An. darlingi HBR measured in Midenangalanti.*

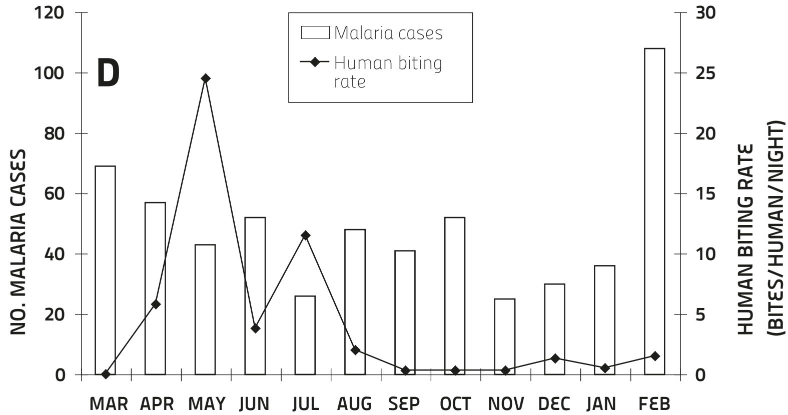

FIGURE 5.3D: *Number of confirmed malaria cases in the health post of Gran Santi in relation to the An. darlingi HBR measured in Flavien Campou.*

The parity rate of 947 female *An. darlingi* was determined. An overall 46.6% of these females was parity positive, meaning that they had had a blood meal. The overall parity rate per study site was 0.309 in Jamaica, 0.453 in Flavien Campou, 0.470 in Midenangalanti and 0.556 in Langatabiki. Monthly parity rates (calculated on a minimum of 5 females), are shown in Figure 5.4.

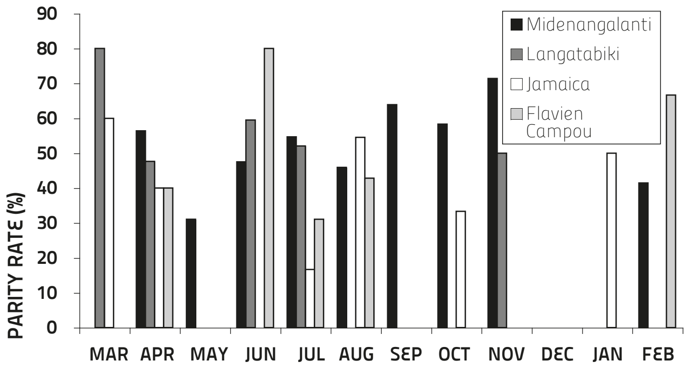

FIGURE 5.4: *Monthly parity rates in Midenangalanti, Langatabiki, Flavien Campou and Jamaica, from March 2004- Feb 2005; [Parity rate=0 means not enough mosquitoes to determine parity rate].*

Of the 2,045 *An. darlingi* mosquitoes analyzed on *Plasmodium* infection, 13 turned out positive, 8 of which came from Jamaica. Of the positive mosquitoes 10 were infected with *P. falciparum*, 2 with *P. malariae* and 1 specimen had a mixed infection of *P. malariae/P. vivax*. Except for 3 specimens all positive mosquitoes were collected in the period of high mosquito densities (April-June 2004). None were collected in the peak period of malaria incidence (Oct-Dec 2004). The resulting overall Circumsporozoite Index and Entomological Inoculation Rates are given in Table 5.2. All *An. nuneztovari* and other *Anopheles* species were CSP negative.

TABLE 5.2: *Overall An. darlingi Circum-Sporozoite Index (CSP) and overall Entomological Inoculation Rate (EIR) in Midenangalanti, Langatabiki, Jamaica and Flavien Campou from March 2004 to February 2005.*

STUDY SITE	NR. OF POSITIVE AN. DARLINGI FEMALES (PLASMODIUM SPECIES)	CSP INDEX (95% CI)*	ANNUAL EIR
Midenangalanti	2 (Pf)	0.00 (0.00-0.01)	152
Langatabiki	1 (Pf)	0.01 (0.00-0.04)	87
Jamaica	8 (6Pf, Pm, Pmv)	0.01 (0.04-0.16)	664
Flavien Campou	2 (Pf, Pm)	0.01 (0.00-0.11)	152

Pf: P. falciparum; Pmv: mixed infection P. vivax and P. malariae; Pm; P. malariae
** : exact binomial method*

DISCUSSION

The entomological data collected in this study are unique in this sense that it is the only study of this kind available for this high malaria risk border area. The high percentage of *An. darlingi* collected in the peri-domestic situation of the study locations is consistent with previous findings in the border area in Suriname by Rozendaal (1987). The results of his study indicated that 85.2% of peridomestic anophelines were *An. darlingi,* while in forest situations *An. nuneztovari* was predominant. Hudson (1984) found 99.36% of the peridomestic anophelines in nearby villages to be *An. darlingi*. This confirms that *An. darlingi* is the most anthropophilic anopheline species in the area.

The results of this study provide some very useful baseline data on *An. darlingi* behavior and malaria transmission in the area but also give rise to a number of questions. We show that the *An. darlingi* population density increase promptly after heavy rainfall in only two of the study sites (Jamaica and Midenangalanti), and not in the other two (Langatabiki and Flavian Campou). In Jamaica the presence of a swampy area on the small island, which fills up after heavy rainfall, may well explain the increase in mosquito density. In Langatabiki the same could be expected, since it is also surrounded by swamps, but the increase did not occur. In the on higher ground situated Flavian Campou no obvious breeding places were found close to the habitations, which could explain the absence of seasonal fluctuations. Midenangalanti lies on a river bank and is surrounded by forest. It is likely that with heavy rainfall and increasing river levels the nearby forest floors will flood, providing temporary breeding places for the *Anopheles* mosquitoes. This may explain the prompt increase in densities after the rains.

This study did not include a detailed survey of the breeding sites in any of the study locations. As known, rain may very well provide new breeding sites in one location, while flushing away breeding sites (and larvae) in another. In turn, dry periods may decrease the available breeding sites in one location by drying them up, while increasing them in another due to the creation of pools on river sides after decreasing of the water level (Gil et al., 2007). A future detailed study of breeding sites in the four locations may provide some answers, especially on the causes of seasonal fluctuations in mosquito population densities. This information is valuable for vector control programs.

Seasonality of *An. darlingi* is discussed for instance by Charlwood (1980) who found peak densities in the transition period between the wet and dry season in Brazil. Rozendaal (1987) could not confirm this for the Stoelmans-island region in the 1980s, but acknowledges that fluctuations in river level will for a substantial part determine the availability of breeding sites (Rozendaal, 1992). Hudson's results confirm that variability in local situations (ecology) may result in variation in the seasonal availability of breeding sites (Hudson, 1984).

Our results also show that the health posts of Langatabiki and Stoelmans-island report increased numbers of malaria cases in periods that have both low rainfall, and low number of mosquitoes. The hypothesis that the mosquitoes, even though they are fewer in numbers, have a higher proportion of infectivity can not be validated with the results of our ELISA tests. A similar study performed a few years earlier in Amerindian villages more south along the Surinames-French Guianese border shows a comparable situation with a significant number of malaria cases in the dry season. Infected mosquitoes were found in these periods of low mosquito densities indication that *An. darlingi* (the primary vector in this area) can maintain malaria transmission even in less favorable seasons (Girod et al., 2008). One other explanation for continued transmission could be that the human population would be less inclined to use impregnated bed nets in periods of low mosquito densities and high temperatures, thus decreasing personal protection measures. Similar problems with bed net compliance in the dry season have occurred elsewhere (Frey et al., 2006). The study by Hudson (1984) in this same area showed a comparable situation with malaria incidence reaching its peak between December and March when the number of mosquitoes caught at the study location were very low or zero. Traveling of people between villages and camps increases towards December, as many are involved in end-of-the-year family celebrations. This may aid malaria transmission as well. Global experience shows that movement of people can have a significant impact on malaria transmission on a local or regional scale (Martens and Hall, 2000).

Only *An. darlingi* mosquitoes were found Plasmodium infected, which confirms its role as primary vector in this area. Of the 13 infected mosquitoes 8 were collected in Jamaica, resulting in an EIR in this location of 66.4. this is consistent with data of the Stoelmans-island health center which shows that Jamaica is a location where many malaria cases originate from. The population in all four study locations consists of Maroons, Maroons are not receptive to *P. vivax* malaria, which explains the high proportion of *Plasmodium falciparum* infected mosquitoes.

That parity rate and thus age composition of the *An. darlingi* population may depend on the seasons and the environment (geographical variance) is discussed by Monteiro de Barros et al.(2007). He found that in the Amazon Basin (northern Brazil) age composition in forest situations differ from savanna (alluvial) situations, and that population stability (high parous rates) in forest situations is highest in the dry season, while in 'alluvial' situations the parity rate increases in the wet season. This allows for the hypothesis that long-lived females may be responsible for dry season malaria transmission in forest situations as found along the Maroni River. In Venezuela monthly parous rates were studied for *An. marajoara* and *An. darlingi* in forested areas with environmental impact due to gold mining activities. They were found similar for the two species throughout the year, with two peaks that coincided with the dry-rainy transition period and the period of less rain (Moreno et al., 2007). This seems to support the hypothesis. In comparison, data originating from the Amerindian villages further south along the Surinamese border show no seasonal fluctuations in parity rates (Girod et al., 2008). It is suggested that in this area vectorial capacity throughout the year is regulated by mosquito densities rather than longevity of the mosquitoes. The Flavien Campou data suggest a peak in parity rate in the rainy season in May and June. The Midenangalanti data show an increasing trend in parity rates in the dry season. Neither period*locality coincides with increased numbers of malaria cases in the nearby health posts. The overall mean parity rate of 0.466 is well below that found in the southern Amerindian villages.

Anopheles darlingi, contrary to *An. nuneztovari*, bites mostly at a time when people are generally sleeping. The use of impregnated bed nets for malaria control may prove very effective in decreasing transmission risk, provided that the population of the villages and settlements are reached by an awareness campaign on consistent use. In Langatabiki and Midenangalanti bites are numerous around 06:00 hr. on the morning. A situation similar to findings in the Amerindian villages south of the Maroni River (Girod et al., 2008). In this situation only bed net use is not sufficient. A combination with IRS could be useful. But the efficacy of IRS has to be investigated, and related to the exophily/endophily of *An. darlingi*. Previous IRS activities in the same region proved ineffective due to a number of reasons, among which traditional housing and cultural issues (Girod et al., 2008). Re-introduction of IRS and large-scale introduction of Long Lasting Impregnated Nets (LLINs) were initiated in Suriname after 2005 as part of a 5-year Malaria Program funded by the Global Fund to fight Aids, Tuberculosis and Malaria. French Guiana is not (yet) planning on a large–scale introduction of impregnated bed nets for malaria control. Using the impregnated nets on only one side of the border while both sides are high risk area and a continuous movement of people across the border exists, will significantly reduce the effect of this control measure.

The high level of variability found in *An. darlingi's* behavior and characteristics seems a returning factor in the many studies done on this malaria mosquito (Hudson, 1984; Rozendaal, 1992; Gil et al., 2007; Monteiro de Barros et al., 2007; Girod et al., 2008). Local differences in ecology, (micro) climate, human impact, and other factors and their influence on the vector will need further investigation to come to some understanding of how this species depends on and is influenced by its surroundings. The Maroon and Amerindian villages along the Maroni River, where *An. darlingi* proofs a very efficient vector and where malaria transmission still needs to be controlled, provide good opportunity for continued research in order to obtain the necessary information on which to base vector control measures.

ACKNOWLEDGMENTS

The work of P. Rabarison, who was responsible for the coordination of the project, is much appreciated. We also would like to thank the populations of Jamaica, Stoelmans-island, Langatabiki, Flavien Campou and Midenangalanti for their support. Prof.Dr. W. Takken is thanked for his comments on the draft manuscript.

Collapse of *Anopheles darlingi* populations in Suriname after introduction of insecticide-treated nets (ITNs); malaria down to near elimination level

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Submitted as: Hiwat H, Mitro S, Samjhawan A, Sardjoe P, Soekhoe T, Takken W. Collapse of Anopheles darlingi populations in Suriname after introduction of insecticide-treated nets (ITNs); malaria down to near elimination level

ABSTRACT

A longitudinal study of malaria vectors, aiming to study *Anopheles darlingi* population dynamics, man-biting and sporozoite rates, was carried out in three villages in the interior of Suriname between January 2006 and April 2010. During 13,392 man hours of human landing collections, a total of 3,180 female mosquitoes were collected of which 33.7% were anophelines. *Anopheles darlingi* accounted for 88.1% and *Anopheles nuneztovari* for 11.1% of the total anophelines collected. The highest mean human biting rate (HBR) observed per survey for *An. darlingi* was 1.43 bites/man/hour outdoors and 1.09 bites/man/hour indoors. Individual ELISA assays of 683 anophelines yielded two *An. darlingi* females infected with *Plasmodium falciparum*. The anopheline HBR decreased to zero in all sites after the onset of malaria intervention activities in 2006, which included the mass distribution of ITNs. Malaria transmission decreased significantly and Suriname reached the Millennium Development Goal for malaria in 2007. It is concluded that the combination of ITN introduction and climatic events have led to a collapse of malaria vectors in the study sites in the interior of the country. The results are discussed in relation to the stability of malaria transmission in areas with low-density human populations.

INTRODUCTION

The human malaria parasite species prevalent in Suriname are *Plasmodium falciparum*, *P. vivax*, and *P. malariae*. Transmission occurs only in the interior of the country, which consists mostly of tropical rainforest. The area is sparsely populated, with people living mostly in discrete communities. Here, malaria prevalence has increased gradually since 1990. *Plasmodium falciparum* is predominant among the Maroon population while the Amerindian and immigrant populations may also be infected with *P. vivax* and *P. malariae*.

