Aspects of spatial and habitat ecology of multiple *Anopheles* species (Diptera: Culicidae): malaria vectors in the highlands and foothills of Ecuador

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Dedication

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Abstract

The resurgence of malaria in highland regions of Africa, Oceania and recently in South America underlines the importance of the study of the ecology of highland mosquito vectors of malaria. Since the incidence of malaria is limited by the distribution of its vectors, the purpose of this PhD thesis was to examine aspects of the ecology of *Anopheles* mosquitoes in the Andes of Ecuador, South America. A historical literature and archival data review (Chapter 2) indicated that *Anopheles pseudopunctipennis* transmitted malaria in highland valleys of Ecuador prior to 1950, although it was eliminated through habitat removal and the use of chemical insecticides. Other anopheline species were previously limited to low-altitude regions, except in a few unconfirmed cases. A thorough larval collection effort (n=438 attempted collection sites) in all road-accessible parts of Ecuador except for the lowland Amazon basin was undertaken between 2008 - 2010 (Chapter 3). Larvae were identified morphologically and using molecular techniques (mitochondrial CO1 gene), and distribution maps indicated that all five species collected (*Anopheles albimanus*, *An. pseudopunctipennis*, *Anopheles punctimacula*, *Anopheles oswaldoi s.l.* and *Anopheles eiseni*) were more widespread throughout highland regions than previously recorded during the 1940s, with higher maximum altitudes for all except *An. pseudopunctipennis* (1541 m, 1930 m, 1906 m, 1233 m and 1873 m, respectively). During larval collections, to characterize species-specific larval habitat, a variety of abiotic and biotic habitat parameters were measured and compared between species-present and species-absent sites using chi-square tests and stepwise binary logistic regression analyses (Chapter 4). *An. albimanus* was significantly associated with permanent pools with sand substrates and *An. pseudopunctipennis* with gravel and boulder substrates. Both species were significantly associated with floating cyanobacterial

mats and warmer temperatures, which may limit their presence in cooler highland regions. *Anopheles punctimacula* was collected more often than expected from algae-free, shaded pools with higher-than-average calculated dissolved oxygen. *Anopheles oswaldoi s.l.*, the species occurring on the Amazonian side of the Andes, was associated with permanent, anthropogenic habitats such as roadside ditches and ponds. To address the hypothesis that human land use change is responsible for the emergence of multiple highland *Anopheles* species by creating larval habitat, common land uses in the western Andes were surveyed for standing water and potential larval habitat suitability (Chapter 5). Rivers and road edges provided large amounts of potentially suitable anopheline habitat in the western Andes, while cattle pasture also created potentially suitable habitat in irrigation canals and watering ponds. Other common land uses surveyed (banana farms, sugarcane plantations, mixed tree plantations, and empty lots) were usually established on steep slopes and had very little standing water present. Using distribution and larval habitat data, a GIS-based larval habitat distribution model for the common western species was constructed in ArcGIS v.10 (ESRI 2010) using derived data layers from field measurements and other sources (Chapter 6). The additive model predicted 76.4 - 97.9% of the field-observed collection localities of *An. albimanus*, *An. pseudopunctipennis* and *An. punctimacula,* although it could not accurately distinguish between species-absent and species-present sites due to its coarse scale. The model predicted distributional expansion and/or shift of one or more anopheline species into the following highland valleys with climate warming: Mira/Chota, Imbabura province, Tumbaco, Pichincha province, Pallatanga and Sibambe, Chimborazo province, and Yungilla, Azuay province. These valleys may serve as targeted sites of future monitoring to prevent highland epidemics of malaria. The human perceptions of malaria and mosquitoes in relation to land management practices were assessed through an interview-based survey (n=262) in both highlands and lowlands, of male and female land owners and managers of five property types (Chapter 7). Although respondents had a strong understanding of where the disease occurs in their own country and of the basic relationship among standing water, mosquitoes and malaria, about half of respondents in potential risk areas denied the current possibility of malaria infection on their own property. As well, about half of respondents with potential anopheline larval habitat did not report its presence, likely due to a highly specific definition of suitable mosquito habitat. Most respondents who are considered at risk of malaria currently use at least one type of mosquito bite prevention, most commonly bed nets. In conclusion, this interdisciplinary thesis examines the occurrence of *Anopheles* species in the lowland transition area and highlands in Ecuador, from a historic, geographic, ecological and sociological perspective.

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Abbreviations

APHA: American Public Health Association
BLR: Binary Logistic Regression (statistics)
CO1: Cytochrome Oxidase 1 (mitochondrial gene)
CCV: Constant Coefficient of Variation
DDT: dichlorodiphenyltrichloroethane (synthetic insecticide)
DEET: *N,N*-Diethyl-*meta*-toluamide (synthetic insecticide)
DEM: Digital Elevation Model
df: Degrees of Freedom
DNA: Deoxyribonucleic Acid
DNTPs: Deoxynucleotide Triphosphate (A, C, T, G)
DO: Dissolved Oxygen
ENSO: El Niño Southern Oscillation cycle
EROS: Earth Resources Observation & Science (of USGS)
ESRI: Environmental Systems Research Institute (makers of ArcGIS software)
F: Forward primer
GDP: Gross Domestic Product
GIS: Geographic Information Systems (software)
GPS: Geographic Position System
HIMAL: HIghland MALaria project (of MARA)
HydroSHEDS: HYDROlogical data and maps based on SHuttle Elevation Derivatives at multiple Scales
ITCZ: Intertropical Convergence Zone
IDRC: International Development Research Council (Canada)
IDW: Inverse Distance Weighted technique (in GIS)
IPCC: Intergovernmental Panel on Climate Change
IRS: Indoor Residual Spray (insecticide)
ITN: Insecticide Treated Net (bed net)
KAP: Knowledge, Attitudes and Practices study
MARA: Mapping Malaria Risk in Africa
MERIS: MEdium Resolution Imaging Spectrometer
MI: Moran's Index (statistical technique in GIS)
NASA: National Air and Space Agency (U.S. Government)
NBI: National Brands Index
NDVI: Normalized Differential Vegetation Index
PHDM: Predicted Habitat Distribution Map
PCA: Principal Components Analysis (statistical analysis)
PCR: Polymerase Chain Reaction
R: Reverse primer
SBLR: Stepwise Binary Logistic Regression (statistics)
SD: Standard Deviation
SNEM: Sistema Nacional de Eradicaccion de Malaria (new name: Servicio Nacional de Control de Enfermedades Transmitidas por Vectores Artrópodos) (Ecuador)
TDR: Special Programme for Research and Training in Tropical Diseases (WHO/UN)
UN: United Nations
USGS: United States Geological Survey
WFD: Water-Filled Depression
WHO: World Health Organization

Chapter One

Literature Review: Highland malaria and malaria in Latin America

Lauren Pinault and Fiona Hunter

1.1 Introduction

Malaria has been one of the most devastating infectious diseases throughout human history: a parasitic insect-borne disease that still affects an estimated 247 million people and causes one million deaths worldwide each year (Carter & Mendis 2002; WHO 2010). Many of the fatalities and severe complications, such as anemia, occur among very young children, particularly infants, who lack acquired immunity to the infections (Imbert et al. 1997; Gupta et al. 1999; Reyburn et al. 2005; WHO 2010). The effects of widespread malaria-related illness are extremely detrimental to the quality of life and to the economic status of affected countries (Gallup & Sachs 2001; WHO 2010). Although the majority of the malaria burden is in Africa, malaria occurs worldwide, throughout tropical and subtropical regions of Asia and the Americas (Attenborough et al. 1997; Roberts et al. 1997; Erhart et al. 2004).

Human malaria is spread by the bite of an infected *Anopheles* spp. adult female mosquito. Some *Anopheles* spp. are therefore the vectors for malaria, meaning the arthropods capable of transmitting disease-causing organisms (in this case, *Plasmodium*), among hosts (Harwood & James 1979). Female mosquitoes of many species must take a blood meal to provide suitable nutrition (protein) for the development of their eggs and can ingest *Plasmodium* gametocytes in the blood of infected humans. Sexual reproduction of the parasite occurs when male and female *Plamodium* gametocytes fuse to form a zygote on the mosquito midgut wall. Asexual division, or sporogony of the parasites, then occurs within an oocyst inside the mosquito midgut wall, a process that requires a minimum ambient temperature for completion of development (Gullan & Cranston 2000). The resulting sporozoites are shed into the mosquito haemolymph and migrate to the salivary glands, where they are passed to a human host during the pre-feeding ejection of saliva containing an anticoagulent (Gullan & Cranston 2000).

Mosquitoes (Culicidae) are members of the order of the true flies (Diptera), of which there are three subfamilies: Culicinae, Anophelinae, and Toxorhynchitinae (Gwadz & Collins 1996; Harbach 2004). This particular thesis focuses on the Anophelinae, of which some species are known to transmit the parasites of the human malarias, as well as the filarial parasites *Wuchereria bancrofti* and *Brugia malayi*, and some arboviruses including o'nyony-nyong fever in East Asia (Gwadz & Collins 1996). Human malaria vectors are concentrated in the Anophelinae subgenera *Nyssorhynchus* and *Anopheles* (Harbach 2004) Species complexes are common within the Anophelinae, where there are morphologically similar or identical individuals that differ genetically and/or behaviourally (Gwadz & Collins 1996).

Species identification in mosquitoes is difficult based on morphology alone, since many species form species complexes, or may simply be difficult to identify due to small or damaged physical characters (Besansky 1999). Charlwood (1996) described substantial morphological (e.g., wing length), and behavioural (e.g., biting behaviour) differences among populations of *An. darlingi* in different regions of the species' range, leading to the scientific re-evaluation of the species as a complex of species. As an example of misidentification, a recent study by Cienfuegos et al. (2011) of Colombian anophelines compared genetic sequences obtained by polymerase chain reaction (PCR) of the ribosomal internal transcribed spacer 2 region (ITS2) to morphological identifications and found that these were previously improperly identified for *Anopheles nuneztovari s.l.*, *Anopheles neomaculipalpus*, *Anopheles apicimacula*, and *Anopheles* *punctimacula*, although morphological identifications were accurate for *Anopheles albimanus*. Therefore, discrimination among species ought to include the use of molecular gene sequencing to confirm morphological species identification (e.g. Paskewitz & Collins 1990; Besansky 1999).

Molecular techniques involving the amplification of specific DNA regions through PCR are commonly used alongside classic morphological techniques. Sequences of the mitochondrial "barcoding" gene region have been used to distinguish among species and populations of neotropical anophelines (Conn et al. 1999; Harbach 2004; Angêlla et al. 2007) as well as mosquitoes living in temperate regions (Cywinska et al. 2006). At a finer scale, such as among cryptic species, egg morphology and gene sequences of the ITS2 region, nuclear *white* gene, and microsatellite markers have been shown to be useful to distinguish among different subspecies or incorrectly synonymized species (Besansky & Fahey 1997; Lounibos et al. 1998a; Conn et al. 2006; Matson et al. 2008; Bourke et al. 2010). Matson et al. (2008) provide a methodology for rapid identification of *Anopheles* species using sequencing of the ITS2. Mapping of the salivary gland polytene chromosome banding patterns have also been used to distinguish populations of Amazonian *An. darlingi* (Rafael et al. 2010). Based on preliminary specimens collected for this thesis, it was decided that morphological identifications supplemented with the coarser-scale molecular sequencing of mtDNA would be sufficient to identify the species encountered.

Following the completion of the laboratory work for this thesis, it was determined that *An. punctimacula* is frequently misidentified using morphological characters and may possibly be *An. calderoni* (González et al. 2010) or *An. apicimacula,* as in Colombia (Cienfuegos et al. 2011).

Mosquitoes undergo complete metamorphosis, which includes an egg stage, four larval instars, and a pupal stage prior to adulthood (Harwood & James 1979). Among *Anopheles* spp., eggs are laid singly either on the surface of stationary water bodies or in periodically flooded areas, and individuals of some species (typically those of drier climates) can remain in the egg stage to endure long periods of desiccation (Harwood & James 1979). Anopheline eggs are characteristically boat-shaped and retain bubbles of air for buoyancy in a matrix of chorion (Gwadz & Collins 1996). Approximately 50 - 200 eggs are produced by a female *Anopheles* mosquito after taking its blood meal (Gwadz & Collins 1996). Larvae are elongate with a schlerotized head capsule, thorax, and nine abdominal segments with a short pair of respiratory spiracles on the eigth segment (Gwadz & Collins 1996). Larvae reside within unmoving or slow-moving water bodies and feed on the surface of the water at the bacteria and detritus-rich neuston layer, using brushes that bring water and suspended particulate matter into their mouths (Harwood & James 1979; Gwadz & Collins 1996). Some *Anopheles* larvae also feed by scraping the surfaces of the substrate or floating vegetation or algae (Gwadz & Collins 1996). Generally, *Anopheles* larvae are known to occur in relatively unpolluted water and reside in a wide variety of species-specific habitats including the margins of water bodies, both temporary and permanent pools, and even water pooling in bromeliads (Gwadz & Collins 1996). Habitat choice by females is mediated by optical cues, salinity, cues from predators such as fish, as well as the presence or absence of shade or specific types of vegetation (Clements 1999). *Anopheles* larvae are distinguished by their lack of long respiratory siphons (as in other mosquitoes) and they rest horizontally on the surface of the water, held in place by the palmate hairs along their dorsa (Harwood & James 1979). The developmental time of larvae can be quite variable and often lasts a few weeks (Harwood & James 1979). In *Anopheles gambiae,* larval developmental time is shortened by warmer temperatures up to 28°C, after which survival declines (Bayoh & Lindsay 2003). *An. gambiae* developmental time is also density-dependent and is lengthened by greater larval densities (Gimnig et al. 2002). The pupal stage is quite active and lasts for 2-3 days, after which adults emerge from the water surface (Harwood & James 1979). Mating of many mosquito species, including *An. gambiae s.s.*, occurs when females enter male mating swarms, usually formed during dusk and dawn (Downes 1969; Charlwood et al. 2002). However, some species of neotropical *Anopheles*, such as *An. darlingi*, mate in the first few hours following female emergence without exhibiting swarming behaviour (Lounibos et al. 1998b). Males have a specialized plumose mechanosensory organ for female location, the Johnston's organ, which is sensitive to the wingbeat frequency of females in flight (Belton 1989) and is also extremely well-tuned to species-specific frequencies (Pennetier et al. 2010). Most species of anopheline mosquitoes are anautogenous, meaning that adult females must take a blood meal to provision protein for each clutch of eggs (Gwadz & Collins 1996). It is during this blood meal that females may become infected with malaria gametocytes.

The human malarias include four widespread parasites of the genus *Plasmodium*: *P. falciparum*, *P. vivax*, *P. malariae*, and *P. ovale* (WHO 2010). A fifth parasite, *Plasmodium knowlesi*, has recently caused human illness in remote forested regions of the Malay archipelago (Greenwood et al. 2008; Taylor et al. 2010). Parasitological diagnosis of malaria is based on repeated thick blood smears for parasites, antigen testing (rapid diagnostic tests) and/or the use of PCR assays, which are often unavailable tools to poor, rural health centres (Lalloo et al. 2007; Taylor et al. 2010; WHO 2011). Misdiagnosis of malaria is known to be widespread, especially among marginalized populations, in part due to the existence of subclinical and asymptomatic malaria, and contributes to the ongoing cycle of continued transmission and poverty (Amexo et al. 2004). In addition, worldwide epidemiological monitoring of malaria depends on passive case reporting, which is often unreliable, at least for *P. falciparum* (Snow et al. 2005).

Malaria prevalence is inextricably linked to global poverty in a cyclical manner (Sachs & Malaney 2002). Gallup & Sachs (2001) estimated that the Gross Domestic Product (GDP) and the growth of income per capita (1965-1990) is five times lower in malaria-endemic than malaria-free countries. Even when initial income level, initial health situation, and the tropical location of many malaria-endemic countries were removed as factors for an economic model, malaria was still responsible for a 1.3% yearly growth reduction within malaria-endemic countries (Gallup & Sachs 2001). Within developing countries, the majority of malaria usually occurs in the 20% poorest demographic, often since the poorest citizens are less likely to own insecticide treated nets (ITNs), less likely to seek medical help, and less likely to be able to overcome the financial barriers to obtaining medical aid, even when provided with subsidies (Barat et al. 2004). In turn, illness can affect the economic status of the individual through school and work absenteeism, and impaired cognitive development from poor nutritional status (Amexo et al. 2004; Sachs & Malaney 2008). Families with malaria incur significant costs through hospital stays and treatment, often paid out from lifetime savings (Ayieko et al. 2009). The cost to caretakers is usually the greatest, since these family members are often unable to work and sometimes must travel a great distance for assistance (Ayieko et al. 2009; Asenso-Okyere et al. 2011). Caretakers are usually women, who are responsible for child care, food production, and are more vulnerable themselves to malaria-related fatalities or complications arising from pregnancy (Asenso-Okyere et al. 2011). On a macroeconomic level, malaria costs can also be detrimental to a country's wealth through effects on tourism, trade, and investment (Sachs & Malaney 2002).

Although malaria eradication programs were very promising in the 1950s (e.g., Russell 1957), the last twenty years have seen a global resurgence of malaria in many tropical and subtropical regions due to changes to malaria control programs and ecological changes (Lindsay & Birley 1996; Roberts et al. 2000; IPCC 2001a; WHO 2010). However, there have been some recent, notable malaria declines in some countries, such as in the Gambia (Ceesay et al. 2010). Some authors have discussed the possible role of global climate change in contributing to malaria vector dispersal into higher latitudes and altitudes than they occur presently (Martens et al. 1999; Epstein 2001; Moreno 2006; Mills et al. 2010). Global circulation climate models predict a substantial expansion of vectors into temperate regions (Zell 2004). In addition to temperature and rainfall change, the Intergovernmental Panel on Climate Change (IPCC) predicts an increased rate of extreme flooding events in coastal regions (IPCC 2001a; IPCC 200b), leading to a possible increase in available larval mosquito habitat (IPCC 2001a). Interestingly, drought events in tropical regions also modify potential larval habitat in ways that may improve suitability for other mosquito species (IPCC 2001a). In practice, the effects of climate change have been very difficult to relate to malaria incidence, and many authors have criticized this proposed link (e.g., Reiter 2001; Hay et al. 2002a; Zell 2004). The trends in malaria resurgence have been more strongly related to many other factors, including a decline of funding during the 1970s and 1980s that led to a reduction of vector control programs, widespread insecticide and drug resistance, human conflict, population growth and increasing urbanization in malaria-endemic regions, and extensive land use alteration leading to larval habitat creation and modification of local microclimate (e.g., Lindsay & Birley 1996; IPCC 2001a; Molyneux 2001; Hay et al. 2002a). No matter the mechanism, the presence of highland malaria has been documented globally, strongly warranting entomological investigation (IPCC 2001b; Ferguson et al. 2010). As well, since altitudinal temperature gradients are stronger over a shorter distance, altitudinal differences have been suggested as a more interesting system for climate-based study than latitudinal gradients (Colwell et al. 2008; Mills et al. 2010).

1.2 Characteristics of global highland malaria

Highland malaria can be defined as malaria that is transmitted in higher-than-average altitudes. The altitude at which malaria is declared "highland" varies in the literature, but lies generally 600 - 1000 m above sea level, depending on the known historical endemic range (Bruce-Chwatt 1985; Attenborough 1997). Cox et al. (1999) and Craig et al. (1999) propose that a continuum of endemic malaria exists along an altitudinal gradient. Lower altitudes are regions with ongoing, permanent transmission, whereas higher altitudes may see seasonal or occasional epidemics. The highest altitudes are characterized by a complete absence of malaria transmission. A similar pattern of malaria transmission was observed along an altitudinal transect in Kenya as researchers moved away from the bottom of a valley (Githeko et al. 2006). Similarly, in Tanzania, *P. falciparum* infection (entomological inoculation rate), and the prevalence of moderate anemia decreased at progressively higher elevations along three altitudinal transects (Drakeley et al. 2005). Therefore, many researchers consider highland regions to be fringes of an endemic area, where unstable epidemics of malaria occur. However, many highland occurrences of malaria may not represent true epidemic situations, but may instead be due to strongly seasonal (though permanent) highland endemism (Hay et al. 2002b).

There has been a resurgence of highland malaria since the 1970s - 1990s on a global scale. Figure 1-1 outlines the global incidence of past and present highland malaria. *Anopheles gambiae* (occasionally with *Anopheles funestus*) has been observed to transmit malaria in the highlands of Kenya, Uganda, Tanzania, Rwanda, Burundi, and Madagascar with a maximum altitude of ca. 2600 m (Loevinsohn 1994; Mouchet 1998; Lindblade et al. 1999; Bødker et al. 2000; Zhou et al. 2004; Protopopoff 2008). In the area of Mount Kilimanjaro, Tanzania, *Anopheles arabiensis* has been incriminated as the most common vector (Kulkarni et al. 2006). The relationship between altitude and malaria has also been explored in coastal Cameroon, where *An. gambiae*, *An. funestus*, and *Anopheles hancocki* were incriminated as vectors up to a maximum altitude of 1200 m, above which the mountain becomes too steep (Wanji et al. 2003). In most of these countries, malaria was formerly endemic in highland regions prior to the 1950s, when large-scale eradication measures were implemented with variable success rates (e.g. Garnham 1948; Roberts 1964c). Before the mid-1960s, highland malaria had also been observed in the Himalayas, transmitted by the vector *Anopheles superpictus* (Garnham 1948; Ahmad & Khan Tariq 1967) (Figure 1-1). Impressively, this vector was observed to transmit malaria at approximately 3000 m altitude, which represents the most extreme high-altitude record to date (Ahmad & Khan Tariq 1967). Highland malaria has also been reported from Papua New Guinea and parts of Indonesia, transmitted by the vectors *Anopheles farauti* and *Anopheles punctulatus* (Sharp 1982; Attenborough et al. 1997; Bangs & Subianto 1999) (Figure 1-1). Within the last ten years, transmission of *P. vivax* has been reported from Bolivia at an altitude of 2300 m (Rutar et al. 2004). The vector incriminated in this epidemic was *Anopheles pseudopunctipennis*, a known highland vector that was widespread in Andean highland regions prior to 1950 (Rutar et al. 2004; Levi-Castillo 1945b) (Figure 1-1).

In Tanzania, Cameroon, and Papua New Guinea, the densities of adult anopheline mosquitoes decline with increasing altitude, with dense populations in malaria-endemic low-altitude regions (Attenborough et al. 1997; Wanji et al. 2003; Balls et al. 2004). Similarly, in Kenya, the number of malaria cases is inversely related to altitude, up to a current limit of occurrence at about 1800 m, above which significantly fewer malaria cases were reported (Brooker et al. 2004). Highland malaria occurs in a clustered pattern, within limited foci, usually near anopheline larval habitat (Lindsay & Martens 1998; Brooker et al. 2004). One of the other mechanisms of clustering may be that adult mosquitoes are limited by steep, wind-swept topography in highlands, and therefore are associated with flatter areas and valley bottoms (Balls et al. 2004; Githeko et al. 2006).

Highland malaria also shows localized seasonal trends (e.g., Bødker et al. 2003; Wanji et al. 2003). For example, on Mount Cameroon, *An. gambiae* is more abundant at higher altitudes during the dry season, where mosquitoes are found up to 800 m (Wanji et al. 2003). Relatively low-altitude "highland" malaria cases occur in Ethiopia and Eritrea as well (up to 1066 m), associated with periods of heavy rainfall (Bruce-Chwatt 1985). Seasonal epidemics also occur in Kenya, Uganda, Tanzania, and Angola up to 1828 m in altitude, and are also related to increased localized rainfall (Bruce-Chwatt 1985).

The composition of the vector population often differs in highland regions. In Papua New Guinea, *Anopheles koliensis* is the main vector recorded below 650 m, whereas above that elevation, *An. punctulatus* becomes the primary human malaria vector (Attenborough et al. 1997).

Finally, in the early part of the twentieth century, malaria was endemic in many highland regions where it is presently re-emerging, including Kenya, Tanzania, Uganda, Madagascar, Papua New Guinea, and the Northern Andes (Levi-Castillo 1945b; Garnham 1948; de Zulueta et al. 1964; Brookfield 1964; Some 1994; Mouchet 1998; Bødker et al. 2000). The various possible reasons for this resurgence will be discussed in the following sections.

1.3 Possible Causes of Global Highland Malaria

Several mechanisms have been proposed as causative factors contributing to highland malaria. In a study by Hay et al. (2002c), a 95-year climate data set for relevant highland sites in Africa showed no significant changes between average temperature, rainfall (except at one site), and vapour pressure during the period of study, suggesting that the observed highland resurgence must be due to non-climate factors. In a separate study in the western Kenyan highlands, malaria rates increased between 1966 - 1995, while there was no significant change in monthly temperature and rainfall (Shanks et al. 2002). However, some highland malaria epidemics have been associated with the El Niño Southern Oscillation (ENSO) warm events (Uddenfeldt Wort et al. 2004). Several reviews have also implicated the following contributing factors as regional mechanisms for highland malaria resurgence: deforestation and/or land use change, including the widespread use of irrigation, new practices for cattle rearing that promote indoor resting sites for mosquitoes, resistance of the parasite to anti-malarial drugs, human migration patterns, human conflict, urbanization within endemic regions, and complacency with regards to vector control and/or insecticide resistance, all of which vary among latitudes and continents (Lindsay & Martens 1998; Patz et al. 2000; Kovats et al. 2001; Molyneux 2001; Hay et al. 2002a; Sutherst 2004; Zell 2004; Patz et al. 2005; Reiter 2008). There is evidence that all of these factors influence the patterns of resurgence in different regions, depending on the vector and its relationship with the local environment, as well as several human factors such as habitat elimination practices (Kovats et al. 2001; Hay et al. 2002a). The following section discusses the evidence for each of these potential causes as found in the published literature.

One major consideration regarding highland malaria, as well as a possible mechanism by which highland malaria may more successfully become established, is a lack of resistance to the parasite among highland residents. While lower altitude dwellers in malaria-endemic regions would have been exposed to the parasite since childhood and thereby gained some immunity to the parasite (Alles et al. 1998; Gupta et al. 1999; Hviid 2005), highland dwellers would not have received regular exposure to the parasite. Indeed, a lack of immunity was observed among highlanders in two studies, in both Kenya and Rwanda (Loevinsohn 1994; Minakawa et al. 2006). In highland Kenya, children living further from a malaria focal point in a valley bottom were also slower to suppress the density of parasites (Githeko et al. 2006).

Another potential cause of highland malaria resurgence is the reaction of medical practitioners to malaria, who may be under the impression that they live in a malaria-free region, and may therefore not recognize symptoms among highland populations with individuals who have not recently traveled to endemic regions. In highland Uganda, Lindblade et al. (2001) reported that highland dwelling doctors only diagnosed 36.5% of malaria cases correctly among their local patients, with more successful diagnosis among patients who presented with high fevers. Similarly, treatment was delayed in patients presenting with malaria symptoms in highland Bolivia, since doctors did not consider malaria to be a possibility at that elevation, allowing the epidemic to continue for a period, untreated (Rutar et al. 2004).

Human migration to highland regions from the malaria-endemic lowlands provides a path for introduction of both the parasite and the vector. Many studies cite the role of imported malaria cases into otherwise unaffected highland communities from travellers returning from lowland areas (Sharp 1982; Rutar et al. 2004). Similarly, the accidental transportation of malaria vectors into highland regions, especially during the 1950s, has been observed along newly-built railways and roads (Garnham 1948; Levi-Castillo 1945a; Reiter 2008). More recently, *Culex quinquefasciatus* mosquitoes have been introduced and become established in the Galapagos

Islands from continuous air transport (Bataille et al. 2009). Human migration triggered by conflict has also led to malaria outbreaks (e.g., Pitt et al. 1998), which may potentially exacerbate highland malaria in some countries.

The continued presence of malaria in highland regions may also be due to drug and prophylaxis resistance. In the Kenya highlands, malaria was reintroduced to tea estates by migrant workers during the 1980s. The ongoing transmission among workers in this region was attributed to the failing of pyrimethanine and chloroquine as a drug treatment for afflicted workers, allowing for transmission cycles to continue uninterrupted (Malakooti et al. 1998; Shanks et al. 2000).

One of the most frequently-cited possible causes of highland malaria is deforestation and/or land use alteration. Deforestation has also been associated with a global resurgence in other vector-borne diseases such as yellow fever (Norris 2004). Changes to land cover, including deforestation, can alter local microclimate (mainly temperature) through changes to albedo, vegetation structure, carbon assimilation, canopy conductance, and evapotranspiration (Bounoua et al. 2002; Norris 2004). Slight increments of temperature warming are known to shorten larval development substantially (Paaijmans et al. 2010). As well, slightly warmer temperatures result in an exponentially larger transmission intensity, through its effect of shortening both the mosquito gonotrophic cycle and the parasite development period (Lindsay & Birley 1996). In highland Kenya, wetland conversion to cultivated fields have led to slightly warmer surface temperatures, leading to a substantial increase in malaria rates (Minakawa et al. 2006). A raised temperature of as little as 0.5°C can shorten the gonotrophic cycle of *An. gambiae* by as much as two days (Afrane et al. 2005). In a deforested area, *An. gambiae* fecundity was 65-80% higher and vectorial capacity two times higher than in an adjacent forested region (Afrane et al. 2006).

In Uganda, greater temperatures (daily maximum and nightly minimum) were recorded in areas of swamp converted to cultivation than nearby unconverted swamp, possibly leading to a the doubled human biting rate of *An. gambiae* (Lindblade et al. 2000). Similarly, land use change with canopy opening has been observed to cause microclimatic warming in Madagascar in anopheline habitat (Mouchet 1998). Deforestation therefore often also include a change in the composition of the anopheline vector community (Patz et al. 2000; Póvoa et al. 2003).

Another mechanism by which land use can affect highland malaria is by the creation of larval habitat through irrigation practices that promote standing water. In highland Tanzania, forests have been cleared to create irrigated terraces for vegetable cultivation, which may have provided suitable habitat for larvae (Bødker et al. 2000). Similarly, anopheline larvae have also been associated with abandoned fish ponds (Mouchet 1998; Mouchet et al. 1998; Yomiko Vittor et al. 2006), reservoirs for irrigation (Chhabra et al. 2006), highland rice farming (Kulkarni et al. 2006), and cattle pasture (Minakawa et al. 2005). Habitat availability may locally be more limiting than climate, although in Tanzania, Bødker et al. (2003) stated that potential habitat availability did not decline at higher elevations.

Aside from land use development, the intensification of human populations in highland regions may also contribute to highland malaria. Highland regions are often densely populated in some tropical regions. Cohen & Small (1998) even suggest that the greater densities of human populations residing at high altitudes in the northern tropics may have been historically due to avoidance of vector-borne diseases occurring in lowlands. Urbanization in more populated areas creates heat islands that favour the development of the vector and parasite (Patz et al. 2005). Growing human populations require greater and more elaborate road networks, which also contributes to highland mosquito proliferation by creating potential larval habitats in road ditches or stream breakages, as with *An. gambiae* in highland Kenya (Patz et al. 2000; Koenraadt et al. 2006). In one study of lowland Kenya, larval habitat was most commonly associated with sites converted from nonurban to urban, as well as with unplanned artificial water bodies such as reservoirs, tire tracks, and puddles (Jacob et al. 2003). Finally, intensification of human development often brings humans in closer contact with introduced agricultural and pet species, such as cattle or monkeys, that may serve as reservoirs (Patz et al. 2000; Cormier 2010).

Several authors have related specific epidemics of highland malaria to either seasonal climate changes or to ENSO cycles. In Tanzania, *P. falciparum* malaria cases were positively related to ENSO-event years, resulting in lower average birthweights five months following the epidemic (since *Plasmodium* spp. can accumulate in the placenta of pregnant women) (Uddenfeldt Wort et al. 2004). ENSO cycles have been linked with monsoon rains and increased malaria incidence in northwest Rajasthan, India (Bouma & van der Kaay 1994). Another study in Tanzania attributed only 50% of the variance in anopheline mosquito populations to seasonal temperature indices (Bødker et al. 2003). Minimum temperature and rainfall patterns were strongly related to malaria incidence in highland Rwanda, with a 2-3 month delay between rainfall and malaria due to the extrinsic development time of *P. falciparum* and the time for rainfall to form suitable larval habitat (Loevinsohn 1994). Rainfall was a better explanatory factor in lower-elevation parts of the Rwandan highlands (Loevinsohn 1994). Importantly, Loevinsohn (1994) ruled out other contributing factors such as land use change, immigration, road construction, and parasite resistance to chloroquine as possible causes of highland malaria resurgence in Rwanda. Similarly, Lindblade et al. (1999) correlated malaria incidence to rainfall in the Ugandan highlands, observing a one-month delay between rainfall and peak *Anopheles* spp. density, followed by an additional two to three-month delay before a peak incidence of malaria. Localized rainfall and temperature abnormalities have also been related to malaria incidence in the Kenyan highlands (Zhou et al. 2004), although gradual changes to overall climate have not been observed (Shanks et al. 2002). ENSO-associated droughts in highland regions have also been associated with highland malaria in Africa (Lindsay & Birley 1996). Similarly, in 1997, a very fatal malaria epidemic of *P. falciparum* passed through highland villages in Irian Jaya, Indonesia, following a severe drought. *Anopheles punctulatus* was incriminated as the vector in this case, passing through larval stages in pools that formed in dried highland streambeds (Bangs & Subianto 1999).

The role of climate change in modifying highland malaria has been strongly debated by many authors (e.g. Shope 1991; Epstein 2001; Hay et al 2002a; Reiter 2008), although it has been predicted through the use of several models. In an African-based climate change model, it was predicted that highlands would become endemic regions for malaria by 2050, especially with an increase of incidence in Madagascar and Southeast Africa (Thomas et al. 2004). In a different statistical model based on current global *P. falciparum* range, there is very little predicted change to the malaria parasite distribution even under the influence of extreme climate change scenarios (Rogers & Randolph 2000). A third African model predicts a 16-28% increase in latitudinal distribution, while only a 5-7% increase in altitudinal distribution (Tanser et al. 2003). A model by Craig et al. (1999) predicts that northern African malaria incidence is influenced by rainfall only, whereas the rest of the continent is influenced by the combined effects of rainfall and temperature. Although data from the Himalaya region are scarce, a simplistic climate change model of temperature on the duration of malaria transmission, or transmission window, predicts that transmission windows will increase in these highland regions by 2030 (Dhiman et al. 2011). However, Dhiman et al. (2011) excluded some possibly important factors such as larval habitat availability. For specific vectors, the link between anopheline distribution for some vector species and rainfall is also well established. For example, Coetzee et al. (2000) has shown that the distribution of *An. gambiae* corresponds to areas where rainfall exceeds 1000 mm.

Increased temperatures from global climate change are expected to shorten the parasite and mosquito development times, and thereby intensify transmission (Lindsay & Birley 1996; Githeko et al. 2000). Anopheline mosquitoes occupy specific temperature ranges and also require a minimum rainfall to allow them to complete larval development in permanent water pools (e.g. Lindsay et al. 1998). To successfully complete parasite sporogony during the lifetime of the adult mosquito, and therefore complete the malaria transmission cycle, temperatures must not drop for an extended period of time below minimum temperatures of approximately 14.5-16°C for *P. vivax* and 16-19°C for *P. falciparum* (Detinova et al. 1962; Martens et al. 1995; Lindsay & Birley 1996; Martens et al. 1997). Detinova et al. (1962) determined this duration for sporogony based on the degree-days necessary for parasite development. In Africa, transmission is considered unlikely at ambient temperatures less than 18°C, since adult mosquito survivorship drops below the period necessary for sporogony (Craig et al. 1999). Despite this assertion, transmission has historically been recorded at lower temperatures in highland regions (Garnham 1948; Ahmad & Khan Tariq 1967). Extremely cold temperatures may also inhibit blood feeding behaviour, as has been shown for *Aedes aegypti* (Christophers 1960; Lindsay & Birley 1996). Higher-altitude areas too cold for *Plasmodium* sporogony could therefore theoretically become more suitable by the effects of climate change, and allow for a greater extent of highland endemism (Githeko et al. 2000) or an increase in the number of suitable months for transmission (Guerra et al. 2008). In most cases, the areas most at risk for malaria epidemics are expected to be those with an established vector but where *Plamodium* cannot yet complete development (Jetten et al. 1996).

Since both larval development and sporogony of the parasite are strongly limited by minimum temperature incubation period thresholds (Lindsay & Martens 1998), the minimum nightly temperature is most likely the biologically significant temperature measurement for the spread of malaria and its vector into highland regions (Epstein et al. 1998). Hay et al. (2002c) concluded that the resurgence of highland malaria could not be due to climate change, since there have been no significant increases in average temperatures over a 95-year period in malaria-endemic highland sites in Africa. A separate study by Small et al. (2003) relates historical changes in the malaria transmission climate suitability index to increases in local rainfall, but not the effects of long-term average global warming. However, Easterling et al. (1997) had calculated that minimum temperatures have increased approximately 1.81°C in the Southern Hemisphere during the last century, while average and maximum temperatures have changed much less. Part of the difficulty in analyzing malaria patterns in relation to minimum or average temperatures is that meteorological stations are sparsely distributed in Africa and long-term data sets are uncommon (Thomas 2004). A rapid increase in minimum nightly temperature coincided in time with a shift in the ENSO cycle during the 1970s (Easterling et al.1997; Wang & An 2006; An et al. 2006). At present, the Andean highlands are characterized by a high diurnal amplitude in temperature due to a large input of solar radiation during the day (Buytaert et al. 2006). Additionally, the relationship between altitude and temperature is complex, with a strong influence of latitude and continent. Therefore, a simple relationship in one area cannot be extrapolated to another region (Cox et al. 1999).

A model for altitudinal range shifts in plants and animals has been proposed by Colwell et al. (2008). According to this model, a change of temperature leads to an increase in average altitude (δ) for both the lower and upper altitudinal limits of a species. At the lower-altitude limit, biological attrition occurs where lowland habitat becomes unsuitable. Near the upper altitude limit, range contraction or local extinction occurs due to a lack of available highland habitat on mountain peaks. With the *Anopheles* in Ecuador, there are two possible mechanisms by which highland malaria may be extended through climate change: either by range shift (Figure 1-2a), or range expansion (Figure 1-2b). Although *Anopheles* spp. currently occur in lowland areas at or near sea level, the lowland habitat may become too hot to support their own survival. Therefore, warming would provoke a range shift by moving the lower-altitude limit up by δ m, and shifting the entire elevational range up-slope by δ m (Figure 1-2a). Current estimates show extensive inhibition of parasite development above ca. 35°C, which interferes with transmission (Lindsay & Birley 1996). However, if high temperature thresholds are not reached by climate change, or if mosquitoes make use of lowland cool refugia microclimates (which is difficult to predict), a range expansion is expected, with an expansion of δ m due to the change of the maximum altitudinal limit and an unchanging minimum elevation limit (Figure 1-2b). Since maximum temperature tolerance differs among species, different combinations of these mechanisms are expected to occur for the various anopheline species. The malaria-endemic region and the incidence of the parasite is expected to differ slightly from the range of the vector, since the precence of malaria also depends on a variety of socio-economic and health-related factors, and is not dealt with in this thesis.

One of the difficulties in relating surface air temperature to malaria vectors is that larvae develop in pools of water that are generally warmer than the air but are not well predicted by air temperature in models. Water temperature is related to surface area and depth of the pool, as well as air temperature and incident sunlight (Paaijmans et al. 2010). Therefore, climate models that base total vector distribution on air temperature may underestimate the true extent of vectors (Paaijmans et al. 2008).

Of additional concern is a behaviour observed among adult mosquitoes that may allow them to disperse to colder, higher-altitude regions, and successfully transmit *Plasmodium* spp.. Many adult mosquitoes rest inside human dwellings, where the air temperature is consistently warmer and may allow for completion of parasite sporogony. *Anopheles gambiae* have been observed in historical, as well as in more recent epidemics resting inside human dwellings, in areas where the outdoor minimum temperatures should exclude *Plasmodium* but where transmission cycles still occur (Garnham 1945; Lindsay & Martens 1998).

Finally, the presence of highland malaria has also been associated with the reduction in the implementation of vector control programs (Mouchet et al. 1998). In Madagascar, the elimination of DDT used in the 1960s led to a slow re-emergence of the vector, *An. funestus,* in highland regions. Highland malaria epidemics began in the 1980s that were controlled a second time by indoor residual spray (IRS) and chloroquine prophylaxis (Romi et al. 2002).

Malaria control programs have been relatively successful at controlling highland malaria, especially in the early twentieth century. Success was likely due to the clustered pattern of highland malaria incidence, allowing for control measures to focus on affected areas. In 1964, Roberts (1964a-c) documented the successful control measures undertaken in a localized highland epidemic, where mass chemoprophylaxis was handed out to the affected villages, alongside educational meetings and yearly IRS. In Uganda, an outbreak of malaria transmitted by *An. funestus* occurred in a highland village that surrounded a lake (de Zulueta et al. 1964).

When it was determined that the only larval habitat was the lake margin itself, a larviciding program was implemented and malaria eliminated from the area (de Zulueta et al. 1964). As in lowlands, historically, DDT was frequently used to control highland epidemics, but was generally discontinued when DDT was phased out from widespread global use during the 1970s and 1980s (Mouchet et al. 1998). As an alternative to chemical insecticides, predatory fish that consume larvae have also been used historically with some success in the highlands of Madagascar (Mouchet 1998).

More recent studies have shown that insecticide-treated nets (ITNs) are effective in highland regions, with a 74% reduction in anopheline biting rate (Maxwell et al. 2003; Lindblade et al. 2006). Additionally, ITNs have reduced the density of vectors in highland communities, possibly since some species (*An. gambiae*, in this case) are particularly sensitive to the coating of pyrethroid insecticides (Lindblade et al. 2006). Protopopoff et al. (2008) determined that within the malaria-prone highland valley bottoms, the use of IRS reduced malaria rates by 12-64% and may be more useful than ITNs, although ITNs may provide appropriate protection when funding for IRS is lacking. Indoor Residual Spray has also been very effective in highland communities of Kenya and Burundi (Some 1994; Protopopoff et al. 2008).

Beginning in 1996, the World Health Organization (WHO), TDR (Special Programme for Research and Training in Tropical Diseases) and the International Development Research Council of Canada (IDRC) have jointly formed the MARA (Mapping Malaria Risk in Africa) project to develop spatial models for malaria risk in Africa. One of the projects of this group is the HIMAL (HIghland MALaria Project), which collaborates to develop models, collect data on highland malaria, determine how altitude is related to malaria rates, and determine risk factors for highland malaria in Sub-Saharan Africa (Cox et al. 1999). Presently, it remains difficult to determine if climate change is the major influential factor in highland malaria, since most highland epidemics are short-lived (1-2 years). The Intergovernmental Panel on Climate Change (IPCC) has recommended the ongoing monitoring of highland regions to determine the relationship between malaria ecology and meteorological influences (IPCC 2001a; IPCC 2001b). In the most recent IPCC report, it is concluded that very little is known about the expected effect, if any, on climate change and malaria in Latin America and that more research is required on all continents (Confalonieri et al. 2007). Even with these powerful scientific collaborations in place, present-day highland malaria is still attributed to a complex mixture of short-term meteorological forcings such as ENSO cycles, confounded with non-climatic factors including land use alteration, urbanization, socio-economic factors, etc. Due to the wide variety of causes described in the literature, it is obvious that different factors are more influential in different highland regions of the world, although there are some commonly-observed trends.

1.4 Malaria in Latin America

Although malaria incidence is lower in the Americas than in Africa, and *P. falciparum* is more prevalent in Africa, malaria remains endemic in low-altitude regions of many Latin American countries, with approximately 20% of people in Latin America at risk from malaria (WHO 2010). Malaria in Latin America is most commonly caused by *P. vivax*, although more frequent cases of *P. falciparum* and *P. malariae* occur locally (e.g., Branquinho et al. 1993; Lounibos & Conn 2000). The most well-established primary malaria vectors in Latin America include *Anopheles darlingi* and *An. albimanus* (Lounibos & Conn 2000). Anopheline mosquitoes colonized the New World during at least two separate occasions (Conn 1998) prior to the introduction of the *Plasmodium* parasite. A more recent, post-colonial introduction of malaria has been inferred from the patchy range of introduced primate malarias, including *P. brasilianum* and *P. simium* among South American monkeys (Dunn 1965), and the records of the gradual spread of malaria north throughout the United States from Central America and the Caribbean (Boyd 1941). It is further evidenced by the lack of reference to a similar-sounding illness among the records of Pre-Columbian groups throughout Latin America (Joralemon 1982), and the lack of malaria immunity among indigenous American populations, including the lack of genetic polymorphisms that confer malaria resistance such as the sickle-cell trait (Shear Wood 1975; Joralemon 1982; Cormier 2010). New World *P. vivax* differs genetically from Old World *P. vivax,* likely due to geographic and vector species isolation for long periods (Li et al. 2001), and has also adapted to different vectors encountered in the neotropics, evidenced by genetic variation (Joy et al. 2008). Similarly, yellow fever, an insect-borne flavivirus infection that also infects monkeys, is believed to have been introduced to the Americas during the same period as malaria (Cormier 2010). Although there is some anthropological evidence suggesting that *P. vivax* may have been endemic in the Amazon during Pre-Columbian times, such as the widespread use of traditional anti-malarial medicines, the evidence is presently considered insufficient when compared to the other evidence listed above (Carter et al. 2002; Cormier 2010). There is some uncertainty regarding if *P. malariae* was present in the Americas prior to European colonization (Carter et al. 2002). In tracing the records of malaria incidence, it is most likely that the human malaria parasite was introduced during the activities of the African slave trade during the 16th century, through the Caribbean (Curtin 1968).

Malaria resurgence in Latin America has been directly linked to the decrease of the use of DDT (Roberts et al. 1997; Curtis 2002). Countries in Latin America that phased out DDT in the 1970s observed an almost immediate surge in malaria rates, while Brazil and Ecuador used

DDT until the mid-1990s and did not observe a similar increase (Roberts et al. 1997). A similar surge in malaria rates occurred in Madagascar and Sri Lanka following the removal of DDT programs (Roberts et al. 2000). Although the ecologically detrimental effects of DDT following outdoor agricultural use have led to its decline, some authors still discuss its potential use in Latin America for anti-malarial IRS (Curtis 2002).

Anopheles species in Latin America have been classified by generalized ecoregions by Rubio-Palis & Zimmerman (1997) as occurring in coastal regions, piedmonts, savannas, interior lowland forests, and occasionally in highland valleys. Many of the principal malaria vectors of Central and South America are widely distributed members of the subgenus *Nyssorhynchus*, including *Anopheles albimanus* and *Anopheles nuneztovari* on the western coast, and *An. darlingi* and *Anopheles trinkae* in parts of Amazonia (Lounibos & Conn 2000). *Anopheles albimanus* is a widespread primary malaria vector on the coast of Central and South America, often associated with higher salinity and sun-exposed sites (Marten et al. 1996; Rubio-Palis & Zimmerman 1997). The most well-known malaria vector of Amazonia is *An. darlingi*, which transmits malaria year-round due to its location in the Amazon (Roper et al. 2000). *Anopheles pseudopunctipennis* is a primary vector in highland regions of both Central and South America (Levi-Castillo 1945b; Rutar et al. 2004). Different *Anopheles* species have different vectorial capacities (i.e., ability to transmit malaria successfully), different preferences for blood meal types (e.g., Elliott 1972; Lardeux et al. 2007), and also carry out primary or secondary vectorial roles depending on the other species present in the same area (Antonio-nkondjio et al. 2006; Rubio-Palis & Zimmerman 1997). Interestingly, in Mexico, *An. albimanus* is associated with the VK210 phenotype of *P. vivax* while *An. pseudopunctipennis*, found at higher altitudes, is associated with the VK247 of *P. vivax* (Rodriguez et al. 2000; Joy et al. 2008).

Malaria in Latin America is monitored at the national level by entomological and epidemiological governmental organizations in each country that organize and carry out interventions such as sprays and educational preventive programs (Lipowsky et al. 1992; Brochero & Quiñones 2008; Espinzoa Lopez 2009). These programs often rely on passive case reporting, where underreporting of malaria cases is common (e.g., San Sebastián et al. 2000). For example, in a study of communities of the Peruvian Amazon, the actual numbers of malaria cases were 4.3 times higher for *P. falciparum* and 1.8 times higher for *P. vivax* than reported passively to authorities (Branch et al. 2005). Misdiagnosis of malaria is also common in Latin America, especially in poor, marginalized areas such as remote parts of Amazonia (e.g., Lipowsky et al. 1992; Alves et al. 2005). Often, medical facilities are not available in rural areas and many patients cannot or do not seek treatment (Peters et al. 2008). Part of the problem is that both *P. vivax* and *P. falciparum* can be symptomatic in some patients and symptomless in others, with asymptomatic persons serving as an infection reservoir for the other members of the community for longer periods of time than if the patient was treated (Greenwood 1987; Alves et al. 2002; Alves et al. 2005). Asymptomatic patients may not be reported and may also provide negative results on blood smears; therefore, detection of asymptomatic malaria cases is of particular difficulty to public health officials (Rodrigues Coura et al. 2006).

Some authors have speculated about the possible risk of malaria dispersing to highland cities in Latin America, such as Mexico City, in Mexico or Quito, in Ecuador (Reiter 2001; Moreno 2006). Dengue fever (a similarly low altitude-occurring disease) has also been reported from an elevation of ca. 1700 m in Mexico (Herrero-Basto et al. 1992). Malaria has recently been transmitted in the highlands of Bolivia, vectored by *An. pseudopunctipennis* and transmitting *P. vivax* at altitudes below 2800 m (Rutar et al. 2004; Lardeux et al. 2007), and is the most abundant anopheline species collected above 500 m in Argentina (Dantur Juri et al. 2005). However, *An. pseudopunctipennis* is somewhat zoophilic in its preferences, preferring donkey, sheep and goat blood to human blood, which may be due to the scarcity of humans in the rural Andean highlands (Lardeux et al. 2007).

Although highland malaria has not been linked to long-term climate change in Latin America (Confalioneri et al 2007), ENSO has been linked to malaria outbreaks in lowland parts of some countries (Bouma et al. 1997; Gagnon et al. 2002). Models linking the 3-7 year ENSO cycle to malaria in Colombia have been developed, with El Niño and El Niño+1 years leading to as much as a 35% greater malaria incidence (Bouma et al. 1997; Poveda et al. 2001; Ruiz et al. 2006). Since warmer temperatures speed up oocyte development, *An. albimanus* gonotrophic (egg development) cycles can be up to 13 hours shorter during El Niño years (Rúa et al. 2005). In Argentina, *An. pseudopunctipennis* population increases have been related to warming temperatures of as little as 1°C , with maximum population occurrence in spring (Dantur Juri et al. 2009). The most recent IPCC climate change scenarios for Latin America predict a 0.4 - 1.8°C warming by 2020 and 1.0 - 7.5°C warming by 2080, and it is not yet established how this warming will affect malaria distributions (Magrin et al. 2007).

1.5 Malaria in Ecuador

Similar to the rest of Latin America, the malaria parasite has been likely introduced to Ecuador during the colonial period (Newson 1993). It is unlikely that malaria has occurred in the Ecuadorian Amazon during the colonial period, due to the barrier of the Andes (Newson 1993). As well, explorations of the upper Ecuadorian Amazon during colonial periods until the early twentieth century did not report malaria nor documented large numbers of biting mosquitoes (Sinclair & Wasson 1923; Newson 1993).

The public health movement in Ecuador did not begin until 1908, when bubonic plague, yellow fever, and malaria became problematic in Guayaquil (Izquieta Pérez 1941). Highland cases of malaria were widely reported from the highland valleys in the vicinity of Quito, as well as in the provinces of Carchi, Imbabura, Pichincha, Chimborazo, Cañar, Azuay and Loja during the 1940s (Levi-Castillo 1944b; Levi-Castillo 1945b; Montalvan 1948; Gómez de la Torre Serrano et al. 1991). A successful campaign was carried out beginning in 1938 to eliminate highland malaria, by draining larval habitats, filling canals and pools, and spraying with DDT and kerosene (Levi-Castillo 1945b; Levi-Castillo 1947; Levi-Castillo 1949; Levi-Castillo 1950). During the 1960s, extensive road-building resulting from agricultural colonization on the coast expanded the reported coastal range of malaria epidemics (Burt et al. 1960), possibly due to the expansion of suitable larval habitat, as well as the presence of non-immune highland immigrants. The list of all *Anopheles* species reported from Ecuador from the literature is found in Table 1-1, with the most commonly reported species being *An. albimanus*, *An. pseudopunctipennis* and *An. punctimacula* (Levi-Castillo 1949; Rubio-Palis & Zimmerman 1997).

The Ecuadorian government manages the Servicio Nacional de Control de Enfermedades Transmitidas por Vectores Artrópodos (SNEM), which has worked against malaria and its vectors since its establishment in 1956 (Espinoza López et al. 2009). The SNEM has been responsible for carrying out extensive anti-vectorial sprays of DDT, with the later use of malathion, sumithion, and pyrethroid insecticide (Espinoza López et al. 2009). The cost of malaria prevention by the SNEM was estimated at USD $0.40 per person in 1994, while the cost of treatment was USD $10.40, mainly due to treatment and travel costs (Ruiz & Kroeger 1994).

During the early 1990s, chloroquine resistance led to an increase of malaria on the coast of Ecuador, including the more severe cerebral malaria (Guzman et al. 1995). However, during the last fifteen years, strong efforts by the SNEM, accompanied by the introduction of free health care to larger communities by the federal government, managed to lower the malaria-attributed death rate in the country to zero by 2008 (with ca. 5000 malaria cases) (Espinoza López et al. 2009; Min. Salud Publica 2009). Impressively, the World Health Organization estimates that there has been at least a 50% decline in malaria cases since 2000 due to national malaria elimination efforts (WHO 2010). Both *P. vivax* and *P. falciparum* are present throughout Ecuador, with the majority of cases reported from the coastal plain. *Plasmodium vivax* infections are more widespread in the country, but *P. falciparum* infections occur in almost equal numbers where malaria foci occur, such as in the provinces of Santo Domingo de los Tsáchiles and Esmeraldas (SNEM 1995-2009).

The present government of Ecuador operates an extensive network of public health centers funded by all levels of government. Private hospitals are also available in urban centers (Min. Salud Publica 2009). As of 2008, new malaria control protocols were proposed that would link the Ministery of Public Health to the SNEM in a joint effort to treat malaria in local health centers and eventually eliminate *P. falciparum* from Ecuador (Min. Salud Publica 2009).

Even with the optimism surrounding an effective malaria control program, there is still some concern that climate change and land use alteration may push malaria back into the environs surrounding Quito and other highland valleys where it is presently not monitored (e.g., Moreno 2006). Unlike in Colombia, no ongoing link between ENSO cycles and epidemics of malaria in Ecuador is apparent (Gagnon et al. 2002), although a government document by Vos et al. (1999) showed a steep increase of malaria rates in coastal Ecuador following the El Niño event of 1982-3. As recently as the early 1990s, a few *An. pseudopunctipennis* larvae were reportedly collected from the Guayllabamba river north of Quito, providing support for this possibility (Gómez de la Torre Serrano et al. 1991).

In Ecuador, a moderate portion of the population, particularly in the Andean highlands and among Amazonian Indigenous groups, attributes diseases and illness to religious or cultural causes, sometimes using traditional medicines and shaman healers (Pedersen & Coloma 1983; Pan et al. 2010). Although Ecuadorian primary health care in larger communities encompasses private and pubic health centres, dual employment of doctors is common and favours hours in private clinics, limiting doctor availability in public health centres, particularly in rural regions (Tejerina Silva et al. 2009), which may contribute to use of traditional medicine in rural areas. In the Ecuadorian Amazon, malaria is transmitted year-round, with the less severe *P. vivax* representing 92% of cases, and *P. falciparum* 8% of cases (San Sebastián et al. 2000). Indigenous residents of the northern Ecuadorian Amazon are four times more likely to become ill with vector-borne disease than neighbouring colonists from the highlands due to socio-economic factors, and this disparity is particularly strong among those of the Shuar ethnicity (Pan et al. 2010). In the Amazonian province of Napo, traditional healers describe "Mal aire", or bad air, as an illness caused by nature with characteristics of a human curse, sometimes caused by the soul of an aborted fetus inhabiting the body of a person (Yánez del Pozo 2005). The similarity in name and symptoms, namely fever and vomiting, are suggestive that this particular Mal Aire may, in fact, be malaria:

> "Walking by the mountain, I started to smell a strange odour, terrible. I never smell this normally. It smelled as if rotten. Then I felt bad and I ran. I arrived at the house with a shaking, with fever, and just after I couldn't stand up. I started to die then..." (Yánez del Pozo 2005; Translated to English by L.P.).

Similarly, the origin of the word malaria was later derived from the Roman description of 'mal aire' associated with the swamps providing habitat for *Anopheles* mosquitoes (Heggenhougen et al. 2003).

For the purpose of this study, it is first important to define the term highland in the context of highland malaria in Ecuador. In Figure 1-3, altitudinal profiles of the Andes (sliced along latitude) in a) Northern Ecuador, b) Central Ecuador, and c) Southern Ecuador are provided. The Andes mountains in the south shift toward the west (Figure 1-3c), and the uphill slope begins and ends at different latitudes, making a longitudinal definition for "highlands" inappropriate. A biologically relevant definition of highlands would be an area where topography is steep rather than relatively flat, since steeper topography can limit mosquito dispersal and also the availability of larval habitat (Balls et al. 2004; Githeko et al. 2006). This occurs conservatively at approximately 500 m altitude on the western, coastal side of the Andes, around which the gradual, flat incline of the coast gives way to a hilly, irregular surface (Figure 1-3). On the Amazonian (right) side, a large, flat, higher-altitude plateaux is observed in central Ecuador, where the majority of larger settlements are located and where malaria is presently endemic, ranging from 500 - 900 m in altitude (Figure 1-3b). Therefore, for the purpose of this thesis, the "highland" region of Ecuador is defined as above 500 m on the coastal side, and above 900 m on the Amazonian side of the Andes.

1.6 Relevant aspects of Ecuadorian geography and climate

As its name suggests, Ecuador lies on the equator, between Colombia to the north and and Peru to the south. It is divided geographically into four main regions: the Pacific coast, the Andes highlands, Amazonia (Figure 1-4), and the Galapagos Islands. The most populous city is

Guayaquil, on the coast, followed by Quito, the capital, at 2800 m. Figure 1-4 is provided for reference, since it includes all of the cities and towns mentioned in this thesis.

Ecuadorian climate varies little seasonally due to its situation on the equator. In the Andes, rainfall increases with altitude up to 1200 - 1500 m, after which it declines, with the interior valleys receiving less rainfall (McGregor & Niuwolt 1998). Precipitation is highly variable due to irregular topographic influences (Buytaert et al. 2006). Figure 1-5 presents climate diagrams (drawn from 2008 tabulated data) for four localities in Ecuador that represent a range of climates in the country. Quito, Pichincha, lies in a northern Inter-Andean valley at 2800 m, and is characterized by cool but consistent temperatures (Ave. = 20.6°C, Min. = 9.9°C) and moderately higher rainfall in January to May (Figure 1-5a). Baños, Tungurahua, is a small city located in the eastern Andes at an altitude of 1639 m, providing consistently warmer average and minimum temperatures (Ave. = 24.3°C, Min. = 14.3°C) and an opposite timing of seasonal rainfall to Quito, during May-August (Figure 1-5b). Throughout the high Andes of Ecuador, the rainy seasons lie from February-May and from October-November, depending on the influence of both oceanic currents and Amazonian continental air currents (Vuille et al. 2000). Highland regions receive reliable water flow from the highland páramo (Buytaert et al. 2006). Guayaquil, Guayas, lies on the Pacific coast, with consistently hot temperatures (Ave. = 30.7°C, Min. = 22.2°C) and a strong seasonal trend in rainfall, with peak rainfall during January-April and a dry season from May-December (Figure 1-5c). Although this coincides with the timing of the field work undertaken for this thesis, much of the coastal area around Guayaquil is near or below sea level, and larval habitat for anopheline mosquitoes is extremely abundant. From January until March, the strong rains on the coast wash out many of the roads every year, making travel to the coast from the highlands nearly impossible. Since the focus of the thesis was on highland regions, which are far more variable in the timing of rainfall, we decided to undertake fieldwork and data collection during July until late December, when roads would more likely be passable. Finally, Figure 1-5d provides the climate of Puyo, Pastaza, which lies on the aforementioned Amazonian highland plateaux. Temperatures are consistent year-round and cooler than on the coast (Ave. = 26.5°C, Min. = 17.5°C), with uniformly high rainfall year-round, especially in May (Figure 1-5d). Generally, within Ecuador, rainfall increases from west to east and from south to north (Buytaert et al. 2006). It is difficult to determine the extent of climate change throughout Ecuador, since a network of weather stations in the country was only established after 1960 (Vuille et al. 2000).

In terms of global climate change, IPCC analyses suggest that there has been increased rainfall in many regions of Ecuador between 1960 and 2000 (Magrin et al. 2007). However, temperature has overall increased less than other regions in South America, between +0.08 and +0.27°C between 1930 and 1990 (Magrin et al. 2007). Although rainfall has increased in many regions, some areas are expected to become drier in the future due to the observed retreat of mountain glaciers (Magrin et al. 2007).

Highland regions of Ecuador were forested although inhabited at least until colonial times, since timber was used to build many colonial buildings in highland cities (Sarmiento 2002). However, fragmentation patterns suggest that highland montane forests were replaced with pasture during pre-Columbian times, and are now predominantly montane grasslands (Sarmiento 2002). Modern highland land uses are also characterized by permanent and temporary crops, with extensive pasture grasslands closer to roads (de Koning et al. 1998). The wet tropical coastal forests of Ecuador were modified during the cocoa boom in the 1920s and the subsequent banana boom in the 1950s, accelerated by a road system built to link farms with port cities and the highlands (Wood 1972; Bromley 1981). Fruit crops and sugarcane were planted along roads linking the coast to highland cities and are still the common land uses in valley bottoms in present day (Bromley 1981; de Koning et al. 1998). The Amazon region, also characterized by wet tropical forest was sparsely settled between 1850-1940 (Bromley 1981). Following the oil boom in 1970, road building encouraged informal settlement, leading to widespread conversion of forest to pasture grassland (Bromley 1981; Southgate et al. 1991; de Koning et al. 1998; Sarmiento 2002).

Aside from malaria, parts of Ecuador are endemic for dengue and yellow fever, as well as leishmaniasis in Zamora-Chinchipe and Esmeraldas provinces and Chagas disease in Manabí, Los Rios and El Oro provinces (Guzman et al. 1995). The presence of multiple vector-borne diseases within Ecuador highlights the importance of the study of the essential ecology of disease vectors, how the distribution of the disease may be affected by land use changes and climate change, and how vector-borne diseases might be controlled.

1.7 Outline of thesis

The purpose of this thesis was to a) determine the current geographic extent of *Anopheles* species in Ecuador, particularly in highland regions, and b) determine relevant aspects of the vector ecology of these *Anopheles* species in highland regions. Ecuador was chosen as a representative country for study due to its small size, extensive road network, relative safety and ease of travel, cooperation with local research organizations, and great diversity of possible habitats for *Anopheles* species.

Since highland malaria was once widespread in Ecuador, a thorough historical review using archival evidence and statistics was undertaken for "Chapter Two: an historical review of highland malaria in Ecuador (1900 – Present)". Chapter two presents historical evidence for the role of the Guayaquil-Quito railway in promoting vector transport to highland regions, as well as discussing the possible role of the inconsistencies of vector control programs in malaria outbreaks during the last century.

"Chapter Three: New highland distribution records of multiple *Anopheles* species in the Ecuadorian Andes" presents the results of a survey-based study to determine the geographical distribution of *Anopheles* species in Ecuador. Larval collections were attempted throughout Ecuador from 438 potential habitats, and larvae identified to species and mapped using a GIS. Although we had correctly predicted that *An. pseudopunctipennis* would be collected from highland regions (i.e., Gomez de la Torre Serrano et al. 1991), we also collected *Anopheles albimanus, Anopheles eiseni, Anopheles punctimacula* and *Anopheles oswaldoi s.l.* in higher-altitude regions than previously recorded. Chapter Three highlights the current presence of multiple malaria vectors in highland regions of Ecuador and provides a baseline of distribution data upon which to base the other chapters.

Figure 1-6 provides photographs of the dorsal aspect of the larval stages of all species collected, except *An. eiseni.* Although the morphological characters used to differentiate the species are usually only visible under strong magnification and therefore not visible in these photographs, the long, filamentous protrusions of the posterior spiracular plates, typical of *An. pseudopunctipennis,* are visible in Figure 1-6c.

Chapter Four outlines a descriptive study undertaken to characterize the larval habitats of *An. albimanus, An. pseudopunctipennis*, *An. punctimacula,* and *An. oswaldoi s.l.* in the entire extent of their Ecuadorian range, including highland areas. Unlike many studies which simply describe habitat in larval-present sites, our study statistically compares larval-present sites to larval-absent sites, which represent the availability of different types of potential habitat.

In Chapter Five, we tested the hypothesis that human land use in highlands provide suitable habitat, and therefore, land use change is at least partially responsible for the establishment of multiple highland vectors. A variety of human land uses that are common in highland regions, as well as roads and rivers (which are highly modified by human land use) were assessed for the presence of standing water and parameters that may provide suitable habitat for anopheline species. Most of the land uses were very dry and were unsuitable for larval habitat, which did not support our hypothesis. However, the presence of many larvae in river-associated habitat suggested that rivers may be an important habitat for highland *Anopheles* and may, in fact, provide a corridor for dispersal to highland regions.

In Chapter Six, a geographic distribution model of the range of *An. albimanus*, *An. pseudopunctipennis*, and *An. punctimacula* was constructed in ArcGISv.10 (ESRI 2010), using habitat and climatic parameters determined in Chapters Three, Four and Five. The modeled distribution explained over 90% of the actual collection sites. The effect of 1, 2, and 3°C warming on the species' distribution was also modeled and discussed.

Finally, Chapter Seven presents an assessment of human perceptions of malaria and mosquitoes, as well as management practices used by land owners/managers to reduce standing water. Highland and lowland land owners and managers both had a strong knowledge of the general geographic occurrence of malaria in Ecuador and understood that a link existed between standing water and malaria. However, risk was often underestimated by respondents even in lowland areas, and many at-risk respondents did not use malaria prevention methods such as ITNs. Standing water was not always managed on properties, likely since respondents had a specific, too-narrow definition of mosquito larval habitat, or due to practical difficulties in eliminating the water.

Table 1-1 List of possible *Anopheles* species referenced in the scientific literature as occurring in Ecuador, by subgenera, with region, and its vectorial role for malaria, if known. Primary vectors are the species implicated in the majority of transmission in a given region, while secondary vectors may transmit malaria but do not play as large of a role as the primary vector in a specific region. This vectorial role is generally region-specific and depends on the local community of *Anopheles* species. The continued existence of species was verified using Mosquito Catalog (Gaffigan et al. 1999).

Subgenus	Species	Region	Vector	Reference
Anopheles	*An. apicimacula*	Amazon	?	Gorham et al. 1973
	An. calderoni	Amazon	?	González et al. 2010
	An. eiseni	Coast	?	Gorham et al. 1973
	An. mattogrossensis	Amazon	?	Gorham et al. 1973
	An. mediopunctatus	Amazon	?	Gorham et al. 1973
	An. neomaculipalpus	Amazon	secondary	Gorham et al. 1973 Moreno et al. 2005
	An. pseudopunctipennis	Coast, Sierra	primary	Gorham et al. 1973
	An. punctimacula	Coast	secondary	Gorham et al. 1973
Kertezia	*An. boliviensis*	Bromeliads	?	Gorham et al. 1973
	An. neivai	Bromeliads	secondary	Gorham et al. 1973
Lophopodomyia	*An. gomezdelatorrei*	Amazon	?	Gorham et al. 1973
	An. squamifemur	Amazon	?	Gorham et al. 1973
	An. vargasi	?	?	Gorham et al. 1973
Nyssorhynchus	*An. albimanus*	Coast	primary	Gorham et al. 1973 Lounibos & Conn 2000
	An. albitarsis	Amazon	?	Lounibos & Conn 2000
	An. aquasalis	Coast	?	Gorham et al. 1973 Lounibos & Conn 2000
	An. darlingi	Amazon	primary	Gorham et al. 1973 Lounibos & Conn 2000
	An. evansae[1]	Amazon	secondary	Gorham et al. 1973 San Sebastián 2000
	An. nuneztovari	Amazon	secondary	San Sebastián 2000
	An. oswaldoi	Amazon	primary/ secondary	Gorham et al. 1973 San Sebastián 2000; Lounibos & Conn 2000
	An. rangeli	Amazon	?	Gorham et al. 1973 Fritz et al. 2004, San Sebastián 2000
	An. triannulatus	?	secondary	Gorham et al. 1973
	An. trinkae	Amazon	secondary	Fritz et al. 2004 Lounibos et al. 1998a
Stethomyia	*An. kompi*	Amazon	?	Gorham et al. 1973

[1] syn. noroestensis.

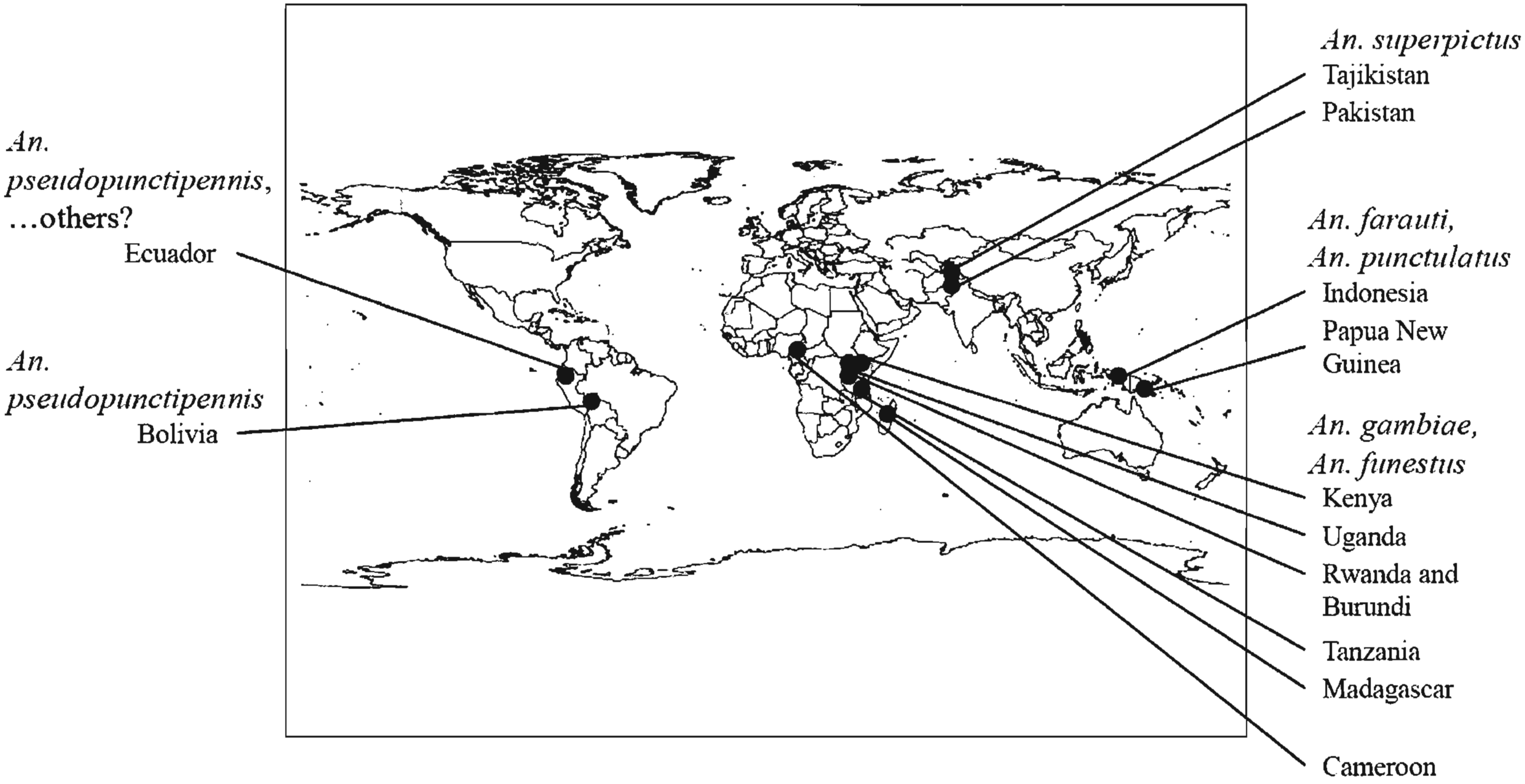

Figure 1-1 Map of the global incidence of highland malaria appearing in the scientific literature, with known highland-occurring malaria vectors. Compiled from: Garnham 1948; Ahmad & Khan Tariq 1967; Sharp 1982; Some 1994; Loevinsohn 1994; Mouchet 1998; Lindblade et al. 1999; Bangs & Subianto 1999; Wanji et al. 2003; Maxwell et al. 2003; Rutar et al. 2004; Protopopoff et al. 2008.

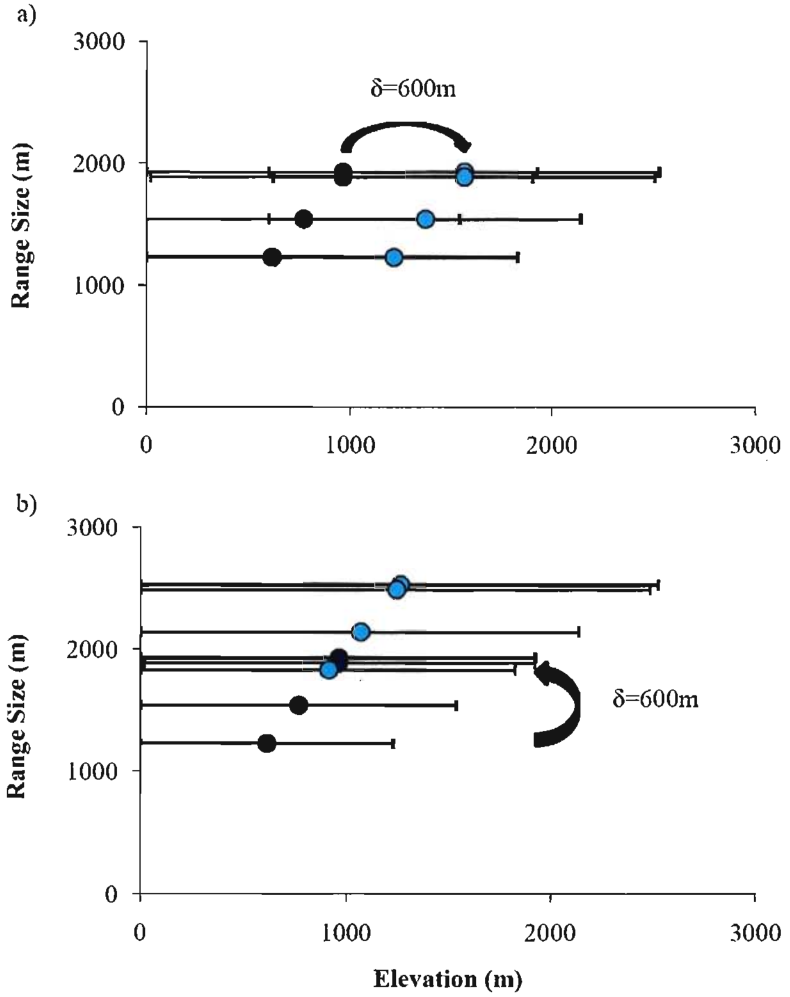

Figure 1-2 Application of Colwell et al. (2008)'s model of climate change to altitudinal change in *Anopheles* spp. in Ecuador. Dark blue circles indicate current altitudinal means for each species (Chapter Three), with bars representing the existing altitudinal range. Light blue circles indicate altitudinal increase due to a shift of δ=600 m with bars representing the new altitudinal range. In (a), low-elevation habitats of *Anopheles* spp. are nearly unsuitable, and a 3.2°C warming followed by a δ=600 m altitudinal shift (Predicted by the IPCC for the next century) leads to a range shift upslope of 600 m. In scenario (b), *Anopheles* species near sea level are able to tolerate the climate warming (i.e., have not yet reached their maximum temperature tolerance level or sought refuge in cooler microclimates), and only the maximum altitude shifts upslope by 600 m, causing a range expansion of +600 m. Both of these mechanisms are possible for all species that occur in lowlands and highlands, with an expected range expansion until lowlands become unsuitable, followed by a range shift. Upper altitudinal limits depend primarily on the availability of larval habitat at higher altitudes.

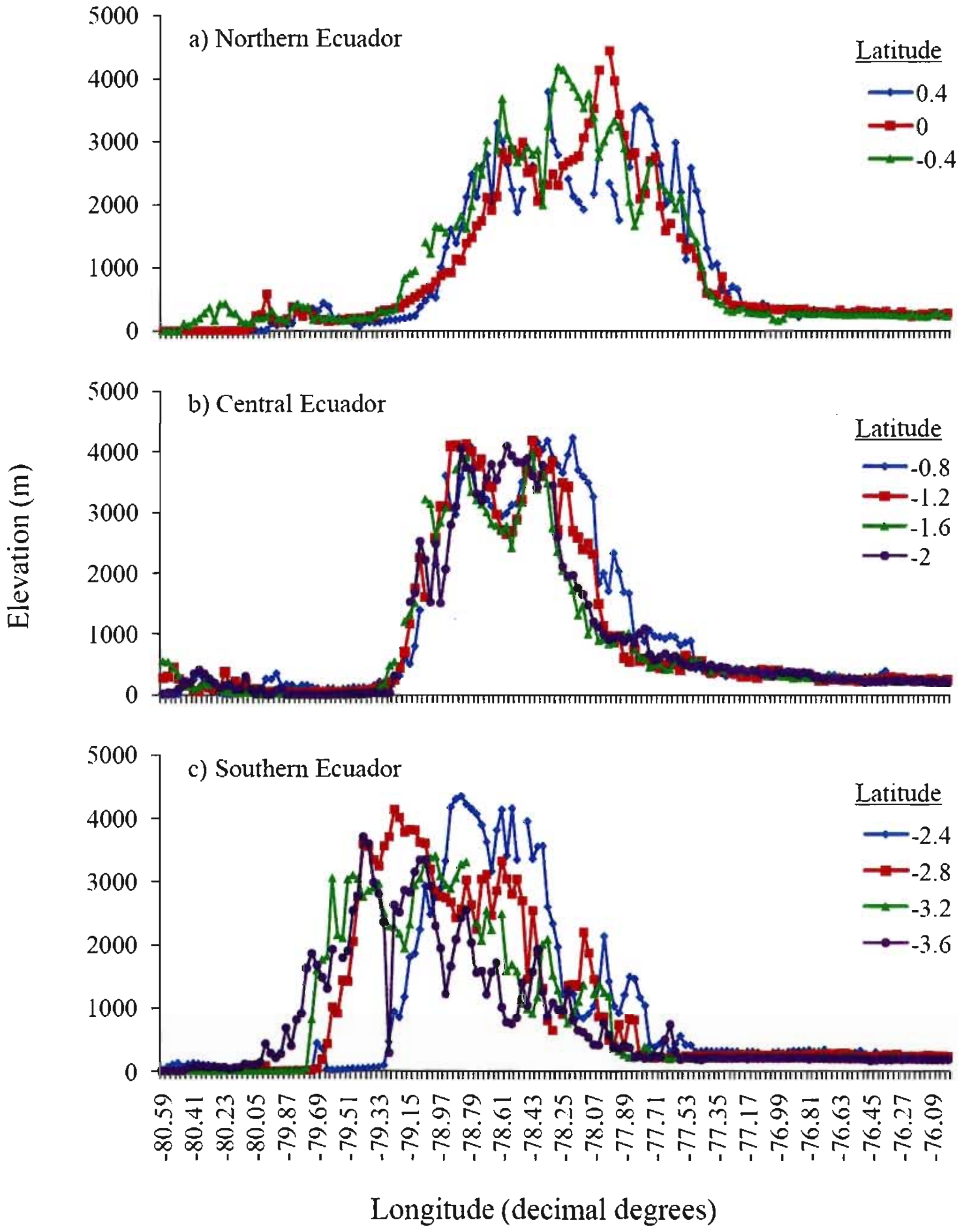

Figure 1-3 Profiles of the a) Northern, b) Central and c) Southern Ecuadorian Andes taken as slices along longitude in decimal degrees (see legends). The left side represents the coast, followed by the Andes, and the right side is the Amazon. Data: Digital Elevation Model (90-meter NASA database (SRTM3)), accessed on the GPS Visualizer online utility (June 2011).

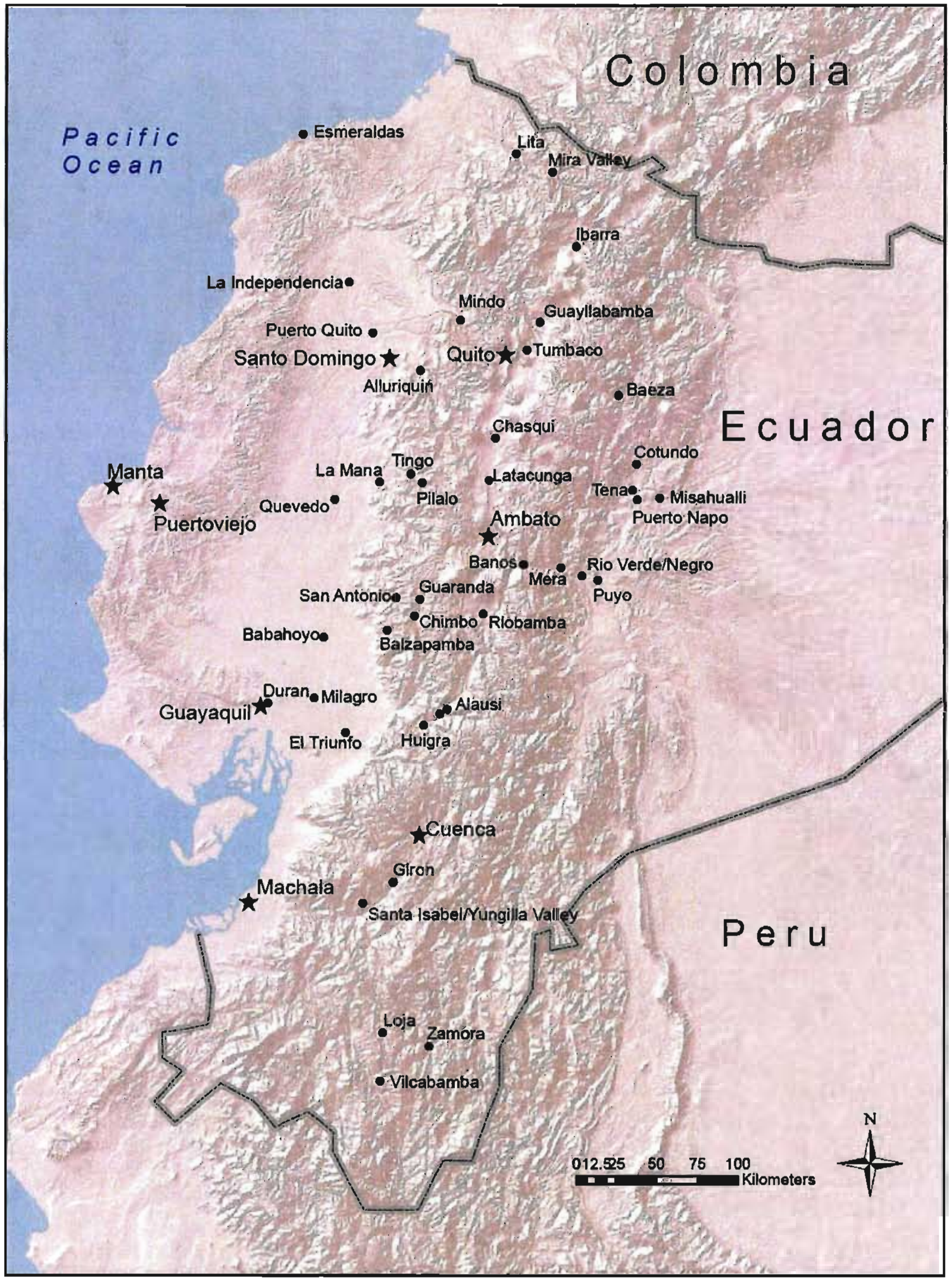

Figure 1-4 Reference map of extent of the Andes, as well as cities and towns in Ecuador. Major cities are indicated with a star symbol, and smaller cities and towns are indicated with black circles. Map prepared in ArcGIS v.10 with ESRI World Terrain base (ESRI 2011).

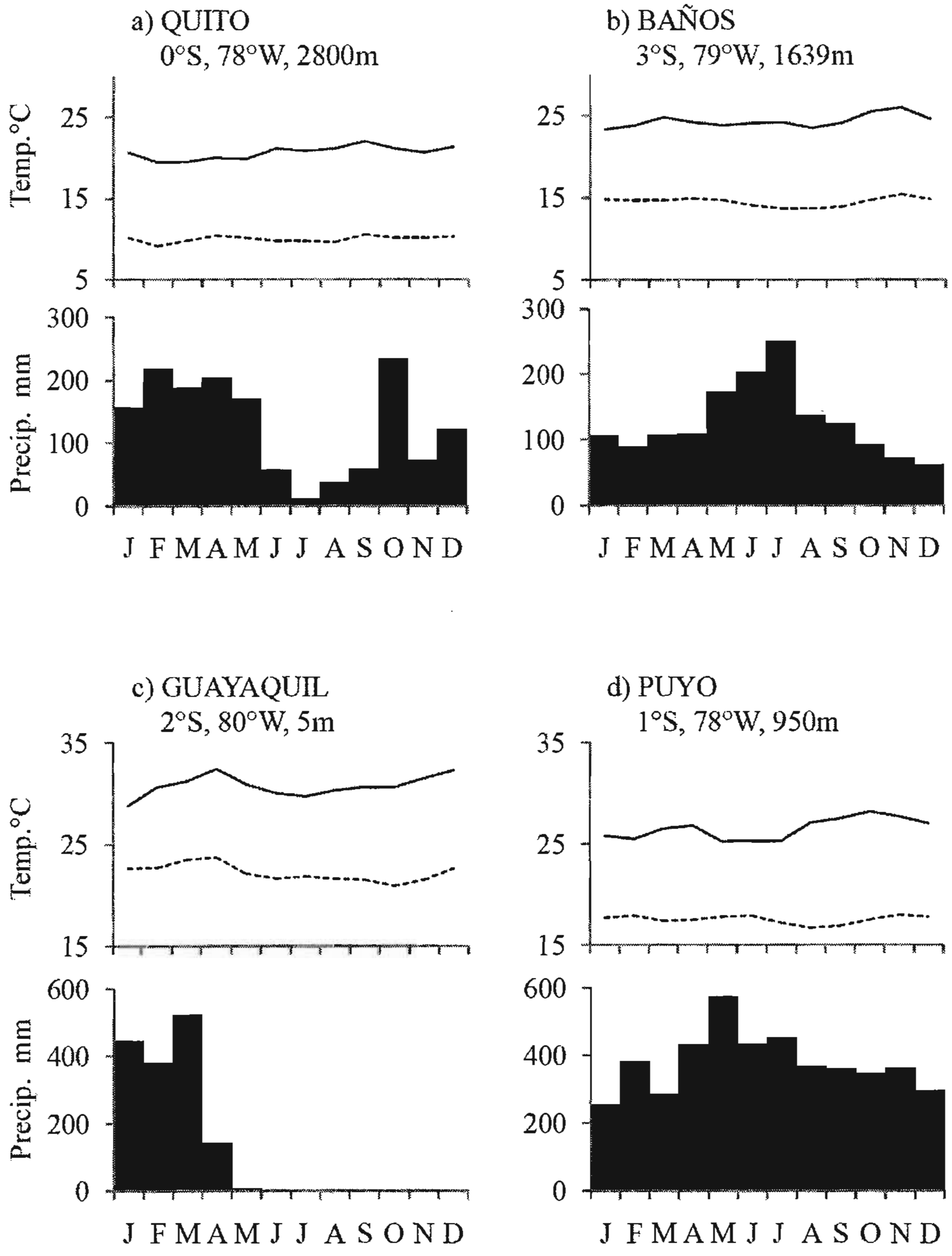

Figure 1-5 Climate diagrams for a) Quito, Pichincha, b) Baños, Tungurahua, c) Guayaquil, Guayas, and Puyo, Pastaza. Mean and minimum temperatures (°C) and rainfall (mm) are provided for each month based on 2008 data (INAMHI 2010).

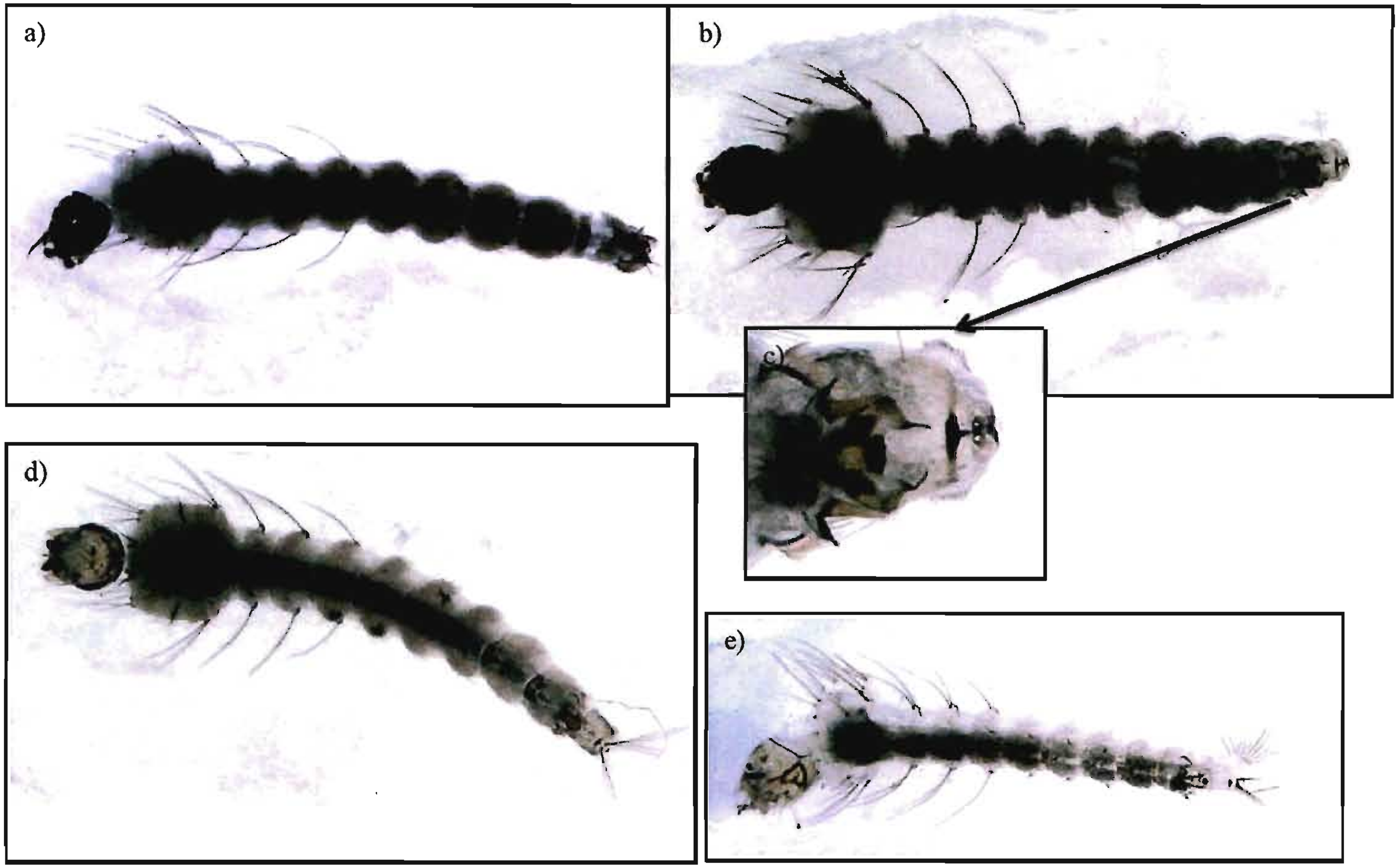

Figure 1-6 Dorsal aspect of anopheline fourth-instar larvae collected in Ecuador during 2008-2010: a) *An. albimanus*, b) *An. pseudpunctipennis*, c) detail of black filaments on posterior spiracular plates, characteristic of *An. pseudopunctipennis*, d) *An. oswaldoi s.l.*, and e) *An. punctimacula*. Photos taken using a three-dimensional microscope imaging system at the Royal Ontario Museum, Toronto.

Chapter Two

An historical review of highland malaria in Ecuador (1900 – 2011)

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2.1 Introduction

Some authors have discussed the possibility that *Anopheles* mosquitoes may once again begin transmitting malaria parasites (*Plasmodium* species) at higher altitudes in the South American Andes (e.g., Reiter 2001; Moreno 2006). Within Africa, highland malaria resurgence has been attributed to land use alterations, malaria treatment resistance, changes to vector control measures, the El Niño Southern Oscillation Cycle (ENSO), and human migration into foothill and mountainous regions (Linsday & Birley 1996; Hay et al. 2002a). In South America, prior to 2004, a short-lived epidemic of *Plasmodium vivax* malaria was recorded in a Bolivian village at an altitude of 2300 m, transmitted by *Anopheles pseudopunctipennis* Theobald (Rutar et al. 2004). Although uncommon, highland incidents of malaria in the Andes have occurred during the last century. As well, multiple *Anopheles* spp. have become established in the highlands of Ecuador (Chapter Three).

In this review, we summarize the documented incidents of highland malaria which occurred in Ecuador during the twentieth century. We define the term "highland malaria" to mean malaria that occurs in steep-topography regions. These occur ca. >300 m and >700 m in elevation on the coastal and Amazonian sides of the Andes, respectively. Using Geographic Information Systems (GIS) and tabulated data from historical sources, we reconstruct the extent of the incidence of malaria during several time periods of interest. Finally, we outline malaria control efforts and attempts at malaria elimination from Ecuador during the last century, as well as the beginning of the present century.

2.2 Methodology

Historical data were acquired from published sources in libraries and archives in Ecuador as well as the Instituto Nacional de Estadísticas y Censos and the Servicio Nacional de Control de Enfermedades Transmitidas por Vectores Artrópodos. To reconstruct the extent of highland malaria during the 1940s, towns listed as malaria-endemic were located by the geographic location of their town square in Google Earth imagery (Google 2010), and malaria extents (hatched regions) plotted using a topographic map at an upper altitudinal limit of 2500 m, in ArcGIS v.10 (ESRI 2010). Two linear regressions were used to relate average daily and minimum nightly temperatures from historical meteorological records in Quito by year.

2.3 The state of malaria in Ecuador at the beginning of the twentieth century

Although malaria was very prevalent on the coast of Ecuador at the beginning of the twentieth century, it was considered by public health officials of Guayaquil to be a minor problem (Pineo 1990). Until 1908, Guayaquil was affected by the constant menace of mosquitoes transmitting yellow fever, and flea-borne bubonic plague reoccurred regularly in all areas of the city and surrounding countryside (Izquieta Pérez 1941; Parks & Nuermberger 1943; Pineo 1990). Due to the 'unhealthy' image of Guayaquil as a major port city, Ecuadorian officials signed an international sanitation convention in 1906 to combat outbreaks of yellow fever, bubonic plague and cholera (Anon. 1906a). Under the terms of the convention, officials were to notify other trading countries when epidemics of these diseases occurred, and to take measures to prevent their ongoing transmission, such as through the use of mosquito screenings on the windows and doors of hospitals (Anon. 1906a). During that time, officials also experimented with the use of mosquito larvae-eating fish as a bio-control method in an attempt to control yellow fever, which was considered more of a public health priority than was malaria (Parks & Nuermberger 1943).

As of 1908, the public health movement became active in Ecuador, and a special Sanitary Commission was formed for the city of Guayaquil (Izquieta Pérez 1941; Parks & Nuermberger 1943). At the time, malaria was still considered a minor health problem, with many residents allowing the anopheline mosquitoes to bite them to provide them with long-term immunity to malaria (Meitzner 1938). The treatment for malaria was quinine (10 grams three times daily), as well as holistic cures incorporating quinine, arsenic, and eucalyptus oil (Meitzner 1938). Malaria prevention measures included bed nets, window and door screenings, and anopheline larval habitat destruction (Meitzner 1938; Parks & Nuermberger 1943). Several medical entomologists became active during this time period in Ecuador, including the French entomologist Paul Rivet, and Ecuadorian entomologists F.R. Campos, Luis León, and J. Rodríguez (Astudillo 1971).

In 1919, many Ecuadorian medical doctors began to receive foreign training, particularly in the United States of America through grants from the Rockefeller Foundation, in an attempt to eliminate yellow fever and malaria from Ecuador (Parks & Nuermberger 1943; Bustamante 1950; Pineo 1990). With the elimination of yellow fever, attention inevitably turned to malaria, which still accounted for a large percentage of mortality on the coast even by 1930 (Espinoza López et al. 2009). In 1940, malaria remained a priority, and was the second leading cause of death in Ecuador after whooping cough (Izquieta Pérez 1941). At the time, it was recommended that a campaign against malaria should be initiated throughout the entire country (Izquieta Pérez 1941).

2.4 Highland malaria during the construction of the Guayaquil – Quito railway (1890-1945)

Around 1886, construction began on the railway line that was to link some of the low-altitude regions of the country near Guayaquil to highland regions and eventually to Quito (García Idrovo 2008). The train was constructed on a route that began in Guayaquil (at sea level), passed through Milagro, following the valley bottom up toward Huigra, in Chimborazo province (1250 m). After Huigra, the railway continued higher towards Alausí, Chimborazo (2340 m), after climbing the infamous Devil's Nose switchbacks (García Idrovo 2008).

When construction of the railway reached an altitude of only 200 m, workers reported being bitten by an unidentified bush-dwelling flying insect and many subsequently died of high fevers (Vásconez & Bravo Silva 1992). At higher altitudes, workers began to report cases of fever attributed to malaria (García Idrovo 2008). In 1906, the Guayaquil newspaper "Grito del Pueblo" reported that railway workers affected by "fevers" were removed from the building site and brought to the highland village of Chasqui, Pichincha, just south of Quito, for their recovery (Anon. 1906b). The following quotation from Daniel Barragán, one of the engineers on the railway, provides strong evidence that mosquitoes (Culicidae) were present on the worksites: "The mosquitoes were our eternal companions, during all of the night, their melodious and incessant humming many times did not let us find sleep" (translated to English by L.P.) (García Idrovo 2008).

The medical doctor M. Meitzner described treating many of the railway workers for malaria during 1911 (Meitzner 1938). In the winter of that year, malaria incidence was so high that construction halted completely (Meitzner 1938). Patients were usually brought to higher-altitude towns such as Huigra for treatment since there were insufficient medical facilities at lower altitudes (Meitzner 1938). Malaria cases among the railway workers were treated by Dr. Meitzner using a combination of castor oil with quinine, and a diet that excluded meat (Meitzner 1938). Prior to the operation of the railway, transportation between the coast and highland regions was limited. Therefore, during the early operation of the railway to Quito, it is possible that the malaria parasite could have been repeatedly introduced by infected passengers and workers to higher-altitude regions, including the valleys around Quito. For example, Dr. Meitzner documented treating the train conductor for malaria a day prior to leaving for Quito (Meitzner 1938).

The presence of malaria rather than other similar febrile illness among railway workers was further supported by the multiple collections of *An. pseudopunctipennis* larvae (the highland malaria vector) in the valley of Chiripungo, near Alausí, Chimborazo (<2400 m) (Levi-Castillo 1945a). As early as 1911, Dr. Meitzner made recommendations to the railway engineers to construct drainage ditches along the sides of the tracks to prevent the establishment of further larval habitats in the pools that formed there (Meitzner 1938). Despite his efforts, *An. pseudopunctipennis* remained in highland valleys of Chimborazo along the the railway at least into the mid-1940s. In 1943, Levi-Castillo collected *An. pseudopunctipennis* larvae along the railway tracks up to an altitude of 1250 m, and in 1944, he collected larvae in pools associated with rivers in the towns of Huigra and Sibambe, Chimborazo (Levi-Castillo 1945a). These entomological collections are consistent with the epidemiology of continued malaria transmission; in 1944, 154 (17.8%) of cases of malaria were documented in Huigra (Montalvan 1948). Aside from habitats associated with the railway line itself, as seen in a photograph circa

1940s (Figure 2-1), a partial blockage of the river below the newly-built Devil's Nose tracks would likely have also produced suitable pools for *An. pseudopunctipennis* larval habitat.

The train itself was likely a carrier for the continued introduction of anopheline mosquitoes to highland regions from the coast (Levi-Castillo 1945a, Levi-Castillo 1944a). At Milagro station, adult mosquitoes were observed to fill the train cars bound for higher altitudes (Levi-Castillo 1947). As well, Levi-Castillo documented the presence of pools of water in the ceiling portion of the train that served as mobile larval habitats for anopheline mosquitoes (Levi-Castillo 1945a). At higher altitudes (>1100 m), *An. pseudopunctipennis* was documented as the sole highland vector although *Anopheles albimanus* Wiedemann larvae were collected at lower altitudes along the path of the railway (Montalvan 1948). It is likely that the train continued to provide a mechanism for the introduction of anopheline mosquitoes to highland regions until the 1960s and 1970s, when the railway fell into disuse (Vásconez & Bravo Silva 1992).

2.5 Malaria foci in Highland Ecuador (1900 - 1950)

Malaria in highland regions of the northern Andes was not exclusive to Ecuador and was observed in Colombia, in the Cauca, Manizales/Cali, and Medellín valleys, as well as in Peru in the Rimac, Urubamba, and Laurin-Orcocota valleys (Levi-Castillo 1947). Within Ecuador, although malaria became more studied and likely more widespread during the 1940s, the vector *An. pseudopunctipennis* has been present for a much longer time period in highland regions prior to its study (Levi-Castillo 1945b). The malaria parasite (likely *Plasmodium vivax*) is believed to have been introduced to a handful of highland valleys in the 1800s, since there are no records of it before that time (Aguilar & Yepez 1994).

In 1905, students at the University of Guayaquil listed the following highland valleys as malaria-endemic in Ecuador: in Imbabura, Chota and Pinampiro valleys, in Pichincha, Tumbaco and Guayllabamba valleys, in Tungurahua, the Patate valley, and in Azuay, the Yungilla valley (Huerta 1905). All of these except Patate were regarded as malaria-endemic into the 1940s (Levi-Castillo 1944b). In almost every highland valley, *P. vivax* was implicated as the only malaria parasite, transmitted by *An. pseudopunctipennis* (Levi-Castillo 1944b; Astudillo 1971).

Highland malaria was very widespread in the early 1940s, when it seemed to reach its most widespread distribution (Levi-Castillo 1944b). In addition to the valleys listed above and the highland valleys in Chimborazo associated with the railway, malaria transmission was observed in Imbabura (Mira valley and Salinas), Pichincha (widespread in all highland valleys), Cañar (all valleys <2500 m), Chimborazo (Pallatanga valley), Azuay (Yungilla valley) and Loja (Catamayo valley) (Levi-Castillo 1944b; Astudillo 1971).

In 1938, Hanson and Montalvan documented a new epidemic of malaria (of *P. vivax* and *Plasmodium falciparum*) in Balzapamba, Azuay (pop. 700), in an orange-growing region at an elevation of 650 m (Hanson & Montalvan 1938). The residents had reportedly never experienced malaria until 1935, although they lived in very close proximity to the malaria-endemic coastal plain (ca. 10 km away). In that year, an earthquake and associated landslides diverted the course of the main river and an open canal was constructed to provide the town with drinking water (Hanson & Montalvan 1938). Throughout their search, the authors were only able to locate *An. pseudopunctipennis* larvae in the open canal and also in the algae-covered pools that were formed on the edges of the new river (Hanson & Montalvan 1938). This epidemic highlights the scarcity of available larval habitat in steep topographic regions and the probable role of river pools and man-made canals as habitat for anopheline larvae in highland regions.

Although *An. albimanus* has traditionally been considered a low-altitude (<300 m) vector, it was identified as the main malaria vector in an epidemic in Yungilla valley (ca. 1500

m) in the late 1940s (Levi-Castillo 1945a, Montalvan 1948). *An. pseudopunctipennis* was collected from higher-altitude towns such as Santa Isabel in the same valley during the 1940s (Montalvan 1948). DDT was just beginning to be used at that time in Ecuador, and was successfully applied in the 1940s to the region of Yungilla valley to control the malaria epidemic (Levi-Castillo 1944a). During that time, 5% DDT in a solution of kerosene was applied to the inside of homes as well as to larval habitats (Levi-Castillo 1949).

Very little research was conducted in the south of Ecuador, (e.g. Loja province), although there were confirmed malaria cases in the valley of Catamayo (ca. 1150 m) (Montalvan 1948). Montalvan believed that the main vector in Catamayo might be *Anopheles punctimacula* Dyar, which was otherwise not implicated in highland malaria transmission during the 1940s in Ecuador (Montalvan 1948). Similarly, there were few reports of malaria in highland parts of the Amazonian side of the Andes during the early twentieth century, likely because the region was sparsely settled. Even in lower-altitude Ecuadorian Amazon communities such as Puyo and Napo Settlement (presently Puerto Napo) (700 - 900 m), the residents reported no cases of malaria (Sinclair & Wasson 1923).

2.6 Highland malaria in northern valleys (Pichincha, Imbabura) (1940 - 1950)

Highland malaria in the northern valleys of Ecuador was very well documented during its most widespread period (1940 - 1950) (Levi-Castillo 1944b; Montalvan 1948). Malaria was reported from valleys in Imbabura and Pichincha provinces, although it never reached the city of Quito itself (2800 m) (Levi-Castillo 1944b). Based on the list of valleys affected and maximum altitudes recorded for anopheline species, the probable extent of highland malaria in the northern valleys during its peak is provided in Figure 2-2.

Prior to the widespread occurrence of malaria in the 1940s, Guayllabamba, Pichincha was considered an historically endemic area for malaria (Levi-Castillo 1945b; Montalvan 1948, Aguilar & Yepez 1994). Gradually, the vector and/or parasite spread to other valleys, reaching Tingo and Alangasi by 1917 (Levi-Castillo 1945b). The spread of malaria may have been in part due to an exodus of citizens from Guayllabamba during the maximum infection period of the illness (Gómez de la Torre Serrano et al. 1991). When malaria became more widespread in the 1940s, the Ecuadorian government brought in the U.S. malariologist Henry Hanson, who identified *An. pseudopunctipennis* as the main vector (Gómez de la Torre Serrano et al. 1991). The maximum altitude of the species was estimated to be 2500 - 2700 m (although it has since been observed at 3200 m in other countries) (Levi-Castillo 1945b; Levi-Castillo 1949; Astudillo 1971). *Anopheles pseudopunctipennis* larvae were collected from clean, sunlit, rocky pools associated with rivers, springs of water, irrigation ditches, and even hoof prints from horses (Levi-Castillo 1945a; Levi-Castillo 1945b; Levi-Castillo 1947; Montalvan 1948). Entomologists also noted a strong association with spirogyra algae (Levi-Castillo 1945b; Montalvan 1948; Levi-Castillo 1949).

Although the expansion of anopheline distributions is often attributed to land use changes, the highland valleys of northern Ecuador were cleared and continuously farmed since pre-Columbian times (Sarmiento 2002). As well, their strong association with river edges (i.e., not an anthropogenic habitat) makes land use change unlikely to be the sole explanation (Levi-Castillo 1945a; Montalvan 1948). The spread of the parasite and vector might also be attributed to meteorological causes, especially an increase in minimum temperatures that might otherwise prohibit the parasite's or vector's development. Indeed, a warming of 0.5°C in average daily temperature and, perhaps more importantly, an increase of 1.3°C in minimum nightly temperature was observed in Quito between 1900 and 1930 (Figure 2-3). Therefore, it is not possible to rule out climate causes for the increased highland malaria distribution before 1940.

A widespread campaign was formed in 1940 to reduced malaria rates in the highland valleys of Pichincha and Imbabura (Gómez de la Torre Serrano et al. 1991). Malaria was successfully eliminated in the Los Chillos valley by the Servicio Antipalúdico del Valle de los Chillos, led by Dr. Jaime Rivadeneira, and assisted by Carlos A. Marin and Benjamin Wandemberg (Levi-Castillo 1944b; Levi-Castillo 1945a; Levi-Castillo 1945b; Astudillo 1971). A field laboratory was established in San Pedro de Tingo, to lead an initial systematic larval habitat inspection of the entire valley (Levi-Castillo 1945b). Beginning at the highest altitudes toward the valley bottoms, all pools of water used by anopheline larvae were drained by the construction of small canals and the spraying of crude oil mixed with kerosene and occasionally DDT (Levi-Castillo 1945a; Levi-Castillo 1945b; Gómez de la Torre Serrano et al. 1991). Smaller pools were filled with earth and residents were provided with chemical repellent for personal use (Levi-Castillo 1945a; Levi-Castillo 1947). The campaign was deemed a success and malaria did not return in the following years (Levi-Castillo 1945b).

2.7 Human colonization of coastal foothill tropical forests (1950 - 1970)

In a map of malaria incidence published in 1950, the highest incidence in the country lay in the northern coastal lowland region, vectored by *An. albimanus*, *An. pseudopunctipennis* and *An. punctimacula* (Mackie et al. 1950). The foothills of the northern coast were sparsely populated and land was not substantially developed prior to 1950, since the region was covered in dense tropical forest with very limited access (Burt et al. 1960). However, roads were built linking Quito to the coast in the late 1940s, and settlers moved into the region, forming Santo

Domingo de los Colorados (presently Santo Domingo de los Tsáchiles) (ca. 500 m) (Burt et al. 1960). Settlers converted wide swaths of forest to maize, rice, cocoa and coffee plantations for trade in Quito and in the port cities (Burt et al. 1960). Seasonal workers from highland regions migrated into the region to work during the summers (Burt et al. 1960). In 1958, the first major epidemic of malaria was reported in the region (Burt et al. 1960). It is likely that land conversion provided sunlit habitat that would have been suitable for *Anopheles* mosquito larvae. As well, the immigration of large groups of highlanders lacking immunity likely contributed to the 1958 and subsequent epidemics. Although Santo Domingo itself does not lie in a high-altitude area, the epidemic would have likely affected a few higher-altitude localities in the same valley, due to the migration of humans along trade roads to Quito.

2.8 History of malaria elimination/control efforts in Ecuador

Although there were several regional public health organizations tackling malaria on the coast of Ecuador, the National Institute of Hygiene and Tropical Medicine "Leopoldo Izquieta Perez" was formed in 1940 (Barragán et al. 2009). In 1944, Ecuador retained the largest available hospital facilities of any country of Latin America. Doctors were trained by the Pan American sanitary bureau with funding from the Rockefeller Foundation, and new public health laboratories were constructed (Perez 1944, Dunham 1944). In 1948, the "Servicio Nacional Antimalárico" was formed to campaign against malaria, especially on the coast, and to organize DDT spray operations twice a year (Aguilar & Yepez 1994). In the early 1950s, the U.S. organizations led efforts to "eradicate" malaria from endemic countries, although insecticide resistance was beginning to appear and slow the eradication progress (Russell 1957). By 1956, Ecuador was considered to be in an early attack phase of an eradication program (Russell 1957).

After a recommendation from the Pan American Sanitation Committee (part of PAHO), the Sistema Nacional de Eradicación de Malaria (SNEM) (presently "Servicio Nacional de Control de Enfermedades Transmitidas por Vectores Artrópodos") was founded on July 21, 1956 (Aguilar & Yepez 1994; Espinoza López et al. 2009). Its focus was on insect-borne diseases through vector control, mainly through the use of chemical insecticides, and also larval habitat elimination, public education such as school visits and interviews, and community participation (Espinoza López et al. 2009). Aside from malaria, the SNEM monitors and controls Chagas disease, dengue fever, onchocerciasis, yellow fever (in Amazonia), and leishmaniasis in Ecuador (Espinoza López et al. 2009).

The success of the SNEM in combating malaria has been generally associated with its variable levels of funding. From 1957-1959, dieldrin was sprayed inside houses on a continuous dosing schedule, but was continually under-dosed and therefore not effective (Stivers 1983). Between 1961-1965, DDT was applied to houses under the direction of U.S. AID and the Pan American Health Organization with greater success (Stivers 1983). Funding for medical entomology research was so poor and unreliable that Dr. Roberto Levi-Castillo, who had previously documented many cases of highland malaria, renounced his post at the University of Guayaquil and burned his books in protest in 1962 (Barragán et al. 2009). By the late 1960s, U.S. AID funding had dropped substantially, resulting in a subsequent epidemic (Stivers 1983). The provinces of Azuay and Cañar, and to a lesser extent Pichincha and Chimborazo, observed a small increase of malaria cases in their low-lying valleys (Figure 2-4). Similarly, in 1969, during a peak year, these highland provinces, as well as most areas of the coast of Ecuador were affected by malaria (Figure 2-5).

U.S. AID funding was reinstated in 1973 through a reinvestment with the SNEM, until 1979, when funding deteriorated once more (Aguilar & Yepez 1994; Stivers 1983). By 1980, the SNEM was considered operational and no longer relied on international funding (Stivers 1983). During an assessment in 1983, the SNEM was deemed to be a capable department but with some financial concerns, such as having an aging fleet of boats and jeeps, and a residual house spray schedule of three times a year rather than the recommended four times (Stivers 1983).

Through the latter half of the 1980s, some malaria cases were reported from the highland provinces of Pichincha, Cotopaxi, Bolívar, and to a lesser extent, Chimborazo and Loja (Figure 2-4). The provinces of Tungurahua, Carchi and Imbabura only saw occasional malaria cases (Figure 2-4). During one of the peak years in the 1990s, there was widespread malaria in the coast and Amazonia, as well as in highland provinces (although perhaps only in lowland regions of these provinces) (Figures 2-4, 2-5). Again, in 2000, widespread malaria was observed in the coastal and Amazonian parts of Ecuador (Figure 2-5), but only Pichincha and, to a lesser extent Cotopaxi and Bolívar observed an increase in cases in the highlands (Figures 2-4, 2-5, 2-6).

The SNEM has been a successful organization in preventing malaria epidemics. Between 1957 - 1991, the SNEM has been credited for spraying chemical insecticides in 11.7 million houses within malaria-endemic areas, examining more than 16 million blood smears for parasites, and, through its efforts has substantially reduced the number of cases per year (Espinoza López et al. 2009). Similarly, a decreasing trend in malaria rates has been observed in highland regions as well, with fewer cases of both *P. vivax* and *P. falciparum* recorded in highland cantons during the last five-year period than in the previous 10 years (Figure 2-6). Still, the steep-topography regions of Santo Domingo and the northern border region with Colombia remain endemic foci of malaria to present day (Figure 2-6).

2.9 General conclusions

Historically, highland malaria in Ecuador was certainly present prior to 1900 although it was not well documented (Huerta 1905). In Chimborazo, between 1900-1950, malaria was spread through lowland-highland valleys along the rail line linking Guayaquil and Quito, transmitted by the vector *An. pseudopunctipennis* (Meitzner 1938; Levi-Castillo 1945a; Montalvan 1948). Interestingly, *An. pseudopunctipennis* is still present in some parts of these valleys to the present day (Chapter Three). With renewed plans to reopen the entire length of this historical railway for tourism, it would be important for managers to maintain the tracks for standing water prevention.

Even though malaria was likely present in the northern highland valleys such as Guayllabamba prior to 1900, malaria certainly became more widespread in these valleys during the first thirty years of the century, but was eliminated from these regions in the 1940s through habitat removal and chemical insecticide use (Levi-Castillo 1945b). Although there have possibly been a few highland epidemics since that time, there are no specific regional records documenting them (Gómez de la Torre Serrano et al. 1991). However, Gómez de la Torrei Serrano et al. (1991) documented the presence of *An. pseudopunctipennis* in river-associated habitats of Guayllabamba, although the same larvae were not found in more recent collection attempts (Gómez de la Torre Serrano et al. 1991; Chapter Three). Ongoing monitoring of highland regions is therefore crucial to identify larvae in these highland regions, especially targeting highland valleys that have historically been suitable for both anopheline larvae and the malaria parasite. Many of the formerly malaria-endemic valleys near Quito are now heavily populated, which may create local heat islands and suitable microclimates for mosquito adult and larval establishment (e.g., Patz et al. 2005).

In the northern foothills near Santo Domingo, human colonization and subsequent land use alteration by non-immune settlers in the 1950s likely played a large role in promoting localized malaria epidemics (Burt et al. 1960). These foothill regions continue to the present day to be foci of malaria transmission, often extending into hilly valleys and higher-altitude regions (Figure 2-6). Anopheline habitats in these steep-topography areas are expected to differ from those utilized in flat, low-altitude regions, therefore necessitating further study of the local dynamics of mosquito ecology and transmission cycles. Therefore, targeting these scattered larval habitats for source reduction may be an effective tool in steep regions to reduce the local population of common mosquito vectors.

Figure 2-1 Photograph of railway construction at base of the "Devil's Nose" switchbacks, showing the railway on the left and the stone-lined riverbed on the right, where several pools are observed (likely formed by falling rocks due to construction) that would likely have provided suitable habitat for *Anopheles pseudopunctipennis* larvae. Photo: Historical Archive of Banco Central, also in García Idrovo 2008.

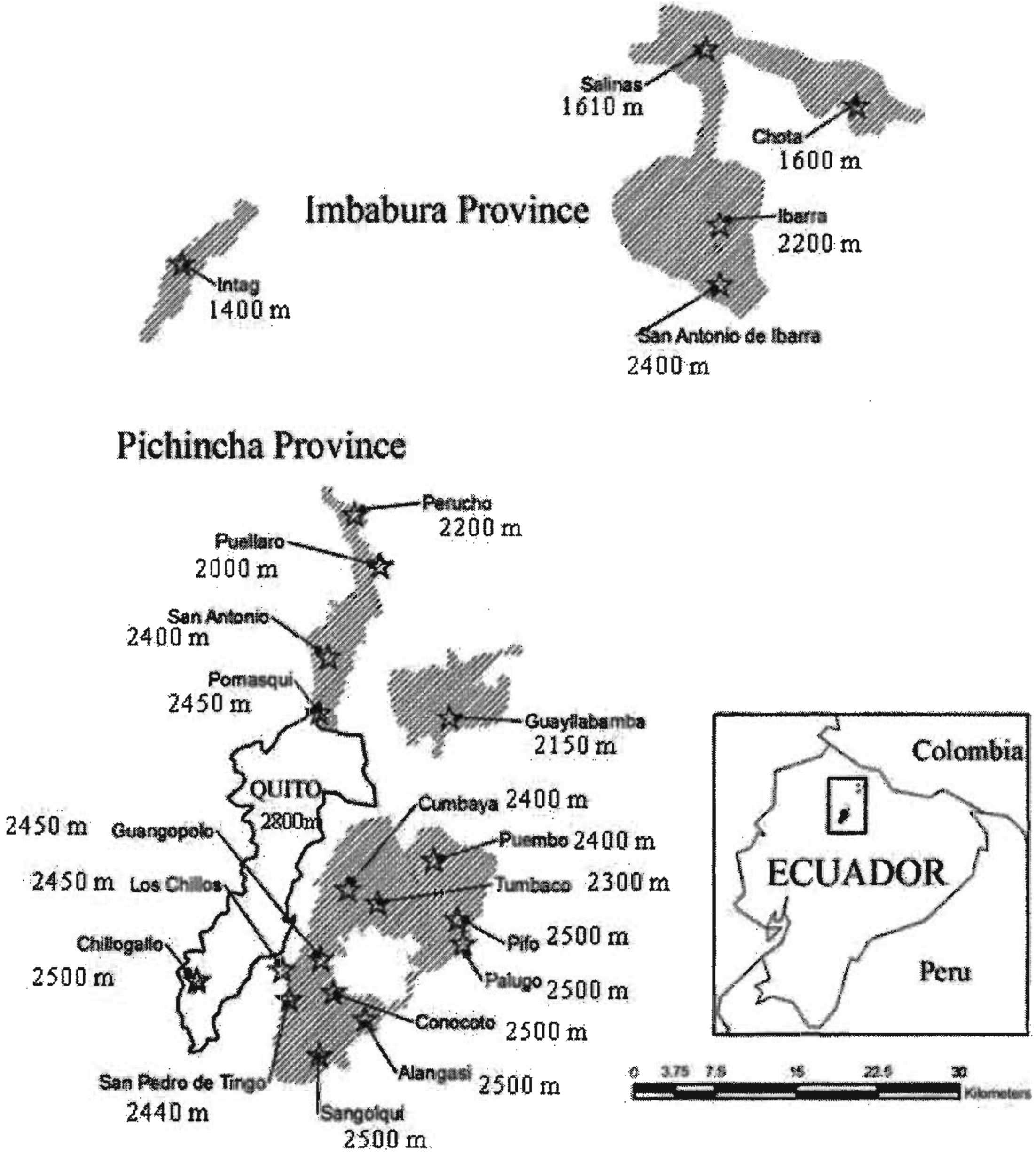


Figure 2-2 Probable extent of highland valley malaria incidence (hatched lines) during the early 1940s in northern valleys in Ecuador. Stars indicate approximate placement of original towns listed as endemic for malaria, judged by the presence of historical town plaza in Google Earth satellite imagery (Google 2010). Hatched extent determined by the valley bottom with an affected town up to an altitude of 2500m. Inset: Approximate location of affected region within Ecuador. (Data: Levi-Castillo 1945a; Levi-Castillo 1945b; Montalvan 1948)

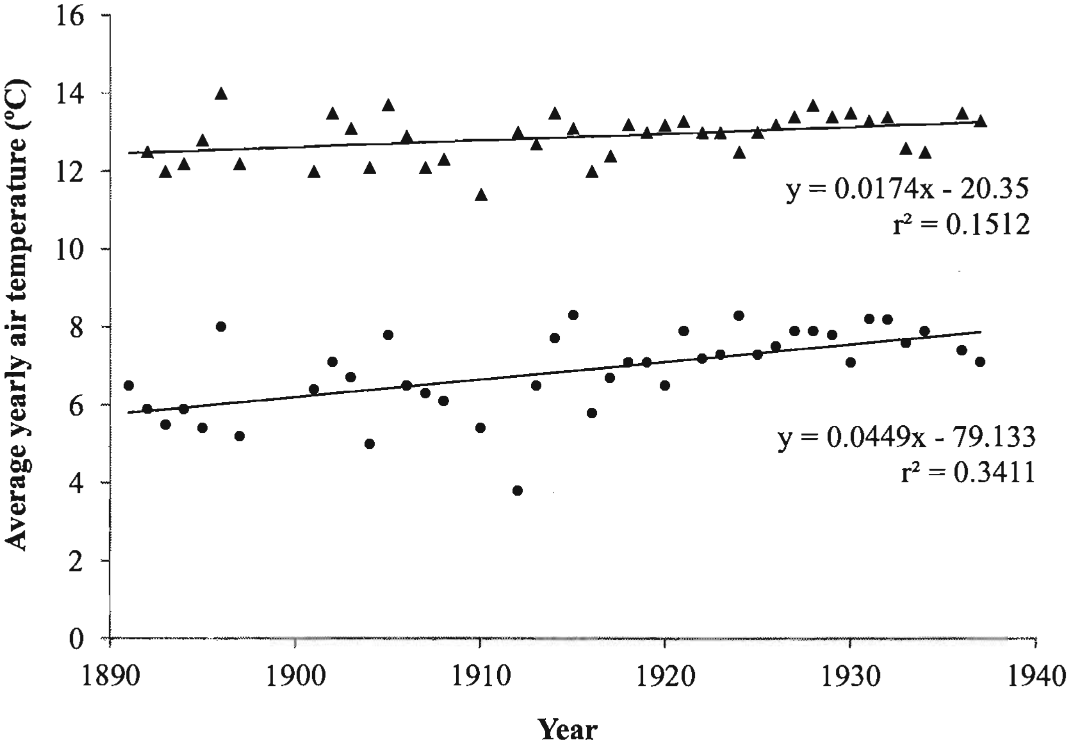


Figure 2-3 Increase in yearly average and minimum temperatures between 1891 and 1937 in Quito, Ecuador. Average 24 hour period temperatures (▲) and average minimum nightly temperatures (●) are indicated, leading up to the years of observation of highland malaria in valleys surrounding Quito. Although average temperature only increased at a rate of 0.017°C a year, minimum nightly temperature, which may be more important for the survival of *Anopheles* species, increased at a rate of 0.045°C a year. (Data: Anon. 1927-1937)

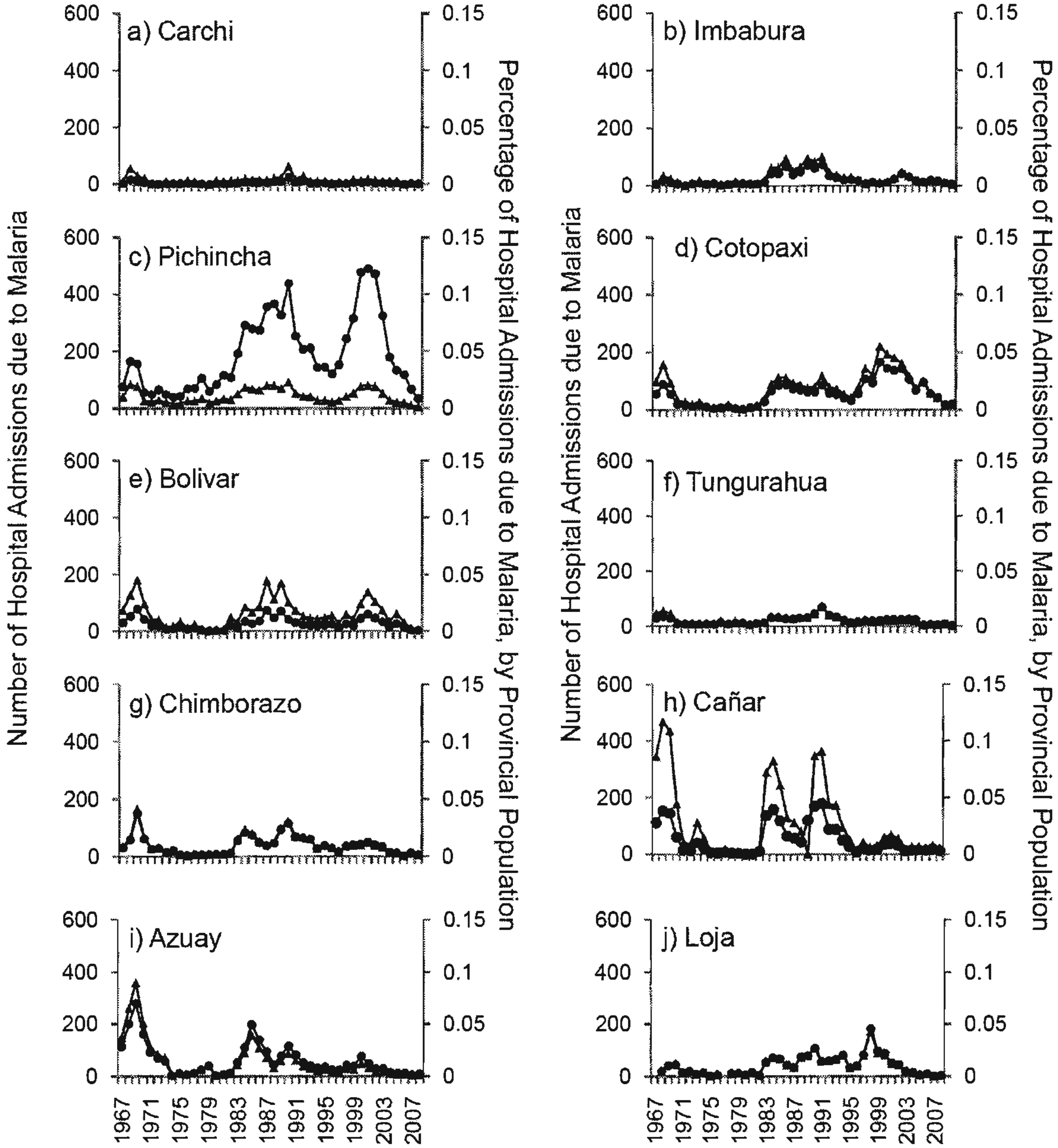


Figure 2-4. Number of hospital admissions due to malaria per year for each province in the highland region of Ecuador: total number of admissions (left axis, ▲) and total number of cases per capita (right axis, •). (Data: INEC 1967-2008; INEC 1960, 2000, 2010).

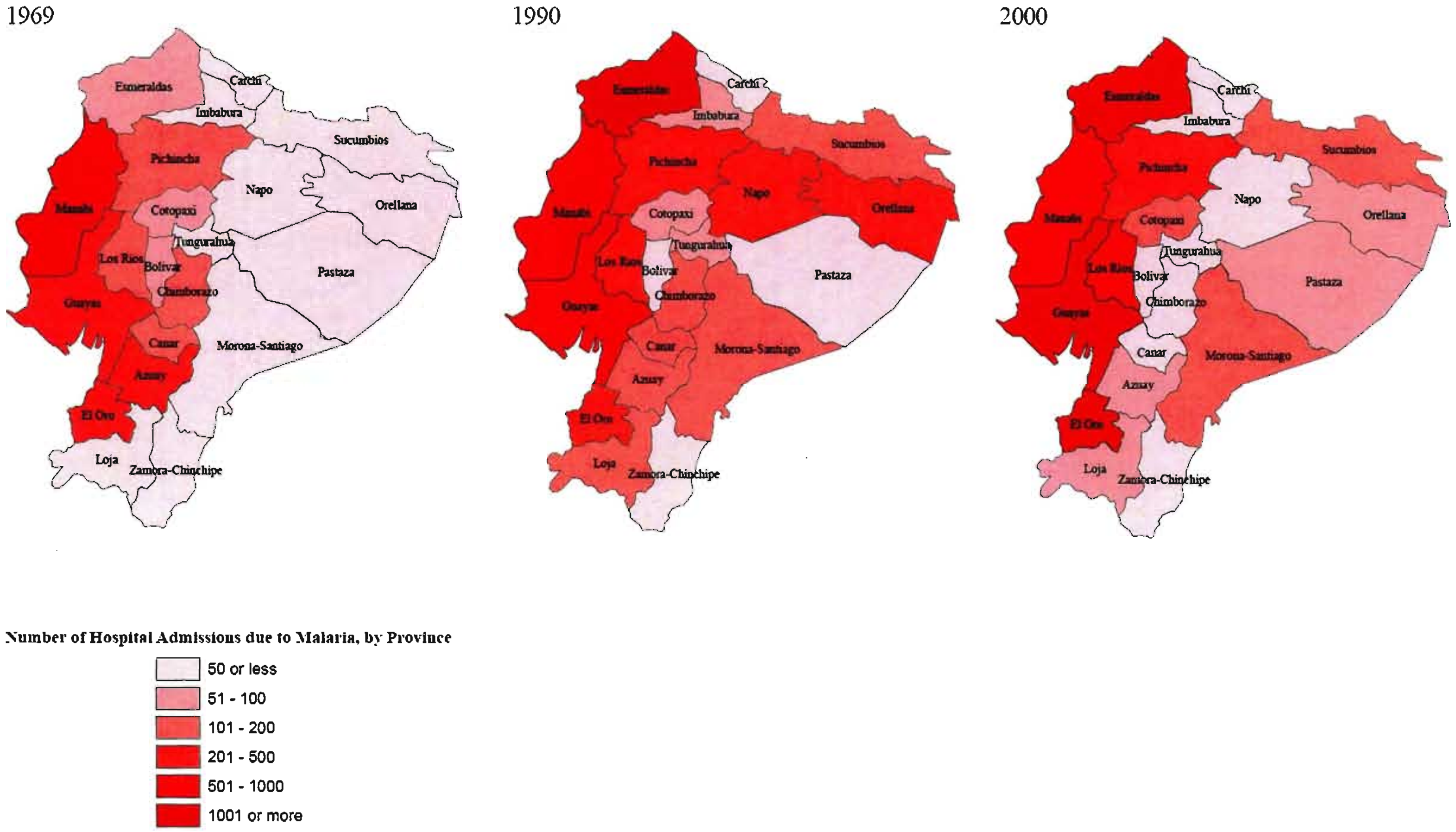
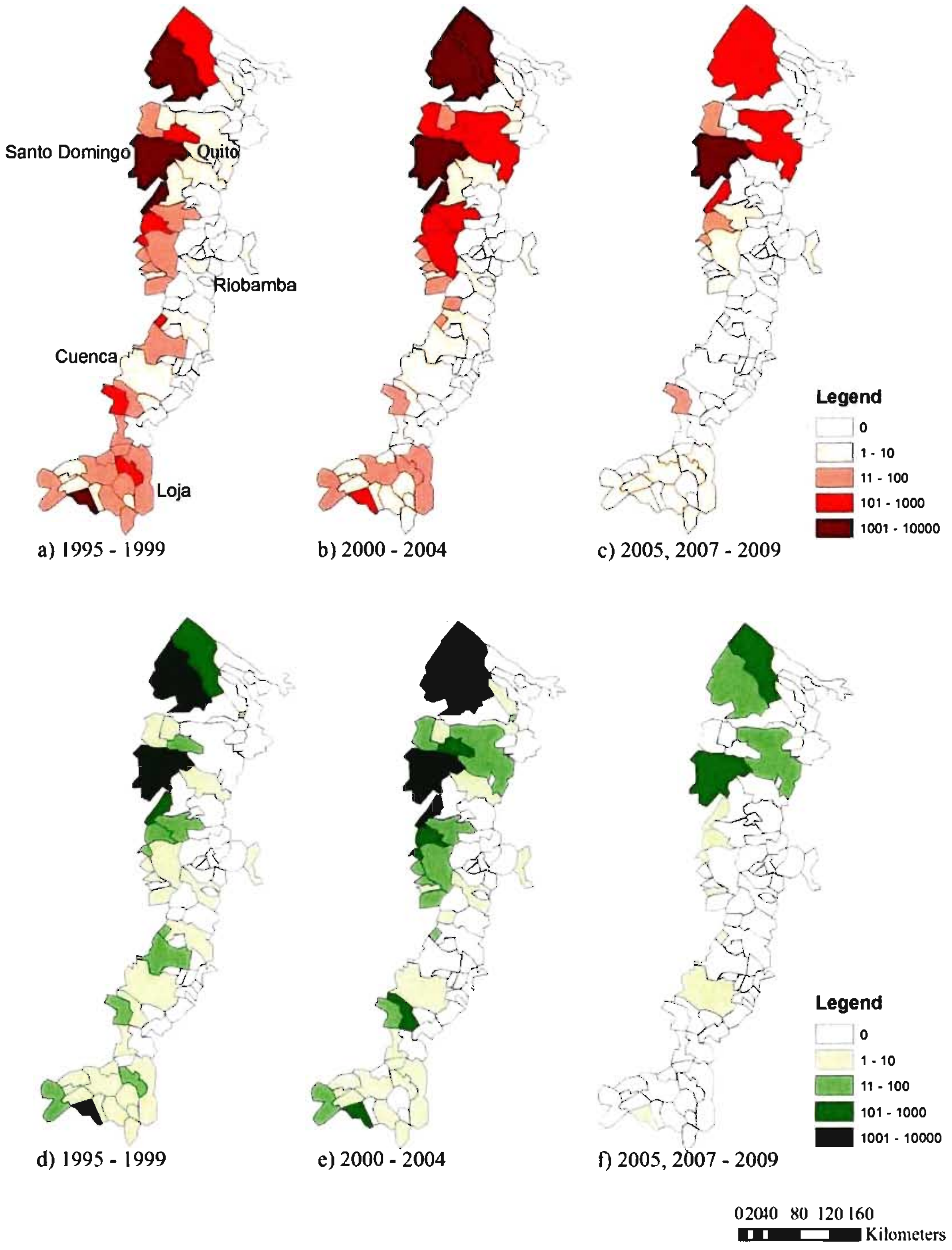


Figure 2-5. Geographic representation of the number of hospital admissions due to malaria in each province of Ecuador (red shading), in the peak malaria years of 1969, 1990 and 2000. Data: INEC (1967-2008).



a) 1995 - 1999 b) 2000 - 2004 c) 2005, 2007 - 2009

d) 1995 - 1999 e) 2000 - 2004 f) 2005, 2007 - 2009

Figure 2-6 Total number of positive malaria tests by the SNEM in highlands of Ecuador during the last ca. 15 years in Ecuador. Cases (per highland canton) attributed to *Plasmodium vivax* between a) 1995-1999, b) 2000-2004, c) 2005, 2007-2009 and *Plasmodium falciparum* between d) 1995-1999, e) 2000-2004, and f) 2005, 2007-2009 are indicated. (Data: SNEM 1995-2009).

Chapter Three

New highland distribution records of multiple *Anopheles* species in the Ecuadorian Andes

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3.1 Introduction

Recently, there has been growing global concern over the shifting distribution of malaria vectors due to land use alteration, changes to vector control measures, insecticide resistance, malaria treatment resistance, as well as local climate change (Mouchet et al. 1998; Lindsay & Martens 1998; Reiter 2001; Zell 2004; Patz et al. 2005; Afrane et al. 2006; Minakawa et al. 2006; Moreno 2006). Many reviews have discussed the hypothetical effects of climate change on insect-borne diseases, and some of these have stated the concern that malaria might be able to return to higher altitudes in the Andes, potentially affecting large cities such as Quito (2800 m) (Shope 1991; Martens et al. 1995; Epstein et al. 1998; Moreno 2006). Already, high-altitude malaria transmission has been recorded in a town in Bolivia at 2300 m, vectored by *Anopheles pseudopunctipennis* (Rutar et al. 2004). However, the effects of climate change, such as rainfall, have only been weakly associated with cases of highland malaria (Loevinsohn 1994; Lindblade et al. 1999), while land use alteration, changes to vector control programs and drug resistance have been more powerful drivers of highland malaria in African countries such as Kenya, Uganda, Tanzania, Madagascar, and Rwanda (Mouchet et al. 1998; Lindsay & Martens 1998;

Reiter 2001; Hay et al. 2002a; Zell 2004; Patz et al. 2005; Afrane et al. 2006; Minakawa et al. 2006).

The resurgence of anopheline vectors in highland regions underlines the importance of studies that determine the distribution of *Anopheles* species in highland areas. The purpose of this Chapter is to identify the extent of previously lowland-restricted malaria vectors within highland regions of the Andes and to update distribution maps of the most common *Anopheles* species in Ecuador. These maps will identify highland areas, as well as low-altitude areas where *Anopheles* were recently collected, and thereby provide valuable data for malaria control in the country. Although other anopheline species have been reported in Ecuador (Levi-Castillo 1945a; Gorham et al. 1973), only five common species were collected during the three years of intensive field work: *Anopheles albimanus* Wiedemann, *An. pseudopunctipennis*, *Anopheles punctimacula* Dyar, *Anopheles eiseni* Coquillett and *Anopheles oswaldoi s.l.* Perryassú. All five species are also found in other countries in Central and South America (e,g, Rubio-Palis & Zimmerman 1997; Lounibos & Conn 2000; Marrelli et al. 2005).

Anopheles albimanus has been considered a low altitude (<500 m) species that is believed to have become more abundant through the irrigation of low-lying areas (Rubio-Palis & Zimmerman 1997). Although there is one historical, anecdotal record of *An. albimanus* transmitting malaria at 1800 m in southern Ecuador in the 1940s, the species has otherwise been considered an exclusively lowland species (Breeland 1972). Currently, it is the main malaria vector on the coast of Mexico and Belize (Breeland 1972, Rejmankova et al. 1993; Rodriguez et al. 1993; Rodriguez et al. 2000). *Anopheles albimanus* is distributed across the northern and Pacific coasts of Colombia (Quiñones et al. 1987; Narang et al. 1991; Marten et al. 1996; Poveda et al. 2001; Gutiérrez et al. 2009) and Peru (Morales-Ayala 1971; Guthmann et al. 2002).

Historically (during the 1940s), *An. albimanus* has been found as far north as Texas and Florida, USA (King 1937), and throughout Central America, where it was collected in one locality at 1000 m altitude (Kumm et al. 1940; Vargas et al. 1941; Kumm & Zúniga 1942; Meyer 1947). It also was a main vector in Cuba and the West Indies (Rozeboom 1941), and was collected in South America along the northern Pacific coast, especially in the area surrounding Guayaquil, Ecuador (Levi-Castillo 1945a; Meyer 1947). *Anopheles albimanus* is not currently considered a species complex although there is some genetic variation among distinct populations from Pleistocene geographic fragmentation, even within Colombia (between the Caribbean and Pacific coasts) and Panama (between eastern and western populations) (Narang et al. 1991; Gutiérrez et al. 2009; Loaiza et al. 2010a; Loaiza et al. 2010b). Further studies are required to determine if internal transcribed spacer 2 sequences from Central America provide evidence of rare cryptic species (Loaiza et al. 2010b).

Anopheles pseudopunctipennis has historically been incriminated as the primary and often only malaria vector in highland Andean and Mexican valleys (Vargas et al. 1941; Levi-Castillo 1944b; Levi-Castillo 1945b; Montalvan 1948). In the late 1940s, Levi-Castillo (1945b) documented the mosquito control efforts against *An. pseudopunctipennis* in highland regions near Quito. Since then, *An. pseudopunctipennis* has been documented as a coastal and also piedmont vector that is usually found up to 1500 m in altitude (Rubio-Palis & Zimmerman 1997). Recently, *An. pseudopunctipennis* has been documented at extremely high altitudes (up to 2800 m) in Bolivia (Rutar et al. 2004; Lardeux et al. 2007). Along with *An. albimanus*, *An. pseudopunctipennis* is one of the main vectors in the southern USA (Rueda et al. 2004), in the foothills of Mexico (Rodriguez et al. 2000), and is also a main vector across Central America (Rejmankova et al. 1993; Rueda et al. 2004), western South America, Trinidad and Tobago, Haiti and other Caribbean islands (Rueda et al. 2004) When the population structure is examined across its distribution, *An. pseudopunctipennis* is a group of two sibling species with the greatest genetic differences being between the Central and South American populations (Estrada-Franco et al. 1993; Manguin et al. 1995).

Anopheles punctimacula is considered a possible secondary malaria vector along the coast of northern South America into Bolivia (Morales-Ayala 1971; Rubio-Palis & Zimmerman 1997; Lardeux et al. 2009). It was collected in the 1940s on the coast of Peru (Villalobos & Valderrama Delgado 1944).

Anopheles eiseni was historically distributed across much of South and Central America, northward to southern Mexico (Kumm et al. 1940; Rozeboom 1941; Vargas et al. 1941; Kumm & Zúniga 1942), as well as in Pará, Brazil (Kumm & Novis 1938). It is currently reported from Amazonian Brazil (Marrelli et al. 2005), Bolivia (Lardeux et al. 2009) and Central America (Wilkerson & Strickman 1990).

Anopheles oswaldoi s.l. is an important malaria vector and species complex in the Peruvian, Colombian and Brazilian Amazon, where it often co-occurs with *An. darlingi* Root (Morales-Ayala 1971; Lounibos & Conn 2000; Schoeler et al. 2003; Monteiro de Barros et al. 2007; Rodriguez et al. 2009), as well as occurring north into Panama (Loaiza et al. 2008). Genetically, *An. oswaldoi s.l.* does not differ substantially among the northern parts of the Amazon, including Colombia, Venezuela and northern Brazil, although it has been reported to be a species complex over the larger extent of its overall distribution (Quiñones et al. 2006). Historically, *An. oswaldoi* has been collected in Panama, Trinidad and Costa Rica (Rozeboom 1941).

This Chapter outlines current distributions for the five most frequently collected species of *Anopheles* in Ecuador. For all species except *An. eiseni*, Getis-Ord Hotspot Analysis (Getis & Ord 1992) was conducted to determine clusters of high and low-density larval populations. Additionally, to place the species distributions in an historical context (when highland malaria was last widespread in Ecuador), we compare current distributions to maps adapted from those compiled by Levi-Castillo (1945a) and Montalvan (1948).

3.2 Materials and methods

Extensive field collections of *Anopheles* larvae were made in 2008, 2009 and 2010 throughout Ecuador (Figure 3-1). A strong effort was made to collect *Anopheles* larvae from sites at higher altitudes (i.e. >500 m) than normally would be considered. Larvae were collected in 438 potential habitats that were encountered in all road-accessible regions of the country, except in the lowland Amazonian basin where road access was poor (Figure 3-1). Potential habitats were defined as any water source where the surface of the water was not moving, or moving very slowly (e.g., pond, river edge with algae). In each habitat, researchers sampled larvae using a 13 cm-diameter plastic dipping cup by skimming the surface of the water a standardized 30 times, extracting *Anopheles* larvae with a plastic pipette, and placing the larvae in 95% ethanol for transportation to the laboratory. In each habitat, latitude, longitude and altitude were recorded with a GPS unit (Garmin GPSmap76).

Large intact *Anopheles* larvae were identified using the morphology-based key of Gorham et al. (1973) supplemented with the description of *Anopheles trinkae* in Faran (1979). To confirm the morphological identifications of large, damaged larvae and small larvae too difficult to identify, a small (800 bp) region of the cytochrome oxidase 1 (CO1) gene was sequenced using the following procedure, and compared the sequences to those of easily identifiable large, intact larvae.

Larval bodies were ground with a small pestle in Lysis C solution and DNA was eluted into 30 μL elution buffer, using the GeneElute TM Mammalian Genomic DNA Miniprep Kit (Sigma-Aldrich). Primers for the CO1 800 bp region were C1-J-2195 (F) and UEA (R) (Simon et al. 1994; Lunt et al. 1996). The PCR reaction mixture was composed of: 2.3 μL of 10x tris-borate EDTA (TBE) buffer, 1.3 μL x 50 mM $MgSO_4$, 0.25 μL x 10 μmol F and R primers, 0.3 μL x 10 mM DNTPs, and 0.3μL x 5000 U/ml Taq DNA Polymerase with ThermoPol II (Mg-free), and was run with 5.0 μL DNA extraction mixture. The PCR ran for 30 cycles for 2.5 minutes at 95°C, 45 seconds at 51°C, 1 minute at 72°C, and a final cycle of 10 minutes at 72°C. DNA that had been degraded by field conditions (as determined by previously weak PCR results), was run for 40 cycles of the same regime.

PCR products were sent for sequencing at Genome Quebec, McGill University and aligned after trimming in ClustalW (European Bioinformatics Research Institute 2010). Consensus sequences for each species were determined from five larvae that were easily identified morphologically and provided high-quality sequences for comparison with unidentifiable larvae. Aligned sequences among specimens were matched at a rate of 97-100% within species and 84-90% among species, except for very low-quality sequences. In these cases, a positive match for the same species was approximately 10% greater than among species. Consensus sequences have been submitted to GenBank (accession numbers JN412826-JN412843) and are provided in Appendix 1. All specimens not destroyed for molecular work were deposited in the Ecuadorian National Insect Collection at the Pontificía Universidad Católica del Ecuador (PUCE) in 2009 and 2010.

All distribution maps were made using ArcGIS v. 9.3 software (ESRI 2009). The background layer used for all maps is the ESRI World Terrain Base (2009). For the current collection data, density (number of larvae per 30 dips) is indicated on the maps by different sizes of map points. For each species' current distribution, Getis-Ord Hot Spot Analysis (Gi*) (Gertis & Ord 1992) was conducted using larval density as the weighting factor. Getis-Ord analysis identifies clusters of high and low relative density larval-present sites by comparing the local value of larval density (as a weighting factor) to other sites nearby in georeferenced space. Significant hot spots and cold spots (high and low-density clusters of larval populations) are indicated in each distribution map. Hot spots may indicate proximity to other sites, including those of higher relative density (ca. >50 larvae per 30 dips), while cold spots indicate proximity to multiple low-density sites (ca. <50 larvae per 30 dips). Historical collection data for four of the species were adapted from hand-drawn maps by Levi-Castillo (1945a) and Montalvan (1948), by using the original town square in the city names when provided, or by using rivers and landmarks in the original maps to determine approximate coordinates in Google Earth (Google 2008).

3.3 Results

Current distribution maps for all five species are presented in Figures 3-2 to 3-6. *Anopheles albimanus* larvae were collected in three highland localities in Ecuador: in the northern Mira River valley, Imbabura Province (767 and 832 m) near La Hesperia Biological Station, Pichincha Province (1366 m) and in the south of the country, near Girón, Azuay Province (1541 m) (Figure 3-2, Table 3-1). *Anopheles albimanus* was collected predominantly in the coastal region of Ecuador, most notably along the northern coast proper, and in the vast rice-growing region northeast of Guayaquil (Figure 3-2). At all latitudes, *An. albimanus* was also found in foothill regions, up to an altitude of 283 m (Figure 3-2). Gi* Hot Spot Analysis identified five significant hot spots for *An. albimanus* larvae (i.e., sites among clusters of other high-density sites): two in the Mira valley, Imbabura Province (Gi* = 4.37; $p < 0.001$), one in Puerto Quito, Pichincha Province (Gi* = 2.16; $p = 0.03$), one high-altitude site near La Hesperia Biological Station, Pichincha Province (Gi* = 2.56; $p = 0.01$) and one near Santo Domingo, in Santo Domingo de los Tsáchiles Province (Gi* = 3.056; $p = 0.002$) (Figure 3-7a). The analysis also identified 15 cold spots (i.e., sites among clusters of other low-density sites), all located along the north-western coast (Figure 3-7a). In the 1940s, *An. albimanus* was ubiquitous on the coast of Ecuador, particularly in many areas surrounding Guayaquil (Figure 3-8a). However, it is important to note that in historical entomological collections, *An. albimanus* was not recorded at elevations higher than approximately 500 m (Figure 3-8a).

Anopheles pseudopunctipennis larvae were most common along the highway linking El Triunfo, Guayas Province, to Alausí, Chimborazo Province, up to a maximum altitude of 1558 m, as well as the road linking Quevedo, Los Rios Province, to Pilaló, Cotopaxi Province, to a maximum altitude of 858 m (Figure 3-3; Table 3-1). Other high-altitude localities include the Mira River valley, Imbabura Province (1273 m), San Antonio, Bolívar Province (1321 m), Unión del Toachi, Pichincha Province (835 m), Santa Isabel, Azuay Province (1353 m) and Chilchil, Cañar Province (1930 m) (Figure 3-3, Table 3-1). *Anopheles pseudopunctipennis* larvae were collected in a few localities on the coastal plain of Ecuador, but the vast majority were collected in the lower parts of the Andes on the coastal side (Figure 3-3). Hot spot analysis did not identify any hot spots, but 23 significant cold spots (low-density clusters) were identified in the northern coastal region, with groups of these in the regions of Alluriquin, Pichincha Province, and La Maná, Cotopaxi Province (Figure 3-7b). Historical maps show that *An. pseudopunctipennis* was widespread in almost all coastal regions, as well as in highland areas, particularly in the south (Loja Province) and in the northern valleys surrounding Quito, Pichincha Province, and Ibarra, Imbabura Province (Figure 3-8b).

Anopheles punctimacula larvae were collected predominantly in the coastal-side foothills of the Andes within an approximate elevation range of 147 - 1300 m, although they were also collected in a few localities on the coast (Figure 3-4). The species was often collected in areas near Mindo, Pichincha Province (1105 - 1312 m), but was also collected in Mira valley, Imbabura Province (1234 m) and in a high-altitude site near Sibambe, Chimborazo Province (1906 m) (Figure 3-4; Table 3-1). No hot spots were identified in the Gi* analysis, although 19 cold spots were identified spread across most of *An. punctimacula*'s Ecuadorian distribution (Figure 3-7c). Interestingly, historical distributions of *An. punctimacula* place the species in four localities: three on the coast proper, and one on the coastal plain (Figure 3-8c).

Anopheles eiseni was collected at three high-altitude sites (1206 - 1873 m) on the coastal side of the Andes (Figure 3-5; Table 3-1). Gi* Analysis was not possible due to the small number of localities. Interestingly, historical collections place *An. eiseni* in one locality only (Figure 7d), very close to the current collection site near Tingo, Cotopaxi Province (Figure 3-5).

Anopheles oswaldoi s.l. was the only species collected on the Amazonian side of the mountains at altitudes greater than 416 m (Figure 3-6; Table 3-1). The lower-altitude Amazonian plain was excluded from our study since these areas were not road-accessible and were also beyond the scope of this study (i.e., highland focus). Two higher-altitude localities were recorded for this species: Mera, Pastaza Province (1233 m), and Río Verde, Tungurahua Province (1230 m) (Figure 3-6; Table 3-1). The species was abundant in areas surrounding Tena and Archidona, Napo Province, and Puyo, Pastaza Province, and was collected once in Zamora, Zamora-

Chinchipe Province (Figure 3-6; Table 3-1). Gi* Analysis did not identify any hot spots for *An. oswaldoi s.l.*, although 20 cold spots were identified in areas surrounding Tena, Napo Province and Puyo, Pastaza Province (Figure 3.7d). There are no historical records available for *An. oswaldoi s.l.*.

3.4 Discussion

Historically, *An. pseudopunctipennis* was abundant and widespread in highland areas, while *An. albimanus* and *An. punctimacula* were considered species generally restricted to lowland areas (<500 m) on the coast (Levi-Castillo 1945a; Montalvan 1948). It is clear from the maximum altitudes recorded for each of these species, as well as for *An. oswaldoi* and *An. eiseni*, that all five of the most common species of *Anopheles* are now residing in highland regions of the Ecuadorian Andes. Although no species on the coastal side was collected at altitudes exceeding 2000 m, only a small change in environmental conditions at higher altitudes may be required for species to move into highland valleys (2100 - 2400 m) if suitable habitat is present. *Anopheles pseudopunctipennis* is physiologically able to survive at these altitudes, since it existed in highland valleys during the 1940s (Levi-Castillo 1945a; Montalvan 1948) and recently has been reported at these altitudes in Bolivia (Rutar et al. 2004; Lardeux et al. 2007). Interestingly, *An. pseudopunctipennis* larvae were not observed in extremely high altitudes, as in Bolivia (2000 - 2800m) (Lardeux et al. 2007), likely due to a combination of different land use, topography, and microclimate in highland Ecuador.

Lacking proper data sets, it is difficult to know whether *An. pseudopunctipennis* have occupied highland regions continuously since the 1940s. Levi-Castillo (1945b) documents the successful elimination of *An. pseudopunctipennis* from highland valleys near Quito through the use of powerful chemical insecticides and habitat elimination, and anecdotal evidence suggests that the species has not been present since then. However, it is possible that very small, undetected populations of this species have remained in highland regions since that time, as is believed to be the case for *Anopheles gambiae* in Tanzania (Bødker et al. 2000)

As opposed to Balls et al.'s (2004) study in Tanzania, where steep topography limited available *An. gambiae* and *Anopheles funestus* larval habitat in highland regions, many suitable habitats are available in the Ecuadorian highlands: alongside roads, rivers and irrigation ditches. Although the amount of habitat available to mosquitoes is probably much less in steep areas, the habitat in highland regions is obviously sufficient to allow species to become established.

Hot spots and cold spots both indicate clusters of larval populations, and both are therefore important to identify foci of potential malaria transmission. Particularly troubling are the hot spots for *An. albimanus* in the region between Puerto Quito, Pichincha Province, and Santo Domingo, Santo Domingo de los Tsáchiles Province, where cases of malaria caused by both *Plasmodium vivax* Grassi & Feletti and *Plasmodium falciparum* Welch still occur (SNEM 1997-2008). Most low and moderate-elevation regions of Ecuador are affected by clusters of at least one species of *Anopheles*, which indicates that potential malaria vectors have already become established in many regions that have been previously considered completely malaria-free. As well, favourable climatic conditions during one year could easily cause low larval densities (cold spots) to increase in population size and become hot spots, potentially leading to malaria outbreaks.

The establishment of various *Anopheles* mosquito species in highland regions highlights the importance of awareness by decision-makers and the general population. Those involved in malaria control in Ecuador will need to begin to consider higher-altitude regions (i.e., 1000 - 2000 m) as potential regions of malaria transmission by multiple vectors and to monitor suspicious illness accordingly. Further studies are necessary to determine the exact nature of the highland *Anopheles* larval habitat and the extent to which these species might further establish themselves in even higher-altitude regions in the future.

3.5 Conclusions

Even though *An. pseudopunctipennis* has been historically incriminated as the only widespread malaria vector in highland regions of the Ecuadorian Andes during the 1940s, present collections indicate that the distributions of *An. albimanus*, *An. punctimacula* and *An. oswaldoi s.l.* are encroaching into higher-altitude regions, in some cases reaching higher maximum altitudes (1541 m, 1906 m, and 1230 m, respectively). For all species, larvae were collected in highland regions in multiple localities within Ecuador. The establishment of multiple malaria vectors in the Ecuadorian highlands will add a greater degree of complexity to the prevention and/or eradication of malaria in highland regions since each vector retains a unique ecological, behavioural, and vectorial role.

Table 3-1 Highland collection localities for *Anopheles* larvae in Ecuador during 2008, 2009 and 2010. Altitudes and habitat types of *An. albimanus* (ALB), *An. pseudopunctipennis* (PSE), *An. punctimacula* (PUN), *An. eiseni* (EIS), and *An. oswaldoi s.l.* (OSW) larvae collected in highland (steep topography, >500 m) regions of Ecuador are provided.

Province	Spp.	Locality	Latitude	Longitude	Alt. (m)	Habitat
Azuay	ALB	Near Girón	S03°15.06′	W79°14.23′	1541	Pond
	PSE	Near Santa Isabel	S03°15.67′	W79°16.41′	1353	River eddy
	PSE	Near El Inca	S02°37.54′	W79°27.74′	805	Pool
	PSE	San Antonio	S01°34.28′	W79°07.76′	1321	WFD[1]
	PSE	Near El Triunfo	S02°15.89′	W79°57.06′	592	Pond
	PSE	Chilchil	S02°26.68′	W79°06.54′	1930	River edge
Bolívar	PUN	Caluma	S01°34.88′	W79°13.18′	517	Pond
Chimborazo	PSE	Near Huigra	S02°19.00′	W79°04.55′	1206	River eddy
	PSE	Near Huigra	S02°18.84′	W78°59.06′	1389	WFD[1]
	PSE	Near Huigra	S02°19.48′	W79°00.49′	1426	Rock pool
	PSE	SW of Huigra	S02°18.89′	W78°59.87′	1387	WFD[1]
	PSE	NE of Huigra	S02°16.09′	W78°57.46′	1558	Road ditch
	PSE	NE of Huigra	S02°16.07′	W78°57.43′	1553	Road ditch
	PSE	Near Suropata	S02°08.55′	W79°02.59′	674	River
	PSE	West of Tingo	S00°52.60′	W79°05.20′	858	WFD[1]
	PSE	Near La Maná	S00°52.61′	W79°05.19′	851	River WFD[1]
	PSE	Near La Maná	S00°52.27′	W79°09.52′	751	River WFD[1]
	PUN	Near Sibambe	S02°14.65′	W78°57.22′	1906	River eddy
	EIS	Near Huigra	S02°19.04′	W79°04.55′	1206	Road ditch
Cotopaxi	EIS	Near Tingo	S00°54.41′	W79°03.51′	1423	Cement pool
El Oro	PUN	Near Uzhcurrum	S03°18.98′	W79°35.69′	539	Road ditch
Guayas	PUN	El Triunfo	S02°16.08′	W79°07.70′	531	River eddy

Province	Spp.	Locality	Latitude	Longitude	Alt. (m)	Habitat
Imbabura	ALB	Santa Rita	N00°48.71′	W78°23.36′	767	Tire tracks
	ALB	Near Lita	N00°48.60′	W78°19.39′	832	Ditch
	PSE	Mira River valley	N00°35.55′	W78°08.25′	1273	Road ditch
	PUN	Lita-Salinas hwy.	N00°43.32′	W78°13.92′	1234	WFD[1]
Loja	PUN	West of Chaguarpamba	S03°51.33′	W79°41.44′	680	River pool
Napo	OSW	Near Archidona	S00°55.45′	W77°44.32′	560	Pond
	OSW	Mondayacu	S00°48.47′	W77°46.64′	907	WFD[1]
	OSW	Mondayacu	S00°49.62′	W77°46.58′	837	Road ditch
	OSW	Cotundo valley	S00°50.00′	W77°47.05′	779	WFD[1]
	OSW	Near Misahuallí	S00°49.21′	W77°48.02′	835	Pond
	OSW	Road to Misahualli	S01°02.15′	W77°46.21′	513	WFD[1]
	OSW	Bajo Talug (Cando)	S01°04.72′	W77°54.63′	567	Road ditch
	OSW	Pano	S01°00.77′	W77°51.27′	566	Road ditch
Pastaza	OSW	North of Puyo	S01°22.64′	W77°57.51′	1067	WFD[1]
	OSW	Near Puyo	S01°25.82′	W77°57.88′	998	Marsh
	OSW	Puyo	S01°28.63′	W77°59.88′	938	Pond
	OSW	Near Puyo	S01°30.06′	W78°01.20′	938	Ditch
	OSW	Near Mera	S01°24.83′	W78°12.45′	1223	Road ditch
	OSW	Sharupi centre	S01°54.55′	W77°49.38′	665	Pond
	OSW	Kunkuki	S01°51.55′	W77°48.99′	894	Pond
	OSW	San Ramon	S01°25.43′	W77°52.46′	991	River eddy
	OSW	Simon Bolivar	S01°26.21′	W77°48.43′	1045	Pond
Pichincha	ALB	San Vicente	S01°20.49′	W77°52.03′	1006	River eddy
	PSE	South of U. Toachi	S00°19.66′	W78°56.91′	835	River eddy
	PSE	Near U.Toachi	S00°19.57′	W78°56.91′	805	Pool
	PUN	Mindo	S00°04.12′	W78°45.74′	1289	Pond
	PUN	Mindo	S00°02.94′	W78°40.44′	1312	Pond
	PUN	Mindo valley	S00°01.48′	W78°48.40′	1105	River pool
	PUN	Road Mindo–Lloa	S00°03.88′	W78°47.60′	1280	Road ditch

Province	Spp.	Locality	Latitude	Longitude	Alt. (m)	Habitat
Pichincha (con't)	PUN	North Alluriquin	S00°14.92′	W79°00.92′	847	River eddy
	PUN	Near Mindo	S00°03.53′	W78°46.77′	1261	Road ditch
	PUN	Near Mindo	S00°03.09′	W78°47.09′	1239	Road ditch
	EIS	Mindo fish farm	S00°04.15′	W78°45.71′	1873	Fish pond
S. Domingo d.l Tsachiles	PSE	City of Santo Domingo	S00°14.92′	W79°08.06′	532	Marsh
	PSE	Alluriquin	S00°19.41′	W78°59.86′	740	River eddy
	PSE	Leila	S00°18.96′	W79°02.00′	778	Pool
	PUN	La Florida	S00°17.20′	W79°00.85′	847	River pool
Tungurahua	OSW	Río Negro	S01°24.84′	W78°11.81′	1230	Road ditch
Zamora-Chinchipe	OSW	Near Zamora	S04°02.85′	W78°54.69′	893	Flooded area

[1] WFD = Water-filled depression

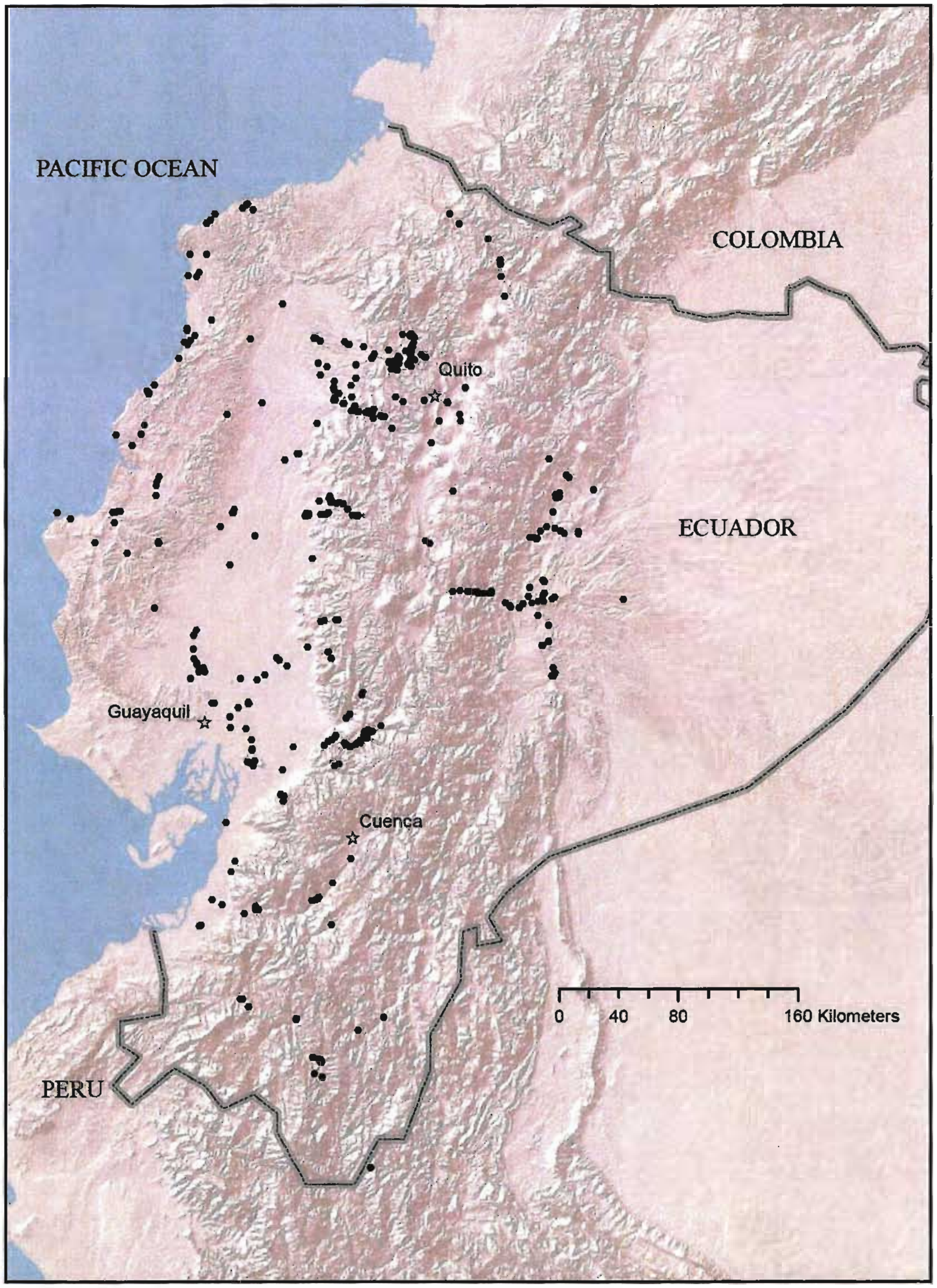

Figure 3-1 Map of all attempted collection localities in Ecuador (black circles) between 2008 - 2010 (n=438). Map prepared in ArcGIS v.9.3 with ESRI World Terrain base (ESRI 2009).

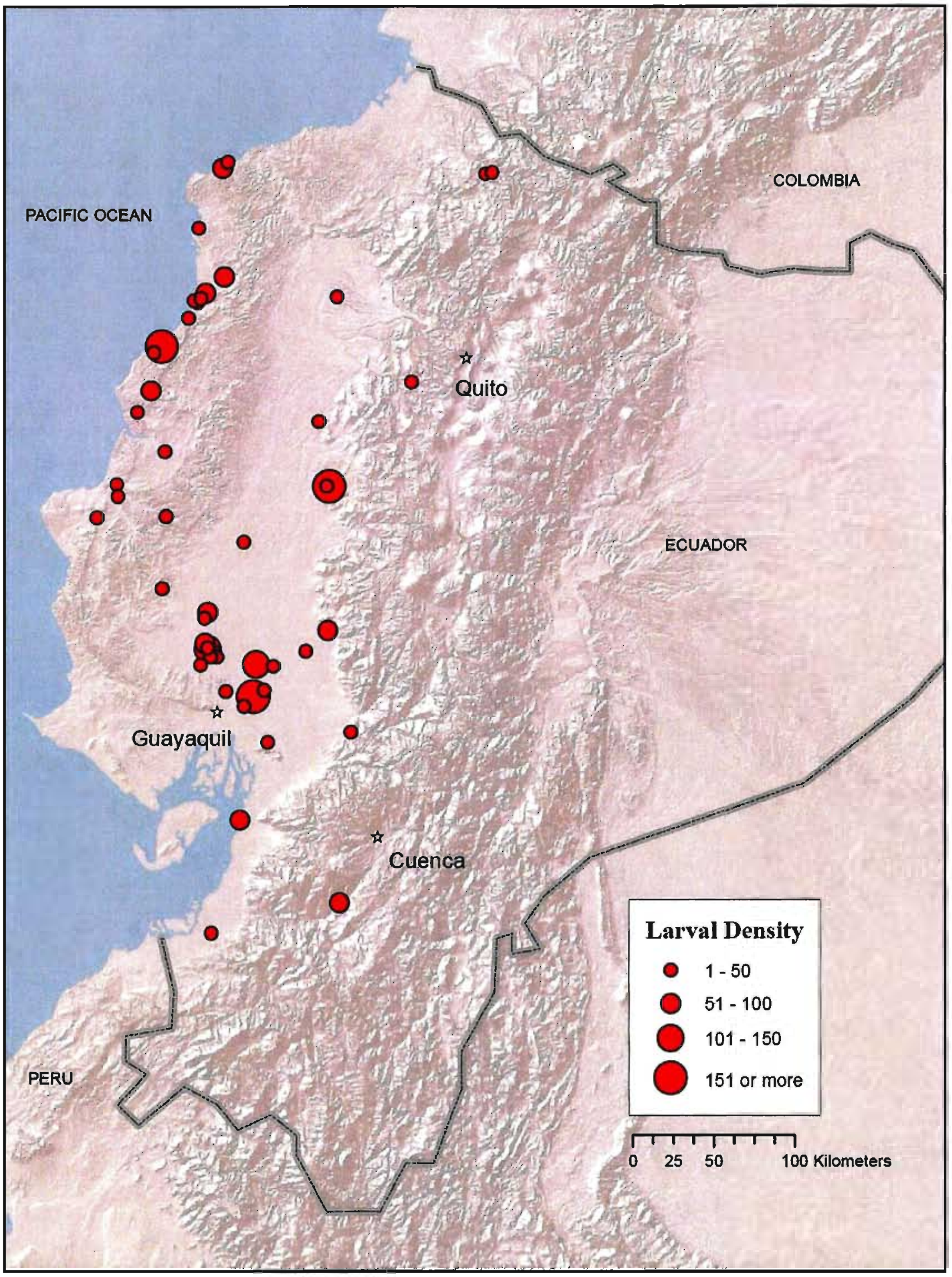

Figure 3-2 Map of *An. albimanus* localities in Ecuador (red circles) from larval collections conducted between 2008 – 2010. Circle size (legend) indicates mean number of larvae per dip in a 30-dip sample. Map prepared in ArcGIS v.9.3 with ESRI World Terrain base (ESRI 2009).

