Leptospire dynamics in its reservoir host in a Brazilian slum setting

Thesis submitted in accordance with the requirements of the University of Liverpool for the degree of Doctor of Philosophy by Amanda Minter

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Photo credit: M. Begon, K.P. Hacker, J.A. Panti-May.

Abstract

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In urban slums, residents often live in close proximity to reservoirs of zoonotic pathogens. Leptospirosis is a zoonosis that humans can contract via contact with animal reservoirs directly or with water contaminated with their urine. The recent population increase in Salvador, a coastal city in North East Brazil, led to the creation of slums, which are overcrowded and lack basic sanitation. The conditions of the slums favour rodent borne transmission of leptospirosis. The Norway rat (*Rattus norvegicus*) is asymptomatic and can transmit the infection for the entirety of its life. It is the main reservoir host for leptospirosis in Salvador. Motivated by the annual outbreaks of human leptospirosis in Pau da Lima, an urban slum community in Salvador, the within population infection dynamics of the Norway rat were investigated.

A mechanistic model of the dynamics of leptospire infection was developed and explored analytically. A global sensitivity analysis of the basic reproduction number to its components was performed.

Using newly obtained age-prevalence data from the field, we sought evidence that would indicate which transmission routes actually occur in the wild. By considering the survival from infection, we created risk curves of infection over time and looked for differences in risk for different demographic factors that were a proxy for transmission.

There are some model parameters which we were unable to estimate and some which we expected not to vary by system. To confirm that proposed values of demographic parameters were sufficient to describe population dynamics in wild Norway rats we present a Bayesian analysis of a mathematical population dynamics model.

These analyses were used to parameterise an age-structured mechanistic model for leptospire infection in the rodent population. