The effect of climate on the epidemiology of plague in Madagascar

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by
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This thesis is based on research carried out in the Department of Epidemiology and Population Health at the University of Liverpool. Except for where indicated, the content of this thesis is my own work.

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Many infectious diseases of humans and animals are influenced by climate and changes in climate have an impact on future disease distribution and intensity. Understanding the processes involved is important to enable better disease prediction and effective prevention.

The studies presented in this thesis aim to investigate the effects of climate on the epidemiology of plague in Madagascar. The influence of climate is explored from different angles aiming to fill some of the knowledge gaps still present in the understanding of the disease.

The effect of large scale climate phenomena on inter-annual cycles of plague in Madagascar showed a change in the relationship between the El Nino Southern Oscillation the Indian Ocean Dipole and the plague incidence.

To investigate the mechanisms by which climate affects the disease further, environmental variables associated with climate were examined for their effects on the spatial distribution of human plague cases in Madagascar. Altitude, vegetation cover and temperature were the main drivers identified when modelling human plague occurrence at the district level.

An investigation into the micro-climate inside rat burrows, variations in flea vector abundance and vector species composition suggested that the climate in the highlands allows plague vectors, for the maintenance of the plague cycle, to be present in fairly high numbers throughout the year.

A subsequent laboratory experiment obtained results on the effect of constant temperatures and humidity, as found in rodent burrows on the development stages of two plague vectors. Evidence for different climatic adaptations of the developmental stages of the species was found and development times and mortality rates differed significantly between species. *X. cheopis* was far more successful under laboratory conditions than the endemic *S. fonquerniei*. However, changes in adult abundance recorded in the field did not reflect predicted changes in development rates estimated using findings from the laboratory study.

CHAPTER ONE

GENERAL INTRODUCTION

1.1 Introduction

Many infectious diseases of humans and animals are influenced by climate. It is therefore likely that changes in climate will have an impact on future disease distribution and intensity. Understanding the processes involved is important to enable better disease prediction and effective prevention. Most infectious diseases are affected by a variety of factors such as changes to human demography and behaviour and pathogen evolution for example, but numerous studies have identified climate as a major disease driver in many cases (Patz et al. 2001; Lafferty, 2009; Cazelles, Hales, 2006). Vector-borne diseases have been associated with certain time periods and geographical areas reflecting the strong influence of climate on both the spatial and temporal distributions. In recent years, many processes have been proposed by which climate might affect vector-borne diseases ranging from direct or indirect effects on the pathogen, the vector, the host to effects on epidemiological dynamics, or the natural environment.

Effects on Pathogens

The rate of development and thus population sizes of certain pathogens or parasites is dependent on temperature as is their survival (Harvell et al. 2002). Lengthening or shortening of periods of favourable climate conditions may therefore increase or decrease the number of cycles of infection possible within 1 year for warm or cold-associated diseases respectively. Any sensitivity to moist or dry conditions may affect pathogens due to changes in precipitation, winds and the frequency of floods.

Effects on vectors

There are several ways in which climate affects disease vectors. Temperature and moisture levels for example frequently limit vector distribution. Low temperatures increase development times and mortality while high temperatures and low humidity lead to excessive moisture loss. Many vectors experience significant mortality during cold winter conditions, while warmer winters may increase their survival (Harvell et al. 2002; Wittman, Baylis, 2000). Most arthropod vectors require warm and relatively moist conditions for development and survival, so their abundance and length of infection season depends on climate. For any specific vector, however, the effect of climate will be significantly more complex than that outlined above. A change in climatic conditions, as a result of changing frequency in

extreme weather events for example, will inevitably lead to increases or decreases in the abundance of many disease vectors. Outbreaks of several vector-borne diseases have been linked to the occurrence of ENSO (Anyamba et al. 2001; Baylis et al. 1999; Gagnon et al. 2002; Hales et al. 1999; Kovats, 2000).

Effects on hosts

Climate affects most animal hosts indirectly through their environment. Climate conditions determine the availability of resources, such as food, nesting material and shelter, and thus population size. Large scale fluctuations in climate were shown to synchronize host population dynamics over large areas (Kausrud et al. 2007), allowing population density to rise over the critical threshold required for disease outbreaks (Davis et al. 2004). Additionally, if changes in climate allow or force potential host species to spread into areas of endemic pathogens and vectors, susceptible hosts with no acquired immunity will be newly exposed, and outbreaks of severe disease could follow. Changes in the distribution of hosts due to climate (Keesing et al. 2010), seasonal, intra-annual or large-scale effects alike may also bring a disease closer to other possible vectors and hosts.

Effects on the environment

Climate may alter epidemiological patterns of a disease by affecting the environment and causing host, pathogen and vector populations to change their spatial and temporal densities and distributions. Human demography, housing, farming practices and movement are known to change due to climate events such as droughts, flooding and storms. All of the above can alter the chances of an infected human or animal coming into contact with a susceptible one (Gigliogli et al. 1965; Kock et al. 1999). For example, the impact of climate on the arthropod vector (Gage, 2009) or climate causing changes in host habitat (Keesing et al. 2010). Potentially all these factors influence the eco-epidemiological plague system, and as a result, plague is an incredibly complex disease. The epidemiological, spatial and temporal distribution of plague is thus the subject of many studies trying to facilitate the development of effective prediction and prevention methods to reduce human plague transmission.

1.3 Plague and climate

Plague is a vector-borne zoonosis, present today in Asia and Africa. Primarily a disease of rodents and enzootic in small mammal wildlife populations, plague is a bacterial infection

caused by *Yersinia pestis (Y. pestis)* transmitted between animals and humans by the bite of infected fleas. Contact with hosts, vectors or infective material can cause accidental infection in humans and transmission of the pathogen is then possible between people. An infection with plague causes serious illness with up to 70 % mortality in human populations if left untreated. The disease is regarded as re-emerging, as it is increasing in frequency throughout the world (Duplantier et al. 2005; Stenseth et al. 2008; WHO, 2005) which has been attributed partly to changes in climate (Stenseth et al. 2006).

Climate affects plague through its influence on ecological relationships often underlying disease transmission such as host and vector population dynamics (Stenseth *et al.*, 2002). The effect of climate on vector-borne diseases includes large scale climate (climate change) and global climate phenomena such as El Niño (Baylis et al. 1999; Ben Ari et al. 2008) as well as inter-annual (one year different to another) climate variation (Anyamba et al. 2001; Pascual et al. 2008) and even seasonal and weather effects (Purse et al. 2005). At the smallest scale, micro-climate influences plague.

Spatio-temporal associations with climate
As plague is seasonal in many parts of the world (Brygoo, 1966; Giles, Peterson, 2011) and transmitted via an ectotherm arthropod vector susceptible to its surrounding climate, early studies in plague endemic areas in India, Africa and Asia suggested annual variations in temperature and humidity to be responsible for the seasonal patterns of human plague incidence (Rogers, 1928; Davis, 1953). Subsequent research yielded more specific results and showed associations between distinct climate patterns and changes in plague incidence (Cavanaugh et al. 1968; Cavanaugh, Marshall, 1972; Kausrud et al. 2007; Neerinckx et al. 2008) in many parts of the world.

The epidemiology of this disease includes complex interactions between vector, host and pathogen prevalence, population dynamics, species composition and distribution, all of which are individually influenced by climate variables (Gubler, 2001; Meserve, 1995). This renders the effects of climate on the epidemiology of plague unique for different geographical areas where plague occurs, challenging researchers with complex and distinctive concepts at each particular location. Today, many questions remain unanswered for some of the most active plague foci, even though plague is one of the oldest and most serious diseases known to man.

1.3.1 A short history of plague

Plague has caused several world-wide pandemics, the most infamous being the "Black Death" which is thought to have started in Central Asia around 1300, reaching Western Europe by the 1340s. All plague pandemics are thought to have killed millions of people. The spread of disease within each was facilitated by ships transporting infected persons, rats and cargo and carrying fleas from continent to continent. The bubonic form of plague is most common, but cases of the pneumonic form are more severe and can spread quickly causing large epidemic outbreaks. The mortality rate varies with the strain of bacteria and between countries.

In 1894, during a pandemic originating in China, Alexandre Yersin discovered and isolated the bacterium from the buboes of deceased victims in Hong Kong and the agent was later named *Y. pestis*. It was found to be one of the most pathogenic bacteria in the world and is currently endemic in rodent populations in Africa, America and Asia (WHO, 2004).

In 2005, when the last comprehensive map was published by the World Health Organisation (WHO) (Map 1.1), 38 countries in Asia, Africa and America were reporting human plague cases every year and in many areas of the world plague is suspected to circulate in wild mammals (sylvatic foci).

Map 1.1: Countries reporting plague (yellow) and areas where plague is suspected to circulate in wild mammals (sylvatic foci) (red). Map: WHO, 2005.

In recent years, several plague outbreaks have occurred in areas that have been free of disease of many years e.g. in Algeria, the Libyan Arab Jamahiriya and Peru. Africa has been most strongly affected, accounting for 97.6% of the total number of cases reported worldwide. Unfortunately, most of the endemic countries in Africa also have the lowest rates of laboratory confirmation of infection, because samples are either not collected, not sampled in an appropriate manner, or are not processed in the laboratory. Within African
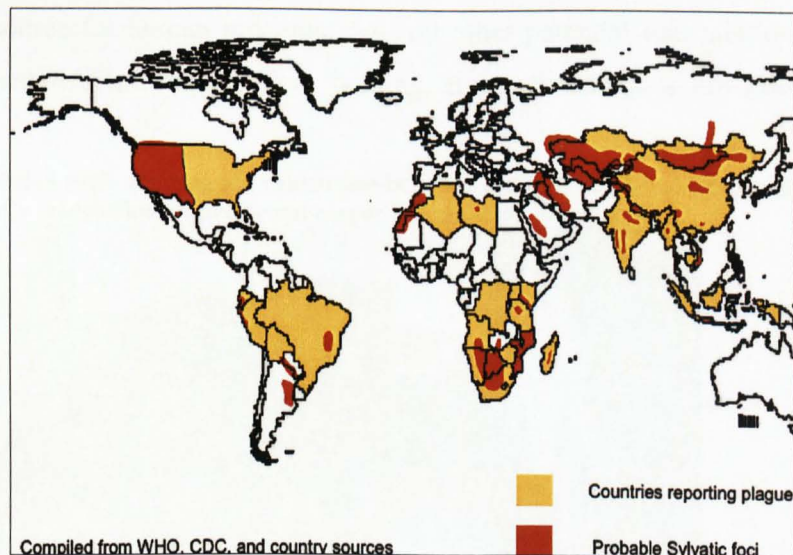
countries, the majority of cases are reported from Madagascar and the Democratic Republic of Congo. Up until 2007, plague was classed as a communicable disease meaning that human cases had to be reported to the WHO. Today, WHO still has to be notified of any event that may constitute a public health emergency of international concern, such as the emergence of plague in an area where it was not known to be endemic (Weekly Epidemiological Record, 2010).

1.3.2   The plague cycle

The transmission cycle

The plague bacterium is transmitted between mammalian hosts via their fleas. The sylvatic cycle where the pathogen circulates in wild mammals usually comprises different rodent species and their fleas, but can also include other mammalian hosts. Within the cycle, the host usually develops septicaemic plague during which the flea vector takes up infective blood. Often the host dies from infection, and its fleas leave the body to find a new host, thus spreading further infection. Epizootics, disease epidemics in animals, often, consequently, precede human epidemics. Some hosts carrying vectors however, stay asymptomatic while others are resistant to infection. Several flea species have been found to be competent vectors with varying vectorial capacities. Typically commensal rats and their fleas, living in and around human habitations, drive the domestic plague cycle, present the link to the sylvatic plague focus and are the source for human infection. Several other potential transmission pathways such as via infective meat consumption, hunting, livestock and pets are also involved (Figure 1.1).

Figure 1.1: The plague transmission cycle showing the connection between the sylvatic plague focus and the domestic cycle which is mostly responsible for accidental plague transmission to humans.
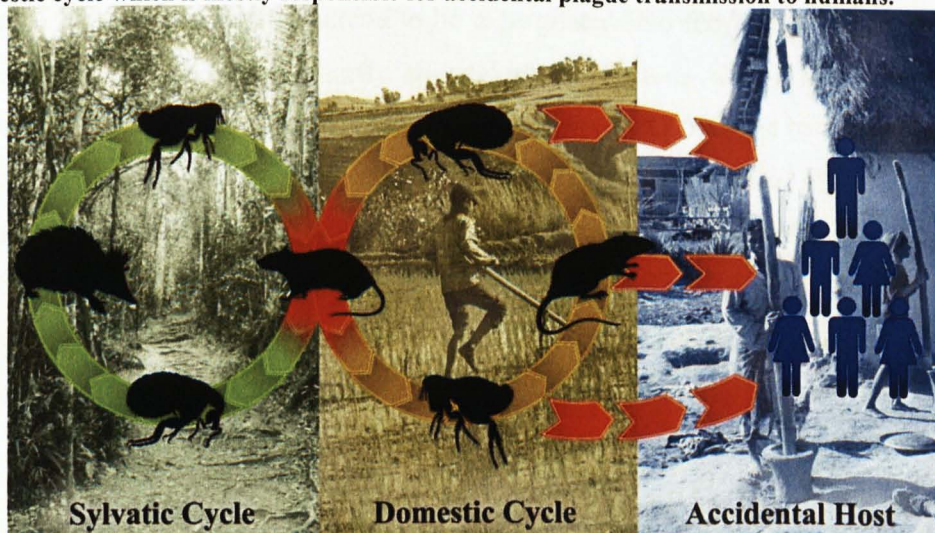
1.3.3.1 The pathogen

The pathological agent of plague, *Y. pestis,* is an enterobacteriaceae from the proteobacteria phylum. It is a gram-negative coccobacillus, which is facultatively anaerobic (capable of making ATP by aerobic respiration if oxygen is present or switching to fermentation) and can develop a protective capsule at temperatures above 33°C. In the laboratory it has been shown to survive at temperatures between 4°C and 40°C; however the optimum temperature for development lies between 28°C to 30°C (Perry, Fetherston, 1997). Various studies show that it can survive several months in soil (Mollaret, Karimi, 1963), but the existence of a viable endogenous form of plague, which permits long-term survival outside, still lacks proof. There are three biotypes of the bacterium, *Antiqua, Orientalis* and *Medievalis,* each responsible for one of the three pandemics (Guiyoule et al. 1997), with *Orientalis* still prevailing today.

Studies in microbiology aiming to find the origin of the plague pathogen, suggest that it is a clone of the enteric pathogen *Yersinia pseudotuberculosis* (Prentice, Rahalison, 2007). Phylogenetically, the two bacteria are closely related but have very different pathogenesis strategies, making *Y. pseudotuberculosis* much more benign (Hinnebusch, 1997). The exceptional pathogenicity of *Y. pestis* could be partly due to its transmission pathway; to be transferred to another host, the bacteria need to be present in adequate numbers in the bloodstream to be taken up by a vector. This is achieved by causing septicaemia in a mammal host (Cazelles, Hales, 2006). A susceptible rodent host develops bubonic plague followed by a septicaemic phase shortly before its death. After infecting the vector, the bacteria cause a blockage between the oesophagus and the stomach, preventing any blood meal reaching the stomach and causing the vector to increase its biting rate. This maximizes transmission potential as feeding causes the bacteria to be regurgitated into the biting wound of the next host. Early phase transmission shortly after taking up bacteria with a blood meal is also possible (Eisen, et al. 2006). At ambient temperatures above 27.5°C, the bacterium often fails to block the flea, decreasing transmission potential (Cavanaugh, 1971). Equally at temperatures below 21°C the extrinsic incubation period of plague in the flea vector gradually increases, therefore decreasing transmission (Kartman, 1956). The abundance of rodents, often governed directly by natural resources strongly affected by climate, is also affected by this route; maximum transmission usually causes the host to die.

The pathogen has temperature-controlled plasmids governing its functions and therefore functions differently in the exotherm insect vector and the mammalian host. To successfully infect an ectotherm vector, the ambient temperature must be below 36°C to activate a plasmid causing bacterial multiplication inside the flea gut. Successful colonisation of the flea vector is therefore dependent upon climatic factors and seasonal temperature changes. Despite this, evidence suggests that in *Y. pestis*, numerous and spontaneous genomic mutations occur enhancing the ability of the pathogen to adapt to new conditions. The bacteria are known to survive cold winters inside a hibernating host such as marmots in Siberia and prairie dogs in the US (Pollitzer, 1954). Any environmental change poses a risk to the human population by forcing *Y. pestis* to change into what is potentially a more pathogenic strain (Duplantier, Duchemin et al. 2005).

1.3.3.2 The principal vector

Fleas are arthropods of the insect class and belong to the order *Siphonoptera*. They are insects which undergo complete metamorphosis from larvae to adult, and adults have a laterally flattened body with strong hind legs used for jumping and mouthparts adapted for piercing skin. Both sexes are haematophagous, and feed on both mammals and birds. A proportion of the rodent flea life cycle is spent inside host burrows, and egg, larval and pupal development is entirely off-host. Depending on the species, the eggs, 0.3 to 0.5 mm in diameter, are laid on the host and fall to the ground or are laid directly in the host burrow. Larvae hatch from eggs deposited inside host burrows and feed on plant debris and flea faeces containing dried blood. Once they reach a sufficient size, the larvae spin an oval shaped cocoon and emerge as adults after metamorphosis. They have a permanent reproduction cycle providing they have access to a host. Depending on the species, they search for a host to feed every few days or they stay on the host permanently. A flea's lifespan depends on its species and habitat conditions. If conditions are not favourable, the flea's life cycle can take up to 20 months to complete (Rodhain, Perez, 1985). Fleas are the primary vector for plague and their role in the transmission of the disease was discovered by Simond in 1898. Thus far, around 120 flea species have been proven to be competent vectors for *Yersinia pestis*, but their vectorial capacity varies. The best known plague vector is *Xenopsylla cheopis* (Rothschild, 1903), the oriental rat flea, which is found all over the world (Rodhain, Perez, 1985). This species readily leaves its rodent host for other mammals including humans.

Susceptibility to climate

Ambient temperature, rainfall, and relative humidity each affect flea development, survival, behaviour and reproduction (Gage et al. 2008; Krasnov et al. 2001, 2002), where warm-moist weather conditions are optimal and are therefore associated with higher flea-abundance per rodent (Cavanaugh, Marshall, 1972; Davis, 1953). The development of *Xenopsylla cheopis (X. cheopis)*, from egg to adult is regulated by temperature via the rate of progression between each metamorphic stage. Fleas are ectotherms in which all of the immature flea stages occur off-host and so are especially sensitive to temperature fluctuations. Flea development rates increase with temperature below a critical value, and decrease above it as if high temperatures are combined with low humidity as the survival of immature stages decrease (Gage et al. 2008). Temperature and relative humidity impact flea survival in addition to development rate (Cavanaugh, Marshall, 1972) survival varies inversely with air saturation deficit at a constant temperature (Bacot et al. 1924). Flea larvae are also susceptible to desiccation (Cavanaugh, 1971) and their survival is reduced in dry air. Survival of immature stages of fleas in rodent burrows is also affected by soil moisture that is partly controlled by outside precipitation (Eisen et al. 2009) even though temperature and humidity fluctuations are reduced by living underground (Krasnov et al. 2001). When combined with a high organic load, excessively wet conditions in rodent burrows (relative humidity >95%) can promote the growth of fungi that diminish larval and egg survival (Cavanaugh et al. 1972; Parmenter et al. 1999).

1.3.3.3 The host

In general, rodents are the most common host for plague facilitating transmission but other mammals can also carry the infection symptomatic and asymptomatic. In total, more than 180 rodent species are sensitive to plague (Pollitzer, 1954). Rodents are divided into 2 sub-orders, both of which are sensitive to plague: Hystricognathi and Sciurognathi (Anderson, Jones, 1984). The two most important rodent families in the epidemiology of the plague are the Sciuridae (squirrels) and Muridea (mice, rats, gerbils and voles) from the Sciurognathi order. The main plague hosts in Eurasia are gerbils (*Meriones spp.*), marmots (*Marmota spp.*) and ground squirrels (*Spermophilus spp*). In North America deer mice (*Peromyscus maniculatus*), ground squirrels (*Spermophilus beecheyi*), chipmunks (*Tamias spp.*) and black tailed prairie dogs (*Cynomys ludovicianus*) are the predominant hosts and for South America guinea pigs

(*Cavia porcellus*) have to be added to the list. On the African continent the main hosts are gerbils (*Tatera* and *Desmodillus* spp) and mice (*Mastomys* spp).

Enzootic hosts

Some rodent species, enzootic hosts, are animals which are relatively resistant to plague and play a key role in the maintenance of the foci. Even though some animals succumb to the disease, population die off does not occur. These hosts are usually found in remote, sparsely populated areas of Asia, Africa and South and North America (Horsburgh, Nelson, 1997). Incidental hosts such as other mammals which are ocasionally infected are not important for the maintenance of the natural plague cycle. These enzootic cycles are referred to as 'sylvatic plague'. Enzootic plague foci have distinct ecological characteristics and vector and host combinations.

Epizootic hosts

Within epizootic host-populations infection is spread rapidly by flea vectors and these more sensitive rodent species succumb to plague in large numbers, greatly increasing the risk of transmission to humans. Widespread die-offs result in the dispersal of flea vectors in search of new hosts often among commensal rodents associated with human settlements or humans directly. However, even within a species, resistance levels vary as epizootics select for more resistant individuals. Individuals that survive infection are immune for a certain period of time. The severity of an epizootic also depends on extrinsic factors such as, for example, virulence of the plague strain involved, rodent population density and vector abundance. The successful coexistence of rodents and plague is most likely due to the high reproductive and re-colonization potential of the host as well as the selection of plague resistant genotypes (Duplantier, 2003).

Susceptibility to climate

Rodent populations are also affected by climate. High intensity rainfall can impact survival by flooding of rodent burrows (Cavanaugh, Marshall, 1972) but the effects of precipitation on rodent densities are mostly indirect consequences of ecological interactions (Meserve et al. 2010), for example by controlling primary production (Letnic et al. 2005). Following wet seasons, an abundance of resources often leads to reproduction periods increasing juvenile populations (Jaksic, Lima, 2003). Consequently, densities of rodent populations show clear

links to annual precipitation and its seasonal distribution as shown in south America (Lima et al. 1999), Africa (Leirs et al. 1996), and Australia (Dickman et al. 2001; Mills, 2005). Effects of temperature on rodent populations are evident; partly because rodents are warm- blooded and hence can withstand a wide range of ambient temperatures. However, in some areas, cold winter temperatures can affect rodent populations negatively either directly or through a lack of resources (Korslund, Steen, 2006).

1.3.3.4 Reservoirs

Two types of reservoirs are distinguished, the sylvatic reservoir and the domestic reservoir. In the sylvatic reservoir the disease circulates amongst wild rodent populations and humans are only at risk of infection when entering such an area. Most sylvatic reservoirs remain unnoticed when human cases are absent and no research takes place. The domestic reservoir combines the infected hosts and vectors from a sylvatic reservoir with commensal rodents which live close to humans. These commensal rodents are the main source of infection for people.

Most human plague foci show only periodic outbreaks, sometimes with long intervals in between. How plague is maintained in foci which are silent for several decades is not fully understood. In certain areas, less sensitive wild rodent populations constitute sylvatic plague reservoirs. It is believed that when these sylvatic reservoirs come into contact with more sensitive rodent species which are closer to human habitation, epizootics occur which pave the way for human epidemics. Another hypothesis suggests the possibility of the survival of bacteria within fleas waiting for re-colonisation in abandoned rodent burrows. Some flea species can stay infected and survive for up to a year (Acha, Szyfres, 1989). To explain long intervals of silence between plague outbreaks, it was also hypothesised that viable *Y. pestis* bacteria survive within the soil of rodent burrows.

1.4 Plague in Madagascar

1.4.1 Climate and geography of Madagascar

Madagascar is the world's fourth largest island, and with an area of 587 000km^2 it is 2.4 times the size of the United Kingdom. It is 1580km long and at its widest point from east to west is 560km wide. The island is situated in the Indian Ocean off the east coast of Africa at the southern limit of the tropics, and is separated from the continent by the Mozambique

Channel. Along its length runs a mountain range with altitudes varying from 700 to 2800m. The land relief shows great variation within relatively small areas (Figure 1.2). While the eastern slopes of the highlands descend abruptly to the Indian Ocean, the western side has gentler slopes interrupted by great plateaus all the way down to the Mozambique Channel.

Figure 1.2: Altitude map of Madagascar showing lowest areas in green and highest areas in red. (Adapted from diva-gis.org).
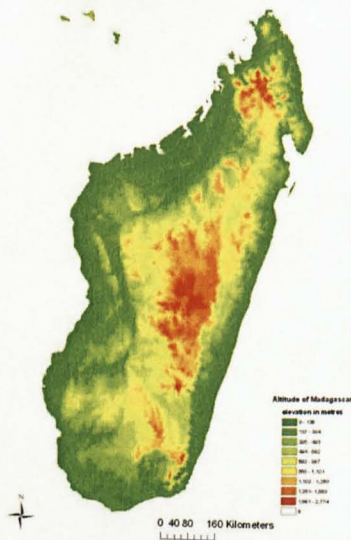
Madagascar, together with the Indian continent, separated from the African continent around 165 million years ago. The island then detached itself from India and arrived in its present position 121 million years ago (Rabinowitz et al. 1983). Madagascar possesses a remarkable variety of natural habitat types from tropical rainforests in the north east to sub-arid areas in the south-west. As a result of the early separation of the island from its continent, a unique fauna and flora developed in isolation.

The UN estimated a human population of 19 million people inhabit Madagascar in 2011, with a growth rate of 2.69% (UN growth rate). The population is relatively young, with 45% under the age of 15. Most people live in rural areas (77.8%) and the highest population density is in the highlands. The main occupation is subsistence farming and as one of the poorest countries in the world, 70% of income is spent on food alone. The staple in Madagascar is rice (Rasambainarivo, 2003). In order to grow more food crops and also to harvest precious hard wood varieties, deforestation is ongoing and forest cover is decreasing rapidly (Bohannon, 2010).

The climate varies greatly between regions but in general it can be described as unimodal tropical, with a hot rainy season from November to March and a cold dry season from April to October. The highlands are governed by temperate climate (Figure 1.3 a and b).

Figure 1.3: Madagascar per administrative district (adapted from Chanteau et al. 2006) a) Annual mean temperature b) Annual mean precipitation .
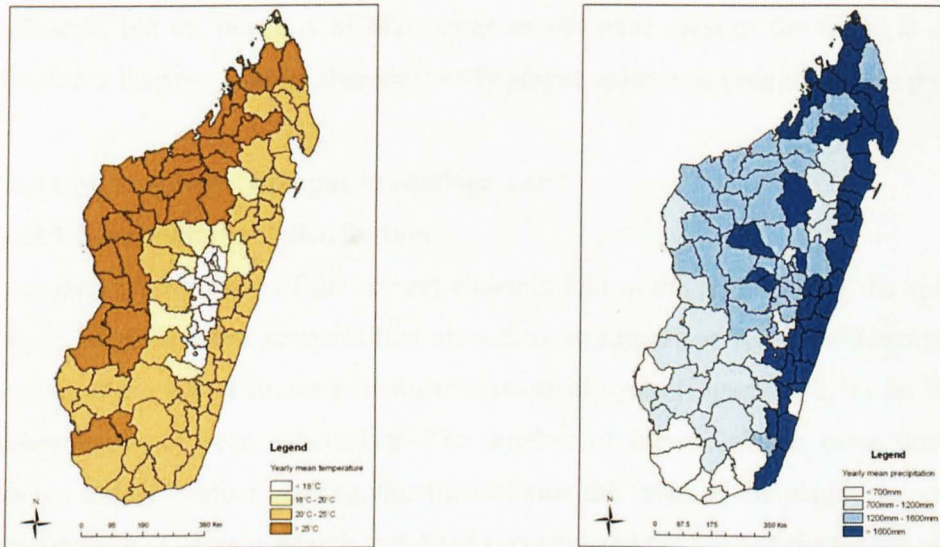
a) Annual mean temperature in degree Celsius
   Darker colours represent warmer temperatures

b) Annual mean precipitation in millimetres
   Darker colours represent more precipitation

Altitude creates temperature differences and the highlands are the coldest area of the country (Figure1.3 a) this is particularly so in the dry season. The East coast, characterised by tropical rainforest areas, is wettest with more than 2000mm rainfall annually, while the South is much drier with only 275mm of rainfall annually (Figure 1.3 b). The Malagasy climate has two seasons: a hot, rainy season from November to April; and a cooler, dry season from May to October. Between January and March, Madagascar is regularly hit by cyclones.

1.4.2 History of plague in Madagascar

Plague arrived around 1898 with rats and fleas in ships from India and spread rapidly within the rodent population. By 1921, it had reached the highlands where it became endemic (Chanteau et al. 2006); human plague cases have been recorded every year since then. In Madagascar, considerable work has been conducted about plague. Research has revealed new vectors for plague – one of them endemic. Other work has led to the development and use of antibiotics, instigation of vaccination campaigns, and the introduction of surveillance and the development of new diagnostic methods. Today, human plague still has a high prevalence in Madagascar and is undeniably its most important rodent-linked disease (Chanteau et al. 2006). Since the late 1980s, between 800-1500 suspected cases have been reported every year of which around 250 are confirmed using laboratory analysis. In 2003, the first successful

rapid diagnostic test for both pneumonic and bubonic plague antigens was developed by the Institute Pasteur de Madagascar (IPM). The majority of clinical cases of disease occur in the highlands, but the port city of Mahajanga on the west coast of the island is also a hotspot; after more than 60 years of absence, yearly plague epidemics returned to this port in 1990.

1.4.3 Epidemiology of plague in Madagascar
1.4.3.1 Seasonality and distribution

The spatial distribution of the current endemic foci in the highlands of the epidemiology of plague in Madagascar suggests that climate is an important factor influencing this disease. Plague on the island shows a distinctive seasonal cycle (Figure 1.4). So far the underlying causes have not been established. The number of human plague cases doubles between August and September marking this time of year the "start of the plague season". There is a rapid decline of cases in March and April is considered the "end of the plague season".

Mean number of monthly plague cases 1955-2009
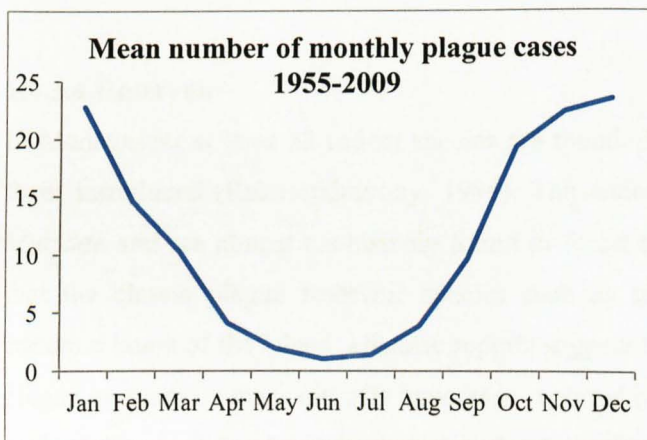
Figure 1.4: Monthly mean number of confirmed human plague cases recorded in Madagascar from 1960 to 2009.

With regards to the current plague foci there appears to be a very distinctive altitudinal cut-off point as human plague cases are mostly reported from regions above 800m with Mahajanga being the only exception. The cause for this altitudinal limitation is not yet understood but speculations include differences in climate, human-rodent contact and rodent immunity (Chanteau et al. 2006).

1.4.3.2 Pathogen

The plague strain found in Madagascar is from the biotype *orientalis*. New ribotypes are constantly being discovered including some which are antibiotic resistant (Guiyole et al. 1997; Vogeler et al. 2011). Studies found that the pathogen is maintained in Madagascar in multiple geographically separated subpopulations suggesting local cycling with limited gene flow from one another. The pathogen can be detected by three different methods, all of which are employed in Madagascar to confirm suspected human plague cases.

1.4.3.3 Vector

In Madagascar, the pathogen is predominantly transmitted by two rat flea species of the *Pulicidae* family, the endemic *S. fonquerniei* (*Synopsyllus fonquerniei*; Wagner, Roubaud, 1932) and the ubiquitous *X. cheopis* (*Xenopsylla cheopis*; Rothschild, 1903). The two main plague vectors coexist on the island, but occupy different niches – the endemic flea *S. fonquerniei* dominates rodent flea communities on rats caught outdoors, while *X. cheopis,* the extensively studied ubiquitous oriental rat flea (Klein, 1973; Margalit, Shulov, 1972; Mellanby, 1932; Sharif, 1949) is mostly found on rats caught in houses. The fleas' pre-imaginal development is also thought to take place in different locations; in burrows found outdoors and indoors, respectively. Below 800m altitude, however, and in coastal areas, *S. fonquerniei* is absent and *X. cheopis* is present both in- and outdoors. The reasons for the difference in distribution are poorly understood and present a knowledge gap in the epidemiology of plague in Madagascar.

1.4.3.4 Reservoir

In Madagascar at least 23 rodent species are found. They are divided into 10 genera, two of them introduced (Rakotondravony, 1996). The endemic rodents are all from the family of *Muridae* and are almost exclusively found in forest areas. One peculiarity in Madagascar is that the classic plague reservoir species such as squirrels or gerbils are not part of the endemic fauna of the island. Historic reports suggest that the endemic rodents are sensitive to plague as sporadic mass die-offs have been reported on forest edges. However, the sensitivity varies with species. Of the two introduced genera, *Rattus* and *Mus,* only the first is implicated in the transmission cycle of *Y. pestis.* It is believed that the black rat, *Rattus rattus,* arrived with the first settlers to Madagascar 2000 years ago. Without many predators, it rapidly established itself all over the island in many ecological niches including human habitations, forests and fields up to an altitude of 2000m. Presently, the black rat (*Rattus rattus*) is the dominant rodent species everywhere except in the tropical rainforest and big cities (Duplantier, Duchemin, 2002). In the transmission cycle in rural areas, *Rattus rattus* (*R. rattus*) plays a central role as the dominant host species of plague vectors. In urban areas and coastal cities, this role is taken over by the brown rat (*Rattus norvegicus*). The exact date of the arrival of this sub-species is unknown, but it arrived after the black rat and the first specimen was caught in Madagascar not before 1941 (Brygoo, 1966). The bigger brown rat

gradually replaced the black rat in big cities on the coast and in the highlands. It differs from the black rat in its fear of people and its relative resistance to plague. Despite this, it has managed to populate big cities due to its affinity to water and thus lives happily in urban sewage systems. Survival of rat populations, despite plague, is most likely related to their high reproductive rate and re-colonization potential, as well as selection for plague resistant genotypes (Tollenaere et al. 2011). In favourable conditions, female rats can have a litter of up to 15 young every three weeks, all year round. Other small mammals which have tested positive for plague include lagomorphs and insectivores such as tenrecs and hedgehogs, but their role in the sylvatic plague cycle is not fully understood. However, the only introduced insectivore, the shrew *Suncus murinus,* which is present in all habitats except tropical forests, was the main host for *X. cheopis* after an epizootic killed most rats in the port city of Mahajanga (Boisier, Rahalison, 2002). A human plague epidemic followed and research showed that the shrew is not only sensitive to plague, but lives in close proximity to humans and was also carrying a new strain of the pathogen.

1.5 Objectives of the thesis

The studies presented in this thesis aim to investigate the effect of climate on the epidemiology of plague in Madagascar. The influence of climate is explored from different angles aiming to fill some of the knowledge gaps still present in the understanding of plague. Throughout the thesis consideration is given to the impact findings could have on ecological processes associated with the epidemiology of the disease in addition to the study of climatic effects on plague. Several different methodologies have been employed.

Chapter two investigates the effect of global, large scale climate phenomena on inter-annual cycles of plague in Madagascar.

Chapter three models the spatial distribution of human plague cases in Madagascar using remotely sensed environmental variables.

Chapter four presents a descriptive study of the micro-climate within host burrows and of vector species composition and abundance in an area where plague is known to be endemic. This includes a closer look at vector habitat and regional differences in annual change of abundance.

Chapter five reports the results of a laboratory experiment undertaken to establish basic relationships between climatic factors and the endemic flea species for the very first time. The study also explores possible differences between the two main flea vector species found in Madagascar.

Chapter six reviews the information gained from these studies and discusses them in the context of other research

CHAPTER TWO

THE RELATIONSHIP BETWEEN

CLIMATE AND PLAGUE INCIDENCE

IN MADAGASCAR

THROUGHOUT THE LAST FIVE DECADES

ABSTRACT

Global climate phenomena such as the El Niño Southern Oscillation (ENSO) and the Indian Ocean Dipole (IOD) have been found to affect the periodicity of infectious diseases. It is widely accepted that both climate phenomena have wide-ranging implications for public health. Therefore a possible association between ENSO, the IOD and human plague incidence between 1956 and 2008 in Madagascar has been investigated.

The results suggest a link with a changing relationship between plague incidence and climate most likely mediated by variations in strength and timing of ENSO and the IOD. The examination of climatic patterns before and during six periods of especially high plague incidence provided further insight into possible connections.

Results imply that periods of high plague incidence between 1956 and the early 1990's are linked to negative ENSO (La Niña) and negative phases of the IOD which caused cooler temperatures during the usually hot boreal winter months in Madagascar. In the months before and during the subsequent epidemics however, a positive IOD followed by effects of a positive ENSO (El Niño) promoted warmer and wetter conditions during the usually cold and dry boreal summer and autumn.

A strong El Niño event in 1997, combined with a positive phase of the IOD, caused wetter and warmer than usually observed climatic conditions in autumn, and shows an association with high plague incidence in the same year. This climatic pattern of both, El Niño and a positive IOD, were again recorded together in 2003 and 2004 with similar results. Yet, by which mechanism large scale climate phenomena affect the epidemiology of plague in Madagascar could not be ascertained in this study. It is to be expected that a multitude of factors affecting host and vector ecology as well as transmission potential are involved.

2.1 INTRODUCTION

Connections between weather and vector-borne diseases are well established and various global climate phenomena such as the El Niño Southern Oscillation (ENSO) have been identified to be a strong influence on the epidemiology of vector-borne diseases including plague. Its effect is often enhanced or reduced by other large scale climate phenomena affecting the same geographical region such as sea surface temperature cycles causing unique conditions. One such example is the western pole of the Indian Ocean Dipole (IOD), situated

close to Madagascar and influencing the climate of the island. Together ENSO and the IOD have been found to affect the periodicity of infectious diseases in several places (Ashok et al. 2003; Zell, 2004) and it is widely accepted that both these phenomena are dominant drivers for year-to-year climate variability on earth with wide-ranging implications for public health (Gagnon, 2001; Kausrud, Viljugrein et al. 2007; Kovats, Bouma et al. 2003; Luo et al. 2010). The El Niño- Southern Oscillation (ENSO) is a quasi periodic climatic phenomenon occurring over the Tropical Pacific Ocean and is a strong climate driver in many parts of the world. The extreme weather conditions related to the ENSO are also associated with the incidence of several infectious diseases (malaria and Rift Valley Fever among others). The Indian Ocean Dipole (IOD) is a climate driver occurring independently from the ENSO phenomenon (Ashok, 2003) although considerable correlation between the two phenomena has been demonstrated (Nichols, 2001). The IOD is an irregular oscillation of sea surface temperatures in the Indian Ocean by which the western part of the basin is alternately warmer or cooler than the eastern part (Saji et al. 1999). A positive phase of the IOD is characterised by warmer sea surface temperatures in the western part and cooler temperatures in the eastern part of the basin. This leads to cooler and drier conditions in the east (Indonesia, Australia) and warmer and wetter condition in the west (Black, 2005). Both the ENSO and the IOD are affecting the climate in Madagascar. As a country with one of the highest reported human plague incidence globally, it seemed important to investigate if here too associations between large scale climate and disease incidence can be found. Climate can play an important part in disease transmission by affecting host reservoir and vector distribution and abundance (Gage, Burkot et al. 2008). However, the effects of large scale climate variables on disease cannot always be generalised as complex disease epidemiology may be affected by both short-term and long-term effects. Additionally, in the last 30 years, differences in the ENSO have been observed (Rodo et al. 2002). Thus after investigating large scale patterns, the human plague epidemics in Madagascar over the last fifty years have been investigated separately.

2.2 METHODS

Associations between plague incidence, the IOD and ENSO and their related climate variables temperature and precipitation were investigated. Wavelet analysis has been chosen as a qualitative method to identify periods in the last 50 years where links between climate and plague incidence can be suggested. This method represents a widely used tool for the analysis of epidemiological and climatological data sets and for the detection of statistical

relationships between them (Cazelles et al. 2005; Colwell, 1996; Constantin de Magny et al. 2007; Grinsted et al. 2004; Thai 2010).

Any links between climate and plague incidence in Madagascar were investigated in two steps. First, the global climatic ENSO and IOD phenomena together with data on human plague incidence were analysed at the country level to identify any relationship between them. Second, the mechanisms by which ENSO and IOD impact plague in Madagascar were explored by assessing their effects upon temperature and rainfall, and in turn, then examining the contribution of temperature and rainfall to plague incidence. Finally periods of unusually high plague incidence anomaly were examined in detail to collect information on the climatic circumstances and relate them to any patterns detected with the previous analysis. It seemed more appropriate to use anomaly of incidence, temperature and precipitation in the analysis as this removes seasonality and takes short term trends into account. Any identified correlations between climate variables and disease are hence more likely to be based on real links as opposed to coincidental congregation of data.

2.2.1 Data

Incidence anomalies

Data of confirmed human plague cases from 1956 to 2008 were made available by the WHO plague reference laboratory of the Institut Pasteur de Madagascar. Bodily fluid or a tissue sample of a person suspected of plague infection is used to confirm an infection with *Y. pestis*. Until 2000 cases were recorded as confirmed when a bacteriological test showed the growth of cultured *Y. pestis* and a laboratory mouse inoculated with an isolate from the culture succumbed to an infection with all symptoms of a plague infection. From 2000 onwards, cases were confirmed by bacteriology and by using a dip-stick test which detects the F1 antigen of the plague bacterium (Chanteau et al. 2003). Using the date of onset of symptoms for each confirmed case and the latest UN population growth estimates a monthly time-series from 1960 to 2008 of human plague incidence was created. The anomalies for the entire time-series were calculated to facilitate analysis in the context of any trends and to get a representation of periods with unexpected plague incidence values. As the average periodicity of ENSO is 4 years (Mac Mynowski, 2008), a four year moving average for each month was applied to the human incidence data and subtracted from the original incidence value to establish the deviation of the data point from the expected value.

El Niño Southern Oscillation

During a warm phase (El Niño), the sea surface temperature in the western Pacific is warmer than average, thus increasing surface pressure. During a cold phase, (La Niña), the opposite takes effect. These sea surface temperatures anomalies are associated with large modifications of the atmospheric circulation over the tropics and the ENSO has an impact at a global scale. (La Niña for example, is associated with wetter winter conditions over southern Africa, heavy rains in Malaysia and Indonesia and droughts in Peru and Chile).The ENSO phenomenon is represented here by an index devised by the Japan Meteorological Agency (JMA) of spatially averaged monthly sea surface temperature (SST) anomalies over the tropical Pacific (4°N to 4°S and 150°W to 90°W) with an applied 5-month running mean to smooth out possible intra-seasonal variations for the period 1960-2010.

The Indian Ocean Dipole

The Dipole Mode Index (DMI) represents the Indian Ocean Dipole variability as a measure for this east-west sea surface temperature gradient. The DMI is calculated as the difference between the Western Tropical Indian Ocean sea-surface temperature index and the South-eastern Tropical Indian Ocean sea-surface temperature index.

Temperature and precipitation anomalies and rates

Temperature and precipitation anomalies were used to describe climate variability more accurately and to allow for more precise calculations of climatic trends over the 50 year period. Monthly surface temperature and precipitation anomalies for the area of Madagascar (42.18°E – 49.68°E; 24.76°S to 11.42°S) from 1956 to 2010 were obtained from the NCEP-NCAR reanalysis (Kalnay et al. 1996) for seasonal regression maps on precipitation rate and air temperature.

2.2.2 ANALYSIS

Plague incidence anomaly time-series

After plotting the plague incidence anomalies time-series, periods of low and high incidence anomalies were identified by looking for monthly incidence anomaly values which remained negative or positive, after adding two standard deviations. A period is retained as a low or high incidence period if there is a minimum of three consecutive months of low or high incidence respectively. Seasonality of the plague incidence anomalies dataset was established

by averaging within months from 1960 to 2008 and subsequently plotting the mean seasonal cycle.

Coefficient of variation

The coefficient of variation (CV) was calculated for each data set to identify the months with the greatest variation throughout the time-series of each dataset. The CV is the ratio of the standard deviation of a dataset to its mean and hence looks at the standard deviation in the context of the mean of the data.

Wavelet analysis and phase angle evolution

In order to establish any correlations in time between monthly plague incidence anomaly and monthly climate variables, time-series analysis was employed. Due to the variability of both plague incidence anomalies and climate, it was suspected, that possible relationships may vary over time. Thus, transforming the data into signals and studying any co-variations between them seemed appropriate. Wavelet analysis is a time-series analysis method whereby time-series data are transformed into signals in time-frequency space, providing the means to look for dominant modes of variability and their variability over time, suggesting statistical relationships between signals (Cazelles, 2008; Grinsted et al. 2004). Different to the windowed Fourier transform, wavelet analysis is used for analysing data series with non-stationary power at many different frequencies such as discontinuities and sharp spikes (Daubechies, 1990; Graps, 1995). While the windowed Fourier transform is limited by its fixed "window of detection", the wavelet transform uses a function depending on a non-dimensional time parameter and allows the study of each component with a resolution matched to its scale (Graps, 1995). Unlike the Fourier transform, a wavelet transform is not based on sine and cosine functions only but on an infinite set of basis functions. Therefore, information which is missed by a time-frequency method such as the windowed Fourier transform is retrieved by wavelet analysis.

Wavelet analysis is a widely recognised tool to investigate temporal dynamics of infectious diseases (Ben Ari, Gershunov et al. 2008; Cazelles et al. 2007, 2008; de Magny 2007; Thai, 2010). Here, wavelet analysis was carried out to detect temporal patterns, their variations and coherence by introducing an established model of Grinsted (2004) into Matlab. For a guide to wavelet analysis see Cazelles et al. (2008) and Torrence and Compo (1998).

Prior to analysis, the datasets were tested for normality and where necessary normalised using a Johnson transformation (Farnum, 1996). In addition, a low-pass Gaussian filter was used to de-seasonalise the data. The continuous wavelet decomposition showed all time-series data in time-frequency space. The non-stationarity of a signal was determined and the presence and absence of periods of a single persistent mode of variability throughout time detected within each dataset. Subsequently, cross-wavelet analysis was applied using two datasets at a time, to identify any time periods with high common power, indicative for possible association between variables. This was followed by wavelet coherency analysis to find significant locally phase locked behaviour, suggesting local correlation between the time-series in time frequency space (Grinsted et al. 2004). The direction of the arrows not only gives an indication of the direction of the correlation between signals but also of the time lag between signals. A cone of influence (COI) is applied to the cross-wavelet and wavelet coherency transforms to mark the limits of the time scale within which signal behaviour can be discussed with confidence. The statistical significance level of the wavelet coherence is estimated using Monte Carlo methods (Torrence, Compo, 1998).

Phase angle evolution

Phase angle statistics complement wavelet analysis by showing the phase relationship between signals of a chosen periodicity. This can be used to gain confidence in causal relationships between the time series (Grinsted et al. 2004) and to obtain information on any time lags between signals. Here, from the wavelet coherency plot, certain periods of interest were identified and investigated in more detail by analysing the phase relations between signals.

Linear regression maps and composite maps

Linear regression maps were created to confirm established effects of a given independent variable on temperature and precipitation during the plague season. The interactive website www.esrl.noaa.gov/psd/data was used for this purpose. Using the same website, seasonal composite maps for temperature and precipitation anomalies were created for periods of high plague incidence anomalies to support findings from the wavelet analysis. The composite shows the average value of the anomalies of a variable for the time period entered and to investigate specific years. The temperature and precipitation anomalies of the months before an epidemic have been explored and the periods with the strongest anomalies were identified.

2.3 RESULTS

2.3.1 Seasonality

The mean seasonal cycle in Figure 2.1 illustrates the strong seasonality of human plague in Madagascar. The plague season coincides with the wet and warm months of the rainy season from September to March.
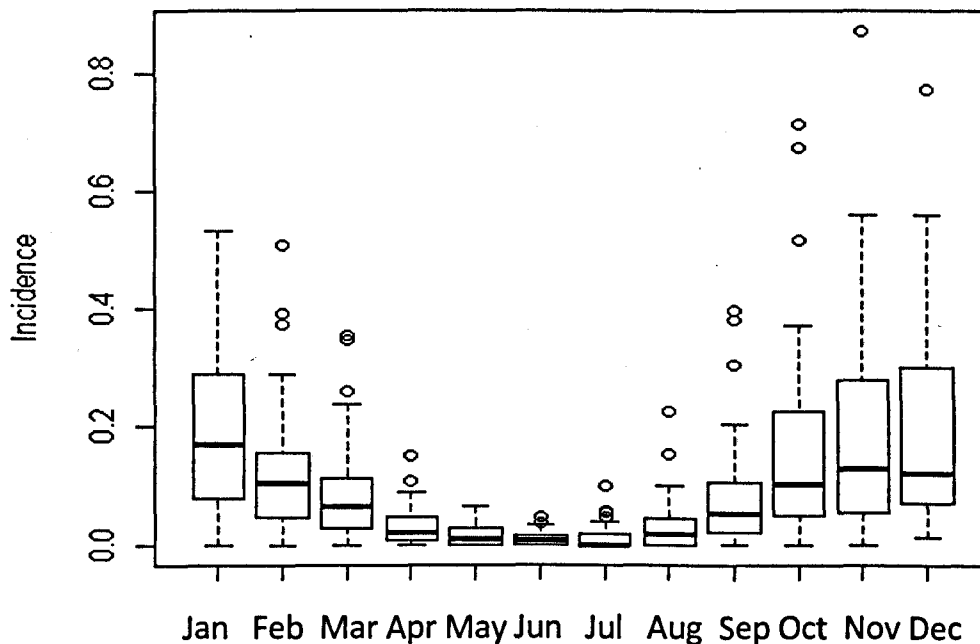
Figure 2.1:
Box plot of seasonal human plague incidence from January 1960 to December 2008. The dark bold line in each box represents the median value, the bottom and top of the box indicates the 25th and 75th percentiles, respectively. The whiskers show either the the maximum value or 1.5 times the interquartile range of the data, whichever is the smaller. Outliers are plotted individually as dots.

2.3.2 Monthly plague incidence, temperature and precipitation anomalies

The time-series in Figure 2.2 (next page) shows monthly plague incidence anomalies from 1960 to 2008. One period of low incidence was identified during the plague season from October 2005 until February 2006 (ONDJF). Periods of high plague incidence were highlighted for the seasons: December to March (DJFM) 1968-69, November to February (NDJF) 1988-89, November to February (NDJF) 1989-90, October to December (OND) 1997, October to February (ONDJF) 2003-04 and October to December (OND) 2004. The time-series in Figure 2.3 and 2.4 (following pages) show monthly precipitation and temperature anomalies from 1960 to 2008.
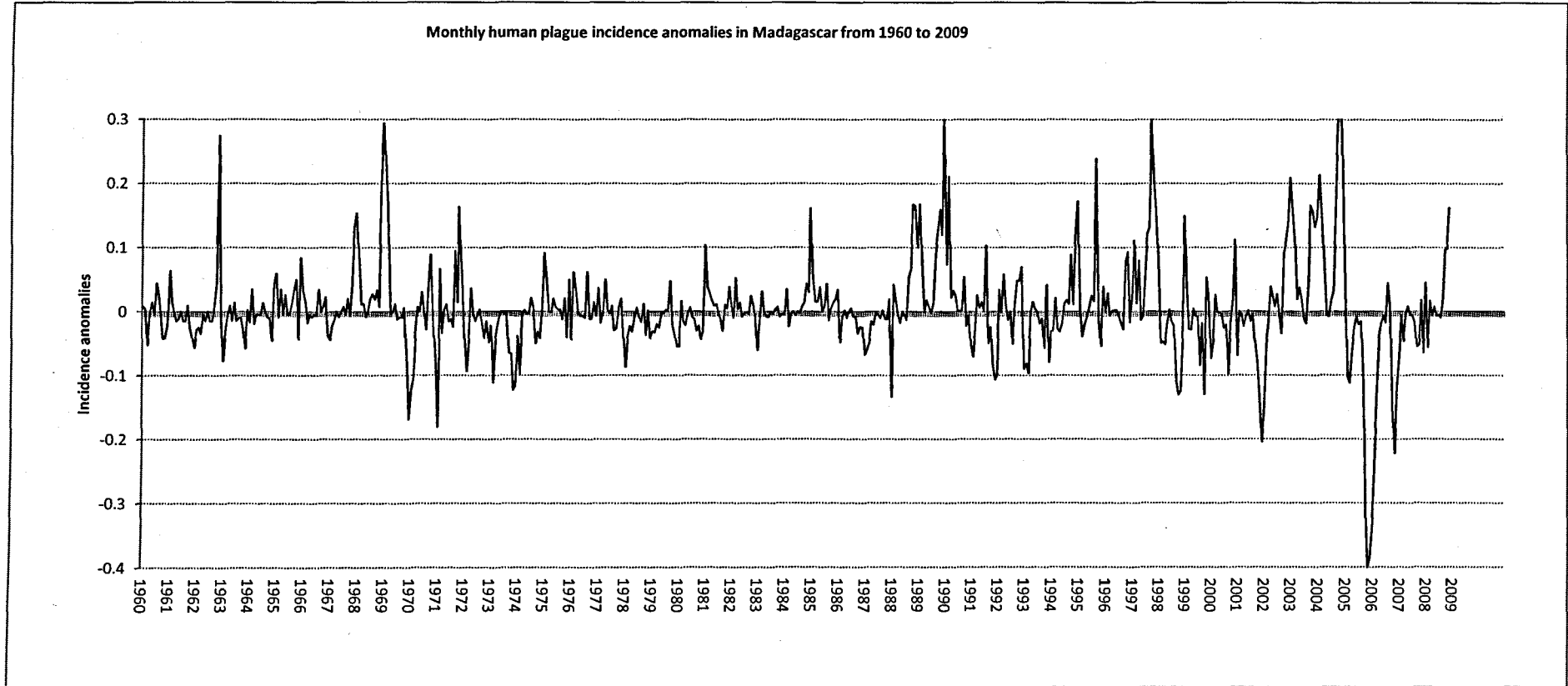
Figure 2.2: Monthly human plague incidence anomalies from 1960 to 2008 for Madagascar computed from data on confirmed human plague cases from 1960 to 2008, made available by the WHO plague reference laboratory of the Institut Pasteur de Madagascar and UN population estimates for Madagascar.

Figure 2.3: Monthly precipitation anomalies from 1960 to 2008 for Madagascar computed from data on monthly precipitation values from 1960 to 2008 published by NOAA.

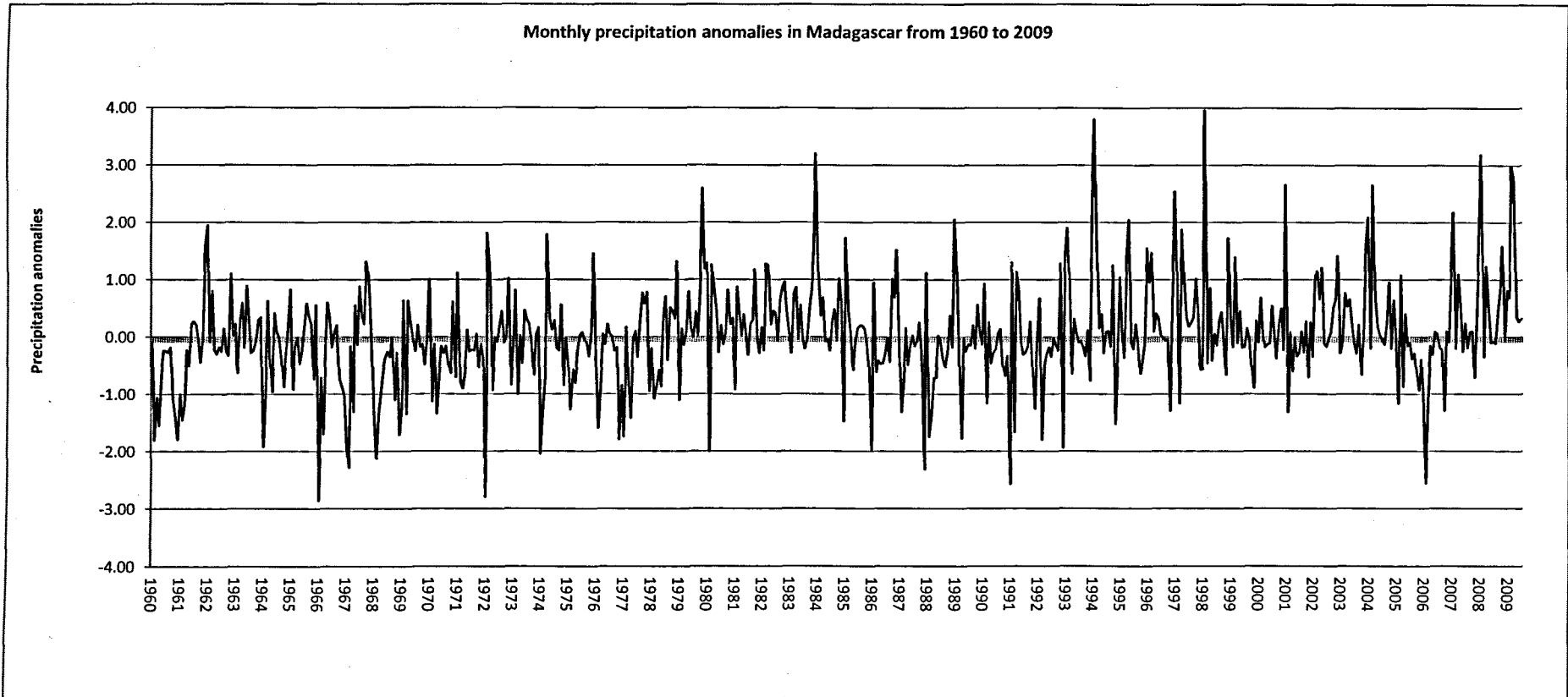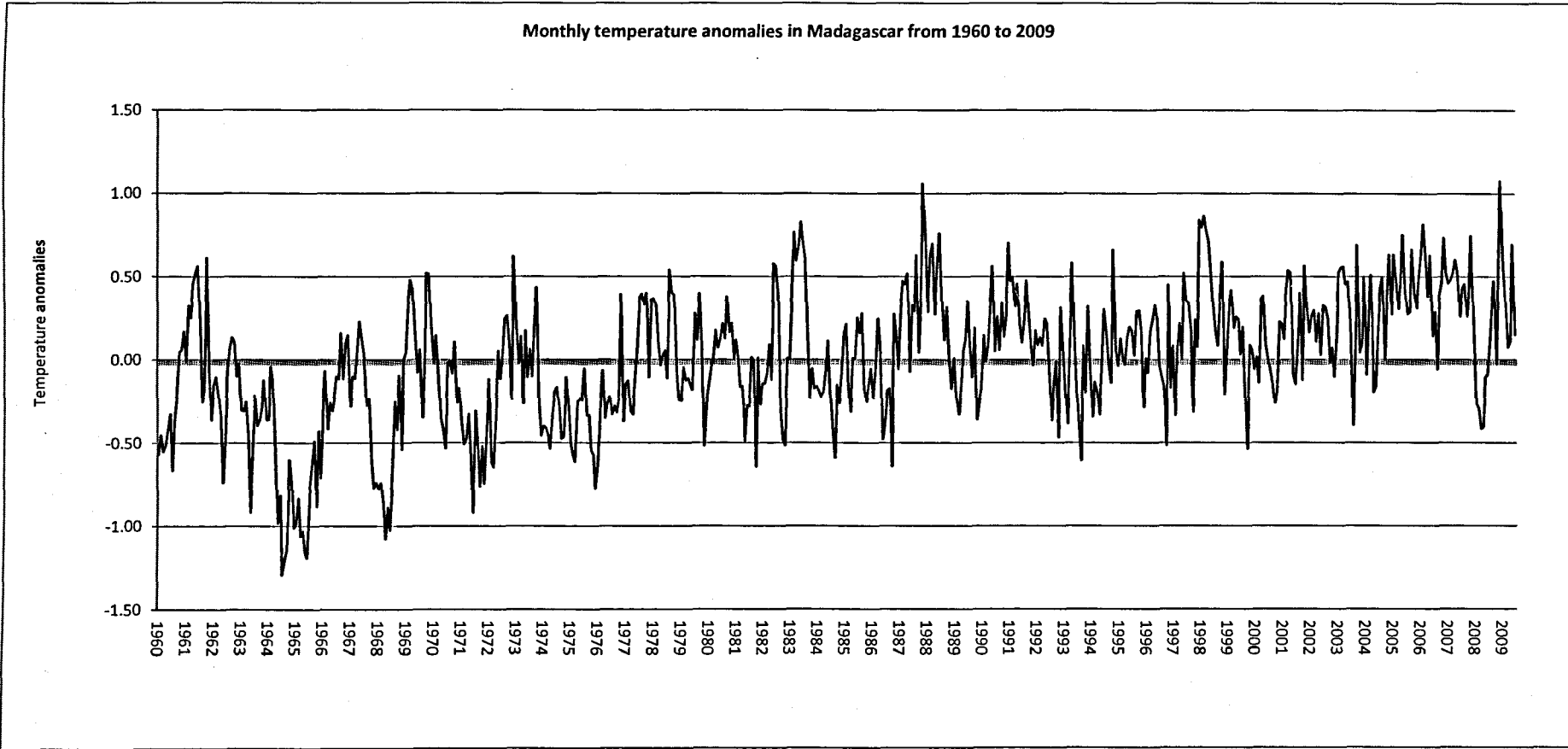**Figure 2.4: Monthly temperature anomalies from 1960 to 2008 for Madagascar computed from data on monthly temperature values from 1960 to 2008 published by NOAA.**

2.3.3 Coefficient of variation

The global climate index, El Nino Southern Oscillation (JMA) has its greatest variation from November to January during the height of the rainy season (Figure 2.5 a), while the Indian Ocean Dipole (DMI) has its greatest variation a little earlier, from September to November (Figure 2.5 b). Incidence anomalies have their greatest variation from October to February (ONDJF) during the plague season. The temperature anomalies do not show great differences in their variation, but vary most from March to June and again in December, while the precipitation anomalies have their greatest variation from December through to March during the wet season.

Figure 2.5 a; b: Monthly coefficient of variation of the a) El Niño Southern Oscillation and b) Indian Ocean Dipole index.
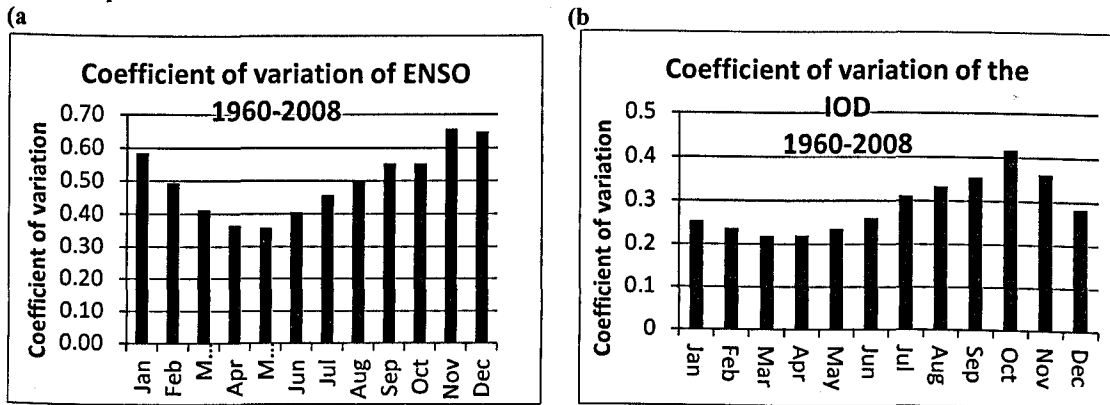
2.3.4 Wavelet analysis and linear regression and composite maps

2.3.4.1 Continuous wavelet transform

The continuous wavelet transform identifies the periodicity (band) or cycle of high variance within a dataset. The plague incidence anomalies for Madagascar show two periods (cycles) of high significant variance (Figure 2.6). Initially a periodicity of about 2 to 4 years is present until 1975 which is followed by a wider 2 to 8 year band. The wavelet power spectrum for the ENSO index (JMA) depicts high variance in the 2-5 year band from 1960 onwards, which slowly shifts to a 2-7 year band until the end of the time-series (Figure 2.7 a). The IOD index (DMI) shows significant variance in the 2 to 6 year band with increased significance from 1990 onwards (Figure 2.7 b). Precipitation anomalies time-series do not show much variance throughout the last 50 years. However, some variance for Madagascar is shown around 1-2 years denoting inter-annual variability in the time series (Figure 2.7 c).

The time-series of temperature anomalies, shows significant variance throughout, but especially during the first 30 years from 1960 to 1990. The periodicity shifts from a range of 1 to 7 years until 1972 to a slightly smaller range of 3 to 5 years until 2000 (Figure 2.7d).

2.3.4.2 Cross-wavelet transform and wavelet coherence

Cross-wavelet analysis indicates significant high common spectrum power of two signals. Wavelet coherence can be understood as correlation between signals in the power spectrum space. Thus, anti-phase coherence is also called negative correlation, while in-phase coherency may be called positive correlation. The relationship between variables was explored by analysing two signals at a time with cross-wavelet and wavelet coherence transforms.

Influence of ENSO on plague incidence anomalies

Cross-wavelet analysis indicates that ENSO and plague incidence anomalies show significant high common spectrum power throughout the time-series (red areas) with a 2 to 6 year cycle suggesting a relationship between the variables (Figure 2.8). The subsequent wavelet coherence plot in Figure 2.9 reveals 3 highly significant periods of correlation with differing relationships.

From 1965 to 1976, the vectors indicate that plague incidence anomalies lag 9 months behind the ENSO signal with a 2.5 to 4.5 year cycle with a weak positive correlation. This is followed by a period of strong negative correlation in the 80s with plague incidence anomalies now lagging by only 6 months. The final period of significant correlation only lasts from 1995 to 1999 when incidence anomalies lag by 9 to 10 months behind ENSO with a 1.5 to 3 year cycle. The direction of correlation depends on the cycle and is positive for a 1.5 to 2.5 year cycle.

The phase angle evolution of the 2 to 5 year band in Figure 2.10 confirms the slight shift of time lags throughout the last 50 years between plague incidence anomalies and ENSO. The first box outlines the period when incidence anomalies follow ENSO with a 9 month lag, while the second box illustrates the negative correlation and 6 months lag from 1981 onwards. Finally the third outline denotes the 9 to 10 month lag between the signals.

Influence of the IOD on plague incidence anomalies

As with ENSO, incidence anomalies also show significant high common spectrum power throughout the time-series with the IOD in the 2-7 year period band in Figure 2.11 However, unlike with ENSO, the subsequent wavelet coherence plot in Figure 2.12 shows no significant periods of coherency until the late 1990s when a short link between plague incidence anomalies and the IOD emerges, cycling almost in-phase with a 1.5 to 3 year periodicity. The emergence of a link between the variables is most likely due to the dramatically changing sea surface temperatures from 1997 to 1998 (Fendorov, 2000; Murtugudde, 1999).

Figure 2.6: The plague incidence anomalies time-series decomposed and presented as a continuous signal in time-frequency space. The thick black contour designates the 5% significance level against red noise. The cone of influence (COI) where edge effects might distort the results is shown as a lighter shade
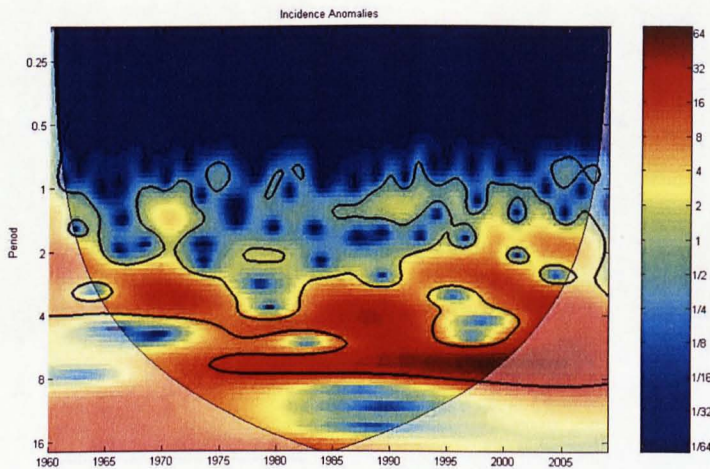
Figure 2.7a; b; c; d: The anomalies time-series decomposed and presented as a continuous signal in time-frequency space. a) ENSO b) IOD c) Precipitation d) Temperature. The thick black contour designates the 5% significance level against red noise. The cone of influence (COI) where edge effects might distort the results is shown as a lighter shade

a) ENSO

b) IOD

c) Precipitation
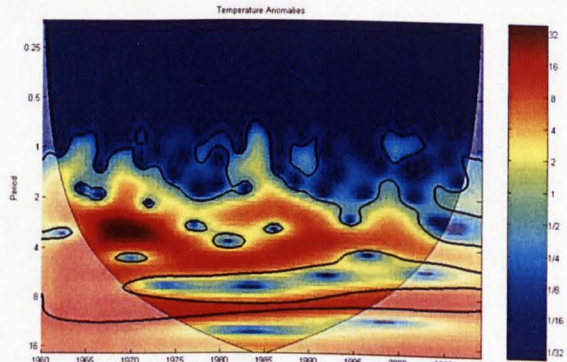
d) Temperature

Figure 2.8: Cross-wavelet plot showing common spectrum power of ENSO and plague incidence anomalies time-series. Red denotes areas of high common power, blue of low common power. The vectors indicate the phase difference between the time-series. The x-axis refers to time. The y-axis is the wavelet period in years. The thick black contour designates the 5% significance level against red noise. The cone of influence (COI) where edge effects might distort the results is shown as a lighter shade
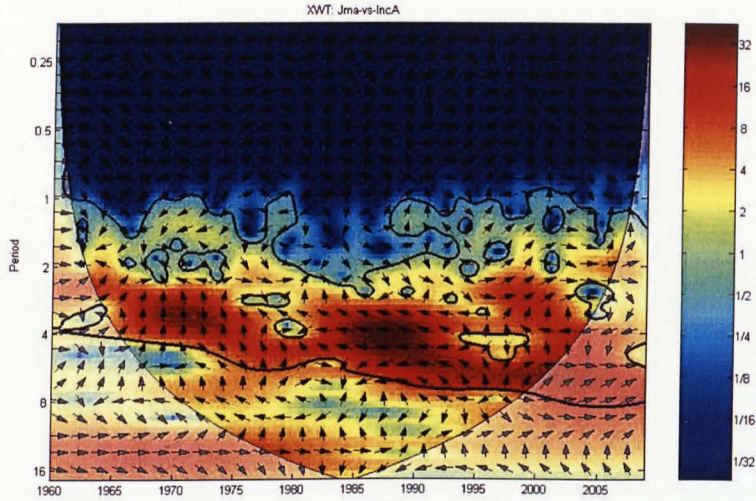
Figure 2.9: Strength of the association between the periodicities of human plague in Madagascar and ENSO, 1960-2008. Wavelet transform coherency plot of ENSO and plague incidence anomalies time-series showing periods of coherency between the signals. Red denotes areas of high coherency, blue of low coherency. The vectors indicate the phase difference between the time-series. The x-axis refers to time. The y-axis is the wavelet period in years. The thick black contour designates the 5% significance level against red noise. The cone of influence (COI) where edge effects might distort the results is shown as a lighter shade.
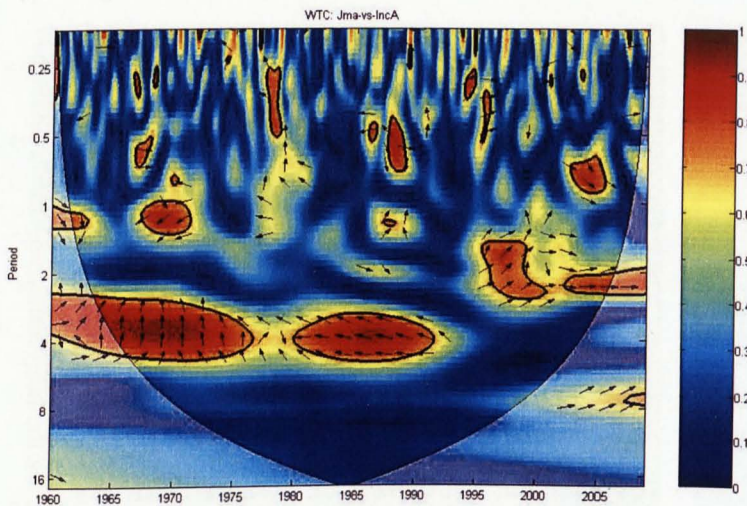
Figure 2.10: Time lag of association between ENSO and human plague incidence in Madagascar 1960-2008. Phase angle evolution showing the phase differences between the ENSO and plague incidence anomalies signals at the 2-5 year band. The red line represents incidence anomalies, the blue line the ENSO index. The x-axis is the wavelet location in time. The y-axis denotes the phase angles.
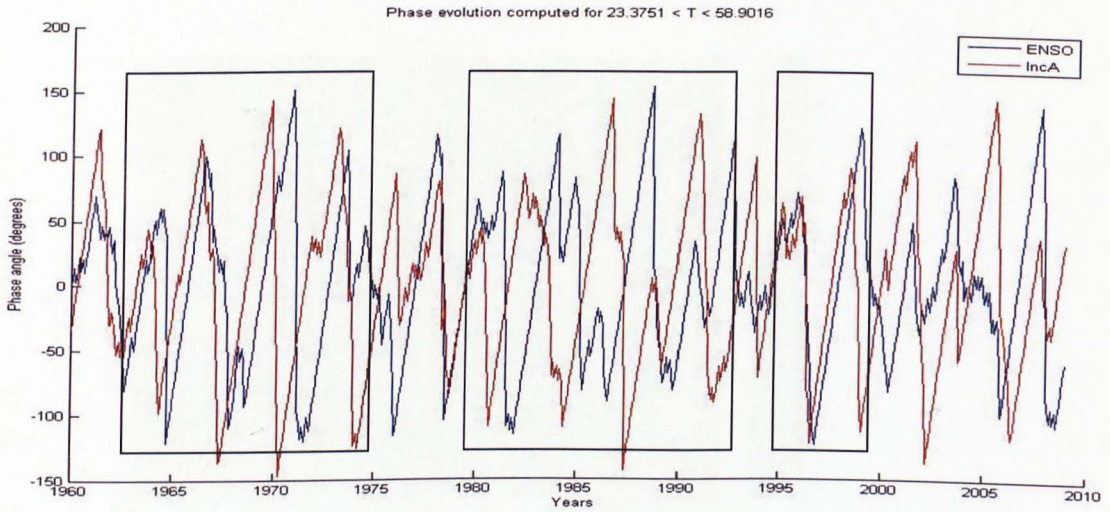
Figure 2.11: Indication of the association between the periodicities of human plague in Madagascar and IOD, 1960-2008. Cross-wavelet plot showing common spectrum power of the IOD and plague incidence anomalies time-series. Red denotes areas of high common power, blue of low common power. The vectors indicate the phase difference between the time-series. The x-axis refers to time. The y-axis is the wavelet period in years. The thick black contour designates the 5% significance level against red noise. The cone of influence (COI) where edge effects might distort the results is shown as a lighter shade.
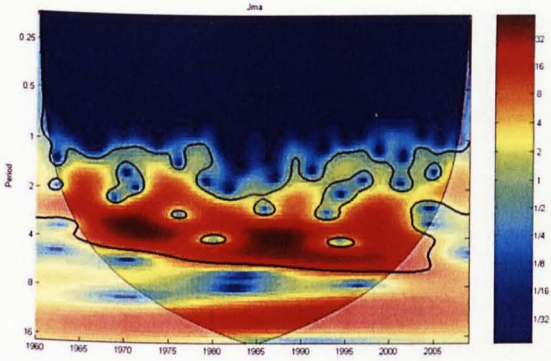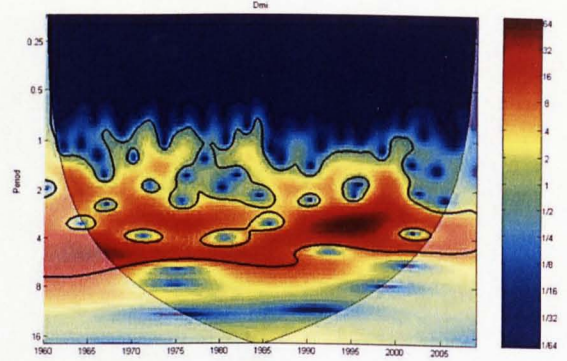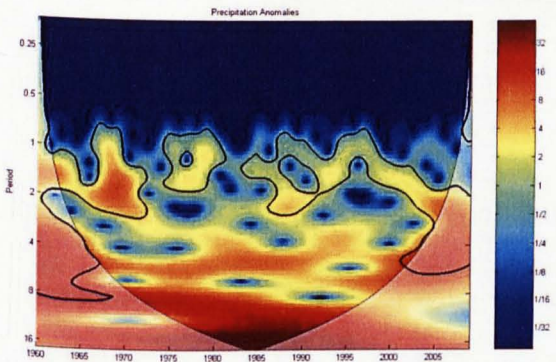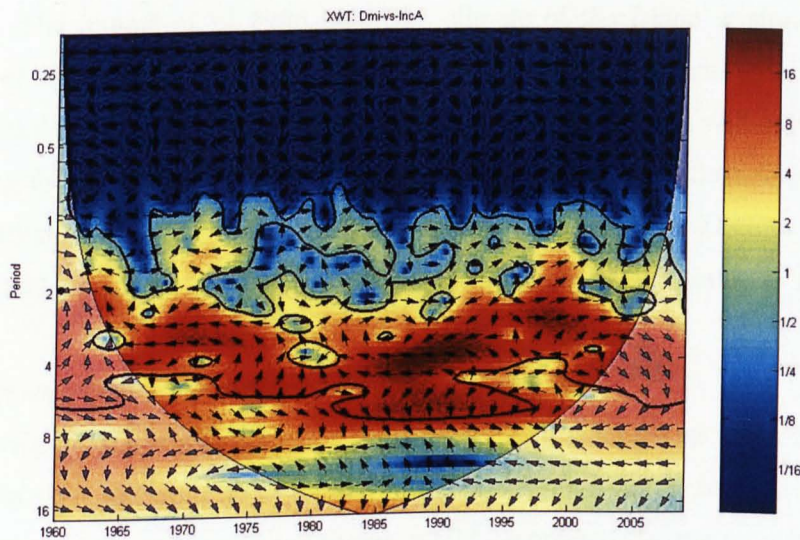
Figure 2.12: Strength of the association between the periodicities of human plague in Madagascar and the IOD, 1960-2008. Wavelet transform coherency plot of the IOD and plague incidence anomalies time-series showing periods of coherency between the signals. Red denotes areas of high coherency, blue of low coherency. The vectors indicate the phase difference between the time-series. The *x*-axis refers to time. The *y*-axis is the wavelet period in years. The thick black contour designates the 5% significance level against red noise. The cone of influence (COI) where edge effects might distort the results is shown as a lighter shade.
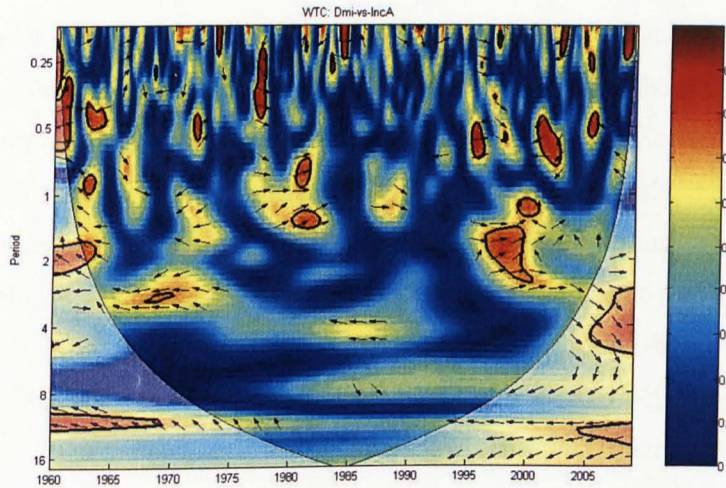

ENSO influence on temperature and precipitation of Madagascar

Generally warm (cold) phases of the ENSO are associated with a warming (cooling) of the whole troposphere over the Tropics (Neelin, 1998). Figures 2.13a, and 2.13b, show the relationship between ENSO and temperature and precipitation conditions over Madagascar respectively. The impact of El Niño upon the climate of the island is similar to the one observed over southern Africa by Nicholson and Selato (2000). A spring-summer El Niño causes drier and warmer than usual boreal winter conditions. El Niña will have the opposite effect leading to wetter and cooler than average conditions. This influence on temperature and precipitation starts six to nine months in advance, thus a summer El Niño will affect the following boreal winter climate which is the rainy season and plague season in Madagascar.

IOD influence on temperature and precipitation of Madagascar

As the western pole of the IOD is located nearby the island, IOD events affect the convection locally and in turn influence the climate in Madagascar. Thus, a positive IOD event is associated with warmer and wetter conditions, while the opposite is true for a negative event. 2.14a, and 2.14b, in turn show the relationship between the IOD and temperature as well as

precipitation conditions on the island. The IOD influences temperature and precipitation three to six months later, so a summer IOD impacts on the following autumn and winter temperature and precipitation.

Relationship between precipitation anomalies, temperature anomalies and incidence

The cross-wavelet analyses show periods of common power of plague incidence anomalies and precipitation anomalies as well as temperature anomalies throughout the time-series. For temperature anomalies and incidence anomalies several periods of coherency between the signals are confirmed in Figure 2.15a. Plague incidence lags about 6 months behind temperature, confirming a strong positive correlation between spring-summer temperature and winter incidence for most of the time-series. The periodicity however changes slightly over time from 2 to 5 years until 1977 to 3.5 to 4.5 years until 1992. The last period of coherency with incidence anomalies lagging by 9 months starts in 1994 and lasts until the COI limits it in 2004. For precipitation anomalies, the subsequent wavelet coherency-transform then shows a positive correlation with incidence anomalies from around 1993 to the end of the time-series with a periodicity of 4.5 to 8 years (Figure 2.15b).
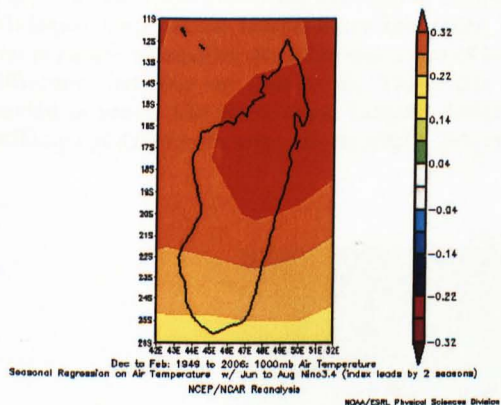
Figure 2.13a: Linear regression between the summer ENSO index and the following boreal winter temperature from December to February (NCEP-NCAR reanalysis). The ENSO index is leading by two seasons (6 months). The green to red colour spectrum denotes positive correlation while the blue to purple spectrum denotes negative correlation.
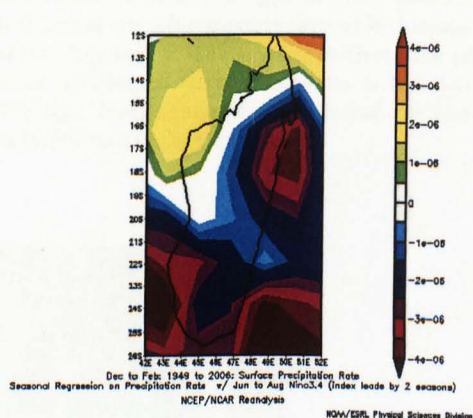
Figure 2.13b: Linear regression between the summer ENSO index and the following boreal winter precipitation rate from December to February (NCEP-NCAR reanalysis). The ENSO index is leading by two seasons (6 months). The green to red colour spectrum denotes positive correlation while the blue to purple spectrum denotes negative correlation.
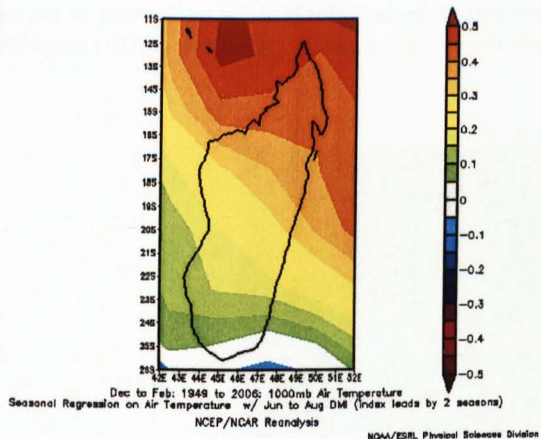
Figure 2.14a: Linear regression between the summer IOD index and the following winter temperature (NCEP-NCAR reanalysis). The IOD index is leading by two seasons (6 months). The green to red colour spectrum denotes positive correlation while the blue to purple spectrum denotes negative correlation.
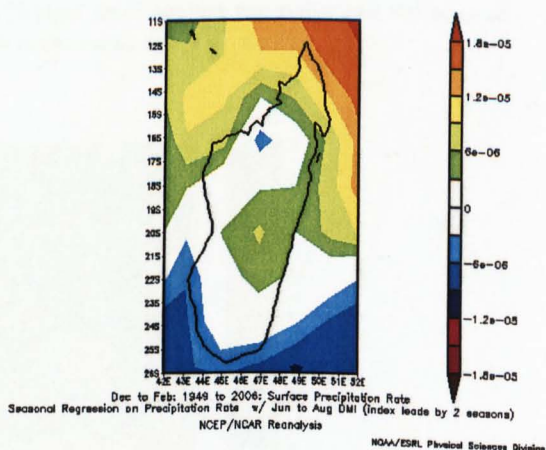
Figure 2.14b: Linear regression between the summer IOD index and the following winter precipitation rate (NCEP-NCAR reanalysis). The IOD index is leading by two seasons (6 months). The green to red colour spectrum denotes positive correlation while the blue to purple spectrum denotes negative correlation.

Figure 2.15a: Strength of the association between the periodicities of human plague in Madagascar and Malagasy land surface temperature anomalies, 1960-2008. Wavelet transform-coherence of incidence and temperature anomalies. Red denotes areas of high, blue of low coherency. The vectors indicate the phase difference between the time-series. The *x*-axis is the wavelet location in time. The *y*-axis is the wavelet period in years. The thick black contour designates the 5% sign. level against red noise and the cone of influence (COI) where edge effects might distort the results is shown as a lighter shade.
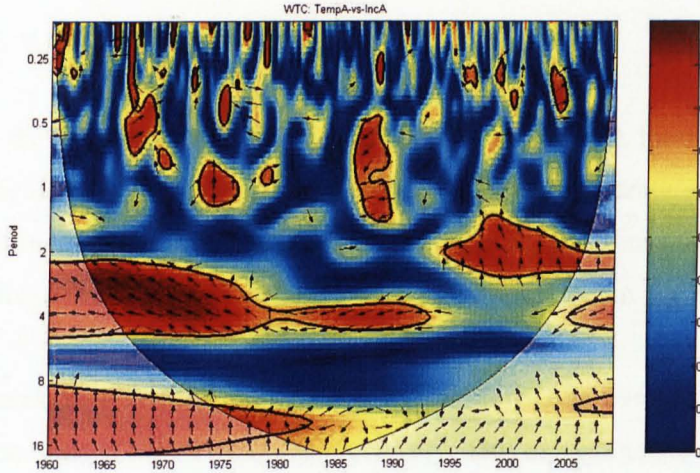
Figure 2.15b: Strength of the association between the periodicities of human plague in Madagascar and Malagasy precipitation anomalies, 1960-2008. Wavelet transform-coherence of incidence and precipitation anomalies. Red denotes areas of high, blue of low coherency. The vectors indicate the phase difference between the time-series. The *x*-axis is the wavelet location in time. The *y*-axis is the wavelet period in years. The thick black contour designates the 5% sign. level against red noise and the cone of influence (COI) where edge effects might distort the results is shown as a lighter shade.
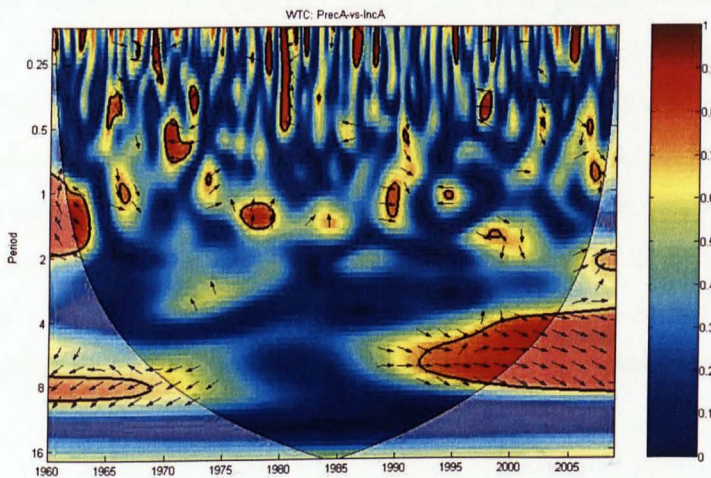
Epidemics

The relationship between the ENSO and plague incidence anomalies is strongest for winter-spring ENSO and the following plague season 9 to 12 months later, whereas the positive correlation between the IOD and plague incidence anomalies is almost immediate (in-phase) or with plague incidence lagging by one season (3 months). As both phenomena impact on the climate of Madagascar a detailed summary of each high plague incidence period identified earlier, is shown in Table 2.1.

For each period the preceding ENSO and IOD index together with local climate anomalies during the particular plague season were explored. The temperature and precipitation anomalies of the months before an epidemic have been explored and the periods with the strongest anomalies were identified. All anomalies found were within September to February.

The high incidences observed in from December 1968 to March 1969 can be related to the negative ENSO conditions seen 12 months previously. This contributed to cooler and slightly wetter conditions during the following rainy season.

Starting 9 months before the occurrence of the high number of plague cases from November 1988 to February 1989, a strong El Niña event prevailed throughout all of 1988 and is associated with colder and wetter climate over Madagascar during the plague season. The following year, the effects of the strong La Nina in 1988/89 still contributed to cooler temperatures and increased precipitation during the next plague season with increased incidence from November to February 1990. The strongest El Niño on record was documented in 1997. Coupled with a positive IOD event, the usually cold and dry winter and spring was warmer and wetter than usual and can be related to an increase in plague incidence and an earlier onset of the plague season. This combination of a positive ENSO and a positive IOD was repeated in the plague season from October 2003 to February 2004. Again the winter and spring was warmer and wetter than usual. At the end of 2004 the positive ENSO effects from the preceding 3 to 12 months are associated with a warmer and wetter spring.

Table 2.1: Months of high human plague incidence and their correlated ENSO and IOD conditions as well as composite maps of temperature and precipitation before.
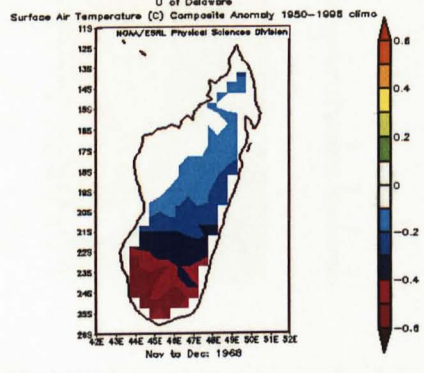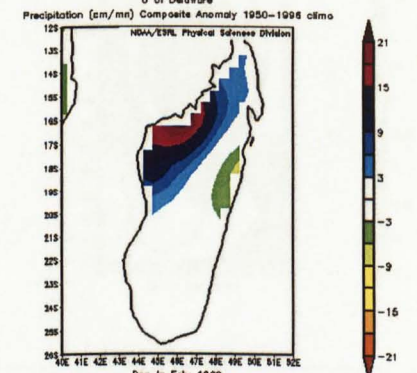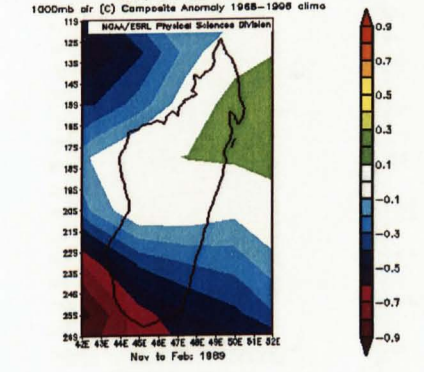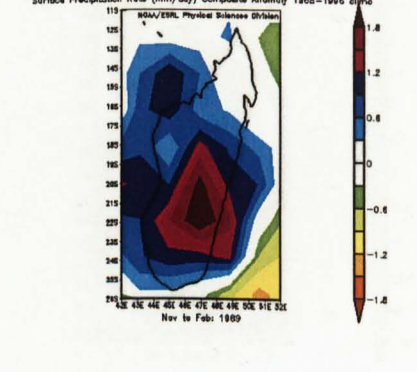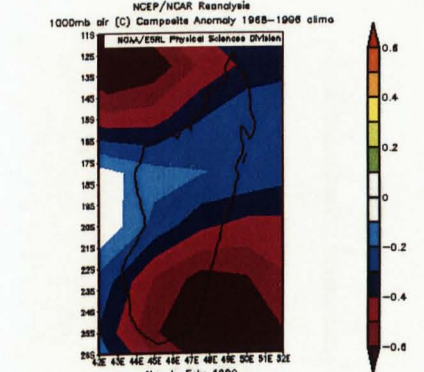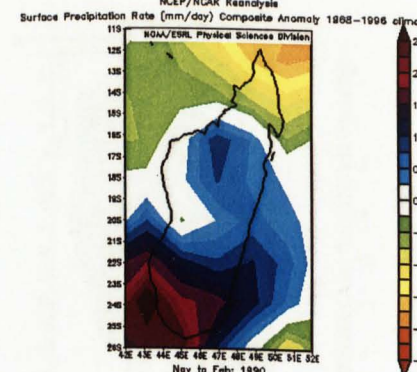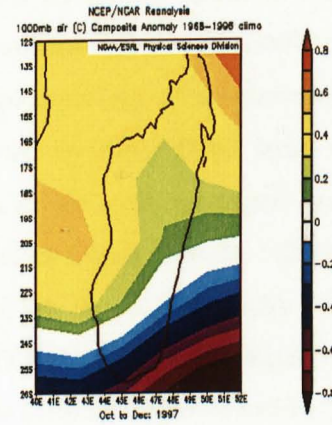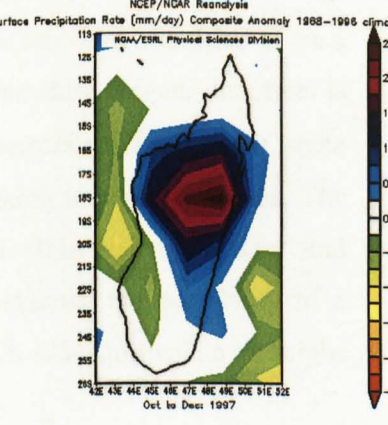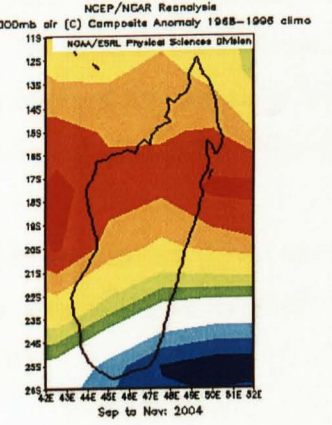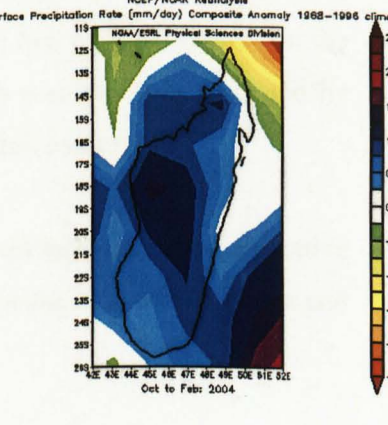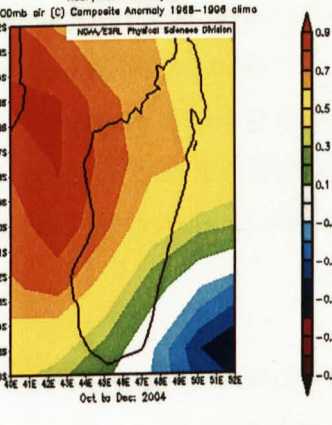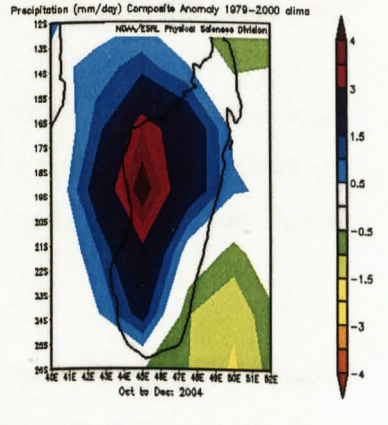
Season	ENSO and IOD values	Temperature	Precipitation
DJFM 1968-69	Negative ENSO 9-12 months before Negative IOD	Colder Nov-Dec	Wet Northwest Dec-Feb
NDJF 1988-89	Negative ENSO 0-9 months before Negative IOD	Colder Nov-Feb	Wetter Nov-Feb
NDJF 1989-90	Negative ENSO 12 months before Negative IOD	Colder Nov-Feb	Wetter Nov-Feb

Table 2.1 continued: Months of high human plague incidence and their correlated ENSO and IOD conditions as well as composite maps of temperature and precipitation anomalies.

OND 1997	Positive ENSO 3-12 months before Positive IOD 3 months before	Warmer Oct-Dec	Wetter Oct-Dec
ONDJF 2003-04	Positive ENSO 3-12 months before Positive IOD 3 months before	Warmer Sep-Nov	Wetter Oct-Feb
OND 2004	Positive ENSO 3-12 months before Negative IOD 3-6 months before	Warmer Oct-Dec	Wetter Oct-Dec

2.4 DISCUSSION

Global climate phenomena have been found to affect the periodicity of several infectious diseases. The El Niño Southern Oscillation (ENSO) for example has been identified to be a strong influence on the epidemiology of vector-borne diseases including plague. Its effect is often enhanced or reduced by other large scale climate phenomena affecting the same geographical region such as sea surface temperature cycles causing unique conditions. The western pole of the Indian Ocean Dipole (IOD) is situated close to Madagascar and influences the climate of the island. This study therefore investigated the possibility of a combined effect of ENSO and the IOD on plague incidence in an effort to explain the highs and lows of human plague incidence recorded from 1960 to 2008.

Associations between ENSO, the IOD and plague incidence over time

The results suggest that there is indeed an association of both, ENSO and the IOD with human plague incidence. The relationship however, between the two climate drivers and the disease over the period examined appears to be changing. This is most likely mediated by variations in strength and timing of ENSO and the IOD over the last century.

Generally, El Niño leads to warmer and drier conditions over Madagascar, while a positive phase of the IOD leads to warmer and wetter conditions. The opposite is true for La Niña and a negative phase of the IOD.

Results of this study imply that periods of high plague incidence between 1956 and the mid 1990's are associated with negative ENSO events (La Niña) and negative phases of the IOD, a climate driver combination known to cause cooler temperatures during the usually hot boreal winter months over most of Madagascar.

The relationship changes throughout time and subsequent periods of high plague incidence show associations with a positive IOD followed by effects of a positive ENSO event (El Niño) promoting warmer and wetter conditions during the usually cold and dry boreal summer and autumn.

Within this changing relationship, further analysis identified three significant periods of signal coherency: The first in the 1960s shows that plague incidence is negatively correlated to ENSO following a 9 month lag. Hence a La Niña preceding the boreal winter by 9 to 12

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months corresponded to colder temperature and increased rainfall during that winter and an increased plague incidence.

The next period of coherency starts in the 1980s and only differs from the first period by a reduced lag between the ENSO and plague incidence anomalies. Then, from the time of the strongest El Niño ever recorded (in 1997) onwards, evidence suggests a change in the direction of the relationship between plague incidence and ENSO from a clearly negative to a positive correlation.

Associations between plague epidemics and large scale climate

A more detailed examination of climatic patterns before and during six periods of especially high plague incidence throughout the time period investigated provided further insight into the nature of these associations. The analysis of the epidemic in 1968/69 shows a strong La Niña event 9 months before and subsequent increased rainfall patterns and colder temperatures during the plague season. A colder and wetter climate during the plague season of 1988/89, caused by a strong and long lasting La Niña event which stretched from 9 to 0 months previously was followed by high plague incidence and an elevated number of cases the year after in 1989/90. Then the relationship seemed to change and the strong El Niño event in 1997 together with a positive phase of the IOD caused wetter and warmer than usually observed climatic conditions in the boreal autumn preceding a higher than usual plague incidence in the same year. This climatic pattern of both, El Niño and a positive IOD were again recorded together in 2003 and 2004 again followed by a high plague incidence.

Implications of the results for the plague focus in Madagascar

The results shown here strongly suggest that the two large scale climate drivers ENSO and IOD have an effect on human plague incidence in Madagascar. It also becomes clear that the relationship between climate and disease is exceedingly complex and on a large scale and that further research is needed to clarify via which mechanisms climate influences the epidemiology of plague. Results of this study link in with previous studies on the epidemiology of plague and are presented here. Yet it becomes clear that in order to be able to make reliable predictions of human plague dynamics more information is needed to interpret the implications of the associations found here in detail.

Influences on the epidemiology of plague

As studies of climate and plague in other areas pointed out, a coherency of the ENSO and incidence is most likely due to the influence ENSO has on temperature and precipitation which in turn are known to affect host and vector ecology and transmission potential (Kartman, 1969; Parmenter et al. 1999; Stenseth et al. 2008). Not only do temperature thresholds exist for pathogen survival and its transmission potential but the ecology of host and vector is also directly and indirectly influenced by temperature and humidity. A direct effect for example, occurs when high intensity rainfall causes flooding of rodent burrows (Cavanaugh, Marshall, 1972) but the effects of precipitation on host populations are mainly affected by resource availability and hence abundance (Meserve et al. 2001).

The results of this study imply that in Madagascar ENSO and the IOD also influence the disease incidence via their effect on temperature and precipitation; yet the associations show great changes over the years in terms of the direction and timing of any relationships detected between plague and climate. Changes in the climate–disease relationship over time have also been found for other diseases (Rodo et al. 2002) most likely mediated by long-term climate change effects and their influence on epidemiological systems. ENSO events have been progressively stronger in recent years, increasing in both, frequency and amplitude.

Due to its unique location and landscape, Madagascar has many distinct climate zones within its main plague focus and studies confirmed an especially complex plague system (Duplantier, Duchemin et al. 2005; Duchemin, 2001) involving two vector species showing very different climate preferences (chapter 4 and 5). Additionally cultural differences especially in housing and farming vary throughout regions. Global climate is most likely to influence an array of factors of the epidemiology of plague many of which will depend on ecological and anthropogenic characteristics.

It is to be expected that a multitude of other factors affecting host and vector ecology as well as transmission potential are also involved. The spatial analysis of human plague incidence will provide more insight into direct and indirect effects of climate and is discussed in the following chapter.

CHAPTER THREE

INVESTIGATION INTO THE

SPATIAL DISTRIBUTION OF HUMAN PLAGUE

IN MADAGASCAR USING

SATELLITE-DERIVED ENVIRONMENTAL DATA

ABSTRACT

Human plague cases in Madagascar show not only temporal, but also spatial variation. The primary objective of this piece of research was to determine if remotely-sensed environmental variables are useful predictors of the spatial distribution of human plague incidence in Madagascar. Such information could then be used to identify areas suitable for disease persistence or potentially at risk from disease emergence now or under future conditions.

Using a set of satellite-derived environmental data and altitude as predictor variables, models were built with data on human plague occurrence and incidence as the dependent variables. Altitude, vegetation cover and temperature were the main drivers identified when modelling human plague occurrence at the district level in Madagascar. The models suggested the existence of a threshold, determined by environmental characteristics, for human plague occurrence. Plague incidence in the plague-endemic area of the island was best predicted by temperature and vegetation cover variables. The results support the hypothesis that satellite data can be used when empirical data is lacking and that a statistical approach represents a way to reveal patterns in large scale epidemiological processes which may have been overlooked when concentrating on small scale studies.

Future studies should investigate human plague data at higher resolution format and consider infection prevalence rather than disease.

3.1 INTRODUCTION

Many vector-borne diseases such as malaria, trypanosomiasis and dengue show substantial spatial variation in incidence across geographical areas. Possible causes have been the subject of many studies and regional environmental variables often prove a valuable tool to provide better insight into drivers (Yasui, Lele, 1997). The identification and mapping of environmental factors using satellite imagery and subsequently linkage of them with the distribution or incidence of various diseases has been comprehensively described and reviewed by Beck et al. in 2000 and Kalluri et al. in 2007. Eisen and Eisen (2010) also recently reviewed the advances, amongst others, in the exploitation of Geographic Information Systems and spatial modelling for the control of vector-borne diseases.

Prerequisites of such analyses are data on disease occurrence or incidence and information on environmental characteristics such as climate and topography as well as vegetation type and vegetation abundance. However, as empirical data of environmental characteristics are frequently unavailable or insufficient, remotely-sensed data derived from satellites are often used to model disease distribution and the incidence of disease (Hay, 1997; Manangan, Schweitzer, 2007; Rogers, 2002). The data originate from a series of observations recorded by satellites in intervals which are transformed into a set of sine curves, or harmonics, of different frequencies, amplitudes and phases. The resulting data corresponds to parameters reflecting environmental conditions and their seasonal changes which can be interpreted biologically (Scharleman et al. 2008). In several endemic plague areas of the world satellite derived data sets have been employed in order to explain its distribution and incidence (Ben Ari, Gershunov et al. 2010; Debien et al. 2010; Eisen et al. 2007; Enscore et al. 2002; Holt et al. 2009; Parmenter et al. 1999).

Human plague incidence in Madagascar shows not only temporal, but also spatial variation and this chapter represents an investigation into the environmental drivers affecting the spatial distribution. Past works examining spatial variation of plague in the USA have identified an association with winter-spring precipitation (Parmenter et al. 1999), and a link between plague risk, vegetation type and altitude (Eisen et al. 2007). Kausrud, Viljugrein et al. (2007) demonstrated that in the deserts of central Asia under certain climatic conditions the dynamics of great gerbils (*Rhombomys opimus*), a rodent host for plague, are synchronized over large geographical areas in the PreBalkhash plague focus of south-eastern Kazakhstan, and Winters et al. (2009) linked plague incidence to altitude and remotely-sensed variables of soil type and vegetation. A reasonable hypothesis is therefore that plague risk in Madagascar is also influenced by environmental variables.

At 587,000 km², Madagascar is a large island which shows substantial environmental gradients and highly varied climate regions. The centre of the island is dominated by a high plateau with summits of up to 2876 m. Some information is already known about the spatial distribution of plague in Madagascar. The island is divided into 116 administrative districts of varying size, but only 44 of these have had human plague cases confirmed in the last 34 years (Figure 3.11). This spatial distribution pattern seems to be limited by an altitudinal cut-off, as with only a few exceptions, all districts affected by plague are situated in the highlands

(Migliani, Chanteau et al. 2006) as illustrated when looking at the elevation structure (Chapter 1 Figure 1.2). The altitudinal cut-off coincides with the distribution characteristics of one of the two main vector species of plague in Madagascar, an endemic flea vector, which as Duchemin (2001) stated, has its highest abundance on rodents caught above 800 metres. Within the plague endemic zone, there is also considerable spatial variation in the incidence of disease (Migliani, Chanteau et al. 2006; Ratsitorahina, Rabarijaona et al. 2000). But how, and to what extent, do environmental characteristics such as altitude, climate and vegetation affect the distribution of the disease?

More insights are needed as to what variables can be used to better predict if an area could maintain a plague focus. Yet, even though anthropogenic variables linked to geographical areas such as farming practises, cultural differences and demographics are also likely to affect plague incidence, they have not been included here mainly due to lack of reliable data. This allows more focus to be given to an area that has not been explored yet for Madagascar but for which data is available.

This chapter explores whether (1) the spatial distribution of plague within Madagascar and/or (2) spatial variation in the incidence of plague within the endemic region are related to environmental variables. The new approach used in this study is also a tool to validate reasons suggested by previous studies as to why human plague is found in some areas in Madagascar and not others. To address these questions, regression models were built using satellite-derived environmental variables and elevation.

3.2 METHODS

3.2.1 Data

Plague incidence data

Data on confirmed human plague cases from 1975 to 2008 at a district level were made available by the WHO plague reference laboratory of the Institut Pasteur de Madagascar. A change in political leadership in 1975 brought a restructuring of the districts, so for this study, plague data before 1975 was omitted. The average yearly human plague incidence per district was calculated using 5-yearly population growth estimates from the UN and the last population census from 1993 (Anonymous, 1996).

Geographical data

District-level geographical information systems (GIS) shapefiles of Madagascar were acquired from map cruzin.com (www.mapcruzin.com/free-madagascar-arcgis-maps-shapefiles.htm). Altitude data (Figure 3.2) for the island was downloaded from the DIVA website (http://www.diva-gis.org/Data). Altitude was averaged over each district.

Environmental variables

MODerate-resolution Imaging Spectroradiometer (MODIS) imagery from the NASA Terra satellite provided measurements of large-scale global processes. The data products derived from MODIS observations describe features of the land, oceans and the atmosphere that can be used for studies of biological, geological and climatic developments and trends on local to global scales (http://modis.gsfc.nasa.gov/data). Potential predictor variables for distribution modelling are obtained by Fourier transforming a series of observations taken at regular intervals into a set of sine curves of different frequencies, amplitudes and phases that collectively sum to the original time series (Scharlemann et al. 2008). A set of remotely-sensed environmental data for Madagascar with a spatial resolution of 100 kilometres^2 derived from 5 years of MODIS imagery (2001-2005) was kindly provided by David J. Rogers and G.R. William Wint from the University of Oxford.

Five MODIS parameters comprising 14 Fourier variables each were used as explanatory variables within the model. The parameters included the vegetation indices 'Normalised Difference Vegetation Index' (NDVI) (Rouse et al. 1973) and the 'Enhanced Vegetation Index' (EVI) (Huete et al. 2002). These are based on measurements of chlorophyll absorption of light and reflectance providing information on vegetation greenness. Also included are the 'Middle Infrared Reflectance' (MIR) and the 'day/night Land Surface Temperature' (dLST and nLST), which yield measurement information on surface temperature based on infrared reflectance values. The definitions of the 14 variables of each parameter are listed in Table 3.1. 'Amplitude' in this context refers to changes in magnitude of a given variable for example while 'phase' or 'peak timing' indicates the rhythm with which a cycle occurs. The average altitude of each district was used as an additional explanatory variable. For the 116 administrative districts of Madagascar, complete data sets were available for 106 districts. MODIS values were averaged over each district.

3.2.2 Maximum Entropy distribution modelling software

The software MaxEnt was used for analysis to determine the spatial distribution of human plague in Madagascar. MaxEnt is a machine learning algorithm designed to automatically recognize and generalize complex patterns based on examples and then produce predictions in new cases. It is a software package mainly used for species distribution modelling based on geographically referenced records of the species' presence only. It does not require absence points, but uses data on the environmental conditions of the region of interest of sites of known occurrence and sites of possible absence (Phillips et al. 2009). MaxEnt uses a maximum entropy method. In Bayesian probability, the principle of maximum entropy is that, subject to known constraints, the probability distribution representing the current state of knowledge best is the one with largest entropy (Jaynes, 1957). The software also identifies which and to what extent entered explanatory variables contributed to the distribution model.

3.2.3 Data analysis

In each analysis, mean altitude and a total of 70 separate environmental variables were used as explanatory variables. To examine the possibility of non-linear relationships between disease presence or incidence and environmental variables, the square of each environmental variable was also considered.

Binary logistic regression

A binary logistic regression model with logit link function was used to examine relationships between environmental factors and plague distribution. Based on the occurrence of disease, the 106 districts were divided into two categories: 'plague-endemic' and 'plague-non-endemic'. Stepwise elimination backwards and subsequently forwards was performed, with 0.05 and 0.051 entry and exit p-values respectively to identify significant predictor variables. The fit of the final logistic model was assessed using a Hosmer-Lemeshow goodness-of-fit-test (Hosmer, Lemeshow, 2000) and sensitivity and specificity values were measured; sensitivity measured the proportion of actual plague-endemic districts which are correctly identified as such, and specificity measured the proportion of plague-non-endemic districts correctly identified. To measure the proportion of all possible cases of plague presence or absence in a district that are predicted correctly by the model after accounting for chance effects, Cohen's Kappa value was calculated (Manel et al. 2001). The responses of regression models can be spatially correlated (autocorrelation) due to non-independence of variables,

which may result in the underestimation of the variance of coefficients and incorrect parameter estimates of statistical models (Anselin, Bera, 1998). The deviance residuals of the model were therefore visualized using Arc Map to check for spatial autocorrelation.

Linear regression

Linear regression was used to explore relationships between environmental variables and mean annual plague incidence within the endemic area (44 districts). The mean plague incidence was log transformed to normalise the data before a linear regression was undertaken. Stepwise elimination backwards and subsequently forwards was performed, with 0.05 and 0.051 entry and exit p-values respectively to identify significant predictor variables. The importance of each environmental variable was first screened individually using Pearsons correlation and variables with low or no correlation to the dependent variable ($p \geq$ 0.1) were removed. Additionally, the explanatory variables were examined for co-linearity and where high correlation ($P \geq 0.2$) was detected; the variable contributing most to the R square value was included in the analysis. The fit of the final linear model for variation between districts in plague incidence was determined using the R-square value and the distribution of the residual versus fitted values.

Maximum Entropy modelling

To address the detected spatial autocorrelation in the binary logistic regression model, the freely available machine-learning software Maximum Entropy algorithm (MaxEnt; version 3.3.1; Phillips et al. 2006) was employed to investigate the results further. The detailed algorithms used by MaxEnt are described by Phillips et al. (2006). A logistic model was built using the same explanatory variables (n=71) and plague presence data (n= 44 records). According to Phillips et al. (2006), MaxEnt does not make the assumption that variables have no interaction and is hence robust to co-linearity between variables and also not hampered by spatial autocorrelation. The MaxEnt model was run with 90% of plague endemic district records (90% training data); 10% of the records were used for testing of the final model (10% test data). To account for over-fitting due to the large amount of predictor variables, the data was smoothed by setting the 'regularization multiplier' in MaxEnt to level 2. The software automatically considered an expanded set of transformations of the original variables: linear, quadratic and hinge terms. Hinges are piecewise linear responses with a positive or negative coefficient (slope) before or after a certain point of the linear line (Figure 3.7). For model

assessment the area under the receiver operating characteristic (ROC) curve (AUC) was calculated by the software, relating relative proportions of correctly and incorrectly classified predictions.

3.3 RESULTS

3.3.1 Environmental variables

Table 3.1 shows the definition of the abbreviations used for the 14 Fourier variables used.

Figure 3.1 illustrates the differences between annual, biannual and triannual cycles.

Table 3.1 Definition of the Fourier variables

Fourier Variable	Explanation
A0	Overall mean amplitude
A1	Amplitude of the annual cycle
A2	Amplitude of the biannual cycle
A3	Amplitude of the triannual cycle
p1	Phase (peak timing) of the annual cycle
p2	Phase (peak timing) of the biannual cycle
p3	Phase (peak timing) of the triannual cycle
d1	Proportion of variance explained by the annual cycle
d2	Proportion of variance explained by the biannual cycle
d3	Proportion of variance explained by the triannual cycle
da	Proportion of variance explained by the annual, biannual and triannual cycle combined
mn	Minimum of the seasonal cycle
mx	Maximum of the seasonal cycle
vr	Variance

Figure 3.1: Fourier transformed sine waves of the annual, bi-annual and tri-annual cycle
Extracted from: "Modis imagery processing and data files" (Rogers D. and Wint W.)

Fourier Sine Waves (Components)

Annual — Biannual — Triannual
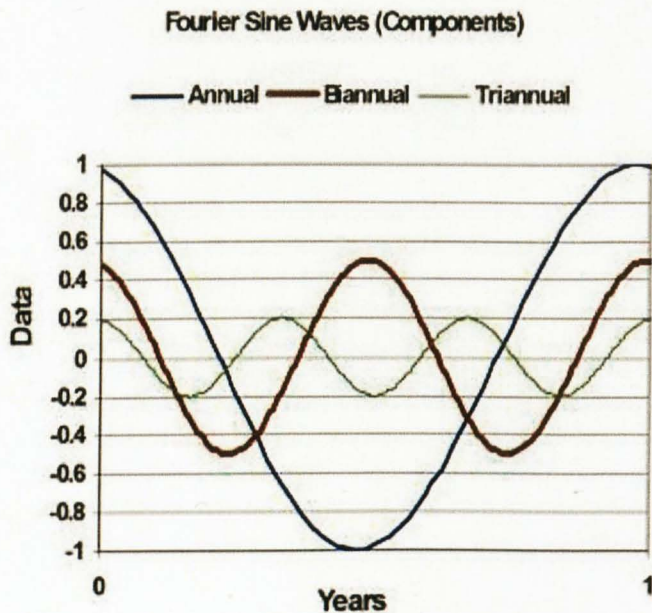
3.3.2 Spatial distribution of plague

Table 3.2 lists all the districts where human plague cases have been confirmed in the last 34 years and provides additional information on each district. Districts with plague with an average altitude below 800 meters are highlighted.