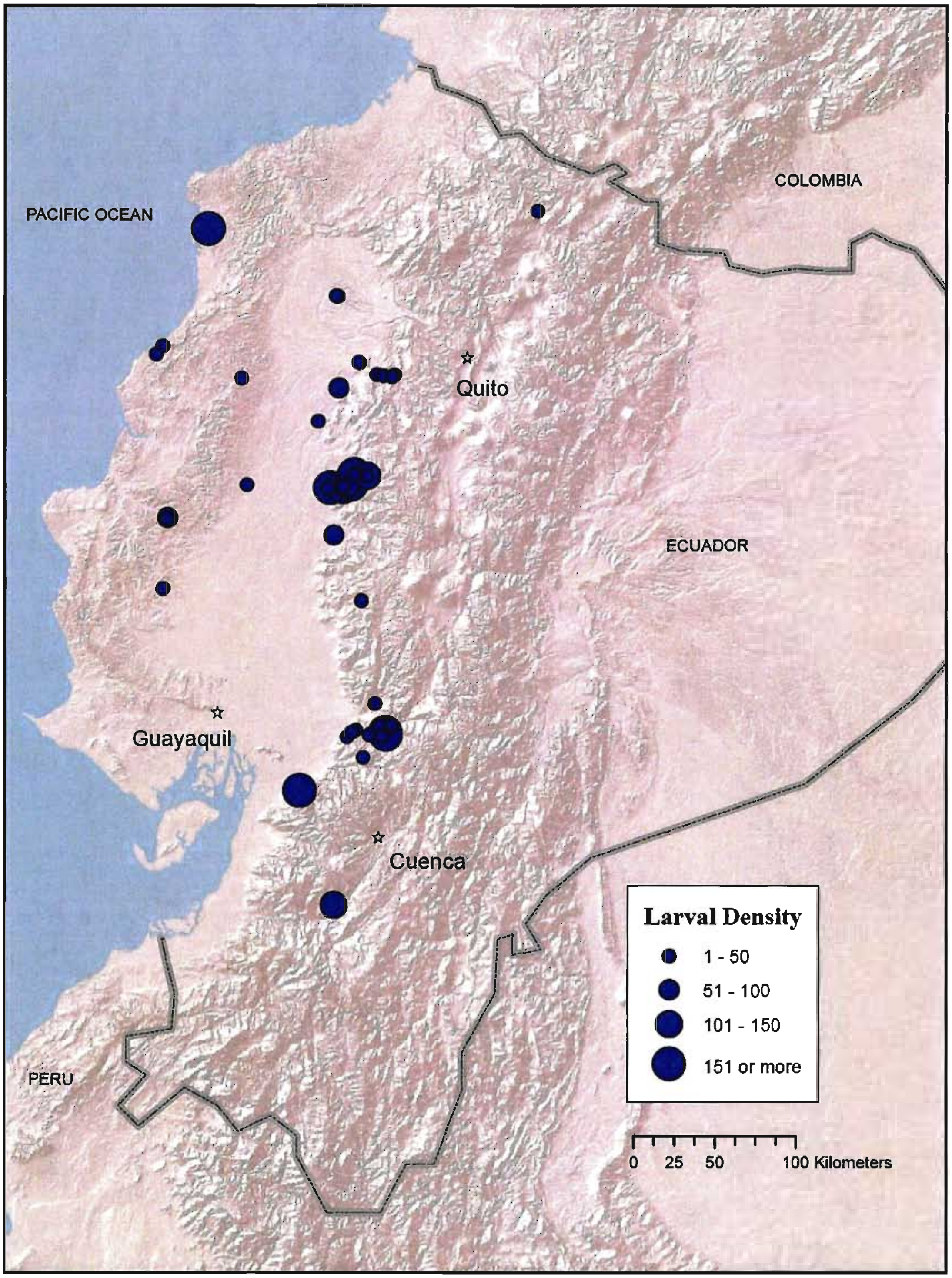

Figure 3-3 Map of *An. pseudopunctipennis* localities in Ecuador (blue circles) from larval collections conducted between 2008 – 2010. Circle size (legend) indicates mean number of larvae per dip in a 30-dip sample. Map prepared in ArcGIS v.9.3 with ESRI World Terrain base (ESRI 2009)

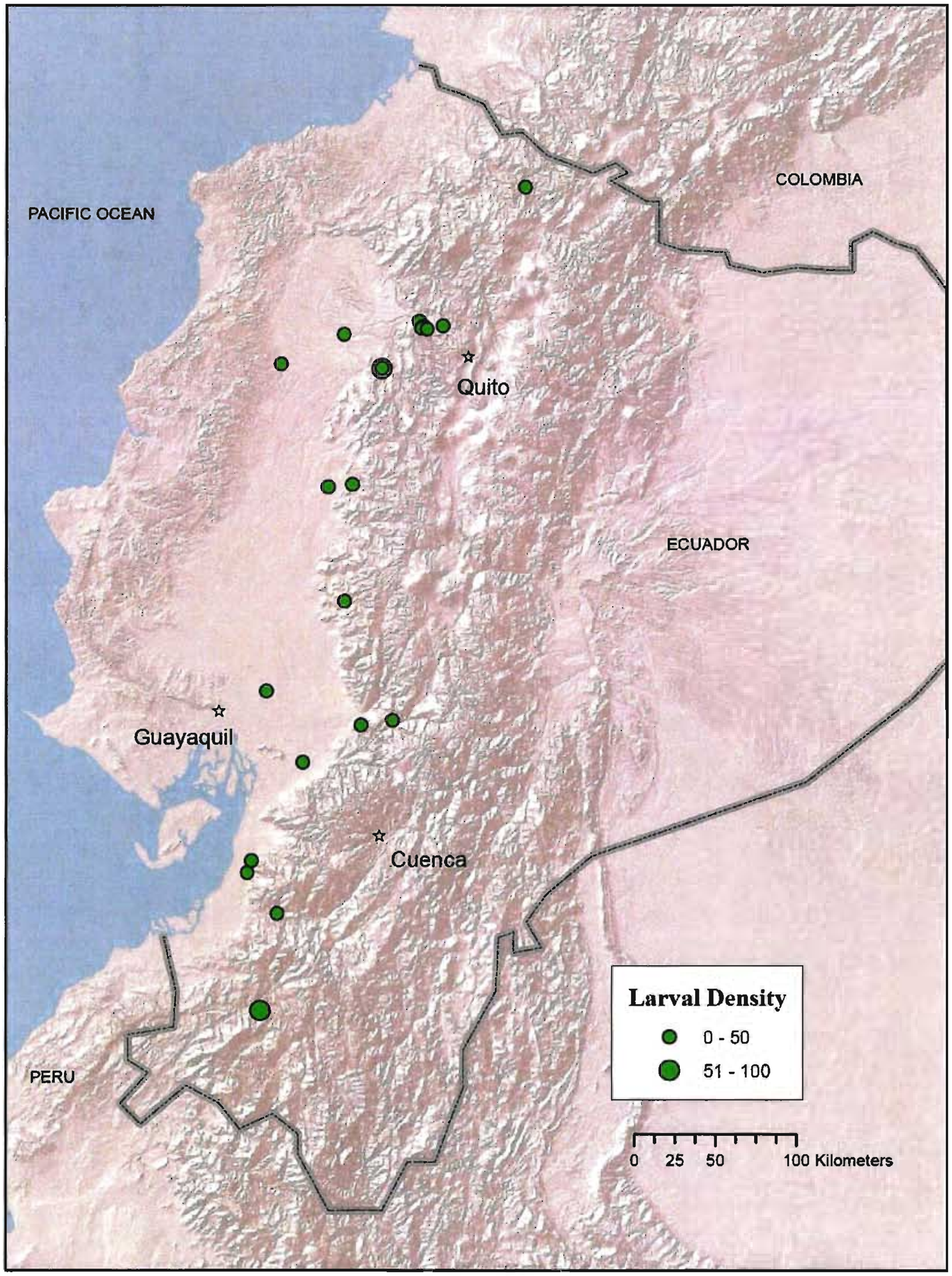

Figure 3-4 Map of *An. punctimacula* localities in Ecuador (green circles) from larval collections conducted between 2008 – 2010. Circle size (legend) indicates mean number of larvae per dip in a 30-dip sample. Map prepared in ArcGIS v.9.3 with ESRI World Terrain base (ESRI 2009).

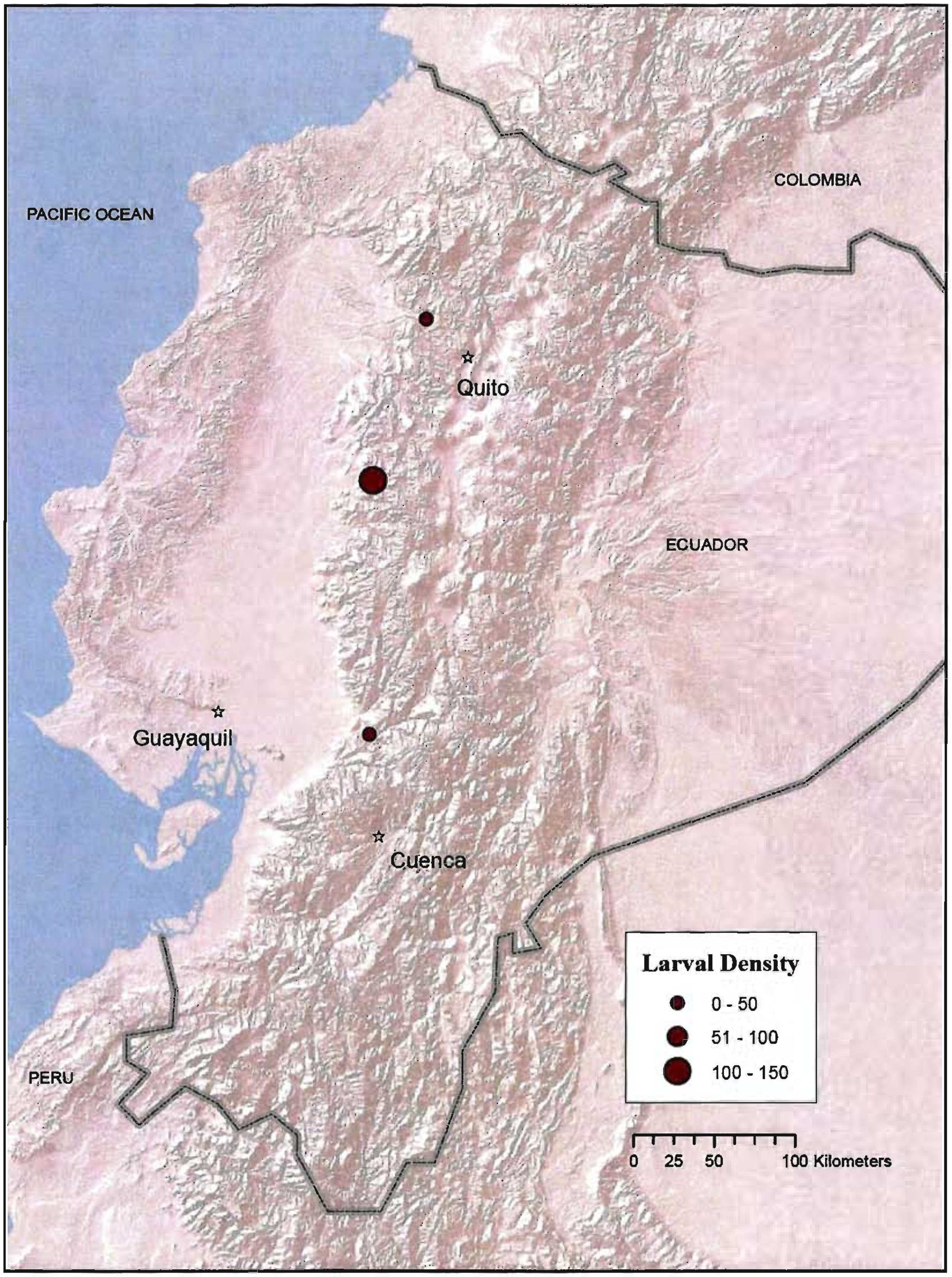

Figure 3-5 Map of *An. eiseni* localities in Ecuador (maroon circles) from larval collections conducted between 2008 – 2010. Circle size (legend) indicates mean number of larvae per dip in a 30-dip sample. Map prepared in ArcGIS v.9.3 with ESRI World Terrain base (ESRI 2009).

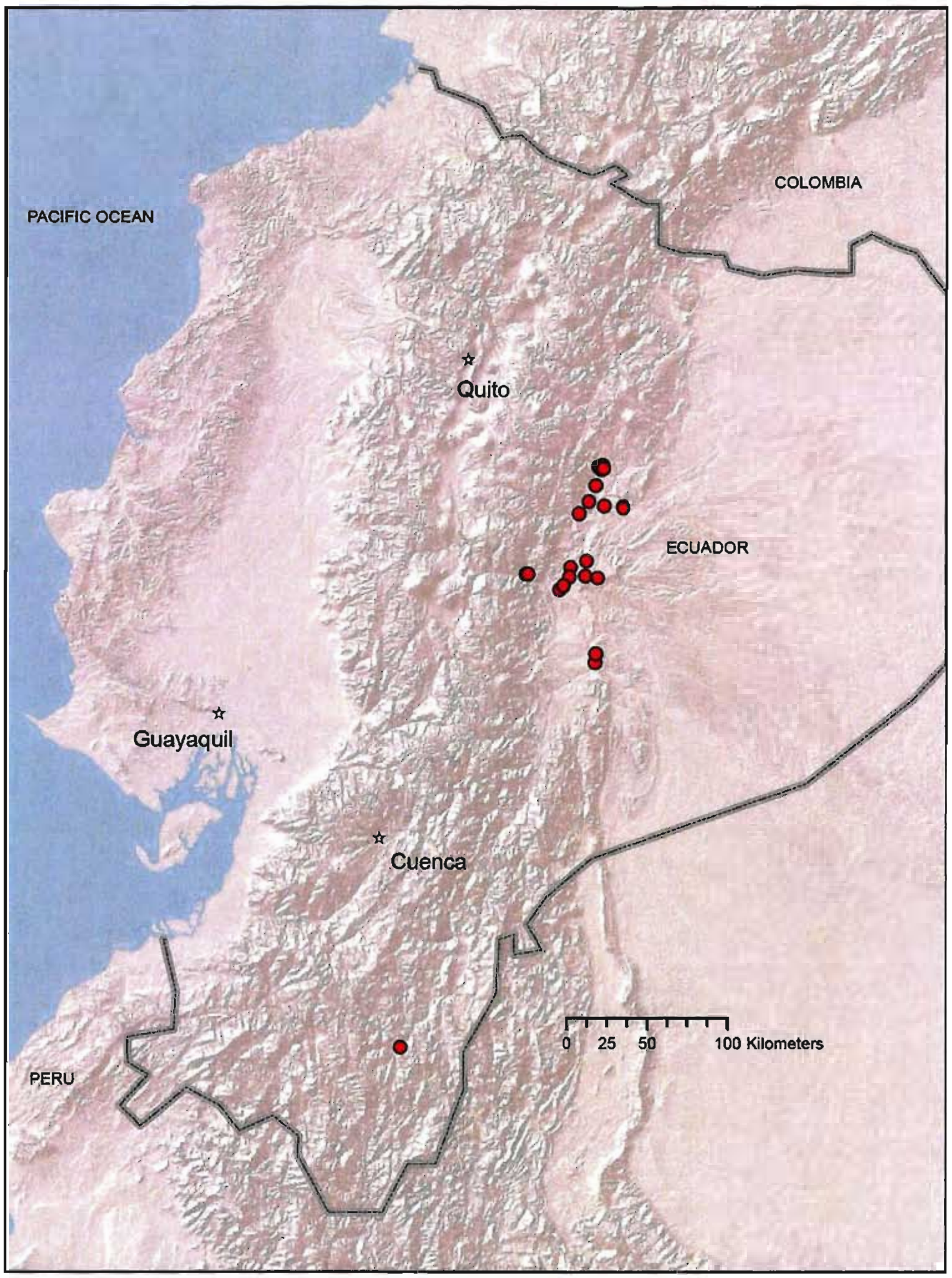

Figure 3-6 Map of *An. oswaldoi s.l.* localities in Ecuador (orange circles) from larval collections conducted between 2008 – 2010. All sample sizes are within 1-50 larvae per 30-dip sample. Map prepared in ArcGIS v.9.3 with ESRI World Terrain base (ESRI 2009).

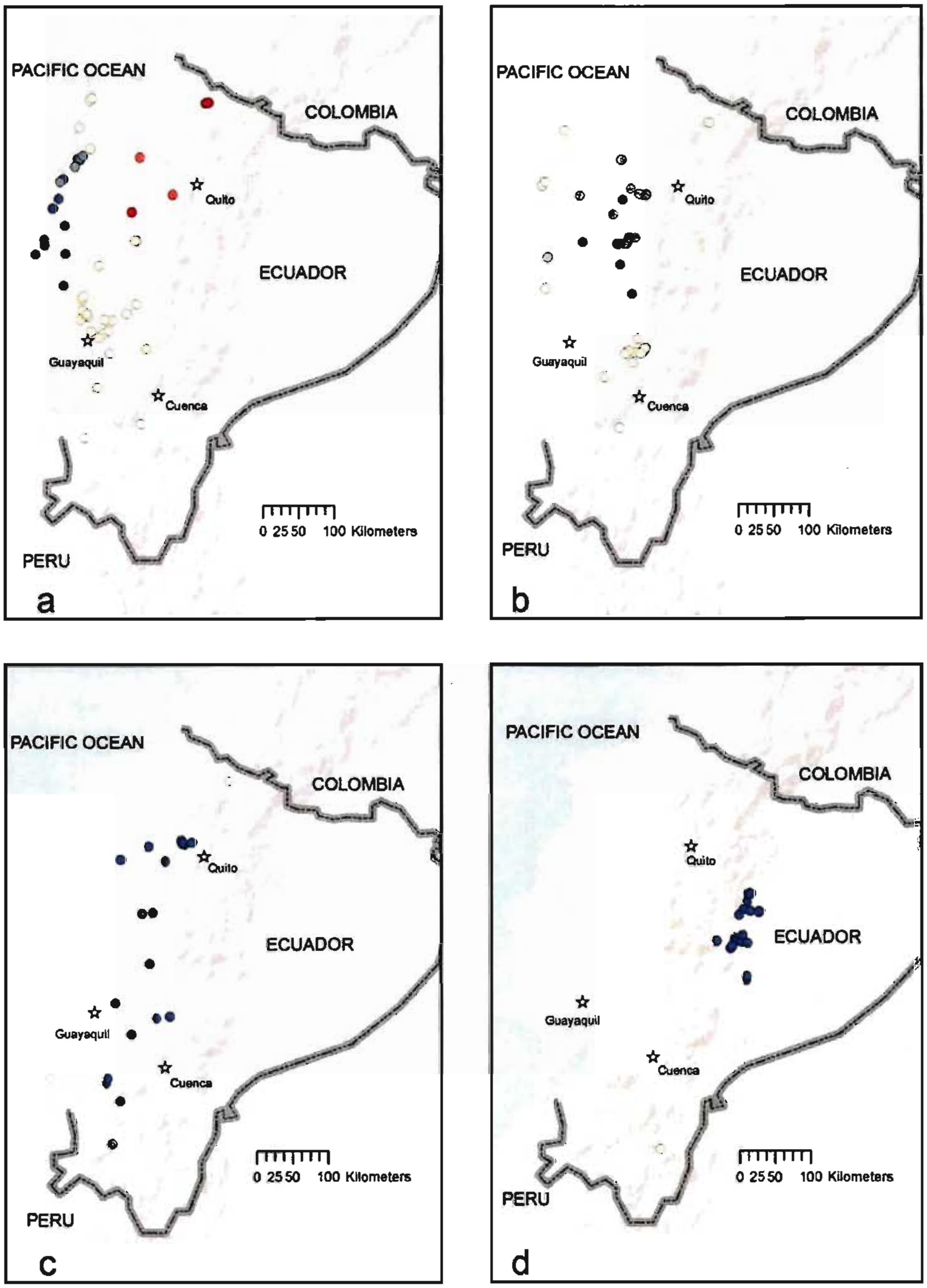

Figure 3-7 Hot Spot Analysis (Getis-Ord) results for a) *An. albimanus*, b) *An. pseudopunctipennis*, c) *An. punctimacula* and d) *An. oswaldoi s.l.*. Red and dark orange circles indicate significant clusters of high larval density sites, whereas blue circles indicate significant clusters of low larval density sites ($p<0.05$). Map prepared in ArcGIS v.9.3 with ESRI World Terrain base (ESRI 2009).

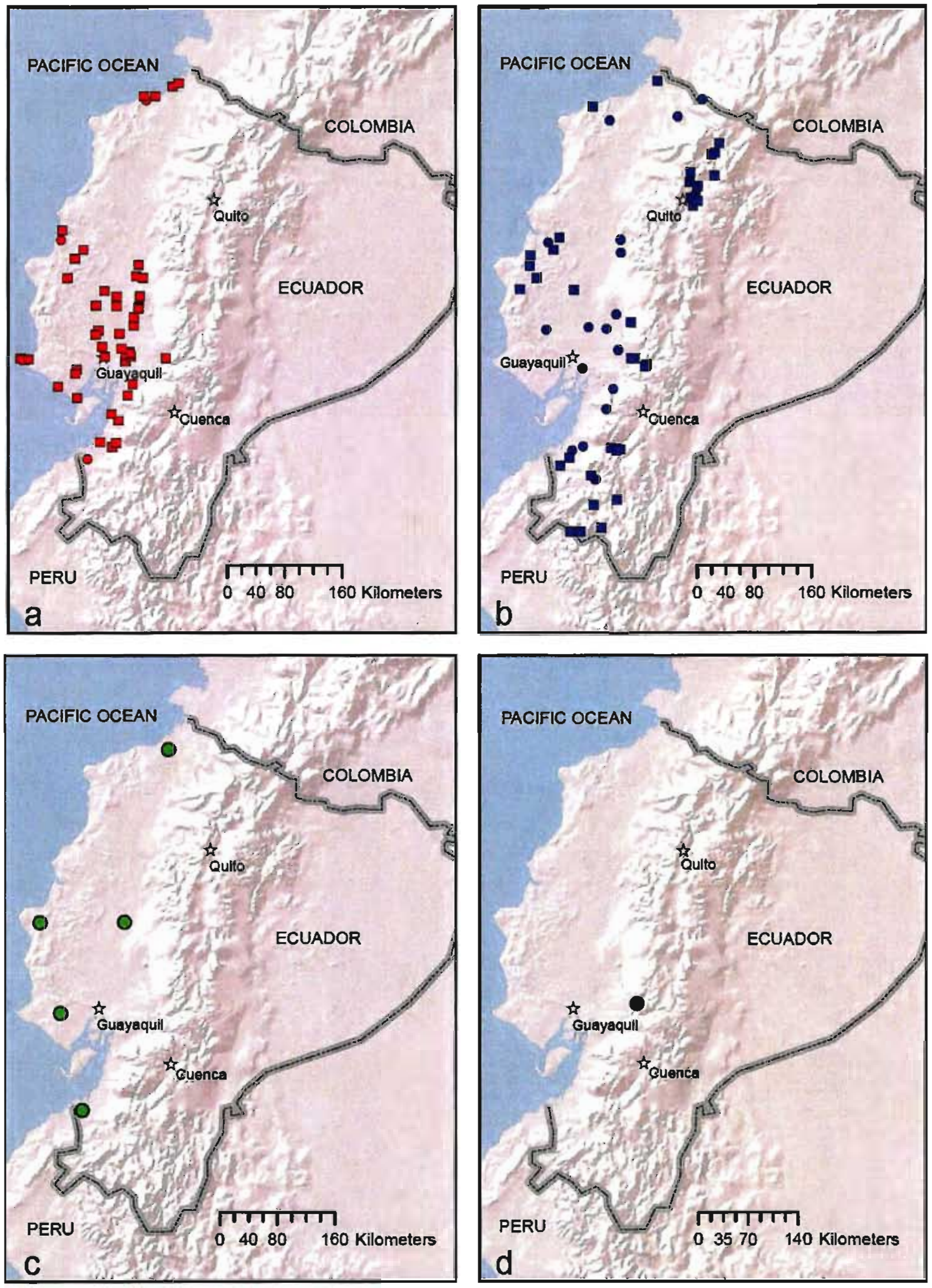

Figure 3-8 Historical distribution during 1940s of a) *An. albimanus*, b) *An. pseudopunctipennis*, c) *An. punctimacula* and d) *An. eiseni.* Data reproduced from historical maps by Levi-Castillo (circles) (1945a) and Montalvan (squares) (1948). Map prepared in ArcGIS v.9.3 with ESRI World Terrain base (ESRI 2009).

Chapter Four

Characterization of larval habitats of *Anopheles albimanus*, *Anopheles pseudopunctipennis*, *Anopheles punctimacula* and *Anopheles oswaldoi s.l.* populations in lowland and highland Ecuador

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4.1 Introduction

Medical professionals and scientists are increasingly concerned about the presence of malaria vectors (some *Anopheles* spp.) and malaria parasites (*Plasmodium* spp.) in previously malaria-free highland regions (e.g., Martens et al. 1999, Epstein 2001, Reiter 2001, Moreno 2006). Although many of these review articles have linked future highland malaria to climate change, only a few studies in Africa provide data-derived evidence for a meteorological cause for highland malaria (e.g., Loevinsohn 1994, Lindblade et al. 1999). Other reasons provided for the phenomenon include drug resistance, changes to vector control methodology, the El Niño Southern Oscillation cycle (ENSO), and human land use alterations in highland regions (e.g., Lindsay & Birley 1996; Bødker et al. 2000, Shanks et al. 2000, Hay et al. 2002a, Afrane et al. 2006). ENSO may play a particularly strong role in the South American Andes since its timing has been strongly correlated with malaria epidemics (Poveda et al. 2001).

In the South American Andes, *Anopheles pseudopunctipennis* Theobald is a known malaria vector residing in highland valleys, collected frequently prior to 1950 (Levi-Castillo 1945). Recently, *An. pseudopunctipennis* has been implicated in *Plasmodium vivax* malaria transmission at an altitude of 2300 m in Bolivia (Rutar et al. 2004). In another recent study, the authors highlighted new, widespread highland distributions in Ecuador of three other common anopheline species: *Anopheles albimanus* Weidemann, *Anopheles punctimacula* Dyar & Knab and *Anopheles oswaldoi s.l.* Perryassú (Chapter Three). It is therefore important to characterize the larval habitat of these four species within their current range, as well as within potential future highland habitats.

Anopheles albimanus is considered a coastal vector occurring below 500 m in altitude throughout Central and South America (Rubio-Palis & Zimmerman 1997). The habitats of *An. albimanus* have been well described for Central American populations. In the wet season, larvae are known to occupy rivers, marshes, estuaries, irrigation canals and rainwater pools, whereas in the dry season, larvae are restricted to permanent habitats such as marshes (Breeland 1972, Rejmankova et al. 1991, Rejmankova et al. 1995, Roberts et al. 2002). In Mexico, larvae have been collected from transitional pastures (Rodriguez et al. 1993). Larvae were positively associated with floating cyanobacterial (blue-green algae) mats, which provide a warmer minimum temperature and lower dissolved oxygen level in the evenings (Rejmankova et al. 1995, Rejmankova et al. 1996, Grieco et al. 2006) and also attract female oviposition through volatile compounds (Rejmankova et al. 2005). *Anopheles albimanus* larvae have been variably associated with macrophyte vegetation according to the literature (e.g., Meyer 1947, Rodriguez et al. 1993, Grieco et al. 2006), including positive associations with mangrove forests, naiads (*Najas* spp.), water hyacinths (*Eichnornia* spp.) (Rodriguez et al. 1993), submersed macrophytes (Rejmankova et al. 1993) and the floating fern (*Salvinia matans*) (Meyer 1947). Much less is known regarding South American populations of *An. albimanus*. In Peru, larvae have been collected from rice fields and drains (Guthmann et al. 2002), and in Colombia, larvae have been associated with sun-exposed sites with a temperature range of 27.5 - 30.0°C (Marten et al. 1996).

Anopheles pseudopunctipennis is a coastal and piedmont malaria vector distributed widely across Central and South America (Rubio-Palis & Zimmerman 1997). In two studies of *An. pseudopunctipennis* habitat across its entire range, Manguin et al. (1996) and Rueda et al. (1994) observed that larvae were associated with drier environments (or frequent droughts), agricultural lands, stream pools, rocky substrates, and clear, shallow, sun-exposed waters with filamentous green algae. In Belize, larvae were associated with greater altitudes and shallower water depth than other species, as well as the presence of filamentous algae (Rejmankova et al. 1993).

Anopheles punctimacula is a secondary coastal vector of Central and South America (Rubio-Palis & Zimmerman 1997). In Belize, *An. punctimacula* larvae have been positively associated with macrophyte vegetation cover, detritus debris, shallow depth, and partial shade (Rejmankova et al. 1998).

Anopheles oswaldoi is a species complex distributed throughout Amazonia (Lounibos & Conn 2000, Monteiro de Barros et al. 2007). Larvae occupy both temporary and permanent pools in Venezuela (Rojas et al. 1992) and clean forest pools in Brazil (Monteiro de Barros et al. 2007).

Female mosquitoes are known to selectively oviposit in habitats to avoid predation by specific predator types (Stav et al. 1999, Spencer et al. 2002), or to place eggs and subsequent larvae in close proximity to preferred food items (Reiskind et al. 2009) or in suitable habitats for temperature requirements (Rejmankova et al. 1996; Rejmankova et al. 2005). We assume that females tend to choose oviposition sites that are favourable for larval development. However, it is reasonable to predict that at the edge of a species' range, such as at very high elevations, females might be provided with fewer site choices and therefore suitability might be far more variable (e.g. Koenraadt et al. 2006). Therefore, we made an effort to incorporate all possible larval habitats that we encountered within the entire current range of these species in Ecuador, including both highland and lowland sites.

Since most studies of larval habitat characterization of these species have been conducted in Central America (e.g. Rejmankova et al. 1993), we expect available larval habitats for South American populations to be sufficiently different to warrant investigation. Many of the South American studies (e.g. Manguin et al. 1996, Martens et al. 1996) only describe sites where *Anopheles* are present, which could be biased by the actual proportion of these parameters in all existing potential habitats. The presence of a species within a specific type of habitat might be a factor of availability rather than suitability. Therefore, we compare *Anopheles*-present sites to *Anopheles*-absent sites within the current range of each species. We also compare *Anopheles*-present sites to highland *Anopheles*-absent sites that could serve as future larval habitats. Each species requires specific habitat characteristics for larval habitat suitability, therefore, we predict that some specific characteristics would be associated with the presence of each species when compared statistically to the habitat availability. For each species, the daily minimum, average, and maximum temperatures were determined and future maximum altitudes based on daily minimum temperatures were estimated, since other authors cite the importance of daily temperature fluctuations for anopheline larval habitat suitability (Huang et al. 2006; Paaijmans et al. 2008).

4.2 Materials and Methods

4.2.1 Larval sampling

Habitat data and *Anopheles* larvae (when present) were collected from 276 sites (Figure 4-1) encompassing a wide variety of habitat types in Ecuador, from September-December 2008 and August-December in 2009 and 2010. We sampled all potential habitats encountered, characterized by a stationary water surface, including river edges clogged with vegetation. At each site, we sampled for *Anopheles* larvae by skimming the water surface a standardized 30 times, using a 13 cm diameter plastic mosquito dipper (BioQuip). For each dip, the total number of *Anopheles* larvae and non-*Anopheles* mosquito larvae were recorded. *Anopheles* larvae were individually collected from the dipper with a pipette and placed in 95% ethanol for transportation to the laboratory for subsequent identification.

Late instar *Anopheles* larvae were identified morphologically using the key of Gorham et al. (1973) with Faran (1979). *Anopheles* larvae too small to be identified morphologically were compared to a subsample of large, identifiable larvae using molecular comparison of the 800 base pair region of the cytochrome oxidase 1 gene (CO1) as described in Chapter Three, with concensus sequences provided in Appendix 1. Intact specimens were submitted to the Ecuadorian National Collection (PUCE, Quito) and genetic sequences were submitted to GenBank (accession numbers JN412826-JN412843).

4.2.2 Data collection of habitat characteristics

Data recorded at each site included the following large-scale factors: latitude/longitude and altitude (Garmin® GPS map 76), water body type (9 categories), primary human site use (7 categories), presence or absence of pollution or contamination (e.g. detergent, oil, garbage), general ecosystem type (8 categories), and water bodies were categorized as natural vs. anthropogenic and permanent vs. temporary. Abiotic factors that were recorded included: pond surface area and depth where the larvae were residing (using a measuring tape), water temperature, pH, and conductivity (Hanna Instruments HI98129 combination water tester), water clarity and incident sunlight (10% classes from visual estimates), and dominant substrate (8 categories). Although we also measured air temperature, we only used surface water temperature in the analysis since air temperature has been shown to be a poor indicator of habitat suitability for *Anopheles* species (Paaijmans et al. 2008). All parameters are summarized in Appendix 2. Theoretical dissolved oxygen was estimated using the following equations:

First, barometric pressure was calculated using the equation in APHA (1965):

$$lnPr = 5.25 \times \ln\left(1 - \frac{h}{44.3}\right)$$

Where Pr = barometric pressure (atm) and h = altitude (km). Then, after converting pressure to units of torr, theoretical dissolved oxygen was calculated using the equation adapted from APHA (1965):

$$0-30°\mathrm{C}:\quad DO = \left(\frac{(P-p)\times 0.678}{35+t}\right) \times s \qquad 30-50°\mathrm{C}: DO = \left(\frac{(P-p)\times 0.827}{49+t}\right) \times s$$

Where DO = calculated dissolved oxygen in mg/L, P = barometric pressure (torr), calculated from altitude, p = vapour pressure (torr), s = salinity correction factor (from measured conductivity), t = temperature (measured) (Weiss 1970).

Biotic factors that were recorded included woody debris and detritus debris (present/absent), vegetation (presence or absence of macrophytes: emergent, rooted floating, submergent, and free floating and of algae: floating, filamentous, attached or slimes/crusts), and invertebrates (presence/absence of invertebrate order observed in 30 dip-samples).

4.2.3 Determination of daily temperature profiles

Since all temperature measurements in study sites were obtained during the day during the daily temperature maxima, we sub-sampled a total of 36 sites (between 5-8 sites for each *Anopheles* species and 10 high-altitude localities) for daily temperature profiles (minimum, daily average, and maximum temperatures). At each site, a Thermochron iButton® (Embedded Data Systems Model DS1921G) was wrapped in an aLoksak® airtight dive bag with all air removed, and permitted to float on the surface of the water on the edge of the pool, where larvae were typically found. Bags were tethered to a rock placed at the bottom of the pool. In a pilot study, the bags were found to have a small warming effect of 0.1°C when exposed to full sunlight. Temperature readings (±0.5°C) were measured at 10-minute intervals for at least two consecutive 24-hour periods and minimum, average, and maximum temperatures determined for each site.

4.2.4 Statistical analyses

Larvae of *An. albimanus* co-occurred with *An. pseudopunctipennis* in six sites and there were no other co-occurring species; therefore, we analyzed the data at the population level rather than community level. Since many previous studies only characterize habitats presently used by *Anopheles* larvae (e.g. Marten et al. 1996; Manguin et al. 1996), and might therefore be biased by the frequency at which these parameters occur in all potential habitats, the proportions of parameters observed in *Anopheles*-present sites were compared to the proportions of parameters in larval-absent sites, to establish differences in habitat usage relative to the actual habitat availability.

Two series of analyses were undertaken to compare larval-present sites to larval-absent sites for each species. First, to define current larval habitats, we compared larval-present sites to larval-absent sites within the current distribution of the species. Although many species-absent sites were sampled throughout Ecuador, we chose species-absent sites that were relevant for habitat availability within the current species range. In the choice of absent sites for the analysis, we chose sites that were present on the same side of the Andes as the species' own occurrence, and absent sites were eliminated from the analysis when they were >300 m higher than the species' altitudinal maxima. Absent sites for *An. albimanus* and *An. pseudopunctipennis* in the vicinity of Mindo, Pichincha (ca. 0°01'60 S; 78°47'60 W) were also eliminated from analysis since these species are not distributed in these general regions (Chapter Three).

Second, to determine the differences between current habitat and potential future habitat in highland regions, larval-present sites were compared to highland larval-absent sites, defined as areas of relatively steep topography (conservatively, above 300 m on the coast and 800 m in Amazonia). As above, absent sites were sampled on the same side of the Andes as the species' distribution, and sites at altitudes higher than 2000 m were not included, since the highest altitude for *Anopheles* larvae recorded in Chapter Three was 1930 m. An example of site selection for larval-absent sites for *An. punctimacula* is provided in Figure 4-1.

For each species and series of analyses, Pearson's Chi-square tests were used to compare natural vs. anthropogenic, permanent vs. temporary, and polluted (presence of garbage, oil, detergent, etc.) vs. not polluted, and among categories of dominant substrates, woody debris and detritus debris, between observed sites (number of *Anopheles*-present sites) and expected sites (calculated from the proportions of *Anopheles*-absent sites). Since multiple Chi-square tests were used, we used a Bonferroni correction for multiple comparisons, which reduces the significance level of α by α/n, where n is the number of tests in the series, and thereby reduces the probability of false positive results (Whitlock & Schluter 2009). Dominant water body type and human land use associated with each species are described graphically rather than statistically since there were too many categories observed for each to permit meaningful statistical analysis.

For both current distribution sites and potential future highland sites, association with algae type and macrophyte vegetation type (presence or absence of four categories) were analyzed using a Binary Logistic Regression (BLR) model where the response variable was the presence or absence of a given mosquito species. To determine which abiotic factors (water temperature, conductivity, dissolved oxygen, water clarity, incident sunlight, surface area, depth and pH) contributed to the presence or absence of a species in a larval habitat, a Stepwise (Backward) Binary Logistic Regression (SBLR) was used on data transformed using the Box-Cox method for normalizing data (Box & Cox 1964). Altitude was included as an additional factor for sites only within the species' current distribution.

Finally, in order to determine the relationship between altitude and larval habitat (water body) surface temperatures, minimum, average, and maximum daily temperatures from Thermochron iButton readings were regressed to altitude. The line of best fit was determined for each of the temperature measurement types.

4.3 Results

4.3.1 Comparison of larval-present habitats to absent sites within current species distribution

Anopheles albimanus was collected predominantly from the northwest littoral wet tropical forest region, which includes banana plantations, with one locality from the cloud forest region. In permanent habitats, a higher proportion of larvae was collected (83.3%) than expected

(63.8%) (Table 4-1). *An. albimanus* larvae were often associated with roads, human habitation, cattle pasture and plantations (Figure 4-2). Larvae were collected from a large variety of water body types, including roadside ditches, rice paddies, water-filled depressions, marshes, ponds, and river edges (Figure 4-3). *Anopheles albimanus* larvae were associated more often with sand substrates (54.2%) than expected from potential habitat availability (7.9%) (Table 4-1). There was no significant association of larvae with natural or anthropogenic habitats, woody debris, detritus debris, or pollution (Table 4-1). Larvae were positively associated with the presence of floating algae, which included cyanobacterial mats, with 64.6% of sites positive for floating algae (expected value = 31.7%) (Table 4-2). Larvae were not significantly associated with the presence of different types of macrophyte vegetation. Water temperature and conductivity were significant factors in the SBLR model (Table 4-3). *Anopheles albimanus* larvae were associated with warmer temperatures (29.9 ± 3.5°C > 26.5 ± 3.8°C; mean ± standard deviation) and higher conductivity (1347 ± 1329 µS > 707 ± 980 µS), the latter possibly due to larval tolerance of higher salinity in coastal sites (Table 4.3).

Anopheles pseudopunctipennis larvae were collected from various ecosystems in Ecuador: in the northwest littoral wet tropical forest, cloud forest, inter-Andean valleys and a semi-desert. Larvae were collected more often along roads, near human habitations, and in areas without an obvious human land use (Figure 4-2). *Anopheles pseudopunctipennis* larvae were collected more often in roadside ditches, water-filled depressions and river edges than in other water body types (Figure 4-3). Of all the Chi-square comparisons, none of the factors was significant (Table 4-1). As with *An. albimanus*, *An. pseudopunctipennis* larvae were positively associated with floating algae in 75.9% of sites versus the expected 27.2% (Table 4-2), as well as negatively associated with the presence of emergent macrophyte vegetation (41.4% of sites <

66.0%) (CCV=1.12, *Z*=-2.38; *df*=1; *p*=0.017). Altitude, water temperature and water clarity were significant factors in the final SBLR model (Table 4-3); larvae were associated with slightly higher altitudes (482 ± 528m > 448 ± 563m), warmer temperatures (26.6 ± 3.5°C > 25.8 ± 3.4°C) and higher water clarity (63.8 ± 30.4% > 42.0 ± 29.4%) than expected from *Anopheles*-absent sites within their distribution (Table 4-3).

Anopheles punctimacula larvae were collected in the north-western littoral wet tropical forest, cloud forest, and inter-Andean valley regions of Ecuador. Larvae were associated mainly with roads and plantations (Figure 4-2) and were collected predominantly from ponds and river edges (Figure 4-3). None of the Chi-square comparisons were significant (Table 4-1). *Anopheles punctimacula* larvae were not significantly associated with macrophyte vegetation but were negatively associated with the presence of floating algae (Table 4-2). Larvae were collected in water bodies with floating algae in only 11.1% of sites while 32.5% of sites had floating algae present. Calculated dissolved oxygen and incident sunlight were significant factors in the SBLR, with larvae associated with greater estimated dissolved oxygen (7.81 ± 0.42 mg/L > 7.53 ± 0.47 mg/L) and less incident sunlight (67.2 ± 34.9% < 84.9 ± 24.0%) (i.e. they were collected from shaded pools) (Table 4-3).

Anopheles oswaldoi s.l. larvae were collected almost exclusively in the Amazonian tropical rainforest, although a few sites were in low-altitude cloud forests. Approximately half of *An. oswaldoi s.l.* sites were associated with roads (Figure 4-2), and were most often found in roadside ditches, water-filled depressions and ponds (Figure 4-3). None of the Chi-square comparisons were significant, except *An. oswaldoi s.l.* larvae were found more often than expected in permanent habitats (Table 4-1). There were no significant associations between larvae and algae or macrophyte vegetation types. Similarly, the stepwise logistic regression did not provide any significant factors to explain the presence vs. absence of *An. oswaldoi s.l.* within its current distribution.

4.3.2 Comparison of larval-present habitats to potential future highland habitats

When *An. albimanus*-present sites were compared to expected values derived from highland absent sites, larvae were positively associated with natural and permanent water bodies than expected from larval-absent sites (Natural: 56.3% > 31.0%; Permanent: 83.3% > 63.8%) (Table 4-4). As before, larvae were positively associated with sand substrates (54.2%), but these were less common in potential highland habitats (17.2%) (Table 4-4). *Anopheles albimanus* larvae were found more often associated with floating algae (64.6% of sites > 36.2% expected), negatively associated with attached algae (6.3% of sites < 22.4% expected) (Table 4-5), and positively associated with emergent vegetation (77.1% of sites > 51.7% expected) (CCV=1.15; *Z*=2.54; *df*=1; *p*=0.011) when compared to species-absent sites in highland regions. The SBLR identified water temperature and calculated dissolved oxygen as significant factors (Table 4-6), with larvae associated with warmer temperatures (29.9 ± 3.5°C > 23.6 ± 3.2°C) and very slightly higher levels of dissolved oxygen (7.55 ± 0.39 mg/L > 7.52 ± 0.52 mg/L) than would be found in potential highland habitats.

Anopheles pseudopunctipennis larvae were associated with natural habitats (55.1%) and gravel/cobble substrates (44.4%) more often than expected from potential highland habitats (27.7% and 17.0%, respectively) (Table 4-4). Larvae were positively associated with floating algae more often than estimated from highland sites (in 75.9% of sites > 29.7% expected) (Table 4-5) but were not significantly associated with macrophyte vegetation. The significant predictors identified by the SBLR were water temperature, conductivity, dissolved oxygen and water clarity

(Table 4-6), with larvae associated with warmer temperatures (26.6 ± 3.5°C > 23.1 ± 2.9°C), higher conductivity (389.3 ± 356.8 μS > 197.9 ± 156.1 μS), higher calculated dissolved oxygen levels (7.62 ± 0.39 mg/L > 7.54 ± 0.53 mg/L) and clearer water (63.8 ± 30.4% > 47.65 ± 31.4%) than available in highland sites.

Anopheles punctimacula larval habitats did not differ significantly from potential highland habitats with regards to any of the predictors listed in Table 4-4, algae types, or vegetation types. In the SBLR, only water temperature and calculated dissolved oxygen were identified as significant factors (Table 4-6), with larvae associated with warmer temperatures (24.5 ± 2.7°C > 23.6 ± 3.7°C) and higher calculated dissolved oxygen levels than expected (7.80 ± 0.42 mg/L > 7.48 ± 0.50 mg/L) during comparison to potential highland sites.

Anopheles oswaldoi s.l. larvae were not significantly associated with any of the Chi-square comparison factors listed in Table 4-4. There were no significant associations between larvae and algae or macrophyte vegetation. As with *An. punctimacula*, only temperature and estimated dissolved oxygen were identified as significant factors in the SBLR (Table 4-6), with larvae associated with warmer temperatures (28.9 ± 4.1°C > 26.3 ± 3.9°C) and lower estimated dissolved oxygen (7.04 ± 0.42 mg/L < 7.08 ± 0.44 mg/L).

4.3.3 Threshold temperatures

Maximum, average and minimum temperatures from Thermochron iButton readings are presented in Table 4-7, with *An. albimanus* found in the warmest habitats, on average, and *An. punctimacula* in the coolest. The effect of the bag may have inflated daytime temperatures in full sun by approximately 0.1°C, but this effect is not expected during cloudy or shaded periods, nor during the evening. Maximum temperatures are highly variable among sites and do not decline significantly with higher altitudes (Figure 4-4). The decreasing relationship between average and minimum temperatures and altitude was non-linear (Figure 4-4).

Since minimum temperature is likely able to restrict larval survival to lower altitudes, it is therefore important to establish it accurately for each species. Therefore, although there were a small number ($n = 5\text{-}8$) of recorded minimum temperatures for each species, we also estimated minimum temperature using the recent maximum altitude recorded for each species (Chapter Three; Table 4-7). The equation of the relationship between minimum water temperature and altitude was translated using a constant of 25 (+2.142) to include all data points as an estimate of the upper limit for minimum temperatures at high altitudes. The minimum temperature was used to calculate approximate maximum altitude predictions for an average of 1 and 2°C habitat warming (Table 4-7). Based on minimum nightly temperatures only, a small warming would have a stronger effect on lower-altitude species than on those situated at higher altitudes (Table 4-7).

4.4. Discussion

The present study has identified habitat characteristics that are associated more often with anopheline larvae than expected, given the range of possible habitats available within both the species' current geographic distributions and within a highland distribution.

Both *An. albimanus* and *An. pseudopunctipennis* larvae were related to warmer habitat temperatures in both SBLRs. Although an association with warmer temperatures would be expected during comparison to highland sites (since the absent sites are located at higher altitudes), larvae were present more often in warmer temperatures than available within the current range of the species. This suggests that *An. albimanus* and *An. pseudopunctipennis* may be restricted by temperature to specific altitudes at present. Even though *An. pseudopunctipennis* is generally associated with warmer average temperatures, the species appears to be more tolerant of colder minimum temperatures than the other species studied (16.0-16.7°C). Interestingly, although the recent maximum recorded altitude is 1930, the predicted approximate altitude limit based solely on minimum temperature increase is 2102 m for 1°C and 2267 m for 2°C. Historically, *An. pseudopunctipennis* occupied several inter-Andean valleys in northern Ecuador (approx. 2100m) (Levi-Castillo 1945b; Chapter Two).

Anopheles albimanus larvae were associated with floating algae in both comparisons, a result that confirms the studies conducted on populations in Central America (Rejmankova et al. 1995, Rejmankova et al. 1996, Grieco et al. 2006). Similarly, floating algae likely plays a role in promoting thermal stability during cold overnight periods (Rejmankova et al. 1995, Rejmankova et al. 1996, Grieco et al. 2006), and is likely an important habitat parameter for the larvae of this species throughout its very broad geographic range. However, unlike the population in Belize that was associated with filamentous algae (Rejmankova et al. 1993), the *An. pseudopunctipennis* population in Ecuador was significantly associated with floating algae. Future research could determine if *An. pseudopunctipennis* larvae also use floating algae for the function of thermal stability during nights, perhaps as an adaptation to their more widespread high-altitude distribution than in Belize.

In both comparisons, *An. albimanus* was positively associated with permanent habitats, as well as positively associated with sandy substrates, which were both less common in highland habitats than in lowland habitats. However, the presence of *An. albimanus* larvae in water bodies of different dominant substrates may mean that it is adaptable to regions where sandy substrates are uncommon. In lowlands, larvae were collected from roads, human habitations, cattle pastures and plantations, most often rice, in roadside ditches, rice paddies, water-filled depressions, marshes, ponds and river edges. While highland rice farming is not practiced in Ecuador, cattle pasture is an abundant land use in highland regions (de Koning et al. 1998), and ought to be evaluated in future studies as a potential source of highland *An. albimanus* larval habitat. Indeed, Central American populations of the species have also been associated with pasture (Rodriguez et al. 1993).

Anopheles punctimacula larvae were not significantly associated with many habitat characteristics within their current distribution, although they were negatively associated with floating algae. Larvae were collected along roads, on plantations, in ponds, road ditches and river edges, all of which are habitats also available in highland regions. The SBLR model identified calculated dissolved oxygen as a significant factor in both comparisons. Calculated dissolved oxygen is weakly negatively correlated with altitude (0.3 mg/L less per 1000m increase, $R^2 = 0.073$), therefore *An. punctimacula* may be prevented from developing at extremely high altitudes by low levels of dissolved oxygen. Except for one *An. punctimacula* habitat where calculated DO was very low (6.63 mg/L), all other sites had calculated DO values greater than 7.20 mg/L. Further studies are required to properly verify our calculations by collecting field measurements of dissolved oxygen as a possible habitat characteristic of *An. punctimacula* and to evaluate the relationship between altitude and actual dissolved oxygen levels.

Anopheles oswaldoi s.l. habitats were much more difficult to characterize, perhaps due to the wide variety of characteristics in potential habitats available on the Amazonian side of the Andes. In collections, *An. oswaldoi s.l.* larvae were often associated with roads and collected from roadside ditches, water-filled-depressions and ponds, all habitats widely available in human-colonized highland regions. Incidentally, in 2010, a single *Anopheles* larva was collected from a site at 1412 m in Río Negro, Tungurahua, up-valley from known *An. oswaldoi s.l.* sites, but the specimen was not identifiable to species and therefore not included in this analysis.

One of the major limitations of this study was the difficulty in obtaining a large sample size of larval-present sites that would be suitable for a meaningful statistical analysis of the many different water body types and human land uses available (7+ categories). As well, the small number of larval-present sites available in highland regions made it statistically impossible to compare highland *Anopheles*-present sites to highland *Anopheles*-absent sites. However, we feel that the current sampling effort of 276 sites in Ecuador represents a strong sampling frequency within the confines of a relatively small country that already provides a limited number of sites accessible for sampling. Another limitation was that we did not consider seasonality in this analysis, which has been shown to significantly affect habitat usage by *An. albimanus* and *An. psuedopunctipennis* larvae in Mexico (Rejmankova et al. 1991). On the coast of Ecuador, the wet season occurs from approximately late January-April and the dry season from May-December, whereas in other regions of Ecuador, the timing of the dry season is variable. We were constrained to sampling during the long dry season due to the practical difficulty in collecting during the wettest parts of the year, when many (if not all) roads to the coast are washed out by frequent landslides and flooding. As a final concern, although we did not formally consider the presence of fish as a factor, and fish are often used as natural predators and biological control of larvae (Rojas et al. 2004). We noted the presence of fish in all habitats when present, and we did not observe any consistent absence of larvae associated with fish. In most habitats with fish present, larvae were associated with algae, vegetation, debris, or even the edge of the pool, and were likely difficult for fish to locate and consume.

As colonization of highland regions by multiple malaria vectors continues, it will be vital to characterize new habitat usage by *Anopheles* species. Since larval habitat in highland regions is generally more scarce than on flat terrain, the elimination of highland habitats can be a particularly effective malaria control measure alongside mosquito bite prevention and prophylaxis (Roberts 1964a-c).

Table 4-1 Summary of chi-square tests comparing observed ratios of categorical characteristics (number of categories provided in parentheses) among sites where *An. albimanus*, *An. pseudopunctipennis*, *An. punctimacula*, and *An. oswaldoi s.l.* larvae were collected in Ecuador, to expected frequencies determined from subsets of species-absent sites from within the same geographical distribution. Significant p-values with the Bonferroni-corrected $\alpha=0.008$ are presented in bold. Please note that some possible categories were excluded from analysis if the expected frequency counts were less than 5.

	An. albimanus			*An. pseudopunctipennis*			*An. punctimacula*			*An. oswaldoi s.l.*		
	$n_{present}=48$			$n_{present}=29$			$n_{present}=18$			$n_{present}=21$		
Characteristic	χ^2	*df*	*p*	χ^2	*df*	*p*	χ^2	*df*	*p*	χ^2	*df*	*p*
Natural vs. Anthropogenic (2)	4.44	1	0.035	1.06	1	0.302	0.23	1	0.639	4.06	1	0.044
Permanent vs. Temporary (2)	8.15	1	**0.004**	0.05	1	0.821	0.07	1	0.792	9.05	1	**0.003**
Dominant substrate (7)	20.39	4	**<0.001**	7.50	2	0.024	0.25	2	0.882	1.38	2	0.502
Woody debris (2)	3.34	1	0.067	0.12	1	0.731	0.61	1	0.437	2.62	1	0.106
Detritus debris (2)	0.48	1	0.489	2.18	1	0.140	1.27	1	0.259	0.53	1	0.466
Polluted/Garbage (2)	0.10	1	0.747	1.27	1	0.260	*Insufficient df*			<0.01	1	0.968

Table 4-2 Summary of Binary Logistic Regression analysis of the relationship between the presence (1) or absence (0) of algae architectural categories to the presence (1) or absence (0) of *An. albimanus*, *An. pseudopunctipennis*, *An. punctimacula*, and *An. oswaldoi s.l.* larvae within their existing geographical distributions in Ecuador. Significant p-values to α=0.05 are presented in bold. Coefficients of variation (CCV), standard error of the coefficient of variation (SE CCV), Z-scores (the distance from the mean in a normal distribution), and Odds Ratios (a measure of effect size) are presented.

Species	Predictor	*CCV*	*SE CCV*	*Z*	*p*	Odds Ratio
An. albimanus	Constant	-1.54	0.28	-5.47	<0.001	
	Floating	1.41	0.36	3.87	**<0.001**	4.11
	Filamentous	0.09	0.43	0.22	0.823	1.10
	Attached	-0.76	0.70	-1.08	0.281	0.47
	Slimes/Crusts	-0.48	0.62	-0.78	0.436	0.62
An. pseudopunctipennis	Constant	-2.63	0.45	-5.85	<0.001	
	Floating	2.06	0.49	4.17	**<0.001**	7.81
	Filamentous	0.68	0.51	1.33	0.183	1.97
	Attached	0.22	0.67	0.34	0.737	1.25
	Slimes/Crusts	0.38	0.62	0.61	0.541	1.46
An. punctimacula	Constant	-1.81	0.34	-5.26	**0.001**	
	Floating	-1.72	0.79	-2.17	**0.030**	0.18
	Filamentous	-0.03	0.69	-0.04	0.969	0.97
	Attached	0.82	0.79	1.04	0.299	2.27
	Slimes/Crusts	0.78	0.63	1.25	0.213	2.18
An. oswaldoi s.l.	Constant	-1.13	0.39	-2.85	0.004	
	Floating	1.79	0.93	1.94	[a]0.052	6.03
	Filamentous	1.05	0.76	1.39	0.165	2.87
	Attached	0.76	0.91	0.84	0.403	2.15
	Slimes/Crusts	1.19	0.99	1.20	0.230	3.31

[a] Weakly insignificant factor, significant to α=0.06

Table 4-3 Summary of significant abiotic predictors for the presence (vs. absence) of *An. albimanus*, *An. pseudopunctipennis*, and *An. punctimacula* larvae within their geographical distribution, using Backward Stepwise Binary Logistic Regression. The original abiotic predictors used were: altitude, surface area, depth, water temperature, pH, conductivity, calculated dissolved oxygen (DO), water clarity, and sunlight, and the most insignificant predictor was removed during each step until all predictors provided significant p-values. When necessary, predictors were normalized (transformed) using a Box-Cox lambda estimate. Coefficients of variation (CCV), standard error of the coefficient of variation (SE CCV), Z-scores (the distance from the mean in a normal distribution), and Odds Ratios (a measure of effect size) are presented.

Species	Predictor	*CCV*	*SE CCV*	*Z*	*p*	Odds Ratio
An. albimanus	Constant	13.45	8.07	1.67	0.096	
	Water temperature	0.02	0.05	4.21	<0.001	1.26
	Conductivity^-0.02	-23.67	8.95	-2.64	0.008	0.00
An. pseudopunctipennis	Constant	-10.82	3.33	-3.25	0.001	
	Altitude^0.11	1.66	0.82	2.01	0.044	5.25
	Water temperature	0.18	0.80	2.27	0.023	1.20
	Clarity^0.58	0.20	0.07	2.84	0.005	1.22
An. punctimacula	Constant	-9.87	4.68	-2.11	0.035	
	Calc. DO	1.22	0.59	2.05	0.040	3.40
	Incident sunlight	-0.02	0.01	-2.28	0.020	0.98

Table 4-4 Summary of chi-square tests comparing observed ratios of categorical characteristics (number of categories provided in parentheses) among sites where *An. albimanus*, *An. pseudopunctipennis*, *An. punctimacula*, and *An. oswaldoi s.l.* larvae were collected in Ecuador, to expected frequencies determined from subsets of species-absent sites from highland sites (within 300-2000 m altitude for the coast, and 800-2000 m altitude for Amazonia). Significant Bonferroni-corrected p-values to α=0.008 are presented in bold. Please note that some possible categories were excluded from analysis, or categories were meaningfully combined if the expected frequency counts were less than 5 units.

	An. albimanus			*An. pseudopunctipennis*			*An. punctimacula*			*An. oswaldoi s.l.*		
	$n_{present}=48$			$n_{present}=29$			$n_{present}=18$			$n_{present}=21$		
Characteristic	χ^2	*df*	*p*	χ^2	*df*	*P*	χ^2	*df*	*p*	χ^2	*df*	*p*
Natural vs. Anthropogenic (2)	14.26	1	**<0.001**	10.97	1	**<0.001**	0.61	1	0.434	0.94	1	0.332
Permanent vs. Temporary (2)	7.93	1	**0.005**	0.12	1	0.733	0.03	1	0.872	5.69	1	0.017
Dominant substrate (7)	47.69	4	**<0.001**	13.79	3	**0.003**	1.05	1	0.306	0.99	2	0.627
Woody debris (2)	4.71	1	0.029	0.94	1	0.332	0.84	1	0.360	0.03	1	0.862
Detritus debris (2)	1.82	1	0.177	0.12	1	0.733	0.91	1	0.339	0.16	1	0.690
Polluted/Garbage (2)	2.58	1	0.108	0.01	1	0.919	0.22	1	0.640	1.39	1	0.239

Table 4-5 Summary of Binary Logistic Regression analysis of the relationship between the presence (1) or absence (0) of algae architecture categories to the presence (1) of *An. albimanus* and *An. pseudopunctipennis* larvae within their existing geographical distributions or absence (0) within potential highland sites (within 300-2000 m altitude for the coast, and 800-2000 m altitude for Amazonia), in Ecuador. Significant p-values to α=0.05 are presented in bold. Coefficients of variation (CCV), standard error of the coefficient of variation (SE CCV), Z-scores (the distance from the mean in a normal distribution), and Odds Ratios (a measure of effect size) are presented.

Species	Predictor	*CCV*	*SE CCV*	*Z*	*P*	Odds Ratio
An. albimanus	Constant	-0.49	0.33	-1.52	0.130	
	Floating	1.36	0.43	3.13	**0.002**	3.89
	Filamentous	-0.21	0.49	-0.42	0.675	0.81
	Attached	-1.62	0.73	-2.24	**0.025**	0.20
	Slimes/Crusts	-0.85	0.67	-1.27	0.203	0.43
An. pseudopunctipennis	Constant	-1.58	0.48	-3.27	0.001	
	Floating	2.01	0.55	3.67	**<0.001**	7.45
	Filamentous	0.34	0.59	0.57	0.567	1.40
	Attached	-0.39	0.72	-0.54	0.589	0.68
	Slimes/Crusts	-0.02	0.67	-0.04	0.971	0.98

Table 4-6 Summary of significant abiotic predictors for the presence of *An. albimanus*, *An. pseudopunctipennis*, *An. punctimacula*, and *An. oswaldoi s.l.* larvae vs. species-absent highland sites (300-2000 m for the coast, and 800-2000 m for Amazonia sites), using Backward Stepwise Binary Logistic Regression. The original abiotic predictors used were: altitude, surface area, depth, water temperature, pH, conductivity, calculated dissolved oxygen (DO), water clarity, and sunlight, and the most insignificant predictor was removed during each step until all predictors provided significant p-values. When necessary, predictors were normalized (transformed) using a Box-Cox lambda estimate. Coefficients of variation (CCV), standard error of the coefficient of variation (SE CCV), Z-scores (the distance from the mean in a normal distribution), and Odds Ratios (a measure of effect size) are presented.

Species	Predictor	*CCV*	*SE CCV*	*Z*	*P*	Odds Ratio
An. albimanus	Constant	-370.89	77.99	-4.76	<0.001	
	Water temp.^0.12	209.90	43.88	4.78	<0.001	<0.01
	Calc. DO	7.75	1.93	4.01	<0.001	<0.01
An. pseudopunctipennis	Constant	52.49	14.56	3.61	<0.001	
	Water temp.^-0.31	-120.76	32.47	-3.72	<0.001	<0.01
	Conductivity^-0.07	-38.69	11.99	-3.23	0.001	<0.01
	Calc. DO	2.20	0.88	2.50	0.012	9.04
	Clarity^0.66	0.13	0.07	1.98	0.048	1.14
An. punctimacula	Constant	-27.07	7.83	-3.46	0.001	
	Water temp.	0.31	0.12	2.60	0.009	1.36
	Calc. DO	2.41	0.74	3.26	0.001	11.12
An. oswaldoi s.l.	Constant	-77.2	29.28	-2.64	0.008	
	Water temp.	0.92	0.34	2.74	0.006	2.5
	Calc. DO	7.32	2.87	2.55	0.011	1514.51

Table 4-7 Maximum, average and minimum larval habitat temperatures for *An. albimanus, An. pseudopunctipennis, An. punctimacula*, and *An. oswaldoi s.l.* in a subsample of sites within Ecuador in 2010. Temperatures were measured using Thermochron iButtons and minimum temperatures were also calculated using maximum altitudes from Chapter 3. Based on the minimum temperature for each species, the maximum altitude of occurrence was also estimated for 1°C and 2°C warming of the water surface – air interface.

	iButton readings							
Species	n	Maximum temperature (°C)	Average temperature (°C)	Minimum temperature (°C)	Maximum altitude[1] (m)	Minimum temperature[2] (°C)	Maximum altitude[2] +1°C	Maximum altitude[2] +2°C
An. albimanus	6	37.5	25.7	20.0	1541	18.7	1731	1911
An. pseudopunctipennis	8	40.0	23.8	17.5	1930	16.6	2102	2267
An. punctimacula	7	36.5	22.3	16.0	1906	16.7	2119	2283
An. oswaldoi s.l.	5	42.5	24.6	19.5	1230	20.3	1447	1639

[1] From Chapter Three.

[2] Calculated from currently-observed maximum altitude using equation $y = -7E\text{-}7x^2 + 0.003x + 25$.

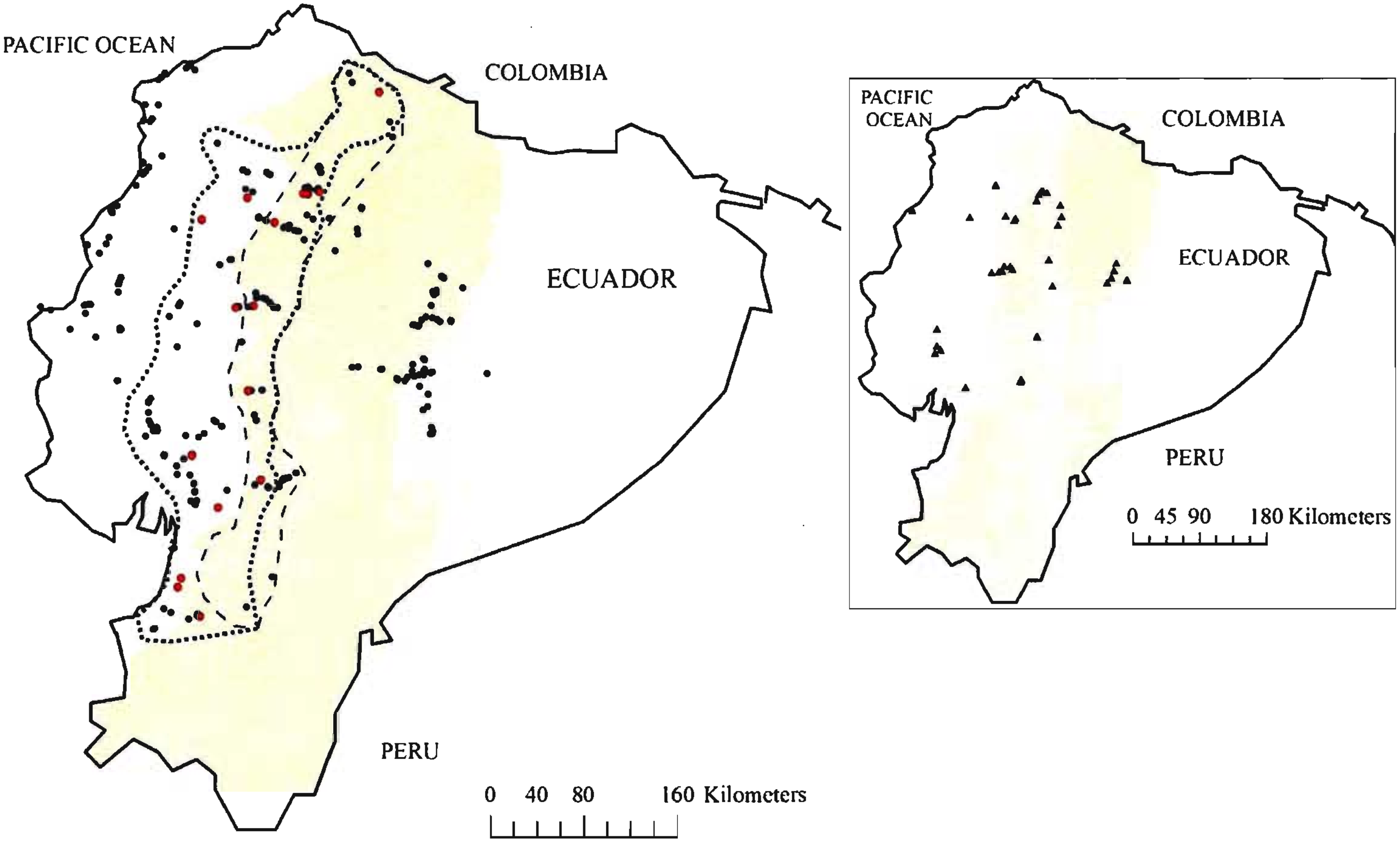

Figure 4-1 Map of all study sites sampled in 2008, 2009 and 2010 (black and red circles) in Ecuador. As an example of larval absent site selection, *Anopheles punctimacula*-positive sites are indicated with red circles, absent sites (●) within the species' current distribution are outlined with a thick dotted line, and absent sites (●) within the highland distribution are outlined with a dashed line. Inset: Map of Thermochron iButton® deployment sites (▲). In both maps, the approximate extent of the Andes is shaded in green. Maps made in ArcGIS v. 9.3 (ESRI).

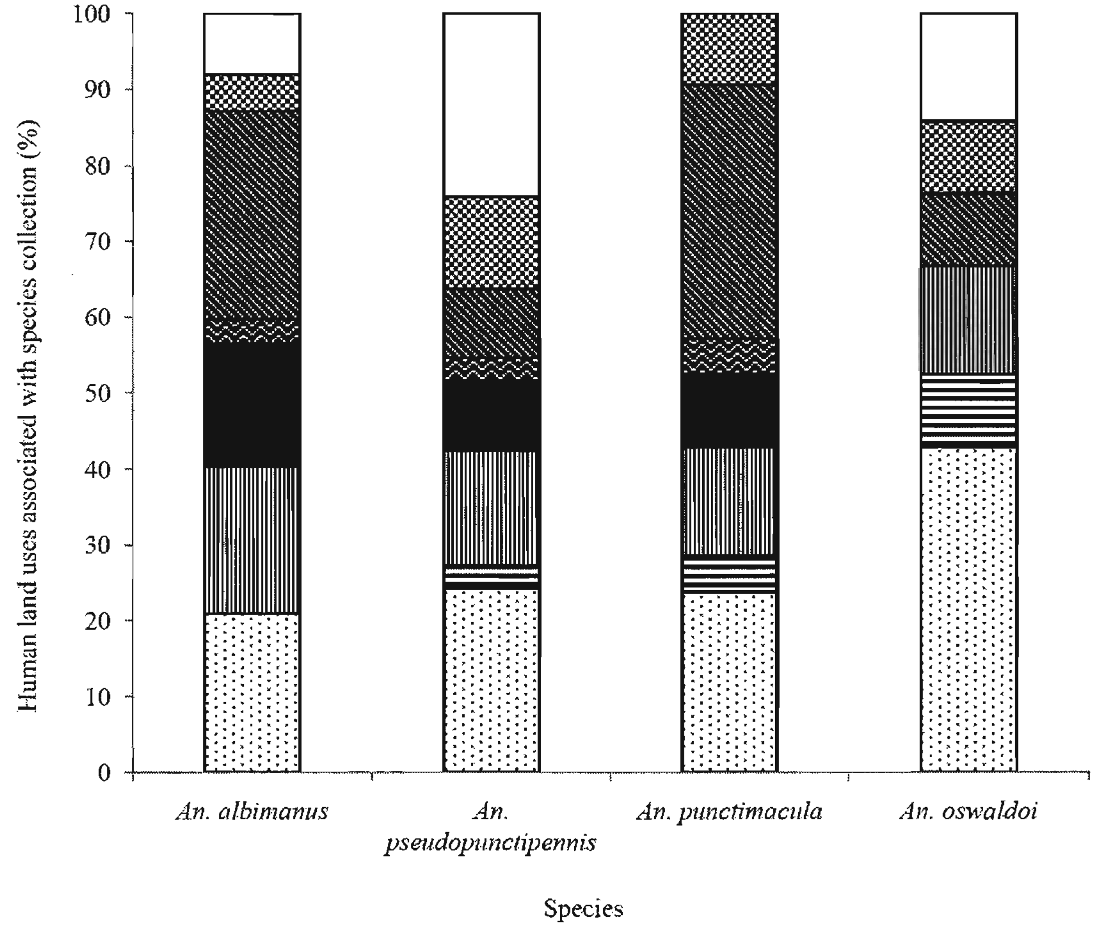

Figure 4-2 Frequencies of human land uses on sites where *Anopheles albimanus* (n=48), *An. pseudopunctipennis* (n=29), *An. punctimacula* (n=18), and *An. oswaldoi s.l.* (n=21) larvae were collected in 2008, 2009 and 2010, for dominant human land use categorizations at a given site. Land uses were categorized from top to bottom as: no land use (solid white), tourism (checker), plantation (diagonal lines), fish farm (waves), cattle pasture (solid black), human habitation (vertical lines), construction (horizonal lines), and road (speckled).

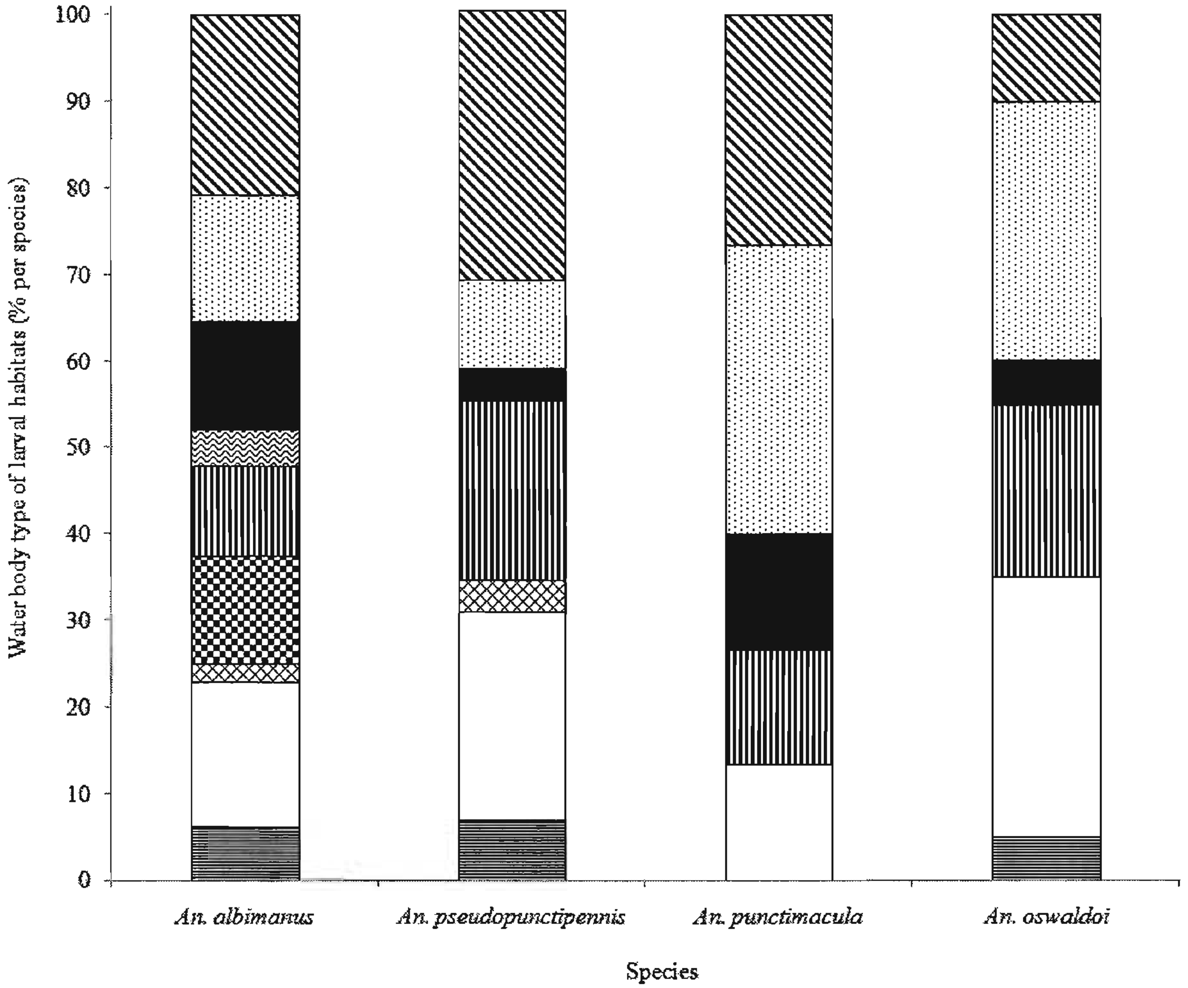

Figure 4-3 Water body types where *Anopheles albimanus* (n=48), *An. pseudopunctipennis* (n=29), *An. punctimacula* (n=18), *and An. oswaldoi s.l.* (n=21) larvae were collected between 2008-2010, in Ecuador, expressed as a cumulative percentage. Water body types were categorized as: river edge (diagonal lines), pond (speckled), marsh (solid black), lake (waves), water-filled depression (vertical lines), rice paddy (checkerboard), irrigation canal (diamond), roadside ditch (solid white), and inland ditch (horizontal lines).

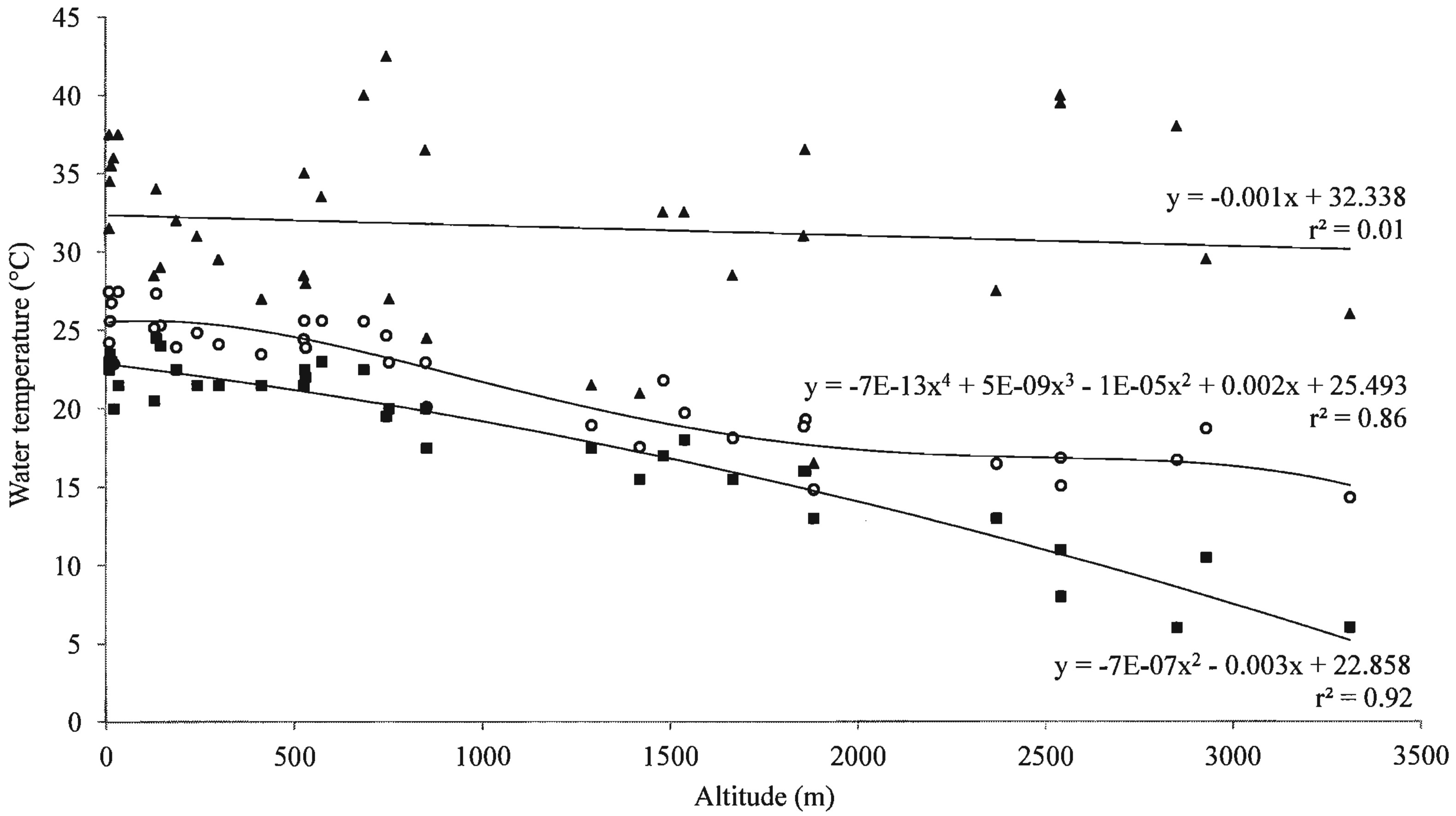

Figure 4-4 Relationship between maximum temperature (▲), average temperature (○) and minimum temperature (■) and altitude recorded at 10-minute intervals by Thermochron iButtons®. Measurements taken at the surface-air interface of current and potential *Anopheles* larval habitats, during a three day period in Ecuador in November and December, 2010, (n=36). Lines of best fit and r^2 values are provided for each temperature category.

Chapter Five

Larval habitat associations with human land uses, roads, rivers and land cover for *Anopheles albimanus*, *An. pseudopunctipennis* and *An. punctimacula* (Diptera: Culicidae) in western highland Ecuador

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5.1 Introduction

Incidences of highland malaria have been observed in recent years on several continents (e.g. Shanks et al. 2000; Hay et al. 2002b; Rutar et al. 2004). Although the link between highland malaria to climate change has been discussed by several authors (Githeko et al. 2000; Epstein 2001; Reiter 2001; Kovats et al. 2001; Hay et al. 2002a; Reiter 2008), the role of climate change is complex and generally difficult to relate to malaria epidemics. On the other hand, land use and/or land cover alteration has been related directly to increasing highland populations of specific anopheline malaria vectors (reviewed in Brinkmann 1994) that thereby increase the transmission of the malaria parasite among highland dwelling human populations (e.g. Afrane et al. 2005).

Conversion of regions to a particular land use can alter habitat parameters for mosquito species (reviewed in: Reiter 2001; Chhabra et al. 2006). Certain land uses are often associated with anopheline larval habitat on multiple continents, including rice farms (Brinkmann et al. 1994; Reiter 2001), fish farms, particularly abandoned fish ponds (Reiter 2001), as well as cattle pastures where footprints create permanent depressions that fill with rainwater (Reiter 2001).

In highland regions, anopheline larval habitat is limited by steep topography to areas where water accumulates (e.g. Balls et al. 2004), often in valley bottoms (Munga et al. 2009). In the Usambara mountains of Tanzania, *Anopheles* larvae live in sunlit pools that offer a warmer microclimate, and may be associated with the irrigated vegetable-growing terraces (Bødker et al. 2000). *Anopheles gambiae* was associated with open habitat in farmlands and pastures in highland Kenya, particularly in water-collecting valley bottoms (Munga et al. 2009).

In some circumstances, reduced canopy cover in the highlands (in disturbed or deforested habitats) influences anopheline larval habitat suitability more strongly than the presence or absence of specific land uses (Patz et al. 2004; Minakawa et al. 2005; Chhabra et al. 2006; Guerra et al. 2006; Pattanayak et al. 2006; Yasuoka & Levins 2007). Deforested areas are approximately 0.5°C warmer than adjacent forested regions in highland Kenya (Afrane et al. 2005; Afrane et al. 2006). *Anopheles gambiae* Giles in highland Kenya have 64.8-79.5% higher fecundity rates, a 40% higher net reproductive rate and a 29% higher vector capacity (Afrane et al. 2006), as well as a shortened gonotrophic cycle in deforested rather than forested regions (Afrane et al. 2005). Increasing temperatures have been shown to have non-linear, positive effects on anopheline larval development rates (Paaijmans et al. 2010). In South America, deforestation has also been associated with malaria incidences, particularly for road-building, gold mining, or permanent colonization or urbanization, most prominently in parts of the Amazon (Pinheiro et al. 1977; Walsh et al. 1993; Tadei et al. 1998; Póvoa et al. 2003; Guerra et al. 2006; Caldas de Castro et al. 2006; Yomiko Vittor et al. 2006; Pan et al. 2010; de Oliveira et al. 2011). In the tropics, there has been a widespread trend of forest conversion to grasslands and grassland conversion to permanent and temporary crops (Chhabra et al. 2006).

Recent studies have identified the presence of multiple *Anopheles* species as well as the occasional small-scale epidemic of malaria in the highlands of the northern Andes (Rutar et al. 2004; Chapter Three). Although land use and land cover are strongly associated with *Anopheles* larval habitat in the highlands of Africa, the associations of the highland-occurring *Anopheles* species in the Andes with land uses have been hitherto unknown. In this study, we evaluate the hypothesis that land use change is responsible for the proliferation of multiple vector species in the western highlands by creating suitable larval habitat. We predict that current land uses would provide potentially suitable habitat for larvae of three species of western-occurring malaria vectors. In this study, we relate the most common land uses, as well as the presence of roads and rivers on the western cordillera of the Andes to potentially suitable larval habitat for *Anopheles albimanus, Anopheles pseudopunctipennis* and *Anopheles punctimacula*. We also compare the distribution of these species based on recent collections to published land cover classification maps. The purpose of this study was to identify human land uses that provide potentially suitable larval habitat for each of the three common *Anopheles* species, as well as to relate any of these species to the presence of roads and rivers that may serve as lowland-highland corridors, and thereby identify priority habitat types for intervention programs.

In Ecuador, highland valleys were afflicted with cases of highland malaria during the 1940s, vectored by *An. pseudopunctipennis* (Levi-Castillo 1945b; Chapter Two). However, Levi-Castillo (1945b) documented the removal of this vector from highland valleys near Quito through the elimination of larval habitat and the use of chemical insecticides. Since that time, highland areas have been generally considered malaria-free (Chapter Three).

Land use changes in the Ecuadorian Inter-Andean valleys, particularly the conversion of highland páramo and forests to crops and pasture, occurred over vast areas during pre-Columbian times (Sarmiento 2002). However, widespread land use changes have occurred in Ecuador during the last century in the coastal and Amazonian regions. The humid tropical forests of the coast were colonized during the cocoa boom in the 1920s and the banana boom in the 1950s, during which an extensive road network was built linking ports to inland coastal cities and highland Andean cities (Wood 1972; Bromley 1981). This road building led to informal colonization of foothill regions (ca. 500-1500 m) to grow sugarcane and oranges (Wood 1972; Bromley 1981). The northern Amazon was heavily colonized during the oil boom in the 1970s, when large-scale road networks were built to accommodate the oil industry (Wood 1972; Bromley 1981). Settler colonies built slash and mulch polycultures to farm rice, yams, corn, plantain and yucca, that were later frequently converted to pasture (Hiraoka & Yamamoto 1980).

More recent studies of land use in Ecuador indicate the presence of permanent crops at lower elevations of the Andes, with denser human populations associated with temporary crops. Grasslands for pasture are associated with fertile soil, whereas economically poor areas are often associated with natural vegetation (no human land use) (de Koning et al. 1998). On the coast, permanent export crops with widespread irrigation are favoured, whereas in the Amazon, there is a trend for agricultural colonists to convert forest to grassland to be used as pasture (de Koning et al. 1998). Spatial models predict an increase of temporary crops in the Andean highlands, and a spreading of grassland in all areas of the country, particularly due to continued deforestation of Amazonia (de Koning et al. 1999). If highland-occurring anopheline species are associated with cattle pasture, this widespread conversion of forest to grassland may provide more suitable habitat in highland regions, thereby facilitating the spread of previously coastal species into highland regions.

5.2 Materials and Methods

5.2.1 Land use and potential anopheline larval habitat availability

In the present study, we attempt to characterize anopheline larval habitat availability in the highlands, i.e., steep-topography areas of the western Andes of Ecuador. These lie conservatively from 500 m to 2000 m in elevation, the latter being the current maximum altitudinal limit for collections of the three most common *Anopheles* species (Chapter Three). To determine potential habitats made available by highland land uses, it was first necessary to characterize the most common land uses present between 500 m to 2000 m. To that end, researchers surveyed land uses present along six altitudinal transects in the western Andes in 2009 and 2010, along the east-west roads from: Quito to La Independencia (ca. N01°02'), Pilaló to Quevedo (ca. S00°53'), Guaranda to Puebloviejo (ca. S01°35'), Chimbo to Babahoyo (ca. S01°45'), Alausí to El Triunfo (ca. S02°18') and Loja to Machala (ca. S03°52'). Researchers progressed very slowly along the roadway in a motor vehicle and stopped at 100 m elevation intervals to record the dominant land use, vegetation type, presence of water bodies, geographical coordinates and altitude observed on the right-hand side of the roadway on the downhill commute (using a Garmin® GPS eTrex Summit). The six most commonly observed land uses were ascertained graphically and used in subsequent analyses.

To determine the potential anopheline larval habitat available on the most common land uses within the highlands (determined above), researchers traveled along accessible roads in the western Ecuadorian Andes in summer, fall and winter during 2009 and 2010 and searched for the following land uses: cattle farms, banana plantations, mixed tree plantations (including citrus fruits), sugarcane plantations and empty lots/cleared land. To introduce randomization, at each potential site researchers flipped a coin to determine if the property would be sampled, then, permission was sought from the landowner or manager to enter the property for the purpose of the survey (except empty lots/cleared land, which were most often public property). Between 21 and 30 sites were sampled for each land use type.

At each site, researchers recorded the geographic coordinates and altitude using a GPS as above, and the air temperature. Researchers then moved about the property and observed every potential anopheline larval habitat (i.e., standing water or slow-moving water bodies), recording the length, width and depth, water temperature (Hanna Instruments HI98129 combination water tester), percentage cover of algae and macrophyte vegetation (10% classes), incident sunlight (10% classes) and water clarity (10% classes). In every water body, water was dip-sampled using a white plastic dipper (BioQuip) for *Anopheles* larvae a standard of 30 times (although five dips were used for very small water bodies). When larvae were collected, they were placed in vials containing 95% ethanol, transported to the laboratory, and identified to species using the morphological key of Gorham et al. (1973) and molecular techniques as described in Chapter Three. Specimens not destroyed for molecular sequencing were deposited in the Ecuadorian National Collection in Quito (PUCE) and nucleotide sequences were deposited in GenBank (accession numbers JN412826-JN412843) When property sizes were greater than five hectares, researchers sub-sampled a portion of the property (approx. two hectares in size).

For sites with water bodies, an average value of the parameters above was obtained for each site and entered into a Principal Components Analysis to determine its relation to land use type (entered individually with dummy variables) (CANOCO 4.5 2002). Data were standardized by conversion of values to Z-scores using the mean and standard deviation of the data set to ensure that factors were similarly weighted, and centered around zero prior to analysis (Whitlock & Schluter 2009). Water bodies were then judged for potential suitability for *An. albimanus*, *An.*

pseudopunctipennis and *An. punctimacula* larvae using the following criteria, derived from field-based observations made during work conducted in Chapter Four. In all cases, all criteria represent values that are inclusive, i.e., are more likely to include a site as potentially suitable for larvae than not, since there is some natural variation in larval habitat site suitability. We therefore chose limiting temperatures and other criteria that would include all observed values from field sites. *Anopheles albimanus*-suitable habitat were permanent water bodies with some floating algae (at least 10% cover) and within a temperature range of 22 - 33°C based on the range of field-measured temperatures (n = 48 sites). *Anopheles pseudopunctipennis*-suitable habitat contained some floating algae (at least 10% cover), 40% clarity or greater, less than 10% cover of emergent vegetation, and temperatures within the field-measured range of 20.3 - 37.0°C (n = 29 sites). *Anopheles punctimacula*-suitable habitat were water bodies with less than 70% incident sunlight and less than 50% algae cover, with temperatures in the range of 19.5 - 28.6°C (n = 18 sites). No larvae of any species were collected from sites with values beyond these parameters.

5.2.2 Rivers and road edges as potential anopheline larval habitats

Water bodies associated with rivers and roads were assessed as potential anopheline larval habitats in highland regions. Since roads and rivers form continuous rather than discrete sample areas, the site-selection process for sites was different than for land uses. Researchers searched in the western Andes for river and road sites that had at least one stagnant or slow-moving body of water that may have been potentially suitable for anopheline larvae, and flipped a coin to determine if the site would be used or not. At each site, all water bodies were scrutinized using the same criteria and sampled for anopheline larvae as above (for land uses) within a 10 m circular study radius. A total of 41 river sites and 38 road edge sites were surveyed. Data were analyzed using a PCA and assessed for potential species suitability as described above.

To determine the spatial distribution of road-edge habitats, researchers undertook three altitudinal transects along the following roads: Ibarra to Lita (ca. N00°50'), Quito to Puerto Quito (ca. N00°06') and Alausí to El Triunfo (ca. S02°18'). We were restricted to these roads due to safety issues regarding conducting research in traffic-heavy sections of other roads. Researchers traveled slowly along the road and stopped at every stagnant or slow-moving water body observed on the right side of the road. Water type, size, coordinates and altitude were recorded as above. To determine the degree of clustering of water bodies along each road, water bodies were plotted in ArcGIS v. 9.2 (ESRI 2008) and analyzed using the Spatial Autocorrelation (Moran's I index) tool, weighted by total water surface area. Moran's I (MI) determines whether spatial data are clustered, dispersed or randomly distributed (Moran 1950).

5.2.3 Land cover classification of collection localities

Land use can affect larval habitat availability through changes to the vegetation architecture and degree of canopy openness. For this reason, we determined the land cover for each of the positive collection localities from Chapter Three for each of the three *Anopheles* species above. Collection localities were plotted in ArcGIS v.10 with the GlobCover© 2009 land cover map, published by the European Space Agency and the Université Catholique de Louvain. The GlobCover© data present mosaics of the 22 land cover classes of the United Nations Land Cover Classification System at 300m resolution, derived from a time series of global MERIS (MEdium Resolution Imaging Spectrometer) (Bontemps et al. 2011) (available at ArcGIS online, 2011). Land use values on raster tiles corresponded to distribution (collection) points were enumerated and compared to expected values derived from the availability in the overall landscape using a Chi-square test.

5.3 Results

5.3.1 Land use and potential anopheline larval habitat availability

Land uses of a total of 263 sites were recorded along five altitudinal transects. Of these, 117 sites were observed to be "natural", i.e., were not used for any discernable purpose and were not recently disturbed in any way, with an average altitude of 1705 ± 654 m (mean + standard deviation). Of natural sites, 47 of these were too steep to be used by humans (i.e., had cliffs), with an average altitude 1617 ± 313 m. Human-use sites observed along the transects are plotted in Figure 5-1. Within the elevations of interest (500 - 2000 m), the most common land uses observed were: cattle pastures (51 sites), human habitations (13 sites), banana plantations (9 sites), sugarcane plantations (8 sites), mixed tree plantations, including citrus species (6 sites), and cleared land/empty lot/construction sites (4 sites) (Figure 5-1). In a concurrent study, human habitations provided almost no standing water other than concrete laundry tanks, rain barrels and septic tanks, all of which are usually unsuitable for anopheline larvae (Chapter Seven). Therefore, the remaining five land uses were used in a subsequent land use survey.

In the survey of properties of pre-established land uses, the largest of the five property types were cattle pastures (n = 30; area 36117 ± 77351 m^2), with an elevation of 1435 ± 517 m, then banana plantations (n = 28; area 13215 ± 32835 m^2), with an elevation of 887 ± 534 m, sugarcane plantations (n = 21; area 6870 ± 8682 m^2), with an elevation of 1249 ± 437 m, mixed tree plantations (n = 25; area 6012 ± 6198 m^2), with an elevation of 795 ± 502 m and finally empty lots (n = 30; area 2335 ± 2504 m^2) with an elevation of 1294 ± 557 m. The five human land uses were related to water body characteristics measured at each water-present site using a Principal Components Analysis (Figure 5-2). In the first bi-plot, banana plantations and mixed tree plantations were strongly related on Axis 1 to depth and negatively related to incident sunlight, while empty lots were strongly associated with incident sunlight (Figure 5-2a). On Axis 2, cattle pasture was strongly related to macrophyte vegetation, increasing surface area, clarity and algae cover (Figure 5-2a). In the second bi-plot, banana plantations and empty lots were associated with incident sunlight and to a lesser extent, depth and higher temperature on Axis 1 (Figure 5-2b). On Axis 3, cattle farms were associated with macrophyte vegetation and algae cover, as well as with altitude, and surface area (Figure 5-2b). A total of 54.7% of the cumulative variance was explained by the first three Axes, with the following eigenvalues: Axis 1: 0.215, Axis 2: 0.183, Axis 3: 0.149.

All sites with standing water were analyzed for potential larval habitat suitability for the three anopheline species and plotted in Figure 5-3a. The human land use type with the most sites with available standing water were empty lots, (40% of sites with some type of standing water), 13.3% of which were potentially suitable for *An. pseudopunctipennis* and 10% potentially suitable for *An. albimanus* (Figure 5-3a). In second place, 33.3% of cattle pastures had some type of standing water, 13.3% potentially suitable for *An. punctimacula* and 10% potentially suitable for *An. albimanus* (Figure 5-3a). *Anopheles albimanus* larvae were collected from one empty lot site and one cattle pasture site. The other land uses in highland regions were quite dry, with only16% of mixed tree plantations, 14.3% of sugarcane, and 7.1% of banana farms with any standing water, most of which was unsuitable for anopheline larvae (Figure 5-3a). *Anopheles* larvae were not collected at any other sites.

5.3.2 Rivers and road edges as potential anopheline larval habitats

Of the three roadside water transects, Ibarra to Lita provided 21 water-present sites, followed by 15 sites for Quito to Puerto Quito and 6 sites for Alausi to El Triunfo. Most water bodies were roadside ditches, although permanent tire tracks, inland ditches, and construction pools were also associated with roads. Spatial Autocorrelation analysis indicated that all three transects were significantly randomly distributed, rather than clustered at specific elevations (Ibarra-Lita transect: MI = 0.21, Z = -0.57; Quito-Santo Domingo transect: MI = -0.02, Z = -0.08; Alausi-El Triunfo transect: MI = -0.22, Z = -0.04). *Anopheles* larvae were only collected at four sites, with *An. pseudopunctipennis* larvae collected at two sites of ca. 1500 m, and *An. albimanus* larvae collected at two sites of ca. 600 m in elevation.

Road and river sites were randomly sampled at mean elevations of 1411 ± 407 m and 1034 ± 537 m, respectively. Figure 5-4 presents the bi-plot results of a Principal Components Analysis relating roads and rivers to water body characteristics. In the first bi-plot, rivers were associated with clarity and to a lesser degree, depth, whereas roads were weakly associated with macrophyte vegetation cover along Axis 2 (Figure 5-4a). The water body characteristics of temperature, incident sunlight and algae were inversely related to altitude (Figure 5-4a). In the second bi-plot, rivers and roads were pulled apart along Axis 3 (Figure 5-4b). Rivers were associated with temperature, depth, algae cover, clarity, and incident sunlight, whereas roads were associated with increasing altitude (Figure 5-4b). The number of active samples in the PCA were 79, and a total of 57.2% of the cumulative variance was explained by the first three Axes, with the following Eigenvalues: Axis 1: 0.296, Axis 2: 0.160, Axis 3: 0.116.

Roads and rivers were judged to have a high proportion of sites that would be potentially suitable for anopheline larvae. Roads were judged to provide the most potential habitat for *An.*

punctimacula (44.7%), followed by *An. albimanus* (26.3%) (Figure 5-3b). Rivers, on other hand, provided the most potentially suitable habitat for *An. albimanus* (58%), followed by *An. pseudopunctipennis* (29.3%) (Figure 5-3b). In terms of actual collections of anopheline larvae, one road site was positive for *An. albimanus* larvae. Rivers provided the most current *Anopheles* habitat, with a total of eight larval-present sites: five *An. pseudopunctipennis* sites, two *An. punctimacula* sites, and one *An. albimanus* site.

5.3.3 Land cover classification of collection sites

Collection sites for the three species were related in ArcGIS to the United Nations land cover classification system. All three species were the most commonly collected in sites classified as "closed/open broadleaved evergreen and/or semi-deciduous forest" (Figure 5-5), significantly more often than expected given its availability in the landscape (*An. albimanus*: $\chi^2 = 18.70$, df = 2, $P < 0.001$; *An. pseudopunctipennis*: $\chi^2 = 8.88$, df = 2, $P = 0.011$; *An. punctimacula*: $\chi^2 = 19.33$, df = 2, $P < 0.001$). *Anopheles albimanus* was also often associated with "mosaic cropland (50-70%)/vegetation", while *An. pseudopunctipennis* was less specifically associated with one land cover type; rather, it was broadly associated with several land cover classifications (Figure 5-5). *Anopheles punctimacula* was more specific and collected from fewer land cover types (Figure 5-5). We could not evaluate the statistical significance of each of these land cover associations individually due to too many land covers associated with too few collection sites.

5.4 Discussion

Our study has identified land uses (including rivers) that may be more amenable to the development of anopheline larvae in the western highlands of Ecuador. Cattle pasture can be considered the managed traditional land use of most concern. Cattle pasture was the land use most commonly observed during the transects (i.e., it is abundant), as well as having the largest property size of all the land uses examined. Thirty-three percent of cattle pastures surveyed contained standing water, with less than 13.3% potentially suitable for each of the anopheline species. Although the water bodies on cattle pasture were associated in the PCA with higher water clarity, which is favourable for all three species, and algal cover, which is very favourable for *An. albimanus* and *An. pseudopunctipennis*, water bodies were also associated with macrophyte vegetation cover, which is unsuitable for all three species (Chapter Three). Given the extent of cattle pasture in the highlands of Ecuador, priority ought to be given to ponds and other "natural" water bodies used to provide cattle with drinking water. The use of drainable troughs, including the frequently used half-tire troughs, is recommended to allow cattle farmers to eliminate potentially suitable pooling of water. Similarly, flooded parts of pasture are of concern as well; in Mexico, *An. albimanus* is associated with regions of flooded cattle pasture (Rejmankova et al. 1991).

Although more standing water was observed on empty lots, these were the least-observed land use in the altitudinal transects and the smallest in size. Incident sunlight is high, favouring *An. albimanus* and *An. pseudopunctipennis* larvae, where water bodies exist. Most often, construction activities and heavy machinery tear up the surface of the earth, creating permanent depressions that fill with water. Better management of these sites would involve ensuring that the ground is carefully levelled prior to its temporary or permanent abandonment by a construction company.

Unlike on the coast, banana, sugarcane and mixed tree plantations are generally dry in highland (steep) regions and do not provide much, if any, potentially suitable standing water for

Anopheles larvae. Therefore, none of these common land uses ought to be considered priority areas for standing water elimination or malaria prevention in the highlands of Ecuador. In Belize, the runoff from sugarcane plantations can lead to a bloom of *Typha domingensis* Pers., which is negatively correlated with the presence of *An. albimanus* larvae (Grieco et al. 2006), and may exclude larvae from sugarcane-adjacent water bodies in Ecuador as well.

Roads were associated in the PCA with macrophyte vegetation cover, which is an unsuitable characteristic for larval habitat of some *Anopheles* spp. However, roads often provide sunlit, open habitat, which were judged in 44% of cases to be potentially suitable for *An. punctimacula* and 26% of cases for *An. albimanus*. Road-associated water bodies were not clustered at specific altitudes with less severe slopes, but rather, randomly distributed along roadways. Cement ditches placed along the roadways often became clogged with debris from adjacent trees and rocks, and subsequently filled with water permanently. Although road workers do periodically remove debris, this study documents the use of these water bodies by anopheline larvae, necessitating more vigilance to ensure that roadside ditches along highways are frequently drained, especially at lower elevations.

River-associated habitat appeared to be important to anopheline larvae in the western highlands of Ecuador. Similarly, in the highlands of Papua New Guinea, *Anopheles farauti* has been observed to breed in high-altitude river flats (Sharp 1982). Rivers were associated with greater water clarity, higher temperatures, the presence of algae and incident sunlight, all of which are very suitable characteristics for *An. albimanus* and *An. pseudopunctipennis*. Although 58% of sites were judged suitable for *An. albimanus* and 29% for *An. pseudopunctipennis*, *An. albimanus* were collected more often in low-altitude river systems, while *An. pseudopunctipennis* were abundant in highland parts of rivers that feed from even higher-altitude watersheds. *Anopheles punctimacula* frequently co-occurs in the same river as *An. pseudopunctipennis,* although the two were never found to cohabit the same pool of water within the river. The presence of *Anopheles* larvae in highland rivers was observed historically in Balzapamba (650 m), where *An. pseudopunctipennis* larvae were collected in river-associated pools formed by an earthquake and subsequent landslide (Hanson and Montalvan 1938). Similarly, Levi-Castillo documented *An. pseudopunctipennis* in highland river edges in the late 1940s (Levi-Castillo 1947).

If rivers produce a large proportion of suitable habitat, they may provide corridors for the passage of anopheline mosquitoes to move into higher-altitude regions exhibiting favourable meteorological conditions. Although rivers are not technically a human land use, rivers in Ecuador are modified by adjacent land uses that sometimes includes the dumping of rocks and boulders into the stream, the construction of off-shoot canals for irrigation and drainage (that are not well-maintained) and the presence of water-collecting tubes that force water to collect in pools and slow-moving streams on the edges of rivers. Another possible source of larval habitat is the reduction of river flow due to either the diminished glacier-fed streams from glacier disappearance due to climate change (Bradley et al. 2006; Vergara et al. 2007) or to the modification of the river flow due to the construction of hydroelectrical dams (Vergara et al. 2007; L. Pinault, personal observation). When the flow is sufficiently reduced, a greater surface area is available on the river edge within the original streambed for the formation of rock pools and slow-moving water streams. It is therefore the author's recommendation that highland rivers and streams (ca. 1500 - 2500 m) be monitored for the presence of *Anopheles* larvae in the western Andes to forecast and prevent future highland malaria incidences. As well, construction and hydrological projects that reduce river flow ought to ensure that the river-edge walls are sufficiently steep, or that boulders and other impediments are removed from the river, to prevent the formation of water pools and slow-moving offshoots on the edges of rivers.

Since land use including roads and rivers is a strong driver of highland malaria, several authors have stressed the importance of relating specific land use changes to the distribution of infectious disease to predict and control future incidences and epidemics (e.g. Patz et al. 2004; Munga et al. 2009). For example, in Nigeria, water bodies related to specific types of farming such as trenches, dams and irrigation have been identified as potential anopheline larval habitat (Oladepo et al. 2010). Farmers in these regions have been provided with these data, resulting in many farmers now managing their property to prevent the formation of standing water (Oladepo et al. 2010). In the management of highland anopheline species, insecticide spray programs used with larval habitat management have been demonstrated to be particularly effective due to the limited number of habitats available (e.g. de Zulueta et al. 1964). The identification of specific highland habitats associated with land uses, such as permanent cattle watering holes/ponds and modified rivers, allows malaria prevention programs to target habitats in highlands and thereby reduce populations of potential malaria-vectoring mosquitoes.

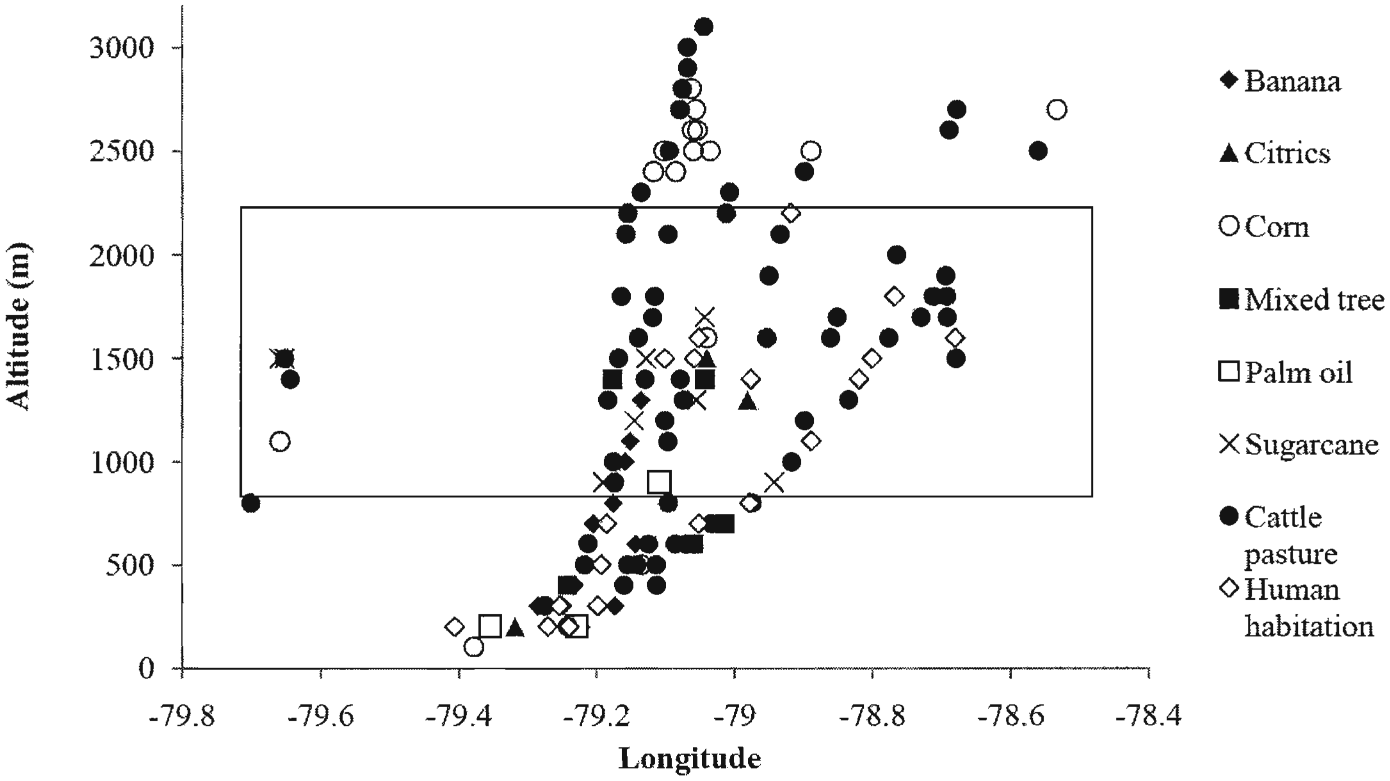
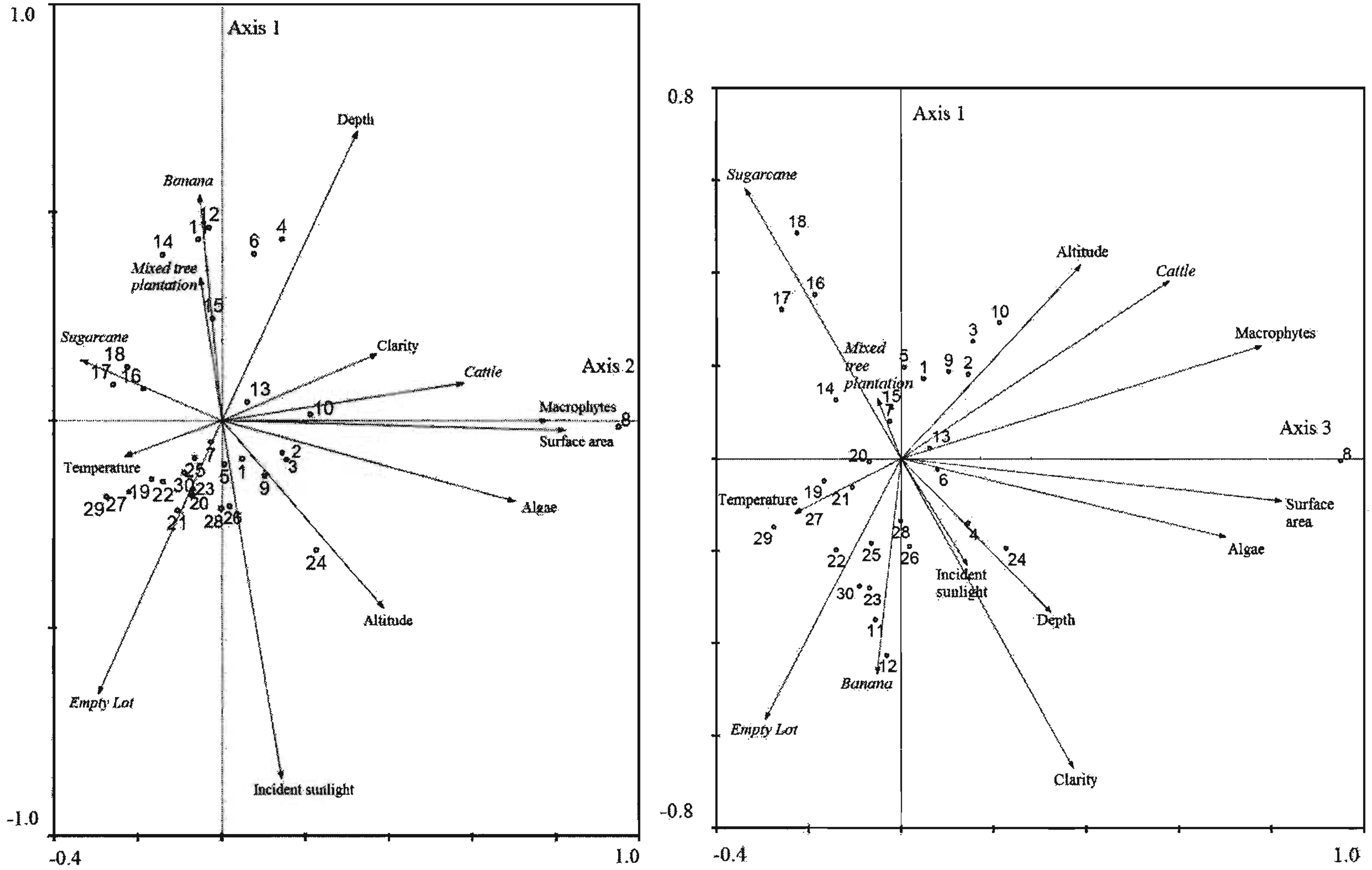


Figure 5-1 Generalized land uses observed along six altitudinal transect in the western Ecuadorian Andes, plotted by longitude. Elevations of biological interest are indicated within a black rectangle and land use types are indicated by symbols (see legend).



a) b)

Figure 5-2 Bi-plot results of Principal Components Analysis of land uses and habitat characteristics for a) Axes 1 and 2 and b) Axes 1 and 3. Output from CANOCO v. 4.5 (Plant Research International 2002).

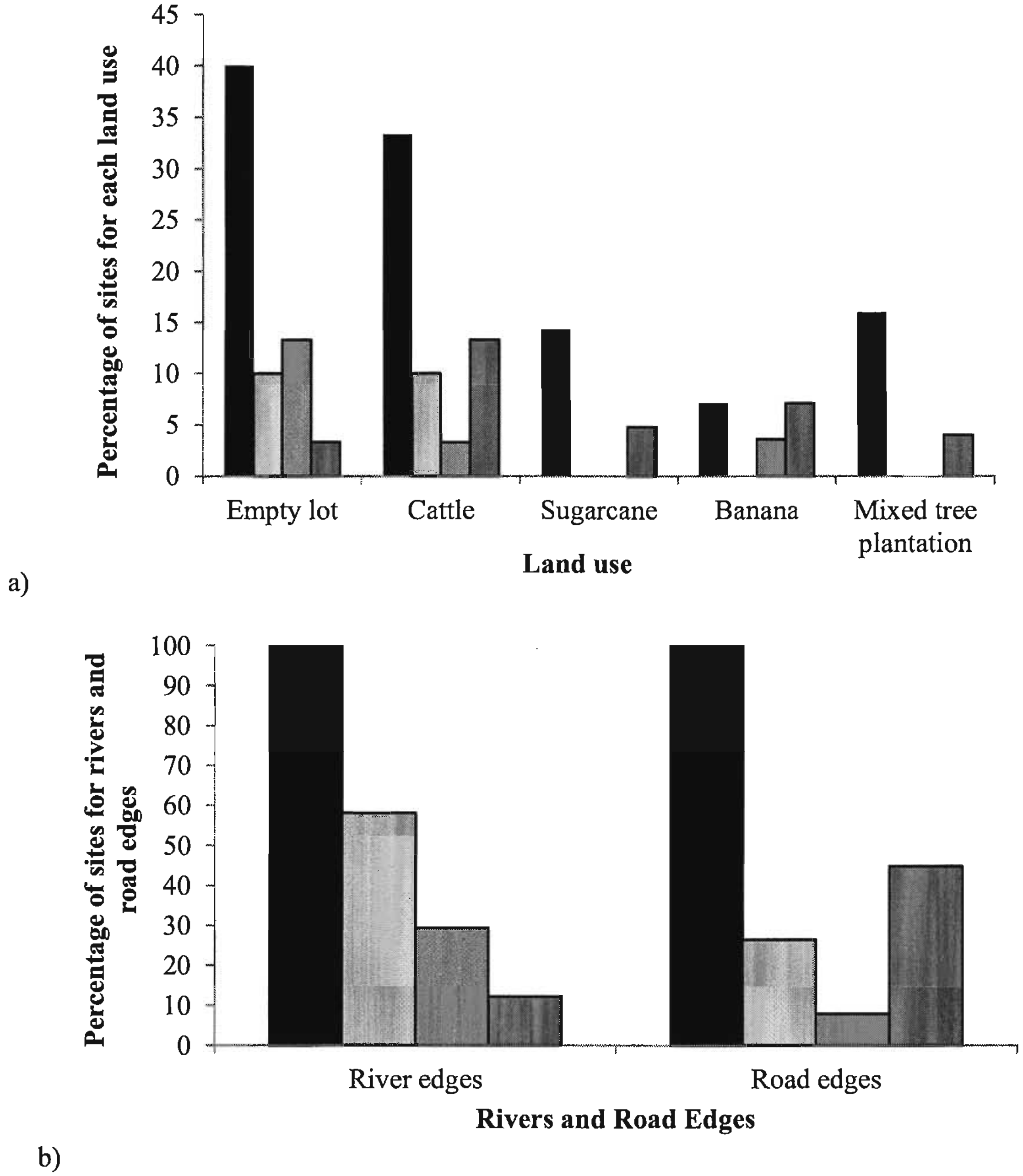
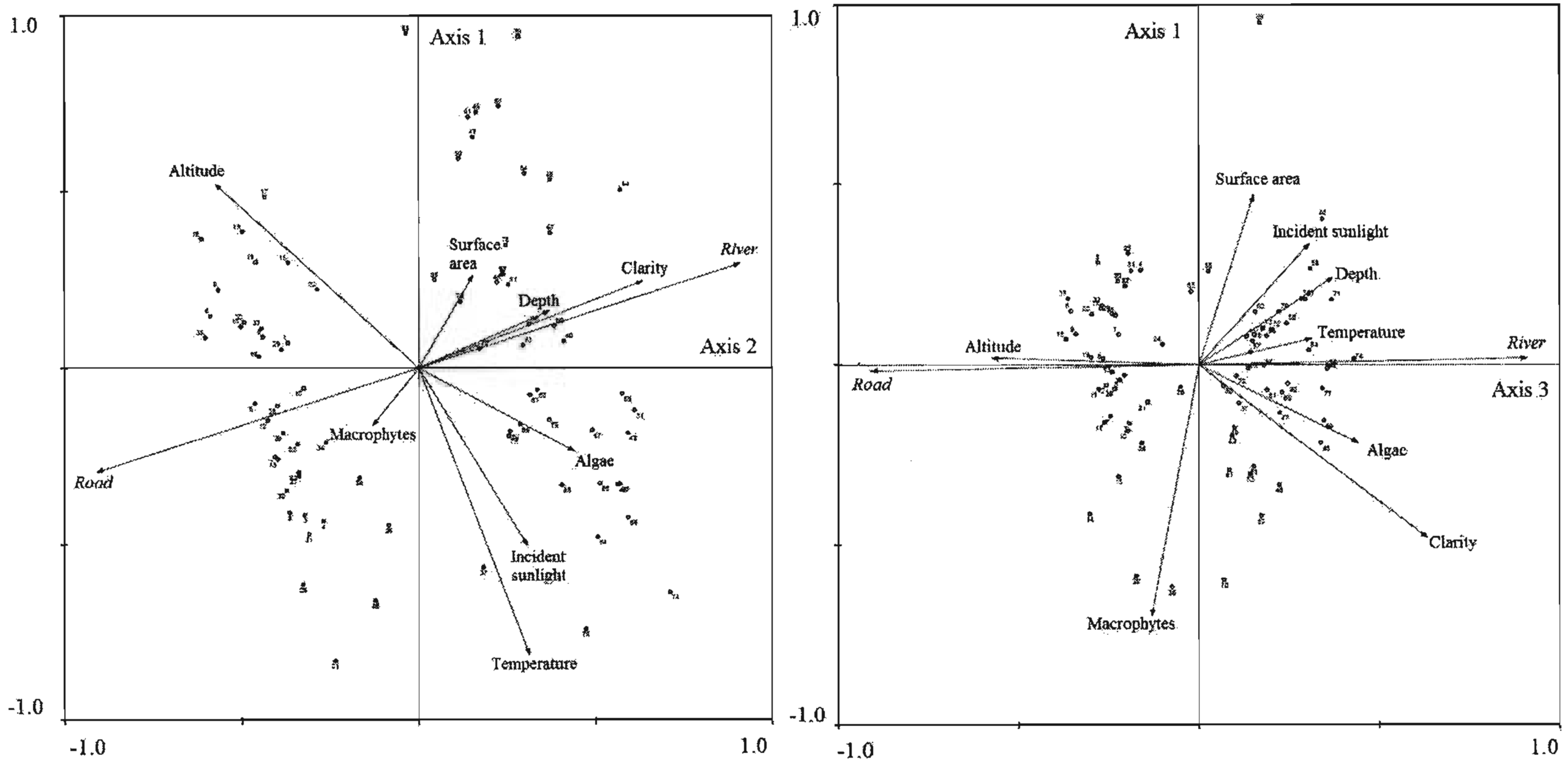


Figure 5-3 Percentage of sites with: standing water available (black bars) and potentially suitable habitat for anopheline larvae: *An. albimanus* (light grey), *An. pseudopunctipennis* (medium grey) and *An. punctimacula* (dark grey); for a) different land uses and b) river and road edges. Note difference in y-axis ranges between a) and b).



a)

b)

Figure 5-4 Bi-plot results of Principal Components Analysis of rivers/roads and habitat characteristics for a) Axes 1 and 2 and b) Axes 1 and 3. Output from CANOCO v. 4.5 (Plant Research International 2002).

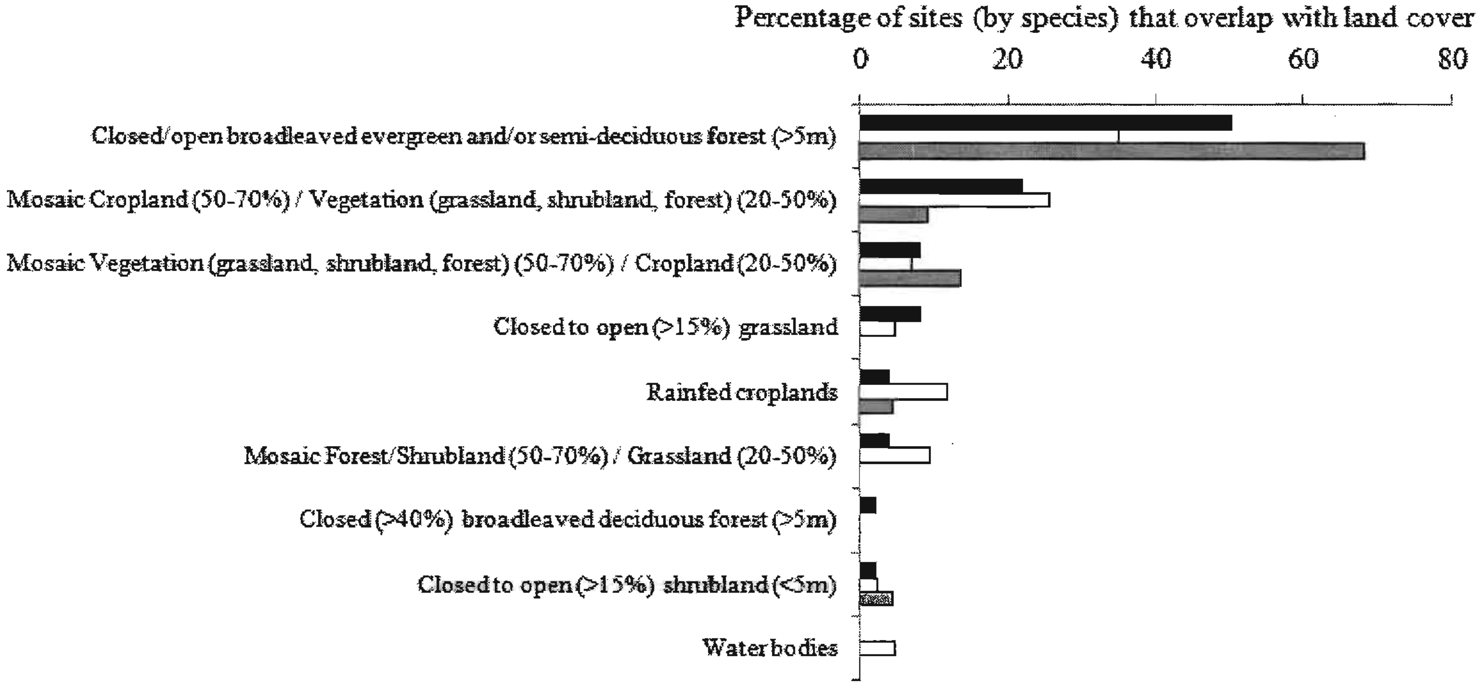


Figure 5-5 Percentage of collection sites of *An. albimanus* (black bars), *An. pseudopunctipennis* (white bars) and *An. punctimacula* (grey bars) larvae in Ecuador, categorized by geographic overlap of land cover categories from GlobCover - 2009 MERIS (MEdium Resolution Imaging Spectrometer FR mosaics for 2009), which uses 22 land cover classes from the UN Land Cover Classification System at 300m raster tile resolution (Data from ArcGIS online, ESRI). Analysis conducted in ArcGIS v.10 (ESRI 2010).

Chapter Six

A predictive spatial model for malaria vector dispersal into highland regions of the Ecuadorian Andes based on larval habitat

Lauren L. Pinault and Fiona F. Hunter

6.1 Introduction

The expansion of the geographic distribution of malaria vectors into higher latitudes and altitudes has been observed globally, attributed to regional climate changes, antimalarial drug resistance, deforestation and land use alteration, and/or the diminishing use of vector control measures (Martens et al. 1995; Zell 2004; Patz et al. 2005). Globally, the effects of climate on vector-borne disease have not been established, since changes in regional average temperatures have not always been substantial (e.g. Hay et al. 2002c). However, a global narrowing of the diurnal temperature range has been observed due mainly to a significant increase in minimum nightly temperatures (Easterling et al. 1997), and this trend is expected to continue based on IPCC scenarios (Meehl et al. 2007). Since both the malaria vector and parasite require temperatures above a minimum threshold to successfully complete development (Martens et al. 1995; Lindsay & Birley 1996; Martens et al. 1997; Lindblade et al. 1999), a change in overall minimum temperatures may have a substantial effect on the potential range of malaria.

Climate has been directly related to malaria epidemics (Bødker et al. 2003; Zhou et al. 2004), as well as to the distribution of malaria vectors in some cases (Lindsay et al. 1998). In highland Kenya, abnormally high temperatures and greater rainfall corresponded with the timing of malaria epidemics, where a 3.5°C increase in temperature was predicted to cause a 27-332% greater transmission intensity (Zhou et al. 2004). Malaria epidemics have been related to El Niño events in India, Colombia, Peru, and Venezuela, though not in Ecuador (Bouma & van der Kaay

1994; Bouma et al. 1997; Gagnon et al. 2002; Ruiz et al. 2006). Warmer temperatures not only affect habitat suitability along the margins of endemic areas, but also reduces the time period necessary for the mosquito's gonotrophic cycle (egg development), larval development, and the digestion of a blood meal (Martens et al. 1997; Rúa et al. 2005; Paaijmans et al. 2010). In highland Uganda, temperature decline with altitude explained 50% of the altitudinal reduction of mosquito populations, while the availability of highland breeding sites was also deemed necessary for mosquitoes to be present in the highlands (Bødker et al. 2003).

Several predictive models, including Geographic Information Systems (GIS)-based models, have been developed to relate land use/land cover and climate parameters to malaria incidence (reviewed in Carter et al. 2000). A global model of climate suitability for *Plasmodium falciparum* transmission combined with water availability predicted an increase in the number of transmission-suitable months in highland regions of Ethiopia, Eritrea, Rwanda, Burundi and parts of Kenya and Tanzania (Guerra et al. 2008). Suitable geographic regions for transmission of *P. falciparum* were identified using a decision-rule model based on climate parameters and parasite data from MARA, for Zimbabwe (Hartman et al. 2002). In the Brazilian Amazon, malaria cases among households at the community level were predicted by the use of Normalized Difference Vegetation Index (NDVI), where proximity to permanent forested areas corresponded with higher household malaria rates (de Oliveira et al. 2011). In Mexico, satellite images were used to quantify wetland and pasture to predict malaria risk for adjacent villages (Beck et al. 1994). A spatial model relating land cover to anopheline distribution in the Tanzanian highlands was developed using maximum entropy software and accurately predicted *P. falciparum* prevalence in young children (Kulkarni et al. 2010). In the Kenya highlands, *Anopheles gambiae* and *Anopheles funestus* adult densities were significantly predicted by canopy cover, derived from land cover spatial layers in GIS (Minakawa et al. 2005). Sophisticated spatial models have also been devised to relate global climate change to vector-borne disease incidence. The MIASMA (Modelling framework for the health Impact ASsessment of Man-induced Atmospheric changes) relates climate change, mainly through rainfall patterns (with a minimum monthly precipitation threshold), to changes in vector-borne disease distributions (Martens et al. 1999). In a separate study, a malaria transmission climate suitability index has been devised and related to local changes in rainfall but not to slow increments of global warming (Small et al. 2003). The U.S. Army has prepared a "mal-area" model that combines vector and parasite presence, human habitation, and climate suitability to predict malaria incidence (Foley et al. 2008). Other climate change/vector-borne disease models are based directly on meteorological predictions for climate change, through use of General Circulation Models (e.g. Patz et al. 1998a). Most models are global in scope and do not assess local environmental parameters that may be important for the presence of vectors and parasites, such as extremely heterogenous rainfall patterns. At a finer scale, in highland Kenya, a local topographic wetness index calculated from a Digital Elevation Model (DEM) was a strong predictor of household malaria (Cohen et al. 2010). The CLIMEX model is an algorithm created to relate the range of a species to climatic constraints that can also be used at a finer scale (Sutherst et al. 2000; Sutherst 2004), but its shortcoming is that it does not consider other possibly important constraints such as land use.

Here, we created a simplified Predicted Habitat Distribution Map (PHDM) for the three most common malaria vectors present on the coast and highlands of western Ecuador, using a combination of land cover and climate (minimum temperature) data layers. Using the PHDM, we are able to predict the effects of incremental minimum temperature changes on the distribution of

Anopheles albimanus, Anopheles pseudopunctipennis, and *Anopheles punctimacula* into highland regions. *Anopheles albimanus* is considered a coastal primary malaria vector, while *An. pseudopunctipennis* is a coastal secondary malaria vector and highland primary vector, and *An. punctimacula* is a secondary coastal vector throughout Central and South America (Rubio-Palis & Zimmerman 1997; Chapter Three).

We focus on the suitability of habitats for larvae rather than for adults, since adult mosquitoes are known to rest inside households in highland regions and can thereby survive very cold outdoor temperatures (Bødker et al. 2003; Paaijmans & Thomas 2011). The role of the *Plasmodium* parasite is not considered in the present model since *P. vivax* and *P. falciparum* require minimum temperatures of at least 14.5-15°C and 16-19°C, respectively, which can be overcome by adult mosquitoes resting inside human dwellings (Detinova et al. 1962; Martens et al. 1995).

While many previous studies base models of vector presence on air temperature, these models are not reflective of the larval habitat, i.e., the surface water temperature (Paaijmans et al. 2008). Therefore, we derive a minimum temperature data layer from a DEM using an equation from field-collected measurements of potentially suitable larval habitat. DEM-derived layers are considered to be more accurate than other data sources for modeling since they are regionally specific and reflect variation from heterogeneous topography (Guisan & Zimmermann 2000).

The western highlands of Ecuador are characterized by continuously rainy conditions, with extremely spatially variable rainfall levels (1000 - 4000 mm), due to an uneven topography (Buyaert et al. 2006). Most of the highland rainfall patterns are influenced by the Intertropical Convergence Zone (ITCZ) from the Pacific Ocean, although the northern highlands are influenced by sea surface temperature abnormalities in the tropical North Atlantic Ocean (Vuille et al. 2000; Buyaert et al. 2006). Unfortunately, long-term climate data from Ecuador is not readily available, since weather stations were only installed in the 1960s throughout the country (Vuille et al. 2000). Therefore, there are no data sets dating back to previous incidents of widespread highland malaria in the country (ca. 1940s) (Levi-Castillo 1945a). However, IPCC estimates for Ecuador predict a 2 - 3°C warming during the next century, with a minor increase in overall rainfall (5%) (Christensen et al. 2007). High altitude regions were heavily modified by humans during the Pre-Columbian age (Sarmiento 2002), and are presently characterized by permanent crops and irrigated cattle pasture (de Koning et al. 1998).

6.2 Materials and Methods

6.2.1 Data layers

The following continuous spatial data layers were incorporated into one or more of the species-specific models: minimum temperatures of larval habitats, slope, canopy closure, presence or absence of algae, presence of suitable land cover or rivers, and estimated dissolved oxygen (Figure 6-1). These data layers were obtained from various well-established sources. All data layers were placed in a matching project, the Geographic Coordinate System WGS-1984 using the Greenwich Prime Meridian prior to analysis.

A 30-arc-second (ca. 1km) Digital Elevation Model (DEM) of South America, GTOPO30, was published by the U.S. Geological Institute Earth Resources Observation and Science (EROS) Data Centre, and was accessed online from DataBasin.org from the Conservation Biology Institute. A data layer for minimum surface water temperature in potential larval habitats (Figure 6-2a) was derived from the GTOPO30 DEM using the ArcGISv.10 (ESRI 2010) Spatial Analyst: Map Algebra function, using a quadratic equation relating elevation to minimum nightly temperature measured in a field study using Thermochron iButtons©

(Embedded Data Systems) (Chapter Four): $y = -0.0000007x^2 - 0.003x + 21.658$, $R^2 = 0.92$, where y = water temperature (°C) and x = altitude (m).

Since very steep topography has been shown in previous studies on highland anophelines to limit the dispersal of adult mosquitoes (Balls et al. 2004), a spatially continuous layer indicating slope (in degrees of inclination) was calculated from the GTOPO30 DEM (USGS 1996) using the Surface: Slope tool in the Spatial Analyst toolbox of ArcGISv.10 (ESRI 2010) (Figure 6.2b).

The presence of algae is extremely important for larval habitat suitablility of *An. albimanus* and *An. pseudopunctipennis* (Chapter Four). A continuous surface layer for the presence of larvae was formed through interpolation of field-observed algae presence or absence points (n=273) using the Inverse Distance Weighted (IDW) technique in the Extraction: Interpolation toolbox in the Spatial Analyst toolbox of ArcGISv.10 (ESRI 2010) (Figure 6-2c). Through this statistical function, raster tiles were assigned intermediate values between 1 (algae present) to 0 (algae absent).

In Chapter Five, the presence of the larvae of different anopheline species was related to specific U.N. land cover classifications, and larvae were also associated with the presence of rivers. Land cover data layers were obtained from the GlobCover© 2009 land cover map, published by the European Space Agency and the Université Catholique de Louvain. The GlobCover© data presents mosaics of the 22 land cover classes of the United Nations Land Cover Classification System at 300 m resolution, derived from a time series of global MERIS (MEdium Resolution Imaging Spectrometer) (Bontemps et al. 2011) (Figure 6-2f). Although Kulkarni et al. (2010) warns against the use of land cover data which was not intended to distinguish larval habitat, all three common species were significantly associated with specific land cover types in Chapter Five. A raster data layer of river coverage was derived in ArcGISv.10 (ESRI 2010) from the polyline river layer from HydroSHEDS (Hydrological data and maps based on SHuttle Elevation Derivates at multiple Scales) for South America. HydroSHEDS was published by the United States Geological Survey in 2006, and is derived from elevation data from the Shuttle Radar Topography Mission at 3-5 arc-second resolution (approx. 0.9-10 km) (Lehner et al. 2006). A combined layer of either a suitable land use (Chapter Five) or the presence of a river was derived using Spatial Analyst: Map Algebra and Reclass tools in ArcGISv.10 (ESRI 2010). Since larval habitat suitability also depends on the presence or absence of incident sunlight (Chapter Four), we derived a second layer from the GlobCover 2009© land cover layer. Land cover classes were reclassified as open canopy, intermediate (half open/half closed) or closed canopy (Figure 6-2d).

Finally, since *An. punctimacula* larval habitats have been significantly related to calculated dissolved oxygen (DO) (Chapter Four), a surface layer for estimated dissolved oxygen was created using calculated point values for dissolved oxygen from Chapter Four (n = 284), which were then interpolated into a landscape layer using the IDW technique as above (Figure 6-2e).

6.2.2 Assignment of data values and model construction

Data layers used in the model were determined by the components of larval habitats deemed essential for larval habitat suitability for each species. Since we were not able to rate relative importance among the factors in the model for larval habitat suitability, we assigned each spatial data layer a contributing factor of one, where a total count of five would represent the most suitable potential larval habitat (Figure 6-1).

Minimum nightly temperatures can limit the survival of species at high altitudes (Lindsay et al. 1998), and were obtained from field observations in highland larval habitats in Chapter Four to extrapolate a spatial layer estimate of minimum nightly temperatures in larval habitats. Since *An. albimanus* was only collected in two sites at altitudes greater than 1000 m, we used the more conservative field-measured minimum temperature of 20.0°C. On the other hand, *An. pseudopunctipennis* is widespread in highland sites, therefore, we used the calculated estimate for minimum temperature of 16.6°C based on maximum recorded altitude. For *An. punctimacula*, the measured larval minimum temperature was lower than the calculated value for a highland site, although it may be an extreme site that dropped below suitable temperatures during the time of measurement. Therefore, the average of the two measures (16.35°C) was taken as a minimum temperature threshold. Exact laboratory-based temperature survival thresholds are not available and may not realistically predict species habitat use in the field. Therefore, the model was based on the best available field approximations from local highland populations, and could be improved by the use of more accurate threshold temperatures. The minimum temperature layer was reclassified for each species, where temperatures equal or greater to the threshold value were assigned a value of 1, and colder temperature were assigned a value of 0 (Figure 6-1). Canopy closure provides shade, which is significantly associated with *An. punctimacula* larval habitat (Chapter Four). Closed and semi-closed canopies were therefore assigned a value of 1, while open canopies were assigned a value of 0 for *An. punctimacula* (Figure 6-1). Although incident sunlight was not significantly related to *An. albimanus* and *An. pseudopunctipennis* larval habitats in Chapter Four, larval habitats generally had a high incidence of sunlight in Ecuador (mean ± SD: 93.3 ± 17.5% and 92.1 ± 15.4%, respectively) and are described as sunlit in other literature (Manguin et al. 1996, Martens et al. 1996). Therefore, open and semi-open canopies were assigned a value of 1, while closed canopies were reclassified as 0 for *An. albimanus* and *An. pseudopunctipennis* (Figure 6-1). *Anopheles albimanus* and *An. pseudopunctipennis* were significantly associated with the presence of floating algae in Chapter four. For these two species, the interpolated raster layer for the presence of algae provides values ranging from 0 = algae absent to 1 = algae present (Figure 6-1). Each of the three species was also related to the land covers in which they occur in Chapter Five. For each species in this model, all land covers associated with larval habitat (e.g., irrigated cropland) and rivers (which are highly modified by humans) were combined into a generalized land cover layer, where the presence of a river or a suitable land cover were reclassified as 1, while unsuitable land covers were assigned a value of 0 (Figure 6-1). Since steep topography can limit the dispersal of adult mosquitoes, as well as the availability of larval habitat (Balls et al. 2004), the most extremely steep regions (cliffs) were assigned a value of 0, or unsuitable, and all other regions assigned a value of 1 (Figure 6-1). Finally, *An. punctimacula* larval habitat was significantly associated with greater calculated value for DO in Chapter Four. Therefore, the range of calculated DO was assigned a value of 1, while calculated DO values too great or too small were assigned a value of 0 (Figure 6-1).

The model raster layer for each species was built using the Weighted Overlay tool in Spatial Analyst in ArcGIS v. 10 (ESRI 2010), where the weights assigned to each layer were one, with an output of a raster with tile values of a potential sum of five, where five presents ideal habitat (Figure 6-1). We considered each layer equally since each of the layers are considered necessary for larval survival. Intermediate values between 1 - 4.9 units may also be considered increasingly suitable for species the model may have some inaccuracy due to its coarse spatial resolution (tile size = 1.515 km^2) and its spatial averaging from interpolation to create DO and algae layers. Output values were ranked by mean raster value (above which habitat was deemed "suitable") and rankings of model values, indicated by different colours on the maps, were based on standard deviations around the mean.

6.2.3 Model validation

The results of the spatial model were compared to a (mostly) independent data set of collection sites from 2008 - 2010, where larvae of the three species were either present or absent (Chapter Three). All layers were independently derived, except the interpolation of algae-present sites (used in the *An. albimanus* and *An. pseudopunctipennis* models), and to a lesser degree, the data used to calculate and interpolate DO (used in the *An. punctimacula* model), which coincide with the verification localities. This may slightly inflate the positive correspondence between the model and its validation data. Model values corresponding to species-present and species-absent sites were compared to the mean raster value using a 1-sample t-test, and between present and absent sites using a 2-sample t-test.

The results of the spatial models for anopheline distribution were also compared to spatially referenced malaria cases recorded in population centers during the last 10 years by the Servicio Nacional de Control de Enfermedades Transmitidas por Vectores Artrópodos (SNEM 1997-2008).

6.2.4 Warmer temperature predictions

Measured water temperatures (y) were related to adjacent air temperatures (x) from Chapter Four (n=283) using a linear regression, where the equation of the line was $y = 1.043x - 1.257$ ($R^2 = 0.64$; $p < 0.001$). Therefore, for each 1°C of air warming, water in larval habitats is expected to increase approximately 1.043°C. The most recent IPCC scenarios predict a 2 - 3°C increase in temperature over the next century, with the greatest increase in eastern Ecuador (Christensen et al. 2007). For a predicted air warming of 1, 2, and 3°C, 1.043, 2.086, and 3.129°C, respectively were added to the minimum temperature layer derived from the DEM using the Map Algebra function of ArcGISv.10 Spatial Analyst, and the models re-run and classified as above. Although precipitation in Ecuador is expected to increase slightly (5%) during the next century (Christensen et al. 2007), the climate predictions are of coarse scale cannot accurately capture the extremely heterogeneous precipiation levels in the western Andes of Ecuador (e.g. Buyaert et al. 2006). Therefore, we do not consider precipitation in the present model.

6.3 Results

6.3.1 Model predictions for *Anopheles albimanus*

The model output for *Anopheles albimanus* is provided in Figure 6-3. Generally, the majority of the western coast of Ecuador may provide suitable habitat, with the greatest suitability in the northern coasts of Esmeraldas and Manabí provinces, within the large low-altitude basin of Guayas Province, as well as along part of the foothill region (Figure 6-3). Potentially suitable habitat occurring on the eastern side of the Andes may be ignored since *An. albimanus* was not collected in that region (Figure 6-3). The raster mean serving as the suitability cut-off value was 3.876, which correctly coincided with 91.8% of the larval collection sites (n=49) (orange + red regions), whereas a more conservative mean + 1 S.D. cutoff value of 4.486 only correctly predicted 65.3% of the larval collection sites (red regions) (Figure 6-3). Species-present site values were also significantly greater than the raster mean value (t=8.65; df=48;

p<0.001) and model values for species-absent sites (t=8.31; df=70; p<0.001). The larval-absent sites did not coincide with lesser values than the cut-off (green regions); only 48.2% of the 307 sites were located within this range (Figure 6-3). Indeed, the model values for larval-absent sites did not differ significantly from the raster mean value (t=-1.07; df=306; p=0.284). The modelled at-risk area (orange + red) also coincided with 78.9% of the malaria-reporting medical centers during the last 10 years (n=90) (SNEM 1997-2008).

Modeled climate change scenarios of 1, 2, and 3°C predict up to a 16.6% increase in potentially suitable *An. albimanus* habitat (Table 6-1). Gradual warming could push an *An. albimanus*-suitable areas to include Mira and Chota valleys in Imbabura province, Pallatanga valley and Sibambe and Huigra in Chimborazo province (Figure 6-4). Over the majority of the lowland-highland interface, steep slopes mostly limit the potential dispersal of vectors, although some valley-bottom communities in the central-western Andes, such as Alluriquin, Pichincha, are expected to become more suitable (Figure 6-4). The southern valleys of Azuay province, including the town of Santa Isabel, are also expected to become more suitable for *An. albimanus* with climate change.

6.3.2 Model predictions for *Anopheles pseudopunctipennis*

The present-state model output for *An. pseudopunctipennis* is presented in Figure 6-5. Potentially suitable habitat occurs on the entire western coast and foothills, including some highland valleys, with strongest suitability in Mira valley, Imbabura, the coast of Esmeraldas and northern Manabí Provinces, and in the southern coastal portion of Guayas Province (Figure 6-5). As with *An. albimanus*, habitat suitability on the eastern side of the Andes can be ignored since the species does not occur there (Figure 6-5). The mean model value was 4.0993, which correctly coincided with 87.2% of the larval collection sites (n=47) (orange + red regions). (Figure 6-5).

Species-present site values were significantly greater than the raster mean value (t=9.72; df=46; p<0.001) and model values for species-absent sites (t=5.96; df=78; p<0.001). Like *An. albimanus*, larval-absent sites did not coincide with values less than the cut-off (green regions); only 40.8% of the absent sites were provided values less than the cut-off value (Figure 6-5). Unlike *An. albimanus*, the larval-absent site values were also significantly greater than the raster mean (t=5.17; df=308; p<0.001), which may reflect a slightly effort to collect larvae in areas that are already potentially suitable versus areas that are obviously unsuitable. Malaria cases reported during the last 10 years coincided strongly (93.8%) with the modeled distribution for *An. pseudopunctipennis*.

Although the range of *An. pseudopunctipennis* is only predicted to expand up to 11.2% at 3°C warming (Table 6-1), many highland valleys would likely become suitable for the larvae of this species. Gradually, the northern Mira, Chota and other highland valleys in Imbabura, including the city of Ibarra, are predicted to provide potential habitat. Highland valleys surrounding Quito, such as Tumbaco, are also predicted to become suitable with a greater change in temperature: these were formerly portions of the *An. pseudopunctipennis* distribution (Chapter Two) (Figure 6-6). The Inter-Andean valleys may provide potential future habitat, including the highland cities of Latacunga, Ambato, Pilaló and Guaranda (Figure 6-6). Many highland valleys of Chimborazo, encompassing the communities of Huigra, Sibambe, and Alausí would also become increasingly suitable, as would Tingo, Cotopaxi and the southern valleys of Yungilla and possibly even Girón, Azuay (Figure 6-6).

6.3.3 Model predictions for *Anopheles punctimacula*

Figure 6-7 provides the present-state model output for *Anopheles punctimacula,* with potentially suitable habitat found at high densities on the eastern coast, and scattered at low densities in the Andean highlands. Amazonian data can be ignored since this species does not occur in that region. The mean raster cut-off value was 4.054, which correctly coincided with 76.2% of the larval collection sites (n = 21) (red regions), whereas the less conservative cut-off of Mean - 1 S.D. of 3.17 (red + orange region) was an even stronger predictor of species presence (95.2% were correctly predicted) (Figure 6-7). Species-present site values were significantly greater than the raster mean value ($t=4.86$; $df=20$; $p<0.001$) and the values assigned to species-absent sites in the model ($t=3.17$; $df=24$; $p=0.004$). Like *An. pseudopunctipennis*, model values for species-absent sites were also significantly greater than the raster mean value ($t=4.61$; $df=336$; $p<0.001$), although were correctly predicted as species-absent in 59.1% of modeled sites. Only a total of 20.7% of the malaria-reporting centers during the last 10 years coincided with the most *Anopheles punctimacula*-suitable model values (cut-off 4.054) (SNEM 1997-2008).

The climate change predictions for *An. punctimacula* show almost no important range shift and/or expansion: 6.7% for a 3°C warming (Table 6-1). In a few highland valley regions, such as: Mira/Chota, Imbabura province, western highland Pichincha province, Pallatanga, Chimborazo province and Yungilla, Azuay province, as well as isolated areas of the Inter-Andean valleys, the most suitable classification (red areas) are expected to become more widespread, possibly promoting more stable highland anopheline populations in these regions (Figure 6-8).

6.4 Discussion

Our models were able to predict the presence of anopheline larval habitat in 76.2 - 91.2% of cases, but were unable to differentiate between sites where larvae were present versus absent. Therefore, for the purpose for which it is intended, errors in prediction occurred in locating 8.2%

of *An. albimanus* sites, 12.8% of *An. pseudopunctipennis* sites, and 23.8% of *An. punctimacula* sites, although error rates could be reduced by taking a less conservative cut-off value and considering more area as potentially suitable. In the field, suitable larval habitats are often found adjacent to completely unsuitable larval habitat, such as rice paddies suitable for *An. albimanus* larvae found next to a saltwater irrigation canal. The spatial resolution of the raster tiles in this model (ca. 1.5 km) is not of an adequately fine scale to differentiate among these suitabilities. Our model is of the mechanistic type, characterized by generality of application and basis in reality, which necessarily suffers from a reduction in precision (discussed in: Guisan & Zimmerman 2000). However, our model can be used to identify the broad-scale distribution where suitable habitats may occur if water were available.

Our model predicts only minor increases in the potential distribution of malaria vectors with the effects of climate change, supporting the idea that observed highland malaria is more heavily dependent on other factors (e.g. Reiter 2008). Even a 3°C increase in air temperature only increases the modeled distribution of species by 6.7 - 16.6%, with the greatest increase shown by *An. albimanus*. Along the western edge of the Andes, the terrain increases in elevation very steeply from sea level to ca. 3000 m: a physical barrier that is not overcome by the effects of climate warming. However, some lower altitude valleys that extend into highland regions have been identified as potential future habitat in the model, including Mira/Chota in Imbabura, lower altitude valleys near Quito, such as Tumbaco in Pichincha, Pallatanga valley, Huigra, Sibambe and potentially Alausí, in Chimborazo, and Yungilla valley, Azuay. Interestingly, these valleys were regions of highland malaria endemism until the 1940s (Chapter Two), which further supports the role of factors other than climate in highland malaria incidence. Unfortunately, widespread, accurate climate data from these sites from the years 1900-1940 are not available for comparison to present day.

As with any simplified version of reality, there are certain sources of variability that are not included in the model. Climate change predictions for Ecuador predict a marginal increase in precipitation over the next century (Christensen et al. 2007) and were not considered due to their spatially unpredictable nature. However, future models may overcome this shortcoming by incorporating modeled soil moisture, which was a stronger predictor of malaria incidence than rainfall in Kenya (e.g. Patz et al. 1998b) Dry and wet seasonality can modify the usage of habitat types by anopheline larvae (Roberts et al. 2002). Although the western highlands of Ecuador up to ca. 2000 m are almost continously rainy (Buyaert et al. 2006), we accounted for seasonality by basing layer data on multi-season field measurements and non-seasonal data such as slope. As well, land cover can modify small-scale microclimate, thereby affecting local temperature through changes to albedo, vegetation structure, and carbon assimilation (Lindblade et al. 2000; Bounoua et al. 2002). Therefore, within each raster tile, it is possible that open or semi-open land covers may locally promote slightly warmer water bodies than in the average landscape estimated from the equation used to generate minimum temperatures from altitude. Finally, the effect of the distance from the forest edge may have an effect on habitat suitability as in Brooker et al. (2004), which is impossible to accurately calculate in models of this resolution.

Our model does not determine the effect of climate warming on the distribution of anopheline mosquitoes in the already-endemic lowlands of Ecuador. Although Ruiz et al. (2006) have determined that malaria begins to disappear from human populations at ambient temperatures above ca. 30°C, it is difficult to determine the effect of climate warming on mosquito larvae in a cautious predictive model without more field-verified data. When conditions are warmer, mosquitoes may utilize cooler, shaded habitats, and thereby survive some degree of climate warming. Since these species are known vectors of a devastating human disease, it is more prudent to continue to monitor mosquito populations in lowland areas rather than to predict that mosquito species may disappear from lowland regions based on uncertain, unverified speculation. However, we do recommend entomological monitoring of the highland valleys that have been identified as future sites in the model in order to predict and prevent introduced malaria incidents in the highlands of Ecuador.

Table 6-1 Predicted changes to surface area of geographic distribution in western Ecuador (percentage and in kms^2) of *Anopheles albimanus, Anopheles pseudopunctipennis* and *Anopheles punctimacula* due to climate warming of 1, 2, and 3°C from models run in ArcGIS v.10 (ESRI).

	Anopheles albimanus			*Anopheles pseudopunctipennis*			*Anopheles punctimacula*		
Scenario	Suitable area (%)[1]	Suitable area (%) increase	New suitable area (km^2)	Suitable area (%)[2]	Suitable area (%) increase	New suitable area (km^2)	Suitable area (%)[3]	Suitable area (%) increase	New suitable area (km^2)
Current	46.29			62.70			35.64		
+1°C	54.54	6.35	11,485	66.44	3.74	6,873	38.09	2.45	4,502
+2°C	58.46	12.17	22,363	69.92	7.22	13,267	40.31	4.67	8,582
+3°C	62.90	16.61	30,522	73.89	11.19	20,563	42.37	6.74	12,385

[1] Based on suitability cut-off of model value >3.876.
[2] Based on suitability cut-off of model value >4.099.
[3] Based on suitability cut-off of model value >4.054.

Data Source / Type	*Anopheles albimanus*	*Anopheles pseudopunctipennis*	*Anopheles punctimacula*
Minimum temperature of small water bodies from DEM*	Minimum temperature >20.0°C = 1	Minimum temperature >16.6°C = 1	Minimum temperature >16.35°C = 1
Open, intermediate or closed canopy from land cover	Open/half open canopy (sunlit pools) = 1	Open/half open canopy (sunlit pools) = 1	Closed/half closed canopy (shaded pools) = 1
Algae present , interpolated from field data	Range 1 (algae present) to 0 (algae absent)	Range 1 (algae present) to 0 (algae absent)	N/A
Land cover + River layer	River or associated land use = 1	River or associated land use = 1	River or associated land use = 1
Slope, from DEM	Steepest (>4 000 000) unsuitable = 0	Steepest (>4 000 000) unsuitable = 0	Steepest (>4 000 000) unsuitable = 0
Dissolved oxygen: Calculated from field data and interpolated	N/A	N/A	Dissolved oxygen range 6.63 – 8.38 = 1

Figure 6-1 Diagram of model structure, where data types and source, and selection parameters for each species are listed. Each layer is given a value of 0-1, where 0 is unsuitable for the species and 1 is most suitable.

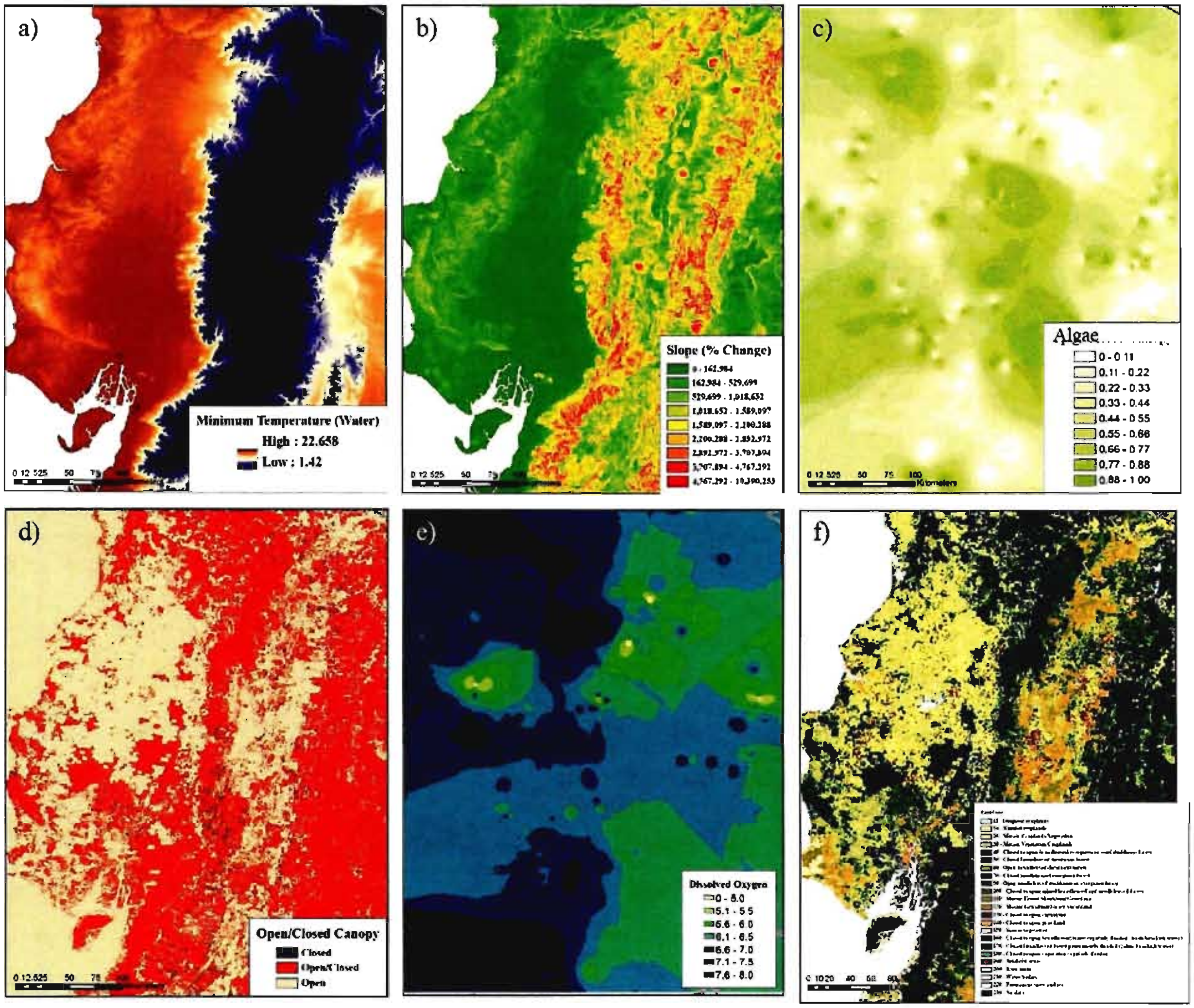


Figure 6-2 Data layers used to create model: a) minimum water temperature and b) slope (% change), both derived from DEM, c) algae (1=present to 0=absent), interpolated from field point measurements, d) canopy closure, derived from land cover, e) estimated dissolved oxygen (interpolated from field measurements/calculations) and f) land cover.

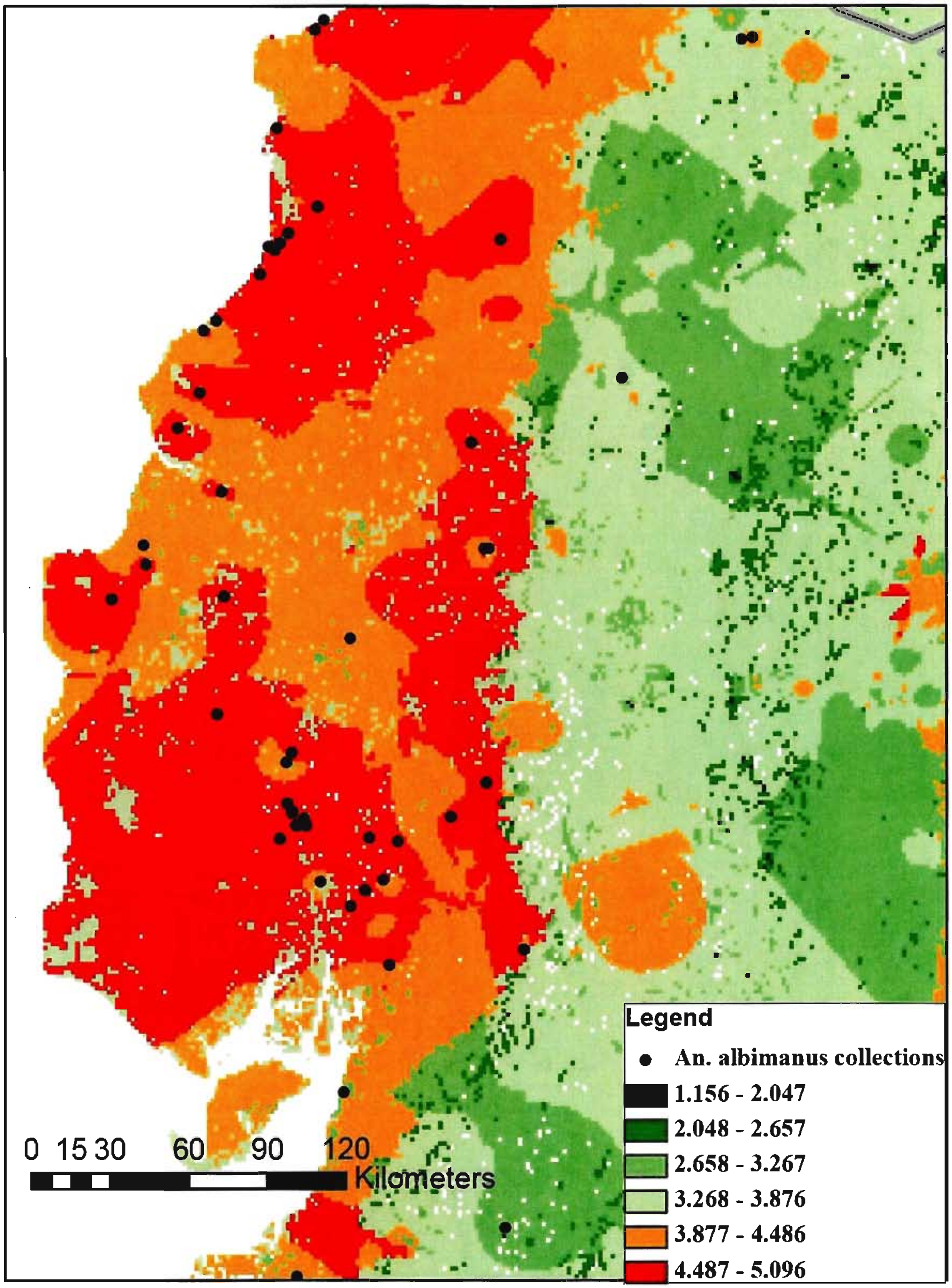


Figure 6-3 Application of the *Anopheles albimanus* model with present parameters, run in ArcGIS v. 10 (ESRI) using the weighted sums function. Actual *An. albimanus* collection localities in Ecuador during 2008 - 2010 are overlaid for reference.

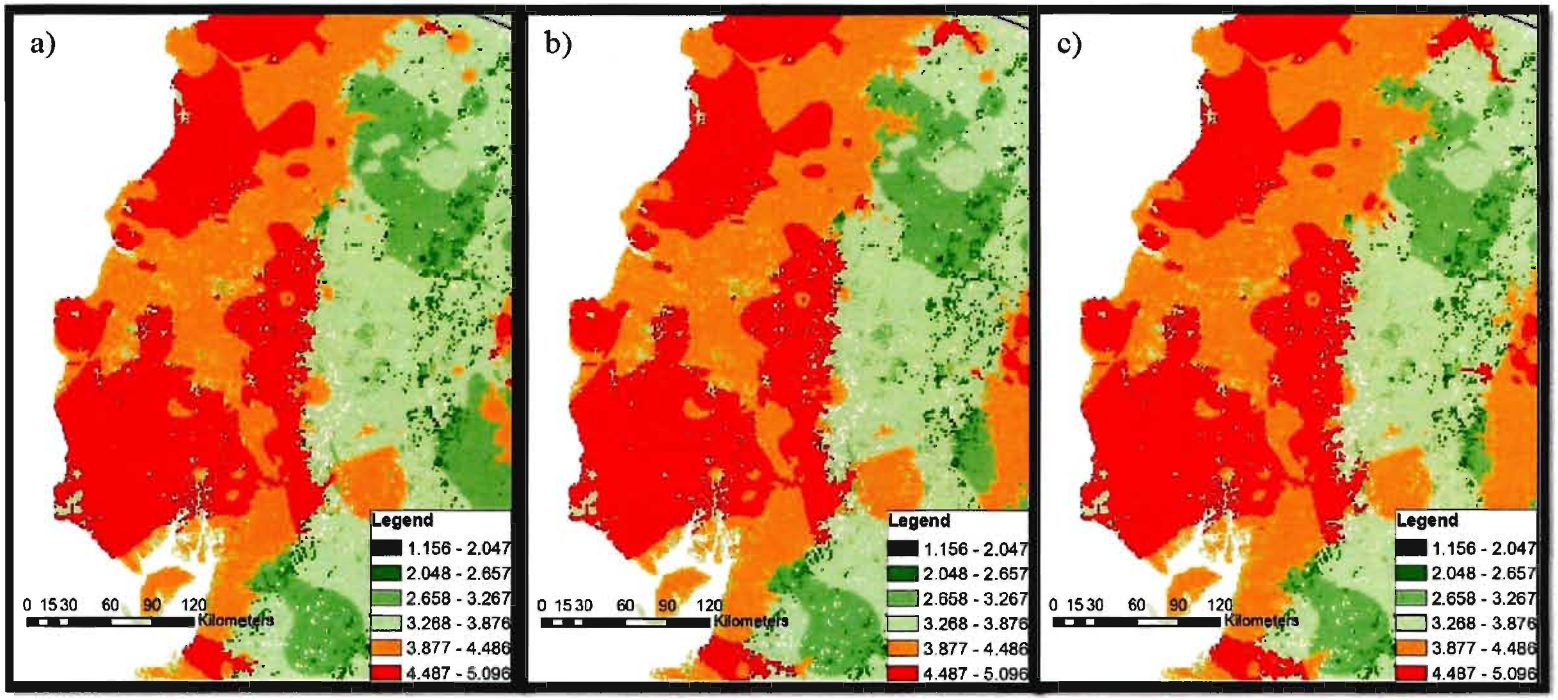


Figure 6-4 Application of the *Anopheles albimanus* model with climate change predictions of a) +1°C, b) +2°C and c) +3°C, run in ArcGIS v.10 (ESRI) using the weighted sums function.

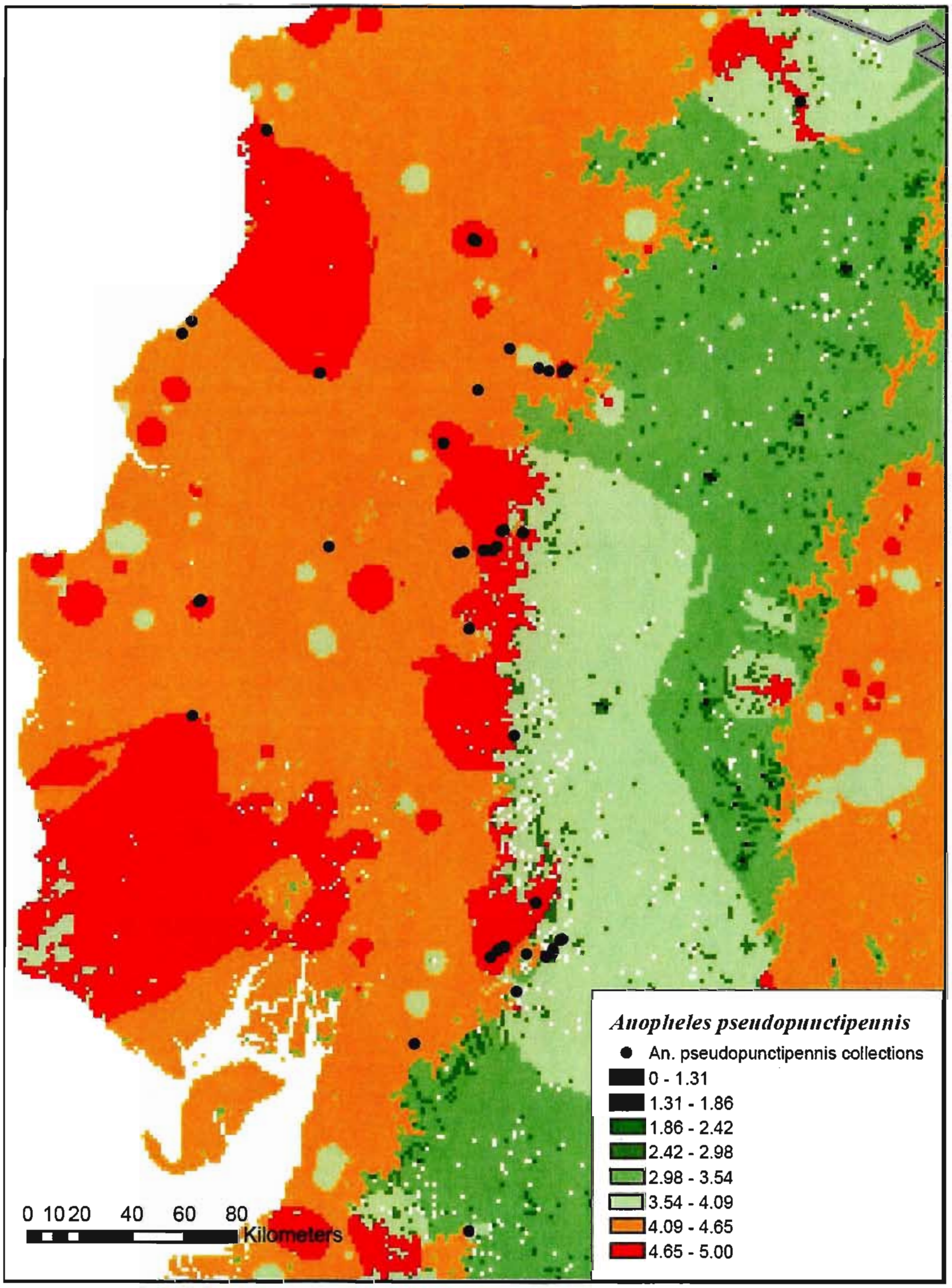


Figure 6-5 Application of the *Anopheles pseudopunctipennis* model with present parameters, run in ArcGIS v. 10 (ESRI) using the weighted sums function. Actual *An. pseudopunctipennis* collection localities in Ecuador during 2008 - 2010 are overlaid for reference.

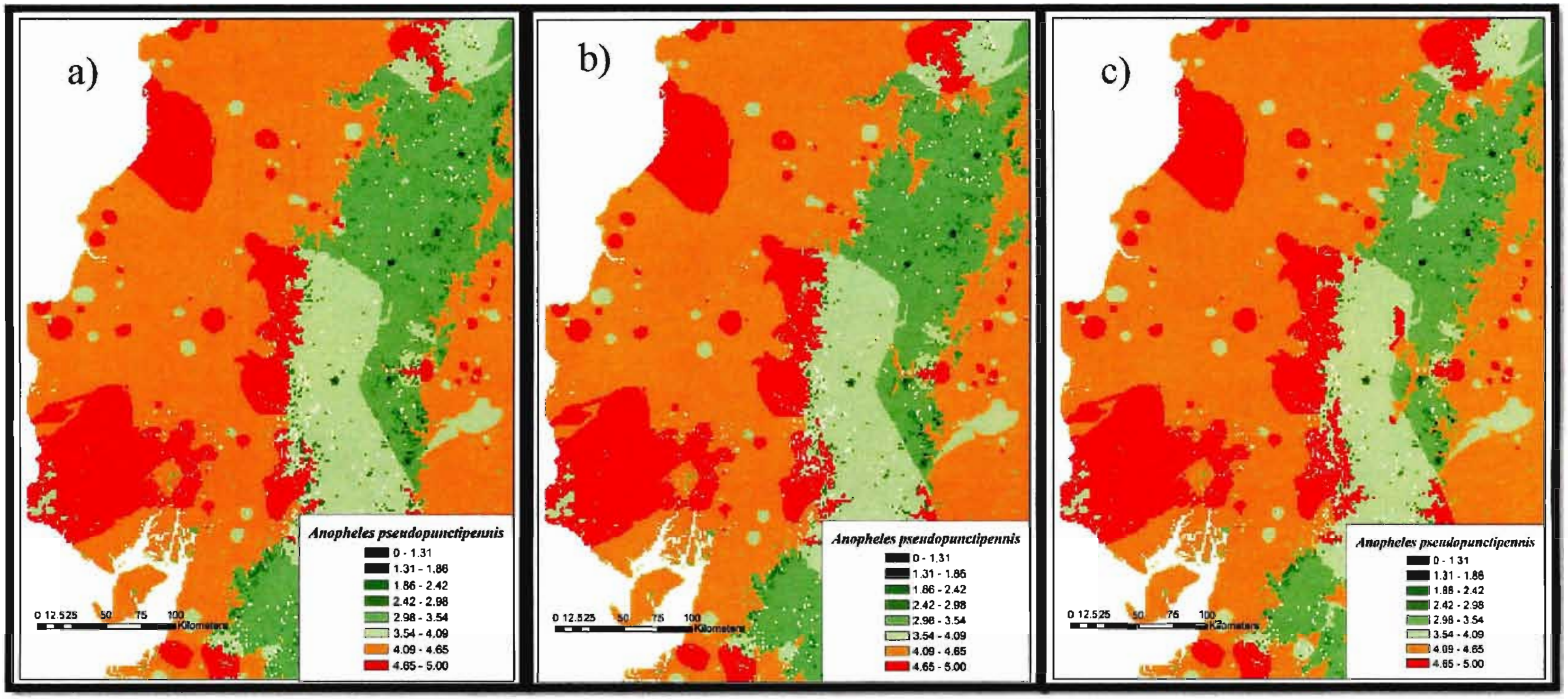


Figure 6-6 Application of the *Anopheles pseudopunctipennis* model with climate change predictions of a) +1°C, b) +2°C and c) +3°C, run in ArcGIS v.10 (ESRI) using the weighted sums function.

