Acta Tropica 115 (2010) 112–118

Contents lists available at ScienceDirect

Acta Tropica

journal homepage: www.elsevier.com/locate/actatropica

Risk of malaria transmission from fish ponds in the Peruvian Amazon

Mathieu Maheu-Giroux[a,e], Martín Casapía[b], Veronica E. Soto-Calle[c], Lea Berrang Ford[d], David L. Buckeridge[a], Oliver T. Coomes[d], Theresa W. Gyorkos[a,e,*]

[a] *Department of Epidemiology, Biostatistics, and Occupational Health, McGill University, 1020 Pine Avenue West, Montreal, QC, Canada H3A 1A2*
[b] *Asociación Civil Selva Amazónica, Urb. Jardín No. 27, Fanning 4ta. Cuadra, Iquitos, Peru*
[c] *Prince Leopold Institute of Tropical Medicine, Nationalestraat 155 – B-2000 Antwerpen, Belgium*
[d] *Department of Geography, McGill University, 805 Sherbrooke Street W., Montreal, QC, Canada H3A 2K6*
[e] *Division of Clinical Epidemiology, Research Institute of the McGill University Health Centre, Royal Victoria Hospital – V Building, 687 Pine Avenue W., Montreal, QC, Canada H3A 1A1*

ARTICLE INFO

Article history:
Available online 25 February 2010

Keywords:
Aquaculture
Cohort study
Geographic information systems
Plasmodium falciparum
Plasmodium vivax

ABSTRACT

Background: The contribution made by fish ponds (pisciculture) to malaria transmission in the Peruvian Amazon remains to be confirmed. Recent entomological evidence indicates that *Anopheles darlingi*, the main malaria vector in the region, is frequently found in fish ponds along the Iquitos-Nauta road (Loreto, Peru). The aim of this study was to quantify the effect of fish pond density on malaria occurrence.
Methods: A retrospective 30-month cohort study was conducted in eight communities along the Iquitos-Nauta road. Malaria incidence was ascertained from malaria registries of the local health post, which consist of data from both active and passive surveillance (247 cases). Fish pond density was measured using an interpreted satellite image and information on potential confounders was collected through interviewer-administered questionnaires.
Results: A total of 1018 individuals from 234 eligible households (90% of the 259 total number of households in the study area) provided complete information on exposures and outcome. Fish pond density was found to be a significant predictor of malaria occurrence (aOR = 1.23; 95% CI: 1.09–1.38).
Conclusion: The association between fish pond density and malaria suggests that fish ponds contribute to malaria transmission in the region. These results have important implications for the prevention and control of malaria and the development of pisciculture as an important economic activity in Amazonia and beyond.

1. Introduction

The Peruvian Amazon was the last region of the greater Amazon to experience the re-emergence of epidemic malaria (Aramburú et al., 1999). 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* (Fernández et al., 1996), the reduction of house spraying control programs over the previous decade (Roberts et al., 1997), and deforestation associated with rural frontier colonization (de Castro et al., 2006; Vittor et al., 2006). The relatively recent development of fish farming activities in the area has sparked renewed interest regarding their role in malaria transmission because *A. darlingi* is generally considered sylvatic and riverine (Alcántara and Bucchi, 2001; Consoli and Lourenço-de-Oliveira, 1994; Faran and Linthicum, 1981; Hudson, 1984; Manguin et al., 1996). However, a recent and exhaustive entomological survey of potential mosquito breeding sites conducted in our study region concluded that fish ponds were the most frequent habitat for *A. darlingi* larvae, compared to other water sources in the area (Vittor, 2003; Vittor et al., 2009). 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 pisciculture has been advocated as an alternative source of fish, providing food security for the local communities (de Jesús and Kohler, 2004; Garcia et al., 2009; Junk et al., 2007; Molnar et al., 2000).

Epidemiological studies conducted to date show mixed evidence regarding the effect of fish ponds on malaria transmission in the Peruvian Amazon. In a cross-sectional survey of malaria prevalence, Vittor (2003) 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 *Plasmodium vivax* malaria. Simpson

---

[*] Corresponding author at: Division of Clinical Epidemiology, McGill University Health Centre, Royal Victoria Hospital Campus (V Building), 687 Pine Avenue West, Montreal, QC, Canada H3A 1A1. Tel.: +1 514 934 1934x44721; fax: +1 514 934 8293.
E-mail address: theresa.gyorkos@mcgill.ca (T.W. Gyorkos).

0001-706X/$ – see front matter © 2010 Published by Elsevier B.V.
doi:10.1016/j.actatropica.2010.02.011

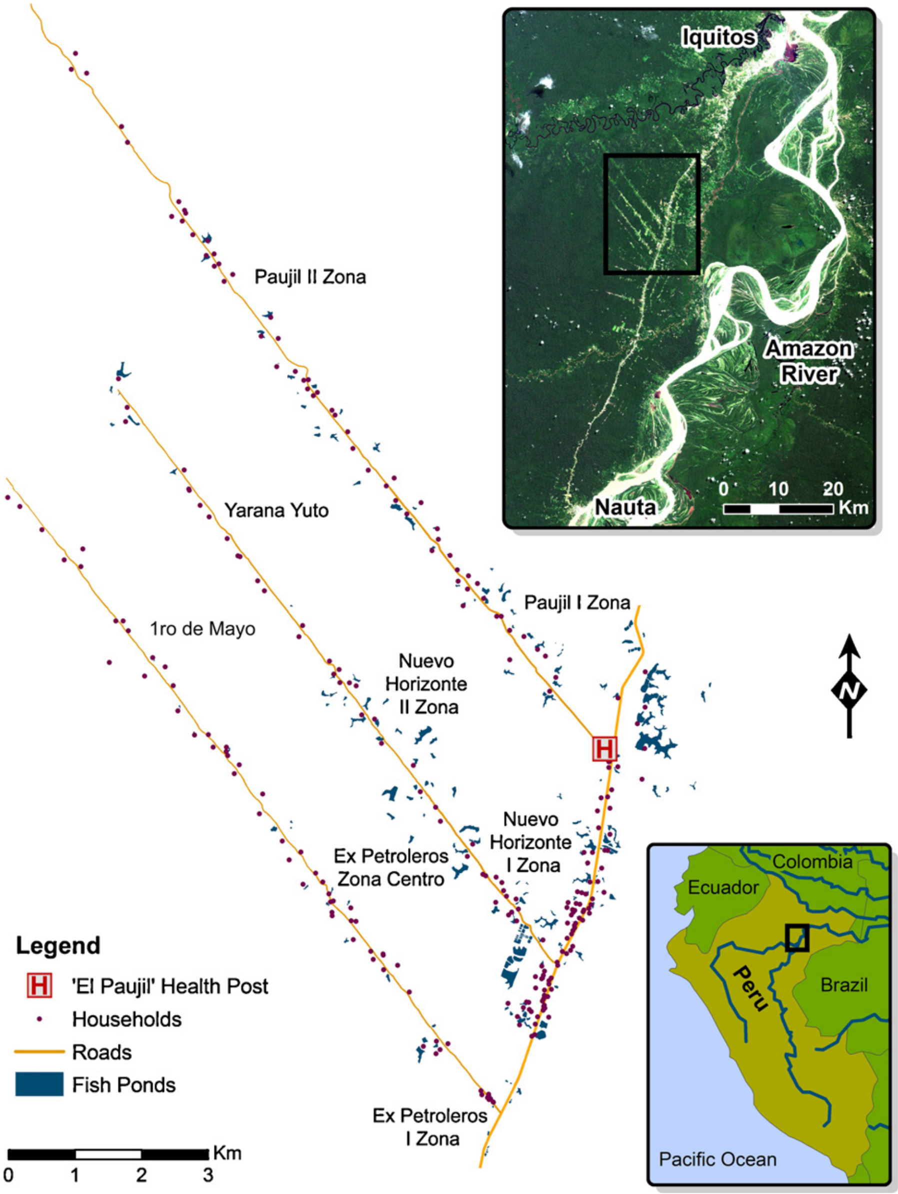

Fig. 1. Study area with location of interviewed households and fish ponds.

(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.

2. Materials and methods

2.1. Study area and population

Iquitos (3°S, 73°W) and neighboring municipalities have a population of 406,000 inhabitants, with the remaining areas of Loreto accounting for another 486,000 (INEI, 2008) (Fig. 1). Malaria is considered hypo-endemic in the region, but even under such low levels of transmission individuals may develop immunological resistance against malaria parasites (Branch et al., 2005; Parekh et al., 2007; Roshanravan et al., 2003). Most cases of malaria are reported from peri-urban and rural areas around Iquitos, especially along the 95-km Iquitos-Nauta road (Mäki et al., 2001). 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 (Mäki et al., 2001). 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 households in the communities were included except those between kilometers 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.

2.2. 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 were recorded using a handheld GPS (Garmin's GPSMAP 60CSx) with a positional accuracy of 3–5 m. A questionnaire was administered in Spanish (by MMG) to the head of each household (or designate) to obtain 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. The study's only exclusion criterion was residing fewer than four weeks in one of the eight study communities between January 2006 and 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.

2.3. Malaria diagnosis

To ascertain malaria incidence we used data routinely collected by the local health post. The health post is open 5.5 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 (MINSA, 2007). 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. Periodic active surveillance of asymptomatic individuals is also carried out by community health workers from the Ministry of Health at different times of the year (MINSA, 2007). 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 second episode was considered as a treatment failure (MINSA, 2007). 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 primary analyses by using a second case definition, as others have done (e.g. Phimpraphi et al., 2008), that considered all *P. vivax* malaria episodes occurring within 90 days of each other as relapses from the first episode.

2.4. Fish pond density

In order to locate and collect information on all fish ponds, we asked each interviewee 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 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 (perimeter method). Fish pond density was also calculated by summing the total area of the fish pond within the buffer zone (area method). 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 (Achee et al., 2006). Thus, we hypothesized that measuring fish pond density using the perimeter method better reflected the vector's breeding behavior since it takes into account only that habitat suitable for oviposition. Preliminary analysis of the density measured at seven scales ranging from 250 to 600 m from a household determined that fish pond density measured at a scale of 500 m 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, we also considered the distance to the closest fish pond in our analysis.

2.5. Statistical analysis

The study design required adjusting for a number of time-dependent covariates including individual's age, fish pond density, and location of residence. Time-dependent covariates can be taken into account within a repeated measures framework where malaria occurrence is modeled by month. A 1-month scale was the minimum observation scale possible given interviewee ability to report dates precisely. Our observations are correlated therefore at two different levels: the household level and the individual level. Adjustment for spatial autocorrelation was not required because inspection of the model's residuals using Moran's I (Moran, 1950) 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. We therefore used a macro function developed by Miglioretti and Heagerty (2007) 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 (Hox, 2002). 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 as an offset in the model. All measured confounders that were judged to have sufficient variation and small measurement errors were included in the analysis. Observations with missing data were excluded from the analysis (1.94% of all observations).

For ease of interpretation, we dichotomized the density of fish ponds at different distance thresholds and calculated population attributable risks (PARs). Adjusted PARs for fish pond density were obtained using the method proposed by Greenland and Drescher (1993). Confidence intervals were estimated by computing 1000 bootstrap replicates using the 'boot' package (Canty and Ripley, 2008) of the R software version 2.7 (R Foundation for Statistical Computing, Vienna, Austria).

2.6. 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.

3. Results

3.1. Cohort characteristics

The leadership of all eight study communities readily accepted to participate in our study. A total of 259 households were visited. One household declined participation, six households could not be interviewed after three attempts and six households were not included because they did not meet the minimum residence time of four weeks. Data were thus collected from 246 households (95%), comprising 1107 individuals between the ages of 1-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 1018 individuals distributed in 234 households, and contributing 2025 person-years to follow-up. The proportion of participants whose follow-up was complete was 61%. Complete follow-up was defined as residing in the study area for the 30 months covered by the January 2006–June 2008 study period. The main reasons for not contributing complete follow-up were immigration into the study area and birth after January 2006. Overall, mean follow-up time was 23.8 months. Baseline characteristics of study participants as well as the person-years at risk and summary of potential confounders are presented in Table 1.

3.2. 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 accounted for 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 (Fig. 2).

3.3. Fish pond density as a risk factor for malaria

Individuals were exposed to a mean fish pond density of 1.4 km (sd = 1.2; perimeter method) or 1.7 ha (sd = 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.

Results from the GEE analysis are presented in Table 2. For total malaria (*Plasmodium* spp.), the adjusted odds ratio for fish pond density measured using the perimeter method is 1.23 (95% CI:

Table 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[a])			
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[b] (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%)	50 (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-m buffer around houses[b]			
≤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 km 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%)

[a] SES = Socio-economic status.

[b] Continuous variables were categorized for display in this table but were included as continuous variables in the GEE analysis.

Table 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[a]		Total area (ha) of fish ponds[a]	
		OR (95% CI)[b]	aOR (95% CI)[c]	OR (95% CI)[b]	aOR (95% CI)[c]
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* do not add to 247 because of one mixed species infection that was removed from the species-specific analyses.

[a] Measured within a 500-m buffer around each household.

[b] OR = odds ratio; CI = confidence interval.

[c] 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.

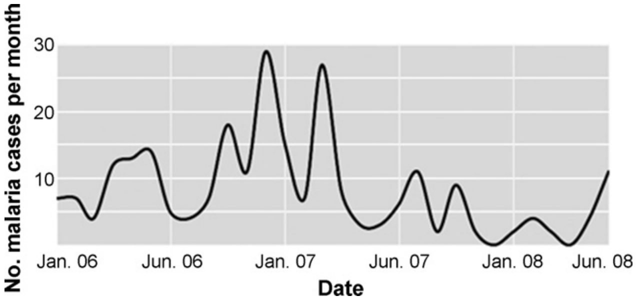

Fig. 2. Number of malaria cases registered from study communities at the El Paujil Health Post each month.

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 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 also seen in the species-specific and symptomatic cases analyses, but none of these estimates reached statistical significance (Table 3).

3.4. *The population attributable risk of fish pond density*

A measure of impact was calculated for the main exposure, dichotomized at different thresholds for ease of interpretation, using the two methods for calculating the density of fish ponds (Table 4). The different adjusted PARs indicate that, using the absence of fish ponds as the reference category, a reduction of 45% (95% CI: 22–69%) of cases could potentially be expected, if there is a causal link between fish pond density and malaria occurrence. 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.

4. 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 research groups (Molyneux, 2003; Norris, 2004; Oomen et al., 1988; Patz et al., 2004). 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.

Table 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[a]	
		OR (95% CI)[b]	aOR (95% CI)[c]
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* do not add to 247 because of one mixed species infection that was removed from the species-specific analyses.

[a] Presented per 500-m unit increment.

[b] OR = odds ratio; CI = confidence interval.

[c] 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 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[a]		Total area (ha) of fish ponds[a]	
Reference categories	PAR[b] (95% CI)	Reference categories	PAR[b] (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.

[a] Measured within a 500-m buffer around each household.

[b] 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.

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 (Matthys et al., 2006). Like the dissertation of Vittor (2003) and the thesis of Simpson (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 do not reach statistical significance even though a trend toward a decreasing risk of malaria with increasing distance was observed. We report our 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 the number of *P. falciparum* cases was small.)

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 km^2). Importantly, given the retrospective nature of the study and the high mobility of the communities (Mäki et al., 2001), 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 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. 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 (Vittor, 2003; Vittor et al., 2009).

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 in order to increase fish production and fish pond development is being widely promoted. 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 showed that about 45% of malaria cases were associated with exposure to fish ponds, appropriate messaging and malaria prevention activities targeting fish farmers are needed. Therefore 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 (Garrett et al., 1997). 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 (de Jesús and Kohler, 2004; Junk et al., 2007) 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 must adapt malaria prevention and control activities accordingly. 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.

Acknowledgments

We are extremely grateful to the study communities for their cooperation and hospitality. We especially thank Jessica Rojas for field assistance, and Salome Chapiama and Evelyn Burga for logistic support. Administrative support was provided by the Asociación Civil Selva Amazónica.

MMG was awarded an 'Ecosystem Approaches to Human Health Graduate Award' from the International Development Research Centre, a Principal's Graduate Fellowship from McGill University, and a scholarship from the 'Fonds de la recherche en santé du Québec' (FRSQ). FRSQ also provides partial institutional support to TWG's research program.

References

Achee, N.L., Grieco, J.P., Masuoka, P., Andre, R.G., Roberts, D.R., Thomas, J., Briceno, I., King, R., Rejmankova, E., 2006. Use of remote sensing and geographic information systems to predict locations of *Anopheles darlingi*-positive breeding sites within the Sibun River in Belize, Central America. J. Med. Entomol. 43, 382–392.

Alcántara, F., Bucchi, M.C., 2001. Pisciculture, Food Security, and Sustainable Development Along the Iquitos-Nauta Highway and the Tigre River – Valorization and Conservation of Our Amazonian Fishes. Instituto de Investigaciones de la Amazonía Peruana y Terra Nuova – Organismo Italiano de Cooperación Internacional, Lima, Peru, p. 83 (in Spanish).

Aramburú, G.J., Ramal, A.C., Witzig, R., 1999. Malaria reemergence in the Peruvian Amazon region. Emerg. Infect. Dis. 5, 209–215.

Branch, O.H., Casapía, M., Gamboa, D.V., Hernandez, J.N., Alava, F.F., Roncal, N., Alvarez, E., Perez, E.J., Gotuzzo, E., 2005. Clustered local transmission and asymptomatic *Plasmodium falciparum* and *Plasmodium vivax* malaria infections in a recently emerged, hypoendemic Peruvian community. Malaria J. 4, 27–42.

Canty, A., Ripley, B., 2008. Boot: Bootstrap R (S-Plus) Functions. R Package Versions 1.2–34.

Consoli, R.A.G.B., Lourenço-de-Oliveira, R., 1994. Principal Mosquitoes of Sanitary Importance in Brazil. Fiocruz, Rio de Janiero, Brazil (in Portuguese).

de Castro, M.C., Monte-Mór, R.L., Sawyer, D.O., Singer, B.H., 2006. Malaria risk on the Amazon frontier. Proc. Natl. Acad. Sci. U.S.A. 103, 2452–2457.

de Jesús, M.J., Kohler, C.C., 2004. The commercial fishery of the Peruvian Amazon. Fisheries 29, 10–16.

Faran, M.E., Linthicum, K.J., 1981. A handbook of the Amazonian species of *Anopheles* (Nyssorhynchus) (Diptera: Culicidae). Mosq. Syst. 13, 1–81.

Fernández, R., Carbajal, F., Quintana, J., Chauca, H., Watts, D.M., 1996. Presence of *A*(N) darlingi (Diptera: Culicidae) in the areas surrounding the city of Iquitos Loreto-Peru. Soc. Peruana Enferm. Infec. Trop. 5, 10–12 (in Spanish).

Garcia, A., Tello, S., Vargas, G., Duponchelle, F., 2009. Patterns of commercial fish landings in the Loreto region (Peruvian Amazon) between 1984 and 2006. Fish Physiol. Biochem. 35, 53–67.

Garrett, E.S., dos Santos, C.L., Jahncke, M.L., 1997. Public, animal, and environmental health implications of aquaculture. Emerg. Infect. Dis. 3, 453–457.

Greenland, S., Drescher, K., 1993. Maximum likelihood estimation of the attributable fraction from logistic regression models. Biometrics 49, 865–872.

Hox, J., 2002. Multilevel Analysis – Techniques and Applications. Lawrence Erlbaum Associates, Inc., Publishers, Mahwah, NJ.

Hudson, J.E., 1984. *Anopheles darlingi* Root (Diptera: Culicidae) in the Suriname rain forest. Bull. Entomol. Res. 74, 129–142.

INEI, 2008. Peru: Population growth and distribution, 2007, in: Instituto Nacional de Estadistíca e Informática. Lima, Peru, p. 46 (in Spanish).

Junk, W.J., Soares, M.G.M., Bayley, P.B., 2007. Freshwater fishes of the Amazon River basin: their biodiversity, fisheries, and habitats. Aquat. Ecosyst. Health Manage. 10, 153–173.

Mäki, S., Kalliola, R., Vuorinen, K., 2001. Road construction in the Peruvian Amazon: process, causes and consequences. Environ. Conserv. 28, 199–214.

Manguin, S., Roberts, D.R., Andre, R.G., Rejmankova, E., Hakre, S., 1996. Characterization of *Anopheles darlingi* (Diptera: Culicidae) larval habitats in Belize, Central America. J. Med. Entomol. 33, 205–211.

Matthys, B., Vounatsou, P., Raso, G., Tschannen, A.B., Becket, E.G., Gosoniu, L., Cissé, G., Tanner, M., N'Goran, E., Utzinger, J., 2006. Urban farming and malaria risk factors in a medium-sized town in Côte d'Ivoire. Am. J. Trop. Med. Hyg. 75, 1223–1231.

Miglioretti, D.L., Heagerty, P.J., 2007. Marginal modeling of nonnested multilevel data using standard software. Am. J. Epidemiol. 2007, 453–463.

MINSA, 2007. Technical norms for the care of malaria and complicated malaria in Peru, in: Ministerio de Salud, Lima, Peru, p. 116 (in Spanish).

Molnar, J.L., Alcántara, F., Tello, S., 2000. Fish culture in the Peruvian Amazon: producer perceptions and practices in three river systems, in: McElwee, K., Burke, D., Niles, M., Cummings, X., Egna, H. (Eds.), Seventeenth Annual Technical Report. Pond Dynamics/Aquaculture CRSP. Oregon State University, Corvallis, OR, pp. 139–152.

Molyneux, D.H., 2003. Common themes in changing vector-borne disease scenarios. Trans. R. Soc. Trop. Med. Hyg. 97, 129–132.

Moran, P.A.P., 1950. Notes on continuous stochastic phenomena. Biometrika 37, 17–23.

Norris, D.E., 2004. Mosquito-borne diseases as a consequence of land use change. EcoHealth 1, 19–24.

Oomen, J.M.V., de Wolf, J., Jobin, W.R., 1988. Health and Irrigation. Incorporation of Disease-control Measures in Irrigation, a Multi-faceted Task in Design, Construction, and Operation. International Institute for Land Reclamation and Improvement, Wageningen, The Netherlands.

Pan, W., 2001. Akaike's information criterion in generalized estimating equations. Biometrics 57, 120–125.

Parekh, F.K., Hernandez, J.N., Krogstad, D.J., Casapia, M., Branch, O.H., 2007. Prevalence and risk of *Plasmodium falciparum* and *P. vivax* malaria among pregnant women living in the hypoendemic communities of the Peruvian Amazon. Am. J. Trop. Med. Hyg. 77, 451–457.

Patz, J.A., Daszak, P., Tabor, G.M., Aguirre, A.A., Pearl, M., Epstein, J., Wolfe, N.D., Kilpatrick, A.M., Foufopoulos, J., Molyneux, D., Bradley, D.J., 2004. Unhealthy landscape: policy recommendations on land use change and infectious disease emergence. Environ. Health Perspect. 112, 1092–1098.

Phimpraphi, W., Paul, R.E., Yimsamran, S., Puangsa-art, S., Thanyavanich, N., Maneeboonyang, W., Prommongkol, S., Sornklom, S., Chaimungkun, W., Chavez, I.F., Blanc, H., Looareesuwan, S., Sakuntabhai, A., Singhasivanon, P., 2008. Longitudinal study of *Plasmodium falciparum* and *Plasmodium vivax* in a Karen population in Thailand. Malaria J. 7, 99–110.

Roberts, D.R., Laughlin, L.L., Hsheih, P., Legters, L.J., 1997. DDT, global strategies, and a malaria control crisis in South America. Emerg. Infect. Dis. 3, 295–302.

Roshanravan, B., Kari, E., Gilman, R.H., Cabrera, L., Lee, E., Metcalfe, J., Calderon, M., Lescano, A.G., Montenegro, S.H., Calampa, C., Vinetz, J.M., 2003. Endemic malaria in the Peruvian Amazon region of Iquitos. Am. J. Trop. Med. Hyg. 69, 45–52.

Simpson, J.M., 2006. Aquaculture Production, Contributing to the Transmission of Malaria in the Peruvian Amazon. MA Thesis. Department of Ecology, Evolution and Environmental Biology, Columbia University, New York, NY, p. 47.

Vittor, A.Y., 2003. Deforestation and Malaria: Associations Between Vegetation, Vector Ecology and Malaria Epidemiology in the Peruvian Amazon. Doctoral Dissertation. Department of Environmental Health Sciences, Johns Hopkins Bloomberg School of Public Health, Johns Hopkins University, Baltimore, MD, p. 480.

Vittor, A.Y., Gilman, R.H., Tielsch, J., Glass, G., Shields, T., Sanchez Lozano, W., Pinedo-Cancino, V., Patz, J.A., 2006. The effect of deforestation on the human-biting rate of *Anopheles darlingi*, the primary vector of falciparum malaria in the Peruvian Amazon. Am. J. Trop. Med. Hyg. 74, 3–11.

Vittor, A.Y., Pan, W., Gilman, R.H., Tielsch, J., Glass, G., Shields, T., Sánchez-Lozano, W., Pinedo, V.V., Salas-Cobos, E., Flores, S., Patz, J.A., 2009. Linking deforestation to malaria in the Amazon: characterization of the breeding habitat of the principal malaria vector, *Anopheles darlingi*. Am. J. Trop. Med. Hyg. 81, 5–12.