Figure 3.2 shows a map of the 44 districts where human plague cases have been confirmed in the last 34 years and illustrates the variation in plague incidence between districts.

Figure 3.3 displays the elevation profile of the island. The districts with the highest incidence are located in the highest parts of the highlands, with the exception of the city-district of Mahajanga on the west coast.

The map in Figure 3.4 highlights eight plague affected districts with an average altitude of below 800m in turquoise. Only two of these, Mahajanga and Ambato-Boina do not incorporate areas of higher altitude.

Figure 3.2: Yearly mean plague incidence of Madagascar from 1975 to 2008. Districts in green do not report human plague cases while areas in red report the highest average number of cases.

Figure 3.3: Elevation profile of Madagascar. Green denotes low altitude while orange denotes high altitude areas.
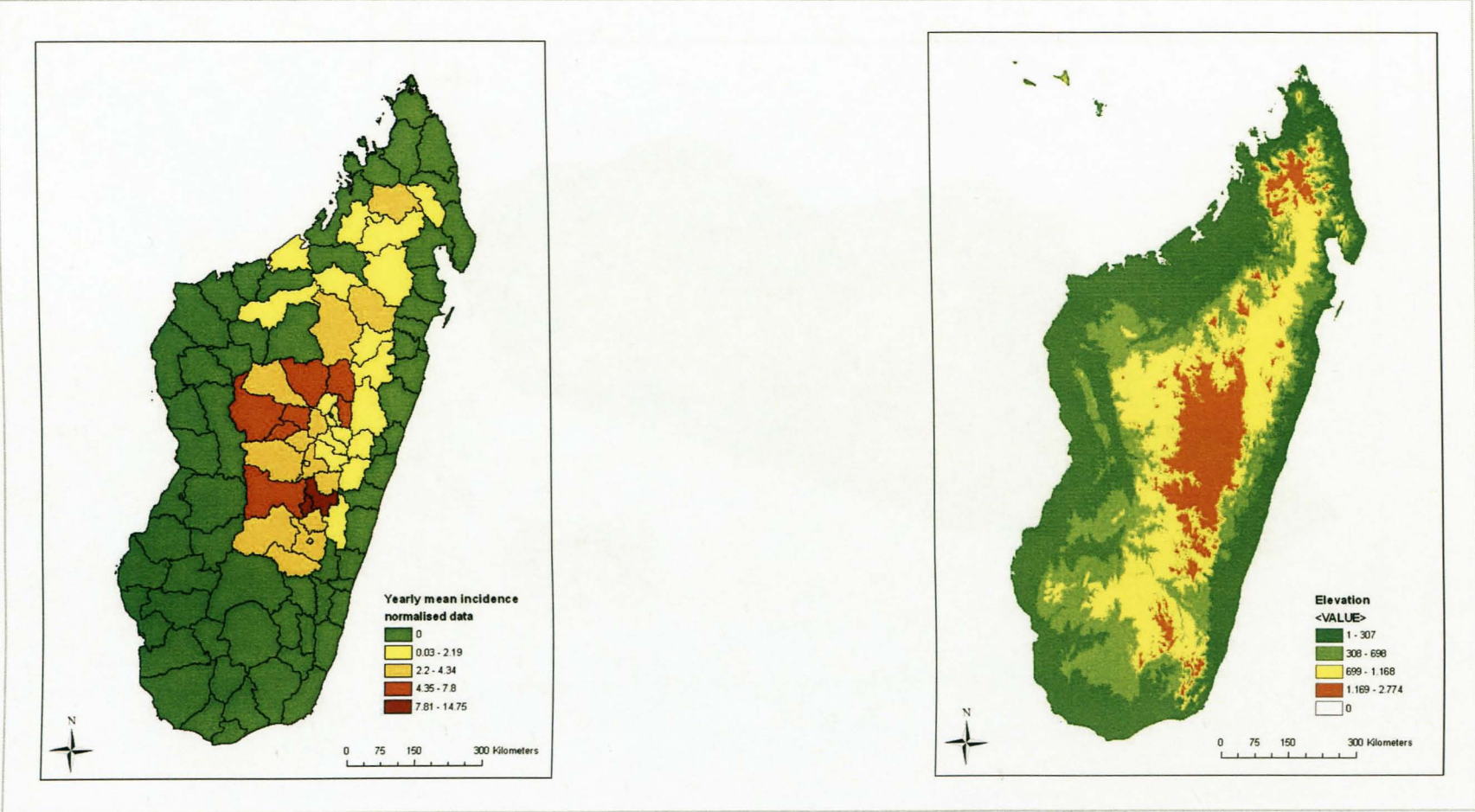
Figure 3.4: Plague affected districts with an average altitude of below 800m have a turquoise contour.
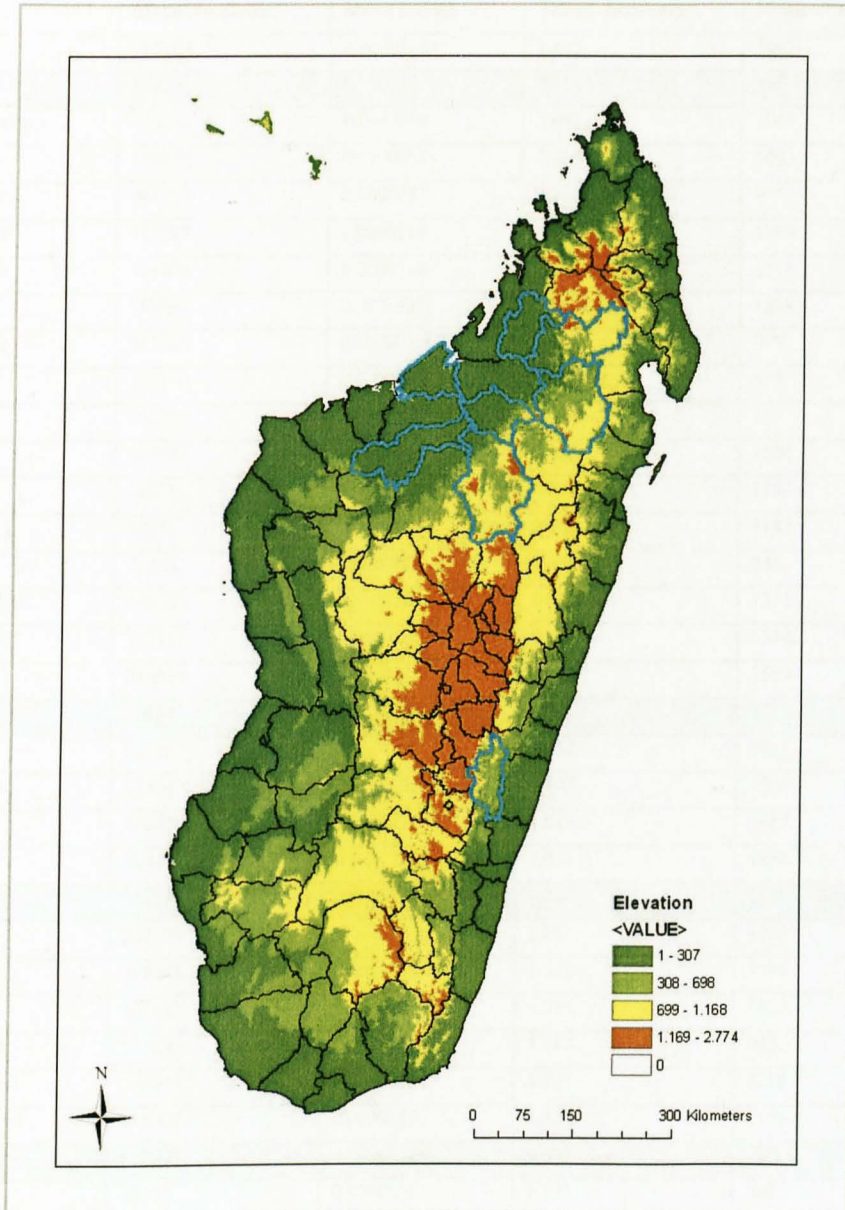
Table 3.2: Altitude, mean population, plague incidence, transformed incidence and altitude of districts in Madagascar. Districts in light blue have reported plague cases between 1975 and 2008

District	Mean population	Mean incidence	Transf. incidence	Mean altitude
Ambalavao	155464	0.0000388	1.687	1042
Ambato-Boina	97137	0.0000005	0.022	146
Ambatofinandrahana	94963	0.0000618	2.686	1041
Ambatolampy	166192	0.0000061	0.265	1603
Ambatondrazaka	204750	0.0000037	0.162	995
Ambohidratrimo	181589	0.0000219	0.950	1289
Ambohimahasoa	161409	0.0000319	1.386	1276
Ambositra	187463	0.0001438	6.243	1338
Amparafaravola	181556	0.0000018	0.080	907
Andapa	136190	0.0000024	0.104	874
Andilamena	35075	0.0000319	1.387	874
Andramasina	107341	0.0000005	0.023	1508
Anjozorobe	112116	0.0000688	2.986	1182
Ankazobe	85637	0.0000499	2.165	1147
Anosibe	72463	0.0000093	0.402	849
Antananarivo-Nord	160330	0.0000128	0.555	1371
Antananarivo-Sud	225186	0.0000104	0.454	1314
Antanifotsy	214919	0.0000141	0.610	1659
Antsihihy	96164	0.0000003	0.012	171
Antsirabe Rural	257509	0.0000311	1.352	1715
Antsirabe Urban	123640	0.0000023	0.102	1506
Arivonimamo	200670	0.0000234	1.018	1487
Bealanana	89944	0.0000240	1.044	1244
Befandriana-Nord	151251	0.0000019	0.081	674
Betafo	232598	0.0000434	1.884	1093
Fandriana	155081	0.0000307	1.332	1504
Faratsiho	127473	0.0000233	1.013	1652
Fenoarivo-Centre	63535	0.0000234	1.017	985
Fianarantsoa Rural	352644	0.0000367	1.594	1218
Fianarantsoa Urban	118002	0.0000284	1.233	1175
Ifanadiana	112008	0.0000007	0.030	628
Ikalamavony	45939	0.0000275	1.193	840
Mahajanga Rural	121110	0.0001214	5.273	859
Mahajanga Urban	41772	0.0000054	0.237	250
Mampikony	75325	0.0000003	0.014	308
Manandriana	74203	0.0001475	6.406	1477
Mandritsara	182569	0.0000022	0.095	639
Manjakandriana	156343	0.0000497	2.160	1411
Marolambo	102366	0.0000003	0.011	809
Miarinarivo	139725	0.0000779	3.385	1260
Moramanga	185846	0.0000166	0.722	944
Soavinandriana	113509	0.0000749	3.252	1076
Tsaratanana	87208	0.0000411	1.787	761
Tsiroanomandidy	167038	0.0000485	2.107	886

3.3.3 Predictors for occurrence of human plague in a district

The final binary logistic regression model (Table 3.3) contains mean altitude and variables of the parameters normalised difference vegetation index (NDVI) and night land surface temperature (nLST) as well as day land surface temperature (dLST). The P-values of all included variables were highly significant ($p \leq 0.05$). All odds ratios are close to 1, suggesting that the significant variables show only small differences between the two groups: plague endemic and plague non-endemic districts. Model coefficients were positive and odds ratios were above 1.0 for four out of the five identified variables. The relationship between altitude and plague occurrence is quadratic and positively correlated to plague occurrence. Peak timing of the biannual dLST and nLST cycle and the proportion of variance in dLST explained by its biannual cycle are positively correlated to the 'plague-endemic' category. Only the NDVI variable correlated negatively with the 'plague-endemic' category indicating that a later peak timing of the triannual vegetation cycle decreases the probability of plague occurrence in a district. The results are listed in Table 3.3.

The P-value of the Hosmer-Lemeshow goodness-of-fit test was $P=0.977$, indicating an adequate model fit; this was supported by a sensitivity of 88.64% (88.64% of plague-endemic districts were correctly labelled as such) and a specificity of 96.77% (96.77% of plague-non-endemic districts were correctly labelled as such) of the model prediction. The model correctly classified the occurrence of human plague in a district 93.4% of the time. The Kappa value, a measure of the proportion of all possible cases of plague presence or absence in a district that are predicted correctly by the model after accounting for chance effects was 0.863 at a 95% significance level. Nevertheless, spatial autocorrelation between districts was indicated when mapping the deviance residuals of the model (Figure 3.5). For this colours were used to assign the deviance residual of each district according to its value.

Figure 3.5: Distribution of the deviance residuals per district computed with a binary logistic regression model. Each colour represents a range of values each individual deviance residual was assigned to in order to detect similar values.

Deviance Residuals

-0.537006
-0.537005 - 0.000000
0.000001 - 0.866414
0.866415 - 2.056839

0   90   180   360 Kilometers
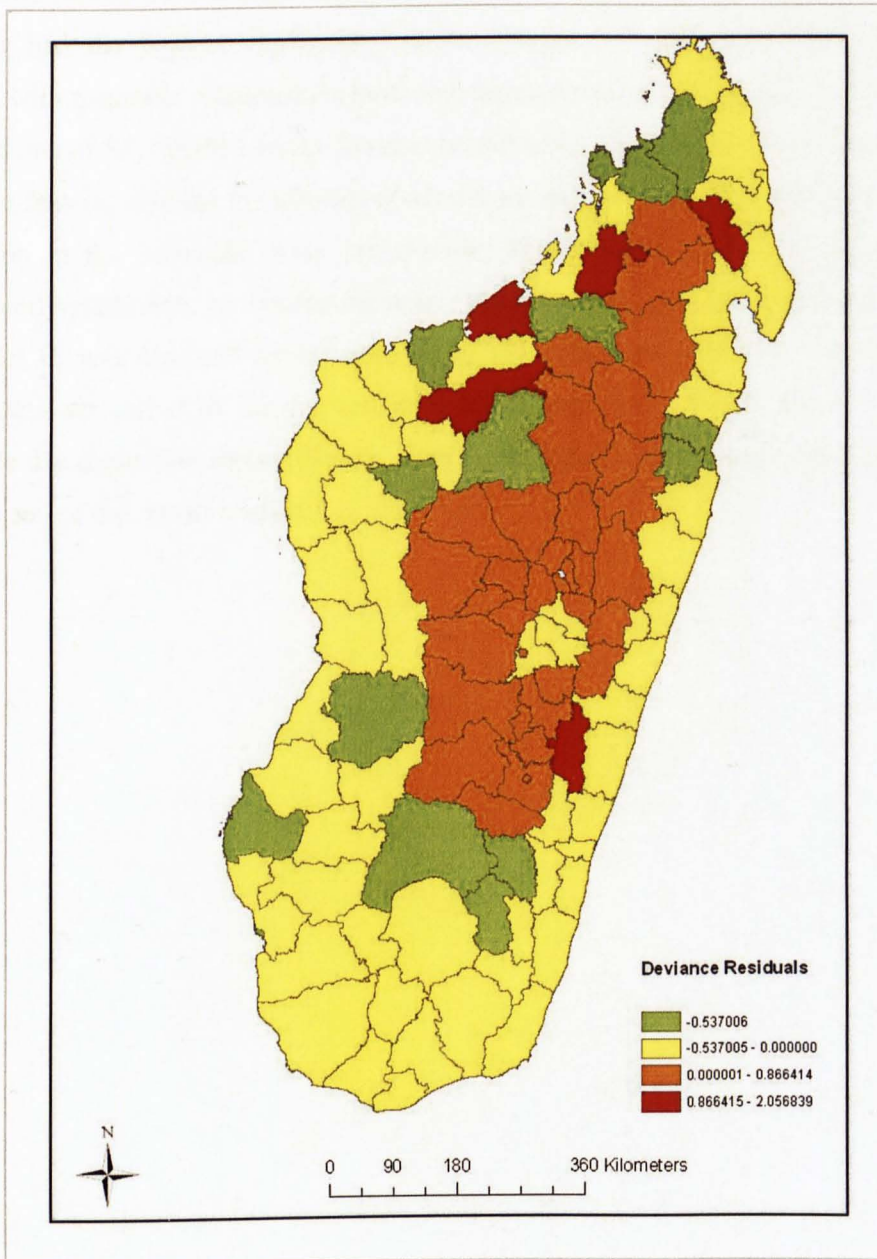
The logistic model built with the MaxEnt algorithm, however, confirmed the results of the binary logistic model, with the same variables identified as main drivers. The coefficients showed differences but matched the binary model in sign. Mean altitude and the NDVI parameter had the highest explanatory power (Figure 3.7 and 3.8). The features were identical, with quadratic relationships between plague occurrences and mean altitude, NDVI, nLST and one dLST variable. In the MaxEnt model mean altitude was additionally identified as a hinge feature, leveling off after its quadratic increase (Figure 3.7), thus providing more information in the threshold value for altitude. Mean altitude was also suggested to be independently predictive, containing the most information not present in the other variables. The model fit was assessed by the area under the curve (AUC) which was 0.79 for the training data set and 0.74 for the test data set (Figure 3.6). Values from 0.70 indicate reasonable discrimination ability (Swets, 1988). The complete results are described in Table 3.5 at the end of the results section.
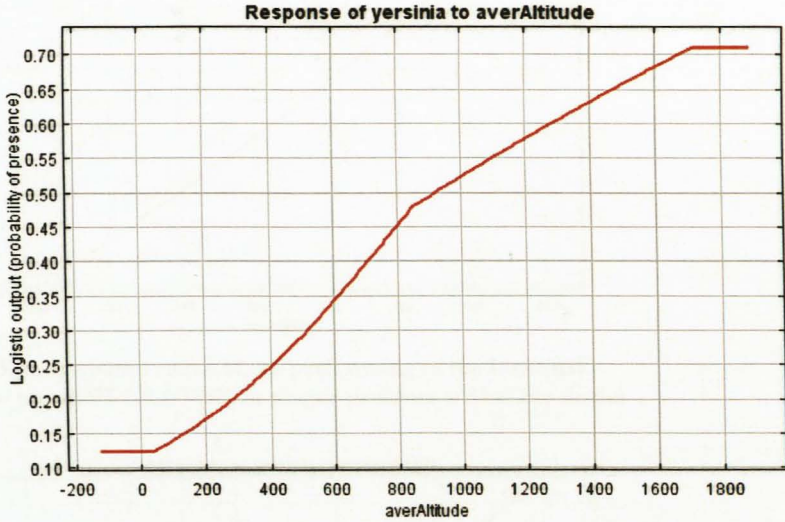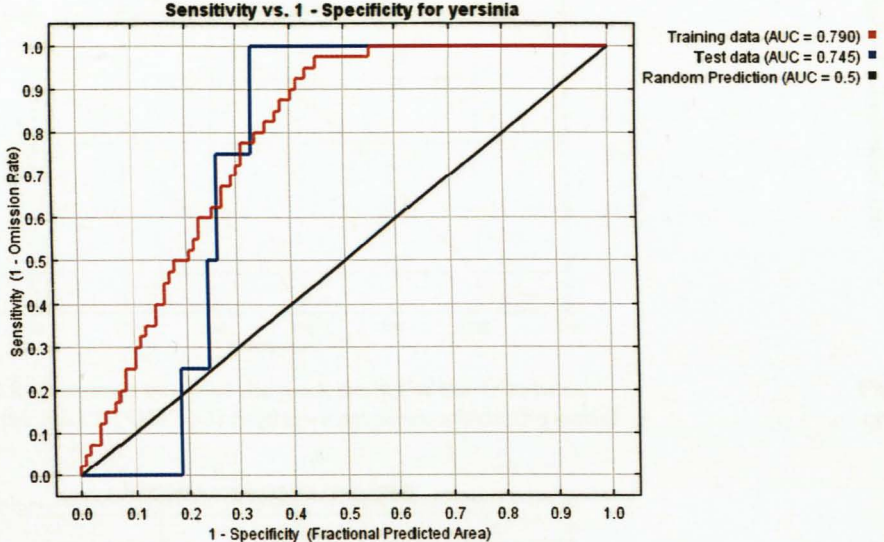
Figure 3.6: Receiver operating characteristic (ROC) curve for the plague occurrence model indicating its sensitivity and specificity
The specificity is defined using predicted area, rather than true 'commission' (accuracy) error (1-specificity = false positive rate representing 'commission' error; Phillips et al., 2006)
x-axis: 1-Specificity = false positive rate
y-axis: Sensitivity = 1 – wrongly predicted rate

Figure 3.7: Response curve of mean altitude to plague occurrence data within the model
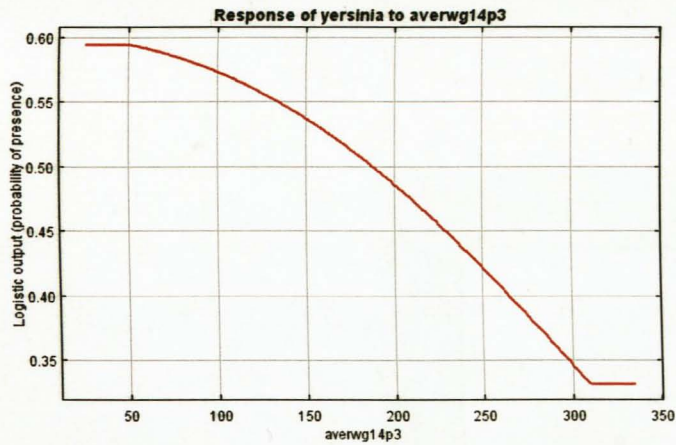
Figure 3.8: Response curve of the peak timing of the triannual cycle of the NDVI (NDVI p3) to plague presence within the model
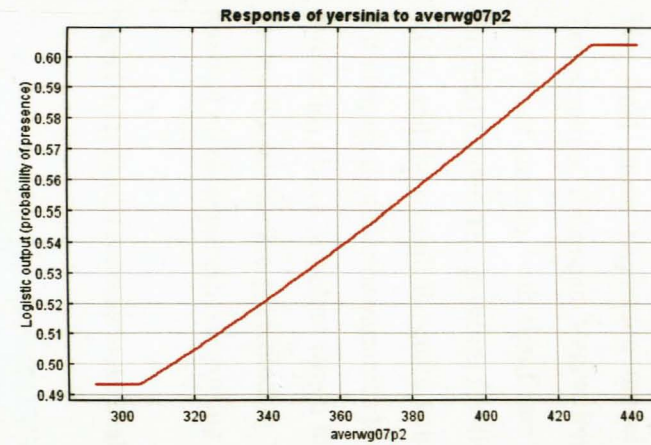
Figure3. 9: Response curve of the peak timing of the biannual cycle of the dLST (dLST p2) to plague presence within the model
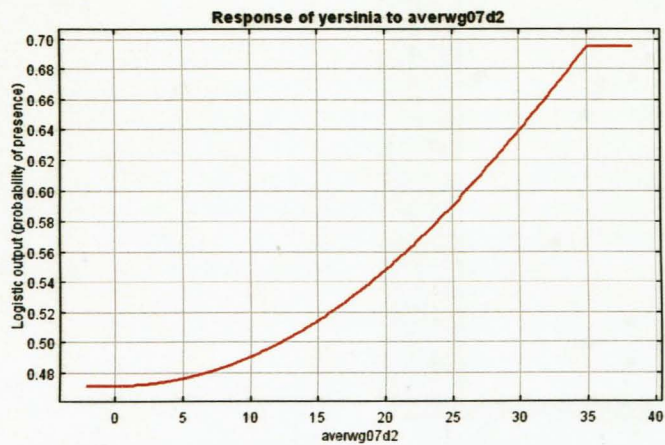
Figure 3.10: Response curve of the proportion of variance explained by the biannual cycle of dLST (dLST d2) within the model
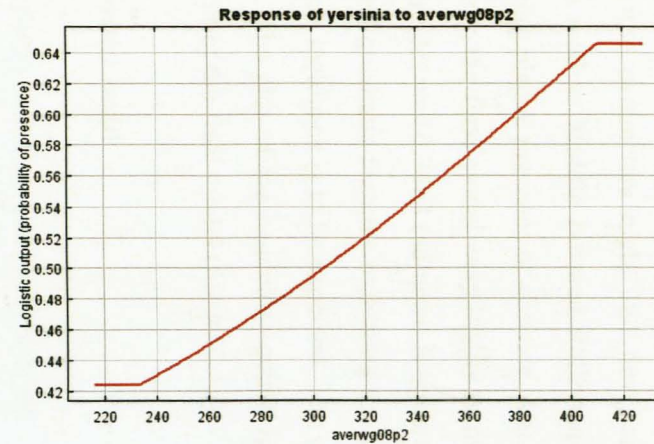
Figure 3.11: Response curve of the peak timing of the biannual cycle of the nLST (nLST p2) to plague presence within the model

3.3.4 Predictors for the plague risk in a district

A linear regression model was built to establish if the environmental variables could be used to predict the magnitude of human plague incidence of each district within the plague-endemic group.

The final linear regression model contains the parameters night and day Land Surface Temperature, Enhanced Vegetation Index as well as Middle Infrared Reflectance. Neither NDVI nor altitude, both significant in predicting plague presence or absence, was retained in the final model. The p-values for all variables in the final model were highly significant ($P \leq 0.05$), and the overall R-square value of the model was 0.7463 (adjusted R-square= 0.6791). All identified predictor variables had linear and quadratic features indicating the existence of thresholds above or below which higher plague incidence is to be expected. The coefficients for each variable are presented in Table 3.4. The plot of the residuals against fitted values showed no clear pattern, supporting the good fit of the final model (Figure 3.12).

Figure 3.12: Plotted residuals of the final linear regression model
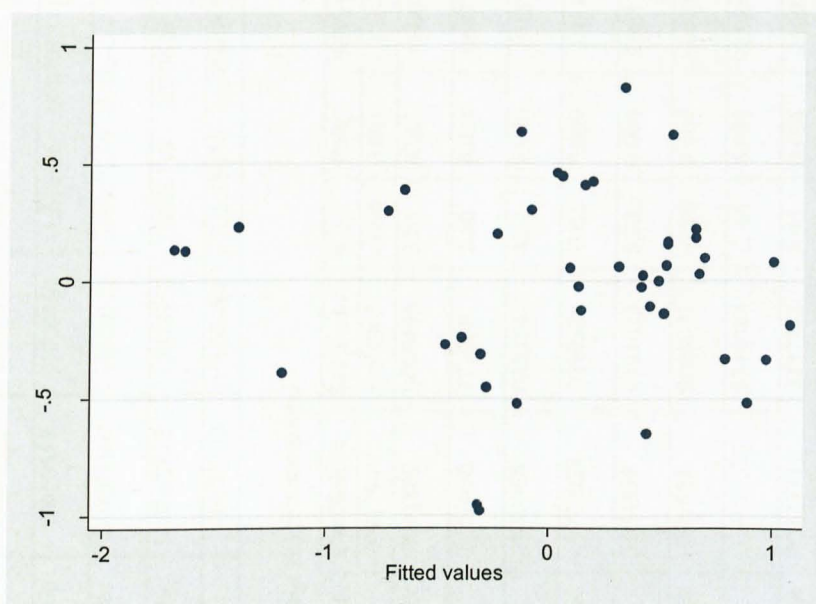
Table 3.3 Results of the binary logistic regression

Variable	Feature	Coefficient	Std Error	95% Conf. Interval		z	P>|z|	Odds Ratio	Std Error	95% Conf. Interval	
Mean altitude	quadratic	9.3e-06	2.45e-06	4.50e-06	.0000141	3.80	0.000	1.000009	2.45e-06	1.000005	1.000014
Average dLST p2	linear	.0283687	.0133456	.0022118	.0545257	2.13	0.034	1.028775	.0137297	1.002214	1.05604
Average nLST p2	quadratic	.0000514	.0000254	1.63e-06	.0001013	2.02	0.043	1.000051	.0000254	1.000002	1.000101
Average NDVI p3	quadratic	-.0001199	.0000572	-.0002319	-7.78e-06	-2.10	0.036	.9998802	.0000572	.9997681	.9999922
Average dLST d2	quadratic	.0042872	.0018752	.0006119	.0079626	2.29	0.022	1.004296	.0018833	1.000612	1.00794
Constant		-14.97333	5.60388	-25.95674	-3.98993	-2.67	0.008				

Table 3.4 Results of the linear regression model

Variable	Feature	Coefficient	Std Error	t	P>|t|	95% Conf. Interval	
Average MIR a2	linear	-.0717949	.0194965	-3.68	0.001	-.1114164	-.0321733
Average MIR a2	quadratic	.0001889	.0000496	3.81	0.001	.0000881	.0002896
Average MIR d2	linear	.3156656	.121508	2.60	0.014	.0687316	.5625995
Average dLST d2	linear	.3680068	.086814	4.24	0.000	.1915796	.544434
Average dLST d2	quadratic	-.0074938	.0019328	-3.88	0.000	-.0114217	-.0035658
Average nLST d1	linear	.1554398	.0291623	5.33	0.000	.0961749	.2147047
Average nLST d1	quadratic	-.001523	.0003071	-4.96	0.000	-.0021472	-.0008989
Average EVI d3	linear	-1.919053	.5244084	-3.66	0.001	-2.984779	-.8533271
Average EVI d3	quadratic	.7311711	.2387242	3.06	0.004	.2460252	1.216317
Constant		-3.123993	.9695764	-3.22	0.003	-5.094409	-1.153577

Table 3.5 Results of the MaxEnt logistic model

Variable	Feature	Coefficient
Average altitude	quadratic hinge	9.016775222 -0.319014010
Average NDVI p3	quadratic	-1.082108818
Average dLSTp2	linear	0.6686996196823227
Average dLSTd2	quadratic	0.9375927181076504
Average nLSTp2	quadratic	0.8755972102210985

3.4 DISCUSSION

Previous studies have identified spatial variation of human plague incidence in Madagascar (Chanteau, 2006; Ratsitorahina et al., 2000), but the environmental factors associated with the distribution of the disease on the island are poorly understood. An altitudinal threshold above which plague is present as well as a temperature threshold has been implicated by various studies before. However, this was based on the conditions recorded at the individual sites investigated. This study represents a first investigation into remotely sensed environmental drivers affecting the spatial distribution and transmission risk to humans. Remotely sensed data provides an alternative to using incomplete empirical datasets. The information gained can then be used to identify areas suitable for disease persistence or potentially at risk from disease emergence now or under future conditions.

This study demonstrates that both the presence and incidence of plague can be modelled based on remotely sensed environmental variables.

Presence and absence of human plague
The binary regression model as well as the Maximum Entropy model identified altitude, land surface temperature and the NDVI as significant drivers for plague presence. For the spatial distribution of human plague in Madagascar results indicate that particularly altitude is a major factor predicting the presence or absence of plague in a district.

Night and day Land surface temperature (nLST and dLST) are both positively correlated with the presence of plague. In both the binary regression model and the Maximum Entropy model the peak timing of the biannual cycle of the temperature variables is important. Additionally, in the Maximum Entropy model the proportion of variance explained by the biannual cycle of the dLST is also significant.

This information implies that it is important at what time of the year the highest and likewise lowest temperatures occur as well as how well the temperatures follow the predicted seasonal means. So an unusual early onset of a season as well as an unexpected hot or cold period out of season is favourable for plague.

The peak timing of the triannual cycle of the NDVI however, is negatively correlated with plague presence suggesting that the timing of extensive plant growth as represented by the NDVI may influence the epidemiology of plague.

Altitude is positively correlated with plague presence in districts showing that higher districts are more likely to report human plague cases. The findings on the importance of altitude are concurrent with the plague data from the last 50 years showing the majority of districts affected by plague in Madagascar to be in the highlands (Chanteau, 2006). The results are also in line with plague distributions in other endemic areas of the world. In Tanzania, Uganda and Vietnam, for example, plague occurs mainly in the highlands (Neerinckx, 2010; Pham, 2009; Winters et al. 2009) and in the western United States the suitability for plague increases with altitude (Eisen et al. 2007). In a similar study by Neerinckx (2010), elevation and vegetation cover derivates have been found to be the most important drivers for human plague occurrence in Tanzania. The majority of the affected districts in Madagascar, 42 out of 44, either have an average elevation of between 693 and 1715 meters or have some mountainous areas (Figure 3.4 and 4a). The presence of plague in the remaining 2 districts, however, particularly the coastal district of Mahajanga, does not correspond with the theory of an elevation threshold and needs further investigation. It is well known that during large pandemics, plague frequently arrived in ports and proliferated in coastal areas before retreating in many places to the highlands. Differences in the epidemiology of plague in the port city of Mahajanga where most cases of this district originate have been previously suggested (Boisier et al. 1997; 2002; Chanteau et al. 1998; Duplantier et al. 2005). So far, however, the reasons why plague resurfaced in the city after 62 years of silence remain unknown.

The results show non-linear relationships between plague presence and altitude, NDVI and day and night land surface temperature suggesting the existence of thresholds above which the conditions are favourable for human plague. The relationship between altitude and plague presence also shows a hinge feature (a leveling-off of the slope) at 820m. The significance of these results for the epidemiology of plague is certainly manifold. This altitudinal threshold for example coincides with the distribution limits of one of the two main vector species for plague as established by Klein et al. in 1966. First studies on the effect of temperature on the larval development of the main flea-vectors propose the existence of temperature thresholds

(chapter five) as found for many flea species (Krasnov et al. 2001). Part of this is due to temperature which varies with altitude and is an important factor in the development and survival of fleas, with different species requiring different conditions for their long-term viability (Krasnov et al. 2001; Sharif 1949). In Madagascar seasonal changes in vector abundance have been observed in the plague-endemic region so it is not surprising that the variables identified as significant for the presence of plague are the biannual and triannual peak timing of temperature and the NDVI.

Yet anthropogenic characteristics represented by environmental variables could also play a role and are discussed in a later paragraph.

Differences in plague prevalence between plague-districts
The model predicting the incidence of plague in affected districts is highly temperature driven with 7 out of 9 variables based on night/day Land Surface Temperature or Middle Infrared Reflectance (another variable describing surface temperature patterns). All variables show a quadratic relationship with plague prevalence indicating the existence of thresholds above which plague incidence increases. The correlation for the proportion of variance of day and night land surface temperature explained to plague incidence is negative suggesting that temperature irregularities increase plague risk within the plague endemic areas. This links in with the MIR variables, both of which are positively correlated to plague incidence as these variables describe the amplitude of temperature, so the more extreme the temperatures the more plague incidence is likely.

The remaining variable identified belongs to the enhanced vegetation index (EVI) parameter. While the EVI is calculated similarly to the NDVI, it gives a more detailed picture of the vegetation cover as it does not become saturated as easily as the NDVI when viewing rainforests and other areas with large amounts of chlorophyll.
Results suggest that vegetation cover characteristics of a plague-endemic district seem to affect its plague incidence. The relationship between the variable and plague is quadratic suggesting a threshold. The more variance of EVI is explained by the triannual cycle the higher the plague incidence. This may partly be due to changes in the resource availability of rodent hosts as found by other studies (Ben Ari et al. 2010; Parmenter et al. 1999), making domestic rodents more reliant on human settlements and hence increasing transmission

potential. It has also been found that vector species composition and abundance is seasonal in plague-endemic regions (chapter four) which might be linked to changes in temperature as well as vegetation cover. The vector and host distributions will be affected strongest by the variables identified here.

Altitude was not identified as a driver of varying plague incidence implying that above a certain elevation threshold, differences in altitude may not be important.

Identified variables as proxy for other characteristics

How the variables identified as determinants for plague occurrence and incidence affect its complex epidemiology is not completely understood. Seasonal temperature cycles and variations not only affect vectors directly but also influence human behaviour and altitude may serve as a proxy for other not identified variables. The results have to be viewed in a wider context including the possibility of anthropogenic factors simply being reflected by certain environmental variables. Possible variables include farming practices, housing, regional traditions and transmission pathways governed by host and vector abundance and distribution. The peak timing of temperatures and their variation throughout the year as well as other factors affecting plant growth will affect crop choice and harvest times. Altitude too is most likely a proxy for one or more unknown variables of the epidemiology of plague. Work on the agro-climatic characterization of Madagascar shows that not only are different crops cultivated in the highlands compared to the lowlands, but that the seasonal availability of plants also varies (Oldeman, 1990; Rasambainarivo, Ranaivoarivelo, 2003). This has an implication for transmission pathways via resource availability and foraging behaviour of rodent hosts and therefore plague infection risk for rural inhabitants as domestic rodents will enter houses in search of food. Differences in socio-economic factors and landscape patterns providing connections between the sylvatic plague foci and the domestic rodent hosts around villages also have to be considered.

The existence of an exact altitudinal threshold however, indicated by the quadratic relationship between plague presence and altitude is most likely the result of the low resolution of the data used. This arose through the necessity to use the 'mean altitude of each district' as well as the occurrence of plague cases in areas below 800m. Yet this only

concerns the existence of an exact threshold and does not contradict the importance of altitude in general.

Limitations and future research

As many of the results of this study resemble findings of the similar plague focus in Tanzania, it would be interesting to see if parallels between the two foci exist and if conclusions can be made through data combination and comparison.

These studies suggests that satellite data can be used when empirical data is lacking and a statistical approach represents a reasonable method of revealing patterns in large scale epidemiological processes which may have been overlooked when concentrating on small scale studies. It also furthers the understanding of the factors involved in the relationship between human plague and the environment, and hence may help to direct further resources at suitable research questions.

As Eisen et al. (2011) remarked model predictions are only as good as the data on which they are based. Increased accuracy is usually achieved using disease incidence and environmental data at a high spatial resolution. The main limitation of this study is its coarse spatial-scale due to the availability of data on human plague. As population estimates for Madagascar are only available at district level, all data was averaged over each district. This reduced local variation and detail in the environmental variables. Additionally, the districts of Madagascar vary greatly in size and show extreme environmental gradients and highly varied climate regions. Different levels of detail, depending on the size of the district were thus incorporated in this analysis. Therefore, an effect on the model fit had to be considered and the validity of any spatial-autocorrelation questioned before assuming an underestimation of coefficient variances and incorrect parameter estimates (Anselin, Bera, 1998). The subsequently analysis undertaken using MaxEnt, software not hampered by inter-dependent variables, confirmed the original estimates, increasing trust in the findings.

CHAPTER FOUR

FIELD STUDY INTO THE EFFECTS OF CLIMATE ON FLEA VECTORS IN THE HIGHLANDS OF MADAGASCAR

ABSTRACT

The seasonal variation and spatial distribution of various vector-borne diseases are known to be influenced by climate (Lafferty, 2009; Rogers, Randolph, 2006; Stenseth et al. 2006) and in many plague-endemic areas of the world, the disease shows specific spatial patterns in its distribution often linked to vector ecology (Haas, 1965; Hubbart et al. 2011; Krasnov, 2002). The vector ecology for Madagascar is unique, due to the presence of an endemic flea vector *Synopsyllus fonquerniei* (*S. fonquerniei*) found only in the highlands (Duchemin et al. 2001) where it is one of the two main vector species. Its presence may affect the spatial distribution of the disease and impact on the seasonality of human plague. Here we explored the micro-climate inside rat burrows, variations in flea vector abundance and species composition to facilitate understanding of the effects of climate on the flea whilst in the host burrow, and therefore indicate whether it could act as a mechanism by which climate might affect plague risk to humans.