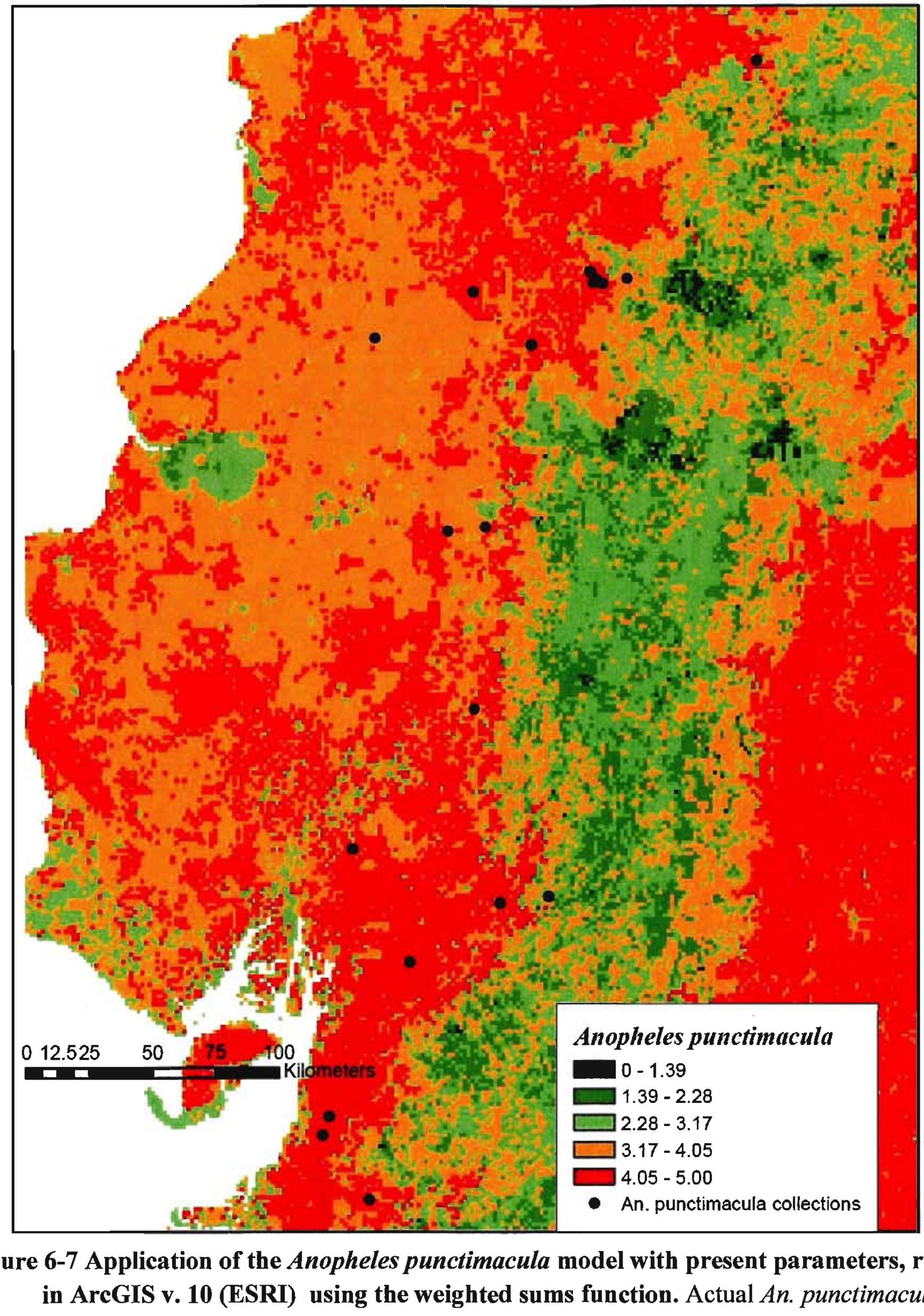


Figure 6-7 Application of the *Anopheles punctimacula* model with present parameters, run in ArcGIS v. 10 (ESRI) using the weighted sums function. Actual *An. punctimacula* collection localities in Ecuador during 2008 - 2010 are overlaid for reference.

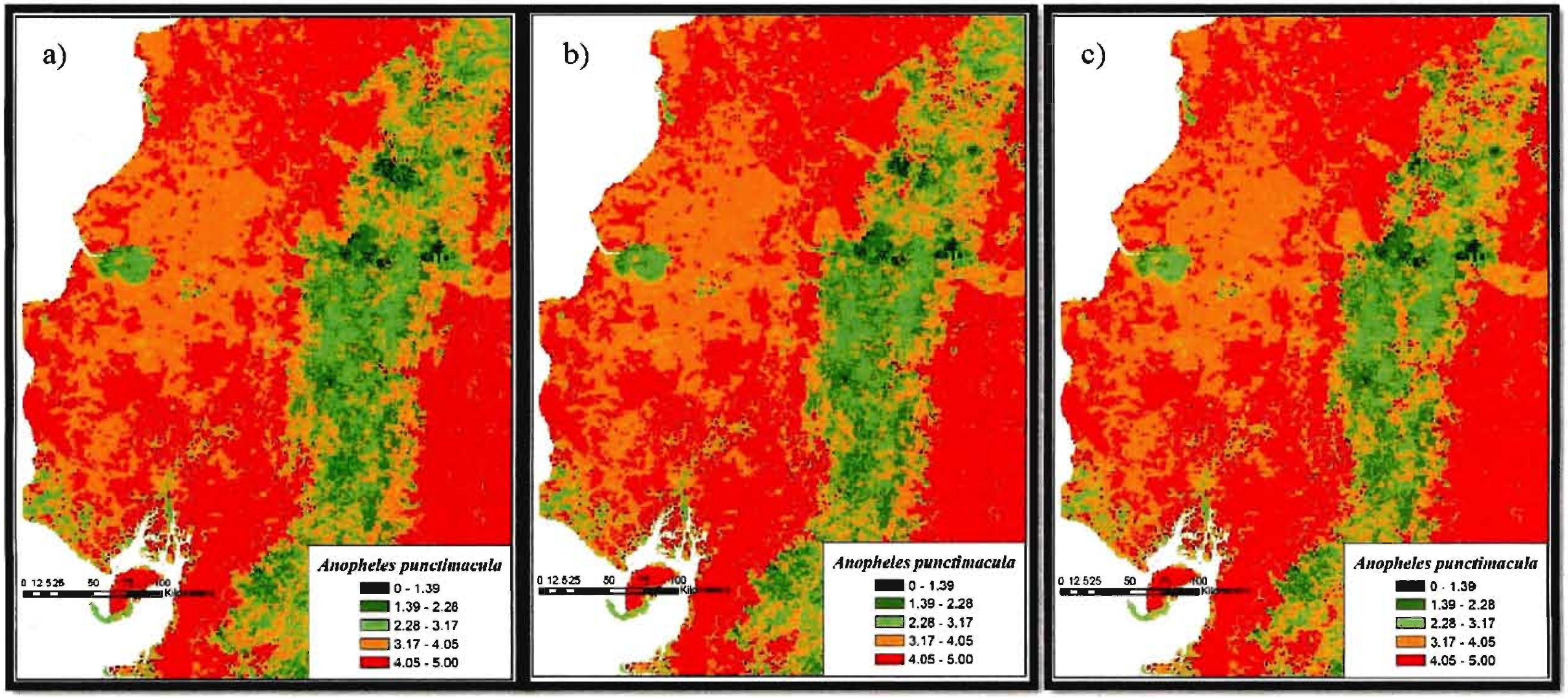


Figure 6.8. Application of the *Anopheles punctimacula* model with climate change predictions of a) +1°C, b) +2°C and c) +3°C, run in ArcGIS v.10 (ESRI) using the weighted sums function.

Chapter Seven

Malaria vector knowledge, concern, land management and protection practices among land owners and/or managers in lowland vs. highland Ecuador

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7.1 Introduction

Malaria remains a significant, debilitating and often lethal disease in many parts of South America, although its incidence, severity and impact is highly region-dependent (Rubio-Palis & Zimmerman 2007; Gagnon et al. 2002). In lowland Colombia, for example, malaria has been rated by focus groups as one of the most important health problems facing communities (Nieto et al. 1999). In Colombian and Ecuadorian communities, the cost of malaria prevention is much less expensive than the sum of treatment costs (e.g., medical treatment, travel, days of work lost) (Ruiz & Kroeger 1994). In both countries, national malaria control programs monitor the disease and carry out mosquito control programs in malaria endemic areas (Brochero & Quiñones 2008; Espinoza López et al. 2009). Due to the success of these programs, statistics in Ecuador indicate that overall malaria incidence has declined over the last fifteen years, with occasional regional-scale epidemics at lower elevations (ca. <1500 m) (SNEM 1997-2008).

At higher elevations, malaria was epidemic in the Inter-Andean valleys of Ecuador (>1500 m) prior to the mid-1940s, when large-scale eradication efforts prevented ongoing malaria transmission in these regions, mainly through the reduction and possible elimination of local populations of *Anopheles pseudopunctipennis* (Levi-Castillo 1945b). Since that time, malaria has only caused illness in highland regions among migrants and travelers returning from low altitudes (Rubio-Palis & Zimmerman 2007; SNEM 1997-2008). Several reviews of the effects of various global changes on the incidence of insect-borne disease have stressed that malaria might move into higher-altitude regions as highland habitats become increasingly suitable for *Anopheles* mosquitoes (Martens et al. 1995; Epstein et al. 1998; Lindsay & Martens 1998; Moreno 2006). Highland malaria has been observed during the last twenty years in higher-altitude regions of Kenya, Rwanda, and Uganda (Loevinsohn 1994; Malakooti et al. 1998; Lindblade et al. 2000), as well as in Andean Bolivia (Rutar et al. 2004; Lardeux et al. 2007). *Plasmodium vivax* has been incriminated as the parasite in highland malaria epidemics in both 1940s Ecuador and recently in Bolivia (Chapter Two; Levi-Castillo 1945b; Rutar et al 2004). Additionally, recent widespread highland records of three coastal malaria vectors: *Anopheles albimanus*, *An. pseudopunctipennis* and *Anopheles punctimacula*, and one Amazonian vector, *Anopheles oswaldoi s.l.*, provide support for the possibility of future highland malaria epidemics (Chapter Three).

In Africa, it is believed that in higher-altitude regions, residents and health care practitioners may lack experiential knowledge of the disease and therefore do not know how to recognize malaria nor protect themselves from mosquito bites (Malakooti et al. 1998). As well, a person's experience with malaria could potentially raise a greater perception of malaria risk. Therefore, highland communities may not rate malaria risk strongly or recognize symptoms in order to seek treatment (Lindblade et al. 2001). Residents may also not approach mosquito habitat elimination and personal mosquito bite protection as seriously as residents of malaria-endemic lower altitudes.

Although there is essentially no data regarding the perceptions of malaria in the South American Andes, there have been several studies conducted in lower-altitude regions. The knowledge of a connection between mosquito bites and malaria is regionally variable, for example, 56% of respondents in a rural community in northern coastal Ecuador versus 85% of respondents in an urban community in Pacific coastal Colombia (Kroeger & Alarcon 1993; Nieto et al. 1999). In Colombia, malaria has been perceived as caused by strenuous activities, ingesting hot foods, poor nutrition, drinking contaminated water and/or proximity to garbage (Pineda & Agudelo 2005). Individual differences in knowledge and perceptions have also been attributed to gender, with women having limited access to malaria-relevant information and treatment, among other factors (Tanner & Vlassoff 1998). Malaria prevention in Colombia incorporates both modern techniques (e.g., bed nets and anopheline mosquito larval habitat elimination) as well as traditional techniques more commonly used in rural areas (Lipowsky et al. 1992; Nieto et al. 1999; Pineda & Agudelo 2005). Urban residents in Amazonian Colombia have also been reported to feel less at risk of malaria than rural residents (Pineda & Agudelo 2005).

Malaria diagnosis by national bodies in South America is often based on passive reporting (Lipowsky et al. 1992), where misdiagnosis of malaria could lead to a general underreporting of the disease over the landscape. The actual risk of malaria over the landscape is complicated by human migration patterns among endemic and non-endemic regions, as well as by proximity to mosquito breeding sites (Sevilla-Casas 1993).

Landowners and land managers, rather than lower-status workers, have the capability to eliminate anopheline larval habitats on their property and to promote and enforce the use of malaria control measures such as bed nets among their families and employees. In this study, we used a quantitative KAP (knowledge, attitudes and practices) approach to assess the degree of knowledge, concerns and beliefs of land managers and landowners in both lowland and highland regions of Ecuador for topics relevant to malaria prevention. We also studied the larval habitat management practices in use and motivations for eliminating larval habitat. Finally, the study assessed mosquito bite protection that is currently in use in both lowland and highland regions and compared it to protection that would be used by respondents for hypothetical travel to a known malaria-endemic area.

7.2 Materials and Methods

To assess the knowledge, beliefs, concerns, land management practices and forms of bite protection used by Ecuadorian land owners and/or managers, we conducted a series of 262 structured, questionnaire-based interviews with individuals throughout all road-accessible regions of Ecuador between 2008 and 2010 (Figure 7-1). Although the regions are endemic for malaria, we did not assess respondents in northern Esmeraldas, Sucumbíos, and Orellana provinces due to travel safety regulations. We attempted to eliminate clustering of respondents by traveling along as many different roads as possible. However, some clusters of respondents occurred (e.g., in Pichincha and Santo Domingo provinces) due to a higher number of potential respondents present outside their home at the time of visiting, as well as a greater population and road density in that particular lowland-highland transition area, which may have led to a regional bias in our analysis (Figure 7-1).

To permit statistical inference, we interviewed a pre-established number of land owners and/or managers from each of five generalized land uses: cattle pasture (whether for meat or dairy), fish farms, plantations, human residences, and nature reserves. Fish farm and nature reserve managers were given fewer interviews than the other categories since they were encountered less frequently (Table 7-1). Only one person was interviewed on the same property for the same land use. Although all five land use categories are present in all general regions of the country, there is some clustering of land uses in particular regions which may have caused small levels of spatial bias due to availability (Figure 7-1).

Land owners and managers were stratified on a second level by gender since women and men are expected to have different access to information and women may more readily apply knowledge toward prevention and treatment than men (Tanner & Vlassoff 1998) (Table 7-1). Although we attempted to interview an equal number of men and women, we were not able to interview many female owners or managers of nature reserves due to the scarcity of women encountered in these positions (Table 7-1).

We further stratified interviews by interviewing an approximately equal number of landowners and/or managers above and below 1500 m (Table 7-1), which travel medical literature suggests as an appropriate altitudinal division for malaria endemism in Ecuador (Bradley & Warhurst 1995). Current statistics from the Servicio Nacional de Control de Enfermedades Transmitidas por Vectores Artrópodos (SNEM) indicate that almost all areas below 1500 m have been affected by at least occasional cases of malaria the last 10 years (Figure 7-1) (SNEM 1997-2008), which we assume should have provided residents of the below 1500 m category with some experiential knowledge of malaria. Entomological data further supports this altitudinal risk division, since densities of *Anopheles* mosquitoes (malaria vectors) are more prevalent below 1500 m, although some larvae have been collected up to 2000 m (Chapter Three). This altitudinal division was used rather than actual malaria rates in reporting centers, since the distribution of malaria varies substantially from year to year, data comes from urban reporting centers rather than rural communities, and we expect rural parts of the country to underreport malaria cases due a lesser access to health centres. Therefore, for the purposes of this study we also assume that all respondents below this altitude are potentially at risk for malaria.

To locate possible respondents, researchers traveled along all roads deemed suitable for travel and potential respondents were approached when visible from the road or other public domain. To introduce randomization, after spotting a potential respondent for a given land use, a coin toss was used to determine if the person would be approached. However, due to the scarcity of fish farm and nature reserve managers/owners, all of these possible respondents were approached when observed. Often, the land owner and/or manager was not present and the researchers did not interview any person on the site.

To avoid a possible feeling of deference toward the interviewer due to any perceived class differences, (e.g. Lenski & Leggett 1960), we included one Ecuadorian interviewer who spoke the local dialects, and a few contradictory statements were placed into the interview to identify respondents who might not be answering truthfully (e.g. all affirmative answers). Yes and no questions were also placed in random order on the questionnaire. Voluntary participation and oral consent were received after we explained the study, including their rights to withdraw at any stage during the interview process. All research was approved Brock University's Ethics in Human Research Committee, file #07-336 (Appendix 3).

Aspects of the interview included: 1) knowledge of where malaria occurs in Ecuador, 2) knowledge of the ecological relationship between standing water and mosquitoes, and malaria, 3) belief in climate change, and belief that climate change *could* cause malaria vectors to move into higher altitudes, 4) concern about insect-borne disease, the presence of any mosquitoes on their property, and the presence of malaria on their property, 5) land management practices for standing water (mosquito habitat) reduction, and 6) mosquito bite protection used and bite protection that respondents would use to travel to an area known to have malaria. A sample questionnaire is provided in Appendix 4.

Respondents answered most questions with the options: yes (1) or no (0). To determine the role of the independent variables altitudinal category and gender on the frequency of positive answers, a Binary Logistic Regression (Logit) was used. Those who answered "do not know" or "maybe" were considered uncertain answers were eliminated from analysis when the frequency was < 10%, although the percentage of uncertain answers is summarized in the text when provided more frequently. Since multiple binary logistic regressions were used in some cases, significance levels were modified using a Bonferroni correction, where α/n (n= number of tests) are used to decrease the probability of false positive results (Whitlock and Schluter 2009).

Some questions, including land management practices and bite protection methods, asked respondents to list all possible answers. Chi-square tests (of equal variances among groups of gender and altitude class, unless otherwise indicated) were used to compare count data from responses among land use and elevation categories. All statistical analyses were conducted in Minitab v.15 software (2007).

7.3 Results

All respondents were land owners, or in the cases of large properties, land managers; therefore respondents in our study were expected to have a higher average economic status and live in rural settings more often than the average Ecuadorian. Only one residential respondent lived in an urban area (Quito), while all other respondents lived in rural regions or small communities. Approximately half (56%) of our respondents were judged to be of a higher-than-average economic status (i.e., living in concrete houses with completed roofing and doors and some signs of disposable income such as televisions, radios, and cars), 23% of respondents were judged to be of a lower socioeconomic status than the other two categories (i.e., living in basic housing sometimes lacking doors and sufficient roofing with no signs of disposable income), and 20% of our respondents were judged to be very wealthy (i.e., living in large, finished homes with some degree of landscaping, on expansive properties). Consistent with our respondents retaining a greater personal wealth than the average citizen, across Ecuador, approximately 38% of households lack sufficient income to provide basic necessities of life and 46% of citizens are rated under the NBI income-poverty index based on statistics collected in 2006 (Cañizares & Lopez 2011). In Ecuador, average monthly income over the last year has been estimated between 188 – 380 USD although 7 – 8% of adults are unemployed at 45% are underemployed (Cañizares & Lopez 2011).

No systematic observable socioeconomic class differences were observed in respondents among any of the land use types or genders. Greater than 90% of our respondents were ethnically identifiable as Mestizo (a mixture of Indigenous, African and Spanish ancestry found in all regions of Ecuador), while fewer than 10 respondents were each of Indigenous, Black, or White. Five people who were approached by interviewers declined to participate in the study, therefore, the interviews did not proceed.

7.3.1 Personal knowledge, belief, and concern

Eleven percent of respondents answering certainly above 1500 m stated that they could become ill with malaria on their property at present, whereas only 52.6% of respondents below 1500 m provided the same answer (Table 7-2). Of all respondents considered together, 13.3% were unsure. However, 96.4% of respondents accurately agreed with the statement that one could get malaria on the coast and/or in the Amazon region of Ecuador, with the responses not differing among genders or altitudinal categories (Table 7-2). However, agreement with the statement that one could get malaria above 3000 m was affected by both gender and altitude category of the respondent (Table 7-2): women and low-altitude dwellers were more likely to inaccurately agree than men and high-altitude dwellers (Women: above 1500 m= 29.7%, below 1500 m = 55.9%; Men above 1500 m = 18.5%, below 1500 m = 30.6%). Thirty-three percent of respondents were uncertain if malaria could occur above 3000 m.

Most respondents answering certainly (89.9%) agreed that eliminating standing water reduces the local population of mosquitoes, thereby acknowledging that they understand where larvae live. A slightly lesser percentage (83.8%) agreed that eliminating standing water reduces the risk of malaria, thereby demonstrating an understanding that mosquito larvae arise from standing water *and* can transmit malaria. There was no effect of gender or altitude category on responses to either statement.

Most respondents (93.3% of those answering certainly, 74.8% total) believed that climate change was occurring, although 19.4% of were uncertain. Fewer (75.6% of those answering certainly, 54.9% total) believed that it may be possible for malaria to move into highland regions with climate change, although a 26.1% were uncertain about the possibility of this phenomenon.

A degree of concern regarding insect-borne diseases was reported significantly more often at elevations below 1500 m (83.8%) than above 1500 m (57.7%), but did not differ among genders (Table 7-2). Concern about the presence of mosquitoes on a person's property was greater for respondents below 1500 m (87.2%) than above 1500 m (61.4%), although there were insignificant differences among genders after the Bonferroni correction (Table 7-2). Concern about the presence of malaria on a person's property was reported more often below 1500 m (82.3%) than above 1500 m (60.0%), regardless of gender (Table 7-2).

7.3.2 Land management perspectives for standing water elimination

Different numbers of respondents reported the presence of some type of standing water on their property depending on their land use (χ^2=14.82; df=4; p=0.005). This significance was mainly due to cattle farmers that reported more standing water and fish farmers that reported less standing water than expected (Figure 7-2). There was no difference in the reporting of standing water presence above and below 1500m (χ^2=0.05; df=1; p=0.816).

During the interview, the presence and type of standing water observed by interviewers was recorded. Figure 7-2 compares the actual presence of some type of standing water to the rate at which it was reported, for each land use. There is a significant association between the proportion that claims to eliminate standing water on each land use type vs. the proportion with standing water present (Two-way Chi-square contingency table: χ^2=12.9, df=4, p=0.012), with strongest statistical effects due to the strong underreporting by fish farmers (Figure 7-2). All other respondents under-report the actual presence of standing water by about half (Figure 7-2).

Types of standing water observed on each of the different land uses are summarized in Figure 7-3. Approximately 77.5% of fish farms had at least one fish pond or tank with an area of stable water surface that could potentially support mosquito larvae (Figure 7-3a). Ponds were the most common type of standing water for nature reserves (Figure 7-3c), while irrigation canals or inland ditches were the most common for cattle farms (Figure 7-3b), residences (Figure 7-3d) and plantations (Figure 7-3e). It should be noted that not all standing water types listed would be suitable for *Anopheles* mosquito larvae, although they may be suitable for other species of mosquitoes.

The percentage of respondents that presently eliminate their standing water differed significantly among land uses (χ^2=16.1; df=4; p=0.003), with the strongest effect due to a smaller number of cattle farmers than expected reporting that they eliminate standing water (Figure 7-4a). However, the proportions of respondents that eliminate standing water for each land use did not differ between altitude categories (above/below 1500 m) (χ^2=2.4; df=1; p=0.121) (Figure 7-4a).

Reasons provided for not eliminating standing water were enumerated (Figure 7-4b), and approximately twice the number of reasons were given by respondents below 1500m than above (Figure 7-4b). The top three reasons provided overall were: logistical difficulty (6.2%), lacking human assistance (3.5%) and lacking time to eliminate the water (3.5%). Water being "natural" was only provided as a reason for not eliminating water by nature reserves. Not being able to eliminate standing water due to practical use was reported for rice farms and fish ponds below 1500 m.

Four possible motivations that might encourage respondents to eliminate standing water were compared and all four appear to be strong motivators to eliminate water: 1) the presence of malaria nearby (95.3%), 2) financial support to eliminate water (93.9%), 3) increased frequency of mosquito bites (93.3%) and 4) recommendations by government or medical authorities (89.9%). There was no statistical difference in agreement with motivational sources between genders or altitude categories.

7.3.3 Mosquito bite protection

Respondents were asked if they currently use any form of mosquito bite protection, and if they would use protection if they visited an area known to have malaria. Use of some type of malaria protection differed among altitude categories as well as current use versus use for a malaria-endemic region, although bite prevention did not differ among genders (Table 7-3). Current use of protection was higher below than above 1500 m (Figure 7-5; Table 7-4). Respondents claimed that they would use malaria protection more often in an area with known malaria than what they currently use, even if they already live in a possibly malaria-endemic area (below 1500 m) (Figure 7-5; Table 7-3).

Methods used to prevent mosquito bites are summarized in Figure 7-6. The most commonly used prevention methods above 1500 m are: 1) insecticides for the home, 2) long sleeves, and 3) chemical repellent (Figure 7-6a). If those respondents visited an area with known malaria, 1) bed nets, 2) long sleeves, and 3) insecticides for the home would be the most commonly used (Figure 7-6b). The most common types of prevention methods used below 1500 m are: 1) bed nets, 2) insecticides in the home, 3) chemical repellent, 4) long sleeves, 5) screen or curtain on doors and windows, and 6) chlorine or chemicals in standing water (Figure 7-6c). The same top six prevention methods are listed by respondents for hypothetical travel to an area with known cases of malaria, although the order in which they are listed is different (Fig. 7-6d). The less-often reported prevention methods for respondents living below 1500 m include calling the Sistema Nacional de Eradicación de Malaria (government agency), use of a fan or air conditioning, or a community effort to eliminate mosquito habitat, all of which have the potential to prevent mosquito bites.

7.4 Discussion

Since the present study focuses on land owners and land managers, who are of a higher socioeconomic status than the average Ecuadorian citizen, it is also likely that respondents had received more education than average, which we expect to relate to better understanding of the topics under discussion. Therefore, the present study should not be extrapolated to the knowledge, attitudes and beliefs of all Ecuadorians, but rather, should provide a baseline of these factors among land owners and/or land managers, i.e., the decision-makers for larval habitat modification in the overall landscape.

Although most participants in this study correctly identified general regions where malaria does and does not occur in their country, it is troubling that 52.6% of respondents below 1500 m did not think that they were at risk of contracting malaria on their property. Possibly, respondents are aware of the anti-malaria campaigns carried out in coastal regions in Ecuador that have successfully reduced deaths from malaria in recent years (Espinoza Lopez et al. 2009). Although a small percentage of these are probably not at immediate risk (SNEM 1998-2008), several respondents appeared to deny personal risk despite anecdotally providing us with examples of neighbours or nearby towns with recent malaria cases. Another explanation might be that respondents feel that malaria is a controllable risk, and they might not be susceptible to infection because they use mosquito bite protection (e.g. Weinstein 1984).

Generally, most respondents understood that standing water could increase mosquito populations, and thereby malaria incidence. Most also believed that climate change is under way and provided anecdotes about personal observations, hearing news reports, or stories from the older generations. It has not yet been established how these scientific ideas are disseminated among the population, which may be worth evaluating. Along the same lines, a large number were also receptive to the possibility of highland regions becoming affected by malaria in the future with climate change. Such an open-mindedness to these scientific ideas would be useful for rapid identification of malaria cases and acceptance of risk should malaria spread to previously-unaffected highland regions.

Substantially fewer respondents reported the presence of standing water on their property than those actually with observable standing water. Although part of this could be due to denial, or to a lack of awareness of their own property, a more likely reason for many respondents to deny the presence of water could be the too-specific definition of "standing water" for mosquito habitat. Anecdotally, several respondents talked about the requirement of swamps and marshes for mosquito habitat, whereas the most commonly observed standing water types on properties were ponds, reservoirs, and irrigation canals. Therefore, we suggest that malaria education programs that target standing water elimination need to define potential larval habitat as any water where the surface of the water is not moving, rather than the more typical "standing water", or "aguas estancadas". Since the type of standing water available is dependent on land use, it is also important for future studies to more extensively define suitable vs. unsuitable habitat types for *Anopheles* larvae to include those that could be considered to flow, such as irrigation canals and roadside ditches. Along those lines, it would be necessary to carry out field surveys to correctly identify larval habitat sources proximate to each community in order to remove or larvicide larval habitats, although the coordination of such activities may be difficult without a top-down organizational approach. Although Ecuadorians could be motivated to eliminate standing water through direct observation or advice from authorities, the largest reason provided for not eliminating the water is difficulty (usually due to topography) – a practical problem that would be expensive to address. Other reasons for not eliminating standing water, such as lack of staff, and lack of time, would be less costly and complicated to remedy.

A large number of respondents below 1500 m used some type of mosquito protection (84.0%). Bed nets were the most prevalent mosquito-bite prevention method in low-altitude regions (66.4%), and were more commonly used than in a community in northern Coastal Ecuador (23%), lowland Colombia (59%), rural Burkina Faso (49%), or Uganda (26%) (Kroeger & Alarcon 1993; Nieto et al. 1999; Nuwaha 2001; Okrah et al. 2002). We did not personally assess whether or not the respondents' bed nets were in good condition, or if they were treated with insecticide, which is important for bite prevention or might help to reduce resting populations of adult mosquitoes within homes (Clarke el al. 2001; Lindblade et al. 2006). Both of these factors could be avenues for future research. The numbers might also have been inflated due to the common use of bed nets to prevent the annoyance of mosquitoes rather than malaria (Miguel et al. 1999). The increased use of mosquito prevention among low-altitude dwellers for hypothetical travel to a malaria-endemic area might indicate that lowland residents would benefit from an educational program in their community that provides updated data concerning their immediate and potential risk of malaria.

Insecticides within the home, including sprays, were also often employed in low-altitude areas (57.5%), similar to communities in the Colombian Pacific coast (57%) (Nieto et al. 1999). Their common usage is positive, since one study in the highlands of Kenya reports that indoor residual house-spraying actually reduces infection rates more than bed nets (Guyatt et al. 2002). Interestingly, mosquito spirals were not presently used by Ecuadorian respondents, despite 60% usage in the Colombian Pacific coast (Nieto et al. 1999) and use in Africa (e.g., Okrah et al. 2002).

Mosquito protection methods that would be employed by Ecuadorian land owners/managers during travel to a malaria-endemic area differ slightly from those reported in the travel medical literature. Although 80-90% of our respondents state that they would use a bed net in a malaria-endemic area of their own country, in one study only 58% of foreign travelers to a malaria-endemic area would do so (Toovey et al. 2004). However, a higher proportion of travelers in the same paper (Toovey et al. 2004) would use long sleeves (93%) and chemical repellent (90%) than our respondents (approx. 50-60%). Travelers from developed countries also rely heavily on chemical prophylaxis for malaria prevention (Van Herck et al. 2003; Wilder-Smith et al. 2004; Toovey et al. 2004), while only <5% of our respondents would use it for travel into a known region with known cases of malaria.

We had expected more folklore-based and traditional medicine and mosquito prevention methods from our respondents, since it is known that plant-based malaria prevention and treatment are still quite common in parts of Ecuador, (e.g. Pederson & Colomba 1983; Kroeger & Alarcon 1993; Ruiz & Kroeger 1994) and in Colombia (Nieto et al. 1999; Pineda & Agudelo 2005), as well as in rural parts of Africa (Nuwaha 2001; Hausmann Muela et al. 1998; Tarimo 2000; Okrah et al. 2002). This may have been in part due to the higher than average socioeconomic status of our respondents, who may have been more able to purchase non-traditional mosquito prevention methods, such as DEET-based chemical repellent. Some of the plant-based repellents that were mentioned by respondents included citrus fruits (e.g., lemon juice) and menthol. The use of smoke as a mosquito repellent included the traditional burning of termite nests in Amazonian regions, as well as burning "palo santo", wood from the *Bursera graveolens* tree, which is common on the coast.

Further studies are required to evaluate the malaria KAP of Ecuadorian citizens who do not own properties in Ecuador or elsewhere in the Andes, and to elucidate cultural and educational differences among perceptions of malaria risk. The present study focused on individual responses, although community approaches to larval source reduction can also play an important role in habitat elimination and highland malaria prevention.

7.5 Conclusions

A large proportion of land owners/managers presented a good understanding of the occurrence of malaria on the Ecuadorian coast or in Amazonia, and were able form a basic causative link between standing water, mosquitoes and malaria. However, about half of the landowners/managers in potential risk areas (areas lower than 1500 m) did not believe that it was possible to become ill from malaria on their own property. Most respondents expressed a belief in climate change and were open-minded to the possibility of anopheline mosquitoes moving into higher-altitude regions.

Different generalized land uses provided different types of standing water, which may vary in suitability for anopheline larvae. Most respondents claimed to eliminate standing water on their property, and were most often unable to do so due to logistical difficulties. In many cases, respondents claimed to eliminate standing water despite observers recording sources of standing water that might provide potentially suitable anopheline habitat.

Most respondents in lowland (at-risk) regions use some type of mosquito bite prevention, most commonly bed nets. During hypothetical travel to a known malaria-endemic region, both lowlanders and highlanders would increase their overall use of bite prevention. Therefore, residents of malaria-endemic regions might be expected to increase their use of prevention if informed that their property lies in a malaria-endemic area.

Generally, it is hoped that the findings of this study will assist malaria control organizations by providing useful data to improve the efficiency of potential educational programs. While knowledge of malaria's occurrence patterns is strong in Ecuador, even in highland regions, it might be necessary to inform residents of actual malaria risk, so that risk does not continue to be underestimated. Although most respondents understand that there is a link between standing water and malaria, the definition of standing water is often too restrictive and does not encompass all potential mosquito habitats on a person's property. Ecuadorian land owners/managers appear to be strongly motivated to eliminate mosquito habitat on their property, and when they do not eliminate standing water, it is usually due to factors beyond their control (practical difficulty or limited financial resources). Most at-risk Ecuadorian respondents currently use mosquito bite protection methods, although they might increase their use if they were informed that they live in a potentially malarious area.

Table 7-1 Summary of sample sizes for interview respondents from 2008-2010, in Ecuador. Numbers of respondents are provided for land uses (cattle farm, plantation, residence, fish farm and nature reserve), genders, and altitude categories (above/below 1500 m).

Land use		Gender		Altitude Category	# Resp. total
Cattle	60	Female	30	Above	15
				Below	15
		Male	30	Above	15
				Below	15
Plantation	60	Female	30	Above	15
				Below	15
		Male	30	Above	15
				Below	13
Residence	62	Female	31	Above	16
				Below	15
		Male	31	Above	15
				Below	16
Fish farm	40	Female	18	Above	7
				Below	11
		Male	22	Above	13
				Below	9
Nature reserve	40	Female	10	Above	5
				Below	5
		Male	30	Above	15
				Below	15

Table 7-2 Summary of binary logistic regression results, from answers to two statements related to knowledge of where malaria occurs and three statements related to concern about malaria and mosquitoes. Gender (reference event = male) and altitude category (reference event = below 1500 m) were included as potential factors. Bonferroni-corrected significance level is $\alpha=0.025$ for "I can get malaria..." and $\alpha=0.016$ for "I am concerned about..."

	Factor	Odds Ratio	C.I.+	C.I.-	*Z*	*P*
I can get malaria...						
... on my property	Gender (*m*)	0.66	1.25	0.35	-1.28	0.202
	Altitude (*b*)	9.10	18.42	9.10	6.15	<0.001
	coefficient				-5.46	<0.001
... above 3000m in altitude	Gender (*m*)	0.41	0.80	0.21	-2.61	0.009
	Altitude (*b*)	2.46	4.82	1.26	2.62	0.009
	coefficient				-2.41	0.016
I am concerned about...						
... insect-borne disease	Gender (*m*)	0.66	1.17	0.37	-1.42	0.156
	Altitude (*b*)	3.80	6.82	2.12	4.47	<0.001
	coefficient				2.22	0.026
... mosquitoes on my property	Gender (*m*)	0.49	0.90	0.26	-2.28	0.022
	Altitude (*b*)	4.33	8.22	2.28	4.47	<0.001
	coefficient				3.38	0.001
... malaria on my property	Gender (*m*)	0.79	1.39	0.45	-0.81	0.415
	Altitude (*b*)	2.87	5.08	1.62	3.62	<0.001
	coefficient				2.18	0.029

Table 7-3 Summary of binary logistic regression results, comparing the use of a type of mosquito protection between genders (male = reference event) and altitude categories (below = reference event); and what is used by the respondent currently vs. what they would use in a region with confirmed cases of malaria (malaria area = reference event).

Factor	Odds ratio	C.I.+	C.I.-	*Z*	*p*
Gender (*m*)	0.88	1.43	0.54	-0.51	0.610
Altitude (*b*)	5.10	8.60	3.02	6.10	[a] <0.001
Currently vs. in malaria area (*malaria area*)	9.31	16.77	5.17	7.43	[a] <0.001
Constant				0.14	0.888

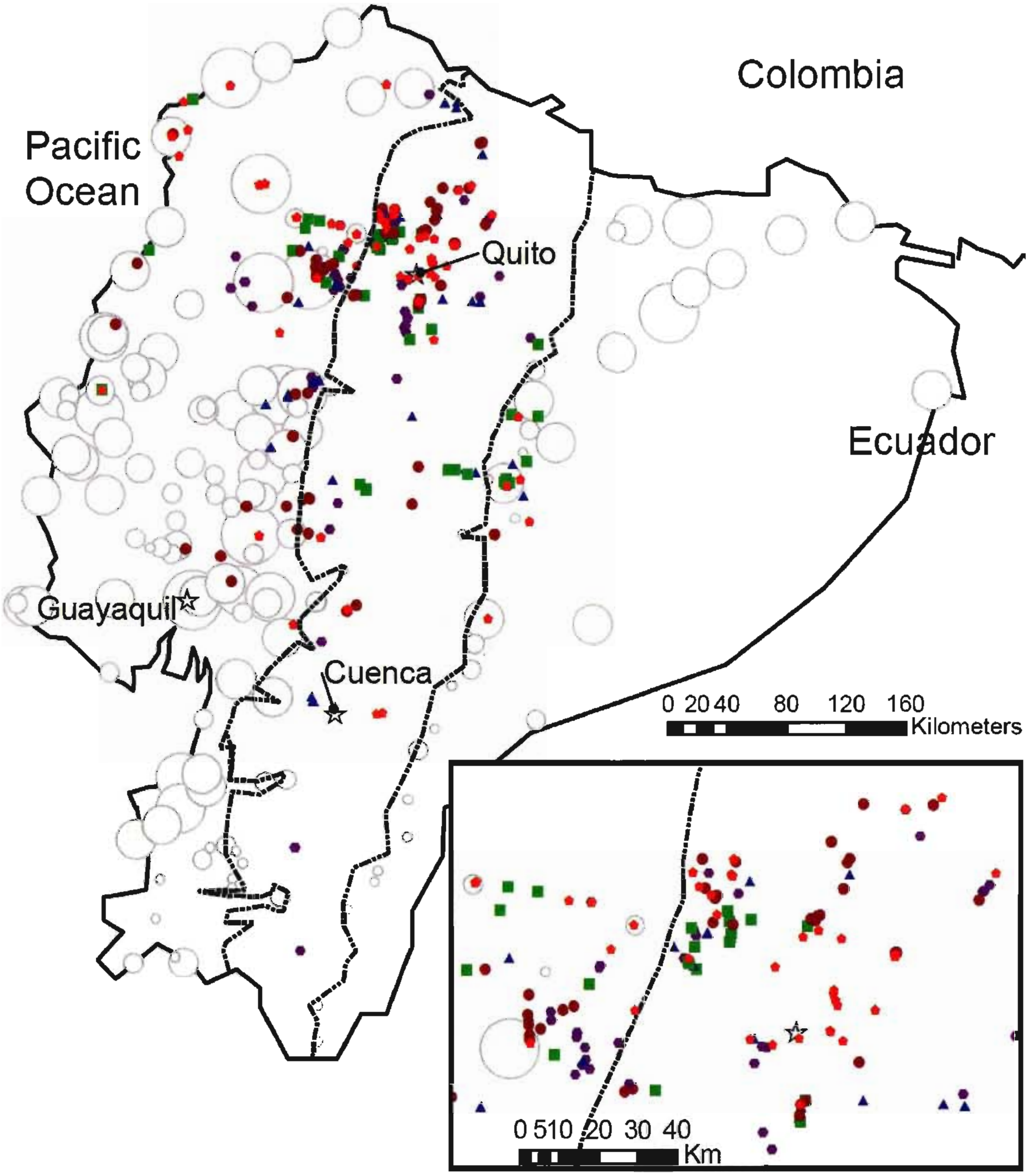

Figure 7-1 Map of interview localities (n=262) in Ecuador, plotted by five general land use types (see legend). Incidences of malaria recorded in population centers during the last 10 years are indicated by progressively larger grey circles, where the smallest circles are 1-49 malaria cases and the largest are 10000-40000 cases. The approximate 1500 m altitudinal division is indicated with hatched lines. Inset: detail of interview localities near Quito, Ecuador. Map prepared using ArcGIS v.10 software (ESRI 2010).

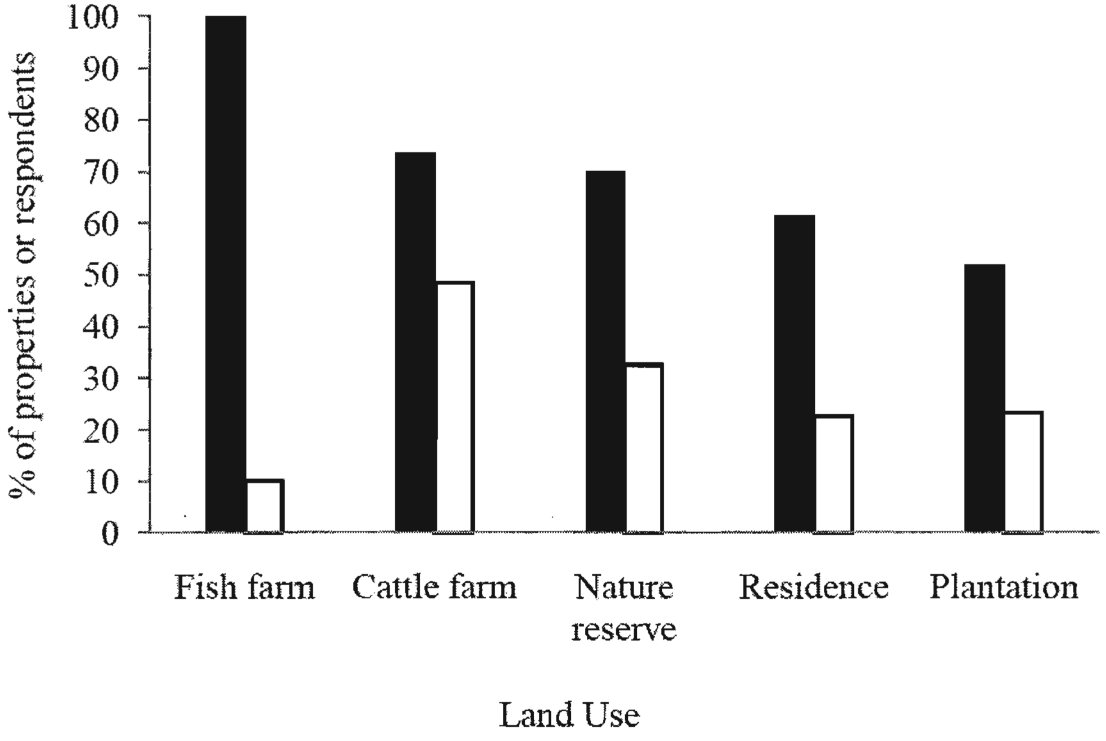

Figure 7-2 Observed standing water and perception of standing water presence on properties, categorized by land use. Black bars indicate the percentage of properties on which standing water was observed by the interviewer. White bars indicate the percentage of respondents who identified that standing water was present on their property.

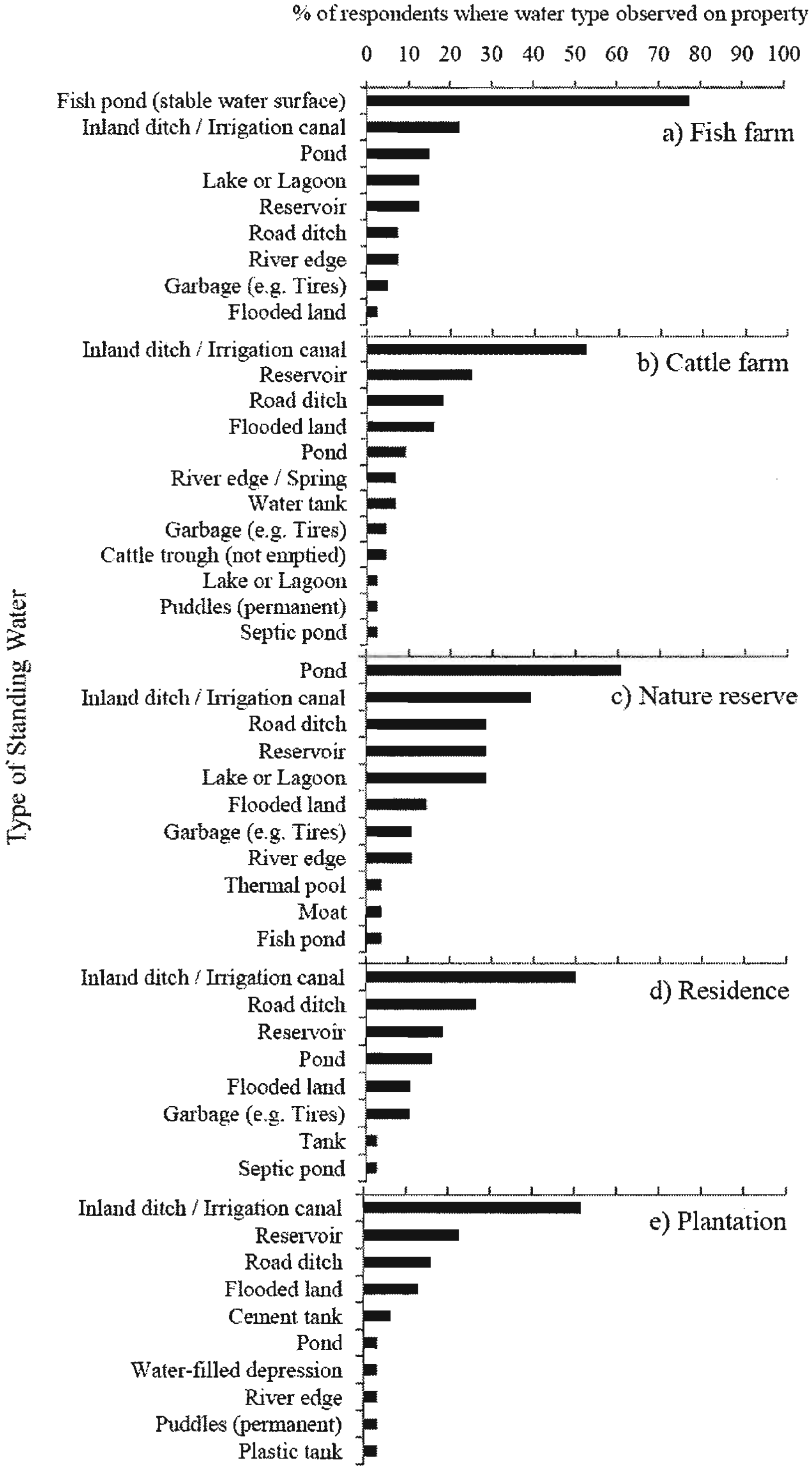

Figure 7-3 Types of standing water observed by interviewers, divided by land use (a-e). For each land use, water type is ordered from most common to least common.

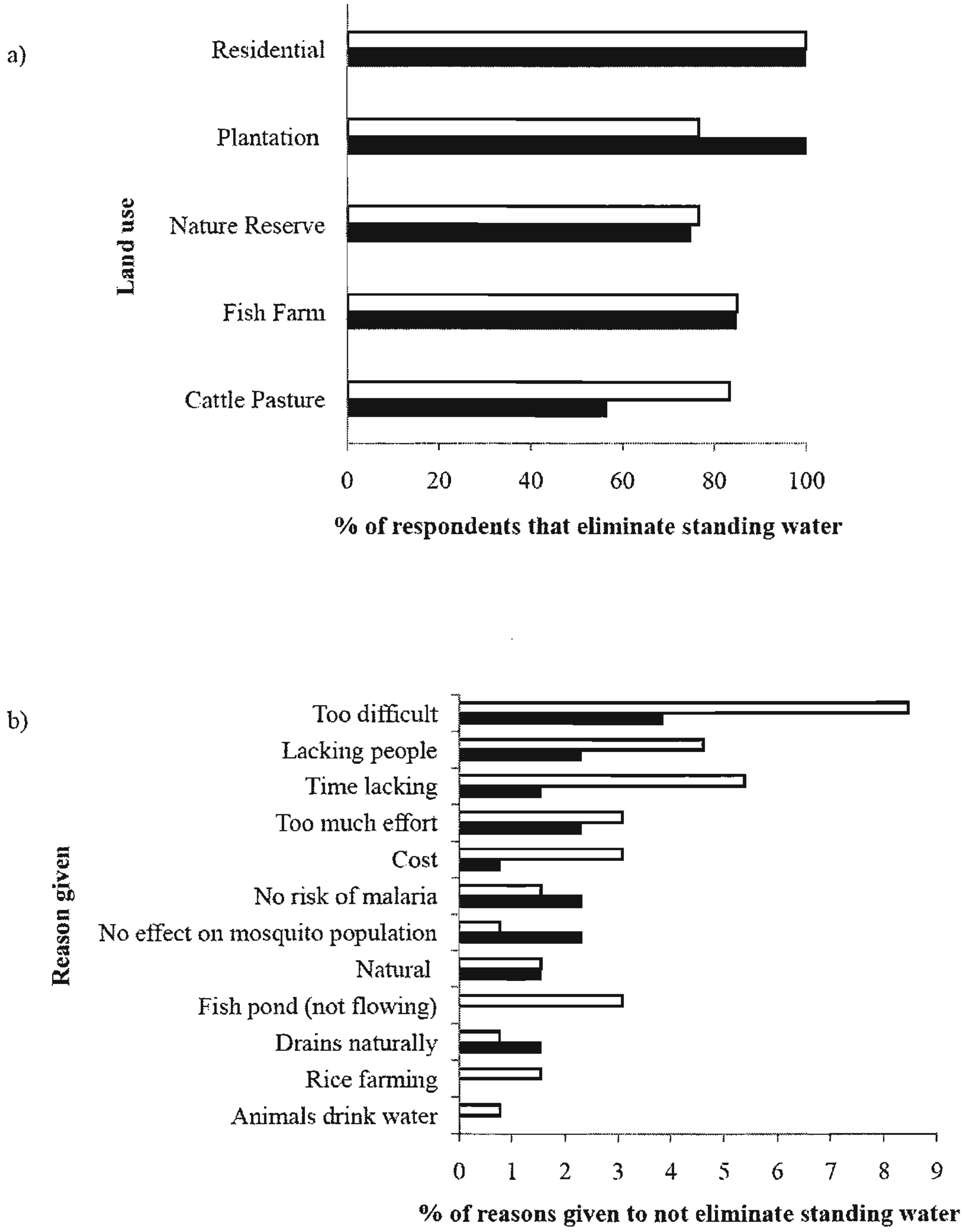

Figure 7-4 Percentage of respondents above (black bars) and below 1500 m (white bars) who: a) presently eliminate standing water, by land use; or b) provide a reason for not eliminating standing water on their property. Reasons are enumerated from most common to least common.

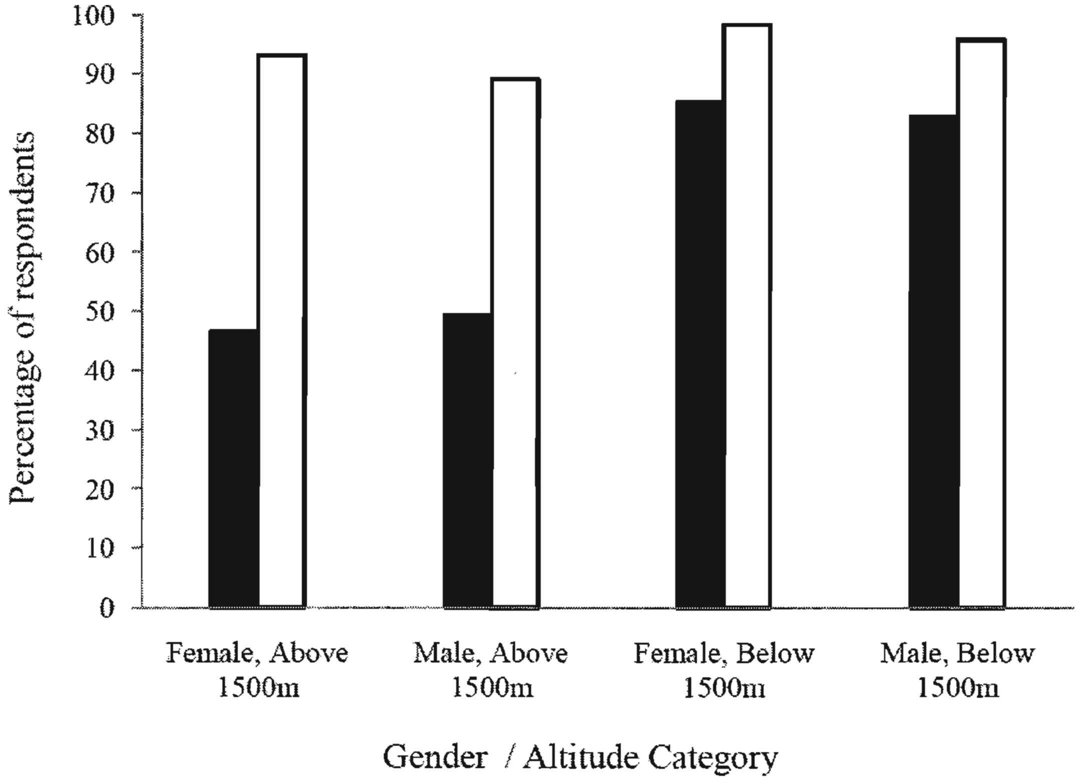

Figure 7-5 Percentage of respondents who use at least one type of mosquito bite-prevention method (black bars) or would use at least one type of mosquito bite-prevention method in an area known to have malaria cases (white bars). Bars are categorized by gender and altitude category.

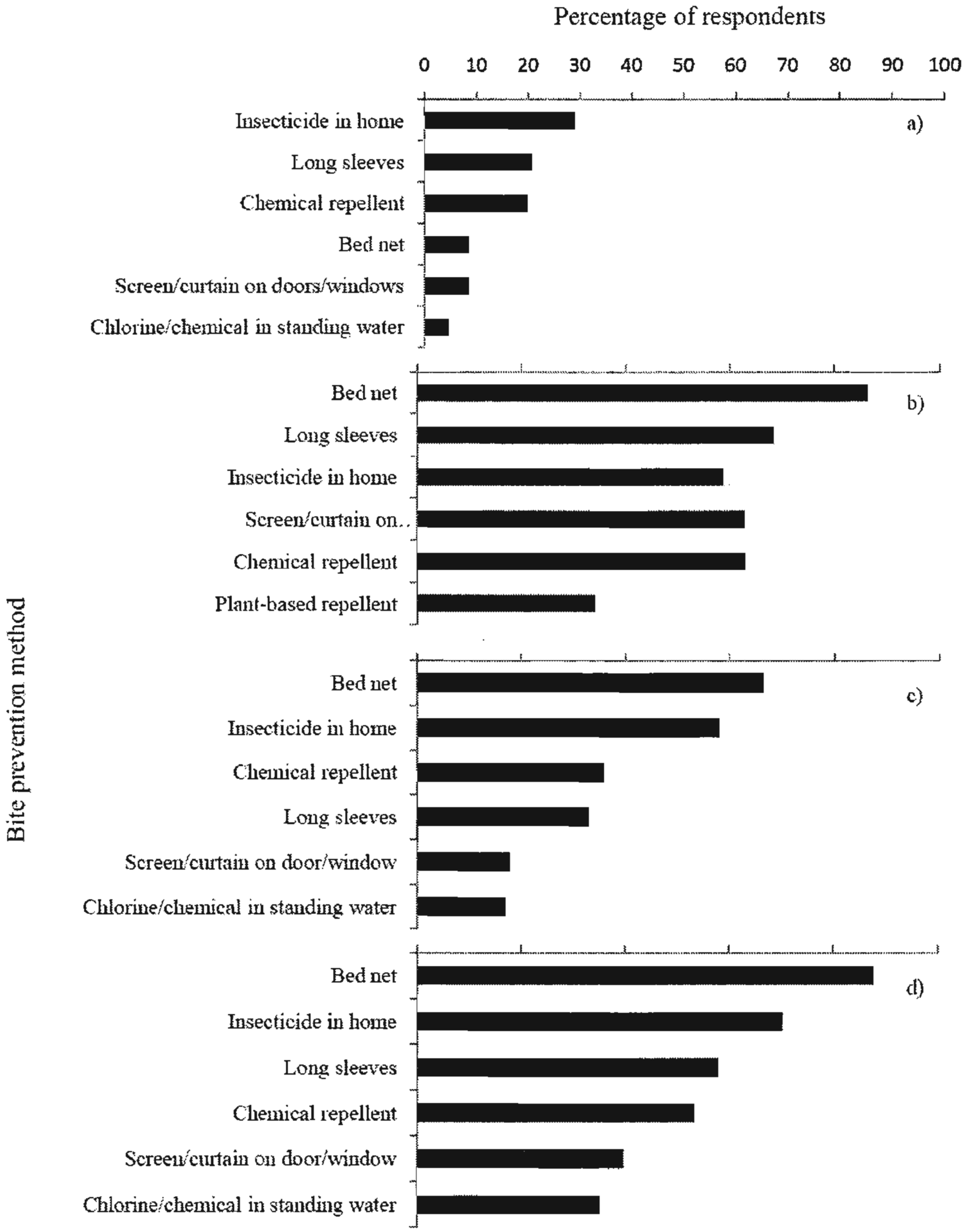

Figure 7-6 Top six bite prevention methods for respondents above 1500 m (a, b) and below 1500 m (c, d), used currently (a, c) and methods that would be used for travel to a known malaria-endemic area (b, d).

