AN ECO-EPIDEMIOLOGICAL STUDY OF FISH FARMING AND MALARIA OCCURRENCE IN THE PERUVIAN AMAZON

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PREFACE

This thesis is formatted as a 'Manuscript-Based Thesis'. It follows the guidelines of the 'Graduate and Post-Doctoral Studies Office' presented below:

> 'As an alternative to the traditional thesis format, the candidate has the option to submit a 'Manuscript-Based Thesis'. This thesis format consists of a collection of one or more papers submitted, or to be submitted, for publication, or the clearly-duplicated text (not the reprints) of which the student is an author or co-author.'
>
> 'The thesis must be more than a collection of manuscripts. All components must be integrated into a cohesive unit with a logical progression from one chapter to the next.'
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> 'When co-authored papers are included in a thesis, the candidate must be the primary author for all papers included in the thesis. In addition, the candidate is required to make an explicit statement in the thesis as to who contributed to such work and to what extent. This statement should appear in a single section entitled "Contributions of Authors" as a preface to the thesis.... The supervisor must attest to the accuracy of this statement.'

This thesis contains one manuscript (Chapter 5). Details on the contributions of each author are outlined on page vii. Some additional results originating from this thesis research have been presented or have been submitted for presentation at scientific meetings. These include the following communications:

- **Maheu-Giroux, M.**, M. Casapia, V.E. Soto-Calle, L. Berrang Ford, D.L. Buckeridge, O.T. Coomes and T.W. Gyorkos. Are fish farming activities contributing to malaria transmission in the Peruvian Amazon? *58th Annual Meeting of the American Society of Tropical Medicine and Hygiene.* Washington, DC (18-22/11/2009) – submitted.

- **Maheu-Giroux, M**., M. Casapia, and T.W. Gyorkos. Adjusting for socio-economic status to estimate a measure of effect in low-income communities – the case of aquaculture and malaria in the Peruvian Amazon. *16th Annual Canadian Conference on International Health.* Ottawa, ON (27/10/2009) – accepted.
- **Maheu-Giroux, M**., M. Casapia, and T.W. Gyorkos. On the validity of self-report in ascertaining malaria prevalence in remote rural communities of the Amazon Basin, Peru. *6th European Congress on Tropical Medicine and International Health.* Verona, Italy (09/09/2009) – accepted.
- **Maheu-Giroux, M**., M. Casapia, and T.W. Gyorkos. Evidence informing a health policy focusing on bathtime to reduce malaria incidence in rural communities of the Peruvian Amazon. *30th Meeting of the Society of Behavioral Medicine.* Montréal, QC. (22/04/2009) – presented.
- **Maheu-Giroux, M**., M. Casapia, and T.W. Gyorkos. Is the landscape context of rural households related to malaria transmission in the Peruvian Amazon? *US – International Association of Landscape Ecology Meeting.* Snowbird, UT. (14/04/2009) – presented.

ABSTRACT

The contribution made by fish ponds to malaria transmission in the Peruvian Amazon is controversial because previous research results have yielded mixed evidence and because the mosquito vector is known to have breeding requirements not typical of fish ponds. To examine the association between fish pond density and malaria occurrence, a retrospective 30-month cohort study was conducted involving 1,018 individuals in eight communities southwest of Iquitos (Peru), using both active and passive malaria surveillance data. Fish pond density (perimeter method) was found to be a significant predictor of malaria occurrence (aOR = 1.23; 95% CI: 1.10-1.38). An estimated 46% (95% CI: 24%-69%) of malaria cases could be attributed to exposure to fish ponds at the population level, if causally linked. These results have important implications for the prevention and control of malaria and the development of aquaculture as an important economic activity in Amazonia and beyond.

RÉSUMÉ

La contribution présumée des étangs piscicoles à la transmission de la malaria en Amazonie Péruvienne est matière à controverse puisque les recherches antérieures n’ont pas été conclusives. De plus, les caractéristiques des habitats de reproduction du principal vecteur de la malaria dans cette région ne sont pas typiques des étangs piscicoles. Afin d’examiner l’association entre la densité de ces étangs piscicoles (méthode du périmètre) et l’occurrence de la malaria, une étude de cohorte rétrospective de 30 mois, incluant 1 018 individus, a été conduite. Les résultats suggèrent que la densité des étangs piscicoles a un effet important sur la transmission de la malaria (aOR = 1.23; IC à 95%: 1.10-1.38) et qu’environ 46% (IC à 95%: 24%-69%) de ces cas peuvent être attribués aux étangs piscicoles, en assument un lien de causalité. Ces résultats ont d’importantes implications pour la prévention et le contrôle de la malaria ainsi que pour le développement de cette activité d’importance économique en Amazonie.

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The first person I would like to acknowledge is my local supervisor in Peru, Dr. Casapia, for finding time for me in his busy schedule. I appreciated his enthusiasm for research and his dedication to the people of Loreto. In addition, I would like to mention the members of my thesis committee (Dr. Berrang Ford, Dr. Buckeridge, and Dr. Coomes) for their support and constructive criticisms.

I would also like to extend my gratitude to the following organizations: the ‘Asociación Civil Selva Amazónica’ and more specifically Evelyn Burga and Salome Chapiama for their logistical support; the PAMAFRO team (‘Control de la Malaria en las Zonas Fronterizas de la Región Andina: Un Enfoque Comunitario’) for their insights and for sharing their vast experience with me; members of the ‘Dirección regional de Salud’ of Loreto for their valuable suggestions; and most importantly the communities involved in the research for their time, help, patience, sense of hospitality, guidance, and for instructing me so much about the ‘selva’.

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CONTRIBUTIONS OF AUTHORS

The original research idea was developed by Mr. Maheu-Giroux following discussions with Dr. Theresa Gyorkos, Dr. Martin Casapia, and Serene Joseph during a workshop of the 'International Development Research Center' held in Montevideo (Uruguay).

Mr. Maheu-Giroux wrote the initial research protocol which was subsequently edited and modified by Dr. Gyorkos and Dr. Casapia. This protocol was presented to Dr. Berrang Ford, Dr. Buckeridge, and Dr. Coomes who provided insightful suggestions. Dr. Soto-Calle also offered precious advice regarding the protocol, the data collection, and the logistics of the field work.

Under the supervision of Dr. Gyorkos and Dr. Casapia, Mr. Maheu-Giroux developed the questionnaire (with help from Dr. Soto-Calle), piloted it, abstracted the data from the malaria registries (with help of field assistant J.T. Rojas), contacted community leaders and stakeholders (with help of J.T. Rojas), conducted the interviews in Spanish, performed the data entry, photo-interpreted and analyzed the remote sensing image, integrated the databases in the GIS, carried out the statistical analyses, and interpreted the results.

All chapters were written by Mr. Maheu-Giroux and suggestions for revisions were made by Dr. Gyorkos. The fifth chapter includes the manuscript entitled 'Risk of Malaria Transmission and Fish Farming in the Peruvian Amazon' which will be submitted to a peer-reviewed journal. This manuscript was written by Mr. Maheu-Giroux and revised by Dr. Gyorkos, Dr. Casapia, Dr. Soto-Calle, Dr. Berrang-Ford, Dr. Buckeridge, and Dr. Coomes.

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LIST OF ABBREVIATIONS

aOR	Adjusted Odds Ratio
CI	Confidence Interval
DIRESA	Dirección Regional de Salud, Loreto, Peru
FONDEPES	Fondo Nacional de Desarollo Pesquero
GEE	Generalized Estimating Equations
GIS	Geographic Information Systems
GPS	Global Positioning System
MINSA	Ministerio de Salud, Peru
MOH	Ministry of Health
MUHC	McGill University Health Centre
OR	Odds Ratio
PAHO	Pan American Health Organization
PAMAFRO	Control de la Malaria en las Zonas Fronterizas de la Región Andina: Un Enfoque Comunitario
QIC	Quasi-likelihood under the Independence Criterion
SES	Socio-Economic Status
WHO	World Health Organization

1- CHAPTER ONE: Introduction

The Peruvian Amazon was the last area of the greater Amazon region to have experienced the dramatic re-emergence of malaria that affected the South American continent in the 1980s (Aramburú *et al.* 1999). Malaria was considered nearly eradicated from the Peruvian Amazon in the 1960s (Velimirovic 1968) but it is now one of the most important public health problems in this country, with the vast majority of cases being reported from the region of Iquitos (Department of Loreto) (Aramburú *et al.* 1999). Throughout this region, the two most pathogenic of the *Plasmodium* parasites which cause malaria are transmitted to humans through the bites of the *Anopheles* mosquito. In 1988, there was not one single *P. falciparum* infection recorded in the Department of Loreto, whereas in 1997, it is a staggering 54,290 cases of *P. falciparum* that were reported by the health authorities (DIRESA (1998) cited in Aramburú *et al.* 1999). In the Amazon region, this dramatic increase in the number of malaria cases is thought to be associated with important socio-environmental changes that occurred regionally through the phenomenon of 'frontier malaria' (de Castro *et al.* 2006). In the Peruvian Amazon, the resurgence has also been attributed to the increased distribution range of the highly anthropophilic and efficient malaria vector *Anopheles darlingi*, which was not present around Iquitos before the early 1990s and is now occupying most of the Peruvian Amazon (Fernández *et al.* 1996; Schoeler *et al.* 2003). It is suspected that invasion of the landscape by *An. darlingi*, following accidental introduction of mosquito larva, resulted from anthropogenic modifications of the environment (Lounibos and Conn 2000). One such modification is the development of fish farming, which became an important economic activity in the 1990s, and is also suspected of contributing to increased malaria transmission (Lounibos 2002). This hypothesis is controversial, however, because the principal malaria vector in the region (*An. darlingi*) is generally considered sylvatic and riverine and its preferred breeding habitat is characterized by clean, partially-shaded, slow-moving waters. Such breeding conditions are not found in fish ponds, which typically hold open stagnant waters with high concentrations of organic matter. Fish farming is an important economic activity and there is a strong cultural dietary preference for fish over beef in this region of Peru. With the anticipated decline of the inland fishery of the Amazon Basin due to overfishing, the expansion of aquaculture has been advocated as an alternative source of fish (de Jesús and Kohler 2004; Garcia *et al.* 2009; Junk *et al.* 2007; Molnar *et al.* 2000). Despite the potentially high consequences fish farming could have on malaria transmission, very little is known on the malaria risk posed by this activity.

In this thesis, an overview of the current knowledge on the epidemiology of malaria in the Amazon region is first presented and the possible links between malaria transmission and fish farming are reviewed (Chapter 2). Then, the general and specific objectives of the thesis research are presented (Chapter 3). The following chapter introduces the methodology adopted to fulfill the research objectives and gives a detailed account of the survey methods, databases used, tools for measuring exposure and confounders, and statistical methods employed (Chapter 4). The next chapter is a manuscript, to be submitted to a scientific journal, which reports the study results and discusses their significance regarding the epidemiology of malaria in the Peruvian Amazon (Chapter 5). Finally, the follow-up visits to the communities and to stakeholders are described (Chapter 6), followed by the concluding remarks (Chapter 7).

2- Chapter two: Literature Review

Introduction

Globally, malaria affects between 189 and 327 million people per year and over 1 million of these infections will be fatal (WHO 2008). Half of the world's population (3.3 billion) lives in endemic areas which are among the poorest regions of the world. Malaria transmission is exacerbated by poverty but the disease itself is also a cause of poverty due to costs for prevention and treatment, labour losses, and deaths.

In recent years, much attention has focused on the impact that human-driven ecosystem changes have had on malaria transmission. Most of this attention has been devoted to large scale environment modifications such as the construction of dams, reservoirs, and irrigation systems, deforestation, and expansion of agriculture. Fish farming is an increasingly common activity in the Peruvian Amazon. There, fish ponds are suspected of contributing to malaria transmission and are posing 'a distinct risk, since they are small in size and rapidly produced, primarily on private landholder properties near homes' (Simpson 2006). Further, the development of this activity has been advocated as a means of poverty alleviation and as a source of nutrition for poverty-stricken communities of the Amazon. Given past and current trends in the development of fish farming, this activity could increase vector-human contact and put local populations at heightened risk of malaria infection. Because of the re-emergence of malaria in the Amazon region and the economic prospects of aquaculture, it is of prime importance for policy-makers to be able to weigh the consequences of this activity. This chapter will give an overview of malaria and the main characteristics of this complex disease. The epidemiology of malaria in the Amazon region will be presented and the potential links between malaria transmission and fish farming will be highlighted.

The main sources of information originated from the peer-reviewed literature. Electronic databases were searched to identify relevant studies using appropriate subject headings and keywords. These databases included PubMed, Web of Science, CAB and LILACS. Furthermore, bibliographies of selected studies were screened as a way to locate studies or reports published in the 'grey' literature. Studies published in English, French, Spanish, and Portuguese were included in this review.

OVERVIEW OF MALARIA IN SOUTH AMERICA

Geographic distribution of malaria

Malaria is endemic in all countries of the South American continent except in Chile and Uruguay. The most recent incidence data from 2006 indicates that endemic countries of South America reported more than 1 million cases of malaria. Owing to the underreporting of malaria cases, WHO estimates the real number of malaria episodes in 2006 for this continent to be close to 2.3 million and that more than 1,800 deaths are directly attributable to this disease (WHO 2008).

In South America, Peru reports the highest number of malaria cases after Brazil, and most cases occur in the Amazon region (Hay *et al.* 2004). The continent's topography (e.g., the Andes) and climate constrain the geographical extent of the disease. Colder temperature affects the development of the protozoan parasite and to a lesser extent the *Anopheles* vector (Molineaux 1988) and precipitation mediates the availability of breeding habitats for the *Anopheles*.

Plasmodium parasites and malaria pathology

Malaria is an acute febrile illness characterized by the cyclical occurrence of sudden chills followed by rigor and then fever and sweating. The most common symptoms are fever, chills, headaches, and excessive sweating (Roshanravan *et al.* 2003). Other symptoms of the disease include dizziness, malaise, myalgia, abdominal pain, nausea, vomiting, mild diarrhea, and dry cough. Those symptoms, however, are nonspecific and cannot be used to differentiate between the different species of malaria parasites or even other febrile illnesses (Luxemburger *et al.* 1996).

More than 200 species of protozoan parasites belong to the genus *Plasmodium* (Perkins and Austin 2009) but only four affect humans (omitting the occasional zoonosis and *Plasmodium knowlesi* which is locally important in Malaysia (Cox-Singh *et al.* 2008)): namely *Plasmodium vivax*, *Plasmodium falciparum*, *Plasmodium malariae*, and *Plasmodium ovale*. These four species of parasites have different morphologies, elicit different immunological responses, have different geographical distributions, and differ in their relapse patterns and drug responses. Because *P. ovale* is not found in South America, this review will not discuss this species.

P. vivax is the most prevalent human malaria parasite and is most common in Asia and Latin America. In Africa, *P. vivax* is absent from western and central sub-Sahara because the absence of Duffy factor on the surface of red blood cells largely protects these populations (Baird 2007). Because *P. vivax* can develop at lower temperatures in the *Anopheles* vector than *P. falciparum,* its geographical range is wider (Greenwood *et al.* 2005). Infections caused by *P. vivax* are generally considered benign, causing mild anemia, and only exceptionally death (generally by rupture of the spleen) (Galinski and Barnwell 2008). However, a number of recent studies concluded that the burden of *P. vivax* malaria, its economic impact, and severity of disease have been underestimated (Baird 2007; Hay *et al.* 2004; Price *et al.* 2007). One important characteristic of *P. vivax* is the occurrence of relapses at different intervals. Relapses are caused by the activation of the liver-stage hypnozoites and relapse patterns differ by geographic areas. In tropical zones, the primary infection occurs early and is followed by frequent relapses at short intervals (Coatney *et al.* 1950; Mason 1975).

P. falciparum is present worldwide in tropical and subtropical areas. In South America about 30% of infections are caused by *P. falciparum* but it is in Africa that the species predominates with 98% of malaria cases belonging to this *Plasmodium* species (WHO 2008). *P. falciparum* can cause severe, potentially fatal, malaria and is the leading cause of malaria deaths in young children in Africa (Snow *et al.* 2004). Compared to other human malaria parasites, *P. falciparum* multiplies itself more rapidly in the blood, which can result in severe anemia and small capillary vessels of the brain can get clogged with infected red blood cells, causing cerebral malaria. Severe *P. falciparum* malaria after-effects include deficits in attention, memory, visuo-spatial skills, language and execution functions (Kihara *et al.* 2006). Furthermore, malaria-induced maternal anemia is suspected to be responsible for 10-20% of low birth weight babies in affected countries (Guyatt and Snow 2001; Steketee *et al.* 2001).

P. malariae has a worldwide distribution, which coincides with that of *P. falciparum*, but occurs at very low frequencies (Collins and Jeffrey 2007). In South America, less than 0.8% of all cases were reported to have been caused by *P. malariae* in 2007 (PAHO 2007). However, studies from the Amazon Basin demonstrated that this species is often confused with *P. vivax* and that the prevalence of *P. malariae* in the region is close to 10-12% (Cavasini *et al.* 2000; Scopel *et al.* 2004). It has also been reported, before the introduction of malaria control measures in a remote community of the Peruvian Amazon, that *P. malariae* accounted for 83% of malaria cases detected (Sulzer *et al.* 1975). *P. malariae* can result in chronic infections, through long-lasting blood-stages, which can persist asymptomatically in the human host for decades (Tuteja 2007).

Life-cycle of malaria parasites

In order to complete their life-cycle, the four human malaria parasites require a female *Anopheles* mosquito and a human host. When an individual is bitten by an infected female *Anopheles*, sporozoites are injected from the mosquito's salivary glands into the blood stream. Within an hour, these sporozoites infect liver cells (hepatocytes) where they multiply into schizonts containing many thousands of merozoites. This phase lasts between 6 to 16 days and infected individuals don't exhibit any symptoms (Tuteja 2007). Contrary to *P. falciparum* and *P. malariae,* some *P. vivax* sporozoites entering hepatocytes mature into a dormant form called a hypnozoite which can cause relapses weeks to years after first symptoms appear. When merozoites are released into the blood stream they infect red blood cells and develop into trophozoites that subsequently mature into schizonts, consisting of many daughter merozoites (asexual reproduction). It is at this stage that infected individuals will manifest symptoms of the disease, a median of 11-15 days after receiving the infective bites (depending on the *Plasmodium* species) (Trampuz *et al.* 2003). Schizonts eventually rupture, releasing merozoites into the blood that will invade new red blood cells to complete a new asexual cycle. Infection can be amplified 20-fold with each cycle in non-immune humans (Trampuz *et al.* 2003). After several cycles, some of the merozoites develop into gametocytes (sexual stage) which cause no symptoms in human. The parasite can complete its life-cycle when a susceptible female *Anopheles* mosquito bites an infected human host and absorbs the gametocytes. In the gut of the mosquito, the parasite will undergo its sexual reproduction phase where male and female gametes will produce a motile, elongated zygote, called an ookinete. This ookinete will penetrate the mid-gut wall where it will grow into an oocyst which will eventually release several hundred to several thousand sporozoites which will migrate to the salivary glands of the mosquito. Depending on the temperature and parasite species, it will take from 10 to 18 days for the transition from gametocytes to sporozoites to occur and the female *Anopheles* mosquito will remain infective for up to 2 months (Tuteja 2007).