Anopheles darlingi is the primary malaria vector in the interior. The role of *An. darlingi* in malaria transmission dynamics in Suriname was studied in the 1980's in Maroon villages along the Upper-Marowijne river, along the border with French Guiana (Hudson, 1984; Rozendaal, 1987; Rozendaal, 1989; Rozendaal, 1992). Between 2000 and 2002 studies were done in Amerindian villages along the Upper-Marowijne River and Upper Lawa River (Girod et al., 2008). *Anopheles darlingi* was the most common anopheline mosquito found in or near the villages. In 2004/2005 vector studies were done in the same area as this was an area of high malaria transmission risk (Rozendaal, 1987; Girod et al., 2008; Hiwat et al., 2009). Both Girod et al. (2008) and Hiwat et al. (2009) found *An. darlingi* specimens infected with *P. falciparum, P. vivax* and *P. malariae*. Among the other anopheline species collected in the villages were *An. nuneztovari*, *An. braziliensis*, *Anopheles neivai* Howard, Dyar & Knab, *An. oswaldoi*, and *An. intermedius*.

In 2006 mass distribution of Long Lasting Insecticide treated Nets (LLINs) were initiated in the entire interior of the country (approx 50,000 inhabitants) together with Indoor Residual Spraying (IRS), Active Case Detection (ACD) and an extensive public awareness campaign. As part of the malaria program a longitudinal study on malaria vectors and transmission dynamics, with special emphasis on *An. darlingi*, was carried out in two Maroon villages and one Amerindian village in the interior from January 2006 until April 2010. In this paper we present the findings of this study and relate these to the change in the malaria situation, the climatic events and the malaria control activities in Suriname.

MATERIAL AND METHODS

STUDY AREA Suriname is part of the northern range of the Amazon forest and borders to French Guiana, Guyana and Brazil. To the north, the country reaches the Atlantic Ocean. Most of the agriculture is located in the coastal plain, and around 90% of the country's population of near 500,000 people lives here (ABS, 2005). The remaining people live in the tropical rainforests of the interior. The population of the interior consists of Amerindians and Maroons, living in tribal communities along the main rivers, and a number of (mostly Brazilian) immigrants working in small-scale gold mines. Due to the limited road system, transportation to the villages is mostly by boat and by plane. The climate is hot and humid, with an average temperature of 27°C and annual relative humidity of around 80%. Four seasons are identified: the major rainy season from mid-April to mid-August; the major dry season from mid-August to November; the minor rainy season from December to January; and the minor dry season from February to mid-April.

STUDY SITES The three sentinel sites of this study are Drietabiki (4° 06'N, 54° 40'W), a Maroon village along the Tapanahony River (3,028 registered inhabitants in 2001, data Medical Mission), Kwamalasamutu (2° 21N, 56° 47'W), an Amerindian village along the south border of Suriname with Brazil (1,068 registered inhabitants in 2001, data Medical Mission), and Jamaica (4°20'N, 54°23'W), a small island with Maroon settlements (about 50 people) in the Lower Lawa River along the border of Suriname with French Guiana (near the medical clinic of Stoelmans-island with 3,111 registered inhabitants in 2001, data Medical Mission). Kwamalasamutu is a riverside village surrounded by tropical rainforest. Most houses have an open structure. A significant number of houses are built on stilts, with the people spending a lot of time underneath the house during the day. Drietabiki is part of a conglomerate of small islands in the Tapanahony River. The island has a surface area of 0.4 km^2.. It is partly vegetated and surrounded by tropical rainforest. The Maroon houses in Drietabiki and Jamaica are mostly ground-level, closed structures. Jamaica is a settlement on a small island in the middle of the Lawa River, situated just south of the larger Stoelmans-Island. The island is partly vegetated and has a small swampy area on the southwestern side, a presumably ideal breeding site for *An. darlingi* (Rozendaal, 1992). In all study sites the people's livelihood depends for a large part on hunting, fishing and slash-and-burn agricultural activities. The people of each study village had access to a medical clinic, where suspected malaria cases were examined and, if positive, treated appropriately.

HUMAN LANDING COLLECTIONS All-night human landing collections were conducted, indoors and outdoors (peri-domestic) simultaneously, during three consecutive nights during each survey. In 2006 and 2007 the surveys were done at two sites per village with two collectors indoors and two collectors outdoors. Collections were done monthly in the first quarter of the year and once in the subsequent quarters. In 2008 and 2009 the collecting effort was decreased to one site per village with one collector indoors and one collector outdoors. Collections were done once per quarter. The collectors were trained local residents teamed up with or supervised by entomology technicians of the Bureau of Public Health. Collectors (males and females of variable age and body mass) were rotated based on availability in two 6-hour shifts between 18.00 and 06.00 hr.. All mosquitoes landing on the lower legs of the collectors were collected with aspirators.

PROCESSING OF SAMPLES The collected mosquitoes were counted, identified, labeled and stored per hour of collection. Identification was done using the keys of Faran & Linthicum (1981), Linthicum (1988), and Gorham (1967). Ovaries of female anophelines were dissected to determine parity rate, observing the tracheolar skeins as in Detinova (1962). Heads and thoraxes were then stored in tubes with silica gel for further processing in the laboratory. In the laboratory of the Bureau of Public Health anopheline females were tested individually for the presence of *P. falciparum, P. vivax* and/or *P. malariae*, using the sandwich (modified) enzyme-linked immunosorbent assay (ELISA) for *P. falciparum, P. vivax* (VK210 and VK247 variant epitopes) and *P. malariae* circumsporozoite protein (CSP) detection according to Wirtz et al. (1987, 1992).

MALARIA CASE DETECTIONS. In the three study sites malaria prevalence was established through passive case detection by the Medical Mission health clinics using microscopic analysis of blood slides (for Jamaica the reference clinic is Stoelmans-island). Active cased detection (ACD) was done in each locality, using rapid diagnostic tests (RDTs; Binax Now®) cross-checked by microscopic analysis of blood slides.

DATA ANALYSIS The human biting rate (HBR) was calculated as the number of female anopheline bites per person per hour. The circumsporozoite protein index (CSP index) was calculated as the proportion of mosquitoes found to be positive for CSP in the ELISA analysis. The entomological inoculation rate (EIR) was calculated as the product of the HBR and the CSP index of mosquitoes collected on humans.

Malaria prevalence data originate from the national malaria database maintained by the Ministry of Health. Malaria diagnostics at the local Medical Mission clinics was done by microscopic analysis of blood slides. Climatology data were provided by the Meteorological Service Suriname.

RESULTS

Between January 2006 and April 2010 a total of 13,392 man-hours of collection (5,185 in 2006; 6,048 in 2007; 864 in 2008; 864 in 2009; and 432 in 2010 (until April)) resulted in 3,180 mosquitoes including *Anopheles* (33.7%), *Culex* (43.3%) and *Aedes* (22.1%) species. The most abundant anopheline species were *An. darlingi* (88.07%) and *An. nuneztovari* (11.09%), which together accounted for 99.16% of the anophelines collected. Other anopheline species found were *An. oswaldoi*, *An. albimanus* and *An. intermedius*. Of the *An. darlingi* 97.04% were collected in 2006 and 2007. The *An. darlingi* mosquitoes caught in 2007 resulted almost all (363 of 372) from one survey at Jamaica in July of that year (Table 6.1). As of late 2007, anophelines were no longer found in any of the three villages. The mean hourly human biting rate of *Aedes* spp. and *Culex* spp. also decreased during the study from 0.581 and 0.200 respectively in 2006 to 0.067 and 0.047 respectively in 2009.

TABLE 6.1: *Collecting effort with human landing collections and number of mosquitoes collected per study site between January 2006 and April 2010.*

	DRIETABIKI	JAMAICA	KWAMALASAMUTU	TOTAL
Human nights	372	372	372	**1,116**
Culicidae	1,331	1,422	400	**3,153**
Anopheles spp.	241	803	29	**1,073**
An. darlingi	220	705	20	**945**
An. nuneztovari	19	93	7	**119**
An. oswaldoi	0	2	0	**2**
An. albimanus	2	2	0	**4**
An. intermedius	0	1	0	**1**
An. spp.[a]	0	0	2	**3**
Culex spp.	686	324	368	**1,378**
Aedes spp.	404	295	3	**702**

[a] *not identified to species due to loss of wings and/or legs*

The highest mean human biting rate per hour (HBRs) for *An. darlingi* was found in Jamaica during the survey of July 2007. For this survey the mean HBR indoors was 1.09 bites/man/hour, while for outdoor biting the mean HBR was 1.43 bites/man/hour. The overall percentage of indoor vs. outdoor biting of *An. darlingi* was similar in all three study sites: Kwamalasamutu 40.0 vs. 60.0%, Jamaica 37.2 vs. 62.8% and Drietabiki 34.6 vs. 65.4%. The pattern of indoor vs. outdoor biting during the nightly hours of collecting for Drietabiki and Jamaica is shown in Fig.6.1. In Jamaica the percentage of outdoor biting showed a peak during the 8th hour of collecting, which is between 01.00 and 02.00 hr.. In Drietabiki biting steadily increased towards 06.00 hr.. By contrast, 71.4% of the total number of *An. nuneztovari* were collected between 19.00 and 20.00 hr..

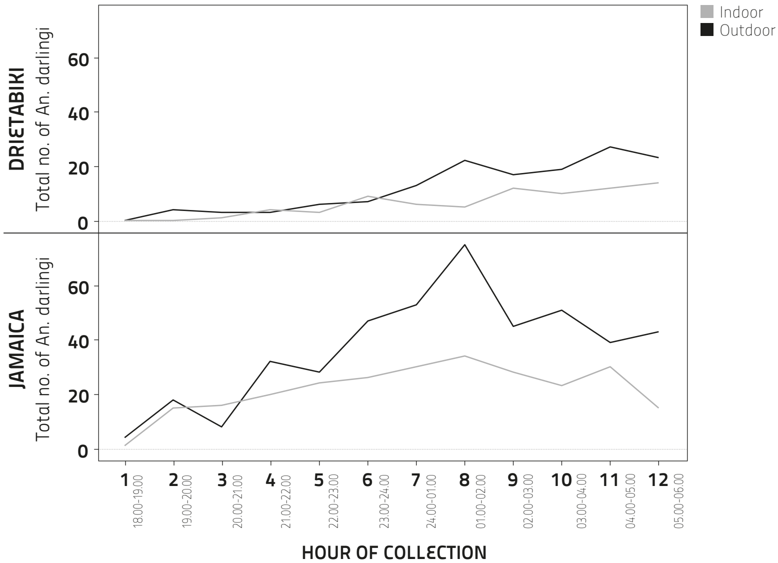

FIGURE 6.1: ***Anopheles darlingi* biting activity.** *Overall nightly indoor (■) and outdoor (■) biting pattern of An. darlingi per hour in Drietabiki and Jamaica per hour of collection, between January 2006 and April 2010.*

Table 6.2 shows the mean hourly HBRs of *An. darlingi* over the years per study site and Fig. 6.2 shows the mean HBR in 2006, 2007 and 2008 for Drietabiki and Jamaica. In Kwamalasamutu the HBR was very low in 2006, one specimen of this species was found in 2007 and since 2008 the HBR has been zero. In Drietabiki the HBR decreased between January and February 2006, was zero in January 2007 and only three specimens have been collected during subsequent surveys until April 2010. In Jamaica the HBR increased between January and February 2006, but decreased again after July 2006. In October 2006 the HBR was zero. It remained near this level except during the survey of July 2007 and the survey of April 2008. From 2009 onwards no *An. darlingi* or other anophelines were found in any of the study sites, except for one *An. nuneztovari* in Drietabiki in 2010. *Culex* and *Aedes* population densities were also very low. Worth mentioning is that during short-term collections in a village about 100 km. southwest of Drietabiki in 2008 and 2009 *An. darlingi* were still found, even though the population had received and were using ITNs (H. Hiwat, unpublished data).

TABLE 6.2: *Mean hourly Human Biting Rate ± SD of Anopheles darlingi mosquitoes per study site per year.*

	MEAN HOURLY HBR		
	DRIETABIKI	JAMAICA	KWAMALASAMUTU
2006	0.1256 ± 0.4157	0.2146 ± 0.4577	0.0094± 0.0782
2007	0.0005 ± 0.0158	0.1606 ± 0.6265	0.0006± 0.0170
2008	0.0069 ± 0.0832	0.0903 ± 0.3525	0
2009	0	0	0
2010[a]	0	0	0
Overall	**0.0428 ± 0.2487**	**0.01410±0.5015**	**0.0039± 0.0499**

[a] *until April 2010*

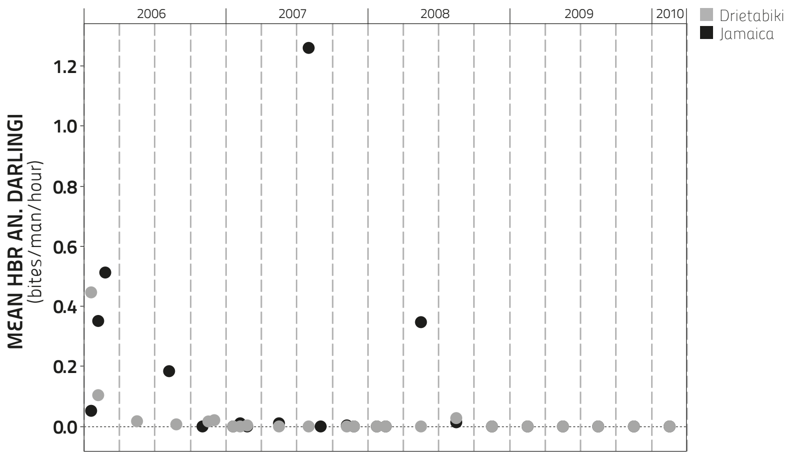

FIGURE 6.2: ***Anopheles darlingi human biting rate.*** *Mean HBR of An. darlingi per survey between January 2006 and April 2010 in Drietabiki (■) and Jamaica (■).*

Rainfall data from Drietabiki show that the rainfall pattern was in line with the annual seasons during the first three years of the study but a bit off during 2009, in which year the month March had a higher rainfall and the month May a much lower rainfall than the previous years (Fig.6.3). Rainfall data from Jamaica and Kwamalasamutu are incomplete, unfortunately, which makes it difficult to relate peaks in HBR to rainfall and availability of mosquito breeding sites.

The overall parity rate of the *An. darlingi* females was 57%. The number of mosquitoes collected and dissected per survey or location was too low to determine seasonal patterns in parity rate. Combining all females of 2006 and 2007 we found a parity rate of 73.1% for January (n=201), 56.7% for February (n=164), 54.6% for March (n=183) and 89.7% for April (n=29). Considering the rain data available for Drietabiki (Fig. 6.3) the numbers suggest a higher parity rate during the wetter months, which may indicate a (partial) dependence of the population on temporal (seasonal) breeding sites.

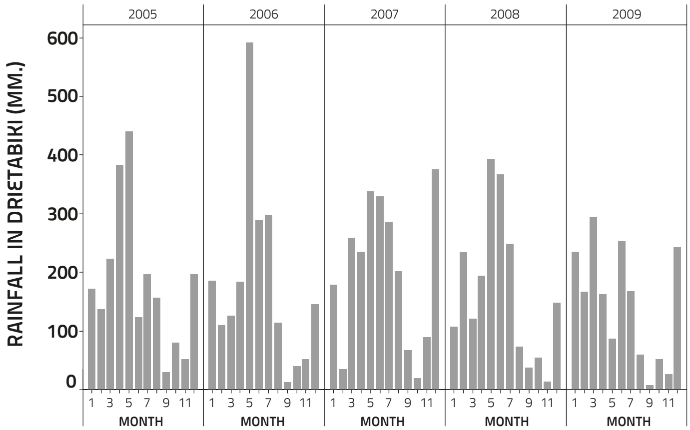

FIGURE 6.3: *Monthly rainfall (mm) in Drietabiki from 2005 – 2009.*

A total of 683 of the anophelines collected were tested for malaria infection. Of these 642 were *An. darlingi*. Two anophelines were found malaria infected, both were *An. darlingi* females and both were infected with *P. falciparum*. One specimen originated from Jamaica and was collected in February 2006. The other specimen originated from Drietabiki and was collected in January 2006. This results in an *An. darlingi* CSP in Drietabiki of 1.02% in January 2006 and of 0.68% for the whole year. Combining this with the HBR data results in an EIR in Drietabiki of 1.7 infected bites per human in January 2006 and 3.7 infected bites per human for the year. The *An. darlingi* CSP in Jamaica was 0.67% in February 2006 and 0.23% for the whole year. The resulting EIR for Jamaica is 0.8 infected bites per human in February 2006 and 2.2 infected bites per human for the year 2006.

The longitudinal entomological study presented here started just before the onset of the main interventions of the national malaria control program.

As part of this program, vector control measures were introduced in the study sites in the second quarter of 2006. This included the introduction and mass distribution of long-lasting impregnated bed nets (LLINs; Permanet™) and a one-time IRS with alpha-cypermethrin (FendonaTM; Drietabiki and Jamaica). In May 2006, just after the distribution of the bed nets and as a result of heavy rains, the water level of the larger rivers of Suriname dramatically increased and the villages along the rivers were flooded. Efforts were made to replace the bed nets people had lost due to the floods and the resulting evacuations.

Active Case Detection (ACD) was done in the Stoelmans-island area (including Jamaica) in July and October 2006. The ACD *Plasmodium* infection rates were 1.57% and 2.17%, respectively. Among the infected people, six asymptomatic cases (*P. falciparum*) were found. In Drietabiki ACD was done in September 2006 and yielded an infection rate of 0.41%. One person was an asymptomatic carrier of *P. falciparum*. The ACD performed in Kwamalasamutu in September 2006 had a focus on people visiting the village for a religious conference. None were found infected with malaria. The number of malaria cases reported by the Medical Mission clinics in Drietabiki, Kwamalasamutu and Stoelmans-island (nearby Jamaica) decreased towards 0 (zero) in 2006 and 2007 and remained around this level until the end of the study (Fig. 6.4).

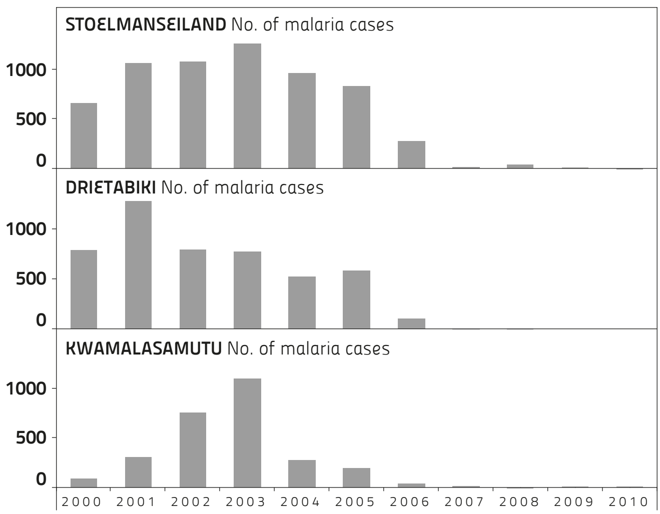

FIGURE 6.4: ***Malaria cases in the study area.*** *Number of malaria cases in Stoelmans-island, Drietabiki and Kwamalasamutu as reported by the Medical Mission clinics for the period January 2000 until April 2010.*

DISCUSSION

Within months after onset of this study, the population densities of *An. darlingi* and other mosquitoes dropped in the three study sites. Except for what seem incidental, possibly seasonal, occurrences, the HBRs observed in all three sentinel sites have been zero bites per man per night since 2007. Although this does not necessarily mean a complete disappearance of anophelines, the long term absence of anophelines in human landing collections and the decrease in number of malaria cases suggests this nevertheless and has not been reported in the country before.

The most interesting site in this study is Jamaica. Not only because this site is situated in (what used to be) the heart of the high-malaria-risk area, but also because mosquito data from Jamaica and locations nearby, from previous studies, exist. One of the study sites of Rozendaal (1987) was Aselikamp, a location almost 7 km. south of Jamaica along the Lawa River. Rozendaal collected mosquitoes during selected hours of the night. The HBR in Aselikamp found during Roozendaal's study varied from monthly means of less than 1 to 5 bites per man per hour. Hiwat et al. (2009) included Jamaica in a one-year mosquito study with all-night collections in 2004/2005 and found an overall mean HBR over this period of just over 1.5 bites per man per hour, compared to HBR values <0.22 and decreasing during the current study. This indicates relatively high mosquito densities in the years prior to the study and prior to the interventions.

The onset of the malaria control interventions unexpectedly coincided with unusual, extensive flooding of the major rivers in the interior of the country. Population density and distribution area of *An. darlingi* often have a positive relation to the availability of breeding sites (Elliot, 1968; Forattini, 1987; Tineo et al., 2003; Cabral et al., 2010). The combination of people on the move as a result of the flood and increased availability of mosquito breeding sites due to the rains was expected to trigger an increase in malaria transmission. As a preventive measure additional bed nets were distributed.

Immediately after the floods, the mosquito densities, as established by HBR, in the sentinel sites were down to zero. This was thought a result of the flooding. Pajot et al. (1977) found similar events in French Guiana where the occurrences of heavy rains were followed by a decrease in and sometimes a total absence of the mosquitoes. Heavy rains can flood breeding places and create flood currents that carry away immature mosquito stages (Charlwood, 1980; Forattini, 1987). Having the floods coincide with malaria control activities makes the impact of target interventions difficult to measure. Drops in mosquito densities may have been due to a combination of both. The continued low density (or even absence) of anophelines as well as *Culex* spp. and *Aedes* spp. in these sites over the course of several years is another matter. This cannot be attributed to the effect of the floods, especially since incidental increases in populations of these mosquito species were observed since then. The role of other macro-climatic factors is not clear. Neither is the role of the LLINs. A mass effect of the introduction of LLINs on the mosquito populations can be hypothesized. Decreased mosquito population densities and biting pressure have been found after use of insecticide treated materials elsewhere and (local) mass effects have been reported (Ilboudo-Sanogo et al., 2001; Hawley et al., 2003). A mass effect on the anopheline population, however, would not explain the peak-occurrences of *An. darlingi* in Jamaica in July 2007 and again in April 2008, which may have been related to the temporal availability of breeding sites. Considering the vigorous washing behavior of the Maroon population, the impression is that the durability of the nets in terms of insecticide levels over time is much lower than advertised by the manufacturers (see also Atieli et al.(2010)). Although this will need to be confirmed the nets may even reach the critical level of insecticide within six months of use, which makes it even more difficult to attribute long-term changes in population densities to the LLINs.

Anopheles darlingi is assumed capable of maintaining a high malaria transmission rate even when found in low densities, and may keep up the transmission by a high human biting rate, combined with a relatively high susceptibility to *Plasmodium* infection (Deane, 1948; Klein et al., 1991; Da Silva-Vasconcelos et al., 2002; Schoeler et al., 2003; Flores-Mendoza et al., 2004; Gil et al., 2007). Finding the asymptomatic malaria cases in the human population is an important aspect of malaria control. Asymptomatic cases with very low parasitaemias can be infective to *An. darlingi*, even if the infections occur at a much lower rate than symptomatic cases (Alves et al., 2005). The ACDs performed within the malaria control program in Suriname showed that asymptomatic cases were present in the Jamaica and Drietabiki areas. ACD is currently the first choice of intervention used in local malaria outbreaks. The difficulty in a country with low malaria transmission is the increasing importance of asymptomatic cases and cases with low (submicroscopic levels) of infection as a source of transmission (Schneider et al., 2007).

The *An. darlingi* biting data from Jamaica show a relatively high biting pressure in the early morning hours. A similar pattern was found by Harris et al. (2006) in the Amazon forest in Bolivia. In contrast, Rozendaal found a decreasing biting pressure towards the morning hours in a location not far from Jamaica (Rozendaal, 1989). Early morning biting peaks have implications for malaria control via impregnated bed nets. Depending on the activity pattern of the local human population it can present a considerable risk of transmission, which needs to be taken into account. Alternative complementing measures for vector control or prevention of malaria transmission need to be considered. These measures may also be of use in the control of malaria transmission in the gold mining areas, where the highly mobile human population is known to have a different daily activity pattern than the village population.

Epidemiological data show that current malaria transmission in Suriname is especially a problem of gold mining areas. Most Maroon and Amerindian villages have been free of malaria for a number of years. The absence of *An. darlingi* from our study sites since 2008 suggests a complete collapse off the local vector populations, and will most certainly have had its effect on local transmission. Considering the events and interventions, this disappearance is either due to the introduction of LLINs or to a combination of climatic events and the bed nets. The challenge will be to prevent malaria transmission in the village populations and to ensure that anopheline populations from elsewhere cannot become established in the former endemic malaria zone(s). In addition, work on decreasing transmission in the mobile gold mining populations should receive high priority.

ACKNOWLEDGMENTS

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7

Novel strategies lead to near elimination of malaria in previously high-risk areas in Suriname, South America

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ABSTRACT

Suriname was a high malaria risk country before the introduction of a new five-year malaria control program in 2005, the Medical Mission Malaria Program (MM-MP). Malaria was endemic in the forested interior, where especially the stabile village communities were affected. The interventions of the MM-MP included new strategies for prevention, case management, behavioral change communication / information, education and communication (BCC/IEC), and strengthening of the health system (surveillance, monitoring and evaluation and epidemic detection system). In the current paper the MM-MP is evaluated both on account of the targets established within the program and on account of its impact on the malaria situation in Suriname.

The interventions of the MM-MP are reviewed and related to the performance indicators established by the donor. The changes in the national malaria situation since initiation of the MM-MP, based on analysis of the national databases, are discussed.

After a slow first year with non-satisfying scores for the performance indicators, the MM-MP truly engaged in its intervention activities in 2006 and kept its performance up until the end of 2009. A total of 69,994 long-lasting insecticide treated nets were distributed and more than 15,000 nets re-impregnated. In high risk areas this was complemented with residual spraying of insecticides. Over 10,000 people were screened with active case detection in outbreak and high risk areas. Additional notification points were established and the national health system was strengthened. Malaria vector populations, monitored in sentinel sites, collapsed after 2006 and

concurrently the number of national malaria cases decreased from 8,618 in 2005 to 1,509 in 2009. Malaria transmission risk shifted from the stabile village communities to the mobile gold mining communities, especially those along the French Guiana border.

The novel strategies for malaria control introduced in Suriname within the MM-MP have led to a significant decrease in the national malaria burden. The challenge is to further reduce malaria using the available strategies as appropriate in the affected areas and populations. Elimination of malaria in the country will require a thorough understanding of transmission dynamics and a dedicated investment in key effective interventions.

INTRODUCTION

The Amazon basin harbors 95% of the total malaria burden in the region and 98% of the *Plasmodium falciparum* infections of the Americas (WHO, 2005; Behrens et al., 2007). The Guyanan Shield area (Suriname, Guyana and French Guiana) was responsible for the highest numbers and concentration of *P. falciparum* cases in the Americas (WHO, 2005). Malaria in Suriname is historically divided in two endemic areas; the coastal belt and the interior (Flu, 1912; Van der Kaay, 1976; Schaapveld, 1984; Rozendaal, 1992). The coastal area was free of malaria by 1968 as a result of a DDT spraying campaign. In the interior spraying was done twice a year, but spray coverage was generally below 40% due to refusals of, and communication problems with the local population and malaria elimination was not achieved (Van der Kaay, 1976; Schaapveld, 1984; Rozendaal, 1990). During the 1990s, a significant increase in malaria incidence in Suriname was observed. This increase was related to the improvement of malaria diagnosis, the increase of antimalarial drug resistance to treatment of *P. falciparum* malaria (4-aminoquinolines (Oostburg, 1973; Van der Kaay, 1976) and population movements due to internal conflicts. Suriname was considered as one of the countries with the highest annual parasite index (API) of malaria in the Americas (WHO, 2005).

Artemisinin-based combination therapy (ACT) was introduced in late 2003. A moderate decline in the number of cases was observed after the nationwide implementation of ACT as first line treatment for uncomplicated *P. falciparum* infections in 2004 and 2005.

The Global Fund to fight AIDS, Tuberculosis and Malaria (GFATM), established in 2002 as a new mechanism to finance a rapid international effort to control the three diseases, approved a malaria proposal submitted by the Surinamese Country Coordinating Mechanism (CCM) in round 4 (R4) (GFATM, 2011a). A five-year grant was provided to the Medical Mission (MM), a local government-supported non-governmental organization as principal recipient for a malaria program. It was termed Medical Mission Malaria Program (MM-MP) and aimed to reduce the transmission of malaria in high-risk communities in the interior of Suriname. The interventions of the MM-MP were in line with the Roll Back Malaria Partnership strategy (WHO, 2005) including activities in prevention, case management, behavioral change communication / information, education and communication (BCC/IEC), and strengthening of the health system (surveillance, monitoring and evaluation and epidemic detection system). In this paper we describe the achievements of the MM-MP with regard to the programmatic performance indicators and evaluate the impact of the MM-MP on malaria incidence and transmission in Suriname.

METHODS

STUDY AREA

Suriname is part of the northern range of the Amazon forest, located between 2° and 6° latitude North and 54° and 58° longitude West along the North coast of South America. The coastal plain is separated from the interior by a so-called savannah belt: poor agricultural land, consisting of a white-sand formation covered with shrubs. The 2004 census showed that Suriname had a population of almost 500,000 people, with 49.3% living in and around the capital, and only 9.8% living in the tropical rainforest of the interior (ABS, 2005). The population of the interior consists of Amerindians and Maroons, living in tribal communities along the main rivers, and a number of immigrants who live and work in small-scale gold mines in the forest. A limited road system exists, which does not extend beyond the Van Blommensteyn Lake, about 150 kilometers into the interior (Fig. 7.1). As a result, transportation to the villages is mostly by boat or plane. The population in the interior varies due to movements during the year and is estimated to be about 48,000 people with additional 15,000 mobile gold miners. The total population at risk for malaria in the country is 63,000 inhabitants. Human migration from and to Suriname is common, particularly along the French Guiana border.

Environmental conditions favor malaria transmission in Suriname. The climate is hot and humid, with an average temp of 27°C and annual relative humidity of around 80%. Four seasons are identified: the major rainy season from mid-April to mid-August; the major dry season from mid-August to November; the minor rainy season from December to January; and the minor dry season from February to mid-April.

Three human malaria parasite species are present in Suriname: *P. falciparum* (Pf), *P. vivax* (Pv) and *P. malariae* (Pm). The majority of malaria cases reported from the interior are due to *P. falciparum* among the Maroons (descendants of African slaves), many of which live in high malaria risk areas, and generally showing a natural resistance against *P. vivax* infections. The Amerindians, the second largest ethnic group in the interior, and Brazilian gold miners ("garimpeiros") proved susceptible to all three malaria species. Mixed infections seldom occur (less than 2%). *Anopheles darlingi* is the primary vector. This species represents over 90% of the anophelines collected in previous entomological surveys (Hudson, 1984; Rozendaal, 1987; Rozendaal, 1990; Hiwat et al., 2009). Rozendaal (1992) established that peaks in biting densities of *An. darlingi* correlated well with periods of (i) high water levels in the long rainy season, (ii) low water levels in the long dry season, and (iii) abundant rainfall in the short rainy season.

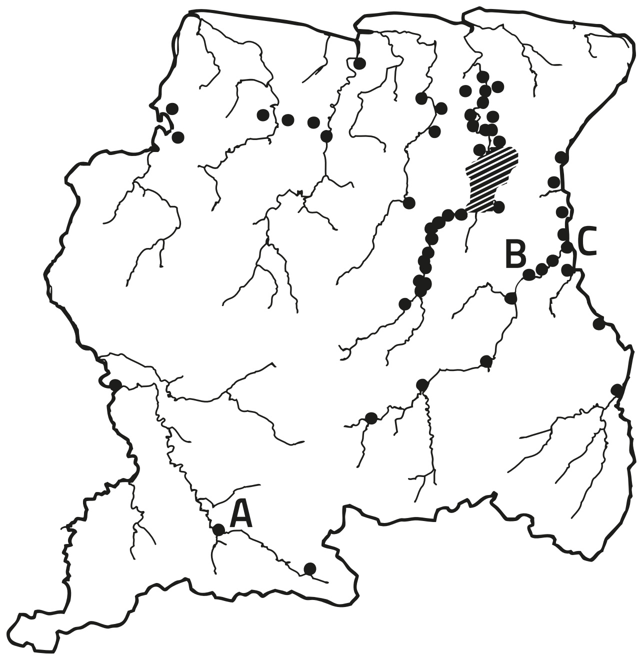

FIGURE 7.1: *Map of Suriname, showing the MM-clinics (black dots) and the sentinel sites for entomological surveillance (**A**: Kwamalasamutu; **B**: Drietabiki and **C**: Jamaica (Stoelmans-island)).*

The Ministry of Health in Suriname (MoH) is responsible for all actions related to disease prevention and control. All but one hospital are located in the capital, Paramaribo. The Regional Health Service provides primary health care in the coastal areas. MM is responsible for the primary health care in the interior with 56 permanent health centers strategically located, which serve as diagnostic and treatment points for the people living and working in this area (Fig. 7.1). The health centers are distributed over five medical regions. Each region has a non-resident medical doctor who supervises and assists local health-care workers through regular visits and radio contact. The health-care workers are able to provide diagnosis and first line treatment for the most common acute diseases; severe cases are referred to the Diakonessen hospital in the capital. Confirmed malaria cases are reported weekly to the MM headquarters via radio, aiming to detect epidemics. Malaria reports are sent monthly to MM headquarters in Paramaribo by the MM health workers. For malaria diagnosis 17 of the 56 health centers have trained microscopists, the remaining health centers rely on the use of rapid diagnostic tests (RDTs) or so-called "dipsticks', which can detect antigens of malaria parasites in the blood of infected individuals. RDT results are cross-checked by microscopic analyses of blood slides at the MM Headquarter in Paramaribo (Derks, 2006). Suriname participates in the Amazon Network for Surveillance of Anti-malarial Drug Resistance - Amazon Malaria Initiative (RAVREDA-AMI) and as such takes part in an evaluation of the use of RDTs as an alternative for microscopy (WHO, 2006a) and as a quality control of microscopic diagnosis.

For technical advice on malaria prevention, diagnosis, treatment and vector control, the MoH relies on the National Malaria Board, which is a national advisory technical group. The Bureau of Public Health (BOG) is responsible for the national malaria control. Data on malaria morbidity and mortality is routinely obtained from health care and malaria providers in the country and centralized at BOG. A malaria information system, which includes a database with weekly recordings of malaria data from the individual health service providers, was set up in 1955. It has been continuously upgraded since then, and represents the backbone of the national surveillance.

PERFORMANCE FRAMEWORK AND MONITORING PROGRESS

The MM-MP was a performance-based program supported by the GFATM. Each GFATM grant consists of two phases, which are continuously monitored with an agreed-upon performance framework. Quarterly audits of grants are usually conducted during the first years, and after that semi-annually. The performance framework is the formal statement of the performance expected over the lifetime of the grant as signed in the grant agreement. It contains a summary of key indicators and targets, which are used to measure i) program outputs and coverage on a routine basis and ii) program outcomes and impact (GFATM, 2011b). The performance rating of a grant program is based on i) the overall progress achieved against time-bound targets for key output indicators; and ii) an assessment of management performance (in the areas of monitoring and evaluation (M&E) financial and program management, and procurements of pharmaceuticals and health products). The program is graded an "A1" or "A2" [exceeding (>100%) or meeting performance expectations (90-100%)], a "B1" (adequate performance 60-89%), a "B2" (inadequate performance but with potential demonstrated, 30-59%) or a "C" (unacceptably poor performance, <30%) (GFATM, 2011c). Evaluation of the MM-MP is done in 23 periods based on the achievements in the performance framework.

INTERVENTIONS

VECTOR CONTROL

Sentinel sites for entomological surveillance were established in three key locations: Kwamalasamutu (near the Brazilian border), Jamaica (Upper Marowijne River, along the border with French Guiana) and Drietabiki (along the Tapanahony River) (Fig.7.1). Simultaneous all-night indoor and outdoor collections were done during surveys of three nights, each quarter of the year. Mosquitoes were collected with the human landing collection method In 2006 and 2007 this was done with 96 man-hours per night, from 2008 onwards this was reduced to 24 man-hours per night.

Over the years conventional nets for prevention of malaria had been distributed in Suriname, with funds from national and international donors. A program of manufacturing by, and marketing through, women group organizations at the local level was established in 1997. Since 2003 these locally-produced bed nets have been impregnated on site with pyrethroids. The MM-MP distributed free long-lasting insecticide-treated nets (LLINs) (PermaNet 2.0®, Vestergaard-Frandsen, Switzerland) to people living in communities in the interior. The target was to distribute 73,000 ITNs to the communities of the interior (Table 7.1, performance indicator 2). One LLIN was available for each person and two LLINs if the person was a pregnant woman. Impregnation kits containing deltamethrin WT 25% (KO-Tab 123®, Bayer Pty., Ltd) were used for impregnation and re-impregnation of nets since 2006 (Table 7.1, performance indicator 8). Insecticide-treated nets

(ITNs), including LLINs, were re-impregnated in high risk areas. Different means of transportation (i.e. cars, boats, airplanes, all terrain vehicles) and stakeholders' participation were used for the implementation of all interventions.

Indoor Residual Spraying (IRS) with alpha-cypermethrin (Fendona®, BASF) was conducted only along the Upper Marowijne and Tapanahony Rivers areas, which were the areas with the highest API in the country. Local men and women were trained in IRS and recruited in an effort to reach high coverage and maximized community participation.

CASE MANAGEMENT

Among the performance indicators were the number and percentage of malaria cases and the number and percentage of severe malaria cases receiving correct diagnosis and treatment (Table 7.1, performance indicator 3 and 5). Paracheck-Pf® (Orchid Biomedical Systems) has been routinely used as a RDT for diagnosis of *P. falciparum* malaria in MM health post without microscopists. Since 2005, Pf/Pan specific tests were introduced as national policy in the country (BinaxNOW® Malaria, Inverness Medical Innovations, Inc.). All health service providers received refreshment training in malaria diagnosis and treatment (Table 7.1, performance indicator 1). A pilot out-reach activity started late in 2005 aiming to provide malaria services to populations without access to health services in remote areas, especially in gold mining areas, via the introduction of Malaria Service Deliverers (MSDs) (Table 7.1, performance indicator 6). MSDs are fixed/mobile local persons trained in diagnosis and treatment of malaria who provide free services in remote areas. MSDs receive supplies and supervision by the malaria control program. MSD points were established as a pilot in key gold mines areas where malaria cases where previously reported. In 2006, a fixed MSD was established in northern Paramaribo to provide malaria service to the large Brazilian ("garimpeiro") community living there. This is the only MSD point with trained microscopists. Northern Paramaribo is the commercial centre for purchasing mining equipment and supplies and to trade off gold. Mine workers move frequently between northern Paramaribo and the mining fields in the interior. An aggressive active case detection (ACD) campaign was initiated in 2006 in the gold mining areas where malaria is endemic. ACD was also performed during confirmed malaria outbreaks. All malaria service providers completed training in routine malaria surveillance and reporting mechanisms (Table 7.1, performance indicators 1 and 9).

TABLE 7.1: *Comparison of performance indicators for the MM-MP: baseline vs. final results.*

PERFORMANCE INDICATORS	BASELINE	FINAL RESULTS			Δ CHANGE^
	VALUE [2004]	TARGET	ACHIEVED	% ACHIEVED	
1) Number of service deliverers trained in the use of LLINs and prompt, effective, anti-malarial treatment #	0	2,333	2,184	93.6	+2,184
2) Number of free nets distributed to the indigenous populations and gold miners	0	73,000	69,994	95.8	+69,994
3) Number (and %) of uncomplicated malaria cases receiving correct diagnosis and treatment @	(60%)	74	112	151	+112
4) Number (and %) of health clinics in the interior with no reported stock-outs of anti-malarial drugs	0	56	2	3.6	+2
5) Number (and %) of severe malaria cases receiving correct diagnosis and treatment	162	8	9	112.5	-153
6) Number of fixed/mobile malaria service delivery points established for gold miners	0	43	31	72.1	+31
7) Number of health facilities reporting information on a regular basis using standardized information systems	0	56	56	100	+56
8) Number of ITNs impregnated and/or re-impregnated	0	600	327	54.5	+327
9) Number of health and community personnel trained in malaria control activities &	0	118	110	93.2	+110
10) Number of communities reached by the malaria media awareness campaign	0	140	58	41.4	+58
11) Number of people living in the interior reached through BCC activities	0	3170	610	19.2	+610

including health workers and community personnel
@ *including children under 5*
& *including epidemiology, entomology, residual spraying, etc.*
^ *compared to baseline value*

BEHAVIORAL CHANGE COMMUNICATION AND INFORMATION, EDUCATION AND COMMUNICATION (BCC/IEC)

The number of communities and the number of people reached by the BCC activities are part of the performance indicators (Table 7.1, performance indicators 10 and 11). A media awareness campaign was launched in 2006. It comprised of mass communication channels to increase the knowledge of malaria control and prevention. Several TV and radio spots, newspapers, flyers, and posters were distributed widely in the coastal areas and points of entry to Paramaribo. Posters, folders and videos (DVDs) were provided in different languages to communities in the interior by a social scientist. Several training sessions with health staff, community leaders and traditional healers in malaria prevention and control activities were conducted.

INTELLIGENT SURVEILLANCE: DETECTION AND RESPONSE TO EPIDEMICS

The epidemic detection and response system was strengthened (Table 7.1, performance indicator 7). Endemic channels were built using weekly malaria reports per each health/malaria reporting unit and standard operation procedures for epidemic containment were developed. Epidemic thresholds were established depending on local situations. In general the threshold for villages was set on three malaria cases per week, but if a village had been malaria free for a significant period of time even one case (as a result of local transmission) was considered an outbreak.

Malaria information is centralized in two databases; one at the MM headquarters and one at the anti-malaria campaign (BOG/AMC) of BOG. BOG/AMC collected all malaria data from blood bank, hospitals and laboratories in Paramaribo. Case administration and reporting was improved by introduction of a new national format for case reporting. All health workers received training in the use of this new national format. Quality control of slides analyzed in the MM clinics in the interior was done at the MM headquarters in Paramaribo. Quality control of the RDT results from the MSDs was done by slide analysis (slides taken simultaneous with RDT tests) at the fixed MSD clinic in northern Paramaribo. Quality control of the fixed MSD clinic was done by regular blind re-reading of slides at the fixed MSD clinic (trained microscopists re-reading 100% of the positive slides and 10% of the negative slides).

MONITORING AND EVALUATION (M&E)

The MM-MP standardized the data collection tools in the country. A new national malaria data collection tool was developed and piloted in 2007 and implemented in 2009. The malaria M&E system for the MM-MP was developed initially in 2006, refined in 2008 and in 2010 was updated to become the national multisectoral M&E plan. An integrated database with reporting system was developed in 2009.

DATA ANALYSIS

Malaria-related data i.e., outpatient and hospital admissions, were collected from notification units and triangulated with the BOG central database. Only confirmed cases were included in the malaria surveillance system. Information on antimalarial drugs stock was collected monthly via radio (Table 7.1, performance indicator 4). Programmatic indicators were assessed using data reported by the MM-MP M&E unit. MM-MP databases on entomological surveillance and IRS and were reviewed. Pearson's chi square tests and Student tests were used to compare two percentages or two means respectively ($\alpha = 0.05$) using SPSS® version 17.0 (IBM Corporation, Somers NY, USA). Δ change (per each performance indicator) was calculated comparing the baseline value of 2004 with final result achieved in the MM-MP. Changes of impact/outcome indicators were used to assess project overall achievements and its implications in public health. Programmatic and financial information were obtained from GFATM web site (grant portfolio SUR-404-G02-M) (GFATM 2011a).

RESULTS

The MM-MP began in February 2005 and ended in October 2010. Performance reports were done quarterly between February 2005 and January 2009 and semi-annually thereafter. There were 19 performance reports during the duration of the project.

INTERVENTIONS

For practical reasons interventions are grouped by strategic areas: vector control (including IRS, LLNs, re-/impregnation of nets and entomological surveillance); case management (diagnosis and treatment); BCC/IEC (mass media, outreach program) and surveillance, monitoring and evaluation (including epidemic detection, passive and active case surveillance, mobile/fixed malaria service deliverers, M&E).

VECTOR CONTROL

Entomological surveillance. Entomology surveys were performed in Drietabiki (372 human-nights (hn)), in Jamaica (372 hn) and in Kwamalasamutu (372 hn) between January 2006 and April 2010. The majority of anophelines collected during the entomological surveys of 2006 (5,185 man-hours (mh)), 2007 (6,048 mh), 2008 (864 mh), 2009 (864 mh) and 2010 (216 mh) were *An. darlingi*. Other anophelines collected were *An. nuneztovari*, *An. oswaldoi*, *An. albimanus* and *An. intermedius*. In all three sites the *An. darlingi* population showed a sharp decrease from 2006 onwards. *Anopheles darlingi* has not been collected in any of the three sentinel sites from 2008 onwards.

ITNs. LLINs distribution began early April 2006 and was temporary interrupted (for two months) due to extensive flooding of the rivers in May 2006 following unusually heavy rains. 55,100 LLINs – were distributed between 2006 and 2007 covering almost all stable communities in the interior. In 2008 and 2009 and additional 14,508 and 386 LLINs were distributed respectively to replace used ones in high risk areas and to supply small communities that had not previously received them. A total of 69,994 LLINs were distributed during the MM-MP. A monitoring and evaluation net survey conducted in 2007 indicated that 83% of the people were sleeping under a net (MM-MP Multiple Indicator Survey report 2007). There were nine gold mining communities (mobile communities) included in the bed net distribution of 2008, which received 1,212 LLINs.

Re-impregnation of nets. Training on the use of KO-Tab® 123 and local impregnation activities were executed in 332 communities. A total of 15,023 nets (conventional/LLINs) were re-impregnated between 2007 and 2009 and targets were achieved during most periods (Table 7.1, performance indicator 8). However, during the last year of the project, only 327 nets were re-impregnated which represented an achievement of 54.5% of the established target of 600 nets in 2010. This was most likely due to a decrease in the number of technical and field personnel employed by the MM-MP during the final year.

IRS. Two rounds of IRS were carried out in 2006 (June-August and September-November) only in communities along the Tapanahony- and Upper Marowijne areas, the highest malaria API areas in the country. These communities had also received LLINs. An overall coverage of 71% was achieved, ranging from 25% to 93%. In 3,672 houses 4,280 rooms were sprayed; 147 refusals were reported.

CASE MANAGEMENT

The expertise of existing microscopy personnel was upgraded with 12 new microscopists trained and 19 working microscopists re-trained by qualified skilled professionals (Table 7.1, performance indicator 1). Only two out of the 56 MM health facilities (3.6%) reported no stock-out of any antimalarial drug at the end of the project and this was evident only at the end of the MM-MP (Table 7.1, performance indicator 4). Antimalarial stocks were at 100% between January 2006 and October 2009. Primaquine, a drug used against *P. vivax* and *P. falciparum* malaria was absent from most health services from November 2009 untill March 2010 due to procurements delays.

MSD. A total of 31 local workers (from target camps or communities) were selected as malaria service deliverer, achieving a 72.1% of the target established at the end of the project. The number of autochthonous malaria cases diagnosed among all MSD increased significantly from 500 in 2006 to 651 in 2009 ($p=0.021$). The fixed MSD in the capital diagnosed 7%, 17% and 22% of the total number of malaria cases in the country in 2007, 2008 and 2009, respectively ($p<0.17$).

ACD. Thirty-four surveys were performed as active case detection in five areas (9 in 2006; 6 in 2007; 16 in 2008 and 3 in 2009). Overall, 10,702 people were screened for malaria with prevalence rates ranging from 0 to 60%. Malaria cases were diagnosed among 265 persons including 126 *P. falciparum,* 117 *P. vivax*, 20 *P. malariae* and two mixed infections. Higher prevalence rates were observed among people screened in gold mining areas (data not shown). In 2010, ACD activities were taken over by a new malaria project under the coordination of the MoH. All infected people were treated according to the national treatment protocol.

BEHAVIORAL CHANGE COMMUNICATION AND INFORMATION, EDUCATION AND COMMUNICATION (BCC/IEC)

Intensive awareness campaigns were initiated in 2006 and training sessions with the local population were conducted, especially with women group organizations. Women group organizations were targeted for education messages in malaria control and prevention, including the use and washing of LLINs, the use of re-impregnation kits for nets and prompt malaria diagnosis and treatment. At the end of the MM-MP, 58 communities and 610 people participated in the outreach media awareness campaign (Table 7.1, performance indicator 10, 41.4% achieved) and BCC/IEC (Table 7.1, performance indicator 11, 19.2% achieved) activities.

SURVEILLANCE, MONITORING AND EVALUATION

A total of 2,184 health service deliverers were trained in the use of LLINs and prompt effective antimalarial treatment at the community level. All health services performing malaria diagnosis (108 centers nationwide) received training in the use of RDTs and in the updated treatment protocol.

Between 2005 and 2009, 156,878 blood samples were taken for malaria diagnosis with an annual average of 26,278 slides taken and 19,962 confirmed malaria cases diagnosed over the five years. While the number of autochthonous cases decreased, the number of imported malaria cases increased significantly (Fig. 7.2), which resulted in a proportional increase from 2.6% in 2005 to 46.0% in 2009 ($p<0.001$; Table 7.2). Most imported cases were diagnosed by the fixed MSD in the capital and by mobile MSDs in mining areas (data not shown). The malaria passive surveillance system reported 17,463 cases over the five years of the project. The total number of autochthonous malaria cases in 2005, 2006, 2007, 2008 and 2009 were 8,618, 3,920, 1,819, 1,597 and 1,509 respectively (Table 7.2). The number of malaria notifications units increased and the surveillance system managed the inclusion of new MSDs into an updated national centralized malaria information system at BOG. The role of the fixed MSD clinic at Paramaribo as a diagnostic center for garimpeiros increased over the years since its establishment in 2007 (Table 7.2). Between 2005 and 2009 ten malaria outbreaks were detected in the interior of the country based on their respective threshold. ACDs performed in those communities resulted in a prevalence varying from 5.7% to 60.0% (data not shown). All epidemics were controlled and malaria cases treated as specified in the national malaria treatment protocol.

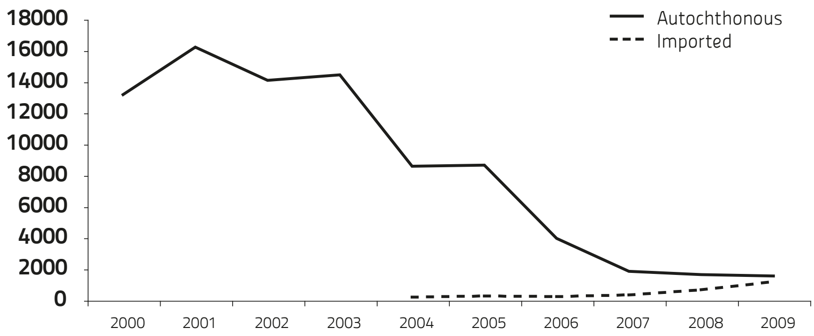

FIGURE 7.2: *Autochthonous and imported malaria cases in Suriname between 2000 and 2009.*

TABLE 7.2: *Overview of the number of malaria cases diagnosed in Suriname between 2005 and 2009 and of the number of malaria cases diagnosed at the fixed MSD clinic (Paramaribo) between 2007 and 2009.*

		2005	2006	2007	2008	2009
No. of malaria cases diagnosed in Suriname	Autochthonous malaria cases	8,618	3,920	1,819	1,597	1,509
	Imported malaria cases	228	200	302	629	1140
TOTAL		**8,846**	**4,120**	**2,121**	**2,226**	**2,649**
No. of malaria cases diagnosed at the fixed MSD clinic (Paramaribo)	Autochthonous malaria cases			122	248	292
	Imported malaria cases			149	515	960
TOTAL				**271**	**763**	**1,252**

PERFORMANCE INDICATORS

Table 7.1 shows the MM-MP indicator values by the end of the MM-MP. By the last reporting period (#23) most indicators substantially reached their targets, with the exception of the number of health facilities reporting no stock out of antimalarials (3.6%), the number of people (19.2%) and communities (41.4%) reached by media awareness campaign and the number of re-impregnated nets (54.5%). The MM-MP was scored B2 in the first four periods, B1 in period 5 and achieved A scores (A1, A2) from 2006 till early 2010; B1 was the final score during closure of the project in periods 22 and 23.

CHANGES IN THE MALARIA SITUATION

Malaria cases declined progressively after the introduction of ACT in 2004. The implementation of effective interventions by the MM-MP in 2005 resulted in a decline of 82% of the total malaria cases in the country and 97% among communities living in the interior of the country under the coverage of MM since 2000. The last malaria-related death was reported in 2007. The national API dropped significantly from 136 per 1,000 population/year at risk to 24 per 1,000 population/year at risk ($P<0.001$). The remaining malaria risk areas in the country are concentrated along the border with French Guiana, specifically in mining areas. Most of the imported malaria cases find their origin here. Continued transmission in these areas, not targeted by the MM-MP, has led to the funding of a new malaria program focused on decreasing malaria in the mining communities. This new program is executed by the MoH with financial support from the GFATM and runs from 2009-2014.

DISCUSSION

In the last decade, malaria control efforts around the globe have gained a significant importance. The implementation of current antimalarial interventions shows promising results in several countries (WHO, 2011). In Suriname, we observed a progressive reduction in the number of malaria cases and deaths, especially after the scaling up of the interventions by the MM-MP starting in 2005. Today, malaria cases reported from the interior are almost all originating from gold mining areas and malaria transmission in the stabile village communities in the interior is almost non-existent. Many of the recent malaria patients in the country work in gold mining areas in French Guiana, but seek diagnosis and treatment in Suriname (imported malaria).

The onset of the decrease in malaria incidence started even before the implementation of the MM-MP and is thought to be due to the introduction of ACT as first line treatment for uncomplicated *P. falciparum* infections, which proved successful in Suriname and elsewhere (Barnes et al., 2005; Sutherland et al., 2005). To further eliminate any residual focus of malaria parasites, primaquine was also added to the ACT in 2007. Prompt diagnosis and treatment are still among the most effective interventions, as well as a continuous monitoring of drug resistance (Oostburg, 1973; Peek et al., 2005).

Whether the decline in malaria in Suriname is due to the impact of the elaborate malaria control activities within the MM-MP, the use of effective drugs or environmental factors is subject of discussion. Between 2005 and 2010 a significant decrease of *Anopheles* population densities in sentinel sites has been recorded by longitudinal surveillance studies. The very low density of *An. darlingi* mosquitoes from the sentinel sites after the excessive flooding of May 2006, suggests an impact of environmental factors on anopheline population densities. It is generally admitted that heavy rains can have an impact on mosquito densities by flooding the breeding sites and creating flood currents that carry away the immature mosquito stages (Pajot et al., 1977; Rozendaal, 1992; Cabral et al., 2010). On the other hand, reports exist on the mass killing of mosquitoes and significantly reduced indoor biting after the introduction of insecticide treated nets (ITNs) in malaria endemic areas (Takken, 2002; Russel et al., 2010). This would support the hypothesis that the decrease of the (local) vector population density, and the ultimate disappearance of the vector from collections, may be a result of the mass distribution of LLINs and it may at the same time explain the lack of recovery of the mosquito populations after the floods.

The decrease of *An. darlingi* populations since the implementation of LLIN/IRS and the flooding in 2006, ultimately led to a total absence of *An. darlingi* from collections in the sentinel sites. Whether or not the *An. darlingi* populations had disappeared or were simply below detection level is undetermined. We can conclude, however that since the collapse of *An. darlingi* malaria transmission has virtually ceased to exist, which supports our observation of a sharp decline in autochthonous malaria transmission.

Even if the effect of the LLINs on *An. darlingi* remains unclear, the use of these nets is a rational choice in areas with anthropophilic and endophagic mosquitoes, like *An. darlingi*. Insecticide-treated nets are used in vector control programs worldwide. Depending on the proportion of insecticide resistance in local vectors results vary, but generally the effects include reduced mosquito survival rates and sporozoite rates (Takken, 2002; Lengeler, 2004). Successful control programs with ITNs are found in Africa. The Gambia (West Africa), for instance, reported a 25% reduction in child mortality after a large-scale bed net impregnation campaign (D' Allessandro et al., 1995). In Kenya, child mortality was reduced by 15-33% (Phillips-Howard et al., 2003). With the current low malaria burden and (locally) low density of malaria vector populations in Suriname the challenge will be to ensure a continued proper use of the bed nets by the people of the interior. The durability of the nets, including the impact of traditional washing methods on insecticide levels and integrity of the netting materials (Graham et al., 2005), needs to be studied in order to estimate at which time the nets should be replaced.

Other possible reasons for the reduction of malaria in Suriname need to be considered. Environmental factors (rainfall changes), changing human population movements (within Suriname and across borders) and increased awareness to fight malaria as a result of the media campaign might all have contributed to the decline of malaria incidence. Nevertheless, from the combined epidemiological and entomological data presented in this paper we conclude that the increased coverage of LLINs is probably among the main reasons of the substantial change in the malaria epidemiology profile in Suriname.

The historical successes of malaria control due to vector control are a motivation to have (again) an increased focus on the role of vectors in malaria transmission and on the opportunities for control and elimination. Considering the diversity of vector species, which can vary considerably in biting and resting behavior, and the occurrence of insecticide resistance

(Takken, 2002; Takken and Knols, 2009), the strategies for control worldwide move towards integrated vector management (IVM), combining the use of ITNs with other tools (Shiff, 2002; WHO, 2004; Fillinger et al., 2009). Success of a vector control strategy will depend on the appropriateness of control measures in a given situation. Knowledge of micro-epidemiology of malaria including ecology and behavior of the vector, social and cultural characteristics of the human population, and changes therein due to interventions or developments, should be guiding factors in deciding the course of action. IRS can be a powerful tool in malaria control, provided that its impact on the mosquito populations is continuously monitored by entomological surveillance, and possible insecticide resistance being detected in time (Sharp et al., 2007; N'Guessan et al., 2007; Pluess et al., 2010). IRS used in combination with other malaria control measures, has led to significant decreases of malaria incidence in, for instance, tropical Asia and South America including neighboring Venezuela and Guyana (WHO, 2006b). Successes with DDT and pyrethroids varied over time in different countries, depending on the changes in biting behavior and insecticide resistance of the vectors. The high costs of IRS programs, as well as the varying successes ultimately led to deterioration of the programs, which in turn led to resurgence of malaria in some countries (for instance in Sri Lanka; (PMI, 2008)). The re-introduction of IRS in Suriname by the MM-MP was based on high malaria incidence and high mosquito pressure in a specific malaria stratum of the country. Halting the IRS within the first year was a sensible decision, considering the decrease in mosquito biting intensity as well as in malaria incidence, the good acceptance of LLINs by the local population and the enormous logistic and financial resources involved in the execution of IRS.

In 2009, 82% of all malaria cases diagnosed in the country (including imported malaria) were carried out by the fixed (50%) and mobile MSDs (32%). This means that malaria cases are no longer reported predominantly from the village communities in the interior, but almost all originate from gold mining areas. MM health centers have a wide area of coverage, but are often out of reach of gold miners. The gold miners, about 15,000 people (WWF, 2005), generally do not seek malaria treatment due to their illegal status and/or the high local transportation costs. Low accessibility to diagnosis and treatment for these gold miners have resulted in a flourishing black market of anti-malarial and other drugs, often of insufficient quality. Gold mining communities are currently the populations most at risk for malaria in Suriname. Improved access to health services and/or malaria services (free adequate diagnosis and high quality effective antimalarial treatment) is necessary. The MM-MP introduced ACD in high risk areas, created new diagnostic points in mining areas and set up the fixed MSDs laboratory in Paramaribo, increasing the access to services, and thus decreasing the number of parasite carriers.

One of the most sensitive areas in terms of malaria control in Suriname is the eastern border region with French Guiana which includes the Upper-Marowijne and Lawa rivers. This area has a high malaria incidence and a semi-mobile population, with many gold miners working on the French Guianese side of the border, but seeking supplies, equipment and health care in Suriname. French Guiana has a hard line policy towards illegal gold mining communities. This is thought to be the cause of the significant number of malaria cases originating from French Guianese gold mining areas, which are treated at the fixed MSD laboratory in the capital of Suriname as these patients are not inclined to visit a health clinic in French Guiana.

The border area has been a focus for treatment and control efforts in both Suriname and French Guiana. Malaria control activities in Suriname led to a decrease of malaria in the French Guianese border region. Both countries recognize the need to come to unity in their approach of dealing with malaria. A cross-border initiative could be instrumental in preventing the re-introduction of malaria from French Guiana into Suriname.

Following the significant reduction of malaria in Suriname, national authorities evaluated the long term goal; elimination. The MoH and the National Malaria Board decided in 2010 to develop a malaria control and elimination Plan 2011-2015 (MC&EP) (Ministry of Health Suriname, 2011). The strategic vision is that the country will be malaria free by 2020 as a result of a full commitment of all stakeholders in further establishing and maintaining the RBM malaria control strategy. The most important strategic directions included in the MC&EP are improved malaria program management and coordination, prompt and adequate case management, evidence-based IVM, continued and directional BCC/IEC, further improvement of the health system integration and its measurement and access.

Our findings support the hypothesis that financial investment in key effective interventions can have significant impact in reducing and even eliminating malaria in countries with low transmission.

CONCLUSIONS

The success of the novel strategies for malaria control employed in the MM-MP in Suriname is evident through the significant reduction in the national malaria burden since their introduction in 2005. Malaria is reduced to pre-elimination levels in the stabile communities, even in previously high-risk areas. The communities considered most at risk nowadays are (mobile) gold miners, especially those working along the Suriname-French Guiana border. The challenge is to further reduce malaria using the available strategies as appropriate in the affected areas and populations. The target established by the Surinamese government to eliminate malaria in the country within a decade, requires a thorough understanding of transmission dynamics and a dedicated investment in key effective interventions. A bi-national approach towards controlling border malaria is necessary.

ACKNOWLEDGMENTS

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Summarizing Discussion

8

Worldwide the availability of funds for malaria control has increased, enabling countries to upgrade and intensify their malaria control activities. Disbursements to malaria endemic countries from agencies like the Global Fund to fight Aids, Tuberculosis and Malaria (GFATM), the US President's Malaria Initiative, the World Bank and others, have gone from 200 million USD in 2004 to 1.5 billion USD in 2009 (WHO, 2011), leading to an increase in malaria intervention activities, which in turn resulted in a progressive decrease of the geographical distribution of malaria (Wernsdorfer et al., 2009). This ongoing "shrinking of the malaria map" has in turn led to a new goal set by the funding agencies and global health organizations, i.e. to eliminate malaria (Das and Horton, 2010).

Suriname has a long history of malaria control with variable levels of success. Indoor residual spraying with DDT and dieldrin was instrumental in the elimination of malaria from the coastal area, but transmission remained in the interior (**Chapter 1**). The funding of a large 5-year malaria control program by the GFATM, the MM-MP in 2005 allowed Suriname to enter a new era of malaria control. New interventions were introduced and incorporated in a strategy which aimed for the most efficient use of the available new tools in combination with 'old' well-known methods. These malaria control activities and the resulting decrease in malaria incidence have led to a ranking of Suriname among nations with a potential for elimination (Tatem et al. 2010). The work presented in this thesis provides some insight into the changes these interventions brought for the *Anopheles darlingi* populations and for malaria incidence in the country.

ANOPHELES DARLINGI ECOLOGY AND BEHAVIOR

The first objective of this thesis was to obtain baseline data on behavioral characteristics, especially host-seeking and biting behavior, of *An. darlingi*, from selected sites in high-malaria risk areas in Suriname. A review of available data on *An. darlingi* throughout its area of distribution (**Chapter 2**) shows that this vector is especially important in the Amazon countries. It exhibits a high variability in breeding site and host selection and in feeding behavior and can adapt to changing environments. The *An. darlingi* population found in one location may very well show different biting peaks or seasonal patterns than a population found 40 kilometers away. Vector population surveillance is difficult due to a lack of efficient monitoring tools. The design of effective control strategies is hampered by the limited availability of information about the (local) characteristics and ecology of the vector.

Baseline-data on vector behavior and malaria transmission were obtained from several sites along the Marowijne (Maroni) River (**Chapter 5**). The data show the highly anthropophilic character of *An. darlingi*, but also confirm its behavioral variability between sites. It is the most abundant malaria vector found in human settlements, causing about 90% of all *Anopheles* bites. The second most common (potential) malaria vector biting the human population was *An. nuneztovari*. Population densities of *An. darlingi* reached their peak in the major rainy season. Biting occurred mainly during late night and early morning hours. Knowledge about the biting behavior of *An. darlingi* provides opportunities for prevention and control of malaria with the use of insecticide-treated nets (ITNs), a tool already employed on a small scale in Suriname before the onset of the MM-MP. The MM-MP included the free mass distribution of long-lasting impregnated nets (LLINs) as one of the new interventions in its control strategy.

Data obtained from sentinel sites during the MM-MP period (**Chapter 6**) confirm the characteristics found during the baseline study. They also show that in the sentinel sites about 40% of *An. darlingi* bites occur indoors. The majority of the information on *An. darlingi* behavior obtained during this longitudinal study was generated in 2006. In the following years the *An. darlingi* population densities were too low for this purpose. The human biting rates of *An. darlingi* ultimately decreased to zero in all three sites by 2008. Whether or not the LLINs were responsible for the mosquito population collapse is subject to discussion. There were a number of other events which may have had an impact on the mosquitoes, including a river flood and indoor residual spraying (IRS) in 2006. It is arguable that the combination of events will have led to the vector population collapse and that LLINs have played a significant part, possibly even by a community-wide killing of *An. darlingi*.

Anopheles nuneztovari females accounted for an overall 11.1% of the bites, but none were found infected with the malaria parasite.The role of *An. nuneztovari* in malaria transmission in the Guyanas, including Suriname, is unresolved. The species is an important malaria vector in several localities in Colombia, Peru and Venezuela and is suspected to play a minor role in transmission in Brazil (Hayes et al., 1987; Rubio-Palis and Curtis 1992b; Scarpassa et al., 1999). Gold mining in the Amazon forest results in excellent breeding sites for this species (H. Hiwat, unpublished data). These and other ongoing ecological changes may increase the importance of *An. nuneztovari* as a malaria vector. Further research and monitoring is necessary to determine if focused control of *An. nuneztovari* needs to be considered.

The current low population densities of *An. darlingi* in Suriname allow for a discussion about the need for continued vector surveillance and control. This is an ongoing discussion in countries in Latin America where malaria incidence is decreasing on a national or local scale (for instance among the member countries of the Amazon Malaria Initiative (AMI)). Considering the limited resources available in terms of (human) capacity and funds, a down-scaling of activities seems called for. The very limited information available about malaria vectors and our inability to predict population dynamics makes it necessary to continue vector surveillance in areas of low malaria risk even if vector control activities may be decreased. Informed decision making in vector control is necessary (Ferguson et al., 2010) and possible only through surveillance. Bundling capacity between national and international vector control programs by exchange of data, experiences and expertise is to be recommended to achieve an optimal use of the available capacity and resources in the control programs.

ALTERNATIVE METHODS FOR VECTOR SURVEILLANCE

The second objective of this thesis was to assess which mosquito surveillance method may be the most representative alternative for human landing catches to monitor densities and biting characteristics of the main malaria vector in Suriname. The baseline data and longitudinal data on *An. darlingi* described in this thesis (**Chapters 5** and **6**) were obtained using the human landing collection method (HLC). The most important advantage perceived for HLC when compared to other methods is that it provides a good estimate of the human biting rate of the malaria vectors. Its disadvantage is that, when the method is applied in a malaria endemic area, it may have risks for the collectors due to exposure to potentially infected vectors. This is something that on ethical grounds is becoming less and less acceptable to health and control authorities worldwide. Other disadvantages include its labor-intensiveness and high costs, and the potential for bias in the data due to differences in personal attractiveness of the collectors (Knols et al., 1995).

A number of alternative collection methods were evaluated and compared to HLC in their efficiency to collect *An. darlingi* in the interior of the country (**Chapter 3**) and *An. aquasalis* in the coastal area (**Chapter 4**). The collection tools tested for *An. aquasalis* (CDC Miniature light trap, BG Sentinel™ trap and Mosquito Magnet® Liberty Plus) were baited with carbon-dioxide. HLC was the most efficient method for collecting this species. The attractiveness of the carbon-dioxide baited traps was far less than the attractiveness of the humans in the HLC. This may indicate that carbon-dioxide is an insufficient stand-alone bait for this vector species in Suriname and that additional host-derived cues may be needed to improve the catches with traps. Human emanations are very attractive to this species, which could be the result of a (local) high degree of anthropophily.

The collection tools tested for *An. darlingi* (CDC Miniature light trap, BG Sentinel™ trap and Mosquito Magnet® Liberty Plus and a custom design trap) were baited with carbon-dioxide or with protected humans. Again, HLC was the most efficient collection method. *Anopheles darlingi* showed no difference in preference for humans or carbon dioxide as bait in the alternative methods. Ground-level traps (BG Sentinel™ and Mosquito Magnet® Liberty Plus) performed better than the higher-level CDC Miniature traps.

An additional study on preferred biting sites on the human hosts (**Chapter 3**) shows that when confronted with a seated human being, the vector has a defined preference for biting its feet. It is hypothesized that this could be the result of a preference for foot odors or for ground-level biting. Considering the trap results and the preference of *An. darlingi* to bite the foot regions when confronted with a seated human being, it may be advantageous to further study the biting preferences of this important vector. Knowledge about the specific cues that attract females to their host can be applied in the design of an efficient alternative collection method. Similar studies are already employed in Africa for *An. gambiae* with promising results for the improvement of collection tools (Schmied et al., 2008; Okumu et al., 2010). Easy-to-use collection methods like the BG sentinel trap, could, in combination with species-specific attractive lures, become valuable tools for the surveillance of host-seeking (female) malaria vectors.