Chapter Eight

General Conclusions

Lauren Pinault

8.1 Summary of research findings

Can and will malaria vectors become re-established in the Andean highlands of Ecuador? This question has been examined through various means in the course of this thesis.

Highland malaria occurred historically throughout Ecuador during the early part of the twentieth century. Although most recent records are sparse, a thorough archival search revealed that malaria was endemic throughout many highland valleys, reaching a peak of incidence during the 1940s. The construction of the railway linking Guayaquil to Quito through Chimborazo province aided the establishment of both the vector, mainly *Anopheles pseudopunctipennis*, and the parasite (believed to be *Plasmodium vivax*), which affected citizens in the towns of Huigra and Sibambe (Meitzner 1938; Levi-Castillo 1945a). Other highland valleys considered malaria-endemic included Yungilla valley in Loja province and several highland valleys in Imbabura and Pinchincha provinces, most notoriously in the valleys adjacent to Quito (Levi-Castillo 1944b; Astudillo 1971). The previous establishment of malaria in these areas demonstrates that the conditions may be at least physiologically suitable for both the successful development of the anopheline vector and the malaria parasite in present day. Highland malaria in the valleys near Quito was successfully controlled by systematic habitat elimination and spray programs (Levi-Castillo 1945b). This activity likely removed almost all suitable habitat that existed at the time, although one more recent unconfirmed report mentions the continued presence of *An. pseudopunctipennis* in Guayllabamba river systems (Gómez de la Torre Serrano et al. 1991).

Examination of malaria statistics from the last century also highlight areas of ongoing transmission (Chapter Two). Although malaria incidence in Ecuador has declined through the activities of the Servicio Nacional de Control de Enfermedades Transmitidas por Vectores Artrópodos (SNEM), areas of continued transmission occur in lowland regions of the coast and Amazon, as well as in the foothill regions of: Santo Domingo, Santo Domingo province and in the northeastern parts of Esmeraldas, including the lower Mira valley (SNEM 1997-2008; Chapter Two).

An updated examination of the distribution of anopheline species in Chapter Three revealed a more widespread highland presence than expected, given previous records (e.g. Rubio-Palis & Zimmerman 1997). *Anopheles albimanus*, a primary malaria vector of the western coast of South America, was collected from highland localities in the Mira valley, Imbabura province, in the valley linking Quito to Santo Domingo, and in the Yungilla valley of Loja at 1541 m. The Gi* hot-spot analysis identified population hot spots in Puerto Quito, Pichincha province, Mira valley, Imbabura province, La Esperia, Pichincha province, and Santo Domingo, Santo Domingo province, which present clusters of high-density larval populations. Interestingly, all of the hot spots are found in foothill or highland regions rather than in flat lowland regions such as the rice-growing region north of Guayaquil, Guayas province, where populations are reputed to be greatest (Chapter Three). *Anopheles albimanus* larvae are associated with naturally-occurring, permanent habitats, but are also collected from anthropogenic habitat, such as flooded rice fields and cattle water ponds. Larvae are also strongly associated with sand substrates, which are more readily available in lowland regions than in highlands. Larvae were significantly associated with temperature and conductivity, the latter likely due to tolerance of high salinity in brackish water. It is likely that *An. albimanus* is limited to lower altitudes by the effects of minimum temperature, with a field-measured threshold of 20.0°C (Chapter Four).

Anopheles pseudopunctipennis was the primary vector of foothill and highland regions and was collected from both lowland coastal and highland regions up to an altitude of 1930 m in Chilchil, Cañar province. Although this species was historically found at even greater altitudes (Levi-Castillo 1945b), its presence in highland regions of multiple valleys along the length of the Andes suggests a very widespread presence and a firm establishment in the highlands (Chapter Three). *Anopheles pseudopunctipennis* larvae were associated most often with roadside ditches, river edges and water-filled depressions, as well as floating algae and boulder/gravel substrates common in these habitats, which coincides well with previous descriptions of the species' habitat associations (e.g., Manguin et al 1996). The species also is associated with warmer habitats than what is available, and might also be limited in altitudinal occurence by minimum nightly temperatures. However, the minimum temperature measured in field habitats was much lower for *An. pseudopunctipennis* than for *An. albimanus*, at 17.8°C (Chapter Four).

Anopheles punctimacula, on the other hand, has been a secondary vector of malaria in lowland coastal regions of Ecuador (Levi-Castillo 1945b; Montalvan 1948; Chapter Three). Currently, it is widespread in a multiple highland valleys along the length of the Andes, including occurrence in Mindo, Pichincha province, Mira valley, Imbabura province, and even near Sibambe, Chimborazo, at an altitude of 1906 m. This widespread occurrence in multiple highland valleys also suggests this vectors' establishment in highland regions (Chapter Three). Interestingly, *An. punctimacula* was not widely collected in coastal regions, where it occurred during the 1940s (Levi-Castillo 1945b; Montalvan 1948; Chapter Three) - suggesting that the coastal plain may have become unsuitable for *An. punctimacula* development, as in Colwell et al.

(2008) and discussed in Chapter One. *Anopheles punctimacula* were associated with plantations, road edges, and shaded pools, and negatively associated with the presence of algae. Unlike *An. albimanus* and *An. pseudopunctipennis*, the species was not significantly associated with warmer-than-average pools, but instead was associated with higher than average calculated dissolved oxygen levels, which may limit its occurrence in higher-altitude regions (Chapter Four).

Anopheles oswaldoi s.l. was the only anopheline species collected in the higher-altitude (>400 m), road-accessible regions of Amazonia where sampling was undertaken. Although *An. oswaldoi s.l.* has not been previously reported in highland regions, it has become established in larval habitats occurring up to approximately 1200 m in Pastaza-Tungurahua provinces (Chapter Three). The difficulty with conducting research in the eastern side of the Andes in Ecuador is the lack of passable roads and sparse human settlement, and it is therefore difficult to know if the species has similarly become established in other eastern highland valleys. Larvae were associated with permanent and man-made water bodies such as roadside ditches with poor drainage (Figure 8-1a,b) as well as ponds that often exist for ornamental purposes (Figure 8-1c) (Chapter Four). Its presence in anthropogenic habitats suggests that the habitats used by this species may be more easily eliminated through drainage activities than in lowland areas where habitat is more widespread.

Land uses, rivers, and roads were examined on the western side of the Andes for their contribution to larval habitat availability of the three western highland anopheline species. Rivers appeared to provide the most suitable habitat, providing water pools and slow-moving eddies with higher clarity, warmer average temperatures, the presence of floating algae, and a greater incidence of sunlight. *Anopheles albimanus* were associated with rivers in lowland regions, while

An. pseudopunctipennis were often collected from highland rivers. Although *An. pseudopunctipennis* and *An. punctimacula* were collected from the same highland river systems, they were never collected from the same water body (Chapter Five), possibly due to exclusion by intraspecific competition (e.g. Paaijmans et al. 2009). Since rivers are continuous sources of water that connect lowland to highland regions, they may form a possible corridor for mosquitoes to migrate and become established in highland regions. Suitable regions of rivers are those where flow is sufficiently reduced to permit pools of water to form on the river edges (Figure 8-2a). The role of streams and rivers as important highland habitat has been observed for anopheline species in Africa as well (Roberts 1964b; Patz et al. 2000). Particularly fatal malaria outbreaks in the highlands of Indonesia have also been attributed to streambeds becoming dry during drought (Bangs & Subianto 1999).

Road edges also provided some habitat for anopheline species in highland regions, which were characterized by greater incident sunlight, but also suitable for the other two western species. Stable water bodies form along road ditches clogged by debris from both vegetation and landslides (Figure 8-2b). Roadside habitat is not clustered along roadsides, but is instead randomly distributed, highlighting the importance of regular maintenance of roadside ditches to prevent the formation of permanent habitat (Chapter Five).

Of all the human land uses in the western lowland-highland transition area, cattle pasture was the most abundant and properties are the largest. Thirty-three percent of sites surveyed provided standing water, with 13% potentially suitable for anopheline species, such as the cattle watering lagoon in Figure 8-2c, which is highland habitat for *An. albimanus* larvae. Cattle farms contain standing water used for watering cattle as well as small water-filled depressions resulting from hoofprints or tire tracks, as in Figure 8-2d. Other common land uses in the highlands, which included banana, sugarcane and mixed tree plantations, did not provide much standing water, since they are often situated along well-drained, steep slopes, as in Figure 8-2e. Therefore, it is unlikely that the observed highland incidence of multiple anopheline species can be attributed to the creation of larval habitat through land use alteration in highland regions, as is the case in many African countries (e.g., Bødker et al. 2000; Chhabra et al. 2006). The neglect of road maintenance and river modification due to construction and hydrological dam-building activities, on the other hand, likely play a larger role in providing suitable habitat for anopheline species in the western highlands of Ecuador.

The construction of a simple predictive habitat distribution model for the three western highland-occurring species of anopheline vectors allows us to predict potential distributions for the three species with climate warming. The model output for *Anopheles albimanus* successfully predicted 91.8% of the current collection localities (n=49), and predicted a slow movement into the Mira and Chota valleys of Imbabura province, Pallatanga valley, and the towns of Huigra and Sibambe in Chimborazo province. The final model for *An. pseudopunctipennis* predicted 87.2% of larval-present sites. It predicts the possible presence of the species in the same regions as *An. albimanus*, as well as the highland valleys near Quito, including Tumbaco, Pichincha province, and Yungilla, Azuay province. The model for *An. punctimacula* predicted 76.2% of the larval-present sites (n=21) and also predicted an increase of its distribution into the same valleys as *An. albimanus*: Mira/Chota valley, Pallatanga valley and Yungilla valley. Importantly, the models of predicted anopheline distributions correspond to the same highland valleys from which endemic malaria was reported during the 1940s, and provide target points for future monitoring and malaria prevention in the highlands.

In Chapter Seven, the human perceptions of mosquitoes and malaria were considered in the context of highland versus lowland malaria risk. Land owners and managers in both highlands and lowlands understood that it was possible to get malaria on the coast or in Amazonia in 96.4% of cases, and understood the link between malaria and mosquitoes, and malaria and standing water in 89.9% and 83.8% of cases, respectively. Consistent with our findings in Chapter Five, cattle farmers reported more standing water than other land uses, with irrigation canals and inland ditches the most common water body type. The main reason not to eliminate standing water was most often logistical difficulty - in most cases (90 - 95%), landowners and managers were motivated to eliminate standing water in both highlands and lowlands due to both external sources of information or from personal observations. Above 1500 m, land owners and managers did not use bed nets as a main mosquito bite prevention method, but highland respondents reported that they would use mosquito bite prevention methods in a similar manner to lowland respondents during travel to a known malaria-endemic area (Chapter Seven). This readiness to adopt new bite prevention methods, such as bed nets, may assist in preventing ongoing transmission of malaria should it arise in highland communities. Therefore, should malaria be detected, educational programs to promote bite protection in highland communities may be particularly effective, similar to Rojas et al. (1992b).

To summarize, the malaria vectors *An. albimanus*, *An. pseudopunctipennis*, *An. punctimacula* and *An. oswaldoi s.l.* are already established in the highlands of the Ecuadorian Andes, in many separate valleys and encompassing regions from the north to the south of the country. While *An. pseudopunctipennis* was implicated in highland malaria transmission during the 1940s at even higher elevations than it is collected at present, the other species were not previously established in highland regions during the last major entomological collection (Levi-

Castillo 1945b; Montalvan 1948). In an examination of habitat, *An. albimanus* and *An. pseudopunctipennis* seem to be excluded from higher elevations since they are found in warmer-than-average habitats, while *An. punctimacula* may be excluded due to its presence in habitats with higher calculated dissolved oxygen, which is also a potential limiting factor at very high altitudes. Other larval habitat characteristics, such as the association of *An. albimanus* with sand substrates, are also limited at higher elevations and may contribute to the species' distribution limits. Since most human land uses in the western highlands do not provide standing water, it is unlikely that land use alteration has contributed to the species' establishment in highland regions. However, neglectful roadside management practices and the modification of rivers do provide highland habitat to the three common western species and may provide mosquito dispersal corridors into higher-altitude regions. Models of climate change predict very little overall change in species distributions with global warming, but the highland valleys of Mira and Chota, Tumbaco, Guayllabamba, Pallatanga, Huigra and Yungilla are all predicted to become more suitable for two or more species of *Anopheles* mosquitoes in the future.

8.2 Recommendations for future study and preventive action

The role of water impoundment and hydrological dam construction in highland regions of the Andes would be an interesting avenue of future study, especially given the role of rivers as a habitat for three western-occurring species. Hydrological dams are constructed readily and frequently in Ecuador, often reducing the river flow of highland-lowland rivers by diverting water to reservoirs through underground enclosed tunnels (e.g. Figure 8-3a). Downstream communities receive a vastly-reduced river flow, often leading to abandoned riverbeds. There, rainfall forms ideal anopheline larval habitat in pools, such as in La Maná, Cotopaxi, where continued transmission of malaria still occurs (Figure 8-3b; SNEM 1997-2008).

Traditionally, water impoundment in lowland regions has been associated with malaria incidence around the flooded areas, and is controlled in tropical regions by varying the water level (Baxter 1977; Goldman 1976; Hunter et al. 1983; Dégallier et al. 1989; Guimarães et al. 2004; Tubaki et al. 2004). Dams can also cause a decline in anopheline mosquitoes but paradoxically an increase in malaria due to the intensification of human settlement near the dam (Fearnside 1999; Guimarães et al. 2004). To the best of our knowledge, malaria has not been associated often with dams in highland regions, although dams built in the piedmont of the USA along the Atlantic Ocean during the turn of the century led to endemic malaria in the region (Boyd 1941). In Ecuador, a Before-After-Control-Impact study of anopheline population changes resulting from reduced river flow due to damming may be used to evaluate the possible role of hydroelectric dams in the establishment of anopheline vectors in highland regions.

The successful elimination of malaria from a country requires a continued effort to monitor, prevent and treat epidemics and eliminate or control larval breeding sites, a task that has been rather successfully undertaken by Ecuador's SNEM. Substantially fewer cases of malaria occur presently than have occurred in the recorded past in Ecuador (SNEM 1997-2008) and in many other regions of Latin America, prompting Behrens et al. (2007) to recommend discontinuing malaria prophylaxis for travel to many regions. However, complete elimination of malaria from Ecuador may be a more difficult goal, especially when one considers that even comparatively wealthy tropical nations such as the United Arab Emirates remain malaria-endemic, while successful elimination has only occurred in temperate regions characterized by cold winters (Gallup & Sachs 2001; Sachs & Malaney 2002). The role of vector ecology in working toward malaria elimination is crucial: it identifies ecological aspects of life stages that can be targeted for life cycle interruption, as well as habitat that can be eliminated or controlled, and the social aspects of these decisions (Ferguson et al. 2010).

Larval habitat can be modified in many ways to reduce its suitability for anopheline species. Drainage through the construction of dykes is a typical method (Carmichael 1972) that may be suitable for roadside ditches that are clogged with debris. On the coast, irrigation systems can link standing freshwater with saltwater during high tide, whereas in the highlands, predacious fish can be introduced to permanent freshwater reservoirs used for irrigation or cattle watering (Carmichael 1972). Alternatively, although *Bacillus thuringiensis israelensis* (*Bti*) is used as an effective and Diptera-specific bacterial larvicide, it is not recommended for *An. albimanus* and *An. pseudopunctipennis* in Ecuador since field tests show resurgence of larval populations after seven days of application (Kroeger et al. 1995). This is likely due to the rapid sinking of *Bti*, while anopheline larvae forage on the surface of water bodies (Kroeger et al. 1995).

The use of insecticide-treated nets (ITNs) and indoor residual spray (IRS) has been examined in the highlands of Africa. In Kenya, the use of (the more economical) IRS reduced the infection rate of *P. falciparum* by 75%, while ITN only reduced the rate by 63% (Guyatt et al. 2002). IRS also reduced malaria rates by 12-64% in highland valleys of Burundi, where ITNs were suggested as an alternative when funding for public spray programs disappears (Protopopoff et al. 2008). Ideally, both methods could be used for malaria prevention should it arise in highland regions, and both may also serve to reduce populations of anopheline adults, as in Lindblade et al. (2006). In highland Kenya, the combination of the use of ITNs and larviciding were more effective in malaria prevention than the use of only ITNs (Fillinger et al. 2009). ITNs should be particularly effective against the western anopheline species of Ecuador since biting activity occurs during nocturnal hours (after 10 p.m.), with the exception of *An. albimanus* that also bites during the hours following sunset (Elliott 1972). IRS, on the other hand, may be complicated by the vertical distribution of mosquitoes on the indoor walls of homes, particularly the higher *An. punctimacula* densities observed resting near floor level, as well as the short duration of contact observed between adult mosquitoes and sprayed surfaces (Elliott 1972).

Chemical prophylaxis has been recommended for highland dwellers during epidemics in Africa, including artemisinin-based combination therapy (ACT), which pairs a long-lasting partner drug with an artemisinin derivative that targets the asexual blood stage of the parasite (Greenwood et al. 2008). Communities might seek western medical treatment and/or make use of naturally-occurring anti-malarial plants such as cascarilla (*Cinchona succirubra*) (Perez 1944; Garavito et al. 2006). Some anti-malaria vaccines show promise as well, including vaccines that target pre-erythrocytic shizonts in liver cells, asexual stages of merozoite attachment to the erythrocyte, and sporozoite development in the mosquito salivary gland (reviewed in Ballou et al. 2004; Greenwood et al. 2008). At-risk populations can be targeted near valley bottoms where the majority of larval habitat is focused (e.g., Githeko et al. 2006).

Regional-scale predictive models may assist malaria control professionals in decision making and targeting of affected highland regions more effectively than large-scale models that do not account for other environmental interactions (Patz et al. 2005). Levi-Castillo (1947) points out that study and control of highland *Anopheles* requires a localized approach since the highland topography, climate and ecosystems are so heterogeneous. Although our model uses land cover to predict suitability, topographic wetness index has been shown in Kenya to be a better predictor (Cohen et al. 2010) and may be worth considering as an alternative model. Satellite imagery analyzed in Geographic Information Systems (GIS), particularly photosynthetic activity estimated using NDVI, could also be used to target potential breeding sites (Carter et al. 2000; Rogers et al. 2002).

Although habitat elimination and/or habitat modification, IRS, and ITNs may successfully eliminate malaria from highland valleys, ongoing vigilance is necessary to prevent its resurgence from permanently endemic lowlands (Roberts 1964c). Active entomological surveillance may be recommended in potentially affected regions, such as the Mira and Chota valleys, Imbabura province, Tumbaco and Guayllabamba valleys, Pichincha province, Pallatanga, Huigra and Sibambe, Chimborazo province and Yungilla, Azuay province. Already, a few highland larval habitats have been identified in these regions and an increase in population densities may contribute to a future malaria epidemic. Community involvement in surveillance and habitat elimination may play an important role in effective highland malaria control. Many smaller Ecuadorian communities still maintain the tradition of carrying out Mingas, in which all community members come together to acheive a common goal. A Minga may be a culturally acceptable and relatively economical way for communities to come together to reduce available larval habitat and/or the suitability of existing water bodies for locally-occurring anopheline larvae. Community participation has the ability to quite powerfully influence health outcomes. For example, in lowland Pacific Colombia, community educational programs resulted in community members actively reducing larval habitat and seeking independent training to identify malaria parasites in blood smears (Rojas et al. 1992c). Therefore, the ongoing involvement of reaching out to highland communities so that they can sustainably prevent local transmission of malaria is also strongly recommended, and will likely play a key role in prevention and elimination.

Figure 8-1 Photographs of *An. oswaldoi s.l.* habitats in highland Amazonia: a) New road construction in valle Cotundo, Napo province with insufficient water drainage, b) Edge of same road with algae-filled *An. oswaldoi s.l.* habitat; c) An ornamental pond outside a jungle lodge in Napo province also serves as *An. oswaldoi s.l.* larval habitat.

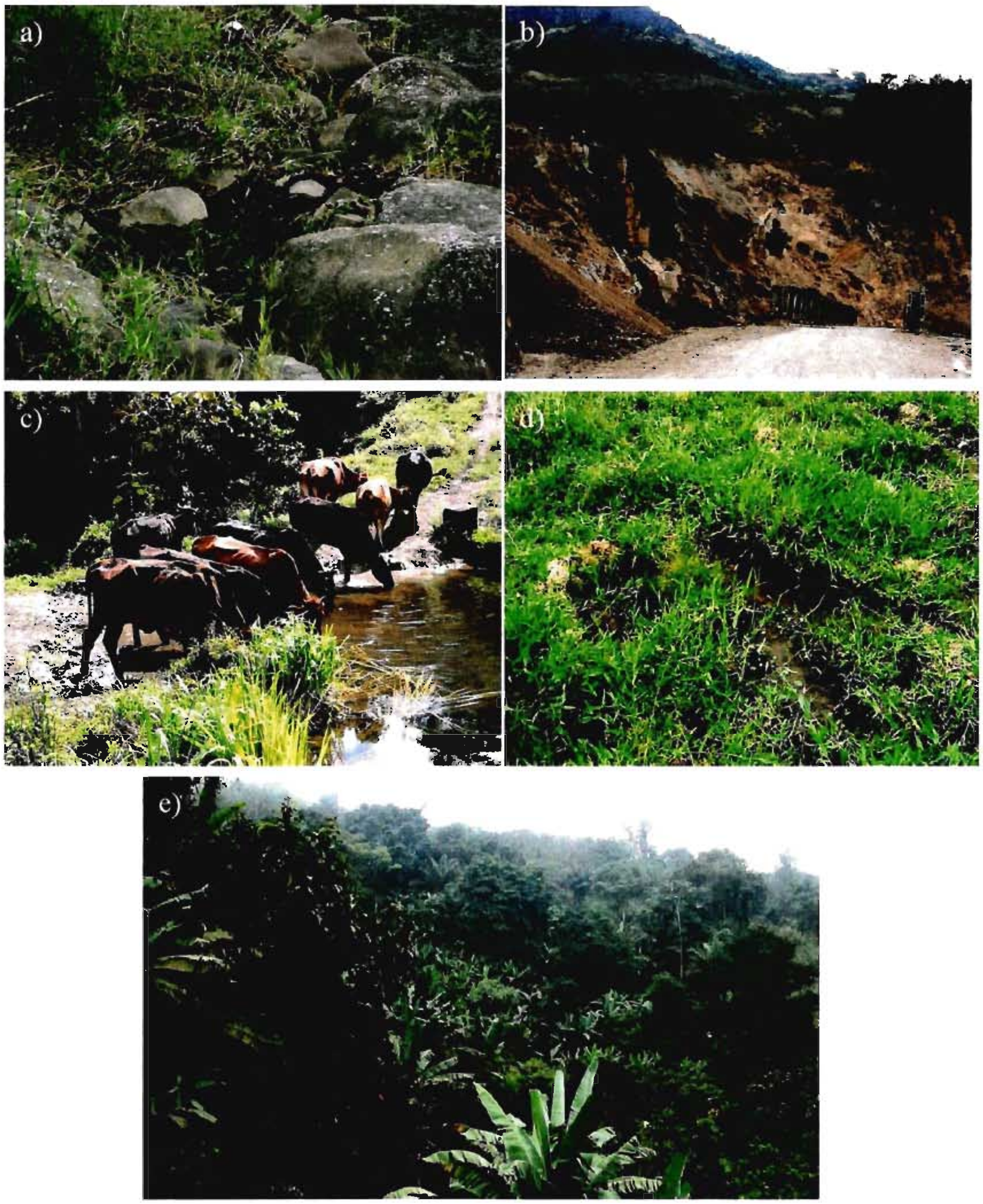

Figure 8-2 Photographs of land uses, rivers, and roads in highland Ecuador: a) *An. pseudopunctipennis* habitat pool formed on river edge in La Florida, Santo Domingo province, b) Construction next to a road edge near Huigra, Chimborazo forms permanent *An. pseudopunctipennis* habitat along the slope, c) Cattle lagoon in La Esperie, Pichincha province forms highland habitat for *An. albimanus*, d) Permanent water-filled depression in Santa Rosa, Imbabura forms habitat for *An. albimanus*, e) Highland plantations of banana occupy steep cliffs and do not accumulate much water for anopheline habitat.

Figure 8-3 Rivers before and after damming in Ecuador: a) The Pilatón river in Pichincha province to be dammed in the coming years for the Toachi-Pilatón hydrological project, with planned reduction in river flow; b) Dry riverbed resulting from upstream hydroelectric projects, where pools of rainwater in the unmanaged riverbed form high-density larval populations of *An. pseudopunctipennis*. La Mana, Cotopaxi, is adjacent to this riverbed and is an area of continued malaria transmission (SNEM 1997-2008).

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Appendix 1. Concensus gene sequences for *An. albimanus*, *An. oswaldoi s.l.*, *An. punctimacula*, *An. eiseni* and *An. pseudopunctipennis* derived from the 800 bp region of the mitochondrial CO1 gene used to distinguish unidentifiable larvae.

Sequences aligned in ClustalW2 (European Bioinformatics Institute 2010).

```
albimanus               GAGGTAAAAAGGAAACATTTGGAAATTTAGGGATAATTTATGCTATGTTAGCAATTGGAT 60
oswaldoi                GAGGTAAAAAGGAAACATTTGGAAATTTAGGGATAATTTATGCTATATTAGCAATTGGAT 60
punctimacula            GAGGTAAAAAGGAAACTTTTGGAAATTTAGGAATAATTTATGCTATATTAGCAATTGGGC 60
eiseni                  GAGGTAAAAAAGAAACATTTGGAAATTTAGGTATAATTTATGCTATATTAGCTATTGGTT 60
pseudopunctipennis      GTGGAAAAAAAGAAACTTTTGGAAATTTAGGAATAATTTATGCAATATTAGCAATTGGAT 60
                        * ** ***** ***** ************** *********** ** ***** *****

albimanus               TATTAGGATTTATTGTATGAGCTCATCATATATTTACTGTTGGAATAGACGTAGATACTC 120
oswaldoi                TATTAGGGTTTATTGTTTGAGCACATCATATATTTACTGTTGGGATAGACGTTGATACTC 120
punctimacula            TATTAGGATTTATTGTTTGAGCCCATCATATATTCACAGTAGGAATAGACGTGGATACCC 120
eiseni                  TATTAGGATTTATTGTTTGAGCTCATCATATATTTACAGTAGGAATAGACGTAGACACAC 120
pseudopunctipennis      TATTAGGATTTATTGTTTGAGCTCATCATATATTCACAGTAGGAATAGACGTTGACACTC 120
                        ******* ******** ***** *********** ** ** ** ******** ** ** *

albimanus               GAGCTTATTTCACATCAGCTACTATAATTATTGCTGTACCAACAGGGATTAAAATTTTTA 180
oswaldoi                GAGCTTATTTTACATCAGCTACTATAATTATTGCTGTTCCTACAGGAATTAAAATTTTTA 180
punctimacula            GAGCTTATTTTACTTCTGCAACTATAATTATTGCAGTACCTACAGGAATTAAAATTTTTA 180
eiseni                  GAGCTTATTTTACTTCAGCTACTATAATTATTGCTGTACCAACAGGAATTAAAATTTTTA 180
pseudopunctipennis      GAGCCTACTTTACATCAGCAACAATAATTATTGCGGTACCCACAGGTATTAAAATTTTTA 180
                        **** ** ** ** ** ** ** *********** ** ** ***** *************

albimanus               GTTGATTAGCTACCTTACATGGTACACAATTAACATATAGTCCTGCTATATTATGAGCCT 240
oswaldoi                GTTGATTAGCTACTTTACATGGAACACAATTAACTTACAGTCCCGCAATACTTTGAGCAT 240
punctimacula            GATGATTAGCTACCTTACATGGAACACAACTTACTTACAGCCCAGCAATACTTTGATCAT 240
eiseni                  GTTGATTAGCTACTCTTCATGGAACTCAATTAACTTATAGCCCAGCTATACTATGAGCCT 240
pseudopunctipennis      GTTGACTAGCAACAATACACGGTACACAATTAACATATAGCCCCGCTATACTTTGAGCCT 240
                        * *** **** **  * ** ** ** *** * ** ** ** ** ** *** * *** * *

albimanus               TTGGATTTGTGTTTTTATTTACTGTAGGAGGATTAACTGGAGTTGTTCTAGCTAATTCTT 300
oswaldoi                TTGGGTTTGTATTTTTATTTACTGTAGGAGGACTAACAGGAGTAGTTTTAGCTAATTCTT 300
punctimacula            TCGGATTTGTATTTTTATTTACAGTAGGAGGATTAACTGGAGTTGTTTTAGCCAATTCTT 300
eiseni                  TTGGATTTGTTTTTTTATTTACAGTAGGGGGATTAACAGGAGTTGTTCTTGCTAATTCAT 300
pseudopunctipennis      TTGGATTTGTATTTTTATTTACTGTAGGAGGATTAACTGGAGTAGTATTAGCTAACTCTT 300
                        * ** ***** *********** ***** *** **** ***** **  * ** ** ** *

albimanus               CTATTGACATTGTACTACACGATACTTATTATGTAGTTGCTCATTTCCACTATGTTTTAT 360
oswaldoi                CAATTGATATTGTATTACATGATACTTATTATGTAGTAGCTCATTTCCATTATGTATTAT 360
punctimacula            CACTTGATATTGTTCTTCACGACACTTATTATGTTGTAGCCCATTTTCATTATGTACTAT 360
eiseni                  CTCTTGATATTGTTTTACATGATACTTATTATGTTGTTGCACATTTTCATTATGTTTTAT 360
pseudopunctipennis      CTATTGATATTGTTCTTCATGATACATATTATGTAGTTGCACACTTTCATTATGTATTAT 360
                        *  **** *****  * ** ** ** ******** ** ** ** ** ** *****  ***

albimanus               CAATAGGAGCAGTATTTGCAATTATAGCAGGGTTTATTCATTGATACCCATTATTAACAG 420
oswaldoi                CTATGGGAGCAGTATTTGCTATTATAGCTGGATTTGTTCATTGATATCCACTTTTAACTG 420
punctimacula            CTATAGGAGCCGTATTCGCAATTATAGCAGGGTTTATCCATTGATACCCCTTATTAACTG 420
eiseni                  CTATAGGAGCCGTATTTGCAATTATAGCAGGATTTATTCATTGGTACCCTTTATTAACTG 420
pseudopunctipennis      CAATAGGAGCGGTATTTGCTATTATAGCAGGATTTATTCATTGATATCCTTTATTAACCG 420
                        * ** ***** ***** ** ******** ** *** * ***** ** **  * ***** *

albimanus               GATTAACAATAAATCCTACTTGATTAAAAATTCAATTTTCTATAATGTTTGTTGGAGTAA 480
oswaldoi                GATTAACTATGAACCCTACATGATTAAAAATTCAATTTTCTATAATATTTGTTGGAGTAA 480
punctimacula            GATTAACAATAAACCCAACATGATTAAAACTTCAATTTGCAATAATATTTATTGGAGTAA 480
eiseni                  GATTAACTATAAATCCATCTTGATTAAAATTACAATTTGCAATAATATTTATTGGAGTAA 480
pseudopunctipennis      GACTAACAATAAATCCAACTTGACTAAAACTTCAATTTGGAATAATGTTTATTGGAGTAA 480
                        ** **** ** ** **  * *** ***** * ******   ***** *** *********

albimanus               ATTTAACTTTCTTCCCTCAACATTTCTTAGGATTAGCTGGAATACCTCGACGATATTCTG 540
oswaldoi                ACTTAACTTTTTTCCCTCAACATTTTTTAGGATTAGCAGGAATACCACGACGATATTCAG 540
punctimacula            ATTTAACATTTTTCCCACAACACTTTCTAGGATTAGCAGGAATACCTCGTCGATACTCTG 540
eiseni                  ATTTAACTTTTTTTCCTCAACATTTTTTAGGGTTAGCAGGTATACCTCGTCGATACTCTG 540
pseudopunctipennis      ATTTAACATTTTTCCCTCAACATTTTCTAGGATTAGCTGGAATACCTCGACGATATTCAG 540
                        * ***** ** ** ** ***** **  **** ***** ** ***** ** ***** ** *
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albimanus               ATTTTCCTGACAGCTACTTAACTTGAAATATTGTATCTTCATTAGGTAGAACAATTTCAT 600
oswaldoi                ATTTCCCTGATAGTTACTTAACTTGAAATATTGTTTCTTCTTTAGGAAGAACAATTTCAT 600
punctimacula            ATTTCCCCGATAGTTATTTAGCTTGAAATATTGTATCTTCATTAGGTAGAACAATTTCTT 600
eiseni                  ATTTTCCTGACAGTTATTTAACTTGAAATATCGTTTCTTCTTTAGGAAGAACAATTTCTT 600
pseudopunctipennis      ATTTTCCAGACAGATATTTAACATGAAATATTGTGTCATCATTAGGTAGTACAATTTCAT 600
                        **** ** ** ** ** *** * ******** ** ** ** ***** ** ******** *

albimanus               TATTTGCTATTTTATACTTCTTATTTATTATTTGAGAAAGTATAATTACTCAACGTACTC 660
oswaldoi                TATTTGCTATTTTATACTTTTTATTTATTATTTGAGAAAGTATAATTACTCAACGAACTC 660
punctimacula            TATTTGCTATTTTATACTTTTTATTTATTATTTGAGAAAGTATAATCACTCAACGTACCC 660
eiseni                  TATTTGCTATTTTATATTTTTTATTTATTATTTGAGAAAGTATAATTACACAACGAACCC 660
pseudopunctipennis      TATTTGCAATTTTATACTTCTTATTTATTATTTGAGAAAGTATAATTACTCAACGAACTC 660
                        ******* ******** ** ************************** ** ***** ** *

albimanus               CTGCATTCCCAATACAATTATCTTCTTCTATTGAATGATACCATACATT 709
oswaldoi                CAGCATTCCCAATACAACTATCTTCATCTATTGAATGATATCACACACT 709
punctimacula            CAGCATTTCCAATACAACTGTCTTCTTCTATTGAATGATACCACACCCT 709
eiseni                  CTGCTTTTCCTATACAACTATCTTCATCTATTGAATGATACCACACTTT 709
pseudopunctipennis      CTGCCTTTCCAATACAACTATCATCATCAATTGAATGATACCACACTCT 709
                        * ** ** ** ****** * ** ** ** *********** ** **  *
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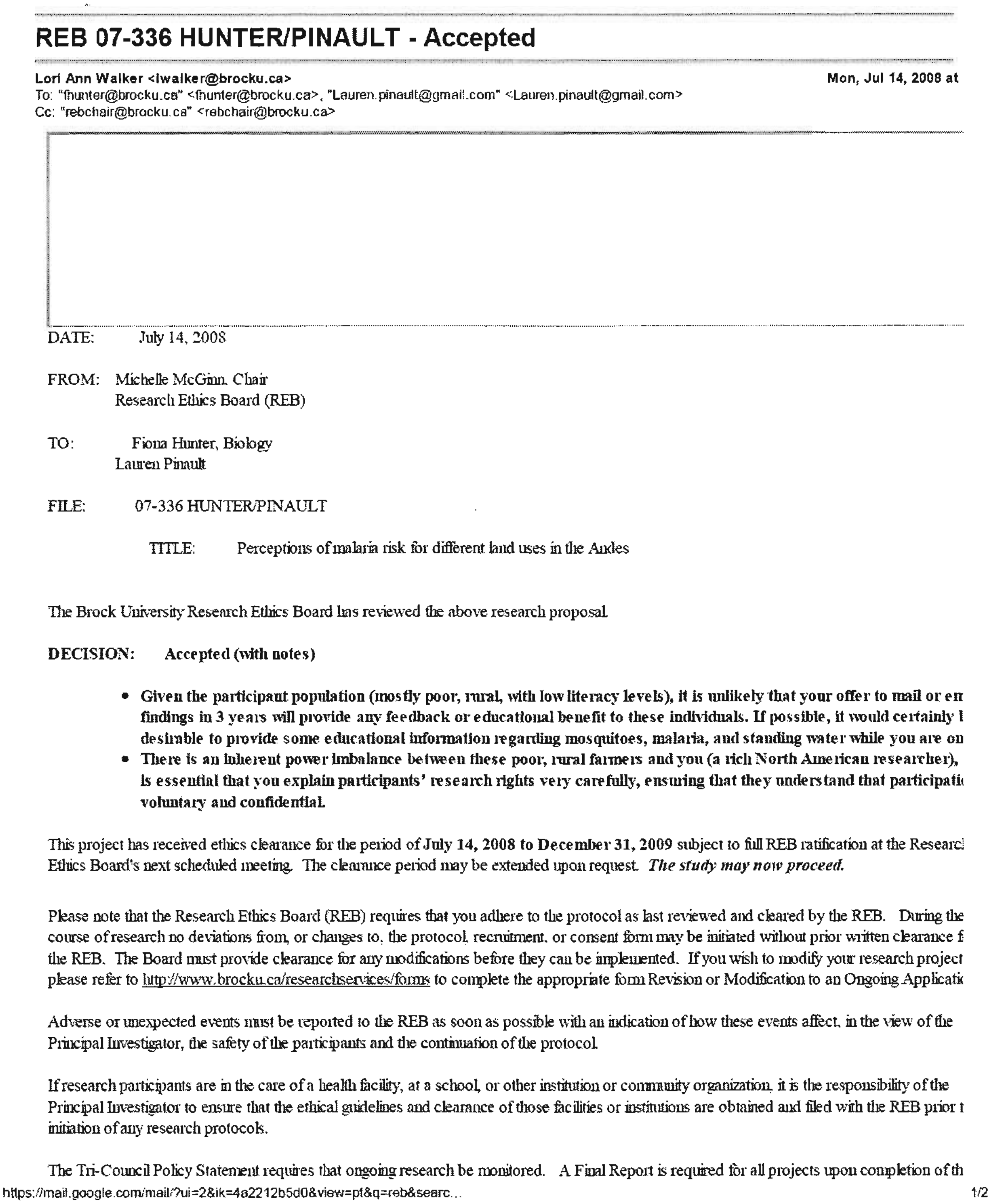
Appendix 2. Habitat characteristic list and categories measured at larval habitats in Chapter 4.

Type	Habitat Characteristic	Category (where applicable)
General	Latitude	
	Longitude	
	Altitude (m)	
	Ecosystem Type	Northwest littoral wet tropical forest
		Inter-Andean valley
		Banana farm
		Cloud forest
		Dry tropical forest
		Amazonian tropical forest
		Semi-desert/Desert
		High-altitude ecosystem/Paramo
Anthropogenic	Water Body Type	River edge
		Pond
		Marsh
		Lake
		Water-filled depression
		Rice paddy
		Irrigation canal
		Roadside ditch
		Inland ditch
	Human Site Use	No land use
		Tourism
		Plantation
		Fish farm
		Cattle pasture
		Human habitation
		Construction
		Road
	Pollution/Contamination	Road
		Garbage
		Sewage
		Manure
		Pesticides
		Detergent
		Fish farm effluent
		Oil
		Mine
	Natural/Anthropogenic	Natural
		Anthropogenic
Abiotic	Permanence	Permanent
		Temporary

Type	Habitat Characteristic	Category (where applicable)
Abiotic	Surface area	
	Depth	
	Water temperature	
	pH	
	Conductivity	
	Water clarity	10% classes - visual estimate
	Incident sunlight	10% classes - visual estimate
	Dominant substrate	Clay
		Silt
		Sand
		Gravel
		Cobble
		Boulder
		Bedrock
		Cement
Biotic	Woody debris	Present/Absent
	Detritus debris	Present/Absent
	Macrophyte Vegetation	Emergent
		Rooted floating
		Submergent
		Free floating
	Algae	Floating
		Filamentous
		Attached
		Slimes/Crusts
	Invertebrates	Coleoptera
		Hemiptera
		Trichoptera
		Ephemeroptera
		Odonata
		Crustaceans
		Mites
		Mollusca
		Diptera
		Oligochaetes
		Hirudinae
		Collembola
		Nematodes

Appendix 3. Human ethics clearance from Brock University Research Ethics Board (Chapter Seven). Both original approval and modification approval forms are attached.



REB 07-336 HUNTER/PINAULT - Accepted

Lori Ann Walker <lwalker@brocku.ca> **Mon, Jul 14, 2008 at**
To: "fhunter@brocku.ca" <fhunter@brocku.ca>, "Lauren.pinault@gmail.com" <Lauren.pinault@gmail.com>
Cc: "rebchair@brocku.ca" <rebchair@brocku.ca>

DATE: July 14, 2008

FROM: Michelle McGinn, Chair
Research Ethics Board (REB)

TO: Fiona Hunter, Biology
Lauren Pinault

FILE: 07-336 HUNTER/PINAULT

TITLE: Perceptions of malaria risk for different land uses in the Andes

The Brock University Research Ethics Board has reviewed the above research proposal.

DECISION: Accepted (with notes)

- **Given the participant population (mostly poor, rural, with low literacy levels), it is unlikely that your offer to mail or en findings in 3 years will provide any feedback or educational benefit to these individuals. If possible, it would certainly l desirable to provide some educational information regarding mosquitoes, malaria, and standing water while you are on**
- **There is an inherent power imbalance between these poor, rural farmers and you (a rich North American researcher), is essential that you explain participants' research rights very carefully, ensuring that they understand that participati voluntary and confidential.**

This project has received ethics clearance for the period of **July 14, 2008 to December 31, 2009** subject to full REB ratification at the Researc Ethics Board's next scheduled meeting. The clearance period may be extended upon request. ***The study may now proceed.***

Please note that the Research Ethics Board (REB) requires that you adhere to the protocol as last reviewed and cleared by the REB. During the course of research no deviations from, or changes to, the protocol, recruitment, or consent form may be initiated without prior written clearance f the REB. The Board must provide clearance for any modifications before they can be implemented. If you wish to modify your research project please refer to http://www.brocku.ca/researchservices/forms to complete the appropriate form Revision or Modification to an Ongoing Applicati

Adverse or unexpected events must be reported to the REB as soon as possible with an indication of how these events affect, in the view of the Principal Investigator, the safety of the participants and the continuation of the protocol.

If research participants are in the care of a health facility, at a school, or other institution or community organization, it is the responsibility of the Principal Investigator to ensure that the ethical guidelines and clearance of those facilities or institutions are obtained and filed with the REB prior t initiation of any research protocols.

The Tri-Council Policy Statement requires that ongoing research be monitored. A Final Report is required for all projects upon completion of th project. Researchers with projects lasting more than one year are required to submit a Continuing Review Report annually. The Office of Researc Services will contact you when this form *Continuing Review/Final Report* is required.

Please quote your REB file number on all future correspondence.

MM/law

Lori Walker
Research Ethics Officer
Brock University, Office of Research Services
500 Glenridge Ave, St. Catharines, ON L2S 3A1
phone: (905) 688-5550 x4876
fax: (905) 688-0748
email: lori.walker@brocku.ca

REB 07-336 - Continuing Review Accepted

Research Ethics Board <reb@brocku.ca> **Fri, Jan 16, 2009 at 3:40 PM**
To: "lauren.pinault@gmail.com" <lauren.pinault@gmail.com>, "fhunter@brocku.ca" <fhunter@brocku.ca>
Cc: Michelle McGinn <rebchair@brocku.ca>

FROM: Michelle McGinn, Chair
Research Ethics Board (REB)

TO: Fiona Hunter, Biology
Lauren Pinault

RE: Continuing Review

FILE: 07-336 - HUNTER/PINAULT
Ph. D.
Original clearance date: July 14, 2008
Date of completion: December 31, 2010

DATE: January 16, 2009

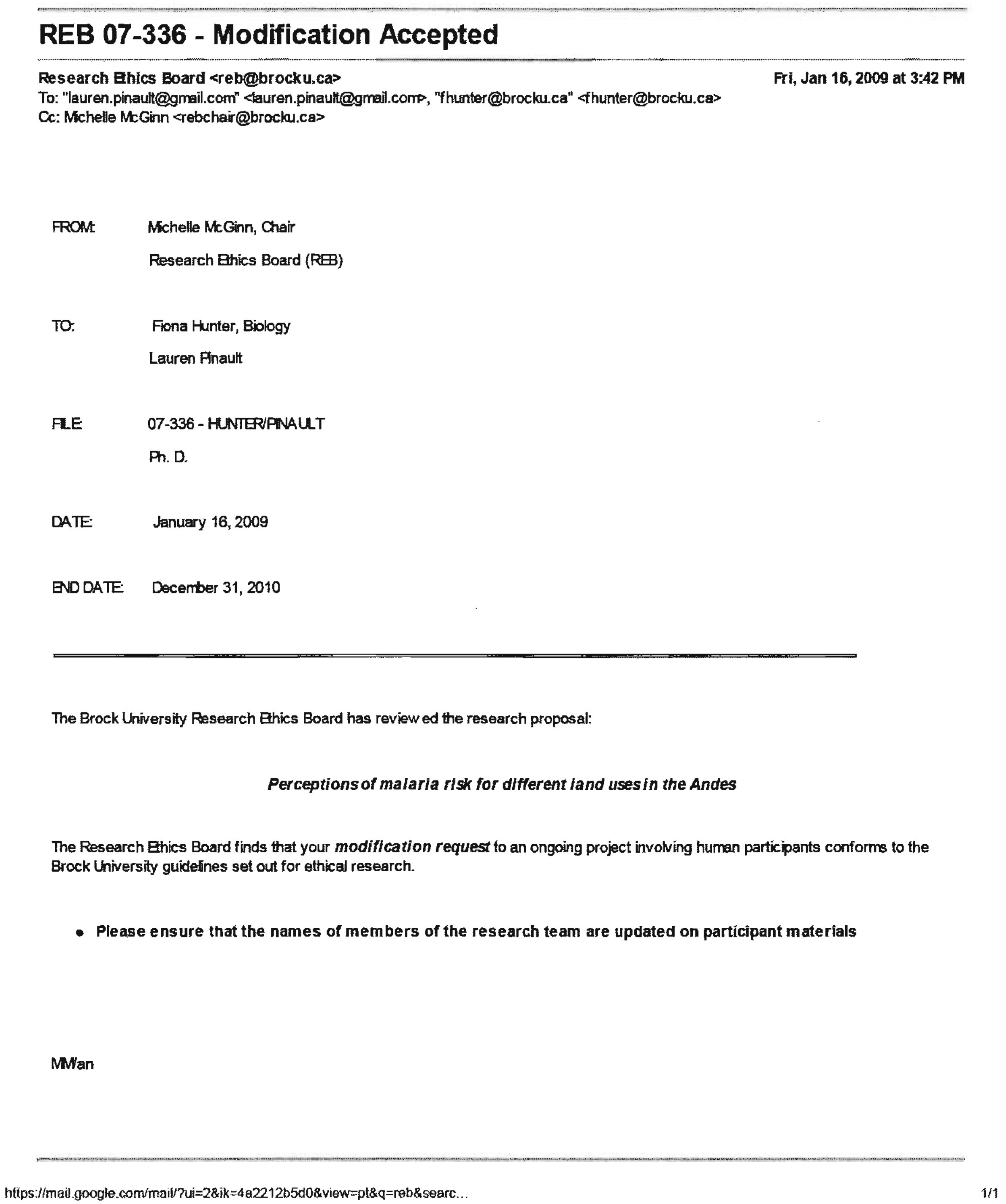
Thank you for completing the ***Continuing Review*** form. The Brock University Research Ethics Board has reviewed this report for:

Perceptions of malaria risk for different land uses in the Andes

The Committee finds that your original proposal and ongoing research conforms to the Brock University guidelines set out for ethical research.

*** Continuing Review Accepted.**

MM/an



REB 07-336 - Modification Accepted

Research Ethics Board <reb@brocku.ca> **Fri, Jan 16, 2009 at 3:42 PM**
To: "lauren.pinault@gmail.com" <lauren.pinault@gmail.com>, "fhunter@brocku.ca" <fhunter@brocku.ca>
Cc: Michelle McGinn <rebchair@brocku.ca>

FROM: Michelle McGinn, Chair
Research Ethics Board (REB)

TO: Fiona Hunter, Biology
Lauren Pinault

FILE: 07-336 - HUNTER/PINAULT
Ph. D.

DATE: January 16, 2009

END DATE: December 31, 2010

The Brock University Research Ethics Board has reviewed the research proposal:

Perceptions of malaria risk for different land uses in the Andes

The Research Ethics Board finds that your ***modification request*** to an ongoing project involving human participants conforms to the Brock University guidelines set out for ethical research.

- **Please ensure that the names of members of the research team are updated on participant materials**

MM/an

https://mail.google.com/mail/?ui=2&ik=4a2212b5d0&view=pt&q=reb&searc... 1/1

Appendix 4. Sample questionnaire used to interview landowners and land managers in Ecuador (Chapter 7).

1. Please state the degree to which you agree to the following statements, rating them as 1: strongly agree, 2: agree, 3: neither agree nor disagree, 4: disagree, 5: strongly disagree, 6: don't know

Statement	**1**	**2**	**3**	**4**	**5**	**6**
I am more often bitten by mosquitoes on my property, compared to other places.						
I will eliminate standing water on my property if scientific reports recommend that I do so.						
I am concerned about other diseases spread by insects, such as leishmaniasis and dengue.						
It is presently *possible* for me to get malaria on my property.						
It is presently *likely* for me to get malaria on the coast or jungle.						
Eliminating standing water on my property will reduce my chances of getting malaria.						
Many scientists believe that with climate change, malaria will become a problem at higher altitudes. I agree and think that it is possible for malaria to become a problem at higher altitudes in the future.						
If more mosquitoes start biting me in future years, I will ensure that all standing water is eliminated from my property.						
If malaria becomes more of a problem in a village nearby, I will make sure to eliminate standing water on my property.						
My type of land use does not create very much standing water compared to other uses of land nearby.						
If I was provided financial assistance, I would eliminate standing water if I was asked to.						
I am concerned about malaria on my property.						
I presently eliminate standing water on my property.						
It is presently *likely* for me to get malaria in the high mountains (altitude >3000m).						
It is presently *likely* for me to get malaria on my property.						
I am concerned about mosquitoes on my property.						
It is presently *possible* for me to get malaria in the high mountains (altitude >3000m).						
I agree with many scientists that the average temperature of the earth is gradually becoming warmer, a process referred to as climate change.						
It is presently *possible* for me to get malaria on the coast or jungle.						
Eliminating standing water on my property will control mosquito populations on my property.						

2. What reasons would stop you from eliminating standing water on your property today? (please check all that apply):
__ cost __no time __too much effort __ lack of people to do it
__would not affect mosquito population __malaria is not enough of a risk
__too difficult to drain a specific area
__other (please specify): ____

3. a) Do you use mosquito protection on your property?
__yes __no

b) If yes, what types of mosquito control do you use on your property? (please check all that apply):
__mosquito bed net __mosquito jacket __chemical repellent (e.g. Deet)
__"natural" repellent (plant base) __eating different foods (vitamins) __mosquito coil
__chemicals in breeding ponds __special screening areas __insecticide spray inside homes
__other (please specify):____

C) Would you use mosquito protection in an area with reported cases of malaria?
__yes __no

d) if Yes, what types of mosquito control would you use in an area with reported cases of malaria? (please check all that apply):
__mosquito bed net __mosquito jacket __chemical repellent (e.g. Deet)
__"natural" repellent (plant base) __eating different foods (vitamins) __mosquito coil
__chemicals in breeding ponds __insecticide spray inside homes
__other (please specify):____

4. The following are the most important sources of standing water for breeding mosquitoes in your area (please check all that apply):
__ponds __ditches on roads __irrigation on farming properties
__flooded land __lakes __garbage and refuse
__other (please specify):____

Land use type:__________________________ Respondent Identifier: ____________________
Altitude:__________________ Latitude:______________________ Longitude:______________________
General location:____
Date:______________________ Time:______________________ Interviewer:______________________

1. Por favor indique que tan de acuerdo esta usted con las siguientes declaraciones según el siguiente criterio: 1: fuertemente de acuerdo; 2: de acuerdo, 3: ni de acuerdo ni en desacuerdo, 4: en desacuerdo, 5: fuertemente en desacuerdo, 6: no se.

Declaraciones	**1**	**2**	**3**	**4**	**5**	**6**
Soy picado por zancudos mas frecuentemente en mi casa/propiedad que en otros lugares.						
Eliminaria las aguas estancadas de mi propiedad si las autoridades de salud lo recomiendan						
Me preocupan otras enfermedades transmitidas por insectos, como la uta y el dengue.						
Actualmente es posible contraer malaria en mi propiedad.						
Es posible para mi que pueda contraer malaria en la costa o en la selva.						
Eliminar agua estancada de mi propiedad reduciria las probabilidades de contraer malaria.						
Muchos científicos creen que con el cambio climático, la malaria va a llegar a ser un problema en zonas de altura. Estoy de acuerdo, y creo que es posible que la malaria llegue a ser un problema en zonas de altura en el futuro.						
Si soy picado por zancudos mas frecuentmente en los proximos años, voy a asegurarme de que toda fuente de agua estancada que exista en mi propiedad es eliminada.						
Si la malaria llegar a ser un gran problema en una comunidad cercana, voy a asegurarme de que toda fuente de agua estancada que exista en mi propiedad es eliminada.						
La forma en que uso la tierra no crea mucha agua estancada, comparada con otras formas de uso de la tierra.						
Si se me ofrece ayuda economica, eliminaría el agua estancada de mi propiedad si me lo pidieran.						
Me preocupa que exista malaria en mi propiedad.						
Actualmente yo elimino fuentes de agua estancada de mi propiedad.						
Actualmente, es muy probable que yo pueda contraer malaria en las montañas a alturas por encima de los 3000 metros sobre el nivel del mar.						
Actualmente, es muy probable que yo pueda contraer malaria en mi propiedad.						
Me preocupa que existan zancudos en mi propiedad.						
Actualmente, es posible que yo pueda contraer en las montañas a alturas por encima de los 3000 metros sobre el nivel del mar.						
Estoy de acuerdo con muchos científicos que afirman que la temperatura media del planeta esta gradualmente aumentando, haciendose mas caliente, un proceso llamar cambio climático o calentamiento global						
Actualmente, es posible que yo pueda contraer malaria en la costa o en la selva.						
Eliminar el agua estancada de mi propiedad va a ayudar a controlar los zancudos						

2. Qué razones le impiden actualmente eliminar fuentes de agua estancada en su propiedad? (por favor indique todas las que se apliquen a su caso):
__ muy caro __no tengo tiempo __demasiado esfuerzo __ faltan personas para hacerlo
__no va a afectar el numero de zancudos __la malaria no es un riesgo serio
__es demasiado difícil drenar las áreas con aguas estancadas
__otro (por favor especificar): ______________________________

3. a) Usa algun tipo de protección contra zancudos en su propiedad?

__si __no

b) Si respondio que si usa algun tipo de proteccion contra zancudos, Que tipos de control usa usted? (por favor indique todas las que se apliquen a su caso):
__tul o mosquitero alrededor de mi cama y/o ventanas __ropa contra zancudos __repelente químico (como Deet)
__repelente natural (plantas) __comer alimentos que previenen de ser picado __espiral contra zancudos
__vertimiento de quimicos en aguas estancadas __insecticida en la casa __trampas de luz violeta
__mallas en las ventanas y puertas.
__ otro (por favor especificar):______________________________

C) Usaría algun metodo de protección contra zancudos en un área donde hay malaria?
__si __no

d) Si respondio que si a la pregunta anterior, Que tipos de control usaria usted en un área donde hay malaria? (por favor indicar las que sean pertinentes):
__tul o mosquitero alrededor de mi cama y/o ventana __ropa contra zancudos __repelente químico (como Deet)
__repelente natural (plantas) __comer alimentos que previenen de ser picado __espiral contra zancudos
__vertimiento de quimicos en aguas estancadas __insecticida en la casa __trampas de luz violeta __mallas en las ventanas y puertas.
__ otro (por favor especificar):______________________________

4. Por favor indicar todos los orígenes de aguas estancadas que existen en su propiedad y que podrian potencialmente servir como criadero de zancudos.
__estanques, pozas o puquiales __zanjas al lado de los caminos __asequias o canales de irrigación __reservorios de agua
__tierras inundables __lagos o lagunas __basura (pilas de llantas abandonadas, contenedores en deshuso u otros artefactos que puedan acumular agua de lluvia de manera mas o menos temporal)
__ otro (por favor especificar):______________________________

Land use type:____________________ Respondent Identifier: ____________
Altitude:__________ Latitude:____________ ___ Longitude:______________
General location:______________________________________
Date:______________ Time:______________