Finally, it is important to mention that all three species of *Plasmodium* parasites encountered in South America can occur as zoonoses in the Amazon Basin. Wild or domesticated monkeys of the genus *Alouatta* and *Ateles* can act as reservoirs for human plasmodia and contribute to malaria transmission (Arruda *et al.* 1989; Duarte *et al.* 2008). However, the magnitude of this phenomenon and its epidemiological significance remain unmeasured.

MALARIA VECTORS OF THE AMAZON REGION

Life-cycle of *Anopheles* vector

Female *Anopheles* cannot complete their oviposition cycle (i.e., lay their eggs) without having taken a blood meal. They feed almost exclusively between dusk and dawn, but the peak in biting activity differs among species and regions. Only *Anopheles* from the South American subgenus *Kerteszia* (*An. cruzzi, An. bellator*, etc.) engages in diurnal feeding and these species are not major malaria vectors in the Amazon (Murillo *et al.* 1988). *Anopheles* females locate their blood meals using carbon dioxide emission and their sense of smell at short distances. Interestingly, recent evidence suggests that malaria infection with *Plasmodium* gametocytes increases the attractiveness of humans to *Anopheles* mosquitoes (Lacroix *et al.* 2005). Once blood-fed, the female will rest until her eggs reach maturity, usually in 24 hours (Pages *et al.* 2007; Roberts *et al.* 1983). Females of the principal South American vector (*An. darlingi*) can breed every three days (i.e., gonotrophic cycle) (Charlwood 1980) and have been observed to oviposition up to six times (Charlwood and Wilkes 1979). The development of the mosquito from egg to adult is highly temperature-dependent but generally varies between 5 to 20 days (Pages *et al.* 2007). Although very difficult to measure in natural conditions, the average life-span of female *Anopheles* is estimated to be between one and two months (Pages *et al.* 2007).

Maximum observed dispersal distances of the main Amazonian vector (*An. darlingi*) are reported to be between 2 and 7 km (Charlwood and Alecrim 1989; Deane *et* *al.* 1948). For most mosquito species, dispersal distances are between 1 and 5 km (Silver 2008). These are maximum dispersal distances and the majority of *Anopheles* mosquitoes disperse over smaller distances (Achee *et al.* 2007, 2005; Service 1997). In fact, one study reported that less than 20% of *An. darlingi* mosquitoes would fly more than 500 metres and less than 1% over 1.2 km (de Barros and Honório 2007).

Epidemiological importance of Amazonian *Anopheles*

More than 460 species of mosquitoes of the genus *Anopheles* (Diptera: Culicidae) have been described so far and about 50 of them are able to transmit the *Plasmodium* parasites to humans (Harbach 1994). In South America, 17 species of *Anopheles* are considered epidemiologically important malaria vectors (Rubio-Palis and Zimmerman 1997). Throughout the Amazon region, *An. darlingi* is considered the principal malaria vector (Table 2.1). Other regionally important malaria vectors include *An. aquasalis* near the north Atlantic coast of Brazil, *An. albitarsis* sensus lato (*s.l.*), *An. rangeli*, and *An. benarrochi* in the Peruvian Amazon. For the latter, it is important to note that *An. darlingi* was not present in the Peruvian Amazon region of Loreto before the 1990s (Fernández *et al.* 1996). The fact that Calderón and colleagues (1995) reported *An. benarrochi* as an important vector may not reflect today's reality because *An. darlingi* is now present in this area. The main factors that affect the vectorial capacity of an *Anopheles* species are its abundance and degree of anthropophily (i.e., preference for human blood). Important factors affecting the epidemiological profile of a region are the degree of endophagy (i.e., preference for indoor feeding) and endophily (i.e., preference for resting indoor) of the principal *Anopheles* vector.

Conditions that make a specific vector species locally important are not well understood but the availability of preferred breeding habitat, food sources, interspecific competition, and environmental conditions are all likely to play a role. The breeding requirements of the main malaria vectors of the interior of the Amazon basin are presented in Table 2.2. Most of them have broadly defined breeding habitat requirements and the main differences are related to luminosity preferences (shade versus full sun) and water quality (clear versus turbid).

Table 2.1: Principal and minor malaria vectors of the Amazon region, as determined by their susceptibility to *P. falciparum* and *P. vivax* infections, identified by microscopy or molecular techniques

Regions (Country)	Principal vector	Minor vector*	References
Acre, Amapá, Amazonas, Pará (Brazil)	*An. darlingi*	*An. aquasalis*† *An. albitarsis s.l.* *An. braziliensis*	(Deane *et al.* 1948)
Pará (Brazil)	*An. darlingi*	*An. albitarsis s.l.* *An. triannulatus* *An. nuneztovari* *An. oswaldoi*	(de Arruda *et al.* 1986)
Rondônia (Brazil)	*An. darlingi*	*An. triannulatus* *An. braziliensis*	(Deane *et al.* 1988)
Rondônia (Brazil)	*An. darlingi*	*An. triannulatus* *An. oswaldoi*	(Tadei *et al.* 1988)
Rondônia (Brazil)	*An. darlingi*	*An. triannulatus* *An. albitarsis s.l.* *An. braziliensis* *An. strodei* *An. oswaldoi*	(de Oliveira-Ferreira *et al.* 1990)
Amapá, Amazonas, Pará, Rondônia, Roraima (Brazil)	*An. darlingi* *An. aquasalis*†	*An. albitarsis s.l.* *An. braziliensis* *An. galvaoi* *An. nuneztovari* *An. oswaldoi* *An. strodei* *An. triannulatus*	(Tadei *et al.* 1998)
Amapá (Brazil)	*An. albitarsis s.l.*	*An. nuneztovari* *An. braziliensis* *An. triannulatus* *An. oswaldoi* *An. rangeli*	(Póvoa *et al.* 2001)
Amapá (Brazil)	*An. albitarsis s.l* *An. darlingi*		(Conn *et al.* 2002)
Roraima (Brazil)	*An. darlingi*	*An. albitarsis s.l.* *An. braziliensis* *An. nuneztovari*	(Silva-Vasconcelos *et al.* 2002)
Rondônia (Brazil)	*An. darlingi*		(Gil *et al.* 2003)
Roraima (Brazil)	*An. darlingi* *An. albitarsis s.l.*		(Póvoa *et al.* 2006)
Rondônia (Brazil)	*An. darlingi*		(Gil *et al.* 2007)
Pará (Brazil)	*An. darlingi*	*An. albitarsis s.l.* *An. rondoni*	(da Rocha *et al.* 2008)

Regions (Country)	Principal vector	Minor vector*	References
Putumayo (Colombia)	*An. rangeli*	*An. oswaldoi*	(Quiñones *et al.* 2006)
Upper-Maroni basin (French Guiana)	*An. darlingi*		(Girod *et al.* 2008)
Loreto, Madre de Dios, San Martin, Ucayali (Peru)	*An. darlingi* *An. benarrochi*	*An. trinkae* *An. nuneztovari* *An. rangeli* *An. oswaldoi* *An. evansae*‡	(Calderón *et al.* 1995)
Loreto, Madre de Dios, San Martin, Ucayali (Peru)	*An. darlingi*	*An. benarrochi*	(Flores-Mendoza *et al.* 2004)
Amazonas (Venezuela)	*An. darlingi*		(Magris *et al.* 2007)

*Secondary malaria vectors are listed in order of decreasing epidemiological importance.
†Only encountered near the Atlantic Ocean coast.
‡Synonym of *An. noroestensis*.

Table 2.2: Breeding site characteristics of the principal malaria vectors of the Amazon region of South America

Species	Breeding habitats	Vegetation type	Shade preference	Other characteristics	Source documents
*An. darlingi**	Streams, ponds, ground pools, and swamps. Shady edges of quiet rivers	Emergent and floating vegetation. Associated with *Pistia* and *Eichomia*	Partial shade	Neutral and unpolluted water. Stagnant to slow running	(Charlwood 1996; Consoli and Lourenço-de-Oliveira 1994; Deane *et al.* 1948; Faran and Linthicum 1981; Forattini 1962; Rachou 1958; Roberts *et al.* 2002)
An. albitarsis s.l.	Great variety of temporary or permanent, natural or artificial, aquatic habitats: freshwater ponds, lakes, marshes, river bays and overflows	Associated with *Pistia* and *Eichomia*	From full sun to complete shade	Clear or turbid water, with or without organic matter	(Castillo 1949; Consoli and Lourenço-de-Oliveira 1994; Gorham *et al.* 1973; Rachou 1958)
An. benarrochi	Ground pools and small streams. Small pockets of stagnant water	Emergent vegetation	Full sun or partial shade	Lots of organic matter. Stagnant to slow running water	(Castillo 1949; Deane *et al.* 1948; Faran and Linthicum 1981; Gorham *et al.* 1973)
An. braziliensis	Ponds and swamp with mud bottoms (or sand), ditches, small streams, pools	Dense algae, emergent and floating vegetation	Full sun to partial shade	Clear stagnant water	(Consoli and Lourenço-de-Oliveira 1994; Deane *et al.* 1948; Faran and Linthicum 1981; Forattini 1962; Gorham *et al.* 1973)
An. nuneztovari	Freshwater of open marshy areas, ponds, lakes, ground pools, stream margins, and hoofprints	Can be associated or not with emergent vegetation	Full sun to partial shade	Turbid water. Stagnant to slow running	(Castillo 1949; Deane *et al.* 1948; Faran and Linthicum 1981; Gorham *et al.* 1973)

Species	Breeding habitats	Vegetation type	Shade preference	Other characteristics	Source documents
An. oswaldoi	Temporary or permanent ponds, margins of ponds, lakes and swamps.	Emergent vegetation	Full shade to partial shade	Tolerant to variations in pH, turbidity, temperature and nutrients. Stagnant to slow running	(Branquinho *et al.* 1993; Consoli and Lourenço-de-Oliveira 1994; Faran and Linthicum 1981; Tadei *et al.* 1998)
An. rangeli	Temporary ground pools, marshy depression, wheel tracks, roadside ditches, hoofprints, margins of stream and lakes	Abundant emergent vegetation but not in forested areas	Full sun or partial shade	Stagnant to slow running water. Turbid waters	(Bates 1945; Deane *et al.* 1948; Faran and Linthicum 1981)
An. triannulatus	Permanent ponds, lakes, canals, slow streams or river margins, ditches, and swamps with abundant floating vegetation	Usually associated with *Pistia* and to a lesser extent to *Eichhornia*, *Azolla*, *Utricularia*, *Jussiaea*, *Elodea* and *Salvinia*	Full sun or partial shade	Clear water. Stagnant to slow running	(Consoli and Lourenço-de-Oliveira 1994; Deane *et al.* 1948; Faran and Linthicum 1981)

*Main malaria vector in the Amazon region of South America

Anopheles darlingi

An. darlingi's range extends from southern Mexico to north-eastern Argentina (Forattini 1962). The high behavioural variation of this species in Latin America and its large distribution range have led some to hypothesize that *An. darlingi* is a species complex (Charlwood 1996). Even if this hypothesis was refuted by Manguin and colleagues (1999), recent studies suggest significant population structure and differentiation within the species (Conn *et al.* 2006; Mirabello *et al.* 2008). In the Peruvian Amazon, however, the population is rather behaviourally and genetically homogeneous (Pinedo-Cancino *et al.* 2006). As previously mentioned, this species was not present in the central region of Loreto before the early 1990s (Fernández *et al.* 1996). The species has now considerably expanded its distribution range and its presence is now reported from many previously uncolonized areas of the Amazon (Schoeler *et al.* 2003). It is believed by some that this expansion range was human-assisted through the construction of artificial larval habitats such as fish ponds (Lounibos 2002; Lounibos and Conn 2000).

An. darlingi is the most anthropophilic, endophagic, and endophilic species present in the Amazon (Alencar *et al.* 2005; Consoli and Lourenço-de-Oliveira 1994; de Oliveira-Ferreira *et al.* 1992; Deane *et al.* 1948; Klein *et al.* 1991, 1992; Rachou 1958; Zimmerman *et al.* 2006). Some degree of zoophily and exophagy, however, have also been reported for this species (Consoli and Lourenço-de-Oliveira 1994; Deane *et al.* 1948; Gil *et al.* 2003). Another factor explaining the vectorial competency of *An. darlingi* is its relatively long life-span. In capture-recapture experiments in Brazil *An. darlingi* was found to be the most long-lived anopheline species (Charlwood and Alecrim 1989). Patterns of biting activity of this species are highly variable and differ between regions (Table 2.3), and although biting occurs throughout the night, the mean peak generally occurred at sunset continuing until midnight. Reasons for explaining this high variability in biting activity are related to seasonality, full moon effects, density-mediated activity, and presence of species clines.

Table 2.3: Biting activity patterns of *Anopheles darlingi* in different regions of South America

Country	Peak biting activity	References
Bolivia	19h00-21h00	(Harris *et al.* 2006)
Brazil	21h00-01h00	(Deane *et al.* 1948)
Brazil	24h00-02h00	(Elliot 1972)
Brazil	Bimodal: 18h00-19h00 & 05h00-06h00	(Charlwood and Wilkes 1979)
Brazil	Bimodal: 17h00-20h00 & 04h00-07h00	(Forattini 1987)
Brazil	Bimodal: 19h00-22h00 & 05h00-07h00	(Roberts *et al.* 1987)
Brazil	Trimodal: Sunset & 20h00-21h00 & Sunrise	(Charlwood and Alecrim 1989)
Brazil	Bimodal: 18h00-20h00 & 05h00-06h00	(Klein and Lima 1990)
Brazil	20h00-01h00	(Rosa-Freitas *et al.* 1992)
Brazil	18h00-24h00	(Voorham 2002)
Brazil	Uniform: 18h00-6h00	(Silva-Vasconcelos *et al.* 2002)
Brazil	Bimodal: 18h00-22h00 & 02h00-06h00	(de Barros and Honório 2007)
Colombia	22h00-24h00	(Elliot 1972)
Colombia	18h00-19h00	(Brochero *et al.* 2005)
French Guiana	Bimodal: 18h00-19h00 & 07h00-08h00	(Pajot *et al.* 1977)
French Guiana	Bimodal 21h30-03h30 & 05h30-06h30	(Girod *et al.* 2008)
Peru	22h00-24h00	(Elliot 1972)
Suriname	22h00-23h00	(Hudson 1984)
Suriname	22h00-24h00	(Rozendaal 1989)
Venezuela	24h00-05h00	(Magris *et al.* 2007)

MALARIA EPIDEMIOLOGY IN THE AMAZONIAN REGION

Frontier malaria

The re-emergence of malaria in the Amazon Basin is attributed to the large-scale, government-sponsored colonization projects that occurred in the 1970s to mid-1980s in Brazil (Deane 1988), and the reduction of DDT spraying activities after the abandonment of the 'Malaria Eradication Program' (Roberts *et al.* 1997). During the same period, the Peruvian Amazon experienced similar socio-environmental changes mainly through oil exploitation and the coca trade (Coomes 1995). The biological, ecological, and socio-demographic phenomena that led to the resurgence of malaria in the Amazon have been named 'frontier malaria'. Frontier malaria is caused primarily by the extensive deforestation that altered the landscape, human clustering close to *Anopheles* larval habitats, and higher vector densities (Guerra *et al.* 2006; Norris 2004; Tadei *et al.* 1998;

Vittor *et al.* 2006; Yasuoka and Levins 2007). Moreover, settlers are generally non-immune, highly susceptible hosts, with limited knowledge of malaria transmission (de Castro *et al.* 2006). Unplanned development with weak institutions, minimal community cohesion, marginalization of settlers, and agricultural failures collectively make attempts to control malaria difficult (de Castro *et al.* 2006; Singer and de Castro 2001). The high rate of malaria transmission further compromised the stability of agricultural settlements because of the chronic exposure to malaria which resulted in high rates of emigration and immigration (Sawyer 1986). Human migration of parasitemic individuals also contributes to the resurgence of malaria by initiating new foci of transmission in previously controlled areas (Machado *et al.* 2003; Marques 1987; Martens and Hall 2000). As settlements became organized, with some degree of urbanization and establishment of appropriate health infrastructures, frontier malaria is eventually replaced by more stable and low levels of transmission (McGreevy *et al.* 1989). Most areas of the Peruvian and Brazilian Amazon have now reached this more stable phase.

Risk factors for malaria

Even though the phenomenon of frontier malaria can generally be described, the risk of malaria transmission is highly heterogeneous in space and time and different epidemiological settings are often encountered within the same region (Bautista *et al.* 2006; Pages *et al.* 2007). Risk factors for malaria are usually related to the behaviours of the local malaria vector and of human hosts. For example, gender-specific activities related to malaria transmission have been investigated. A number of studies found that men were at increased risk of malaria but the majority of them did not find any specific statistically significant differences in determinants between men and women (Table 2.4). A trend was observed, however, for men being at increased risk of malaria infection.

Age is another important variable that could influence the occurrence of malaria infection either through age-specific activities that could increase one’s exposure to the malaria vector or the process of acquired immunity. Malaria in the Amazon is considered hypoendemic, meaning that few individuals develop clinical immunity. However, studies demonstrated that continuous exposure to hypo or mesoendemic conditions may induce clinical immunity in some populations of the Brazilian Amazon (Coura *et al.* 2006;

Ladeia-Andrade *et al.* 2009) and Peruvian Amazon (Bharti *et al.* 2006; Roshanravan *et al.* 2003). Clinical immunity in the Amazon Basin is something relatively new as asymptomatic infections were found to be infrequent in the 1980s (Prata *et al.* 1988). There are contradicting results regarding age as a risk factor: some studies identified children as a high risk group and others described adults as being at increased risk of malaria infection. It is possible to reconcile these views by taking into account the study population in which the studies were conducted: the ones that found that being a child was a risk factor focused on Amerindian populations whereas findings that adults were a risk group originated from studies conducted in 'mestizos' or mixed-heritage populations, possibly because they were resettled (non-immune) populations.

Table 2.4: Potential risk factors for malaria infection (*Plasmodium spp.*) in areas of the Amazon where *Anopheles darlingi* is reported to be the principal malaria vector

Potential risk factors	Studies reporting a statistically significant effect*	Studies that did not report a statistically significant effect*
Male (gender)	(Camargo *et al.* 1994)† (Camargo *et al.* 1996)† (Magris *et al.* 2007)	(Neel *et al.* 1968)† (Castilla and Sawyer 1993)†‡ (Roper *et al.* 2000) (San Sebastián *et al.* 2000)† (Vittor 2003)† (Duarte *et al.* 2004) (da Silva-Nunes *et al.* 2008) (Ladeia-Andrade *et al.* 2009)
Age	**Children are at risk:** (Magris *et al.* 2007) (Ladeia-Andrade *et al.* 2009) **Adults are at risk:** (Castilla and Sawyer 1993)†‡ (Camargo *et al.* 1994)† (Camargo *et al.* 1996)† (Roper *et al.* 2000)† (Vittor 2003)†	(Neel *et al.* 1968)† (San Sebastián *et al.* 2000)† (Duarte *et al.* 2004)
Ethnicity**	(Alexander *et al.* 2005) (Hustache *et al.* 2007) §	(Duarte 1999)
Low SES	(Hustache *et al.* 2007)§	(Vittor 2003)† (Duarte *et al.* 2004) (Alexander *et al.* 2005) (da Silva-Nunes *et al.* 2008) (Castilla and Sawyer 1993)†‡ (Duarte *et al.* 2004)

Potential risk factors	Studies reporting a statistically significant effect*	Studies that did not report a statistically significant effect*
		(Alexander *et al.* 2005)
'Precarious' house characteristics	(Hustache *et al.* 2007)†§ (Ladeia-Andrade *et al.* 2009)	(Castilla and Sawyer 1993)†‡ (Roper *et al.* 2000) (Vittor 2003)† (Alexander *et al.* 2005)
Crowded household	(Hustache *et al.* 2007) § (da Silva-Nunes *et al.* 2008)	(Vittor 2003)†
Poor knowledge of malaria transmission	(Castilla and Sawyer 1993)†‡	
Early wake-up time	(Ladeia-Andrade *et al.* 2009)	(Roper *et al.* 2000) (Vittor 2003)†
Late sleep time	(Roper *et al.* 2000)	(Vittor 2003)† (Ladeia-Andrade *et al.* 2009)
Occupational risk**	(Perez *et al.* 1999) (da Silva-Nunes *et al.* 2008)	(Vittor 2003)† (Duarte *et al.* 2004) (Alexander *et al.* 2005)
Living closer to *Anopheles spp.* breeding habitat	(Castilla and Sawyer 1993)†‡ (Vittor 2003) (Hustache *et al.* 2007) §	
Living closer to forested areas	(Castilla and Sawyer 1993)‡ (Hustache *et al.* 2007) §	(Vittor 2003)†
House located in vegetation	(Vittor 2003) (Hustache *et al.* 2007) §	(Castilla and Sawyer 1993)†‡
No use of bed net	(Alexander *et al.* 2005)	(Vittor 2003)† (Duarte *et al.* 2004)
No use of protective clothing	(Hustache *et al.* 2007) §	(Duarte *et al.* 2004)

*Level of statistical significance reported in the publications is $p<0.05$

†The estimate of effect is not adjusted for other potential confounders.

‡Statistical significance was assessed using chi-square tests calculated from the data presented in the publication.

§Study population is composed of children aged from 1 to 5 years.

**At-risk groups differ in each study.

Ethnicity as a risk factor for malaria has only been considered by three studies, two of which were conducted in Amerindian communities. Ethnicity in these studies referred to an individual's tribal affiliation. The study of Duarte et al. (2004) looked at skin color, possibly because it is a strong cultural concept of identity in Brazil. Only the studies that related ethnicity to an individual's tribal affiliation found any significant effect of this variable on malaria infection.

Malaria is characterized as a disease of poverty. This is true at the macroeconomic scale but at the microeconomic (household) scale the relationship is weaker and somewhat inconsistent. This could be due partly because it is notoriously difficult to precisely quantify an individual's SES in rather economically homogeneous communities. The most commonly used proxy variables have been: if the head of the family has a regular income, the monthly household income *per se*, wealth ranking by community members, asset-based indices, and the education level of the head of the household. In the Amazon Basin region of South America, only one study reported a significant effect of SES on malaria infection and this was an unadjusted estimate (Hustache *et al.* 2007). All studies, however, reported a trend towards households or individuals of low SES as being at increased risk for malaria. Other risk factors related to SES are housing characteristics, household crowding, and poor knowledge of malaria transmission. Here a number of studies reported a significant effect for these three risk factors and studies which did not find any statistical evidence also noted a trend in this direction.

Wake-up time, bed time, and occupational risks have been found to be related to the degree an individual is exposed to mosquito bites (i.e., the pattern of biting activity) and the degree of endophily of the malaria vector. Results are difficult to generalize because of the highly context-specific nature of these risk factors and because different studies used different at-risk groups. Although most studies did not report that these variables were risk factors in the Amazon Basin, significant evidence was nevertheless found for wake-up time, bed time, and some occupational risks.

The location and context of an individual's household within the ecological landscape also appears to be an important factor affecting malaria risk in the Amazon. Individuals living close to potential *Anopheles* breeding sites and close to the forest or surrounded by much vegetation have been found to be at higher risk of malaria infection. These studies are difficult to replicate because of the arbitrarily defined exposures.

Finally, the use of bed nets has been confirmed as one of the best measures to protect against malaria infection in the Amazon Basin (Alexander *et al.* 2005; Kroeger *et al.* 1995). Bed nets are also of very common use, with 98-99% of some populations reporting using bed nets in the Peruvian Amazon (Nawaz *et al.* 2001; Roper *et al.* 2000), and 96% in the Colombian Amazon (Alexander *et al.* 2005). Other protective methods, such as the use of protective clothing (pants and long sleeve shirts), produced mixed evidence.

MALARIA RISK AND AQUACULTURE

Anthropogenic modifications to the land-water interface have been linked to an increase in the density of *Anopheles* vectors and associated increased malaria risk (Molyneux 2003; Norris 2004; Oomen *et al.* 1988; Patz *et al.* 2004; Warrell and Gilles 2002). One such activity, increasingly common in developing countries, is aquaculture. Throughout this thesis, aquaculture is referred to as the organized production of aquatic species (fish, mollusc, algae, etc.) in man-made ponds. By creating year-round breeding habitats for malaria vectors, aquaculture could potentially increase the subsequent transmission of malaria in endemic areas (Garrett *et al.* 1997). Aquaculture may also be practiced in cages or pens in natural bodies of water but this type of aquaculture does not increase the risk of malaria transmission above that caused by the presence of the natural bodies of water alone.

The contribution of aquaculture to the global supplies of halieutic resources has increased exponentially over recent decades from 3.9% of total production by weight in 1970 to 27.1 % in 2000 and 32.4 % in 2004 (FAO 2007). Furthermore, this activity has steadily developed faster than the other sectors of food production and this difference is even more pronounced in developing countries (FAO 2007). In the Amazon Basin of South America, the commercial fishery is beginning to show classical signs of overfishing such that expansion of aquaculture has been advocated as an alternative source of fish and as means of poverty alleviation (de Jesús and Kohler 2004; Junk *et al.* 2007; Molnar *et al.* 2000). The importance of aquaculture in South America is still minor compared to Asia but the potential of this region is considered very high (Alcántara *et al.* 2004; FAO 2007; Junk *et al.* 2007; Saint-Paul 1992). In the Amazon Basin, there is no official database or report that can be used to quantify the importance of aquaculture.

Records of *Anopheles spp*. breeding in fish ponds

In order to pose a malaria risk, fish ponds need to be suitable breeding habitats for *Anopheles* mosquitoes. *Anopheles* larvae have been collected in fish ponds of Africa, Asia, and South America. No records of anophelines breeding in similar habitats were found for North America, Europe, or the Middle-East. In South America, a total of 11 studies contained records of *Anopheles spp*. breeding in aquaculture ponds. Some of these accounts, however, were not based on entomological surveys *per se* and the reported evidence should be considered anecdotal. Moreover, the majority of the studies only reported presence/absence data. To quantify the epidemiological importance of these breeding habitats measures of larval abundance should be used. Based on the number of reports, the main *Anopheles* species breeding in aquaculture ponds of South America is *An. nuneztovari* (Table 2.5). *An. darlingi* was found in fish ponds of Brazil (anecdotal evidence) and Peru but were not found in fish ponds of Colombia. Other species commonly encountered in fish ponds are *An. rangeli*, *An. triannulatus,* and *An. albitarsis s.l.* All of the main malaria vectors of the Amazon tolerant of turbid water (*An. albitarsis s.l., An. benarrochi, An. oswaldoi, An. nuneztovari,* and *An. rangeli*) could be found in fish ponds of South America as well as species who usually prefer clear water (*An. darlingi* and *An. triannulatus*). *An. braziliensis* whose breeding requirements are also characterized by clear water was not encountered in fish ponds. It is important to recognize that the sample of retrieved studies might be biased by the underreporting of the surveyed aquaculture ponds negative for *Anopheles spp*. larvae.

Table 2.5: Records of *Anopheles spp.* breeding in fish ponds of South America

Country	Species	Observations	References
Brazil	*Anopheles spp.*	Anecdotal evidence	(Tadei *et al.* 1998)
Brazil	*An. darlingi*	Anecdotal evidence	(Gil *et al.* 2007)
Colombia	*An. albimanus* *An. nuneztovari*		(Olano *et al.* 1997)
Colombia	*An. nuneztovari*		(López and González 1994)
Colombia	*An. pseudopunctipennis* *An. nuneztovari* *An. rangeli* *An. triannulatus s.l.*		(Brochero *et al.* 2006)

Country	Species	Observations	References
Colombia	*An. albitarsis s.l.* *An. rangeli*	No anophelines found in fish ponds free of marginal vegetation	(Brochero *et al.* 2005)
Peru	*An. darlingi*	Anecdotal evidence	(Lounibos and Conn 2000)
Peru	*An. triannulatus s.l.* *An. nuneztovari* *An. albitarsis*		(Ventosilla *et al.* 1999)
Peru	*An. darlingi* *Anopheles spp.*		(Arata *et al.* 1999)
Peru	*An. darlingi*	Anecdotal evidence	(Roper *et al.* 2000)
Peru	*An. benarrochi* *An. darlingi* *An. nuneztovari* *An. oswaldoi* *An. rangeli* *An. triannulatus* *An. mattogrossensis*		(Vittor 2003)

Regarding the association between fish ponds and *Anopheles* larvae on the African continent, nine accounts were found (Table 2.6). These records suggest that the main species encountered in this habitat is *An. gambiae* and to a lesser extent *An. funestus*. Again, the underreporting of studies that did not find any *Anopheles* larvae in fish ponds must be acknowledged although one report mentioned that no anopheline larvae were encountered in well-maintained and well-stocked ponds (Meschkat 1967). This report should however be considered as anecdotal evidence.

Table 2.6: Records of *Anopheles spp*. breeding in fish ponds of Africa

Country	Species	Observations	References
Cameroon	*An. funestus* *An. gambiae* *An. moucheti*		(Mouchet and Gariou 1966; Mouchet *et al.* 1960)
Congo	*An. gambiae*		(Zoulani *et al.* 1994)
Congo	*An. gambiae* *An. funestus* *An. moucheti*	Anecdotal evidence	(Lemasson 1957)
Côte d'Ivoire	*An. funestus* *An. gambiae*	Abandoned fish farms	(Adja *et al.* 2006)

Country	Species	Observations	References
Côte d'Ivoire	*An. funestus* *An. gambiae*	No species-specific identification except in a few instances	(Matthys *et al.* 2006a)
Kenya	*An. funestus* *An. gambiae*	Abandoned fish farms	(Howard and Omlin 2008; Howard *et al.* 2007)
Kenya Uganda Zambia Zaire	*An. funestus* *An. gambiae* *An. pharoensis*	Anecdotal evidence	(Paperna 1982)
Zaire	*An. coustani* *An. demeilloni* *An. funestus* *An. gambiae* *An. marshalli*	*An. funestus* and *An. gambiae* comprised 48.6 % and 49.7 % of the sample, respectively	(Basabose 1995)
Not specified	None	Ponds surveyed were all well maintained and well-stocked - anecdotal evidence	(Meschkat 1967)

In Asia, aquaculture has a long history and tradition that dates back thousands of years. Most research, however, has been published in local languages and/or are not indexed in accessible databases. In total, nine records from Asia were found (Table 2.7). They indicate that *An. sundaicus* is the species most often encountered in fish ponds followed by *An. subpictus*. In Indonesia, where most of the records originate, fish ponds are generally located near the ocean and most of them contain brackish waters (explaining the reported abundance of *An. sundaicus*).

Table 2.7: Records of *Anopheles spp.* breeding in fish ponds of Asia

Country	Species	Observations	References
India	*An. subpictus* *An. vagus* *An. barbirostris* *An. annularis* *An. hercnaus* *An. aconitus*	Anecdotal evidence from rizipiscicultures	(Hora 1951)
Indonesia	*An. aconitus* *An. sundaicus*		(Separmo and Laird 1954)
Indonesia	*An. sundaicus*	Anecdotal evidence	(Soerono *et al.* 1965)

Country	Species	Observations	References
Indonesia	*An. subpictus* *An. sundaicus* *An. annularis*	*An. subpictus* is most abundant in fish ponds	(Dachlan *et al.* 2005)
Indonesia	*An. sundaicus* *An. subpictus*		(Collins *et al.* 1979)
Indonesia	*An. sundaicus*		(Takagi *et al.* 1995)
Pakistan	*An. culicifacies* *An. stephensi* *An. subpictus*	Anecdotal evidence	(Herrel *et al.* 2001)
Vietnam	*An. sundaicus*	Anecdotal evidence	(Nguyen Tang *et al.* 1993)
Vietnam and Laos	*An. sundaicus*	Shrimp farming	(Trung *et al.* 2004)

Few of the studies listed in Tables 2.5-2.7 mentioned characteristics of aquaculture ponds that favoured anopheline presence or abundance. In the few instances where this type of information was provided, the abundance of vegetation at the margins of the fish ponds seemed to have the greatest influence on larva occurrence (e.g., Brochero *et al.* 2006; Howard and Omlin 2008). In Kenya, the frequency at which the pond was emptied to harvest fish was also found to be negatively associated with the presence of mosquito larva (Howard and Omlin 2008). In Indonesia, the presence of *Tilapia sp*. fish and extreme shading of the pond were associated with lower *An. sundaicus* densities (Takagi *et al.* 1995). In Peru, a survey of different larval habitats (including, but not restricted to, fish ponds) indicated that breeding sites with a leaf litter, algae, emergent grasses, aguaje palms (*Mauritia flexuosa*), slow currents, and clear waters were all positively associated with the presence of *An. darlingi* larvae (Vittor 2003).

Association between fish ponds and malaria transmission

Although it is documented that *Anopheles* can breed in fish ponds in different parts of the world, the epidemiological significance of aquaculture remains unmeasured. Only three studies have investigated the association between fish ponds and malaria infections: the dissertation of Vittor (2003), the thesis of Simpson (2006), and the paper of Matthys *et al.* (2006b).

In a cross-sectional survey of malaria prevalence in the Peruvian Amazon, Vittor (2003) reported that the presence of a fish pond close to the house was a risk factor for *P. falciparum* infection (OR=5.53; 95% CI: 1.83-16.65) but not for the more prevalent *P. vivax* infection (OR=1.2; 95% CI: 0.26-4.05). The main limitation of this study is that it did not take into account the distance, size, and shape of fish ponds in the vicinity of the house. Furthermore, when she considered total malaria (both *P. vivax* and *P. falciparum* infection) in her adjusted model, fish pond presence was not included. This result is puzzling because the main malaria vectors in this study region, *An. darlingi* and *An. benarrochi*, can transmit both *Plasmodium* species.

In the same region, Simpson (2006) documented an association between living in proximity to a fish pond and self-reported malaria incidence (aOR=0.95; 95% CI:0.92-0.98 – per 25 metre increments) (i.e., increasing distance from a fish pond decreases risk). He used a GPS to measure the distance between houses and fish ponds. This is an advantage over the method of Vittor (2003) but again, the number and shape of the fish ponds were not taken into account. Furthermore, the use of self-reported malaria introduces a bias because of its reduced accuracy compared to microscopic diagnosis (sensitivity of 68% and specificity of 95% according to Duarte (1999)) and because asymptomatic cases are not taken into account.

Finally, Matthys *et al.* (2006b) found that living in proximity to a fish pond was a risk factor for malaria in an urban area of Côte d'Ivoire. Living more than 500 metres away was found to be protective against malaria infection (OR= 0.47; 95 % CI 0.22 - 0.99). This result was obtained for *P. falciparum* infections and the comparison group included households living less than 100 metres from a fish pond. Like the study of Simpson (2006) a GPS was used to measure distance between fish ponds and houses, and the density of fish ponds was not taken into account.

In these three studies, the primary exposure (i.e., fish ponds) was inadequately ascertained. In order to have a clear understanding of the impact of fish ponds on malaria transmission, a measure of fish pond density must be used. Density measures take into account not only the closest fish pond, but all fish ponds within a predetermined radius from a house. The public health significance of fish farming on malaria transmission in the Amazon region therefore requires closer scrutiny.

CONCLUDING REMARKS

The main malaria vector in the Amazon basin of South America, *An. darlingi*, can transmit the three *Plasmodium* species present on the continent. *An. darlingi* larvae have been found in fish ponds of several countries of South America despite the fact that this species breeding requirements are not typically encountered in fish ponds. Even though the species presence has been noted in fish ponds, very few studies have reported on its relative abundance in fish ponds compared to other potential breeding habitats. Finally, only three studies have examined at the epidemiological significance of fish ponds on malaria infection (one MA thesis, one PhD dissertation, and one peer-reviewed journal article). None of these studies adequately ascertained the primary exposure and, therefore, the public health significance of fish ponds on malaria infection remains unanswered.

3- CHAPTER THREE: Objectives

General objective

To determine the association between the density of fish ponds and the occurrence of malaria infection in rural communities of the Peruvian Amazon

Specific objectives

The specific objectives are:

1) To quantify the effect of fish pond density on malaria occurrence in the Peruvian Amazon.

2) To quantify the population impact of fish pond density on malaria occurrence.

These objectives were carried out using a community-based participatory approach.

4- CHAPTER FOUR: Methodology

OVERALL STUDY DESIGN

A 30-month retrospective open cohort study design was carried out to examine the effect and impact of fish pond density on malaria transmission (*Plasmodium spp*.) in eight communities located southwest of Iquitos, in the Peruvian Amazon. The study's only exclusion criterion was residing less than four weeks in one of the eight study communities during the January 2006 to August 2008 period. Information on outcome was collected from the 2006, 2007 and 2008 malaria registries of a government health post and information on exposures (and confounders), from household interviews. The latter enabled us to recreate the time sequence of fish pond construction (fish pond density was measured using remote sensing), changes in household location, and changes in household composition.

STUDY AREA DESCRIPTION

The study was carried out in the Peruvian Department of Loreto. This administrative unit is located in the north-eastern part of the country and is entirely encompassed by the Amazon Basin. Iquitos (3^{o} S, 73^{o}W) is the capital of this Department and lies at the confluence of the Amazon and Itaya rivers, about 400 km east of the Andes and 2500 km west of the Atlantic Ocean. In this region malaria transmission is year-round. However, strong seasonal patterns are observed and the number of reported cases is linked to fluctuations in river water levels, caused by the alternation of the dry and wet seasons (Bautista *et al.* 2006). The rainy season begins in November-January and ends in April-May. Months with maximum precipitation are March, April, and November (Marengo 1998). Annual precipitation is high with an average of 3018 mm but inter-annual variation can be as important as seasonal ones. Average monthly temperature varies between 25^{o}C and 27^{o}C and relative humidity maximum and minimum are 96% and 72%, respectively. Environmental conditions are generally typical of the Amazon (Kalliola and Flores Paitán 1998) and local forests are internationally renowned for their biodiversity (Gentry 1988).

Malaria in Loreto is considered hypoendemic. Most cases of malaria are reported from peri-urban and rural areas around Iquitos, the majority of them occurring along the 95-kilometre Iquitos-Nauta road. This is the only road in the region, the city being accessible only by river or air, and the primary site of frontier agricultural colonization in north-eastern Peru (Mäki *et al.* 2001). Construction of the road began in the 1970s but progress was slow; in 1980 the road only extended 20 km from Iquitos. In 1985, the road (unpaved) extended 40 km from Iquitos and it was only in 1989 that the road reached Nauta (Kalliola and Flores Paitán 1998). Pavement of the entire stretch of the road was completed in 2005. Active settlement began in the 1980s and colonization was enhanced by government subsidies (Coomes 1996). During this period, three parallel roads called 'penetraciones' that stretch from the Iquitos-Nauta road to the Nanay River were cleared to facilitate product extraction (timber, charcoal, hunting, etc.) and agricultural colonization. In 1999, the northernmost part of these 'penetraciones' became a protected forest area called the Allpahuayo-Mishana Reserved Zone.

The local population is almost entirely of mixed Indigenous-European heritage ('mestizo'). They engage in subsistence-scale farming, selling their products to local markets around Iquitos. The main cash crops are plantain, yucca roots, rice, and corn. Others activities include hunting, fish farming, raising chicken, and cattle ranching. Soil composition is considered to be of poor to intermediate quality. Most houses are located on small hilltops and surrounding streams ('quebradas') are usually dammed to create fish ponds. The rural population is highly mobile and about a third of the inhabitants don't own the land they cultivate. Not owning their land implies that they would usually work on different properties as guardians, with the consequence that they generally move to work outside of the community after a few years. Within the study region, there are six primary schools and two high schools. There is no electricity and no piped water is available in the area.

Study area justification

The following criteria were defined *a priori* as requirements for the study area and population:

a) The area must be endemic for malaria.

b) The area must exhibit a gradient in fish pond density.
c) The study population must have a certain degree of stability.
d) The study area should be located away from major rivers such as the Itaya, Nanay, and Amazon rivers as to not confound or interfere with the primary exposure.
e) The chosen study communities must be contiguous in order to use remote sensing data to measure the primary exposure and other potential confounders.
f) Communities must be located close to a rural health post and far enough away from the city of Iquitos so that the primary outcome can be ascertained precisely based on the health post's malaria registries.
g) The households comprising the communities must be distant from one another in order for the primary exposure to vary between them.

The chosen study area that fulfilled these requirements lies between kilometre 34 and 42 of the Iquitos-Nauta road (kilometre 0 corresponds to the outskirts of Iquitos) and encompasses the three 'penetraciones' (Figure 4.1). In this area, eight communities are present: Paujil 1ra Zona, Paujil 2^{a} Zona, Nuevo Horizonte 1ra Zona, Nuevo Horizonte 2^{a} Zona, Yarana Yuto, Ex Petroleros 1ra Zona, Ex Petroleros Zona Centro, and 1^{o} de Mayo. Part of the community of Ex Petroleros 1ra Zona, between kilometre 40 and 42, did not meet the last requirement (g) and was therefore excluded from the study. There, the households are built in a row format, side by side. In the rest of the study area, households are generally located on individual parcels of land and are consequently distant from one another. The chosen study area is extensive and covers more than 95 km^2 of a diverse array of land cover and land use.

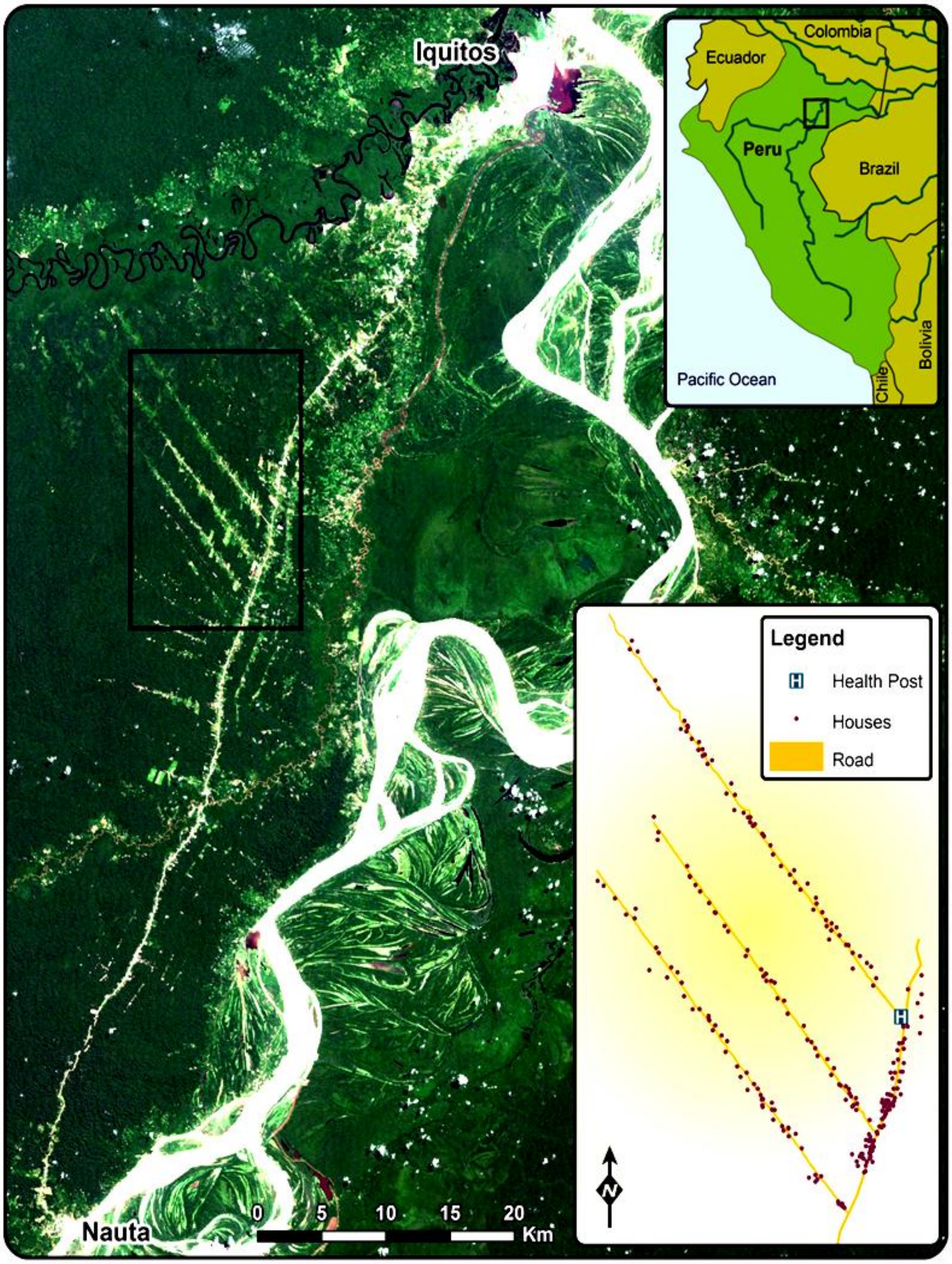

Figure 4.1: Location of the study area and regional context (Landsat ETM+ image from 2001)

SAMPLE SIZE AND POWER CALCULATIONS

The study was designed to include approximately 1,100 individuals. Based on government data, the population of the eight communities in the study area is estimated at 1,536 individuals in 2007 (Table 4.1), including the portion not sampled of 'Ex Petroleros 1ra Zona'.

Table 4.1: Population estimate of communities in the study area (1990s -2007)

Communities	**Population estimates (number of inhabitants)**			
	1990s*	**2005†**	**2006†**	**2007†**
Paujil 1ra Zona	389	69	64	64
Paujil 2a Zona	226	281	225	265
Nuevo Horizonte 1ra Zona	600‡	400	*NA*§	360
Nuevo Horizonte 2a Zona		190	254	123
Yarana Yuto	135	*NA*§	64	70
Ex Petroleros 1ra Zona	300	365	274	415
Ex Petroleros Zona Centro	220	54	156	149
1o de Mayo	128	84	81	90
Total	**1998**	***NA*§**	***NA*§**	**1536**

*Estimates from Mäki et al. (2001)
†Estimates from the DIRESA (unpublished data)
‡Population estimates of Nuevo Horizonte 1ra Zona and 2a Zona are pooled together
§NA = not available

The sample size of 1,100 individuals is based on the following conservative assumptions:

a) Intra-class correlation of malaria infection measured at the household level is estimated to be below 0.1 (based on a study of *P. vivax* in Papua New Guinea (Kasehagen *et al.* 2007)).
b) The average household is composed of 5 individuals (based on the study of Simpson (2006) in the same area who reported an average of 4.6 individuals per household).
c) Approximately 90% of the population attends the 'El Paujil' health post (based on discussions with local health authorities).
d) A response rate of 95% is expected.
e) The 30-month malaria prevalence is approximately 15% (when exposed to the mean density of fish ponds in the area). Given the fact that Simpson (2006)

reported a 12-month malaria prevalence of 27.5% in 2005, this is a very conservative estimate.

Taking into account i) non-responses, ii) individuals attending a health post other than ‘El Paujil’ iii) and intra-household correlation, 1,100 individuals was estimated to result in an effective sample size of 697. The method of Hsieh (1998) was used to calculate power for performing a logistic regression analysis with a normally distributed primary exposure variable. It was estimated that the study had excellent power (94%) to detect a moderate effect that corresponds to an odds ratio of 1.45 when the primary exposure is increased to one standard deviation above the mean (Table 4.2). Furthermore, the study would have good power (80%) to detect an odds ratio of 1.35 and fairly acceptable power (70%) to detect a small effect corresponding to an odds ratio of 1.30.

Table 4.2: Logistic regression power analysis

Odds Ratio*	**Power**
1.15	26.0%
1.20	40.5%
1.25	55.7%
1.30	70.0%
1.35	80.7%
1.40	88.7%
1.45	93.8%
1.50	96.9%
1.55	98.5%

*All odds ratios correspond to a one standard deviation increase of the exposure above the mean (the level of statistical significance considered is 0.05, with an effective sample size of 697, and a malaria period prevalence of 15%, when exposed to the mean density of fish ponds)

COMMUNITY-BASED APPROACH

Community involvement was vital to ensure cooperation of all inhabitants of the selected communities and is considered an efficient way to maximize participation (Macaulay *et al.* 1999). Also of prime importance was the inclusion of decision-makers and stakeholders in the study in order to facilitate the later uptake of study results. Therefore, a community-based research framework was adopted.

A meeting with the members of the DIRESA and the Peruvian team of PAMAFRO (the organization charged with of malaria control in the remote Amazon regions of Ecuador, Peru, Colombia and Venezuela) was organized and the research project was described. Anticipated difficulties were discussed and the selection of the study area was validated. The medical director and health personnel of the 'El Paujil' health post were also met, the project was presented to them, and their suggestions were solicited.

Before beginning data collection in the communities, two preliminary visits were undertaken in each community by the research team (M. Maheu-Giroux and research assistant J.T. Rojas). The objective of the first visit was to present the project and introduce the research team to the community's elected authority figures: the 'Agente Municipal' (i.e., the civil authority) and the 'Teniente Governador' (i.e., the judicial authority). Obtaining the support of these leaders was crucial because they could have refused access of the study communities to the research team. In order to secure their cooperation, the study was explained in detail, all concerns were answered, and any misunderstandings were clarified. Once the relevance of the study was demonstrated, the 'Agente Municipal' and 'Teniente Governador' were asked to invite community members to a 'charla' (i.e., a general assembly). During the 'charlas', the research team introduced itself to the community members and the study's objectives and general framework were presented. The attendees were encouraged to express their concerns and priorities regarding malaria and to provide suggestions to improve the execution of the study. Whenever possible, the research protocol was modified to address the expressed concerns. For example, some fish farmers were concerned that they might be accused of spreading malaria. To avoid this kind of backlash, the protocol was modified to take into account the impact of other potential *Anopheles spp.* breeding habitats (wetlands, lakes, streams, etc.). During the 'charlas', motivated knowledgeable individuals able to help in the planning and execution of the study were identified. For example, some houses were difficult to find because they were located far away from the path/road and help from locals was often needed. Sketches and preliminary maps were also drawn up for that purpose. Finally, the informed consent form (Appendix 1) was read out loud to all the ‘charla’ participants because the illiteracy rate is high in this population. The informed consent form was subsequently re-read to study participants before each interview.

Regarding stakeholders, officials of FONDEPES (National Aquaculture Development Fund) were met and the project was presented. Although they expressed concerns similar to the ones of the fish farmers, they acknowledged the fact that empirical evidence was needed to inform the debate. They were very useful in terms of explaining to the research team how the fish ponds are managed and the implications this could have for mosquito breeding. FONDEPES itself has a fingerling production and capacity-building facilities in one of the communities (Nuevo Horizonte 1ra Zona). The research team was offered to stay at this research facility if needed.

Finally, following completion of the data collection and preliminary analysis of the results, ‘charlas’ were held in all study communities and with members of the DIRESA and PAMAFRO to present them with the project’s results.

DATA COLLECTION PROCEDURES

Outcome ascertainment

To ascertain malaria incidence we used data routinely collected by the local health post. Malaria diagnosis and treatment are provided free of charge at these government-run clinics. The ‘El Paujil’ health post is open $5^{1/2}$ days a week and is staffed with a doctor, nurses, a laboratory technician, and community health workers. This clinic’s catchment area encompasses all communities located between kilometre 25 and 60 of the Iquitos-Nauta road (‘El Paujil’ is located at kilometre 35). Within the study area, access to the health post is generally by foot and individuals of the most remote community (1^{o} de Mayo) must walk six hours to reach the clinic. Even though the three ‘penetraciones’ have dirt roads, motorized vehicles cannot access the area whenever it rains. Due to difficulty of access, the Ministry of Health (MOH) trained a number of community-based health promoters to take blood smears at patients’ houses and subsequently to take the smears to the health post for diagnosis. In the eventuality that the smear was positive for malaria parasites, the health promoter was also trained to administer half a course of the malaria treatment, after which the patient must visit the health post to complete the treatment. This is done in order to avoid the trafficking of malaria drugs.

Patients who come to the health post with symptoms of malaria (fever, headache, diarrhea, malaise, etc.) are tested for *Plasmodium spp.* infection using a Giemsa-stained blood smear that is examined under a microscope using a standard procedure (with 1,000x magnification to read the thick smears and using a minimum of 100 microscope fields) (MINSA 2007). The laboratory technician is employed and trained by the MOH and a minimum of 20% of all blood smears undergo a second quality control reading by the MOH reference laboratory. Since 2007, rapid tests (*Para*Sight® F+V) for the diagnosis of *P. falciparum* and *P. vivax* were also available to health promoters and the health post to help improve the rapidity and accuracy of the diagnosis. Even in the eventuality of a positive rapid test, microscopic examination of blood slides would be carried out to calculate the parasitemia level. Each patient tested for malaria, either at the health post or by a health promoter, is included in the malaria registry where name, age, sex, domicile, date of first symptoms, date of visit at the clinic, diagnostic results, parasitemia level, malaria species, and notification date are recorded. The malaria registry thus reflects symptomatic cases of malaria and represents passive surveillance data.

Active surveillance of asymptomatic individuals is also carried out by community health workers from the MOH who regularly go into the communities and take blood smears of cooperative individuals. All asymptomatic individuals are registered in the 'active-surveillance' registry kept at the health post and the same information as in the 'passive surveillance' malaria registry is recorded.

The case definition therefore comes from both active and passive surveillance methods. The name, age, sex, and community of domicile were used to link participating individuals with malaria cases detected at the local health post or by health promoters. Because it is illegal in Loreto for pharmacies to sell anti-malarial treatment (although this law is not enforced), it is believed that self-treatment is uncommon and that the government-run clinics treat the great majority of cases.

Whenever a case of *P. vivax* is confirmed, the routine treatment administered to adults consists of chloroquine (3 days) and primaquine (7 days) (MINSA 2007). Primaquine is the only drug available that can eliminate hypnozoites from the liver and prevent relapses of *P. vivax*. The risk of relapse is not eliminated completely, however. It was estimated that the current primaquine regime in Peru can decrease the risk of relapse to approximately 80% during the following six months (Goller *et al.* 2007). For *P. falciparum*, the treatments differ by region in Peru but it generally consists of artesunate and mefloquine (3 days) in the Amazon area. If a resistant strain of *P. falciparum* is encountered, three drugs are administered: quinine, clindamicine, and primaquine (7 days).

The study question focuses on malaria transmission and it is therefore of prime importance to take only incident cases into account. The three definitions below were used to define cases that are not considered as incident.

<u>Treatment failures</u>: if two or more malaria episodes occurred within 28 days of each other, the subsequent episodes were considered as treatment failures.

<u>Mixed species infection</u>: if different *Plasmodium* species were found in consecutive slides examined fewer than 28 days apart, the two were considered as a single episode of a mixed-species infection.

<u>*P. vivax* relapses</u>: to take into account possible relapses, analyses were confirmed using a case definition that considered all *P. vivax* malaria episodes occurring within 90 days of each other as relapses from the first episode. This choice of 90 days was based on the fact that the interval between a first and second episode of malaria generally varies between 62 and 110 days (Chen *et al.* 2007; Mason 1975; Rowland and Durrani 1999).

Given the previous definitions, subjects diagnosed with malaria were removed from the person-years at risk denominator for the following 28 days (90 days if the definition of *P. vivax* relapses is used).

Primary exposure

In order to accurately measure fish pond density around households, remote sensing data was used. An image was acquired from the QuickBird® satellite which, at the time of acquisition, was the commercial satellite with the highest spatial resolution available in the multispectral bands. The pan-sharpened image was taken in July 2008 and the resulting image's pixels have a ground resolution of 60-70 cm. The orthorectification of the image was not required because of the relatively flat topography of the study area.

The image was nevertheless georectified in ArcGIS 9.3 (ESRI Inc., Redlands, CA) using a first order polynomial transformation and 58 ground control points. These points were taken using a handheld GPS (Garmin's GPSMAP 60CSx) with a positional accuracy between 3 and 5 metres. It is important to mention here that the absolute spatial precision of the image is not of importance because fish pond density will be measured using the image itself, which has a high relative precision.

Fish pond density was measured by digitizing the fish pond within a GIS. From this layer, the area and periphery of fish ponds were accurately measured. A total of 247 fish ponds were digitized from the satellite image (within a one km buffer zone around each household) (Figure 4.2). The cloud cover of the image was 5.9% and an older satellite image, acquired in 2004, was used to locate and digitize fish ponds covered by clouds. Other land cover and land uses were also digitized and classified into 9 categories (10 including fish ponds): cloud, stream, cropland, forest, grassland, recently cleared area, roads, scrubland, and water bodies (natural ponds and wetlands). The density of fish ponds was calculated by summing the length of the perimeter of each fish pond (i.e., the shoreline) in a predetermined buffer zone around each household. Fish pond density was also calculated by summing the total area of the fish pond within the buffer zone. Whereas both measures were considered in our analyses, results from the perimeter method are considered superior. The *Anopheles* vector is known to oviposition close to the land/water interface and it is also along the shoreline that the *Anopheles* larva develop (Achee *et al.* 2006). Thus, it is hypothesized that measuring fish pond density using the perimeter better reflects the vector's breeding ecology. Preliminary analysis of the density measured at seven scales ranging from 250 to 600 metres from a household determined that fish pond density measured at a scale of 500 metres provided the best fit (i.e.; within a 500 m radius around households). Goodness of fit was assessed using the quasi-likelihood under the independence model criterion (QIC) for Generalized Estimating Equations (GEE) (Pan 2001). In order to compare our results with those of previous studies, the distance to the closest fish pond was also considered.