A satisfactory alternative monitoring tool for host-biting *An. darlingi* females, to replace HLC, has not yet been found. Still, the alternative trapping tools evaluated in this study might, if enhanced with more attractive bait, be used as a proxy for HLC. Studies on alternative trapping tools are ongoing (see for instance Dusfour et al., 2010), but until a suitable alternative is found, HLC remains the mosquito surveillance tool that provides the best depiction and characterization of the vector population targeting humans in a specific area. Though concerns have been expressed with regards to the perceived increased risk for collectors associated with the use of HLC, actual evidence to support this is not available. Considering the awareness of the personnel involved in surveillance programs this conceived risk may be much less than envisioned. On the other hand, failure to conduct adequate and timely entomological assessments may pose a greater risk of malaria transmission to the local populations. For this reason and until a suitable alternative surveillance method is found, the use of HLC is to be recommended to detect and monitor malaria vector populations in a specific area.

IMPACT OF THE MALARIA CONTROL PROGRAM

The third objective identified for this thesis was to study the effect of malaria and vector control measures on the *An. darlingi* populations and malaria transmission dynamics in Suriname. A longitudinal vector study in three sentinel sites (**Chapter 6**) shows that the *An. darlingi* populations in these sites collapsed after the introduction of LLINs by the MM-MP. The human biting rates of *An. darlingi* decreased to zero in all three sites. At the same time malaria transmission decreased both in the sites and nationwide to such an extent that Suriname reached its Millennium Development Goal for malaria in 2007. The strategy of combining new and 'old' malaria control methods, as employed in the MM-MP, has led to near elimination of malaria in Suriname (**Chapter 7**). After a slow first year, the MM-MP fully engaged in delivering the interventions and kept up its performance until the end of 2009. Besides the free mass-distribution of LLINs (and (re-impregnation of nets) the strategy included improved (access to) diagnosis and treatment, IRS, improved case management (including active case detection and the introduction of Malaria Service Deliverers (MSDs)), improved public awareness and education, and the development of an efficient epidemic detection and response system.

As a result of the MM-MP malaria transmission risk decreased and shifted from the village communities (stabile population) towards gold mining communities (mobile populations). The introduction of MSDs in remote gold mining areas is a new pilot strategy which significantly decreased the distance from the major gold mining communities, where the strategy was employed, to diagnosis and treatment. Access to healthcare is a common problem in Latin American countries, not only for gold mining communities, but also for indigenous people (Grenfell et al., 2008). Training local people to provide diagnosis and treatment under (distant) supervision is a viable option to deal with this problem. The pilot showed promising results and efforts will be made to continue and improve the activity. The establishment of a "fixed" MSD point in the heart of the, mostly illegal, garimpeiro communities in the capital of Suriname was an undeniable success and just proves the importance of easy access to health care.

The MM-MP was preceded by introduction of Coartem (artemether and lumefantrine) as a first line treatment for *P. falciparum* infections, which is thought responsible for the onset of the decrease in malaria incidence in 2004. Adequate drugs are needed to combat malaria. By improving access to (free) diagnosis and treatment, the use of (expensive) black-market drugs will decrease, which in turn will decrease the risk of development of drug resistance by the parasites.

The impact of malaria control activities in Suriname was not only apparent in Suriname but also in French Guiana where a significant decrease of malaria incidence was observed along the Marowijne (Maroni) and Lawa Rivers, which constitute the border between the two countries. In 2006 the malaria cases along these rivers accounted for 77.2% of the national total of French Guiana (INVS, 2006). In 2011 the border area is considered a low malaria risk area except for foci in Maripasoula and Antecumpata (Fig. 8.1). Malaria incidence along the Oyapock River, border between French Guiana and Brazil, remains comparably high (142 cases reported within the first 16 weeks of 2011; data La Cire Antilles-Guyane, Cayenne, French Guiana). Data of the fixed MSD point in Paramaribo, Suriname, show that a significant part of the garimpeiros diagnosed and treated in this laboratory obtained their malaria infection in French Guiana, where they work in (illegal) gold mining areas. This import of malaria is becoming increasingly important for Suriname, which is entering the pre-elimination phase. Pro-active prevention of the import of malaria, including border screening of migrants and the development of regional initiatives is essential for a successful strategy of countries considering malaria elimination (Moonen et al., 2010).

Both countries are aware of the need for, and the opportunities which arise from, bi-national cooperation. This has resulted in a more frequent interaction on different levels of authority with regard to health in general and malaria control in particular.

MALARIA IN SURINAME; A NEW ERA

The success of the novel malaria control strategies of the MM-MP in Suriname is evident through the reduction in the national malaria burden to pre-elimination levels since its introduction. The current situation allows for a new vision, as proclaimed by the Ministry of Health in Suriname (MoH): a vision of malaria elimination in the country. With this proclamation the Surinamese government decides against the alternative to have controlled low-endemic malaria and commits itself to long-term political and financial investments in order to achieve and maintain malaria elimination. As a starting point a Malaria Control and Elimination Plan 2011-2015 (MC&EP) was developed (Ministry of Health Suriname, 2011).

Suriname is, with Belize, Honduras and the Dominican Republic, ranked among countries with a high technical and operational feasibility to eliminate *Plasmodium falciparum* and a moderate feasibility to eliminate *Plasmodium vivax* (Tatem et al., 2010). Combined with the nationwide reduction in malaria cases and the continued progress towards local elimination in areas of the interior, this allows Suriname the label of malaria eliminating country.

Malaria elimination is not achieved easily, otherwise the world would be a different one today. It is not something that Suriname can do by itself or that can be achieved with the strategies as defined in the MM-MP. A continued mind-shift towards evidence-based decisions in malaria control and the development of additional activities to promptly eliminate malaria foci is necessary. The current transmission in mobile gold mining communities and the continued import of malaria cases, especially from French Guiana, are important challenges which will require specific intervention measures. Ongoing financial and technical support will be needed to overcome these and future challenges.

The (local) collapse of *An. darlingi* populations during the MM-MP was advantageous for malaria control, but malaria vector populations still exist in the country and recovery of the populations impacted by the MM-MP interventions or by other events is to be expected. As became apparent in this thesis, information on the ecology and behavior of the malaria vector *An. darlingi* in Suriname is very limited. Obviously, it is hard to define an efficient control strategy against a vector for which the details of the life cycle, role in transmission, adaptability to environmental changes, and feeding and resting behavior are not known. And if an effective strategy has been identified, it may well become ineffective over time due to the vectors developing behavioral avoidance or insecticide resistance or due to a changing role of secondary vectors. Understanding the ecology and evolution of the malaria vectors is a key factor in malaria control programs (Ferguson et al., 2010). Investing in long-term vector ecology studies is therefore of strategic importance and needs to be part of the control strategy in Suriname.

The impact of the globally increased availability of funds for malaria control is evident in the large number of countries that achieved a more than 50% reduction in malaria cases between 2000 and 2009 (WHO, 2011). A number of countries in Latin America have achieved this, but Suriname is the only Amazon country to reduce malaria to this extent. The challenge will be to further reduce malaria using available and new strategies as appropriate in the affected areas and populations.

GENERAL CONCLUSIONS

Anopheles darlingi is one of the most important malaria vectors in the Americas. Its close relation with the human host and its behavioral variability and adaptability make it an extremely competent vector, which can transmit malaria even in very low population densities. Knowing its characteristics and role in transmission will help us define control measures. Human landing collections still are the "golden standard" for the surveillance of its human biting rate and will be necessary until an acceptable alternative methodology is identified. Research into the specific host-related cues involved in host-seeking behavior of the *An. darlingi* may be instrumental in developing alternative trapping methods which prove sufficiently attractive to the females.

The malaria burden in Suriname has decreased to near-elimination levels during the implementation of the MM-MP. The mass introduction of LLINs was one of the new tools incorporated in the strategy of combining 'old' en new methodologies for malaria control. *Anopheles darlingi* populations in sentinel sites collapsed after the introduction of the LLINs and have not recovered since. Current malaria "hot spots" are found in the mobile gold mining communities, especially along the Marowijne and Lawa Rivers. The remaining malaria cases recorded in Suriname include a significant amount of import malaria, mostly from migrant gold miners working in French Guiana, but commuting to and trading in Suriname.

The Surinamese government has proclaimed a vision of malaria elimination in the country. Progressive decrease of malaria toward elimination will need a thorough understanding of malaria transmission dynamics, including vector ecology and evolution, and a dedicated investment and prompt application of key effective interventions. Once (local) elimination has been achieved, efforts will need to be made to prevent re-introduction of malaria. This will require a continued investment in interventions in risk areas and a continued mind-shift of both authorities and local communities towards this goal.

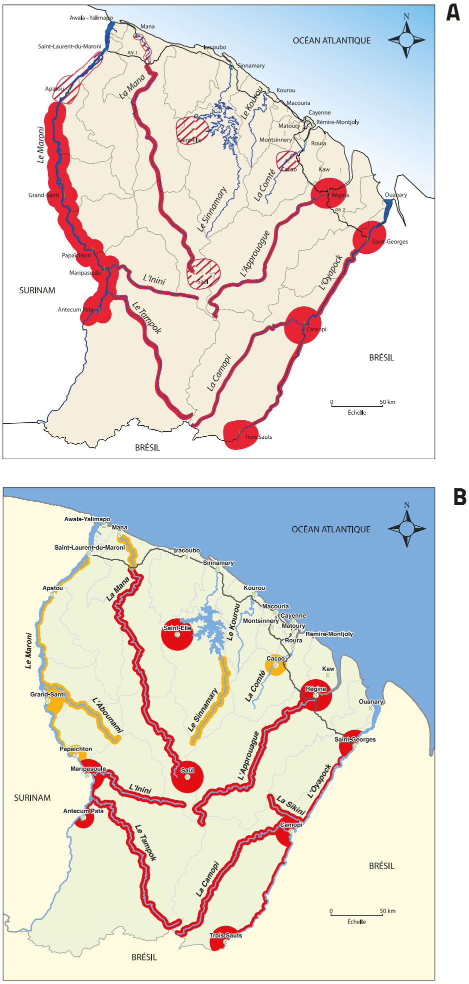

FIGURE 8.1: *Areas of high malaria risk (solid red) in French Guiana as identified in September 2007* ***(A)*** *and March 2011* ***(B)****. After maps by DSDS and La Cire Antilles-Guyane, Cayenne, French Guiana. (Source: Institut de Veille Sanitaire, www.invs.sante.fr).*

Abbreviations

Commonly used abbreviations in this thesis are:

ACD	Active Case Detection
ACT	Artemisinin-based Combination Therapy
API	Annual Parasite Index
BBC/IEC	Behavioral Change Communication/Information, Education and Communication
BOG	Bureau of Public Health (Bureau Openbare Gezondheidszorg)
BOG/AMC	Bureau of Public Health Anti-Malaria Campaign
CCM	Country Coordinating Mechanism
GFATM	Global Fund to fight AIDS, Tuberculosis and Malaria
HLC	Human Landing Collections
IRS	Indoor Residual Spraying
ITN	Insecticide-Treated Nets
IVM	Integrated Vector Management
LLIN	Long-Lasting Insecticide-treated Nets
MC&EP	Malaria Control and Elimination Plan
MDG	Millennium Development Goals
M&E	Monitoring and Evaluation
MM	Medical Mission (Medische Zending)
MM-MP	Medical Mission Malaria Program
MoH	Ministry of Health
MoH-MP	Ministry of Health Malaria Program
MSD	Malaria Service Deliverer
PAHO	Pan American Health Organization
RBM	Roll Back Malaria
RDT	Rapid Diagnostic Test

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SAMENVATTING

Malaria is een infectieziekte die wordt veroorzaakt door *Plasmodium* parasieten. Deze parasieten leven in het bloed van hun gastheer en worden verspreid door *Anopheles* muskieten. Jaarlijks raken zo'n 225 miljoen mensen in de wereld besmet met malaria en sterven er bijna 800.000 mensen aan deze ziekte. Bestrijding van malaria is een complexe aangelegenheid die gericht is op alle drie actoren die betrokken zijn bij de transmissie en op hun omgeving. De mens als gastheer speelt door haar mobiliteit een belangrijke rol in de verspreiding van de ziekte. De *Anopheles* muskieten, ook mobiel, kunnen zich aanpassen aan nieuwe leefomstandigheden en zijn in staat om door middel van bijvoorbeeld het ontwikkelen van insecticide-resistentie het effect van bestrijdingsmiddelen te ondermijnen. De *Plasmodium* parasieten hebben een geweldig aanpassingsvermogen en kunnen op den duur resistentie ontwikkelen tegen de toegepaste medicatie, waardoor de beschikbare malaria-behandelingen ineffectief worden. Het vinden van nieuwe methoden en strategieën om malaria te bestrijden en te elimineren is een voortdurende uitdaging. De geschiedenis leert ons dat malaria bestrijding een zeer veelzijdige aanpak behoeft. Tegenwoordig bestaan malaria bestrijdings-strategieën uit een complex van maatregelen die elkaar versterken en aanvullen. Nieuwe methoden zijn in ontwikkeling.

In 2005 werd in Suriname een nieuw 5-jarig malaria-bestrijdingsprogramma opgestart. Daarin werden een aantal reeds bekende, maar ook verbeterde en nieuwe bestrijdingsmethoden en -strategieën uitgevoerd. De studies in dit proefschrift beschrijven het effect van deze methoden en strategieën op de malaria incidentie en de *Anopheles darlingi* populaties in Suriname en evalueren enkele muskieten-monitoringsmethoden.

Sinds het begin van de vorige eeuw is Suriname actief in de malaria-bestrijding. De bestrijdingscampagnes in de periode 1950-1960 waren zo succesvol dat Suriname erin slaagde malaria in de kustvlakte te elimineren. In het binnenland bleef malaria een probleem en de malaria incidentie nam zelfs toe (**Hoofdstuk 1**). *Anopheles darlingi*, de voornaamste malaria vector in Suriname, speelt een grote rol in de transmissie. Deze muskiet is een belangrijke vector in haar gehele verspreidingsgebied binnen Zuid- en Centraal Amerika. Mede door haar preferentie voor menselijk bloed is zij bijzonder effectief in het overbrengen van malaria, zelfs in lage populatie-dichtheden. Vanwege haar gevarieerd gedragspatroon en een uitstekend aanpassingsvermogen, en onze beperkte kennis daarover, is het erg moeilijk deze vector te bestrijden (**Hoofdstuk 2**).

De dichtheid van bijtende *Anopheles*-muskieten per persoon is een belangrijke factor in het bepalen van het risico van malaria transmissie. Deze dichtheid wordt traditioneel vastgesteld door de muskieten die op de onderzoeker landen om te bijten te vangen, te identificeren en te tellen; de zogenaamde "human landing collecties". Meestal worden de muskieten gevangen voordat ze bijten, maar het is duidelijk dat deze methode een risico van malaria-infectie voor de onderzoeker met zich kan meebrengen wanneer het wordt uitgevoerd in malaria-endemische gebieden. Bovendien is het een zeer arbeidsintensieve en dure methode. Alternative methoden voor muskietenvangst zijn daarom nodig. Het vermogen van de CDC Miniature lichtval, de BG Sentinel™-val en de Mosquito Magnet® Liberty Plus val om als alternatieve methode ter vaststelling van de muskietendichtheid en bijtdruk te dienen, werd gestest voor zowel *An. darlingi* (**Hoofdstuk 3**) als *Anopheles aquasalis* muskieten (**Hoofdstuk 4**). Daarbij werd CO_2 of een (door een klamboe beschermde) mens als lokmiddel gebruikt. Hoewel met name de BG Sentinel ™ en de Mosquito Magnet® Liberty Plus vallen potentie toonden voor de vangst van *Anopheles* muskieten, werd geen van de genoemde valmethoden effectief bevonden ter vervanging van de human landing collecties. De resultaten doen vermoeden dat het gebruik van uitsluitend CO_2 niet voldoende is als lokstof voor de betreffende *Anopheles* soorten. Andere lokstoffen, zoals van de mens-afgeleide geurstoffen, kunnen mogelijk de resultaten van deze vallen verbeteren, waardoor de muskieten-monitoring met minder risico's en kostenbesparend kan worden uitgevoerd. De BG Sentinel™-val bleek efficiënt in het vangen van *Culex* muskieten.

De belangrijkste veranderingen in de malariabestrijdingsstrategie in Suriname vanaf 2004 waren 1) de introductie van een nieuwe op Artemisinin gebaseerde eerste-lijn behandeling voor *Plasmodium falciparum* infectie, kort voor aanvang van het nieuwe malaria programma, en 2) de distributie van gratis, duurzaam geïmpregeneerde klamboes (long lasting insecticide-treated nets; LLINs) aan de gemeenschappen in het hoge risico-gebied. Andere maatregelen waren het uitvoeren van een huisbespuitings-campagne in het hoge risicogebied, het actief opsporen van malaria-patiënten (met name voor de detectie van a-symptomatische infecties) en het trainen van lokale malaria-dienstverleners ("Malaria Service Deliverers") in afgelegen gebieden.

Het effect van het malariabestrijdingsprogramma op de muskieten-populaties werd geanalyseerd in een 4-jarige onderzoek in drie studie-locaties, met behulp van human landing collecties. De resultaten hiervan werden vergeleken met muskietengegevens die waren verzameld in het grensgebied met Frans Guyana voor aanvang van het nieuwe malaria programma (**Hoofdstuk 5**). De *An. darlingi* populaties in de studie-locaties stortten kort na de aanvang van het malariaprogramma in en herstelden zich niet in de daarop volgende jaren (**Hoofdstuk 6**). De mogelijke impact van de geïmpregneerde klamboes op de muskietenpopulaties wordt besproken. Met de beperkte beschikbare kennis over de redenen voor de afname van de *An. darlingi* populaties en over de ecologie van *An. darlingi* in het algemeen, is het niet mogelijk voorspellingen te doen over de populatie dynamica van deze vector. De populatie-dichtheden bleven gedurenden de gehele onderzoeksperiode laag in de studie-locaties, maar een toename van de populatiedichtheid zou op elk moment kunnen plaatsvinden als gevolg van wijzigende omstandigheden. Een voortdurende monitoring van de vectoren en verdere studies naar het bijtgedrag en de ecologie van de vector is daarom noodzakelijk.

De nieuwe malariabestrijdingsstrategie leidde tot een significante afname van malaria in de dorpen in het binnenland, waardoor Suriname in staat was de Milleniumdoelstellingen (United Nations Millenium Development Goals) voor malaria reeds in 2007 te bereiken (**Hoofdstuk 7**). De malaria situatie in Suriname ontwikkelde zich tot de huidige status waarbij de ziekte bijna volledig onder controle is in de stabiele dorpsgemeenschappen in het binnenland, waar malaria is gedaald tot een pre-eliminatie niveau. Dit is een belangrijk succes, maar kan Suriname deze status handhaven? Transmissie vindt nog steeds plaats in de mobiele gemeenschappen in het bos, met name onder de goudmijners. Bovendien is het grensgebied met Frans Guyana erg kwetsbaar door grensoverschrijdende verplaatsingen van mensen. De nieuwe uitdaging voor Suriname is om malaria verder te bestrijden en mogelijk (lokaal) te elimineren door het uitvoeren van een geïntegreerde malariabestrijdingsstrategie met een sterk malaria-monitoringssysteem en snelle interventies in gebieden waar malaria opnieuw opkomt. Hiervoor zijn financiele middelen, toewijding en regionale samenwerking noodzakelijk (**Hoofdstuk 8**).

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Most of the entomology work presented here was done in cooperation with the Entomology Department of the Bureau of Public Health in Suriname.

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Your father and I enjoy so much seeing you grow and find your own interests and joys in life. I hope this work will help you realize that learning is a never ending story. By learning and by keeping to the values of life I am sure you will grow into the fine young men we sincerely hope you will become.

Hélène Hiwat – Van Laar
November, 2011

Curriculum Vitae (CV)

Hélène Hiwat - Van Laar was born in Veenendaal, The Netherlands, in 1973 and grew up in Kesteren. She finished secondary school at the Ichthus College Veenendaal in 1991, after which she started her biology study at the Wageningen University. Hélène graduated in 1996 with a thesis on Digestive strategies and cecal fermentation in rabbits and European hares and a second thesis, done in cooperation with the University of Utrecht, on Resource partitioning of stingless bees in Suriname. After graduation Hélène joined her husband in Suriname and started working as a Curator of Invertebrates at the National Zoological Collection (NZCS), which is part of the Anton de Kom University of Suriname. During the next 8 years Hélène participated in, initiated and coordinated entomological biodiversity research in the interior of Suriname. She also participated in a number of international conferences on biodiversity and entomology. In 2005 she started as a vector entomologist in the Medical Mission Malaria Program (MM-MP), a 5-year national program supported by the Global Fund to fight AIDS, Tuberculosis and Malaria (GFATM). Considering that she lacked the experience in mosquito research Hélène decided to make her work-activities part of a PhD study on the ecology of *Anopheles darlingi*, the most important malaria vector in Suriname. This was supported by the Laboratory of Entomology at Wageningen University and supervised by Prof. Dr. Willem Takken and Prof. Dr. Marcel Dicke. The results of this study are presented in this thesis. The entomological fieldwork was done in cooperation with Entomology Department of the Bureau of Public Health (BOG; Ministry of Health) in Suriname. Since 2007 Hélène is acting head of this department.

The MM-MP was very successful in controlling malaria in the interior of Suriname and resulted in Suriname receiving the "Malaria Champions of the Americas" award by the Pan American Health Organization in 2010. Since 2009 Hélène participates in the follow-up malaria control project in Suriname, which is again supported by the GFTAM. This project, called Ministry of Health Malaria Program, has an aim to decrease the incidence of malaria in the mobile populations of the gold mining areas. For both the new malaria program and the MM-MP Hélène participated, as a national representative for Suriname, in regional entomology meetings of RAVREDA (Amazon Network for the Surveillance of Anti-malarial Drug Resistance) and AMI (Amazon Malaria Initiative) and in annual meetings of the ASTMH (American Society of Tropical Medicine and Hygiene). She also received training by CDC (Centres of Disease Control, Atlanta USA) specialists in various aspects of mosquito monitoring and control, and participated in a short course on Integrated Vector Management at the London School for Hygiene and Tropical Medicine. Hélène will continue working in vector entomology in Suriname.

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List of Publications

PUBLISHED

Hiwat H and Bretas G: Ecology of *Anopheles darlingi* Root with respect to vector importance: a review. Parasites and Vectos 2011, 4:177.

Hiwat H, De Rijk M, Andriessen R, Koenraadt CJM, Takken W. Evaluation of methods for sampling the malaria vector *Anopheles darlingi* (Diptera, Culicidae) in Suriname and the relation with its biting behavior. Journal of Medical Entomology 48(5):1039-1046. 2011. (Presented in poster format at the ASTMH Annual Meeting 2010)

Hiwat H, Andriessen R, De Rijk M, Koenraadt CJM, Takken W. Carbon dioxide baited traps do not correlate with human landing collections of Anopheles (Nyssorynchus) aquasalis in Suriname. Memorias do Instituto Oswaldo Cruz 2011, 106: 360-364

Hiwat H, Issaly J, Gaborit P, Somai A, Samjhawan A, Sardjoe P, Soekhoe T, Girod R. Behavioral heterogeneity of *Anopheles darlingi* (Diptera Culicidae) and malaria transmission dynamics along the Maroni River, Suriname, French Guiana. Transaction of the Royal Society of Tropical Medicine and Hygiene 2009, 104: 207-213

SUBMITTED

Hiwat H, Mitro S, Samjhawan A, Sardjoe P, Soekhoe T, Takken W. Collapse of *Anopheles darlingi* populations in Suriname after introduction of insecticide-treated nets (ITNs); malaria down to near elimination level. (To be presented orally at the ASTMH Annual Meeting 2011)

Hiwat H, Hardjopawiro L, Takken W, Villegas L. Novel strategies lead to near elimination of malaria in previously high-risk areas in Suriname, South America. (To be presented in poster format at the ASTMH Annual Meeting 2011)

PE&RC PhD Education Certificate

With the educational activities listed below the PhD candidate has complied with the educational requirements set by the C.T. de Wit Graduate School for Production Ecology and Resource Conservation (PE&RC) which comprises of a minimum total of 32 ECTS (= 22 weeks of activities).

REVIEW OF LITERATURE

- Ecology of *Anopheles darlingi* with respect to vector importance: a review (2006)

WRITING OF PROJECT PROPOSAL

- The effect of malaria vector control measures on the behavioural characteristics of the main malaria vector, *Anopheles darlingi*; Suriname, South America (2005)

POST-GRADUATE COURSES

- Basic and advanced statistics; WUR (2007)

LABORATORY TRAINING AND WORKING VISITS

- CDC Bottle method for insecticide resistance testing; Medical Mission Malaria Program; CDC, Altanta (2005)
- Mosquito identification and dissection; Medical Mission Malaria Program (2006)
- Mosquito ELISA for plasmodium infection; Medical Mission Malaria Program (2006)
- Anopheles larvae collection, breeding, identification and conservation; Ministry of Health Malaria Program, Suriname (2009)
- Use of colorimetric test for insecticide levels on bed nets; PAHO-Suriname, CDC, Altanta (2010)

DEFICIENCY, REFRESH, BRUSH-UP COURSES

- SPSS; ICTS Training Bureau Suriname (2008)
- Tableau visual data analysis; Ministry of Health Malaria Program, Suriname (2009)
- Integrated vector management; London School of Hygiene and Tropical Medicine (2011)
- Basic science pre-meeting course; American Society of Tropical Medicine and Hygiene (2010)

COMPETENCE STRENGTHENING / SKILLS COURSES

- Basic strategies for Insecticide Residual Spraying (IRS) programmes; Medical Mission Malaria Program (2006)

- Building and Maintenance of a vector reference collection; Medical Mission Malaria Program (2007)
- Scientific writing and presenting; WUR (2007)

DISCUSSION GROUPS / LOCAL SEMINARS / OTHER SCIENTIFIC MEETINGS

- Malaria Control Meetings; Malaria Board Suriname (2006-2011)
- *Anopheles darlingi* along the Maroni River; Institute Pasteur Cayenne (2007, 2010)
- YELREM (2008)

INTERNATIONAL SYMPOSIA, WORKSHOPS AND CONFERENCES

- Reunion de la Iniciativa Intergubernamental de Vigilancia y prevencion de la Enfermedad de Chagas en la Amazonia (AMCHA); Cayenne, French Guiana (2005)
- Reunion Anual Conjunta de la Iniciativa Andina de Control de la Enfermedad de Chagas (IPA) y de la Iniciativa de Vigilancia y Prevención de la Enfermedad de Chagas en la Amazonia (AMCHA); Quito, Ecuador (2006)
- Ravreda/AMI Meeting on the use of entomology in malaria control programs in the America: Reunion sobre el uso adecuado de la entomología en los programas de control de malaria de las Américas; Panama, Panama (2006)
- Malaria cross-border meetings Suriname-French Guyana; Cayenne and St. Laurent, French Guiana / Paramaribo, Suriname (2006, 2007, 2011)
- Annual meeting of the American Society of Tropical Medicine and Hygiene; Atlanta, USA (2006, 2010)
- Workshop on the use of Bioflash (mosquito larval control agent Bti); Qeshm, Iran (2008)
- Annual meeting of the Caribbean Health and Research Council (CHRC); Paramaribo, Suriname (2008)
- Ravreda/AMI Technical meeting on procedures and management of entomological information; Guyaquil, Ecuador (2008)
- AMI Technical Meeting on vector control for Latin America; CDC, Atlanta, USA (2010)

COURSES IN WHICH THE PHD CANDIDATE HAS WORKED AS A TEACHER (2 HRS. WEEKLY/ 3 MONTHS/PER YEAR)

- Medical Entomology; Medical Faculty, University of Suriname (1998, 2002-2005)
- Zoology; Faculty of Technological Sciences, University of Suriname (2005)

SUPERVISION OF 2 MSC STUDENTS (4 MONTH STUDY)

- Trapping methods for and biting preferences of *Anopheles darlingi* mosquitoes