Substantial differences in abundance and the impact of climate on abundance variation were established between the two main plague vectors in Madagascar. The vector species differed in their abundance patterns through the year and showed species specific sex ratios and differing temporal variations in age distribution. The findings suggest that the micro-climate favourable for the development and survival of *Xenopsylla cheopis* (*X. cheopis*) can best be provided in rodent burrows which are located inside houses; while outdoor burrows in the highlands support the endemic *S. fonquerniei*. The conditions in the preferred habitat of each species' developmental stages were found to be distinct from each other. An assumption could therefore be made that the abundance of both vector species is limited by lower temperature thresholds and possibly also by upper humidity thresholds.

In terms of the temporal and spatial distribution of human plague cases in Madagascar, the results imply climate allows plague vectors for the maintenance of the plague cycle to be present in fairly high numbers throughout the year. The peak abundance time for *S. fonquerniei* coincides with the beginning of the human plague season, whilst *X. cheopis* are at relatively high abundance throughout much of the rainy season until the human plague season ends in March.

4.1 INTRODUCTION

The incidence of, seasonal variation in, and global distribution of various vector-borne diseases are known to be influenced by changes in climate (Lafferty, 2009; Rogers, Randolph, 2006) and plague is no exception (Stenseth et al. 2006). In many plague-endemic areas of the world, the disease shows seasonality and specific spatial patterns in its distribution, and several past studies have investigated if such trends are linked to vector ecology (Haas, 1965; Hubbart et al. 2011; Krasnov, 2002).

Different flea species act as vectors of plague worldwide (Duplantier, 2005), and past work has established that the spatial distribution of such vector species can differ significantly even though the parasites share the same hosts and are found in overlapping habitats (Klein, Uilenberg, 1966; Krasnov, 1997). Studies have also frequently indicated seasonality in the abundance of fleas (Hubbart et al. 2011; Krasnov, Burdelova, et al. 2002), including flea species known to be important vectors of plague in Madagascar (Klein, 1966).

The vector ecology for Madagascar is unique, due to the presence of an endemic flea vector *Synopsyllus fonquerniei* (*S. fonquerniei*) found only in the highlands (Duchemin et al. 2001) where it is one of the two main rodent vector species. Its presence may affect the spatial distribution of the disease and impact on the seasonality of human plague, more than a single vector species alone. There is evidence that each vector species occupies a different environmental niche, with *Xenopsylla cheopis* (*X. cheopis*) mostly found on rats caught indoors (Eskey, 1938), and *S. fonquerniei* found on rats (of the same species) caught outdoors, away from houses (Brygoo, 1966; Duplantier, 2005). However, the reasons for these distinct spatial distributions remain to be established.

In other parts of the world, studies have shown that temporal and spatial variation in flea vector abundance and species composition are correlated to temperature and humidity inside host burrows (Figure 4.1; Cooke, 1988; Krasnov et al. 2001; 2002; Longanecker, Burroughs, 1952; Ryckman, 1971; Shenbrot, 2002). It is likely that this is because a large proportion of the flea life-cycle is spent off-host; egg, larval and pupal stages all take place inside the rodent burrow (Klein, 1966; Krasnov 2001; Sharif, 1949) where conditions are known to affect

development and survival (Margalit, Shulov, 1972; Metzger, Rust, 1999; Silverman, Rust, 1953).

Figure 4.1: Flowchart showing the interactions between climate, micro-climate and vector ecology
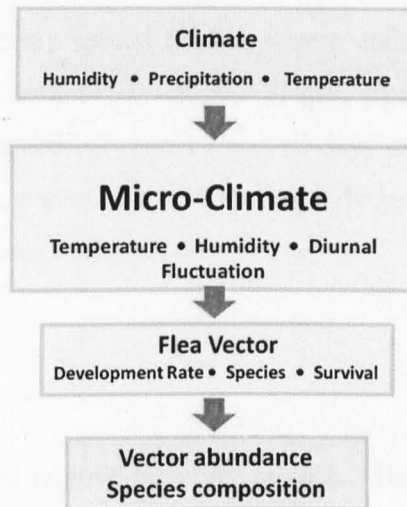
Given that as ectotherms, fleas are dependent upon ambient conditions, it is judicious, therefore, to explore 1) the micro-climate inside rat burrows found in and around villages within the plague endemic area of Madagascar; 2) variations in flea vector abundance and species composition. This would facilitate understanding of the effects of micro-climate on the flea vector whilst it's in the host burrow, and therefore indicate whether it could act as a mechanism by which climate would affect plague risk to humans. Research of this essential part of the flea habitat is scarce. One reason frequently given is that measuring instruments are likely to interfere with the micro-climate conditions by blocking airflow inside the burrow. We felt however, that the latest affordable temperature and humidity recording instruments are slim enough to minimise this possible source of bias.

Within this piece of work, we aim to assess the effect of ambient climate and burrow climate on temporal and spatial variation in the abundance of the two flea vectors. We will also wish to determine the population structure of both species.

4.2 METHODS

4.2.1 Study area

The study was conducted in the central highlands of Madagascar (Figure 4.2), within the plague endemic area (Figure 4.3) in the districts Antsirabe (region A) and Betafo (region B) (Figure 4.4). In 2009 five evenly spaced fieldtrips were undertaken in January, April, July, September and November. A total of 20 remote villages, 10 in region A and 10 in region B were visited. Each fieldtrip lasted between 15 and 18 days during which data was collected from 4 villages, 2 from each region (Table 4.1). All villages were geo-referenced for their later visualisation using mapping software.

4.2.2 Data collection

Climate data

To record air temperature and relative humidity in each village, a numbered Tiny Tag data logger (Tiny Tag Plus 2, TGP-4500) which had been fixed to a square wooden platform (10cm^2) with a small roof, was used. Each platform was mounted on top of a wooden stake which had been hammered into the ground (Figure 4.5). These 'Tiny Tag weather stations' were positioned within open spaces in villages, so that the ambient temperature and humidity at a height of approximately 1m was recorded. One Tiny Tag weather station was established in each village for 12 nights. Every 30 minutes the stations recorded the temperature in degree Celsius (°C) and the relative humidity (RH %) with a typical accuracy of ± 0.5 °C and ± 3 RH%, respectively (Tiny Tag datasheet 2011, Gemini data loggers). The exact date and time of the positioning was noted as well as the date and time of removal.

The micro-climate inside rat burrows was recorded using numbered Easy Log data loggers (EL USB-2). These were inserted at a standard depth of 20cm and fitted comfortably into an average sized rat burrow. A wire cage was used to protect each device from dirt and damage by rats, with each instrument securely fastened to a wire for easy positioning and retrieval (Figure 4.6). Specific climate drivers that were recorded were temperature, relative humidity, diurnal temperature range (DTR) and diurnal humidity range (DHR). Temperature and relative humidity were recorded every 30 minutes, with a typical accuracy of ±0.5 °C and ±3 RH%, respectively (Easy Log USB-2 datasheet 2010, Lascar Electronics). Each burrow was

numbered and the exact date and time of the positioning of the logger was noted as well as the date and time of removal.

At the start of each fieldtrip the first two villages were visited and four 'fixed' easy loggers per village were inserted into rat burrows to record the micro-climate for a total of 6 nights. Two of these loggers were inserted into burrows found outside (exterior burrows) and two were placed into burrows found inside houses (interior burrows). After 6 nights, the 'fixed' loggers were moved to the next two villages and the data recording procedure was repeated. Additionally, four 'mobile' easy loggers were used to record the micro-climate in other randomly selected rat burrows in each village, thus increasing the sample size; however, these loggers were deployed in one village at a time for logistic reasons. These four 'mobile' loggers were inserted into novel burrows every 24 hours, for a total of 3 nights in each village. Two were always inserted into interior burrows while the other two were placed into exterior burrows. The exact date and time of the positioning was noted as well as the date and time of removal.

Figure 4.2: Elevation profile map of Madagascar
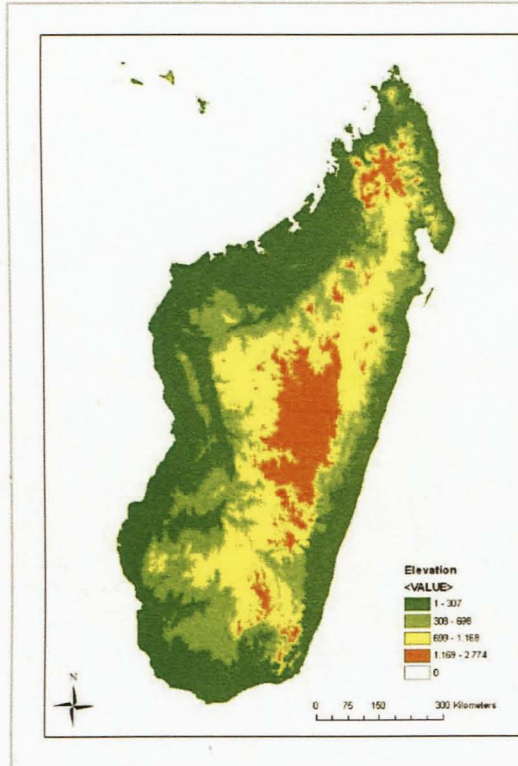
Figure 4.3: Yearly mean human plague incidence per district in Madagascar
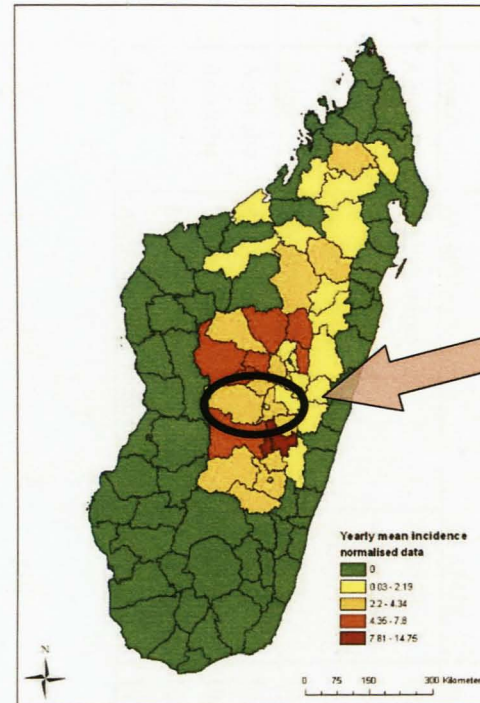
Figure 4.4: Fieldsites in the districts Antsirabe and Betafo in Madagascar
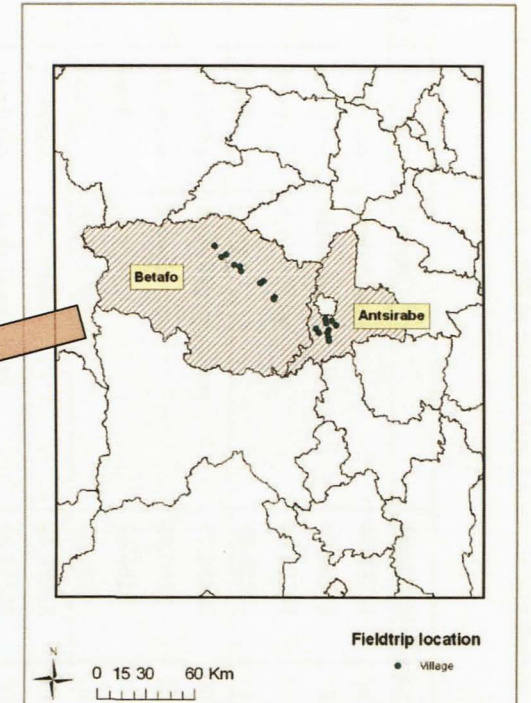
Table 4.1 Study sites visited in 2009 in the districts Antsirabe and Betafo

Fieldtrip	District	Village	Longitude	Latitude	Altitude	Plague
Jan-09	Antsirabe	Ambohitsoa	47.04711	20.01658	1374	Y
Jan-09	Antsirabe	Maharivo	47.00375	20.3451	1398	N
Jan-09	Betafo	Ambatolahy	46.53391	19.65906	1018	Y
Jan-09	Betafo	Ivory	46.42568	19.5537	955	Y
Apr-09	Antsirabe	Ambohitrimanjato	47.04036	20.05592	1354	Y
Apr-09	Antsirabe	Mahamavo	47.0454	20.07666	1376	Y
Apr-09	Betafo	Mazoto	46.4615	19.61561	992	Y
Apr-09	Betafo	Ambohitromby	46.48744	19.5994	1041	N
Jul-09	Antsirabe	Soalafadray	46.98958	20.0315	1537	N
Jul-09	Antsirabe	Ambohimandroso	46.97588	20.0105	1613	Y
Jul-09	Betafo	Ambohimahasoa	46.69043	19.7472	1292	Y
Jul-09	Betafo	Ambohipeno	46.67416	19.76093	1351	N
Sep-09	Antsirabe	Ambohitraivo	47.06586	19.96615	1457	N
Sep-09	Antsirabe	Ampandrotrarana	47.08729	19.99278	1538	Y
Sep-09	Betafo	Ambohimasina	46.7517	19.83855	1545	Y
Sep-09	Betafo	Maromanana	46.74791	19.8478	1599	Y
Nov-09	Antsirabe	Ambohipeno	47.02633	19.95782	1460	Y
Nov-09	Antsirabe	Soaraikena	47.03254	19.98171	1489	Y
Nov-09	Betafo	Malaza	46.56112	19.66603	1184	Y
Nov-09	Betafo	Andratsaimahamasina	46.56896	19.69133	1116	Y
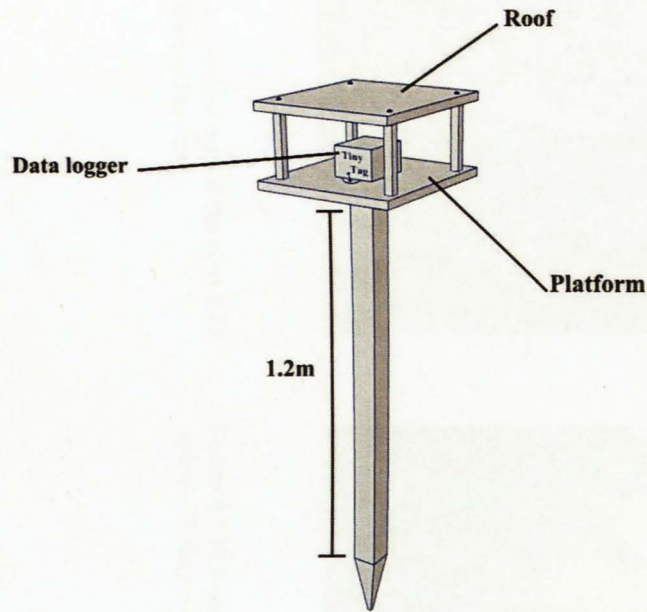
Figure 4.5: Weather station used to record ambient temperature and humidity
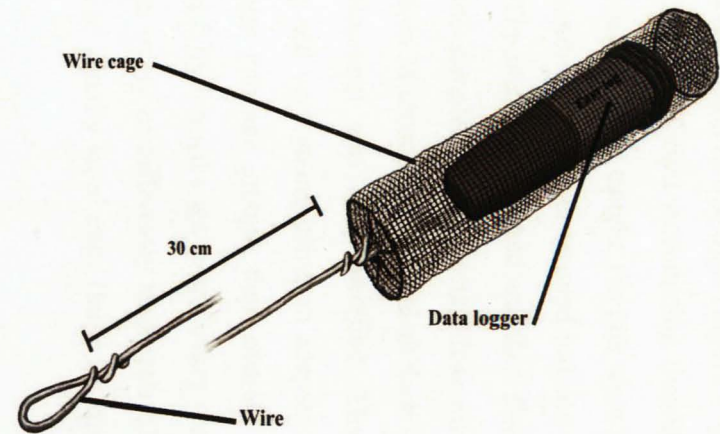
Figure 4.6: Data logger in protective cage recording temperature and humidity inside rat burrows

Host and vector

During each fieldtrip, rats were caught in live traps (Figure 4.7a) that were positioned in three different habitats: houses, hedges of sisal surrounding livestock enclosures or villages and farmed fields adjacent to villages. Once caught, the rats were numbered, identified to species level and their trapping location noted; other trapped rodents such as mice and shrews were not included in this study. The rats were brushed and their fleas collected with an aspirator and counted. A representative sub-sample of the collected fleas was dissected under a microscope (Leica Zoom 200, Stereozoom Microscope) to establish their species and sex. For all female fleas their stage of development was also specified. Three development stages were distinguished: adult, young and very young using an adapted method by J.M. Klein (Klein, 196). To categorise the fleas into age groups, their ovaries were extracted and examined (Figure 4.7b). Fleas without fully formed eggs in any ovary were classed as very young, fleas with only one large egg per ovary or differently sized sets of smaller eggs were classed as young and fleas with a set of equally sized eggs (large or otherwise) were classed as adult females.

Figure 4.7 a) Live rodent trap of the type BTS (Besançon Technique Service)

Figure 4.7 b) Extracted flea ovary of an adult female *S. fonquerniei*

Soil samples

To exclude a possible bias of differences in soil composition between regions, soil samples were collected during the first field trip. Samples were taken from the mouth of a total of 40 rat burrows (10 per village) in four different villages (two from each region). They were immediately weighed, sealed in plastic bags and later analysed by the Laboratory for Radioisotopes in Antananarivo. The samples were tested for their proportional content of clay, silt, coarse silt, sand and coarse sand as well as their humidity.

4.2.3 Data preparation

Climate data

Data from the loggers was downloaded using the appropriate software (Tiny Tag Explorer 4.6 and EL-WIN-USB 4.6). It was assumed that ambient conditions did not change significantly during each fieldtrip, and data from all time-points collected over a total of 12 days was therefore analysed as one continuous day (for an example see appendix, Table A4.1). The climate data from each burrow and weather station was arranged into 24 hour blocks and divided into 'above ground climate data', 'interior burrow data' or 'exterior burrow data'. Data rows with missing records due to insertion times after 8am or problems associated with the logger were completed, where possible, using data records from the same burrow. Only complete data rows (corresponding to 24 hour blocks) were used for analysis to avoid oversampling of a certain time of day. The mean as well as the diurnal temperature range (DTR) and diurnal humidity range (DHR), over a 24 hour period were calculated for each row for temperature and humidity respectively (Appendix, Table A4.2 a and b). Temperature and relative humidity data was processed separately for each burrow, weather station, village, region and fieldtrip. The 5 fieldtrips were numbered 1 to 5 and each village was assigned to region A or region B.

Rat and flea data

Rat and flea abundance data and information on flea species, sex and development stage were processed separately for each village and fieldtrip. Rats were assigned to the habitat where they were caught, indoor or outdoor and the number of rats in each habitat with fleas was recorded. The total number of fleas collected of each species was calculated for each village.

For each separate flea species, dissected fleas were sorted into male and female and all females were categorised as very young, young or adult.

4.2.4 Data analysis

Differences between ambient climate and burrow climate

We hypothesised that (1) the micro-climate in burrows is significantly different from ambient climate conditions and (2) the micro-climate between burrow types also differs significantly. Temperature and humidity data were analysed independently.The data on climate was subdivided according to regions and villages (Figure 4.8a). The micro-climate was then subdivided again according to burrow type (interior or exterior) (Figures 4.8 b).

Figure 4.8 a: Data on ambient climate and micro-climate divided into two subsets according to region and villages
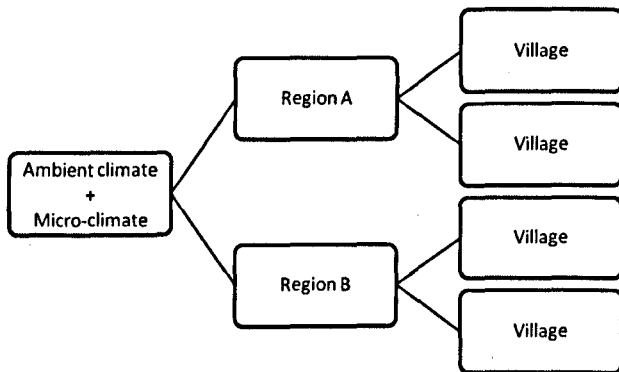
Figure 4.8 b: Data on burrow micro-climate divided into two subsets according to burrow type
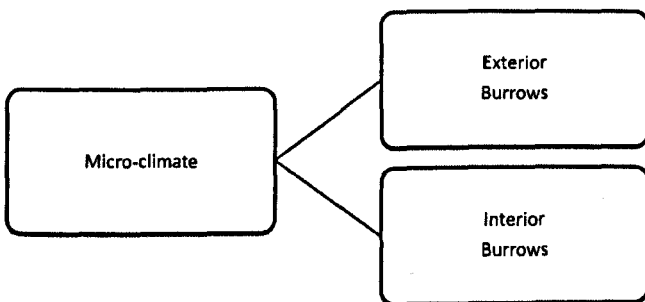
The distribution of all climate datasets was checked using an Anderson Darling test. Statistical differences between datasets were established using student t-tests for normally distributed and Mann-Whitney U tests for non-normally distributed data when comparing two datasets. When comparing groups, a Levene's test compared within-group variance before a one-way ANOVA was used. The significance level for all statistical tests was set at 0.05.

Soil

The aim was to establish if there are significant differences between regions in the clay and humidity content, both of which might affect burrow humidity (Dr. Alain Albrecht, pers. communication, 2009). The ratio of components of the soil samples from the two different regions was compared using an ANOVA test followed by Fisher's individual error rate to ascertain validity.

Flea species biology

A comparison of the sex ratio, abundance and female age distribution of fleas between fieldtrips was undertaken. Significant differences between species were established using a student's t-test or Mann-Whitney U test where appropriate.

The effect of climate on the variation in flea abundance

Three hypotheses were tested:

- Climatic factors explain temporal and spatial variation in the abundance of fleas.

- The effects of climate vary between the two flea species.

- The microclimate within burrows is more strongly related to flea abundance than ambient climatic conditions.

For *X. cheopis* which is mostly found on rats caught in houses, a flea index was calculated based on the total number of *X. cheopis* found on house rats divided by the number of house rats. For *S. fonquerniei*, which is mainly found on rats caught outdoors, the flea index was based on the number of *S. fonquerniei* found on rats trapped outside divided by the number of exterior rats.

Generalised linear models were used to investigate factors influencing variation in vector abundance. For *S. fonquerniei*, negative binomial generalised linear models were used to model the flea index. The total number of *S. fonquerniei* found on exterior rats was the response variable and the number of exterior rats was used as an offset. High levels of overdispersion within the *X. cheopis* dataset prevented this approach. The dataset was heavily affected by clustering of fleas at the individual host level and information on vector abundance for *X. cheopis* was therefore obtained by taking the proportion of rats carrying *X. cheopis* caught indoors of the total number of rats caught indoors. Consequently, the proportion of house rats with *X. cheopis* (i.e. rats caught in houses with ≥1 *X. cheopis*) was modelled using a quasibinomial distribution.

For both flea species four analysis stages were carried out.

Stage1

At the first stage, the temporal and spatial patterns in flea abundance for each species were modelled. Where initial examination of the data suggested a clear and simple seasonal cycle, sin and cosine waves based on the number of days since the start of 2009 were used to model temporal variation in abundance, and results were compared to temporal variation modelled using field trip number as a categorical variable. In both cases, region was added to account for spatial variation.

Stage 2

To test the hypothesis that climate and/or burrow climate influence flea abundance, the effects of mean temperature and mean humidity of each village on flea abundance were explored. In the analysis the climate variables are referred to as current climate conditions. First, to determine whether climatic factors explained additional spatial and temporal variation these variables were added to the best model from stage 1. In addition, to examine the extent which climatic factors explained the temporal and spatial patterns detected in stage 1, explanatory variables related to climate were also added to a model containing only an intercept. As climate and microclimate variables were correlated, especially for exterior burrows, ambient climate and microclimate were considered separately.

Stage 3

As preceding climatic conditions recorded during the previous fieldtrip (≤ 85 days before) may be more important than current conditions for determining the abundance of adult fleas, in a third stage the combined effects of current and previous climate and microclimate were explored. Examining the influence of climate and/or burrow climate further, the effects of ambient and burrow temperature and humidity from the previous fieldtrip on flea abundance were also investigated. Models were based on field trips carried out between April and November (fieldtrip 2-5). Microclimate variables were based on village-means and lagged values were based on regional mean conditions for the previous field trip.

Model selection of the negative binomial generalised linear models used to investigate the abundance of *S. fonquerniei* was based on Akaike's information criterion (AIC) and the principle of parsimony. For *X. cheopis,* model selection was based on F-tests and the principle of parsimony; the quasibinomial distribution applied to the generalised linear model prevented the computation of AICs. House rat abundance was also added to the final climate model for *X. cheopis*. The explanatory variables used related to climate were ambient conditions and exterior and interior burrow conditions.

4.3 RESULTS

4.3.1 Differences between ambient climate and burrow climate

Burrow conditions versus ambient conditions

The mean temperatures of exterior burrows, interior burrows and ambient conditions do not differ significantly from each other. The mean diurnal temperature ranges of burrows (DTR) however, are significantly different ($P \leq 0.001$) from the ambient DTR. The mean DTR of interior and exterior burrows is not significantly different, yet the ambient mean DTR was significantly greater (Figure 4.9a). The mean exterior, interior and ambient relative humidity all differ significantly from each other ($P \leq 0.001$). The mean ambient relative humidity was lower than burrow humidity and also shows the highest variation, while the mean relative humidity of exterior burrows is highest (Figure 4.9b). The mean DHR does not differ significantly between exterior and interior burrows, although the mean DHR of both burrow types are significantly lower than the mean ambient DHR ($P \leq 0.001$) (Figure 4.9c).

Figure 4.9: (a) Mean diurnal temperature ranges of interior burrows, exterior burrows and ambient conditions (b) Mean diurnal temperature ranges of interior burrows, interior burrows and ambient conditions (c) Mean diurnal humidity range of interior burrows, exterior burrows and ambient conditions
The dark line in each box represents the median value, the bottom and top of the box indicates the 25th and 75th percentiles, respectively. The whiskers show either the maximum value or 1.5 times the interquartile range of the data, whichever is the smaller. Outliers are plotted individually as dots.

(a)

(b)

(c)
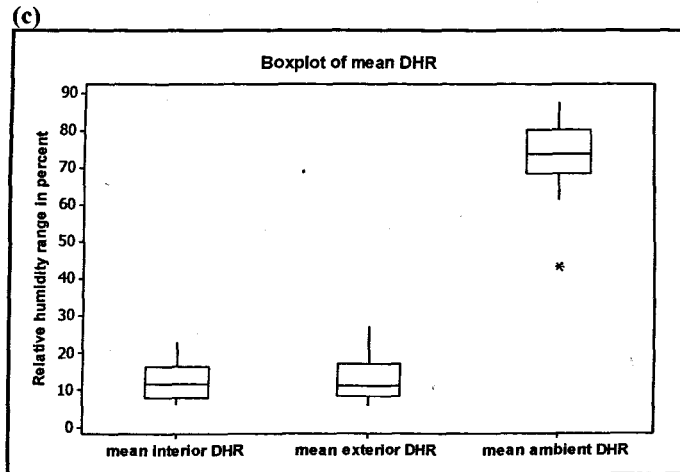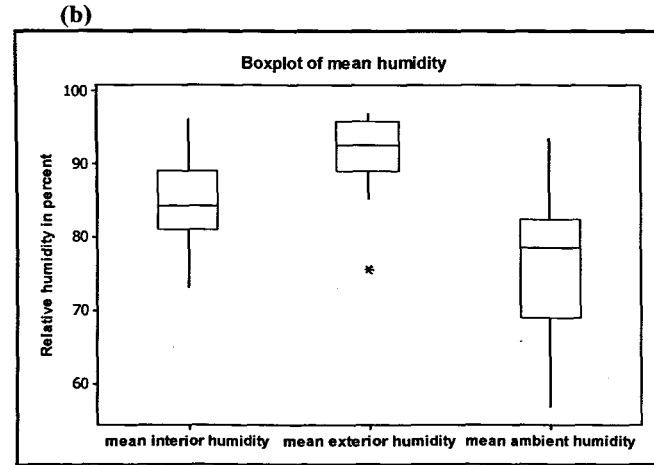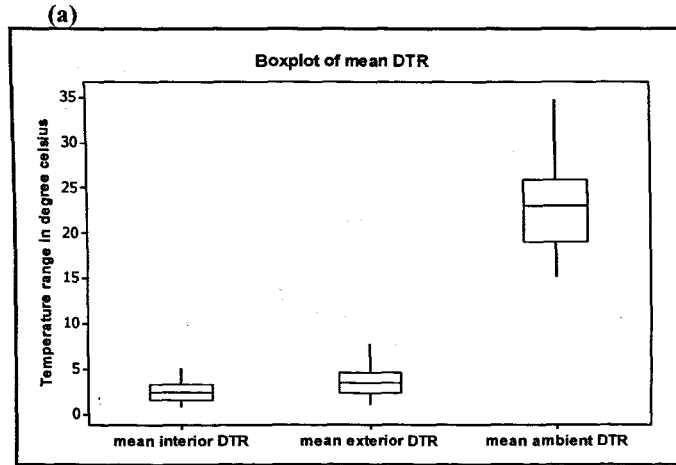
4.3.2 Climate differences between regions

The observed inter-relationships between interior burrows, exterior burrows and ambient conditions in each region separately are the same when both regions are analysed together. Only the mean ambient temperature between regions is significantly different (P=0.011), with region B being warmer than region A (Table 4.2). There were no significant differences between regions in the mean temperatures recorded in burrows, the mean DTR of interior and exterior burrows, nor the ambient values.

The mean ambient humidity is significantly higher in region A than region B (P=0.020). The mean ambient DHR, the mean humidity of the same burrow types and the DHR between regions does not differ significantly between regions (Table 4.3).

Temperature	Mean interior Region A	Mean interior Region B	Mean exterior Region A	Mean exterior Region B	Mean ambient Region A	Mean ambient Region B
Mean interior Region A	x	0.162	0.089*	0.910	0.054*	0.064*
Mean interior Region B		x	0.141	0.212	0.104	0.970
Mean exterior Region A			x	0.473	0.520	0.014**
Mean exterior Region B				x	0.273	0.212
Mean ambient Region A					x	0.011**

Table 4.2 Statistical differences in mean temperature of interior burrows, exterior burrows and ambient conditions between regions. Statistical significance is indicated by * for a p-value of 0.1-0.051; ** for a p-value of 0.05-0.01 and ** for a p- value below 0.01.

Humidity	Mean interior Region A	Mean interior Region B	Mean exterior Region A	Mean exterior Region B	Mean ambient Region A	Mean ambient Region B
Mean interior Region A	x	0.633	0.002***	0.072	0.128	0.003***
Mean interior Region B		X	0.002***	0.037**	0.329	0.007***
Mean exterior Region A			x	0.288	0.000***	0.000***
Mean exterior Region B				x	0.003***	0.000***
Mean ambient Region A					x	0.020**

Table 4.3 Statistical differences in mean humidity of interior burrows, exterior burrows and ambient conditions between regions. Statistical significance is indicated by * for a p-value of 0.1-0.051; ** for a p-value of 0.05-0.01 and *** for a p- value below 0.01.

4.3.3 Seasonal variation in climate

The mean ambient temperature in July is significantly different to the mean ambient temperature recorded during all other fieldtrips (with p-values of 0.011; 0.040; 0.035 and 0.016 for January, April, September and November respectively) (Figure 4.10a).

The mean ambient DTR, mean ambient relative humidity and mean ambient DHR show no significant differences between fieldtrips. The mean temperature in both interior and exterior burrows was significantly colder in July than in January, April, September and November (P=0.003 and P=0.009 respectively) (Figure 4.10b and 4.11a respectively).

The mean DTR of both burrow types shows no significant difference between fieldtrips.
The recorded mean humidity does not change significantly between fieldtrips in interior burrows, but shows a significant difference in the exterior burrows between July and the fieldtrips in January and November (P≤0.001) (Figure 4.11b). The mean DHR of both burrow types does not change significantly between fieldtrips.

4.3.3   Soil analysis

The soil analysis shows no significant differences between the clay and humidity content of the samples between regions (Figure 4.12 a; b).

Figure 4.10: a) Mean ambient temperature values for each fieldtrip (b) Mean interior burrow temperature values for each fieldtrip The dark line in each box represents the median value, the bottom and top of the box indicates the 25th and 75th percentiles, respectively. The whiskers show either the maximum value or 1.5 times the interquartile range of the data, whichever is the smaller. Outliers are plotted individually as dots.
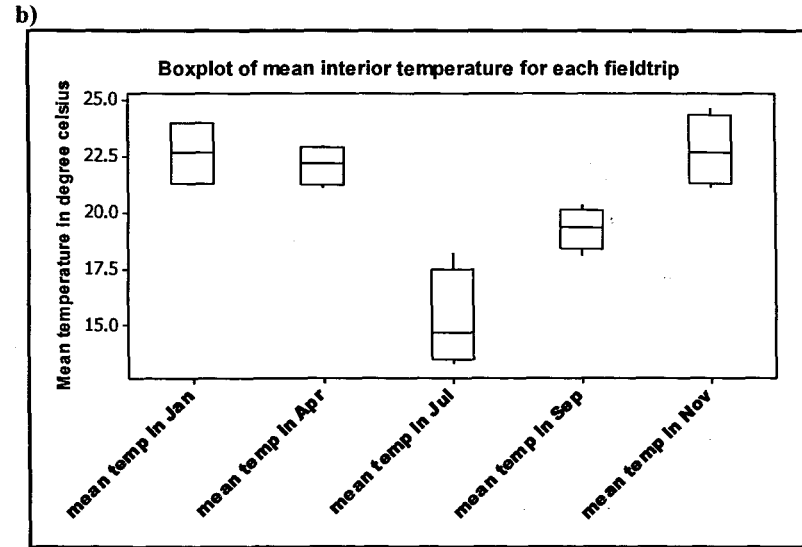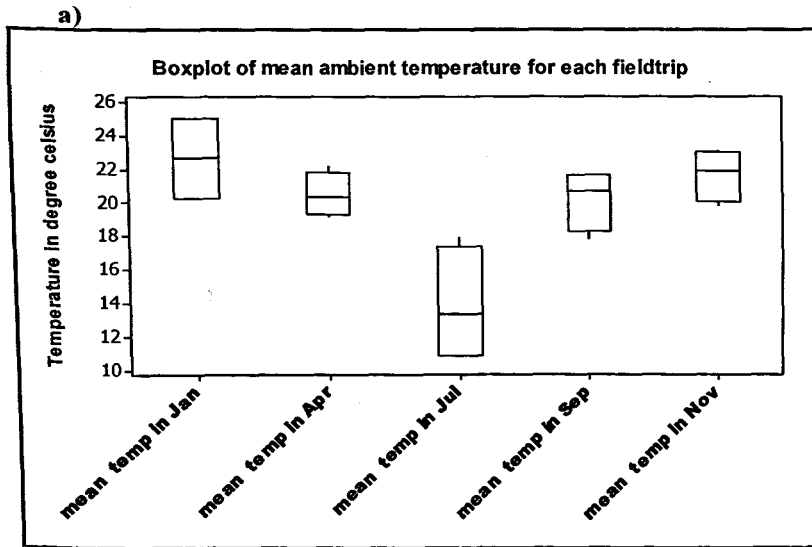
Figure 4.11: a) Mean exterior burrow temperature values for each fieldtrip **b)** Mean exterior burrow humidity values for each fieldtrip. The dark line in each box represents the median value, the bottom and top of the box indicates the 25th and 75th percentiles, respectively. The whiskers show either the maximum value or 1.5 times the interquartile range of the data, whichever is the smaller. Outliers are plotted individually as dots.
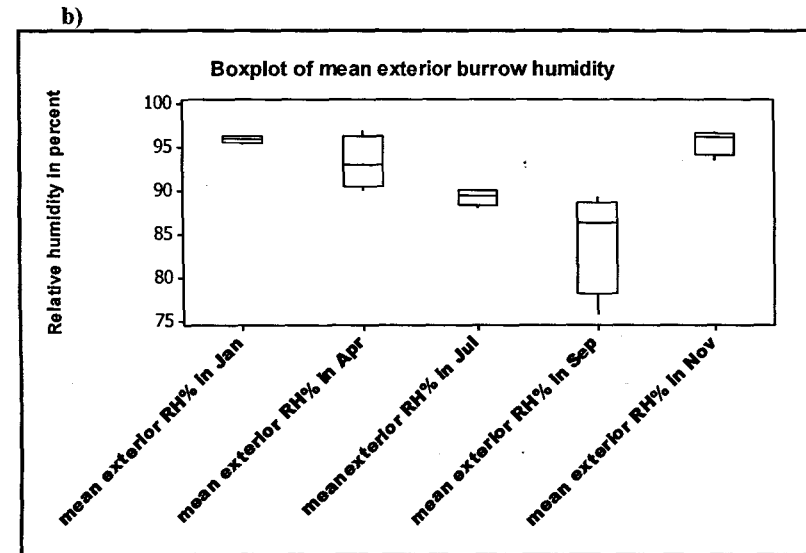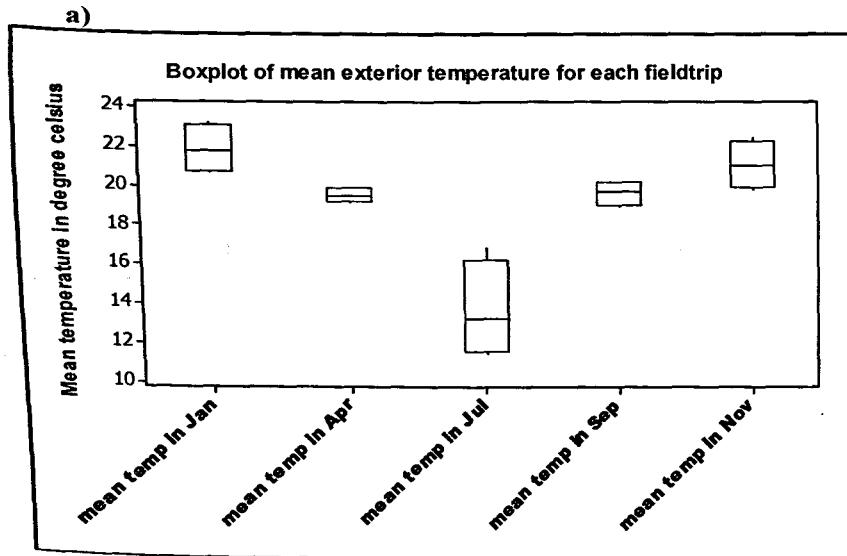
Figure 4.12 a: Mean content of clay and water (humidity) in soil samples
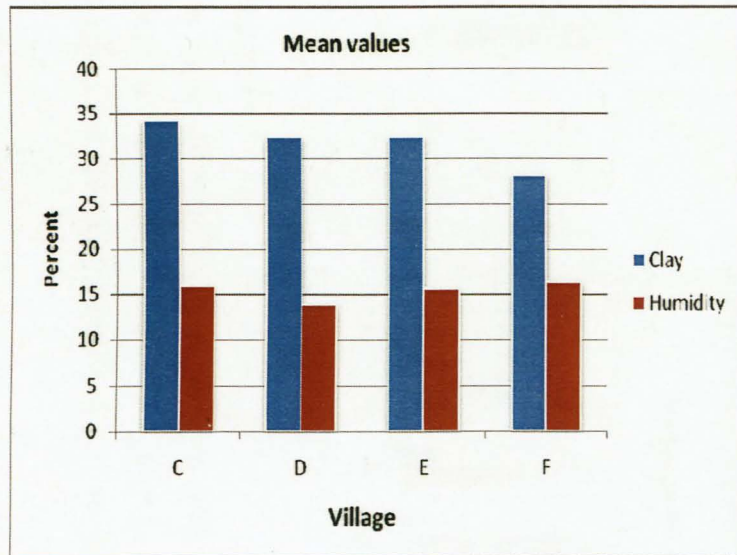
Figure 4.12 b: Mean percentage of clay and water (humidity) in soil content per region. The dark line in each box represents the median value, the bottom and top of the box indicates the 25th and 75th percentiles, respectively. The whiskers show either the maximum value or 1.5 times the interquartile range of the data, whichever is the smaller. Outliers are plotted individually as dots.
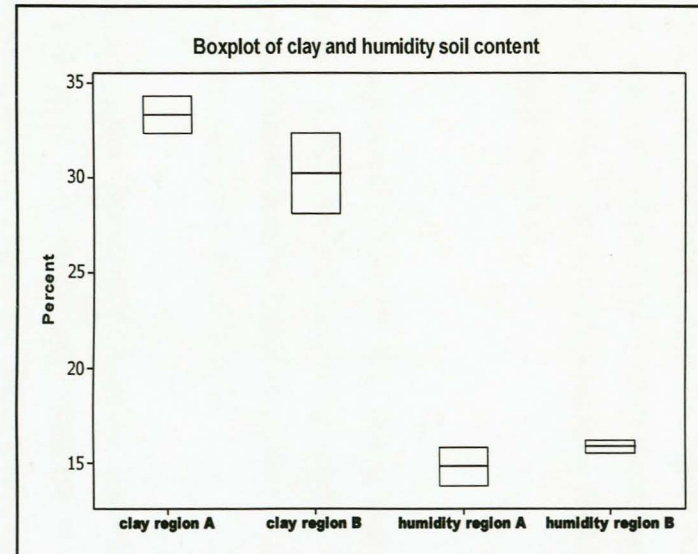
4.3.4   Flea species biology

Variation in abundance

A total of 1733 rats of the species *Rattus rattus* were caught. Fleas were found on 38.8% of these rats. The number of rats caught was highest in July and lowest in January and differed significantly between these two fieldtrips (P=0.028) (Appendix, Figure A4.1). In April the percentage of rats with fleas was only 15% while in November more than half of the rats caught had fleas (53%). On average, 86.9% of all fleas collected were dissected and therefore identified to species level and their age determined.