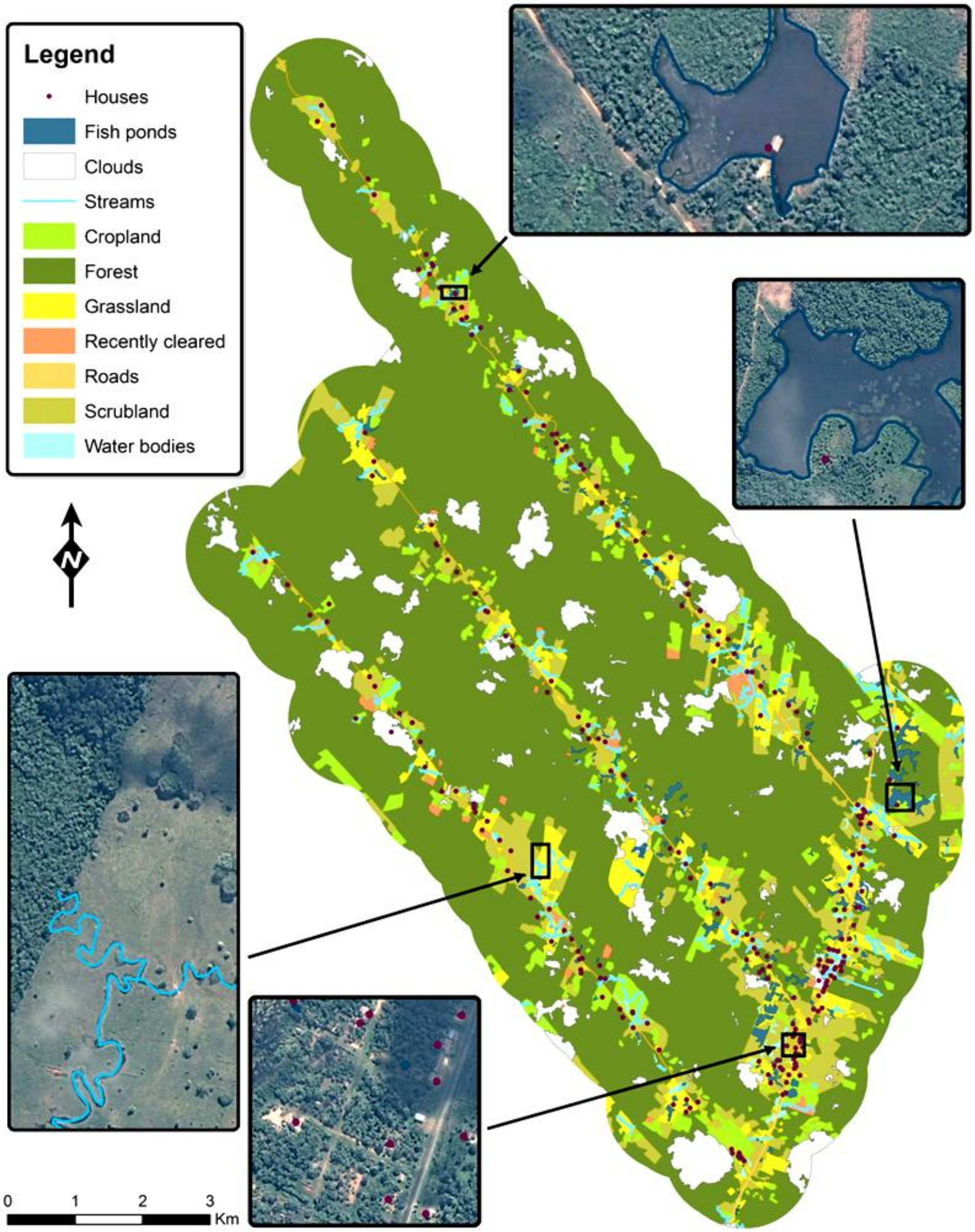

Figure 4.2: Land cover and land use map of the study area with examples of the level of detail that can be attained with the satellite image

Pilot study and questionnaire development

A pilot study was conducted in four communities along the Iquitos-Nauta road (Villa Buen Pastor, 13 de Febrero, Belen de Juda, and 24 de Junio) that shared the same characteristics as the ones in the study area. The main objective was to pre-test the interview questions and to assess the feasibility and logistics of the field work. The questionnaire's goal was to collect information on the main risk factors for malaria in the Peruvian Amazon and potential confounders. This preliminary questionnaire was tested on 12 households which included 64 individuals. As a result of this pilot study, the questionnaire was modified and its validity and reliability was improved (see Appendix 2 for the final version of the questionnaire).

Confounders and household interviews

All households of the selected communities were visited between June and August 2008 by the research team. The geographical coordinates of each household visited were recorded using the handheld GPS. The questionnaire was administered in Spanish (by MMG) to the head of each household (or designate). Information on known risk factors and potential confounders such as age, gender, occupation, house characteristics, socio-economic status, insecticide spraying, use of bed nets, use of protective clothing, and household crowding was collected. In the event that an adult was unavailable at the time of the first visit, two additional visits on different days and at different times were undertaken. Each interviewee was asked if they owned or operated a fish pond. Following an affirmative reply, the geographical coordinates of the fish pond were recorded using the GPS, along with information on its construction date, maintenance status, depth, species of fish raised, and mosquito larval control activities. Fish farms on abandoned properties were also surveyed and information on these was collected by asking a knowledgeable neighbour. A total of 220 fish ponds were located during the field visits.

Individuals who reported attending a health post other than the 'El Paujil' health post were excluded *post-hoc* from the sample.

STATISTICAL ANALYSES

The choice of a particular statistical model was determined after considering a number of factors. First, the presence of a design-effect which causes correlation at the household level was taken into account. Individuals from the same household being more similar, they cannot be regarded as independent observations. Second, a number of time-dependent covariates required consideration (e.g., age, fish pond density, and location of residence). Because the study is longitudinal in nature, either a repeated measure approach or a survival analysis approach could be used. The first option was chosen for the simple reason that the research question focus is not whether or when malaria occurs but where malaria occurs. To use a repeated measures approach, the follow-up time of each subject needs to be divided into a set of (repeated) observations. A one-month scale was the minimum observation scale possible given interviewee ability to report dates precisely. Using this approach, correlation now arises at two different levels, the household level and the individual level (repeated measures on the same subject). The repeated measures framework offers two more options: a population-averaged approach (GEE) and a subject-specific approach (random-effects logistic models). The choice between the two is usually made on theoretical grounds relating to the research question (i.e.; a public health perspective vs. a clinician perspective). Because the research question is posed from a public health perspective, the population-averaged model of GEE was used. Furthermore, estimates of coefficients from a population-averaged approach have the same interpretation as the ones obtained from traditional epidemiologic methods (stratified analysis, logistic regression, etc.) (Hu *et al.* 1998). Using GEE, the correlation within clusters is taken into account by robust estimation of the variance of the regression coefficients. This is an important advantage of this model over random-effects logistic models because in the latter, the choice of a correlation structure is not trivial, making GEE a more efficient approach (Hanley *et al.* 2003; Liang and Zeger 1986).

Another complication arises because a total of 115 subjects (involving 63 households) changed their place of residence within the study area during the study period. In some instances, the whole family relocated to a new house and in other instances there was a rearrangement of the household/family unit; for example, when a woman would move to live with her husband in the house of the in-laws. The clusters are thus non-nested and standard GEE functions of different statistical packages cannot be used. Instead, a macro that invokes PROC GENMOD in SAS version 9.1 (SAS Institute Inc., Cary, NC, USA) was used to estimate the coefficients' standard errors (Miglioretti and Heagerty 2007). This macro assumes a working independence assumption coupled with a multistep method for obtaining empirical standard errors. An advantage of GEE models is that, given a reasonably large sample size, estimators are robust against misspecification of the model and violations of normality assumptions (Hox 2002). All measured confounders that were judged to have sufficient variation, few missing data, and small measurement errors were included in the analysis. Observations with missing data were excluded from the analysis (1.94% of all observations). All covariates were centered on their mean, previous to model estimation.

To deal with the seasonal nature of malaria transmission, an index of intensity of malaria transmission that takes into account the time at risk for each month was included as an offset in the model:

Offset_{ij} = ((Nb. of malaria cases observed at month_i) / (Maximum nb. of malaria cases observed during a month)) * Time at risk for individual_j

Individuals living in households located closer in a geographical space may not be considered independent of one another. This can pose a statistical difficulty if the residuals of the regression model are spatially correlated. However, it is likely that the spatial clustering of cases can be taken into account by including in the model relevant spatially structured variables such as the density of fish ponds, SES, or proximity to potential breeding sites. In order to verify that the model's residuals are spatially independent, the Moran's I statistic for spatial autocorrelation was used (Moran 1950). Residuals autocorrelation was investigated for distances ranging from 1 metre to 1 kilometre. Calculations were performed in the R software version 2.7 (R Foundation for Statistical Computing, Vienna, Austria) using the 'moran.test' function of package 'spdep' (Bivand 2008) on the log-transformed residuals and using row-standardized connectivity matrices. Results indicate that the residuals exhibited negligible autocorrelation and, therefore, accounting for spatial dependence was not required.

To estimate the public health impact of fish ponds on malaria transmission, population attributable risks (PARs) were calculated. Although it is possible to estimate PARs for a continuous exposure, they are difficult to interpret because the reference category is a theoretically shifted distribution of the measured exposure. For ease of interpretation, the density of fish ponds was dichotomized at different distance thresholds to obtain a set of binary variables. Adjusted PARs for fish pond density were obtained using the method proposed by Greenland (Greenland and Drescher 1993). Confidence intervals were estimated by computing 1 000 bootstrap replicates using the 'boot' package (Canty and Ripley 2008) of the R software version 2.7.

Ethical considerations

The study was given ethics approval by the Research Ethics Office of the McGill University Health Centre, Canada (Appendix 3). The study protocol was also approved by the Dirección Regional de Salud of Loreto, Peru (Appendix 4). Written informed consent was obtained from all interviewees. The study procedure included an interviewer-administered questionnaire which did not involve any risk. In the eventuality that a participant was found to have symptoms of malaria (or any other disease), s/he was referred to the 'El Paujil' health post and the local health promoter was informed.

An incentive consisting of a pair of batteries (DD type) worth no more than 2.00 S/. (approximately 0.75 CAD$) was given as compensation to each household after the interview was completed. Furthermore, whenever overnight stays in the communities were required, the household was offered 1/2 litre of cooking oil (approximately 4.00 S/. or 1.50 CAD$) and 1 kilogram of sugar or rice (approximately 3.00 S/. or 1.15 CAD$). If the household provided meals (generally breakfast) the quantity of goods given was increased.

According to Peruvian tradition, whenever a 'charla' was organized for the research project, a sufficient quantity of soft drinks, cookies, and sweets were provided to the participants.

5- CHAPTER FIVE: Manuscript - Risk of Malaria Transmission and Fish Farming in the Peruvian Amazon

Mathieu Maheu-Giroux, Martin Casapia, Veronica E. Soto-Calle, Lea Berrang Ford, David L. Buckeridge, Oliver T. Coomes, and Theresa W. Gyorkos

ABSTRACT

The contribution made by fish ponds to malaria transmission in the Peruvian Amazon is controversial because previous research results have yielded mixed evidence, and because the mosquito vector is known to have breeding requirements not typical of fish ponds. To examine the association between fish pond density and malaria occurrence, we conducted a retrospective 30-month cohort study involving 1,018 individuals in eight communities southwest of Iquitos (Peru), using both active and passive malaria surveillance data. Fish pond density was found to be a significant predictor of malaria occurrence (aOR = 1.23; 95% CI: 1.09-1.38). At the population level, an estimated 45% (95% CI: 22%-69%) of malaria cases could be attributed to exposure to fish ponds, if causally linked. These results have important implications for the prevention and control of malaria and the development of aquaculture as an important economic activity in Amazonia and beyond.

KEYWORDS: Aquaculture, Cohort study, Epidemiology, Fish farm, Geographic Information Systems, Malaria, Peru, *Plasmodium falciparum*, *Plasmodium vivax*.

INTRODUCTION

The Peruvian Amazon was the last region of the greater Amazon to experience the re-emergence of epidemic malaria (1). In this region, the dramatic increase in the number of cases occurred in the 1990s and has been attributed primarily to the extended distribution range of the highly anthropophilic and efficient malaria vector *Anopheles darlingi* (2), the reduction of house spraying control programs in the previous decade (3), and deforestation associated with rural frontier colonization (4). More controversial is the claim that the development of fish farming activities has contributed to this resurgence - controversial because *A. darlingi* is generally considered sylvatic and riverine, and its preferred breeding habitat is characterized by clear, partially-shaded, slow-moving waters with abundant emergent and floating vegetation (5-8). Such breeding conditions are not typical of fish ponds (9). However, a recent and exhaustive entomological survey of potential breeding habitats conducted in this region concluded that fish ponds are the habitat in which *A. darlingi* larva are most frequently found (10). Due in part to the strong cultural dietary preference for fish over beef in this region of Peru, fish farming is now an important economic activity in the area. In addition, the commercial inland fishery of the Amazon Basin is beginning to show classical signs of overfishing, such that expansion of aquaculture has been advocated as an alternative source of fish (11-14).

Epidemiological studies conducted to date show mixed evidence regarding the effect of fish farms on malaria transmission in the Peruvian Amazon. In a cross-sectional survey of malaria prevalence, Vittor et al. (10) reported that the presence of a fish pond close to the house was a risk factor for *Plasmodium falciparum* malaria but not for the more common *P. vivax* malaria. Simpson (MA thesis, Columbia University, 2006) also found that households located closer to a fish pond had a higher number of self-reported malaria episodes in the last year than households farther away. In both studies, only the closest fish pond was taken into account and no attempt was made to control for the size and density of all fish ponds within a specified distance to the households. The public health significance of fish farming on malaria transmission in this region therefore requires closer scrutiny.

The purpose of this study was to quantify the effect of fish pond density on malaria occurrence in the Peruvian Amazon. A 30-month population-based open cohort study was conducted in eight communities located southwest of Iquitos, the capital of the Department of Loreto.

MATERIALS AND METHODS

Study area and population

Iquitos (3^o S, 73^oW) and neighboring municipalities have a population of 406 000 people with the remaining areas of Loreto accounting for another 486 000 (15) (Figure 5.1). Malaria is considered hypo-endemic in the region, but some may develop immunological resistance against malaria parasites (16-18). Most cases of malaria are reported from peri-urban and rural areas around Iquitos, especially along the 95-kilometer Iquitos-Nauta Road (19). Iquitos is accessible only by river or air. The Iquitos-Nauta road is the only highway in the region and the primary site of frontier agricultural colonization in northeastern Peru (19). Along this road, we included all eight communities in the vicinity of a government-run health post ('El Paujil') that provides malaria diagnosis and treatment free of charge. There is no other medical facility in the area; the nearest one being located about 24 km away. All houses of the communities were included except those between kilometer 40 and 42 of the Iquitos-Nauta road; they were excluded because they are built in a row format, side by side, and therefore the main exposure did not vary among them. Within the study region, there are six primary schools and two high schools. Electricity and piped water are not available in the area.

Study design

A population-based open cohort study was conducted retrospectively to quantify the impact of fish pond density on malaria occurrence. Between June and August 2008, one author (MMG) and a trained local research assistant visited all households of the selected communities. The geographical coordinates of each household visited were recorded using a handheld GPS (Garmin's GPSMAP 60CSx) with a positional accuracy of 3 to 5 meters. A questionnaire was administered in Spanish (by MMG) to the head of each household (or designate). Information on potential confounders such as age, gender, occupation, house characteristics, socio-economic status, insecticide spraying, use of bed nets, use of protective clothing, and household crowding was collected. The study's only

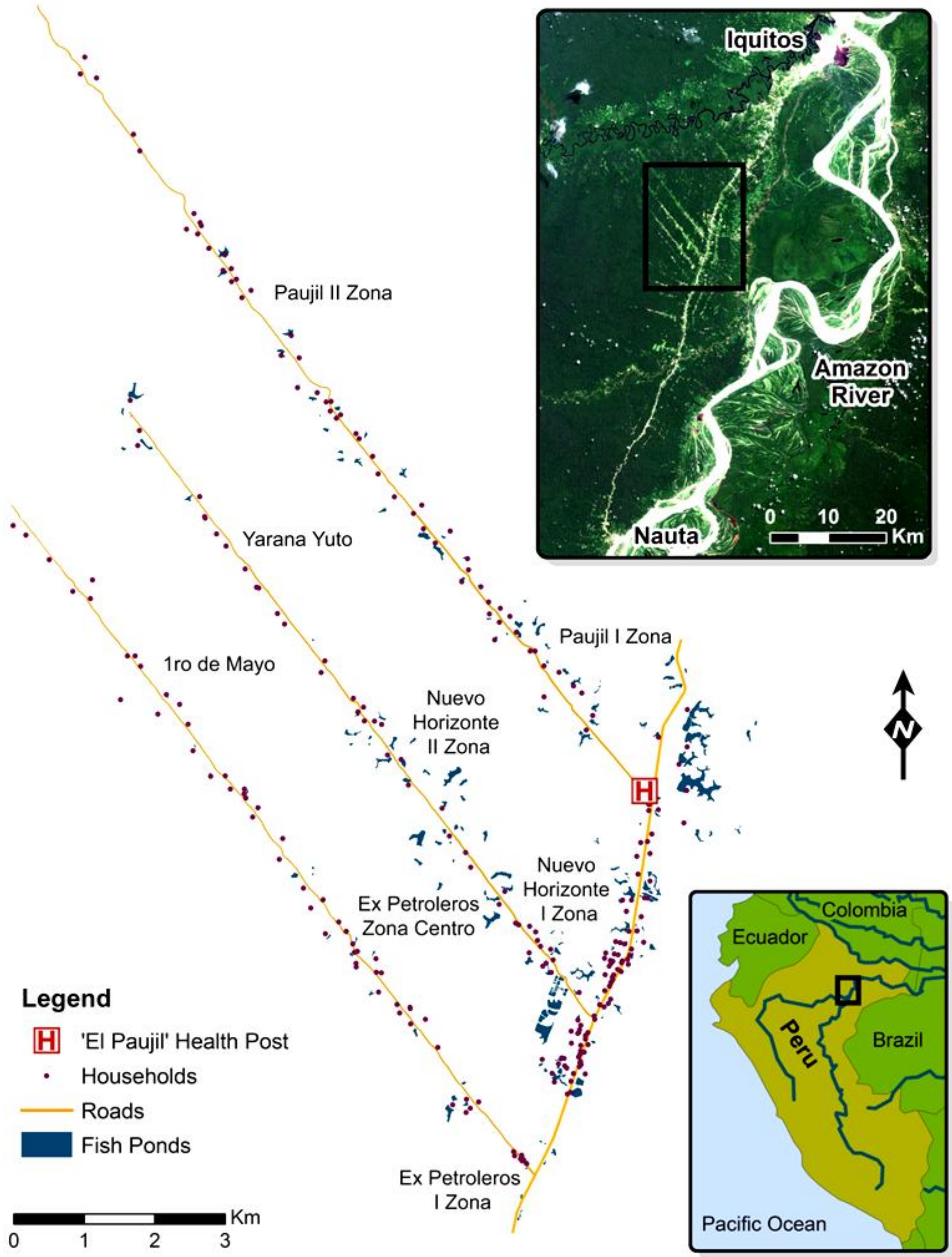

Figure 5.1: Study area with location of interviewed households and fish ponds

exclusion criterion included residing fewer than four weeks in one of the eight study communities during January 2006 – August 2008. In the event that an adult was unavailable at the time of the first visit, two additional visits on different days and at different times were undertaken. Individuals who reported attending a health post other than the ‘El Paujil’ health post were excluded post-hoc from the sample.

Malaria diagnosis

To ascertain malaria incidence we used data routinely collected by the local health post. The health post is open $5^{1/2}$ days/week and is staffed with a doctor, nurses, a laboratory technician, and community health workers. Patients who attend the clinic with symptoms of malaria (fever, headache, diarrhea, malaise, etc.) are tested for *Plasmodium spp.* infection using a Giemsa-stained blood smear that is examined under a microscope using a standard procedure (20). The name, age, sex, domicile, date of first symptoms, date of visit at the clinic, diagnostic results, parasitemia load, malaria species, and notification date of each patient tested for malaria, are recorded in the malaria registry. Active surveillance of asymptomatic individuals is also carried out by community health workers from the Ministry of Health who regularly survey the communities and take blood smears of cooperative individuals (20). Information from these asymptomatic individuals is recorded in a registry kept at the health post. Both databases were used to link confirmed cases diagnosed at the health post to our study participants.

If two or more malaria episodes occurred within 28 days of each other, the subsequent episodes were considered as treatment failures. If different *Plasmodium* species were found in consecutive slides examined fewer than 28 days apart, the two were considered as a single episode of a mixed-species infection. Subjects diagnosed with malaria were removed from the person-years at risk denominator for the following 28 days. Finally, to take into account possible *P. vivax* relapses, we confirmed our analyses by using a second case definition that considered all *P. vivax* malaria episodes occurring within 90 days of each other as relapses from the first episode.

Fish pond density

Each interviewee was asked if they owned or operated a fish pond. Following an affirmative reply, the geographical coordinates of the fish pond were recorded using the GPS, along with information on its construction date, maintenance status, depth, species of fish raised, and mosquito larval control activities. Fish farms on abandoned properties were also surveyed and information on these was collected by asking a knowledgeable neighbor. A high resolution satellite image (Quickbird), with a ground resolution of 60 cm, was acquired in July 2008. This image was geo-rectified to ensure geographical precision. Fish ponds were digitized using ArcGIS Desktop version 9.3 (ESRI Inc., Redlands, CA, USA). From this layer, fish pond density around each surveyed household could accurately be measured. Fish pond density was calculated by summing the length of the perimeter of each fish pond (i.e., the shoreline) in a predetermined buffer zone around each household. Fish pond density was also calculated by summing the total area of the fish pond within the buffer zone. Whereas both measures were considered in our analyses, results from the perimeter method are considered superior. The *Anopheles* vector is known to oviposition close to the land/water interface and it is also along the shoreline that the *Anopheles* larvae develop (21). Thus, we hypothesized that measuring fish pond density using the perimeter better reflects the vector's breeding ecology. Preliminary analysis of the density measured at seven scales ranging from 250 to 600 meters from a household determined that fish pond density measured at a scale of 500 meters provided the best fit (i.e.; within a 500 m radius around households). Goodness of fit was assessed using the quasi-likelihood under the independence model criterion (QIC) for Generalized Estimating Equations (GEE) (22). In order to compare our results with those of previous studies, we also considered the distance to the closest fish pond in our analysis.

Statistical analysis

The study design required control of a number of time-dependent covariates including age, fish pond density, and location of residence. Time-dependent covariates can be dealt with within a repeated measures framework where malaria occurrence is modeled by month. A one-month scale was the minimum observation scale possible given interviewee ability to report dates precisely. Our observations are correlated thus at two different levels: the household level and the individual level. Adjustment for spatial autocorrelation was not required because the inspection of the model's residuals using Moran's I (23) exhibited negligible autocorrelation. Because the research question is posed from a public health perspective, we used the population-averaged model of GEE. A further complication arose when a change of residence occurred within the selected communities, which resulted in the clusters being non-nested. To circumvent this problem, we used a macro function developed by Miglioretti et al. (24) that invokes PROC GENMOD in SAS version 9.1 (SAS Institute Inc., Cary, NC, USA) to estimate the standard errors of the coefficients. An advantage of this GEE model is that, given a reasonably large sample size, the estimators are robust against misspecification of the model, including misspecification of the correlation structure and violations of normality assumptions (25). We dealt with the seasonal nature of malaria transmission by including an index of intensity of malaria transmission and the time at risk for each month (taking into account the case definition considerations outlined above) as an offset in the model. All measured confounders that were judged to have sufficient variation, few missing data, and small measurement errors were included in the analysis. Observations with missing data were excluded from the analysis (1.94% of all observations).

Although it is possible to estimate population attributable risks (PARs) for a continuous exposure, they remain difficult to interpret because the reference category is a theoretically shifted distribution of the measured exposure. For ease of interpretation, we dichotomized the density of fish ponds at different distance thresholds to obtain a set of binary variables. Adjusted PARs for fish pond density were obtained using the method proposed by Greenland (26). Confidence intervals were estimated by computing 1 000 bootstrap replicates using the 'boot' package (27) of the R software version 2.7 (R Foundation for Statistical Computing, Vienna, Austria).

Ethical considerations

Ethics approval was obtained from the Research Ethics Office of the McGill University Health Centre (Canada). The study protocol was also approved by the

Dirección Regional de Salud of Loreto (Peru). Written informed consent was obtained from all interviewees.

RESULTS

Cohort characteristics

A total of 259 households were visited and the interview response rate was 99.96 % (one household declined participation). Six households were not included because they did not meet the minimum residence time of four weeks; another six households could not be interviewed after three attempts. Data were thus collected from 246 households, comprising 1,107 individuals between the ages of one month and 85 years. After excluding a further 89 individuals (from 12 households) because they reported attending another health post, the population analyzed included a total of 1,018 individuals distributed in 234 households, and contributing 2,025 person-years to follow-up. The proportion of participants who had a complete follow-up was 61% and the mean follow-up period was 23.8 months. Complete follow-up was defined as residing in the study area for the 30-month covered by the January 2006-June 2008 study period. The main reasons for not contributing complete follow-up were immigration in the study area and birth after January 2006. Baseline characteristics of study participants as well as the person-years at risk and summary of potential confounders are presented in Table 5.1.

Malaria occurrence

From January 2006 to June 2008, individuals from the surveyed communities had 269 malaria episodes registered at the health post. Of all the malaria episodes found in the registries, we were able to link 56% of them to individuals in our survey. It is likely that the high mobility of the population can account for the cases that could not be linked to an individual. According to our case definition (removing treatment failures), there were 247 malaria cases, mostly *P. vivax* infection (93.5%). There were also 15 *P. falciparum* and one mixed species infection. The transmission of malaria was highly seasonal and exhibited important inter-annual variations (Figure 5.2).

Table 5.1: Baseline characteristics of study participants and their corresponding time at risk for several potential confounders

Baseline characteristics	No. (%) Individuals	No. (%) Person-years	No. (%) Malaria episodes
Gender			
Male	569 (55.9%)	1116 (55.1%)	139 (56.3%)
Female	449 (44.1%)	909 (44.9%)	108 (43.7%)
Age			
< 10 years old	339 (33.3%)	586 (28.9%)	52 (21.0%)
≥ 10 years old	679 (66.7%)	1439 (71.1%)	195 (79.0%)
Median education level of adults in a household (proxy for SES)			
Illiterate	52 (5.1%)	113 (5.6%)	12 (4.9%)
Some primary	341 (33.5%)	607 (30.0%)	97 (39.3%)
Completed primary	365 (35.9%)	773 (38.1%)	78 (31.6%)
Some secondary	218 (21.4%)	463 (22.9%)	58 (23.5%)
Completed secondary	33 (3.2%)	51 (2.5%)	2 (0.8%)
Superior	6 (0.6%)	11 (0.5%)	0 (0%)
Missing	3 (0.3%)	7 (0.3%)	0 (0%)
Number of times the house was fumigated since January 2006* (measured when an individual leaves the cohort)			
0 times	185 (18.2%)	214 (10.6%)	25 (10.1%)
1 times	111 (10.9%)	214 (10.6%)	28 (11.3%)
2 times	597 (58.6%)	1353 (66.8%)	172 (69.6%)
3 times	81 (8.0%)	180 (8.9%)	20 (8.1%)
More than 3 times	20 (2.0%)	41 (2.0%)	0 (0%)
Missing	24 (2.4%)	23 (1.2%)	2 (0.8%)
Household crowding			
< 8 individuals	752 (73.9%)	1500 (74.1%)	185 (74.9%)
≥ 8 individuals	266 (26.1%)	525 (25.9%)	62 (25.1%)
House characteristic			
At least one closed room	800 (78.6%)	1596 (78.8%)	195 (78.9%)
No closed room	210 (20.6%)	418 (20.6%)	20 (20.2%)
Missing	8 (0.8%)	12 (0.6%)	2 (0.8%)
Occupational risk			
Fish farmer (‘piscigranjero’)	78 (7.7%)	165 (8.1%)	27 (10.9%)
Engaging in other activities	940 (92.3%)	1860 (91.9%)	220 (89.1%)
Total length of streams (km) in a 400-meter buffer around houses*			
≤ 1 km of streams	697 (68.5%)	1386 (68.5%)	178 (72.1%)
> 1 km and ≤ 2 km	262 (25.7%)	521 (25.7%)	55 (22.3%)
> 2 and ≤ 3 km	47 (4.6%)	94 (4.6%)	12 (4.9%)
More than 3 km	12 (1.2%)	24 (1.2%)	2 (0.8%)

*Continuous variables were categorized for display in this table but were included as continuous variables in the GEE analysis

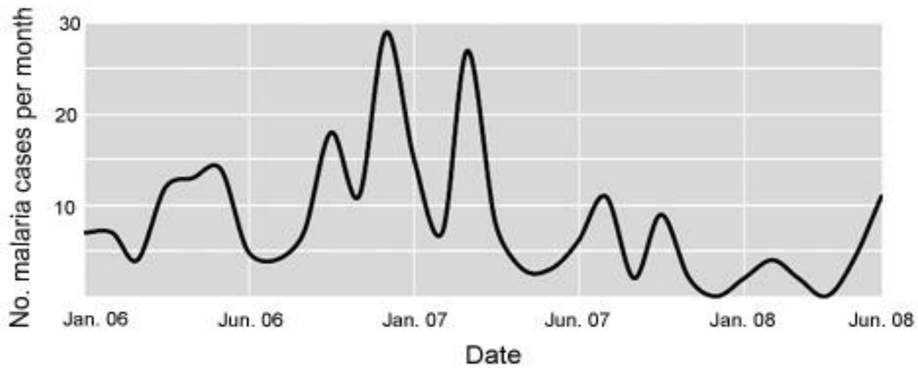

Figure 5.2: Number of malaria cases registered from study communities at the El Paujil Health Post each month

Fish pond density as a risk factor for malaria

Individuals were exposed to a mean fish pond density of 1.4 km ($\sigma = 1.2$) (perimeter method) or 1.7 ha ($\sigma = 1.7$) (area method) within a 500-m radius. Of the 259 houses originally visited, 95 of them (37%) had at least one fish pond on their property. Density plots of the fish pond exposure stratified by malaria status show that the aggregate of person-time without malaria (*Plasmodium spp*.) occurred in areas with lower fish pond densities than the aggregate of person-time with malaria (Figure 5.3).

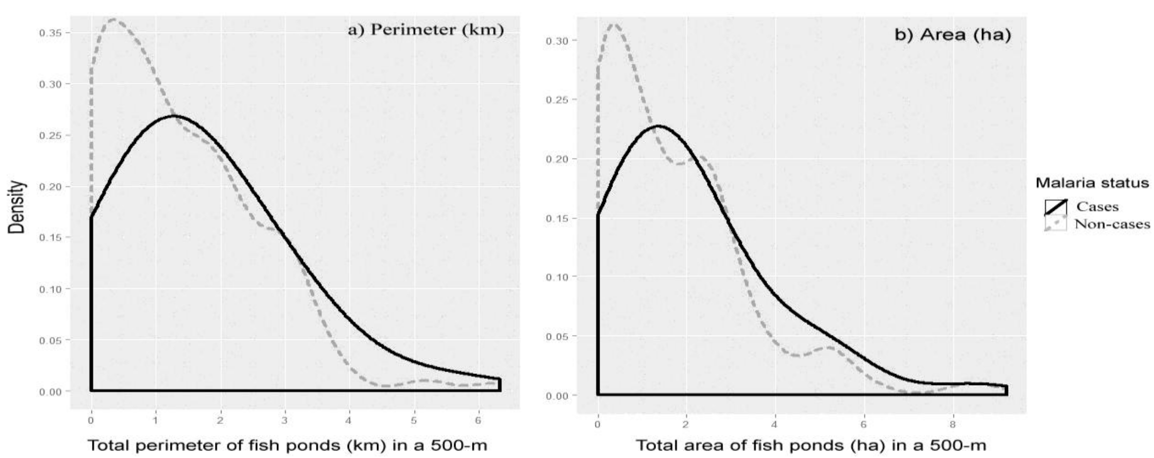

Figure 5.3: Density plots of the primary exposure stratified by malaria status, measured a) using the total perimeter of fish ponds and b) the total area of fish ponds within a 500-m buffer around households

The impression given by the density plots is confirmed by the results from the GEE analysis (Table 5.2). For total malaria (*Plasmodium spp*.) the adjusted odds ratio for fish pond density measured using the perimeter method is 1.23 (95% CI: 1.09-1.38) and does not differ greatly from the unadjusted estimate of 1.22 (1.09-1.36). The same is true if the exposure is measured using the total area method: unadjusted odds ratio of 1.13 (1.05-1.21) and an adjusted odds ratio of 1.15 (1.06-1.24).

For species-specific malaria, a significant relationship between the density of fish ponds and *P. vivax* infection is found. This relationship with *P. vivax* holds even when episodes of *P. vivax* occurring fewer than 90 days from a first episode were excluded. Density of fish ponds (perimeter method) is also a risk factor for *P. falciparum* infection although the confidence interval is wide and crosses the null. If the analysis is limited only to symptomatic cases of malaria, the relationship between fish pond density and total malaria is still significant and the point estimate is slightly higher (perimeter method).

Interestingly, when the distance to the nearest fish pond is used, the unadjusted and adjusted estimates showed a trend towards decreasing malaria risk with increasing distance, as in the species-specific and symptomatic cases analyses, but none of these estimates reached statistical significance (Table 5.3).

The population attributable risk of fish pond density

A measure of impact was calculated for our main exposure, dichotomized at different thresholds for ease of interpretation, using the two methods for calculating the density of fish ponds (Table 5.4). The different adjusted PARs indicate that, considering the absence of fish ponds as the reference category, a reduction of 45% (95% CI: 22%-69%) of cases could potentially be expected. PARs become negligible if the thresholds used are 2 km of fish pond perimeter or more or 2 ha or more of fish pond area.

Table 5.2: Unadjusted and adjusted odds ratios for the association between malaria and measures of fish pond density, using different case definitions

Case definition	No. Cases	Total periphery (km) of fish ponds*		Total area (ha) of fish ponds*	
		OR (95 % CI)†	**aOR (95 % CI)‡**	**OR (95 % CI)†**	**aOR (95 % CI)‡**
Removing treatment failures					
Total malaria *(Plasmodium spp.)*	247	1.22 (1.09-1.36)	1.23 (1.09-1.38)	1.13 (1.05-1.21)	1.15 (1.06-1.24)
P. vivax malaria	231	1.22 (1.10-1.36)	1.22 (1.08-1.37)	1.14 (1.06-1.22)	1.15 (1.06-1.23)
P. falciparum malaria	15	1.13 (0.67-1.92)	1.20 (0.73-1.96)	0.91 (0.63-1.31)	0.96 (0.69-1.34)
Removing relapses					
P. vivax malaria	222	1.22 (1.10-1.36)	1.22 (1.09-1.37)	1.14 (1.07-1.22)	1.15 (1.07-1.23)
Symptomatic cases only					
Total malaria *(Plasmodium spp.)*	217	1.25 (1.11-1.40)	1.25 (1.10-1.41)	1.13 (1.04-1.22)	1.14 (1.05-1.25)

The number of cases of *P. vivax* and *P. falciparum* don't add to 247 because of one mixed species infection that was removed from the species-specific analyses.
*Measured within a 500-m buffer around each household.
†OR = odds ratio; CI= confidence interval.
‡aOR = odds ratio adjusted for all covariates presented in Table 1 and a variable for time. An offset that takes into account the time at risk at each month multiplied by the number of recorded cases of this month was also included in both unadjusted and adjusted estimates.

Table 5.3: Unadjusted and adjusted odds ratios for the association between malaria and the distance to the closest fish pond, using different case definitions

Case definition	**No. Cases**	**Distance to closest fish pond***	
		OR (95 % CI)†	**aOR (95 % CI)‡**
Removing treatment failures			
Total malaria *(Plasmodium spp.)*	247	0.98 (0.95-1.00)	0.78 (0.60-1.01)
P. vivax malaria	231	0.79 (0.59-1.05)	0.79 (0.61-1.02)
P. falciparum malaria	15	0.71 (0.25-2.00)	0.56 (0.23-1.38)
Removing relapses			
P. vivax malaria	222	0.79 (0.59-1.05)	0.79 (0.61-1.03)
Symptomatic cases only			
Total malaria *(Plasmodium spp.)*	217	0.75 (0.53-1.07)	0.76 (0.55-1.05)

The number of cases of *P. vivax* and *P. falciparum* don't add to 247 because of one mixed species infection that was removed from the species-specific analyses.
*Presented per 500-m unit increment.
†OR = odds ratio; CI= confidence interval.
‡aOR = odds ratio adjusted for all covariates presented in Table 1 and a variable for time. An offset that takes into account the time at risk at each month multiplied by the number of recorded cases of this month was also included in both unadjusted and adjusted estimates.

Table 5.4: Adjusted attributable risk fractions for *Plasmodium spp.* malaria (symptomatic and asymptomatic cases) using different reference categories and using the two methods for measuring fish pond density

Total perimeter (km) of fish ponds*		**Total area (ha) of fish ponds***	
Reference categories	**PAR† (95 % CI)**	**Reference categories**	**PAR† (95 % CI)**
Absence of fish pond	0.45 (0.22-0.69)	Absence of fish pond	0.45 (0.22-0.69)
< 250 m of fish pond perimeter	0.39 (0.19-0.60)	< 0.5 ha of fish pond area	0.37 (0.21-0.54)
< 500 m of fish pond perimeter	0.41 (0.24-0.58)	< 1 ha of fish pond area	0.31 (0.18-0.45)
< 1 km of fish pond perimeter	0.32 (0.20-0.44)	< 2 ha of fish pond area	0.05 (-0.05-0.16)
< 2 km of fish pond perimeter	0.06 (-0.02-0.15)	< 3 ha of fish pond area	0.09 (0.02-0.15)
< 3 km of fish pond perimeter	0.07 (0.01-0.12)	< 4 ha of fish pond area	0.07 (0.02-0.13)
< 4 km of fish pond perimeter	0.02 (0.00-0.05)	< 5 ha of fish pond area	0.05 (0.01-0.09)

The outcome used is total malaria (*Plasmodium spp*.) of symptomatic and asymptomatic cases and taking into account treatment failures.

*Measured within a 500-m buffer around each household

†PAR = Population attributable risk adjusted for all covariates presented in Table 1 and a variable for time. An offset that takes into account the time at risk at each month multiplied by the number of recorded cases of this month was also included in both unadjusted and adjusted estimates.

DISCUSSION

The impact of large-scale anthropogenic modifications of the land-water interface such as the construction of dams, reservoirs, and irrigation systems on *Anopheles* vectors and malaria risk has received due attention from scientists (28-32). Fish ponds generally occur at much finer spatial scales and pond development is less ubiquitous than water management schemes. This might explain why few epidemiological studies have directly addressed the public health significance of aquaculture on malaria occurrence. To our knowledge, there is only one published report, from Côte d'Ivoire, that demonstrates a correlation between living in proximity to a fish pond and *P. falciparum* prevalence (33). Like the dissertation of Vittor (10) and the thesis of Simpson (MA thesis, Columbia University, 2006), the Côte d'Ivoire study also did not measure the density of fish ponds, making the reported estimate of effect difficult to interpret. Clearly, the malaria risk posed by living close to a small fish pond is not equivalent to the risk of living close to a large one. In fact, when we used the distance to the nearest fish pond in our analyses, the estimates of effect did not reach statistical significance even though a trend toward a decreasing risk of malaria with increasing distance was demonstrated. In the present study we report results using two methods of calculation to quantify the fish pond density. For theoretical reasons, we favor the perimeter method in assessing effect because, by summing the total length of fish pond, we take into account size, shape and number of ponds, and it better reflects the vector's oviposition behavior. We therefore recommend that future studies use the perimeter method for measuring vector habitat density.

Our most important finding is that fish pond density is associated with malaria occurrence. This association is also robust to the different case definitions considered. Furthermore, species-specific analyses also showed that fish pond density was correlated with both *P. vivax* and *P. falciparum* infection (perimeter method), although the relationship is significant only for the former. (This result was anticipated as, with only 15 *P. falciparum* cases, we knew a priori that we did not have enough statistical power to detect an important odds ratio.)

The strengths of our study include its large sample size, high response rate, accurate measurement of the primary exposure using remote sensing and GIS, use of incident laboratory-confirmed cases of malaria, inclusion of both active and passive surveillance databases, and an extensive study area (95 km2). Importantly, given the retrospective nature of the study and the high mobility of the communities (19), the rate of complete follow-up (61%) can be considered high.

One important limitation of the study lies in its retrospective nature which might have introduced a non-differential recall bias. For example, some households had difficulties identifying the precise date when they moved to their current house with the result that there may be measurement error in their time at risk. Another limitation is our exclusion of 89 individuals, those who did not attend the El Paujil health post. The excluded individuals were generally older and more highly educated than individuals who attended the health post. We also did not sample *Anopheles* larvae from fish ponds and it may be that other secondary malaria vectors are present in the study area. In fact, an entomological survey of potential *Anopheles sp.* breeding habitats along the Iquitos-Nauta road found that four potential malaria vectors (i.e., *A. rangeli, A. triannulatus, A. nuneztovari*, and *A. benarrochi*) are more frequently found in fish ponds compared to the other available habitats. This latter point has important implications for malaria control because different vector species will have different behaviors (biting time, anthropophily, endophily, etc.) that could affect the choice of control strategies.

Our results have important implications for the control of malaria as well as for the development of aquaculture in the Peruvian Amazon. Currently, the ‘Ministerio de Producción’ is subsidizing fish farmers so as to increase fish production and fish pond development is being widely promoted (Figure 4). When we asked fish farmers if they had plans to build more fish ponds, 61% answered positively. Moreover, 89% of them said that fish farming was an economically profitable activity. Because our data show that about 45% of malaria cases could be attributable to fish farming, if causally linked, intersectoral communication and action by all relevant private and governmental institutions is highly recommended. Reducing the relative amount of habitat suitable for oviposition is also advised, for example by minimizing the perimeter area ratio of ponds or constructing few bigger ponds as compared to more numerous small ones (34). The cost-effectiveness, feasibility, and sustainability of this measure and of larvicide use should be assessed locally.

With the anticipated decline of the freshwater fisheries in this region, fish farming activities are expected to become more important (11, 13) and our study suggests that this will increase the malaria burden on local populations. Given the economic benefits and nutrition source this activity provides to the rural poor, local governments face difficult dilemmas. The measures of effect and impact we present in this paper do not take into account habitat characteristics of fish ponds such as water turbidity, the abundance of emergent vegetation, and algae that have been associated with the presence of A. darlingi larvae (10). Malaria risk posed by fish farming might be mitigated by promoting good management practices that would make fish ponds a less favorable habitat for *Anopheles larvae.*

Figure 5.4: A local public advertisement painted along the Iquitos-Nauta Road praising the increased production of common agricultural goods including fish ponds (locally called 'piscigranja')

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MMG is a graduate student in epidemiology at McGill University. His research interests include landscape epidemiology, vector-borne diseases, and global health.

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6- CHAPTER SIX: Post-study community-based follow-up

Within the context of community-based research, presenting the study's results to the participating communities and stakeholders was an important and time-consuming part of this project. In January of 2009, M. Maheu-Giroux returned to the study area and a meeting with health officials of PAMAFRO and local collaborators was arranged. Suggestions about the presentation of the preliminary results to the communities were made. The presentation focus was not restricted specifically to the impact of fish ponds on malaria but more generally on important risk factors for malaria and the impact of the different breeding habitats for malaria transmission (including, but not restricted to, fish ponds). This distinction is important because local collaborators and health officials felt that it would be counter-productive at this time to only target fish ponds as a risk factor for malaria. Furthermore, this could have precipitated a climate of fear and suspicion toward this important economic activity. It should be added that, in order to develop targeted policies and appropriate interventions, the results of this thesis will now be used to spur further research on this topic and also to inform current MOH discussions on how to best mitigate malaria risk in the area.

'Charlas' were organized in each of the participating communities and a giant poster highlighting the study's results was produced and presented at these assemblies (Figure 6.1). The study's results were presented and discussed with the communities and the MOH's prevention recommendations were strengthened (use of bed nets, protective clothing, etc.). A large amount of time at the 'charlas' was also dedicated to answering questions from the participants. The turnout to the assemblies was considerable and it is estimated that more than 20% of the study population, with representatives of more than 50% of surveyed households, attended one of the 'charlas'.

Results were also presented to stakeholders and health officials of the DIRESA and PAMAFRO.

Ex Petroleros Zona Centro

Nuevo Horizonte 1ra Zona

Yarana Yuto

Paujil 2^{a} Zona

Figure 6.1: Photographs taken during the different ‘charlas’ when the study’s results were presented to the participating communities.

7 – CHAPTER SEVEN: Conclusion

The overall results from this research indicate that the density of fish ponds within 500 metres of households has the strongest association with malaria risk, compared to all measured confounders, in the study population (i.e., communities of the Peruvian Amazon located in an area of frontier agricultural colonization under conditions of hypo-endemic malaria transmission). It is further estimated that roughly half the cases of malaria observed in the communities during the study period can be attributed to the presence of fish ponds, demonstrating the important public health impact of this activity on malaria.

The conclusions of the present thesis call for intersectoral communication and action by all relevant private and governmental institutions. The future development of aquaculture in Amazonia should include health impact assessments. Management practices that could mitigate malaria risk should be researched and promoted. Such management practices could include maintaining the pond's periphery free of marginal vegetation, designing ponds with steep banks to prevent vegetation encroachment, emptying ponds when not in production, use of larvivorous fish or larvicides, constructing bigger ponds to minimize the perimeter/area ratio, shading the ponds, etc. The cost-effectiveness, feasibility, and sustainability of the suggested mitigation methods should be assessed locally.

The community-based approaches used resulted in a high participation rate of study participants, stakeholders, and health officials. The post-study presentations informed current MOH discussions on the prevention and control of malaria in Loreto. Finally, community awareness on the impact of fish ponds on malaria was increased and both communities and researchers benefited from the collaboration.

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APPENDIX 1: Informed Consent Form

Asociación Civil SELVA AMAZONICA

McGill

Página 1

Impact of different water sources on malaria transmission in the region of the Iquitos-Nauta road, Loreto

INFROM CONSENT

Investigadors: Dr. Martin Casapia Morales (Asociación Civil Selva Amazonica – Iquitos, Perú), Dr. Theresa W. Gyorkos (McGill University, Canada) and Mathieu Maheu-Giroux (McGill University, Canada).

Hi, I am working for the 'Asociación Civil Selva Amazónica' and McGill University (Canada). We are doing a study that aims to quantify the potential impact of different water bodies (for example: rivers, streams, fish ponds and lakes) could have on malaria transmission in the department of Loreto, Peru. We are asking for the participation of a family representative for an interview. The information you will provide will serve to improve the planning of health activities in the region. This interview will take 30-45 minutes to complete.

The participation to this interview is completely voluntary and you can take the decision to not answer any question without suffering any lost of benefits or privileges in relation to your sanitary assistance. You are totally free to take part or not in this study and you have the right to withdraw of the interview at any moment. However, we wish that you will participate because the information that you will provide is highly valuable to inform and improve malaria control activities. Also, it is important to mention that this study was approved by the Dirección de Salud de Loreto and the Research Ethics Committee of the McGill University Health Center.

The majority of questions in this interview are related to the characteristics of your house and to the malaria experiences of members of you family. Moreover, I would like to take a photography of you house, with your permission. All your information is confidential – this means that nobody but me and my research team will know your answers. Your name and information will not be revealed in any document or report, and nobody will know what your answers were. All interviews and data collected are going to be guard in a safe place under lock or password for electronics archives.

¿At this moment, do you have any question in relation to our interview? If you have any more questions on our study after having completed the interview, we direct you to your health promoter and he will be able to get the information from the El Paujil health post.

I have read and understand the content of the above statement. I had the opportunity to make questions and all were answered for my security. I understand that my participation is voluntary. Also, I understand that I can withdraw of this study at any moment without suffering any lost of benefits in relation to my sanitary assistance. I consent in my participation in this study.

Fingerprint

Name of the family representative	Signature of the representative - date (d/m/a)
Name of the interviewer	Signature of the interviewer - date (d/m/a)
Name of the witness	Signature of the witness - date (d/m/a)

1

ID

APPENDIX 2: Malaria Questionnaire

SELVA AMAZONICA

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Página 2

HOUSE ID NUMBER

COMMUNITY		LOCATION: EAST VS. WEST OF THE ROAD		CODE = COMMUNITY + LOCATION + KM + # ID
☐ El Paujil – Zona I	PA1	☐ East (Rigth - North)	E	
☐ El Paujil – Zona II	PA2	☐ West (Left - South)	O	
☐ Yaranayuto	YT0			
☐ Nvo Horizonte – Zona I	NH1	KM FROM THE NAUTA ROAD		
☐ Nvo Horizonte – Zona II	NH2			
☐ 1° de Mayo	MA0			
☐ Ex Petroleros – Zona I	EX1			i.e.: 'Paujil - Zona 2' + East + Km 11 + casa #1 = **PA2E11-1**
☐ Ex Petroleros – Zona II	EX2	# ID FROM THE LIST		
☐ San Lucas	SL0			

INTERVIEW ATTEMPT

VISIT 1	VISIT 2	VISIT 3
Date (Day, month, year) / /	Date (Day, month, year) / /	Date (Day, month, year) / /
☐ Failed attempt	☐ Failed attempt	☐ Failed attempt

TO BE COMPLETED BY INTERVIEWER

1	A- Date: (Day, month, year) / /	B- Parcel number (if applicable)
2	A- Community or locality	B- Fundo (if applicable)
3	Name of the interviewed participant:	
4	Geographic position of the house (UTM WSG84)	A- Latitud: B- Longitud:

5	From what material is made the roof of the house?	☐ Palm leaves (1) ☐ Calamina (2) ☐ Otro:_____ (3)
6	How many complete walls do the house poses?	☐ No wall (0) ☐ 1 wall (1) ☐ 2 walls (2) ☐ 3 walls (3) ☐ 4 walls (4)
7	Does the house have a minimum of one closed room?	☐ Si (1) ☐ No (0)
8	From what material is the floor of the house made?	☐ Sand or dirt (1) ☐ Wood (2) ☐ Cement (3) ☐ Other:_________ (4)
	Take a photo of the house with ID number	☐ Accept (1) ☐ Refuse (0)

BEGINNING OF THE INTERVIEW

WE WILL BEGIN BY ASKING YOU GENERAL QUESTIONS IN RELATION TO YOUR HOUSE AND MATERIAL POSESSION.

9	A- Since when is your family living in this house?	Time ☐ Month ☐ Year
	B- If less than 3 years, do you know the name of the family who was living year before? What is their family name?	Last Name: .... ☐ Not aplicable (0) ☐ *Don't know* (99)

2

ID

SELVA AMAZONICA

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10	For how long has your house been built?	Time [ ] ☐ Month ☐ Year ☐ *Don't know* *(99)*
11	A- Does your family own another house in another community or city? B- If yes, where?	☐ Yes (1) ☐ No (0) Communidad: .... ☐ Not aplicable (0) ☐ *Don't know* (99)
12	Does your family own the house/parcel?	☐ Yes (1) ☐ No (0)
13	Do you have a light generator?	☐ Yes (1) ☐ No (0)
14	What kind of combustible do you use to cook your food?	☐ Kerosene (1) ☐ Wood (4) ☐ Carbon (2) ☐ Other: ________ (5) ☐ Gaz (3)
15	What water source do you use for your daily necessities?	☐ River (1) ☐ Collected rain water (4) ☐ Stream (2) ☐ Lake (5) ☐ Well (3) ☐ Other: :__________ (6)
16	How much time does it take to you to walk to this water source?	Time [ ] Minutes
17	A- How many chickens do you have? B- How many ducks do you have? C- How many pigs do you have? D- How many cows do you have? E- How many fruit trees do you have? F- How much corn do you cultivate? G- How much yucca do you cultivate? H- How much platano do you cultivate?	A- Chickens [ ] E- Fruit trees [ ] B- Ducks [ ] F- Corn area [ ] C- Pigs [ ] G- Yucca area [ ] D- Cows [ ] H- Platanos area [ ]
18	A- Does one member of your family members has a regular monthly income? B- What is the monthly income of your family? *The total of all family members.*	☐ Yes (1) ☐ No (0) *ESTABLISH MONTLHY INCOME USING THIS SET OFQUESITONS:* Do you fish? *Do carbon? Sow leaves of irapai? Hunt? Sell fruit, vegetables, chickent? What else?* *¿How much can you get for those product?* Average monthly income [ ]
19	Do you have any bed net in your house?	☐ Yes (1) ☐ No (0)
20	How many do you have in the house?	Quantity [ ]
22	Which of your bed nets are impregnated with insecticide?	# 1 ☐ Impregnated (1) ☐ Not impregnated (0) ☐ *Dont' know (99)* # 1 ☐ Impregnated (1) ☐ Not impregnated (0) ☐ *Dont' know (99)* # 1 ☐ Impregnated (1) ☐ Not impregnated (0) ☐ *Dont' know (99)* # 1 ☐ Impregnated (1) ☐ Not impregnated (0) ☐ *Dont' know (99)* # 1 ☐ Impregnated (1) ☐ Not impregnated (0) ☐ *Dont' know (99)* # 1 ☐ Impregnated (1) ☐ Not impregnated (0) ☐ *Dont' know (99)*

ID [ ]

23	A- During the last 3 years, has anyone fumigated your house to protect it from biting insects? B- If yes, how many time has your house been fumigated in the last 3 years?	☐ Yes (1) ☐ No (0) ☐ *Don't know* (99) Number of time ☐	
24	Who fumigated your house?	☐ Health worker (1) ☐ Private company (2) ☐ Household member (3)	☐ Other:_______ (4) ☐ *Don't know* (99)

NOW, I WOULD LIKE TO KNOW ABOUT YOUR FAMILY MEMBERS

How many members does your family have?		Number of members ☐
Complete name of all household members currently living in the house	Individual 1:	
	Individual 2:	
	Individual 3:	
	Individual 4:	
	Individual 5:	
	Individual 6:	
	Individual 7:	
	Individual 8:	
	Individual 9:	
	Individual 10:	
	Individual 11:	
Where there other person who used to live here during the last 3 years but have moved out?	*If yes, note their complete name* Emigrant 1: ____ Emigrant 2: ____ Emigrant 3: ____	
Has any household member died in the last 3 years? *Only the ones that used to live here*	*If yes, note their complete name* Deceased 1: ____ Cause of death: ____ Deceased 2: ____ Cause of death: ____ Deceased 3: ____ Cause of death: ____	

ID ☐

25	Do you have any fish ponds on your property?	☐ Yes (1) ☐ No (0) ☐ *Dont' know* (99)

¡SEGUIR CON ESTA PARTE (26-36) DE LA ENCUESTA SOLAMENTE SI LA REPUESTA ES AFIRMATIVA!

26	How many fish ponds do you have / are you taking care of?	Number of fish ponds ☐
27	A- How many fish pond are in use? *Defined as a pond that has fish inside or where fish are going to be introduced in the next year.* B- How many fish pond are in construction? C- How many fish pond are abandoned?	Number of fish pond in use ☐ Number of fish pond in construction ☐ Number of abandoned fish pond ☐
28	Does your family own the fish pond?	☐ Yes (1) ☐ No (0) ☐ *Dont' know* (99)
29	A- Do you have any contact with an organization that can provide you with technical assistance? B- What is the name of the organization?	☐ Yes (1) ☐ No (0) ☐ *Dont' know* (99) Nombre: ....
30	How many times per day or week do you visit your fish pond?	Number of time ☐ ☐ day ☐ week
31	How much time does it take you to walk to the farthest fish pond?	Time ☐ Minutes
32	A- Has your fish pond ever been treated for mosquito control? B- How many times was it treated in the last 3 years?	☐ Yes (1) ☐ No (0) ☐ *No sabe* (99) Number of times ☐ ☐ Not applicable (0)
33	Do you fertilize your fish pond (with chicken or pigs feces)?	☐ Yes (1) ☐ No (0) ☐ *Dont' know* (99)
34	Do you periodically cut the aquatic vegetation at the periphery of you fish pond?	☐ Yes (1) ☐ No (0) ☐ *Dont' know* (99)
35	Do you plan to construct more fish pond?	☐ Yes (1) ☐ No (0) ☐ *Dont' know* (99)
36	Does raising fish is economically advantageous for you?	☐ Yes (1) ☐ No (0) ☐ *Dont' know* (99)

Comments: ....
....
....

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IN THE NEXT SECTION, WE ARE GOING TO ASK SPECIFIC QUESTIONS ABOUT EACH INDIVIDUAL THAT YOU HAVE LISTED AS LIVING OR HAVING LIVED IN THIS HOUSEHOLD.

A	1- # **INDIVIDUAL** [ ]	2- First and last name: ________
B	Is (NAME) a male or female?	☐ Men (1) ☐ Women (0)
C	How old is (NAME)? *If less than 2 years old, put the number of months.*	Age: [ ] If don't know, approximate [ ]
D	What is the birth date of (NAME)?	Day [ ] Month [ ] Year [ ]
E	**1.** Is (NAME) alive? ☐ Yes (1) ☐ No (0) ☐ *Don't know* (99) **If no,** →	**2.** How long as it been that (NAME) passed away? Time [ ] ☐ Month ☐ Year ☐ Not aplicable (0) ☐ *Dont know* (99)
F	**1.** How long has it been that (NAME) is living in this house? Time [ ] ☐ Mes ☐ Año ☐ *Dont know* (99)	**2.** If the answer is less than 3 years, where did (NAME) leave before? Community: …………… ☐ Not aplicable (0) ☐ *Dont know* (99)
G	*ONLY ASK THIS QUESTION FOR THOS WHO <u>EMIGRATED</u> FROM THE HOUSE* → If (NAME) left the house, how long has it been that he left?	Time [ ] ☐ Month ☐ Year ☐ Not aplicable (0) ☐ *Dont know* (99)
H	**1.** Is this house the principal domicile of (NAME)? (Does he sleep the great majority of nights here?)	☐ Yes (1) ☐ No (0) ☐ *Dont know* (99)
	2. If it is not is principal domicile, where is it located? *(Community)*	Community: …………… ☐ Not aplicable (0) ☐ *Dont know* (99)
	3. If it is not is principal domicile, how many nights per month does he sleep in your house? (average of the last 3 years)	Nigth [ ] per month ☐ Not aplicable (0) ☐ *Dont know* (99)
I	What is the highest level of education attained by (NAME)?	☐ Can not write or read (0) ☐ Primary – incomplete (1) ☐ Primary - complete (2) ☐ Secundary - incomplete (3) ☐ Secundary - complete (5) ☐ Superior (6) ☐ *Dont know* (99)
J	1- What is the occupation of (NAME)?	☐ Agriculture (without chacra) (1) ☐ Cultivate chacra (2) ☐ Extract irapai leaves (3) ☐ Do carbon (4) ☐ Fish farmer (5) ☐ Fishermen (6) ☐ Wood men (7) ☐ Hunter (8) ☐ Vendor (9) ☐ Student (10) ☐ Stay at home mom (11) ☐ Other: ________ (12) ☐ Not aplicable (0) ☐ *Dont know* (99)

ID [ ]

	2- How many nights per month does (NAME) sleeps out of the house for work or study? (average of the last 3 years) 3- How many months per year does he pass away from the house for work or study? (average of the last 3 years)	Nigths [ ] per month ☐ Not aplicable (0) ☐ *Dont know* (99) Months [ ] per year ☐ Not aplicable (0)
K	1- Where those (NAME) work?	Community: .... ☐ Not aplicable (0) ☐ *Dont know* (99)
L	(NAME) walk how much time to go to his place of work or study? (or to the place they take a motocarro or moto)	Time [ ] Minute ☐ Not aplicable (0) ☐ *Dont know* (99)
M	1- At what time does (NAME) wake up? 2- At what time does (NAME) go to bed?	Wake-up time: [ ] Go to bed: [ ]
N	(NAME) bath every day?	☐ Yes (1) ☐ No (0) ☐ *Dont know* (99)
O	1- Where does (NAME) bath? 2- At what time does he bath?	☐ Stream (1) ☐ Lake (3) ☐ Well (2) ☐ Other: ___________ (2) ☐ Before 6h00 (1) ☐ Not aplicable (0) ☐ Between 6h00 and 17h00 (2) ☐ *Dont know* (99) ☐ After 17h00 (incluiding 17h00) (3)
P	Does (NAME) use a bed net?	☐ Yes (1) ☐ No (0) ☐ *Dont know* (99)
Q	Does (NAME) use insect repelent to protect himself from biting insect?	☐ Yes (1) ☐ No (0) ☐ *Dont know* (99)
R	Does (NAME) use long sleeve shirt to protect himself from biting insect? Does (NAME) use pants to protect himself?	☐ Not using neither (0) ☐ Use both (3) ☐ Long sleeve (1) ☐ *Dont know* (99) ☐ Pant (2)
S	Does (NAME) take anti-malaria pill only when he his sick?	☐ Yes (1) ☐ No (0) ☐ *Dont know* (99)
T	How many time did (NAME) had fever this year (2008)? And last year (2007)? And two years ago (2006)?	2008 2007 2006 Fiver: [ ] [ ] [ ]
U	1- Has (NAME) had any malaria episode in the last 3 years? 2- How many time did (NAME) had malaria this year (2008), **confirmed by the health post**? And last year (2007)? And two years ago (2006)?	☐ Yes (1) ☐ No (0) ☐ *Dont know* (99) 2008 2007 2006 Malaria: [ ] [ ] [ ]
V	If (NAME) get sick, where does he go to seek treatment?	☐ El Paujil (1) ☐ Other:_________________ (0) ☐ *Dont know* (99)

ID [ ]

SELVA AMAZONICA

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Página 1

*IF THE ANSWER IS YES IN **25** O FISH FARMERS IN **J** → NOW, WE WOULD LIKE TO SPEAK TO THE FISH FARMERS AND ASK HIM SOME QUESTIONS RELATED TO HIS FISH PONDS*

COMMUNITY: .... KM: .... PARCEL NB: .... FUNDO: ....

OWNER OF REPRESENTATIVE: ....

FISH POND # **P1.** →	#	#	#	#	#
P2. Is it in use, in construction or abandoned?	☐ Use (1) ☐ Construction (2) ☐ Abandoned (3) ☐ *Dont know* (99)	☐ Use (1) ☐ Construction (2) ☐ Abandoned (3) ☐ *Dont know* (99)	☐ Use (1) ☐ Construction (2) ☐ Abandoned (3) ☐ *Dont know* (99)	☐ Use (1) ☐ Construction (2) ☐ Abandoned (3) ☐ *Dont know* (99)	☐ Use (1) ☐ Construction (2) ☐ Abandoned (3) ☐ *Dont know* (99)
P3. How long ago was the fish pond built?	*Dont know (99)*	*Dont know (99)*	*Dont know (99)*	*Dont know (99)*	*Dont know (99)*
P4. How long ago was it abandoned?	☐ Month ☐ Year ☐ Not aplicable (0) *Dont know (99)*	☐ Month ☐ Year ☐ Not aplicable (0) *Dont know (99)*	☐ Month ☐ Year ☐ Not aplicable (0) *Dont know (99)*	☐ Month ☐ Year ☐ Not aplicable (0) *Dont know (99)*	☐ Month ☐ Year ☐ Not aplicable (0) *Dont know (99)*
P5. What is the water source of the fish pond?	☐ Stream (1) ☐ Filtration (2) ☐ *Dont know* (99)	☐ Stream (1) ☐ Filtration (2) ☐ *Dont know* (99)	☐ Stream (1) ☐ Filtration (2) ☐ *Dont know* (99)	☐ Stream (1) ☐ Filtration (2) ☐ *Dont know* (99)	☐ Stream (1) ☐ Filtration (2) ☐ *Dont know* (99)
P6. How was it built?	☐ Excavated (1) ☐ Impounded (2) ☐ Natural (3) ☐ *Dont know* (99)	☐ Excavated (1) ☐ Impounded (2) ☐ Natural (3) ☐ *Dont know* (99)	☐ Excavated (1) ☐ Impounded (2) ☐ Natural (3) ☐ *Dont know* (99)	☐ Excavated (1) ☐ Impounded (2) ☐ Natural (3) ☐ *Dont know* (99)	☐ Excavated (1) ☐ Impounded (2) ☐ Natural (3) ☐ *Dont know* (99)
P7. What is the average depth of the fish pond?	. **Metres**	. **Metres**	. **Metres**	. **Metres**	. **Metres**
P8. To what level is it exposed to the the sun?	☐ Full sun (1) ☐ Partial shade (2) ☐ Shade (3)	☐ Full sun (1) ☐ Partial shade (2) ☐ Shade (3)	☐ Full sun (1) ☐ Partial shade (2) ☐ Shade (3)	☐ Full sun (1) ☐ Partial shade (2) ☐ Shade (3)	☐ Full sun (1) ☐ Partial shade (2) ☐ Shade (3)

1

ID

Asociación Civil SELVA AMAZONICA

McGill

P9. What is the percentage cover of the different vegetation strata in the fish pond?	Emergent (1) Floating (2) Shrub (3) Tree (4)	Emergent (1) Floating (2) Shrub (3) Tree (4)	Emergent (1) Floating (2) Shrub (3) Tree (4)	Emergent (1) Floating (2) Shrub (3) Tree (4)	Emergent (1) Floating (2) Shrub (3) Tree (4)
P10. What species of fish have you raised in the last 3 years? Marks all that applies.	☐ Acarahuazu (1) ☐ Boquichico (2) ☐ Bufurqui (3) ☐ Gamitana (4) ☐ Lisa (5) ☐ Paco (6) ☐ Paiche (7) ☐ Sábalo (8) ☐ Tucunaré (9) ☐ Yaraqui (10) ☐ Other: _____ (11) ☐ **Not aplicable** ***(0)*** ☐ *Dont know (99)*	☐ Acarahuazu (1) ☐ Boquichico (2) ☐ Bufurqui (3) ☐ Gamitana (4) ☐ Lisa (5) ☐ Paco (6) ☐ Paiche (7) ☐ Sábalo (8) ☐ Tucunaré (9) ☐ Yaraqui (10) ☐ Other: _____ (11) ☐ **Not aplicable** ***(0)*** ☐ *Dont know (99)*	☐ Acarahuazu (1) ☐ Boquichico (2) ☐ Bufurqui (3) ☐ Gamitana (4) ☐ Lisa (5) ☐ Paco (6) ☐ Paiche (7) ☐ Sábalo (8) ☐ Tucunaré (9) ☐ Yaraqui (10) ☐ Other: _____ (11) ☐ **Not aplicable** ***(0)*** ☐ *Dont know (99)*	☐ Acarahuazu (1) ☐ Boquichico (2) ☐ Bufurqui (3) ☐ Gamitana (4) ☐ Lisa (5) ☐ Paco (6) ☐ Paiche (7) ☐ Sábalo (8) ☐ Tucunaré (9) ☐ Yaraqui (10) ☐ Other: _____ (11) ☐ **Not aplicable** ***(0)*** ☐ *Dont know (99)*	☐ Acarahuazu (1) ☐ Boquichico (2) ☐ Bufurqui (3) ☐ Gamitana (4) ☐ Lisa (5) ☐ Paco (6) ☐ Paiche (7) ☐ Sábalo (8) ☐ Tucunaré (9) ☐ Yaraqui (10) ☐ Other: _____ (11) ☐ **Not aplicable** ***(0)*** ☐ *Dont know (99)*
P11. How many times did you harvest your fish stock in the last 3 years?	*Dont know (99)*	*Dont know (99)*	*Dont know (99)*	*Dont know (99)*	*Dont know (99)*
Take a photo of the fish pond with its ID number					

ID

MAKE A CROQUIS OF THE HOUSE AND SURRONDINGS WITH THE FAMILY REPRESENTATIVE TO IDENTIFY THE FISH PONDS

ID

APPENDIX 3: Ethics approval from the MUHC

Centre universitaire de santé McGill
McGill University Health Centre

Les meilleurs soins pour la vie
The Best Care for Life

November 19, 2007

Dr. Theresa Gyorkos
Department of Medicine
Division of Clinical Epidemiology
McGill University Health Centre
Montreal General Hospital

RE: GEN#06-050 entitled "Development and application fo an Eco-Epidemiological Decision Support Model to Improve Malaria and Dengue Control Programs in Iquitos, Peru."

Dear Dr. Gyorkos:

The research proposal entitled above received Full Board review at the convened meeting of the MUHC-Montreal General Hospital Research Ethics Committee on April 24, 2007, and was found ethically acceptable for conduct at the MUHC, and was entered accordingly into the minutes of the Research Ethics Board (REB) meeting.

We are pleased to provide you with approval for the research protocol (dated 8 February 2007), via full Board review on May 8, 2007, and the clarifications to the issues raised (November 14, 2007), via review by the Co-Chairman on November 19, 2007. At the MUHC, sponsored research activities that require US federal assurance are conducted under Federal Wide Assurance (FWA) 00000840.

All research involving human subjects require review at a recurring interval and the current study approval is in effect until **April 23, 2008**. It is the responsibility of the principal investigator to submit an Application for Continuing Review to the REB prior to the expiration of approval to comply with the regulation for continuing review of "at least once per year".

The Research Ethics Boards (REBs) of the McGill University Health Centre are registered REBs working under the published guidelines of the Tri-Council Policy Statement, in compliance with the "Plan d'action ministériel en éthique de la recherche et en intégrité scientifique" (MSSS, Qc) and the Food and Drugs Act (17 June, 2001); and acting in conformity with standards set forth in the (US) Code of Federal Regulations governing human subjects research, functions in a manner consistent with internationally accepted principles of good clinical practice. We wish to advise you that this document completely satisfies the requirement for Research Ethics Board Attestation as stipulated by Health Canada.

Should any revision to the study, or other unanticipated development occur prior to the next required review, you must advise the REB without delay. Regulation does not permit initiation of a proposed study modification prior to REB approval for the amendment.

Good luck with your study.

Sincerely,

Cournoyer

Denis Cournoyer, M. D.
Co-Chairman
GEN Research Ethics Board (Genetics/Population Research/Investigator Initiated Studies)
MUHC-Montreal General Hospital

HÔPITAL GÉNÉRAL DE MONTRÉAL • MONTREAL GENERAL HOSPITAL
1650, avenue Cedar, Montréal (Québec) Canada H3G 1A4, Tél: (514) 934-1934

Centre universitaire de santé McGill
McGill University Health Centre

Les meilleurs soins pour la vie
The Best Care for Life

November 11, 2008

Dr. Theresa Gyorkos
Department of Epidemiology
McGill University Health Centre

RE: Amendment to an already approved study
Mr. Mathieu Maheu-Giroux
MSc Candidate

Dear Dr. Gyorkos:

We are writing to inform you that the GEN Research Ethics Board endorsed the expedited approval of June 20, 2008, granting full Board approval on October 28, 2008, for Amendment dated June 2008 to include additional information is needed on malaria, household and pisciculture characteristics from a representative of households in the endemic region.

At the MUHC, sponsored research activities that require US federal assurance are conducted under Federal Wide Assurance (FWA) 00000840.

Should any revision to the study, or other unanticipated development occur prior to the next required review, you must advise the REB without delay.

We trust this meets with your complete satisfaction.

Sincerely,

Denis Cournoyer, M. D.
Co-Chairman
GEN (Genetics/Population Research/Investigator Initiated Studies) Research Ethics Board
MUHC-Montreal General Hospital

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Centre universitaire de santé McGill
McGill University Health Centre
Les meilleurs soins pour la vie
The Best Care for Life

January 20, 2009

Dr. Theresa Gyorkos
Department of Medicine
Division of Clinical Epidemiology
McGill University Health Centre
Montreal General Hospital

RE: GEN#06-050 entitled "Development and application fo an Eco-Epidemiological Decision Support Model to Improve Malaria and Dengue Control Programs in Iquitos, Peru."

Dear Dr. Gyorkos:

We have received an Application for Continuing Review of the Montreal General Hospital GEN Research Ethics Committee for the research study referenced above and the report was found to be acceptable for ongoing conduct at the McGill University Health Centre.

At the MUHC, sponsored research activities that require US federal assurance are conducted under Federal Wide Assurance (FWA) 00000840.

The re-approval for the study was provided on January 20, 2009, via expedited review of the Co-Chairman for a period of six (6) months, valid until July 20, 2009. It is noted in your report that the study is complete and that the data is in final analysis.

All research involving human subjects requires review at a recurring interval. It is the responsibility of the principal investigator to submit an Application for Continuing Review to the REB prior to the expiration of approval to comply with the regulation for continuing review of "at least once per year".

However, should the research conclude for any reason prior to the next required review, you are required to submit a Termination Report to the Committee once the data analysis is complete to give an account of the study findings and publication status.

Should any revision to the study, or other unanticipated development occur prior to the next required review, you must advise the REB without delay. Regulation does not permit initiation of a proposed study modification prior to REB approval for the amendment.

Sincerely,

Denis Cournoyer, M. D.
Co-Chairman
GEN-Research Ethics Committee
MUHC-Montreal General Hospital

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1650, avenue Cedar, Montréal (Québec) Canada H3G 1A4, Tél: (514) 934-1934

APPENDIX 4: Approval of the research protocol by the DIRESA, Peru

MINISTERIO DE SALUD
GOBIERNO REGIONAL DE LORETO
DIRECCION REGIONAL DE SALUD DE LORETO
DIRECCIÓN EJECUTIVA DE SALUD DE LAS PERSONAS
DIRECCION DE SERVICIOS DE SALUD

"AÑO DE LAS CUMBRES MUNDIALES EN EL PERU

Iquitos, 05 Marzo del 2008

CARGO

OFICIO N° 804 2008/GOREL/DRSLoreto/30.13.02

Señor:
Dra. MARINA RIVAS PANDURO
Médico Jefe .C.S San Juan
Presente.-

ASUNTO: Presentación de Profesional
REF. Oficio S/N de Fecha 29-02-08

Es grato dirigirme a usted para saludarla cordialmente y al mismo tiempo Presentarle a la estudiante Mathieu-Giroux, quien realizar la recolección de datos de su estudio en el P.S. El Paujil, los meses Mayo a Julio y Octubre a Diciembre del presente año.

Agrediéndole su atención al presente y por todo el apoyo que brinde al proyecto, me suscribo de usted, no sin antes reiterarle las muestras de mi especial consideración.

Atentamente.

GOBIERNO REGIONAL DE LORETO
DIRECCION REGIONAL DE SALUD LORETO
Dra. Ginna E. Lozano Carozzi
DIRECTORA SERVICIO DE SALUD

Jerson Lynn Sanchez Paucar
Medico Cirujano
CMP 46168

GELC/mmn
C.c.
Archivo

09/03/08