Flea abundance

The total flea index did not differ significantly between fieldtrips or regions, although the index shows strong variation (Figure 4.13a). Results for species specific flea indices show that the July index of *S. fonquerniei* is higher than the September index of *X. cheopis* (Figure 4.13b). Analysis found the difference to be significant (P≤0.001).

The index of *X. cheopis* did not differ significantly between fieldtrips, while the *S. fonquerniei* index in January and April was significantly different to the index of *S. fonquerniei* found in July and November with the latter two indices being higher (P≤0.001).

Figure 4.13 a: Total flea index per fieldtrip   **Figure 4.13 b: Flea index of *X. cheopis* and *S. fonquerniei* per fieldtrip**
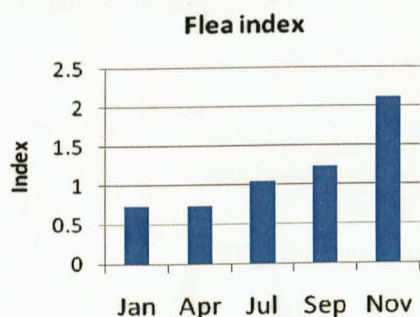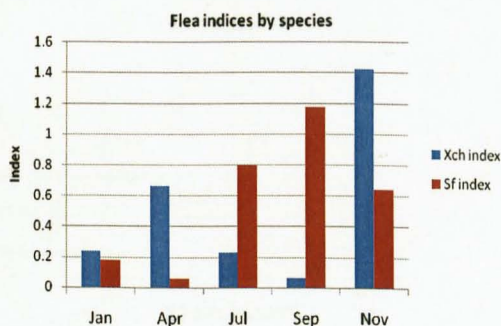
Sex and age structure of the flea population

The species *X. cheopis* has a higher ratio of males to females while the endemic *S. fonquerniei* shows the opposite except in January, where more male *S. fonquerniei* were found (Appendix, Figure A4.2 and A4.3). The age structure of the two flea species, which

was recorded for the dissected females, differed between fieldtrips. Both species show a high young/very young to adult ratio in July. *S. fonquerniei* exhibits its highest ratio of adults in April with more than 80% of the dissected females being adults. For *X. cheopis*, the highest adult ratio was detected in September (Appendix, Figure A4.4 and A4.5). The ratio however, does not give an indication of the abundance of females (Figure 4.14a; b).

Figure 4.14 a: Age structure of female *X. cheopis*. Number of adult, young and very young females dissected per fieldtrip.

Age of *Xch*
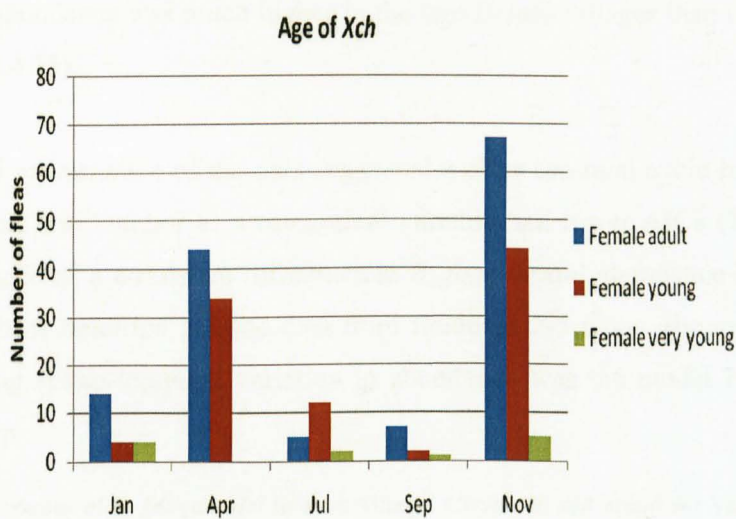
Figure 4.14 b: Age structure of female *S. fonquerniei*. Number of adult, young and very young females dissected per fieldtrip.

Age *Sf*
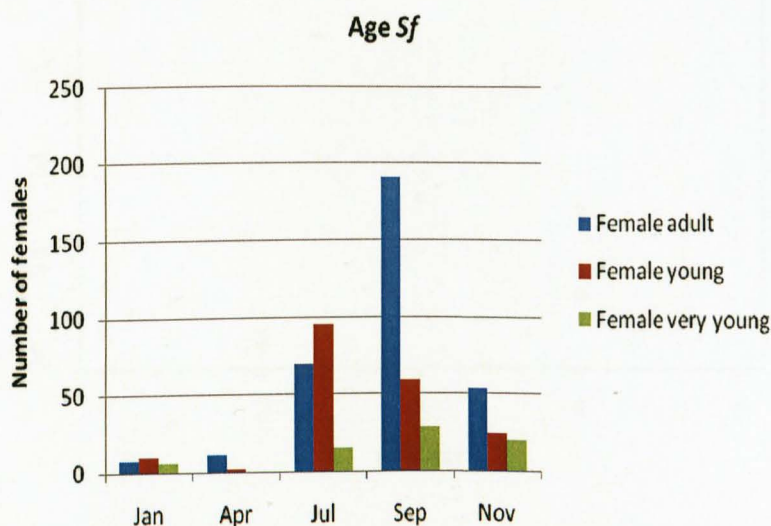
4.3.5 The effect of climate on the variation in flea abundance

For the species S. fonquerniei

The flea index for *S. fonquerniei* showed a large amount of temporal variation, with lowest abundance observed in April (fieldtrip 2) and highest abundance observed in September (fieldtrip 4) (Figure 4.14b). Villages from the same region showed similar abundance levels. Notably, at least some *S. fonquerniei* were found in all villages, irrespective of season. Although, in general, there was not a clear difference between the two regions, in September, *S. fonquerniei* abundance was much higher in the two Betafo villages than the two Antsirabe villages (Figure 4.15).

Although initial examination of the data suggested a clear seasonal cycle in flea abundance, models with field trip number as a categorical variable had lower AICs (Table 4.4). There was little evidence of a consistent difference in *S. fonquerniei* abundance between regions. For both data from fieldtrips 1-5 and data from fieldtrips 2-5 alone, the most parsimonious model describing spatio-temporal variation in abundance was the model including only an effect of fieldtrip.

Figure 4.15: Abundance of *S. fonquerniei* in each village. Circles in red stand for villages in the Betafo region (B) and black circles stand for villages in the Antsirabe region (A).
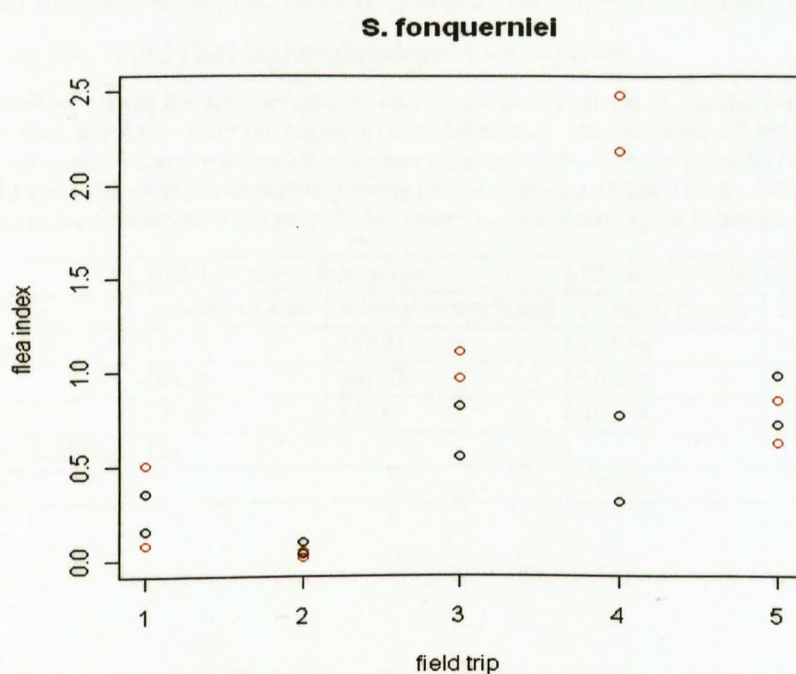
Stage 1

Table 4.4 Modelling results for the temporal and spatial patterns in the flea index of *S. fonquerniei*. AIC models in bold show the most parsimonious models (simplest model within 2 of the lowest AIC value)

	5 fieldtrips	fieldtrip 2-5
Variables	AIC	AIC
Intercept only	194.69	166.23
Fieldtrip	**171.00**	**144.36**
Region	194.89	166.57
Sin+Cos	176.48	148.37
Sin + Cos + Region	177.01	149.49
Fieldtrip+ Region	170.48	143.3

Stage 2

When ambient climate and burrow microclimate variables were added to the best model from stage 1, only burrow humidity resulted in a decrease in AIC of more than 2 (Table 4.5). A similar result was obtained when ambient climate and burrow microclimate variables were added to an intercept only model. In both cases, high humidity is related to low abundance of *S. fonquerniei* (Table 4.5). Although burrow humidity was significantly related to *S. fonquerniei* abundance, the effect of fieldtrip remained strong. Specifically, once the effect of humidity had been accounted for, April (fieldtrip 2) had lower flea abundance than expected and December (fieldtrip 5) had higher abundance than expected.

Table 4.5 Modelling results for ambient climate and microclimate effects on the flea index of *S. fonquerniei*. Values are AIC values for negative binomial models. Microclimate conditions are based on village-mean for exterior burrows. Models were run with and without the best model from stage 1. AIC models in bold show the most parsimonious models (simplest model within 2 of the lowest AIC value) with and without the best model from stage 1. All models included data from fieldtrips 1-5.

5 fieldtrips	With best model from stage 1		Climate variables alone	
Climate variables	Ambient climate	Burrow microclimate	Ambient climate	Burrow microclimate
Mhumid + Mtemp	171.78	169.31	194.74	192.22
Mhumid	169.86	**167.43**	192.86	**190.22**
Mtemp	171.02	172.6	196.66	196.28
Stage 1 model (fieldtrip)	171			
Intercept only			194.69	

Stage 3

When considering only the effects of current climatic variables, the results for the dataset including fieldtrips 2-5 alone (Table 4.6 a) are similar to the results for the full 5 fieldtrip

dataset (Table 4.5), showing an effect of burrow humidity with and without the effect of fieldtrip. When only lagged variables were added to the best model from stage 1, the best model included an effect of exterior burrow temperature. However, lagged air temperature (which is correlated to burrow temperature) also results in a substantial drop in the AIC value. There is no suggestion of a lagged effect of humidity (ambient or microclimate). However, when the lagged effects of climate are added to an intercept only model, there is evidence that both humidity and temperature have an effect, with the AIC of the ambient conditions model being lower than the model with microclimatic conditions.

When current and lagged climatic variables are considered together, the best model has an effect of both current and lagged ambient temperature and humidity and an effect of lagged burrow temperature (Table 4.6 b). Both current and lagged ambient temperature and humidity are negatively associated with *S. fonquerniei* abundance, whilst lagged burrow temperature is positively associated with flea abundance (Table 4.10b). Similarly, in the model that includes fieldtrip, lagged burrow temperature is positively associated with flea abundance. Comparisons of the models with and without the effect of fieldtrip indicate that most of the variation explained by fieldtrip is explained by the effects of current and lagged ambient temperatures (AIC 133.74 vs. AIC 138.79) (Tables 4.6 a and b).

Thus, together, the results indicate that the seasonal abundance pattern of *S. fonquerniei* is related to variation in temperature and humidity, with the highest abundances achieved when temperature and humidity have been low for several months. Humidity appears to have a particularly adverse effect on *S. fonquerniei*. Thus, abundances peak at the end of the winter, in September. However, the positive effects of lag in burrow temperature (in models with and without an effect of fieldtrip), suggest that temperatures that are too low can also be a problem. This effect is probably driven by a difference between the two regions in the minimum temperatures in July.

Table 4.6 Modelling results for current and lagged ambient climate and microclimate effects on the flea index of *S. fonquerniei*. All modelling is based on field trips carried out between April and December. Values are AIC values for negative binomial models. Microclimate conditions are based on village-mean for exterior burrows. Lagged vales are based on region-mean conditions for the previous field trip (an average of 85 days previously). AIC models in bold show the most parsimonious models (simplest model within 2 of the lowest AIC value).

(a) Ambient and burrow climate variables analysed separately with variables of the best model of stage 1 included and without variables of stage 1.

(b) Ambient and burrow climate variables from current and previous fieldtrips analysed together without variables of the best model of stage 1.

a)

Fieldtrip 2-5	With best model from Stage 1				Climate variables alone			
	Current conditions		Lagged conditions		Current conditions		Lagged conditions	
Climate variables	Ambient climate	Burrow micro-climate	Ambient climate	Burrow micro-climate	Ambient climate	Burrow micro-climate	Ambient climate	Burrow micro-climate
Mhumid + Mtemp	143.89	142.53	138.4	135.01	167.73	166.23	**160.31**	163.53
Mhumid	141.96	**140.57**	142.83	146	165.73	**164.27**	162.43	164.8
Mtemp	143.99	146.14	139.32	**133.74**	168.14	168.23	166.29	164.13
Stage 1 (Fieldtrip)	144.36							
Intercept only					166.23			

b)

Fieldtrips 2-5	Current conditions + Lagged conditions		
Variables	Current conditions	Lagged conditions	AIC
Ambient climate Burrow climate	Mtemp +Mhumid	Mtemp1+Mhumid1 Mtemp1	**138.79**
Ambient climate Burrow climate	Mtemp+Mhumid	Mtemp1+Mhumid1 Mtemp1+Mhumid1	139.18
Ambient climate Burrow climate	Mtemp+Mhumid Mhumid	Mtemp1+Mhumid1 Mtemp1+Mhumid1	139.8
Ambient climate Burrow climate	Mtemp+Mhumid Mtemp+Mhumid	Mtemp1+Mhumid1 Mtemp1+Mhumid1	140.43
Ambient climate Burrow climate	Mhumid Mtemp	Mtemp1+Mhumid1 Mtemp1	142.49
Ambient climate Burrow climate	Mtemp Mhumid	Mtemp1+Mhumid1 Mtemp1	144.3

For the species X. cheopis

The flea index values for *X. cheopis* showed a large amount of variation, ranging from 0 to 14.2 (Figure 4.16). Although there was no clear seasonal pattern, abundance did seem to be higher in some months such as April (fieldtrip 2) and December (fieldtrip 5). *X. cheopis*

abundance appeared generally higher in Betafo than Antsirabe. However, a total of 8 villages had a flea index value of 0 for *X. cheopis* and villages from the same region trapped at the same time did not necessarily have similar flea index values, indicating a large amount of spatial variation at a smaller spatial scale than region.

Figure 4.16: Abundance of *X. cheopis* in each village. Circles in red stand for villages in the Betafo region (B) and black circles stand for villages in the Antsirabe region (A).
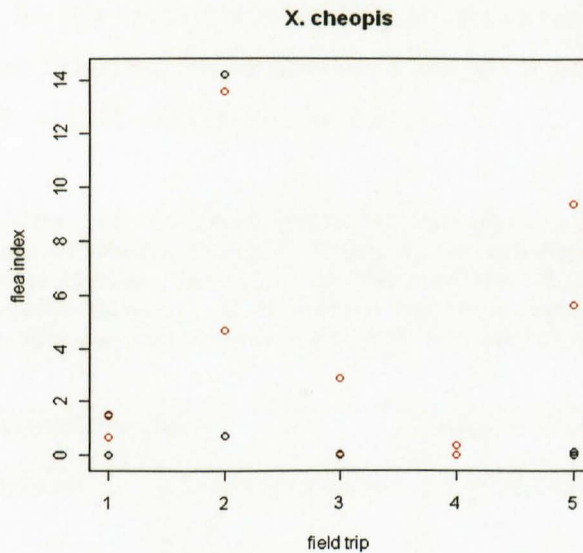
Stage 1

Table 4.7 Modelling results for the temporal and spatial patterns in the proportion of rats caught inside houses carrying *X.cheopis*. Models were quasibinomial models and model selection was based on F-tests. F tests presented are based on removing the variable from the full model.

Variables	fieldtrip 2-5			5 fieldtrips		
	F	df	p-value	F	df	p-value
Fieldtrip	6.32	3	**0.0095**	5.92	4	**0.0053**
Region	10.76	1	**0.0073**	13.38	1	**0.0026**

For data from fieldtrips 1-5 and data from just fieldtrips 2-5, both fieldtrip and region were significant (Table 4.7), with higher abundance in Betafo (coeff: 2.814, SE: 0.949, p-value= 0.010).

Stage 2

When ambient climate is added to the best model from stage 1, no climate variables are significant (Table 4.8). However, when interior microclimate conditions are added to the model, burrow temperature is significant, with region becoming non-significant. Similarly, when ambient climate and interior microclimate variables are added to intercept only models, there is evidence of an effect of burrow temperature but little evidence of effects of ambient conditions (Table 4.8). These results indicate that the effect of region appears to be driven by burrow temperature, but that variation between fieldtrips is still apparent, over and above the effects of temperature. The proportion of rats with *X. cheopis* is positively related to burrow temperature (coeff: 0.3411; SE: 0.1353; p-value=0.0214).

Table 4.8 Modelling results for ambient climate and microclimate effects on the proportion of rats caught inside houses with *X. cheopis*. Models were quasibinomial models and model selection was based on F-tests. F-tests presented are based on removing the variable from the full model. Models were run with and without the best model from stage 1. All modelling is based on current conditions recorded on field trips carried out between January and December. Microclimate conditions are based on village-mean for interior burrows.

5 fieldtrips	With best model from Stage 1				Climate variables alone			
Climate variables	Ambient climate		Burrow microclimate		Ambient climate		Burrow microclimate	
Model	Fieldtrip+region+mhair +mtair		Fieldtrip+region+mhin +mtin		mhair+mtair		mhin+mtin	
	F	p-value	F	p-value	F	p-value	F	p-value
Fieldtrip	3.06	0.0594	5.03	0.0129				
Region	9.14	0.0106	1.23	0.2893				
Mhumid	0.01	0.9233	0.07	0.8010	1.02	0.3277	2.20	0.1564
Mtemp	1.35	0.2674	5.86	**0.0322**	2.20	0.1563	10.72	**0.0045**

Stage 3

The effects of current climatic conditions on flea abundance in fieldtrips 2-5 (Table 4.9a) were similar to those for the full dataset of 5 fieldtrips (Table 4.8); the drop in statistical power resulted in the effect of interior burrow temperature being only close to significance in models that also included fieldtrip and region. When the effects of lagged ambient climate and lagged microclimate conditions were added to the best model from stage 1, no climatic

variables were significant (Table 4.9b). However, when the effects were examined in an intercept only model, a strong effect of ambient temperature in the previous month was found.

When models with both current and lagged climatic conditions were considered, the final best model had an effect of current burrow temperature and lagged ambient temperature (Table 4.9c). The proportion of rats with *X. cheopis* was positively related to both current burrow temperature and lagged ambient temperature (Table 4.10b). As there are significant correlations between ambient and microclimate conditions, all other combinations of temperature variables were also examined (e.g. current ambient temperature and lagged burrow temperature). In all cases, both the current temperature variable and the lagged temperature variable were significant, indicating strong effects of both current and previous temperature on the abundance of *X. cheopis*. As no effect of lagged temperature is found in models that include the effects of fieldtrip and region (Table 4.9b), it appears as if these variables are acting as surrogates for previous temperature.

Together, the results indicate that *X. cheopis* abundance is higher during periods of consistently high temperatures. Humidity appears to have little effect on the abundance of *X. cheopis* in houses.

Table 4.9 Modelling results for current and lagged ambient climate and microclimate effects on the proportion of rats caught inside houses with *X. cheopis*. Modelling is based on field trips carried out between April and December. Models were quasibinomial and model selection was based on F-tests. In (a) and (b), models were run with and without the best model from stage1 and F tests presented are based on removing the variable from the full model. (a) shows results based on current conditions and (b) shows results based on lagged conditions. Lagged values are based on region-mean conditions for the previous field trip (an average of 85 days previously). (c) shows the final model for the analysis of the combined effects of current and lagged conditions and F tests presented are based on removing the variable from the final model after backward selection.

a) All modelling is based on current conditions recorded on field trips carried out between April and December. Microclimate conditions are based on regional-means for interior burrows.
b) All modelling is based on lagged conditions recorded on field trips carried out between April and December. Microclimate conditions are based on regional-means for interior burrows.
c) All modelling is based on combined current and lagged conditions recorded on field trips carried out between April and December. Microclimate conditions are based on regional-means for interior burrows. The grey area shows results of the best model.

a)

Current conditions								
Fieldtrip 2-5	With best model from Stage 1				Climate variables alone			
Climate variables	Ambient climate		Burrow microclimate		Ambient climate		Burrow microclimate	
Model	Fieldtrip+region+mhair +mtair		Fieldtrip+region+mhin +mtin		mhair+mtair		mhin+mtin	
	F	p-value	F	p-value	F	p-value	F	p-value
Fieldtrip	2.81	0.1005	3.93	**0.0480**				
Region	7.48	**0.0231**	1.20	0.3021				
Mhumid	0.01	0.9420	0.01	0.9219	0.98	0.3404	1.29	0.2767
Mtemp	1.17	0.3076	5.05	**0.0512**	2.97	0.1084	10.70	**0.0061**

b)

Lagged conditions								
Fieldtrip 2-5	With best model from Stage 1				Climate variables alone			
Climate variables	Ambient climate		Burrow microclimate		Ambient climate		Burrow microclimate	
Model	Fieldtrip+region+mhairL +mtairL		Fieldtrip+region+mhinL +mtinL		mhairL + mtairL		mhinL + mtinL	
	F	p-value	F	p-value	F	p-value	F	p-value
Fieldtrip	1.43	0.2973	4.82	**0.0287**				
Region	0.17	0.6862	0.05	0.8210				
MhumidL	0.14	0.7186	0.90	0.3684	3.17	0.0983	0.07	0.7955
MtempL	0.83	0.3864	0.11	0.7457	15.29	**0.0018**	1.39	0.2602

c)

Fieldtrips 2-5	Current conditions + Lagged conditions					
Variables	Current conditions	F	p-value	Lagged conditions	F	p-value
Ambient climate				MtempL	16.901	**0.00123**
Burrow climate	Mtemp	10.238	**0.00697**			

Table 4.10: Coefficients, standard errors and p-values from the best climate models for both vector species.

a) best models with current variables for *S.fonquerniei* (Table 4.5) and *X.cheopis* (Table 4.8) on field trips carried out between January and December. Best models for *X. cheopis* are based on backward selection from the full model based on F-tests.

5 fieldtrips	*S. fonquerniei*						*X. cheopis*					
	With best model from Stage 1			Climate variables alone			With best model from Stage 1			Climate variables alone		
Variable	Coefficient	SE	p-value	Coefficient	SE	p-value	Coefficient	SE	p-value	Coefficient	SE	p-value
Intercept	7.62873	3.23654	0.01842	7.94516	3.58495	0.0267	-25.49	11.32	0.0409	-7.7052	2.9447	0.0175
Fieldtrip 2	-1.95048	0.40523	1.49E-06				3.11	1.73	0.0941			
Fieldtrip 3	0.45947	0.37545	0.22102				7.33	2.17	0.0481			
Fieldtrip 4	0.41744	0.50254	0.40617				1.45	0.48	0.6365			
Fieldtrip 5	0.98563	0.3059	0.00127				1.94	1.44	0.1728			
Region												
Burrow hum	-0.09248	0.03371	0.00609	-0.09248	0.03912	0.0181						
Burrow temp							1.04	0.47	**0.0459**	0.3411	0.1353	**0.0214**

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Table 4.10: Coefficients, standard errors and p-values from the best climate models for both vector species.
b) best models with lagged variables and current and lagged variables combined for *S.fonquerniei* (table 6) and *X.cheopis* (table 9) on field trips carried out between April and December.

| Fieldtrips 2-5 | *S. fonquerniei* | | | | | | *X. cheopis* | | |
| | With best model from Stage 1 | | | Climate variables alone | | | Climate variables alone | | |
Variable	Coefficient	SE	p-value	Coefficient	SE	p-value	Coefficient	SE	p-value
(Intercept)	-9.93338	1.30553	2.77E-14	19.8820	1.91041	2.00E-16	-18.14	4.94	0.0028
Fieldtrip 3	3.55479	0.32526	< 2e-16						
Fieldtrip 4	5.74976	0.54233	< 2e-16						
Fieldtrip 5	3.42968	0.32565	< 2e-16						
Region									
Burrow temp							0.30	0.11	0.146
Burrow hum									
Ambient hum				-0.03131	0.00829	0.000158			
Ambient temp				-0.21905	0.03058	7.89E-13			
Burrow temp lag	0.31917	0.05778	3.32E-08	0.831536	0.16465	4.41E-07			
Burrow hum lag									
Ambient hum lag				-0.18095	0.01971	2.00E-16			
Ambient temp lag				-0.84718	0.14174	2.27E-09	0.58	0.23	0.0269

4.4 DISCUSSION

Human plague incidence as well as rodent plague prevalence has been linked to climate in several plague foci around the world. Climate variables influence the dynamics of flea vectors and rodent hosts with responses varying considerably among species (Gubler, 2001; Meserve, 1995). The present study is thus important to establish mechanisms by which plague is linked to climate in Madagascar.

It is widely accepted that vector and host abundance play a crucial part in the epidemiology of plague (Davis, 2006, 2008; Gage, Kosoy, 2005; Lorange et al. 2005). Vector abundance is often assumed to be related to host abundance with some direct modifications by climate. In the US primary production affected by precipitation and temperature was shown to be positively correlated to plague incidence (Ben Ari, Gershunov et al. 2010). This links in with findings by Krasnov et al. (2005) on the positive effects of food availability to the host on fecundity, development rate and longevity of fleas. Other studies also found increased rodent host abundance after above-average winter-spring precipitation in the US (Parmenter, Yadav, 1999) linked to plague. Similarly, the plague focus in Kazakhstan shows an effect of increased winter-spring precipitation on the plague host population (great gerbil, *Rhombomys opimus*) and an effect of warmer springs and cooler, wetter summers on flea numbers (Stenseth, 2006.) Interestingly in both Kazakhstan and the US, late winter-spring precipitation seems important in driving the start of the seasonal flea cycles and the plague season. In Kazakhstan, spring flea burden was negatively correlated with spring frost and positively correlated with spring temperature. Autumn flea burdens were positively correlated with relative humidity and in general humidity was higher in cooler summers. When flea burden was included in the model, the effects of climate became insignificant, strongly suggesting that the climate results were being driven by fleas. This however, does not rule out the possibility that climate was acting as a surrogate for both rodent abundance and fleas. Temperature, rainfall, and relative humidity have direct effects on development and survival of fleas (Gage, 2008; Krasnov, 2001), but they are species specific. In the Negev desert in Israel for example, both perennial flea populations and seasonal flea populations have been found on the same hosts (Krasnov, 2002). In Asia, the US and Africa, the abundance of rodent fleas was mainly linked to temperature, rainfall, and relative humidity, with warm-moist weather providing a likely explanation for higher flea indices (Cavenaugh, 1972; Davis, 1953; Ryckman, 1971).

Chapter two established that plague incidence in Madagascar has seasonal and inter-annual cycles. Yet, is the relationship of human plague incidence with climate also driven by vector species? Here we concentrated on flea abundance and how its seasonal patterns appear to reflect climate based on data collected over one year. Although this already yields valuable information and may be indicative of climatic variables that explain year to year variation in flea abundance, other variables may be involved in year to year variation.

Spatial variation

On the island, the presence/absence of the plague vector *S. fonquerniei* seems to explain broad scale spatial patterns of human plague distribution. Our results indicate that compared to *S. fonquerniei*, *X. cheopis* shows more spatial variation and clear variation between villages within a region, while *S. fonquerniei* only shows clear spatial variation in September when it is more abundant in the generally warmer Betafo region. Many studies noted that the ubiquitous flea vector *X. cheopis* is found mainly on rats caught in houses in the highlands while in other parts of the island it is found on rats regardless of their location (Brygoo 1966; Duplantier, 2001). The investigation into differences in micro-climate between indoor rat burrows and outdoor rat burrows suggests that the micro-climate favourable for the development and survival of *X. cheopis* can best be provided in rodent burrows which are located inside houses in the highlands; outdoor burrows support the endemic *S. fonquerniei*.

Temporal variation

S. fonquerniei shows a clear seasonal cycle, thriving during July, September and November, in the middle and at the end of the dry during the cold season, just before the plague season, similar to findings in Kazakhstan and the US mentioned earlier. Both, the data on reproduction and the flea index indicate that *S. fonquerniei* has its highest development rate between April and September. *X. cheopis* on the other hand, was found to be most numerous at the start and the end of the warm rainy season in November and April respectively, but showed no clear seasonality. Contrary to *S. fonquerniei*, its lowest development rate and adult survival was from April to September and highest from September to November and January to April. In Kazakhstan there is evidence that very hot days with low humidity have a negative effect on flea populations, probably related to adult survival. We were not able to look at interactions between season and climatic variables due to lack of data, but in

Madagascar the ecosystem of the main plague focus means that high temperatures occur at the same time as high humidity.

Habitat conditions

The micro-climate in burrows of the black rat in Madagascar is different from the ambient climate conditions, as previously confirmed by Longanecker and Burroughs (1952) for ground squirrel burrows in the US. In Madagascar the conditions in the preferred habitat of each species' developmental stages were found to be distinct from each other. The results of this study imply that two different climatic habitats exist depending on burrow type. Burrows found in houses and burrows found outdoors show a different micro-climate. In general, burrow climate shows less annual and diurnal variation than ambient climate in both, temperature and humidity, providing much more stable conditions than those prevailing outside. In particular, burrows located in houses are warmer and drier than their outside counterparts, which stay humid even through the driest months of the year.

Effects of climate on S. fonquerniei

Spatial and temporal patterns in the abundance of *S. fonquerniei* were at least partly explained by climatic factors. A particularly strong effect was detected for humidity, which was negatively associated with flea abundance in the majority of models that considered climatic effects. Moreover, where an effect of fieldtrip was not included, both current humidity and humidity 2.5 months ago had a negative effect. In models where an effect of fieldtrip was not included, current and lagged ambient temperatures were also important. Thus, at least some of the temporal variation in abundance observed through the year is attributable to variation in temperature. Again, effects were negative, such that *S. fonquerniei* abundance was highest at the end of the cooler, drier winter. Additionally the female population age structure during the colder months, with more than half the adult females being young or very young in July, suggests favourable micro-climate conditions for eggs, larvae and pupae. Interestingly, the effect of lagged burrow temperature was positive – both in models where fieldtrip was included and in models where the negative effects of ambient temperature and humidity were accounted for. Therefore, although low temperatures are related to higher *S. fonquerniei* abundance, there is evidence that too low temperatures may have a negative effect. Indeed, this lagged burrow temperature effect is probably driven by a difference between the two regions. Temperatures in July were much lower in Antsirabe than

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in Betafo, yet the September peak in *S. fonquerniei* abundance was much higher in Betafo than in Antsirabe.

The field results suggest that there may be a lower temperature threshold, where development time and/or mortality rates are increased to such an extent that future abundance of *S. fonquerniei* is adversely affected. It seems that Antsirabe may have low temperatures in winter, whilst Betafo does not. Together, the results point towards a preference of *S. fonquerniei* for colder temperatures and a possible adverse effect of high humidity. Both factors are consistent with the current temporal and spatial distribution of this species. In Madagascar, with decreasing altitude, temperature increases; *S. fonquerniei* is therefore not found in lower or coastal regions (Duchemin et al. 2001). Although climatic variables largely explain fieldtrip-driven temporal variation, the variables measured here do not fully describe it. Other factors that may be important include host abundance and behaviour patterns. In particular, burrow visiting rates affect access of newly hatched adults to blood meals and opportunities of females to lay eggs inside burrows.

Effects of climate on X. cheopis

The results for *X. cheopis* clearly indicate that temperature is an important variable in determining abundance, explaining some spatial variation (between regions) and temporal variation. The lack of AIC values for the quasibinomial models for *X. cheopis* prevented easy model comparison of the relative importance of fieldtrip compared to climate. However, it appears as if much of the effect of fieldtrip is related to the temperature recorded 2.5 months ago. Together with an effect of current temperature, the results therefore indicate that adult *X. cheopis* abundance tends to be higher in periods that have benefitted from high temperatures for several months. This effect of temperature is consistent with the results from the laboratory experiment (chapter 5), where a drop of 3 °C from 21 to 18 °C almost doubled larval development time and increased mortality by a third. Other studies investigating *X. cheopis* in Sri Lanka and Ethiopia already postulated that the species prefers warm and dry conditions (Traub, 1972 and 1978). This may contribute to the fact that *X. cheopis* is mostly found on rats caught in houses in the highlands of Madagascar, possibly due to the species mainly developing in interior rodent burrows which are significantly drier than their exterior counterparts.

It is clear from the graph of *X. cheopis* abundance (Figure 4.16) that temperature alone cannot fully explain the spatial and temporal variation in abundance observed. Not all villages sampled during warm months had high *X. cheopis* abundance. Further work is necessary to explore the effect of both current and previous host abundance, as well as other factors that may explain the high variation in *X. cheopis* abundance between villages. Measures of flea abundance taken at the same village over time would be particularly helpful in distinguishing the relative importance of spatial and temporal variation.

The lack of evidence of an effect of humidity on *X. cheopis* abundance is consistent with the results of the laboratory experiment presented in chapter 5. Under laboratory conditions, higher humidity showed no clear change in mortality rate and no alteration in larval development times. It should be noted, however, that the field data analysis focussed on factors explaining temporal and spatial variation in the abundance of *X. cheopis* on house rats. Humidity may still play a role in explaining why *X. cheopis* are primarily found on rats inhabiting houses. Indeed, the mean humidity of exterior burrows was significantly higher than the mean humidity of interior burrows.

Limitations of the study

The need to base *X. cheopis* abundance on the total number of *X. cheopis* found on house rats divided by the number of house rats, clearly impeded on the analysis method chosen to analyse its abundance and hindered comparison with *S. fonquerniei*. Also in an ideal situation, a continuous, longitudinal study would be carried out to gain more complete information on year to year variation and to confirm the findings of this study. The vector life cycle in favourable conditions is around 3 weeks from egg to adult (as found in chapter 5), which implies that during the 85 days between fieldtrips, undetected changes in abundance could have taken place, which could explain the effect of climate on flea abundance in more detail.

Implications for the plague cycle and future research

With regards to the effects of climate on the two main vector species on the island it makes sense to assume that the abundance of both is limited by lower temperature thresholds and possibly also by upper humidity thresholds. Such thresholds are likely to be species specific and further laboratory research and field studies are needed to ascertain them. In addition to

the above mentioned research on previous host abundance at a local scale, more information on host behaviour such as house and burrow visiting patterns would allow further investigation into factors affecting changes in vector abundance. Also socio-economic factors should be taken into account such as overall hygiene levels and animal farming practises (animals kept inside the house) as well as harvest times and crop storage which all could assist in explaining the variation in flea abundance via their effects on host abundance.

In terms of the temporal and spatial distribution of human plague cases in Madagascar, the results of this study imply that in the region studied, climate allows plague vectors for the maintenance of the plague cycle to be present in fairly high numbers throughout the year. The peak abundance time for *S. fonquerniei* coincides with the beginning of the human plague season, whilst *X. cheopis* are at relatively high abundance throughout much of the rainy season (until the human plague season ends in March). To verify the importance of the different temporal patterns of the two flea species, a plague-persistence model for Madagascar, as developed for plague foci in California, USA (Foley et al. 2010), could yield valuable information. The model is a multi-host, multi-flea species model. Findings include that increased diversity of hosts increases plague persistence, contrary to an earlier model by Keeling and Gilligan (2000), due to both an increase in host abundance and different breeding times in the two host species. One flea species seems to be the key to persistence in this model, while adding two more flea species to the model decreases persistence. The reason for this could not be established, but it is suggested that higher vector abundance might lead to worse epizootics, reducing the number of susceptible hosts too quickly for further pathogen transmission (burn-out). In Madagascar, evidence indicates the presence of just one host species, the black rat (*Rattus rattus*), but the different seasonality of the vector species could be the key to persistence of plague in the focus. Applying a similar model using the data collected in this study might confirm that it is the co-existence of two vector species and their population dynamics which govern the spatial and temporal distribution of human plague in Madagascar. It was not in the scope of this study however, to develop a plague persistence model for Madagascar. Reliable data on flea distribution, flea host preferences and precise information on plague transmission dynamics are required for the parameter estimation of such a model. Information on the number of host and vector species and particulars on vector development such as hatching rate is also essential. Even though some

of this information is available for the domestic host, little is known about the sylvatic plague reservoir, within the human plague foci on the island.

CHAPTER FIVE

THE EFFECT OF CONSTANT TEMPERATURE AND HUMIDITY

ON THE LARVAL DEVELOPMENT

OF *S. FONQUERNIEI* AND *X. CHEOPIS*

ABSTRACT

It is known that temperature and humidity are important factors in the development and survival of fleas and that different species require different micro-climatic conditions (Krasnov et al. 2001; Sharif 1949). Climatic constraints on the development of the two main vectors of plague could explain the differences in their distribution, but there have been no comprehensive studies examining the developmental needs of the endemic flea species *Synopsyllus fonquerniei* and comparing it to those of *Xenopsylla cheopis*. I examined the two species' temperature and humidity requirements, in terms of larval and pupal development rate and survival under experimental conditions. Evidence for different climatic adaptations of the pre-imaginal stages of the species was found, which could govern their distribution and annual changes in their densities.

Development times and mortality rates differed significantly, with the endemic *Synopsyllus fonquerniei* taking on average 1.79 times longer to complete development and having a 43% higher mortality rate than *Xenopsylla cheopis*. Degree day analysis estimated an average developmental threshold of 9°C for the endemic *Synopsyllus fonquerniei*, 3.48 degrees °C lower than the threshold for *Xenopsylla cheopis*.

5.1 INTRODUCTION

Variation in temperature and humidity is known to influence the development and survival rates of insect disease vectors (Bayoh and Lindsay, 2004; Mellanby, 1933; Padmanabha, 2011; Rueda et al. 1990) and thus often affects their abundance (Barandika, 2010; Buxton, 1933; Kreppel, chapter 4). However, there remains a critical need for information on the basic biology of many disease vector species, partly to be able to predict the timing of eradication methodologies. Ample evidence suggests that the temperature and humidity conditions during the developmental stages are important predictors of survival and adult vector abundance (Krasnov, 2002; Sharif, 1949). In Madagascar, currently the country with the highest annual number of human plague cases however, very limited published data on the biology of one of the two main vector species is available. The two vector flea species coexist on the island, but occupy different niches – the endemic flea *Synopsyllus fonquerniei* (*S. fonquerniei*) dominates rodent flea communities on rats caught outdoors, while the extensively studied ubiquitous oriental rat flea *Xenopsylla cheopis* (*X. cheopis*) (Klein, 1973;

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Margalit and Shulov, 1972; Mellanby, 1933; Sharif, 1949) is found mostly on rats caught in houses (Duplantier, 2005; Brygoo, 1966). The fleas' larval and pupal development is also thought to take place in different locations, in burrows found outdoors and indoors respectively. Below 800m altitude and in coastal areas however, *S. fonquerniei* is absent and *X. cheopis* is present both in and outdoors. The reasons for such difference in distribution are poorly understood and present a knowledge gap in the epidemiology of plague in Madagascar. Adaptation and thus habitat preferences govern the distribution of plague vector species elsewhere (Krasnov et al. 2001), and this could also be the case here, but too little is known about the biology of the endemic vector *S. fonquerniei*.

Here I present the first study investigating the temperature and humidity needs of *S. fonquerniei* under experimental conditions, by examining larval and pupal development as well as survival. Our main aim was to assemble original data on endemic *S. fonquerniei*, to provide information on the effects of climate on its development and survival, and to compare results with those for ubiquitous *X. cheopis*. As the insectarium of the Institut Pasteur de Madagascar (IPM) had colonies of both vectors, *S. fonquerniei* and *X. cheopis*, it seemed logical to use this opportunity test both in terms of their adaptation to climate, even more so as results on *X. cheopis* can be compared to existing research on this species acting as a control.

Larvae of vector species were used, and each individual larva was subjected to one of several temperature-humidity treatments. The impact of a range of temperatures and humidities on the larval and pupal development and larval survival was studied. Results also facilitated the estimation of the average number of thermal units needed by each species to complete the larval and pupal stage using the degree day method (Arnold 1959; Campbell 1974, Jones et al. 2005); a method frequently used to explain development rates of fungi, plants and insects in the wild (Eliopoulos, Kontodimas et al. 2010; Gu and Novak, 2006; Smits et al. 2003; Wermelinger and Seifert, 1998). This enabled us to link results obtained under laboratory conditions to research outcomes on flea abundance using field data on micro-climate conditions in the vector habitat. Finally I compared results on *X. cheopis* to a previous study on the development time of *X. cheopis* in relation to temperature and humidity.

5.2 METHODS

5.2.1 Rearing conditions of the two species

A proportion of the rodent flea life cycle is spent inside host burrows, with egg, larval and pupal development occurring entirely away from host species. Larvae hatch from eggs deposited inside host burrows and feed on plant debris and flea faeces containing dried blood. Once they reach a sufficient size, the larvae spin an oval shaped cocoon and emerge as adults.

Colonies of both vector species originated from adult fleas collected in 2001 from a total of 12 different highland regions (Antananarivo, Manjakandriana, Moramanga, Ambatondrazaka, Fianarantsoa, Ambositra, Ambalavao, Ankazobe, Miarinarivo, Betafo, and Antsirabe) of Madagascar. New specimens of both species from these regions were added every year until 2006. So a population bottleneck effect reducing the genetic variation has to be considered. Since collection, they have been maintained at the Medical Entomology unit at the Institut Pasteur Madagascar (Figure 5.1).
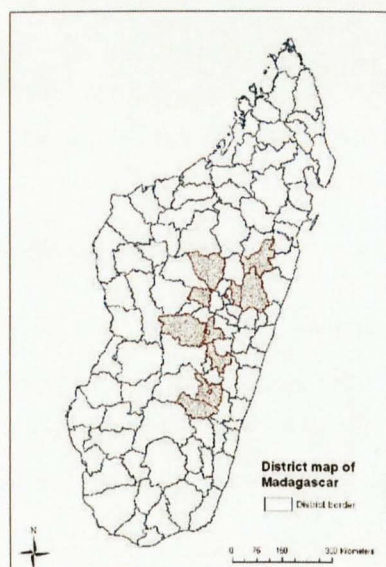
Figure 5.1: Districts of origin of fleas maintained in the insectarium of the Institut Pasteur de Madagascar

The fleas were reared in covered glass jars (Figure 5.2), 1/4 filled with a 50:1 mixture of rice husk and dried beef blood for larva nutrition, under constant conditions at a temperature of 27°C ± 2 and a relative humidity of 70% ± 5. To obtain sufficient numbers of larvae for the

experiment, recently fed, adult male and female fleas were transferred into empty glass jars and 2 gram of rice husk as a surface for oviposition was added. After 24 hours the adults were carefully removed and the jars with the eggs were left to stand in the insectariums for 6 days. The jars were checked once a day until newly hatched larvae were detected; larvae were only collected once from each jar to ensure the same age of the specimen. The humidity conditions under which the eggs are incubated do not affect the development time of larvae subsequently exposed to different conditions (Krasnov, 2001).

Figure 5.2: Covered glass jars containing *X. cheopis* fleas in rice husk with nutrient mix

5.2.2 Setting and monitoring climate conditions

Saturated salt solutions were used to create different relative humidities within airtight plastic boxes. The boxes were each filled with 1.5cm of saturated salt solution to create "humidity chambers" (Figure 5.3a). After a method described in detail by Winston and Bates (1960) and widely used since (Abogast, 1972; Rockland, 1960), Ammonium Sulphate (NH$_4$) SO4 and Sodium Carbonate NA$_2$ CO$_3$ were employed to allow different relative humidities (RH) within the boxes. Temperature treatments employing incubators (NUVE ES 120, UK and Binder KBF, GER) ranged from 18°C to 32°C (18, 21.5, 25, 28.5 and 32°C) and relative humidity treatments were 80 and 90%RH (±2%). The relative humidities reflect conditions recorded inside Madagascan rat burrows during the study presented in chapter 4.

Incubators were each set at one of the 5 constant temperatures and the boxes inserted (Figure 5.3b); thus providing 10 different experimental treatments. Humidity and temperature readings were taken in each box 24 hours before use and intermittently during the experiment with hygrometers (Oregon Scientific 433MHz Cable free Hygrometer) to ensure consistent climatic conditions.

Figure 5.3a: Airtight plastic boxes (humidity chambers) containing saturated salt solutions creating different humidities

Figure 5.3b: Plastic boxes (humidity chambers) with saturated salt solutions and flea larvae inside incubator

5.2.3 Experimental set up

Specimen

Newly hatched *S. fonquerniei* (n=1288) and *X. cheopis* (n=750) larvae up to 12 hours old were transferred onto halved ELISA-plates (NUNC MaxiSorp™ polystyrene 96 well), with one larva per well, 5mg of larval rearing medium (powdered rodent diet, dried beef blood and dried yeast at a ratio of 20:3:1 respectively) was also inserted. As a higher mortality rate became apparent, more *S. fonquerniei* larvae were reared than *X. cheopis*. Before being placed on stands in the humidity chambers within the incubators, the plates containing larvae were covered with fine nylon mesh to prevent escape during the first two days when the larvae were most active.

Protocol of data recording

Survival and cocoon formation (also referred to as pupation) was noted daily and the developmental progress of the larval stages of both species was recorded every second day by placing the transparent ELISA plate under a microscope lit from below. Pupation was taken as an indicator of the completion of the active larval stage, and could be established to within an accuracy of 24 hours. Larvae were observed until they either pupated or died except for those which did not pupate after twice the time of the majority of their counterparts; these although still alive were assumed to have died and were removed from the study population. After pupation, cocoons were individually transferred into 5 ml Eppendorfs and sealed with fine nylon mesh to prevent the adult flea escaping once hatched. Each cocoon was then subjected to the same conditions as the larva before pupation. Wells with cocoons glued to the bottom were sealed with perforated Para film and checked daily for emerged adults. Eppendorfs and plates with cocoons were shaken every day to stimulate emergence (Marshall, 1981). Once emerged, the adults were immobilised using ice, and they were sexed and their species confirmed.

5.2.4 Statistical analysis

Flea development: time to pupation and time to emergence

The data was divided into two main sets for analysis: observed development time to pupation and observed development time to emergence. For each dataset, linear mixed effects models were used to examine differences between species and the effects of temperature and

humidity on development time. Temperature was log transformed. The data collected on larval and pupal development time were initially tested for normality using an Anderson Darling test. Cleveland plots were used to examine for violation of homogeneity. Outliers greater than the mean ± 2 standard deviations were removed from the analysis. There was a potential of non-independence of samples, as the individual larvae, and later pupae of one plate were likely to be from the same batch of eggs. To account for non-independence of egg batches, plate was included as random effect in all models.

The maximal model for each dataset included interactions between species, humidity and log temperature. Model selection was based on the Akaike information criterion (AIC; Akaike 1973) and the principle of parsimony prioritising simplicity. The analysis was carried out using the nlme package (Pinheiro et al. 2007) in R 2.11.1 for Windows R (R Development Core Team, 2007).

5.2.5 Mortality and time of death

For both species, the mortality rate at each development stage was calculated by dividing the number of dead individuals by the number of individuals entered for each treatment and expressed as a percentage. Using the proportion of larvae in a plate that died as the response variable, generalised linear models with binomial errors were used to examine the impact of species, temperature and humidity on mortality rates. Larvae that did not die or pupate by the end of the experiment were excluded from this analysis (n=46 for *S. fonquerniei* and n=9 for *X. cheopis*).

To investigate the effect of species, temperature and humidity on the timing of death, survival analysis was applied to the dataset on larval development. Pupal survival was not studied, as the time of death within their cocoon could not be established. Analysis was based on a cox proportional hazard model. Model selection was based on AIC values. Duration of survival and successful completion of the larval stage by pupating are two different things as one shows the successful completion of larval development while the other shows that survival is possible but the conditions are not good enough to pupate and censoring was applied when larvae left the experiment by pupating. Larvae that had not died or pupated at the end of the experiment were also censored. Survivorship over time was plotted as survival curves for

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each treatment and species and was based on the number of larvae at risk and each day's events of death and pupation.

5.2.6 Degree-day analysis

The thermal units needed by both larvae to reach pupation and by pupae to complete development were estimated using degree-day analysis (Campbell, 1974). This analysis was undertaken for both humidities and for each species separately. As development time decreased with increasing temperature in both species, a regression line was fitted to the linear part of the observed data, thus omitting data at 32°C, where development time started to level off, hence diverging from linearity. This was carried out for both humidities together as well as separately. The linear regression equation, of the form y=a+bx, was extrapolated to the x-intercept to estimate the lower developmental threshold. The reciprocal of the slope of the linear regression was then used to estimate the thermal constant (K-value) of each species (Arnold, 1959). The K-value defines the sum of thermal energy above the threshold required for 50% of the population to complete their development stage. The thermal constant is expressed in degree-days (DD). By using the thermal units calculated for each species together with data on mean temperature conditions in rodent burrows in the field (chapter 4), the predicted development time for larvae in rat burrows for each field trip and species was established.

5.2.7 Comparison with earlier study

Sharif previously published a study on the development time of *X. cheopis* from India in 1949 in relation to temperature and humidity (Sharif, 1949). Part of his data on larval and pupal development time at 90%RH was used to compare to these results on the same species. The mean development times in days until pupation and until pupal emergence for *X. cheopis* of both studies were plotted against each other and significant differences between them examined using a t-test ($P \leq 0.05$).

5.3 RESULTS

Analysis of the raw data revealed that more individuals of the ubiquitous species *X. cheopis* completed both development stages successfully and emerged as adults than the endemic *S. fonquerniei*. Of 100% of *S. fonquerniei* larvae (n=1288) included in the study, 19.02% successfully completed the larval stage but less than 6% of the total larvae included hatched

(Figure 5.4). The sex ratio of the emerged *S. fonquerniei* was 2.5 females to every male; *X. cheopis* had 1 female per 1.27 males (Figure 5.5).

Figure 5.4: Percentage of total individuals completing the larval stage and percentage of total individuals completing the pupal stage of S. *fonquerniei* (in blue) and *X. cheopis* (in red) respectively
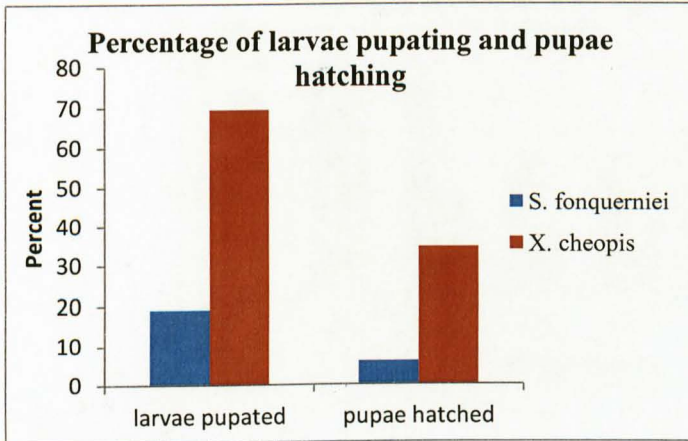
Figure 5.5: Sex ratio of emerged adults of *S. fonquerniei* and *X. cheopis* (males in blue, females in red)
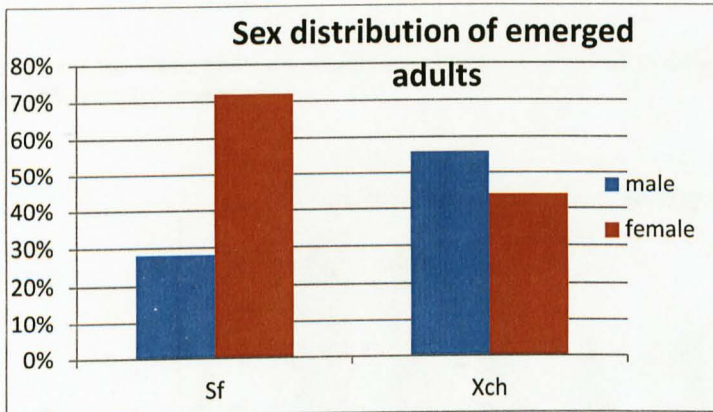
For the mean larval development times regardless of humidity the differences between species were significant at every temperature except at 18°C (Figure 5.5). Larvae of the endemic species *S. fonquerniei* took on average 1.79 times longer to develop across treatments than *X. cheopis*. At 80%RH unlike at 90%RH, the species differed at a temperature of 21°C (Figure 5.6a). At 25°C the reverse was seen (Figure 5.6b) (80%RH no difference, 90%RH significant difference). The pupal development times, were neither significantly different between species nor between humidities at any temperature.

Figure 5.5: Mean time to pupation of *S. fonquerniei* (in blue) and *X. cheopis* (in red) at each temperature regardless of humidity treatment
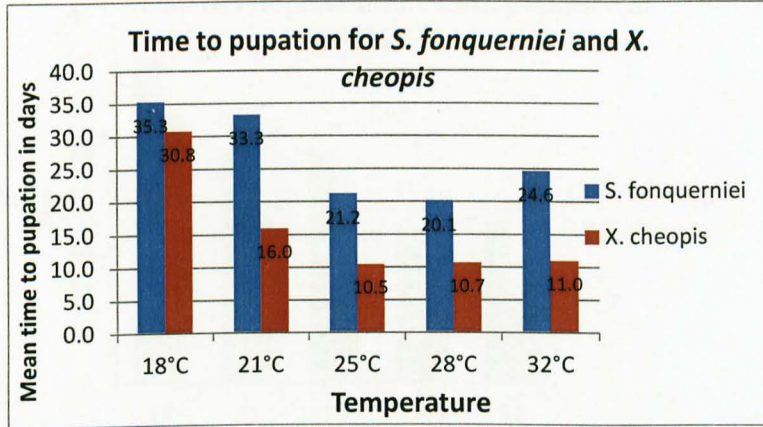
Figure 5.6a: Mean time to pupation of *S. fonquerniei* (in blue) and *X. cheopis* (in red) at each temperature at 80% relative humidity
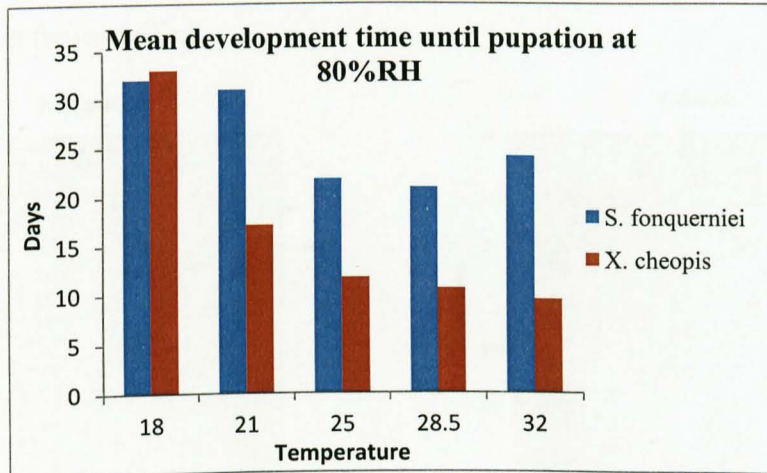
Figure 5.6b: Mean time to pupation of *S. fonquerniei* (in blue) and *X. cheopis* (in red) at each temperature at 90% relative humidity

Mean development time until pupation at 90%RH

■ S. fonquerniei
■ X. cheopis
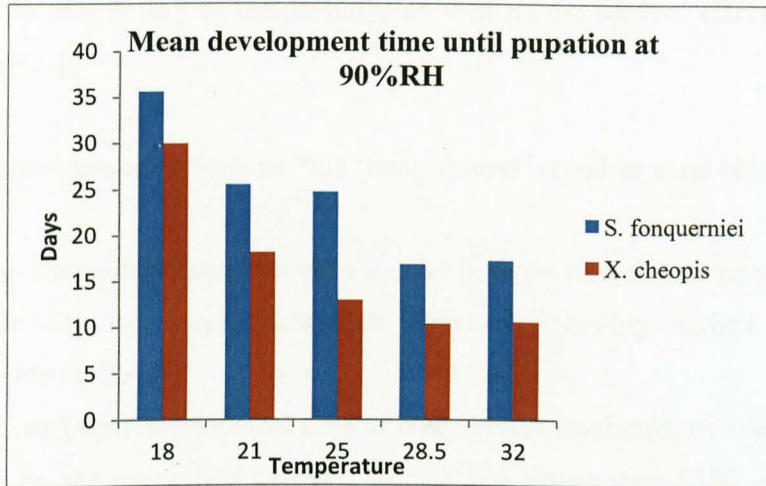
The effects of treatment on development time

Cleveland plots showed strong heterogeneity of variance in both datasets with the variance being larger at treatments with lower temperatures (Figure 5.7a; b). To correct for this, a fixed variance structure as a function of temperature was used by applying a weights argument during the modelling.
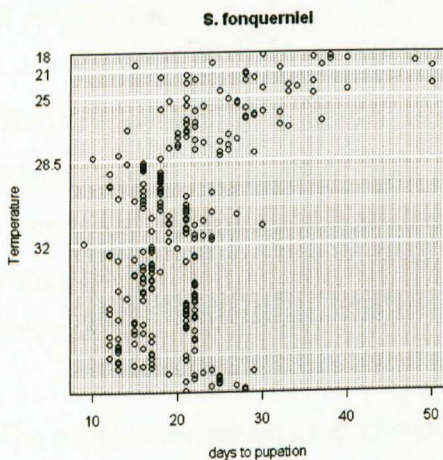
Figure 5.7 a) Cleveland plot of the time to pupation by larvae of *S. fonquerniei* at different temperatures
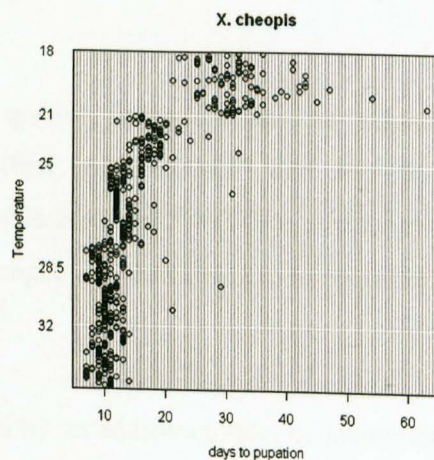
Figure 5.7 b) Cleveland plot of the time to pupation by larvae of *X. cheopis* at different temperatures

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Plate was confirmed to affect model performance and was included as a random effect.

For the data set on larval development of both species combined, development time was best explained by the full model: 3-way interaction between temperature, humidity and species entered as fixed effects (log of temperature) as well as the random effect of plate (AIC: 4352.65) (Table 5.1).

development time =humidity *species *log (temperature) + random plate effect

Results confirm strong differences between species in terms of associations with temperature as well as humidity and development time. However, humidity seemed to be the least important variable for both.

For the data set on pupal development time of both species combined, development time was best explained by the interaction between species and temperature (AIC: 2222.65) (Table 5.2).

development time = species *log (temperature) + random plate effect

Adding an interaction with humidity does lower the AIC (AIC: 2220.76), but not enough to justify adding a variable to the model. The coefficients indicate that development time decreases with increasing temperature but that the decrease is greater for *X. cheopis* than for *S. fonquerniei*.

The development time of the larvae of both species separately can be explained by temperature only, combined with the random effect (AIC: 2894.57 and 1464.56 for *X. cheopis* and *S. fonquerniei* larvae respectively) (Table 5.3a and 5.4a). The development time until pupation for *X. cheopis* and *S. fonquerniei* is negatively correlated to temperature (Table 5.5b and 5.6b respectively).

The pupal development of *X. cheopis* is influenced by an additive model of temperature and humidity with the random effect (Table 5.5a), suggesting that the effect of humidity is the same at all temperatures. Pupal development time is negatively correlated to temperature and is faster at 90% relative humidity than at 80% humidity (Table 5.5b).

The pupal development time of *S. fonquerniei* is best explained by temperature and the random effect only (Table 5.6a). Temperature is negatively correlated to development time (Table 5.6b).

Table 5.1 Modelling results for the effects of humidity, temperature and PCR plate on larval development time until pupation for both vector species combined. a) AIC models in bold show the most parsimonious models (simplest model within 2 of the lowest AIC value). b) best model

a)

LARVAE	
X. cheopis and S. fonquerniei	AIC
hum*species*logtemp,random	**4352.656**
hum+species+logtemp,random	4363.701
species*logtemp,random	4364.47
hum*species,random	4411.236
species,random	4417.476
logtemp,random	4501.787
hum*logtemp,random	4505.539
hum,random	4533.142
hum+logtemp,random	4503.551

b)

LARVAE			
X. cheopis and S. fonquerniei			
Variable	coeff	SE	p-value
(Intercept)	76.84744	21.01972	0.0003
logtemp	-16.331	6.44616	0.015
hum90	61.30743	26.44951	0.0253
speciesX	64.03241	22.72372	0.005
logtemp:hum90	-19.4302	8.113	0.0211
logtemp:speciesX	-22.2348	6.88415	0.0013
hum90:speciesX	-112.964	31.74765	0.0004
logtemp:hum90:speciesX	35.18419	9.63053	0.0003

Table 5.2 Modelling results for the effects of humidity, temperature and PCR plate on pupal development time from pupation until hatching for both vector species combined. a) AIC models in bold show the most parsimonious models (simplest model within 2 of the lowest AIC value). b) best model

a)

PUPAE	
X. cheopis and S. fonquerniei	AIC
hum*species*logtemp,random	2220.76
hum+species+logtemp,random	2224.418
species*logtemp,random	**2222.654**
hum*species,random	2270.709
species,random	2269.508
logtemp,random	2225.624
hum*logtemp,random	2223.683
hum,random	2268.228
hum+logtemp,random	2223.784
hum+species,random	2269.177

b)

PUPAE			
X. cheopis and S. fonquerniei			
Variable	coeff	SE	p-value
(Intercept)	90.8729	19.85436	0
logtemp	-22.55872	6.046875	0.0006
speciesX	55.79563	23.44456	0.018
logtemp:speciesX	-16.50706	7.161924	0.0219

Table 5.3 Modelling results for the effects of humidity, temperature and PCR plate on larval development time until pupation for *X. cheopis*. a) AIC models in bold show the most parsimonious models (simplest model within 2 of the lowest AIC value). b) best model

a)

LARVAE	
X. cheopis	AIC
logtemp*hum,random	2893.922
logtemp+hum,random	2895.874
logtemp,random	**2894.577**
hum,random	2930.12

b)

X. cheopis			
Variable	coeff	SE	p-value
(Intercept)	127.7596	12.35161	0
logtemp	-34.8314	3.870842	0

Table 5.4 Modelling results for the effects of humidity, temperature and PCR plate on larval development time until pupation for *S. fonquerniei*. a) AIC models in bold show the most parsimonious models (simplest model within 2 of the lowest AIC value). b) best model

a)

LARVAE	
S. fonquerniei	AIC
hum*logtemp,random	1463.15
hum+logtemp,random	1466.344
logtemp,random	**1464.56**
hum,random	1488.803

b)

S. fonquerniei			
Variable	coeff	SE	p-value
(Intercept)	108.7598	13.37009	0
logtemp	-26.2734	4.122514	0

Table 5.5 Modelling results for the effects of humidity, temperature and PCR plate on pupal development time from pupation until hatching for *X. cheopis*. a) AIC models in bold show the most parsimonious models (simplest model within 2 of the lowest AIC value). b) best model

a)

PUPAE	
X. cheopis	AIC
logtemp*hum,random	1736.721
logtemp+hum,random	**1736.462**
logtemp,random	1739.948
hum,random	1770.905

b)

X. cheopis			
Variable	coeff	SE	p-value
(Intercept)	142.2746	13.42756	0
logtemp	-37.1067	4.221907	0
hum90	-4.10648	1.678683	0.0221

Table 5.6 Modelling results for the effects of humidity, temperature and PCR plate on pupal development time from pupation until hatching for *S. fonquerniei*. a) AIC models in bold show the most parsimonious models (simplest model within 2 of the lowest AIC value). b) best model

a)

PUPAE	
S. fonquerniei	AIC
logtemp*hum,random	477.6646
logtemp+hum,random	477.9938
logtemp,random	**476.1426**
hum,random	493.6724

b)

S. fonquerniei			
Variable	coeff	SE	p-value
(Intercept)	90.64506	14.88847	0.00E+00
logtemp	-22.531	4.539804	1.00E-04

Mortality

The calculation of mortality rates reveals that *S. fonquerniei* larvae have, on average, a 43% higher mortality rate than *X. cheopis* across treatments. As *S. fonquerniei* showed much higher mortality rates (Figure 5.8 a and b), the analysis of mortality rates was conducted on each species separately (Figure 5.9). Due to over dispersion, quasibinomial models were used for each species. The best model for *X. cheopis* only included an effect of temperature, with the larval mortality rate declining at higher temperatures (β=-2.42, SE=0.68, P=0.002). For *S. fonquerniei,* the best model had an effect of both temperature and humidity, with mortality rates declining at higher temperatures (β=-6.15, SE=1.05, P<0.001) and higher humidities (β=-0.97, SE=0.37, P=0.013). However, an interaction between temperature and humidity was close to significance (β=-3.90, SE=2.06, P=0.067), suggesting that the decrease in

mortality rate at 90% humidity was specifically pronounced at higher temperatures (Figure 5.9).

Figure 5.8: Larval mortality rate per species at each temperature a) at 80% relative humidity b) at 90% relative humidity.

a)

b)
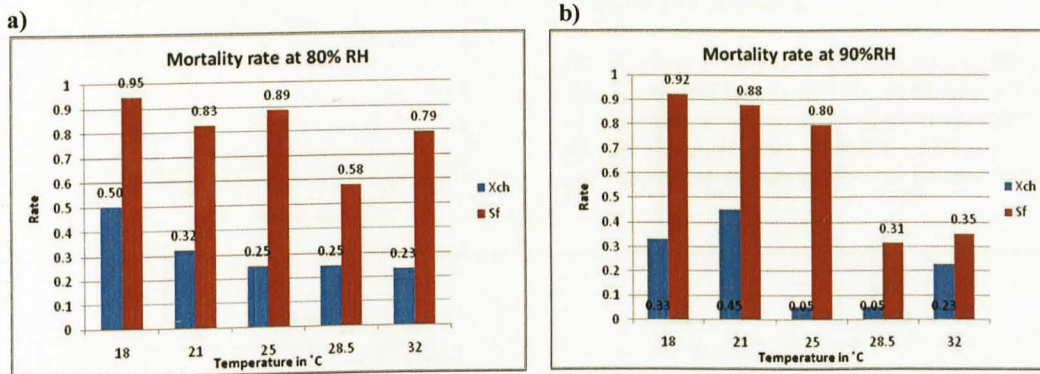
The steeper survivorship curves for *S. fonquerniei* indicate that this species not only has higher overall larval mortality but also that mortality tends to occur faster than for *X. cheopis*. The Cox proportional hazard analysis for *X. cheopis* indicated that time to death was influenced by humidity only, with larvae tending to live longer at higher humidities (90% humidity β= -0.338, SE =0.14, P=0.018; approximate hazard P=0.713). For *S. fonquerniei* the best model had an interaction between temperature and humidity, such that at higher temperatures larvae lived longer at 90% humidity compared to 80% humidity, but that at lower temperatures there was little difference between the two humidity treatments (Table 5.7 and Figure 5.10).

Table 5.7 Results from the Cox proportional hazard analysis on time to death for *S. fonquerniei*. Hazard values are approximate hazard values, calculated by taking the exponential of the coefficient.

Variable	Coefficient	Hazard	Standard Error	P-value
Logtemp	-0.294	0.745	0.213	0.168
Humidity	3.504	33.25	0.968	<0.001
Logtemp * humidity	-1.194	0.302	0.310	<0.001
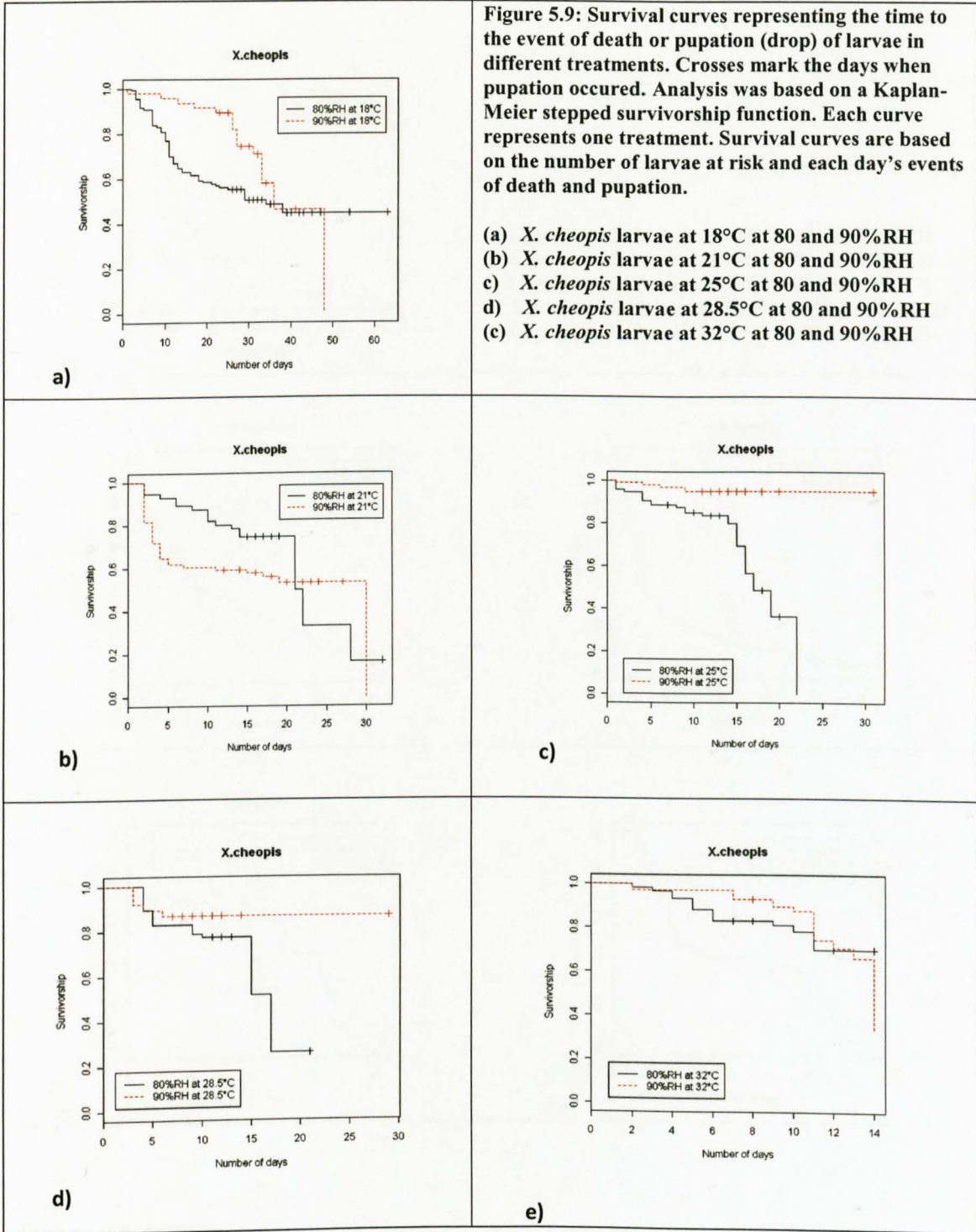
Figure 5.9: Survival curves representing the time to the event of death or pupation (drop) of larvae in different treatments. Crosses mark the days when pupation occured. Analysis was based on a Kaplan-Meier stepped survivorship function. Each curve represents one treatment. Survival curves are based on the number of larvae at risk and each day's events of death and pupation.

(a) *X. cheopis* larvae at 18°C at 80 and 90%RH
(b) *X. cheopis* larvae at 21°C at 80 and 90%RH
c) *X. cheopis* larvae at 25°C at 80 and 90%RH
d) *X. cheopis* larvae at 28.5°C at 80 and 90%RH
(c) *X. cheopis* larvae at 32°C at 80 and 90%RH

Figure 5.10: Survival curves representing the time to the event of death or pupation (drop) of larvae in different treatments. Crosses mark the days when pupation occured. Analysis was based on a Kaplan-Meier stepped survivorship function. Each curve represents one treatment. Survival curves are based on the number of larvae at risk and each day's events of death and pupation.

a) *S. fonquerniei* larvae at 18°C at 80 and 90%RH
b) *S. fonquerniei* larvae at 21°C at 80 and 90%RH
c) *S. fonquerniei* larvae at 25°C at 80 and 90%RH
d) *S. fonquerniei* larvae at 28.5°C at 80 and 90%RH
e) *S. fonquerniei* larvae at 32°C at 80 and 90%RH

Degree-day analysis

By applying the degree-day calculation method (Campbell, 1974), the mean number of thermal units (k-value) needed by *S. fonquerniei* and *X. cheopis* to complete their larval and pupal stage at 80-90%RH was estimated to be 682 (larvae: 362 and 158) and 425 degree-days respectively. The average developmental threshold is 3.48 degrees °C lower for the

endemic *S. fonquerniei*. Table 5.8a and b show the threshold temperatures estimated via linear regression (Figure 5.11a and b) and their corresponding r^2 values and k-value for larvae of each species at both temperatures together and separately.

To be able to use the information on larval development gained in the lab, the thermal units needed by the larvae to complete their development stage were calculated using the degree-day method (Arnold, 1959):

Data were plotted as the developmental rate (i.e. Time $^{-1}$) and departures from linearity at the top or bottom temperatures were excluded (Figure 5.11a and b). It is common practise to find a regression line with an adequate r^2 value and a biological sensible intercept/temperature threshold by omitting data points that do not fit into a linear line (Collier, Finch, 1985; Naves, Sousa, 2008).

Figure 5.11: Linear regression between the rate of development flea larvae and temperature at 80 to 90% relative humidity a) of *S. fonquerniei* b) of *X. cheopis*.

a) *S. fonquerniei*　　　　　　　　　　　　　　b) *X. cheopis*
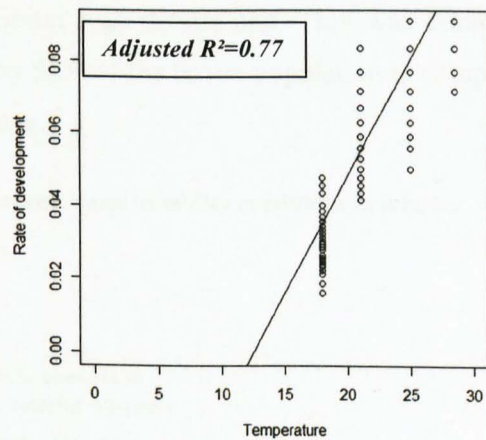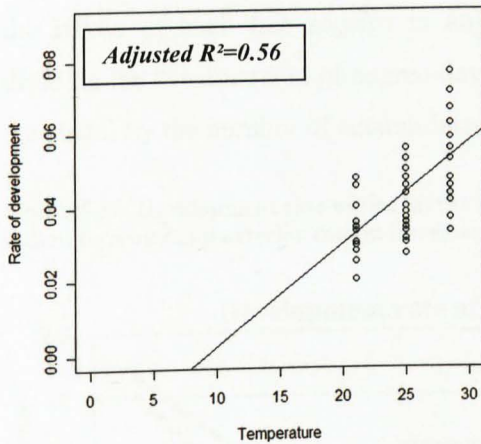
Using the slope and intercept, the K-value, (sum of degree-days required for 50% of the population to complete their stage), and the threshold temperature were established (the temperature below which no development will occur). This method was applied to the data of both humidities together and separately.

By using the thermal units calculated for each species from the laboratory data together with data on temperature in the field, the predicted development time for larvae in rat burrows was

established. For all burrow types - house burrow and outside burrow *X. cheopis* larvae were found to develop faster, especially in the warm and wet season.

Table 5.8 a: Thermal units needed by *X. cheopis* larvae to complete development in rodent burrows

Xenopsylla cheopis larvae			
Humidity	80% + 90%	80%	90%
Threshold	12.36	12.34	12.71
R ² - value	0.77	0.75	0.76
K - value	158.15	158	155

Table 5.8 b: Thermal units needed by *S. fonquerniei* larvae to complete development in rodent burrows

Synopsyllus fonquerniei larvae			
Humidity	80% + 90%	80%	90%
Threshold	9.26	9.14	8.57
R ² - value	0.45	0.40	0.47
K – value	361.6	360.77	345

After establishing the accumulated number of degree days in any given month via the website calculations, using the single triangulation method (Roltsch, 1999), a development ratio for the larvae of each flea species in any given habitat was determined. This was done by dividing the k-value (sum of degree-days needed by 50% of the larvae population to complete the stage) by the number of accumulated degree days.

Figure 5.12: Development rate of flea larvae under temperature and humidity conditions in interior rodent burrows and exterior rodent burrows.
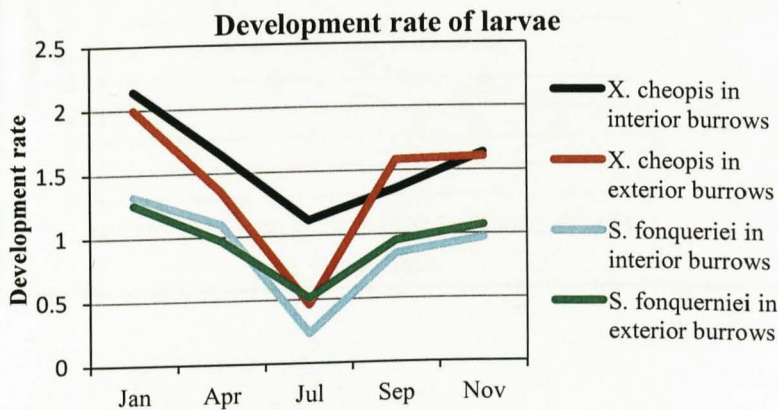
S. fonquerniei larvae development shows strong seasonality in both habitats while *X. cheopis* larvae development is much less seasonal inside, indicating that this species may be adapted to a warmer climate (Figure 5.12).

Comparison with earlier study

When plotting the mean time taken to pupation at 90%RH of *X. cheopis* larvae reared by Sharif and our *X. cheopis* larvae, both curves show remarkably similar trends (Figure 5.13a) and no significant difference (P=0.25). The same was done for the pupal development (Figure 5.15b). Here again the same trend is apparent and there is no significant difference (P=0.36) As the two studies compared here did not use the same temperatures, the crossing of the trend lines is most likely due to the exponential increase in development time at lower temperatures.

Figure 5.13: Development time under different temperatures with 90% relative humidity as recorded for *X. cheopis* larvae by Sharif in black and by this study in red. a) Time of larval development until pupation b) Time of pupal development

a)   Time of larval development until pupation
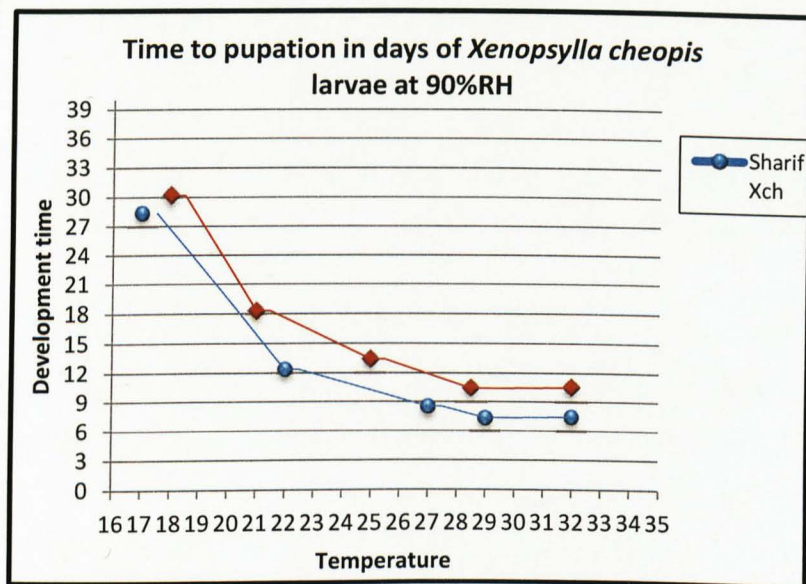
b) Time of pupal development until hatching

Time of pupal development until emergence in days of *Xenopsylla cheopis* at 90%RH
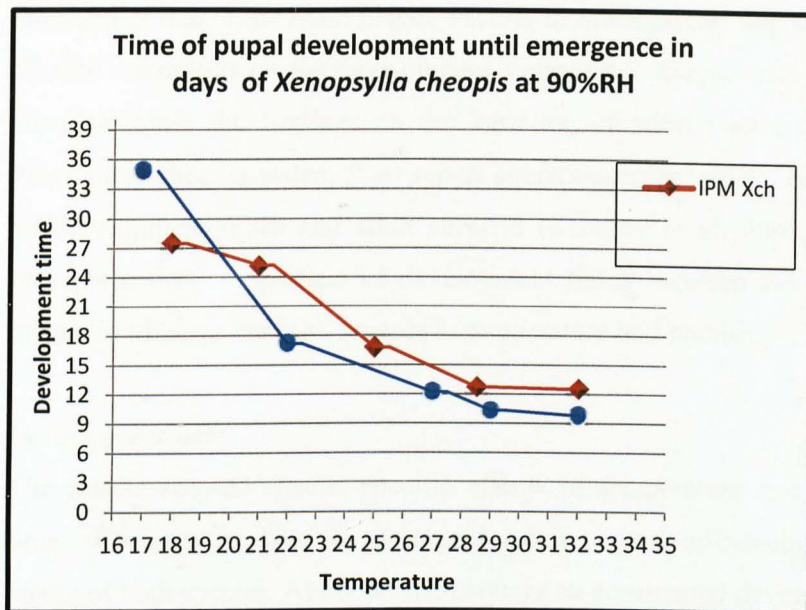
5.4 DISCUSSION

The main aim of this study was to gather basic knowledge on the biology of the endemic *S. fonquerniei* one of the main plague vectors in Madagascar. Especially to gain information of climate constraints on the development stages of *S. fonquerniei*. The laboratory results also elucidate more the findings on the variation of adult vector abundance from chapter 4. Previous studies on rodent fleas report species-specific effects of humidity and temperature on development times and adult survival (Krasnov et al. 2001; Sharif, 1949). This study presents a first comparison of development times between the two key vector species of plague in Madagascar with regards to temperature and humidity.

Development time

The results showed species-specific effects of temperature and humidity on development time. Temperature was the dominant factor overall influencing the development rate of larvae of both species. At higher temperature an accelerated development of larvae and pupae was observed in both species. The effect of humidity was less prominent, with an effect only found on pupal development of *X. cheopis*. In addition to the positive effects of temperature, *X. cheopis* pupae developed faster at higher humidity. Larvae of *S. fonquerniei* developed much slower than the larvae of *X. cheopis* at all temperatures apart from the lowest (18°C). Pupal development time was more similar for the two species, but, as with larvae, pupal development in *X. cheopis* was more sensitive to declining temperatures.

Mortality

The higher temperature treatments showed lower mortality rates of larvae in both species. For *S. fonquerniei*, higher humidity also decreased mortality. This effect of humidity is likely to be pronounced at higher temperatures as the interaction term was close to significance. Results from the field study presented in the previous chapter (chapter 4) on the effect of temperature and humidity on the variation in adult vector abundance, suggested a negative effect of humidity on *S. fonquerniei*. The opposite was found under laboratory conditions, where larvae of *S. fonquerniei* subjected to humidities similar to those recorded in the field showed lower mortality at higher temperatures with higher humidity. This is an indication for the differences of laboratory conditions compared to the field and implies that any conclusions have to be treated with care.

Effects on abundance

Results of this study confirm some of the findings established from the study on the effect of micro-climate in host burrows on the variation in abundance of adult fleas in the highlands of Madagascar. The laboratory experiment presented here and the field studies described in chapter 4, show species specific temperature and humidity preferences and suggest different developmental thresholds. Both, the laboratory and field results indicate that *S. fonquerniei* are more tolerant of low temperatures than *X. cheopis*. However, results from the field also imply a possible lower temperature threshold affecting the abundance of adult *S. fonquerniei* negatively. Such a temperature appears to be below the temperatures used in the laboratory experiment.

Stenseth (2006) suggests that high flea abundance observed in a plague focus in Kazakhstan in spring is mediated by faster development times and increased fecundity due to favourable climatic conditions. Flea abundance in wet summer months is proposed to be the result of increased adult survival. In Madagascar the dry and cold season from April to September seems to be a key period in the seasonality of vector abundance. The temperatures the flea larvae and pupae were subjected to in the laboratory were not low enough to capture mid-winter conditions recorded in the field; however 18°C is reasonable for at least some of this period. The results suggest that at this temperature development occurs but is slowed down. In contrast the humidities used are rather low to mimic the conditions in exterior burrows during the crucial November-January period when *S. fonquerniei* numbers decline.

X. cheopis was found to prefer higher temperatures in the laboratory and in the field alike. This is consistent with results from Sharif (1949) and Mellanby (1933). Chapter 4 suggests that together with an effect of current temperature *X. cheopis* abundance tends to be higher in periods that have benefitted from high temperatures for several months. This effect of temperature is consistent with the results from the laboratory experiment, where a drop of 3 °C from 21 to 18 °C almost doubled its larval development time and increased mortality by a third.

The results of this study propose *X. cheopis* patterns could be driven by low rates of development from May to September and adult survival also seems to be an issue. Krasnov (2001) found that pupae of *Xenopsylla* species survive better at low humidities than adults. This study here does not indicate that effects of relative humidity and temperature on larval

and pupal development can fully explain the spatial patterns of *X. cheopis*; however, low rates of development coupled with high adult mortality during the dry and cool months are unfavourable for abundance.

In the field, the abundance of *S. fonquerniei* adults peaked following the cooler and drier period and declined during the rainy season, and showed a strong negative relationship with humidity. Consequently, changes in adult abundance do not seem to reflect predicted changes in development rates according to laboratory results. When using the results from the laboratory experiment and combining them with data on micro-climate conditions of the larval and pupal vector habitat (interior and exterior rodent burrows), the estimated development times for *S. fonquerniei* predicts slow development rates during the dry cold month of July and faster development rates during the warm, rainy season. There is also no suggestion that high abundance of adults in September and the low abundance in November-January is driven by seasonal variation in survival rates of larvae and pupae, as survival rates and time to death in the laboratory increased with temperature and humidity rather than declined. This seasonal decline of *S. fonquerniei* observed in the field cannot be explained by the results obtained in the laboratory study. Most likely it is due to effects on another part of the flea life cycle such as adult survival or destruction of eggs, larvae and pupae in the host burrow where high humidity during this period is coupled with a high organic load promoting the growth of destructive fungi (Cavanaugh, Marshall, 1972; Parmenter et al. 1999).

Limitations of the study

This laboratory study was a first attempt to quantify the effects of temperature and humidity on the developmental stages of *S. fonquerniei* and compare them to those of *X. cheopis*. It also served to show obstacles and limitations which have to be addressed in any further investigations. The range of temperatures and humidities used in this study certainly is a limiting factor and further laboratory work would be necessary to determine both the upper and lower developmental threshold temperatures for both species. There was not enough data generated from the 10 treatments applied to provide enough information and a study with a wider range of temperatures and humidities and constant as well as fluctuating climate conditions would certainly improve findings. Also from this experiment the importance of humidity could not be ascertained and results were opposing findings from those collected in

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the field. It would be interesting to extend analysis and investigate the effect of saturation deficit, as some of the results on decreased mortality in treatments with higher temperature and higher humidity compared to lower humidity imply a detrimental effect of drying power on the larvae. The fact that we used fleas from established colonies increases the potential to introduce bias, such as adaptation of the flea species to insectarium conditions due to natural selection. The comparison of results by Sharif of Indian *X. cheopis* with the *X. cheopis* from Madagascar however, shows no significant difference in development times, increasing confidence in our results. Yet for *S. fonquerniei*, the sex ratio found in the laboratory study was highly in favour of females, and could have distorted results on development times as females of this species are bigger in size and therefore would need longer to develop. The sex ratio found on adults in the field was also in favour of females, if not as strong, so there is no way of telling if a bias was introduced or not.

General implications of the study

Our results do not offer an explanation for the success of the endemic flea species in the highlands of Madagascar. The larvae of *X. cheopis* developed significantly faster in all treatments but at the lowest temperature, raising the question why it is not more dominant in the exterior rodent flea communities in the highlands. Our results point towards the possibility that the endemic *S. fonquerniei* is better adapted to colder conditions found in the plague regions. This is supported by the degree-day analysis, estimating the developmental threshold of *S. fonquerniei* to be 3.49°C lower than that of *X. cheopis* and thus giving the endemic *S. fonquerniei* an advantage in the colder regions of the island. However, due to the low number of larvae pupating at 18°C, particularly for *S. fonquerniei*, there was only limited power to estimate lower development rates and thresholds and compare them between species. Even though many questions remain, our study provides valuable information adding to findings from field studies. Our results are a first step towards understanding the vector ecology in Madagascar and the methods presented show a way to combine lab data with field data.

CHAPTER SIX

GENERAL DISCUSSION

6.1 GENERAL DISCUSSION

In this thesis the influence of climate was explored from different angles and at different scales. The findings fill some of the knowledge gaps still present in the understanding of the epidemiology of plague. Throughout the thesis consideration is given to the impact findings could have on ecological processes associated with the epidemiology of the disease in addition to the study of climatic effects on plague.

Climate impacts on all elements of plague (host, vector, and pathogen) in various ways and over a wide range of scales from the micro-climate in host burrows affecting vector development to global climate phenomena influencing human plague incidence. Thus it seems reasonable to look at plague from different angles. Studies of many foci aiming to understand the links between climate and plague cover different scales (Ben Ari et al. 2011; Koelle, 2009). Here effects of large scale climate on human plague incidence, associations of climate variables on the spatial pattern of the disease and the influence of micro-climate on flea vectors have all been explored to provide a comprehensive picture.

The effect of large scale climate phenomena on plague incidence

Global climate phenomena, such as the El Niño Southern Oscillation (ENSO) and the Indian Ocean Dipole (IOD), affecting the periodicity of many vector-borne infectious diseases (Ashok et al. 2003; Zell, 2004) were found to be associated with the temporal distribution of plague cases in Madagascar throughout the last fifty years. Interestingly, a changing relationship between plague incidence and climate most likely mediated by changes in the strength and timing of ENSO and the IOD was established. The correlation between ENSO and plague turned from negative to positive and the association with the IOD became stronger with time. Similar non-stationary changes over time have been found for correlations between cholera and the El Nino Southern Oscillation (Rodo et al. 2002). A consistent association between ENSO and cholera outbreaks was found for the time period 1980–2001. This association with cholera becomes weaker and eventually uncorrelated during the periods 1893 to 1920 and 1920 to 1940 respectively.

Changes in the association between ENSO, sea surface temperatures and several diseases are most likely due to changes of ENSO itself in the past decades. The ENSO system is a strong driver of inter-annual variability in global climate (Gagnon, 2001; Kovats, Bouma et al. 2003;

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Kausrud, Viljugrein et al. 2007; Luo et al. 2010). From 1976, a shift towards warmer and wetter conditions in the tropical Pacific was detected, with widespread climatic and ecological consequences (Graham, 1994). This could explain the changes in the detected associations between human plague and global climate in Madagascar.

In other plague foci plague was also linked to global climate. In China, an increased rate of human plague is associated at the province level with the Southern Oscillation Index and Sea Surface Temperature of the tropic Pacific east equator (Zhang et al. 2007). In the US, inter-annual variability of human plague has been linked to global climate phenomena via its effect on the rodent host community (Ben Ari et al. 2010). Human plague incidence was shown to depend both on time-lagged precipitation events, presumably increasing rodent populations, and on relatively cool summer temperatures during the plague transmission season, supporting infectious fleas (Enscore et al. 2002).

Any of these established associations between large scale climate events and plague incidence are most likely due to an influence on temperature and precipitation. These are known to affect host and vector ecology and transmission potential (Kartman, 1969; Parmenter et al. 1999; Stenseth et al. 2008).

Effects of environmental variables on the spatial distribution of plague cases

Plague foci are present over an expanded geographical range including the US, South America, East and South Africa and Southeast Asia (WHO, 2008) and the disease manifests itself under various ecological conditions (Prentice and Rahalison, 2007; Dennis et al. 1999). To investigate the mechanisms via which climate affects plague in Madagascar further, environmental variables associated with climate in other foci were examined. Remotely-sensed environmental variables proved useful predictors of the spatial distribution of the disease in other foci when empirical data were lacking. Debien et al. (2010) found a significant relationship between monthly rainfall in the main Tanzanian plague focus and monthly composite normalised difference vegetation index (NDVI) and then established a strong link between plague incidence and rainfall patterns. In a similar study by Neerinckx (2010) elevation and vegetation cover derivates have been found to be the most important drivers for human plague occurrence in Tanzania. Using a similar method the aim was to gain a more detailed understanding of the temporal and spatial links between climate and plague.

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Human plague cases in Madagascar showed not only temporal, but also spatial variation. Altitude, temperature and vegetation cover were particularly important parameters for the presence and number (incidence?) of plague cases while precipitation was not important. In Tanzania, Uganda and Vietnam, plague also seems to be linked to altitude (Neerinckx, 2010; Pham, 2009; Winters et al. 2009) and in the western United States the suitability for plague increases with altitude (Eisen et al. 2007). Similar characteristics regarding vegetation cover and precipitation have been suggested for other foci in North and East Africa, North and South America and many regions in Asia. Here plague is found primarily in either semi-arid to arid areas or low humidity forest types of habitat, while the disease fails to persist in humid tropical lowland areas were vegetation cover is high (Perry and Fetherston, 1997; Gage and Kosoy, 2005; Barnes, 1982). In Madagascar, however, plague is not found in the lowlands with the exception of the coastal town of Mahajanga where a different host has been implicated (Boisier and Rahalison, 2002).

Using remotely sensed environmental data for the spatial plague distribution in Madagascar represented a way to reveal patterns in large scale epidemiological processes which may have been overlooked when concentrating on small scale studies. The results suggested the existence of a threshold, determined by environmental characteristics, for human plague occurrence. The unique climate profile of the island which varies greatly between regions is responsible for differences in vegetation cover. The environment not only influences the host and vector distribution but also defines the way of life for the majority of Malagasy people (and accidental hosts for plague) who mainly rely on subsistence farming. Anthropogenic factors which may influence the occurrence of plague include the style of construction of houses?. Naturally in the highlands of Madagascar locally available materials are used for building (Thomas, 1998) possibly facilitating contact with rodent hosts more than in other regions. In Tanzania and other plague foci, socio-cultural factors were found to play an important role as determinants of the disease (Kilonzo et al. 1997). This area however is greatly under-researched and could yield essential information on the epidemiology of plague.

Effects of the micro-climate within host burrows on the abundance of plague vectors
In order to understand the effect of environmental variables on the plague cycle, climate conditions inside the burrows have been investigated. Burrow conditions are likely to

influence the distribution of plague vectors so that hosts' distribution becomes insufficient to explain vectors distribution. This has been suggested for plague areas in Madagascar (Klein, 1966; Klein and Uilenberg, 1966; Chanteau, 2006) and plague-free areas in Israel (Krasnov et al. 2002).

The vector ecology for Madagascar is unique, due to the presence of an endemic flea vector *Synopsyllus fonquerniei* (*S. fonquerniei*) found only in the highlands where it is one of the two main vector species. An investigation into the micro-climate inside rat burrows indicated that it could act as a mechanism by which climate affects plague risk to humans.

In the plague endemic region in Madagascar, substantial differences were established in the abundance, and variation in abundance, between the two main plague vectors, namely *S. fonquerniei* and *Xenopsylla cheopis* (*X. cheopis*). The vector species differed in their abundance patterns through the year, and their developmental stages (larvae and pupae) favoured different habitats with distinctively different climatic conditions. The micro-climate in rodent burrows is not only different from the ambient climate conditions, but also differs between burrow types, i.e. burrows found in houses and burrows found outdoors. In general, burrow climate shows less annual and diurnal variation than ambient climate in both temperature and humidity, providing much more stable conditions than those prevailing outside. This was also suggested by previous studies of the micro-climate in rodent burrows elsewhere (Longanecker and Burroughs 1952; Shenbrot, Krasnov et al. 2002; Sumbera, Chitaukali et al. 2004).

In the highlands of Madagascar, burrows located in houses are warmer and drier than their outside counterparts, which stay humid even throughout the driest months of the year. *X. cheopis* can best be sustained in rodent burrows which are located inside houses; while outdoor burrows support the endemic flea. *S. fonquerniei*, which seems to prefer colder temperatures and is possibly adversely affected by high humidity. Both factors are consistent with the current temporal and spatial distribution of this species. The fact that the two flea species are found in different niches even though they share the same host species could not be established in this study, but studies of the rodent population suggest significant genotypic differentiation of *Rattus rattus* ?? populations at a fine spatial scale in Madagascar indicating that indoor and outdoor rats are in fact distinct populations (Gilabert et al. 2007).

Effects of temperature and humidity on the development of both vector species

Basic knowledge on the biology of the endemic *S. fonquerniei*, one of the main plague vectors in Madagascar, was gathered and species-specific effects of humidity and temperature on development times and adult survival were established. Generally *X. cheopis* seemed more robust than *S. fonquerniei*. Temperature was the dominant factor overall influencing the development rate of larvae of both species. At higher temperature an accelerated development of larvae and pupae was observed in both species. The effect of humidity was less prominent, with an effect only found on pupal development of *X. cheopis*. In addition to the positive effects of temperature, *X. cheopis* pupae developed faster at higher humidity. Larvae of *S. fonquerniei* developed much slower than the larvae of *X. cheopis* at all temperatures apart from the lowest (18°C). Pupal development time was more similar for the two species, but, as with larvae, pupal development in *X. cheopis* was more sensitive to declining temperatures. The higher temperature treatments showed lower mortality rates of larvae in both species. For *S. fonquerniei*, higher humidity also decreased mortality. Contrary to results from the field, larvae of *S. fonquerniei* showed lower mortality at higher temperatures with higher humidity under laboratory conditions. This is an indication of the differences between laboratory and field conditions and implies that any conclusions have to be treated with care. Results from the field also imply a possible lower temperature threshold affecting the abundance of adult *S. fonquerniei* negatively. Such a temperature appears to be below the temperatures used in the laboratory experiment. *X. cheopis* was found to prefer higher temperatures both in the laboratory and in the field, consistent with results from Sharif (1949) and Mellanby (1933).

The results of the study on the effects of micro-climate combined with the laboratory results highlight that observed climate effects on plague incidence are multi-factorial. Differences between plague foci could be due to differing responses of flea species to climate, but also different climate patterns. In Kazakhstan low humidity is associated with high temperature while in Madagascar high humidity is associated with high temperature for example. The results of this study do not suggest that the Malagasy plague system is simply driven by flea development rates, but this is unsurprising as other factors also have to be considered such as adult flea survival and vector responses to host abundance.

The relationship between host abundance and fleas however, needs to be further worked on in all systems including plague. The common assumption that high host abundance leads to high flea abundance needs to be challenged and tested. There is some evidence that flea abundance can increase with current host density in some areas, but that the presence of alternative hosts can change that relationship (Telfer et al. 2004). Host community composition also seems important (Foley et al. 2010). Flea abundance may also show lags with host abundance and a dilution effect with current host density (Telfer et al. 2006).

All these are important factors which should be investigated with regards to plague transmission. The question is what is more important: the total number of fleas or the number of fleas per host (which depends on the exchange of fleas between hosts)? Telfer et al.'s (2006) results indicate that for flea-borne Bartonella, the number of hosts is more important than the number of vectors – higher densities of hosts may lead to more exchange of fleas between hosts.

Comparison between foci

The effects of climate on the epidemiology of plague investigated here extended from large scale climate effects to the influence of temperature and humidity on the developmental stages of the main plague vectors in Madagascar. Even though many similarities exist between plague foci in different parts of the world in terms of the effects of climate, it would be unwise to generalise conclusions without differentiating between foci and surveillance method. Also the importance of links between components of the plague system varies greatly depending on the focus described. This is due to focus-specific factors such as different environmental impacts, adaptive hosts as well as diversity in vectors and differences in thresholds.

For the studies presented in this thesis, human plague cases were used because they stand for disease spill-over signifying a very active plague focus and also because it is the most extensive, reliable data source available in Madagascar. Available enzootic plague data for Madagascar were unsuitable for large scale climate analysis due to spatial constraints. Yet not all major plague foci use human incidence data and most studies carried out in Kazakhstan concentrate on plague prevalence in the main rodent host instead. The focus is well

researched and detailed links between local climate and host populations, percolation thresholds and vector abundance have been described (Davis, 2008; Stenseth et al. 2006). Several other studies in Asia on climate and plague mentioned before however, rely on longitudinal data on human plague. For China in particular, mostly large human case data sets are available and little is known about the epidemiology of plague and climate at a finer scale. In the US both data types are used: human case data as well as rodent host infection rates. The origin of cases is well known and the focus profits from very detailed studies of host and vector dynamics and transmission routes. However, linking the relatively small number of human cases to large scale climate phenomena could provide over-analysed results which lack any connection to the plague cycle at a smaller scale.

The study presented here implies large scale as well as small scale effects of climate on the epidemiology of plague in Madagascar. These effects can be linked via the mechanisms by which climate affects certain components of the plague cycle. The information gathered here could be used to identify areas suitable for disease persistence or potentially at risk from disease emergence now or under future conditions.

APPENDIX OF CHAPTER 4

Table A4.1: Example of climate data arrangement. Temperature in degrees Celsius

| Fieldtrip	Burrow	08:00	08:30	09:00	09:30	10:00	...	02:30	03:00	03:30	04:00	04:30	05:00	05:30	06:00	06:30	07:00	07:30	Mean	Max	Min
Jan-09	CTE 3	20	21	21	21	21	...	21	20.5	20.5	20.5	20.5	20.5	20.5	20.5	20.5	20.5	20.5	20.63	21	20
Jan-09	CTE 4	20	21.5	21	21	21	...	20.5	20.5	20.5	20.5	20.5	20.5	20.5	20.5	20.5	20.5	20.5	20.63	21.5	20
Jan-09	CTE 7	21.5	21.5	21.5	21.5	21.5	...	21	21	21	21	21	21	21	21	21	21	21	21.13	21.5	21
Jan-09	CTE 9	20	20	19.5	19	19	...	19	19	19	18.5	19	18.5	18.5	18.5	18.5	18.5	18.5	18.79	19.5	18.5
Jan-09	CTE 10	20	21	21	21.5	22	...	21.5	21.5	21	21	21	21	21	21	21	21	21	21.18	22	21

Figure A4.1: Number of rats and fleas collected per fieldtrip
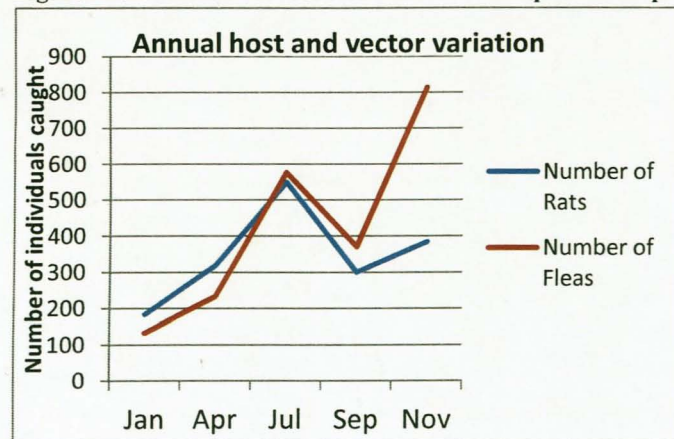
Table A4.2 a: Temperature data (degree Celsius)

region	village	month	Interior burrow temperature				Exterior burrow temperature				Ambient temperature			
			min	max	mean	DTR	min	max	mean	DTR	min	max	mean	DTR
A	C	Jan	20.71	21.64	21.26	0.93	20.25	21.36	20.94	1.11	16.02	31.27	20.22	15.24
A	D	Jan	20.44	24.06	21.37	3.63	20.06	23.22	20.71	3.17	15.87	35.99	20.35	20.12
B	E	Jan	23.22	25.53	23.94	2.31	21.79	25.53	23.29	3.74	17.22	36.26	24.93	19.04
B	F	Jan	23.13	25.63	23.98	2.50	21.84	24.08	22.68	2.24	17.33	43.38	25.07	26.04
A	G	Apr	20.19	23.94	21.63	3.75	18.78	20.12	19.27	1.33	14.45	49.12	19.77	34.66
A	H	Apr	20.51	21.97	21.09	1.46	18.88	23.44	19.76	4.56	14.83	37.06	19.13	22.22
B	I	Apr	21.75	25.08	22.94	3.33	18.56	25.50	19.95	6.94	11.93	40.97	20.84	29.04
B	J	Apr	21.17	23.83	22.71	2.67	17.81	21.43	19.18	3.62	11.07	37.66	22.14	26.60
A	K	Jul	12.88	17.25	14.00	4.38	9.60	17.40	11.42	7.80	4.58	23.68	10.90	19.10
A	L	Jul	11.56	15.06	13.30	3.50	9.71	14.36	12.07	4.64	3.10	25.98	10.85	22.88
B	M	Jul	17.29	22.50	18.18	5.21	15.31	21.19	16.87	5.88	11.14	26.35	15.82	15.22
B	N	Jul	14.61	15.88	15.34	1.28	12.82	16.28	14.36	3.46	9.92	34.92	17.89	25.00
A	O	Sep	18.24	21.38	19.24	3.15	19.20	21.73	20.18	2.53	10.28	36.06	19.71	25.79
A	P	Sep	19.54	22.13	20.34	2.58	17.94	21.48	19.32	3.54	7.16	33.27	17.75	26.11
B	Q	Sep	18.65	20.25	19.45	1.60	18.04	23.94	20.16	5.90	13.63	36.89	21.63	23.25
B	R	Sep	17.27	19.30	18.09	2.02	18.20	20.52	18.92	2.33	12.38	36.17	21.54	23.78
A	S	Nov	20.92	23.03	21.75	2.11	18.55	21.66	19.77	3.11	13.08	37.95	20.92	24.88
A	T	Nov	20.23	21.75	21.12	1.53	19.08	23.00	20.46	3.92	12.71	28.01	19.74	15.29
B	U	Nov	22.81	25.25	23.61	2.44	20.97	24.58	22.48	3.61	17.32	35.25	23.05	17.93
B	W	Nov	23.92	25.58	24.62	1.67	20.58	23.00	21.61	2.42	17.75	39.34	22.75	21.58

Table A4.2 b: Humidity data (relative humidity %)

region	village	month	Interior burrow humidity				Exterior burrow humidity				Ambient humidity			
			min	max	mean	DHR	min	max	mean	DHR	min	max	mean	DHR
A	C	Jan	89.63	96.20	92.95	6.57	92.53	98.44	96.21	5.91	27.01	100.00	91.07	72.99
A	D	Jan	81.88	91.53	89.32	9.66	82.75	97.89	95.85	15.14	38.62	100.00	87.92	61.38
B	E	Jan	87.93	97.77	96.23	9.83	80.50	98.35	95.26	17.85	32.15	100.00	56.45	67.85
B	F	Jan	80.17	89.15	86.00	8.98	87.52	98.63	95.90	11.11	29.22	100.00	55.95	70.78
A	G	Apr	72.88	85.44	82.09	12.56	89.36	98.36	96.88	9.00	12.51	100.00	73.72	87.49
A	H	Apr	84.23	90.83	88.68	6.59	81.38	96.00	94.06	14.63	21.46	100.00	75.15	78.54
B	I	Apr	62.20	81.25	75.26	19.05	72.91	94.41	91.90	21.50	20.38	97.57	69.95	77.19
B	J	Apr	71.58	86.33	81.07	14.75	82.42	93.41	89.93	10.99	26.01	100.00	68.84	73.99
A	K	Jul	75.61	92.50	89.16	16.89	70.63	93.31	89.63	22.69	36.80	99.86	80.66	63.07
A	L	Jul	59.13	82.06	73.26	22.94	79.31	94.25	89.98	14.94	19.19	100.00	80.26	80.81
B	M	Jul	59.29	81.93	78.15	22.64	70.50	92.38	89.17	21.88	39.34	82.59	65.24	43.25
B	N	Jul	77.77	85.62	82.67	7.85	82.34	91.34	87.91	9.00	23.49	91.73	62.36	68.24
A	O	Sep	71.34	84.91	81.21	13.56	83.64	91.68	89.10	8.04	18.95	92.53	67.46	73.58
A	P	Sep	78.56	89.25	86.51	10.69	78.35	90.98	86.97	12.63	14.89	100.00	56.54	85.11
B	Q	Sep	74.24	86.79	82.35	12.55	57.89	84.76	75.74	26.87	10.84	80.19	50.34	69.35
B	R	Sep	66.91	85.21	78.91	18.29	78.09	88.78	85.28	10.69	19.43	92.53	59.67	73.10
A	S	Nov	76.66	85.00	82.46	8.34	89.11	95.84	93.46	6.74	12.87	100.00	69.85	87.13
A	T	Nov	80.44	88.00	85.90	7.56	87.21	98.36	96.67	11.14	22.21	100.00	70.16	77.79
B	U	Nov	82.17	88.44	86.21	6.28	91.07	97.77	96.03	6.70	19.34	100.00	80.40	80.66
B	W	Nov	74.61	87.33	83.64	12.72	89.50	97.79	95.96	8.29	23.14	100.00	72.56	76.86

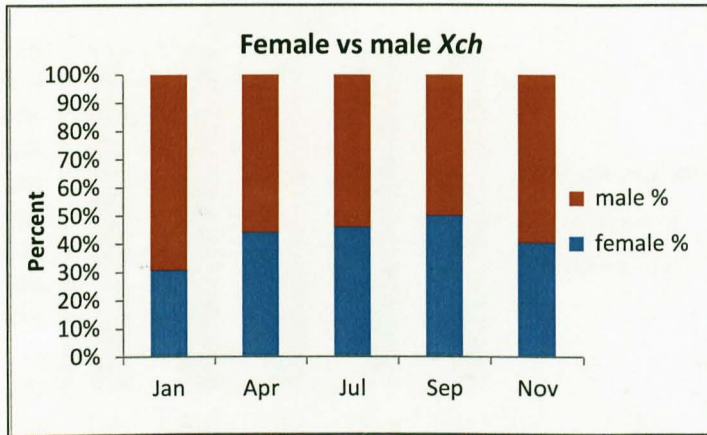**Figure A4.2: Sex ratio of collected *X. cheopis***

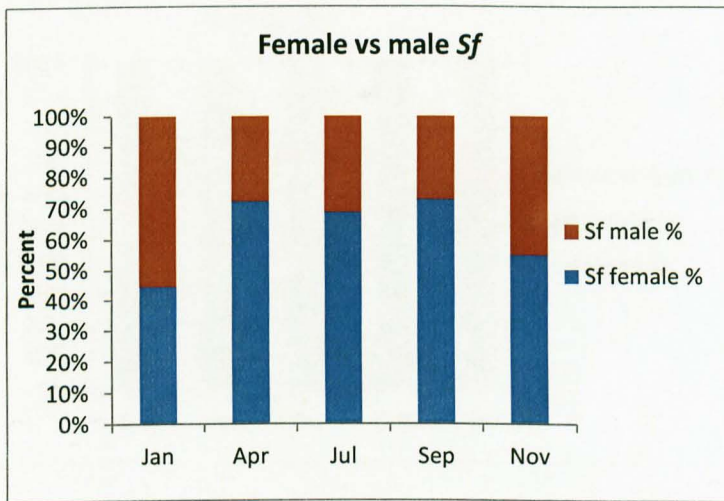Figure A4.3: Sex ratio of collected *S. fonquerniei*

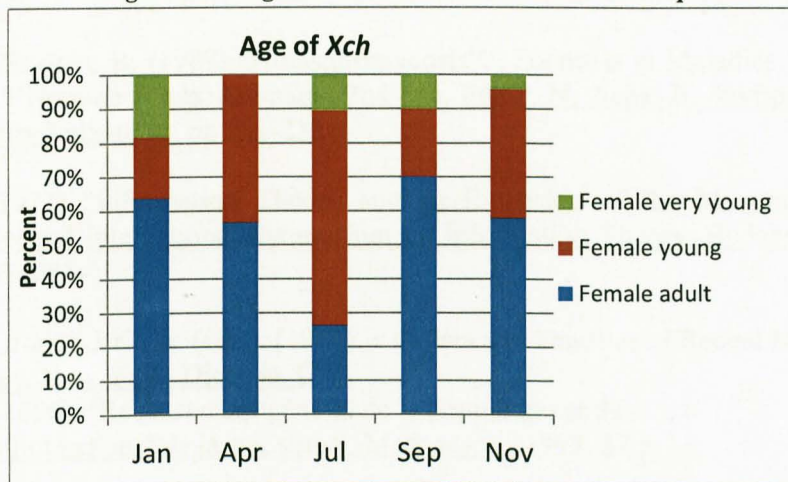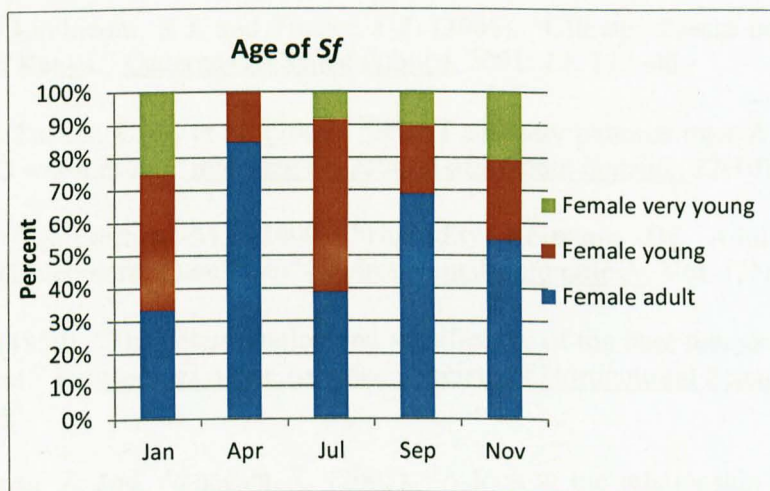**Figure A4.4: Age structure of collected female *X. cheopis* fleas**

Figure A4.5: Age structure of collected female *S. fonquerniei* fleas

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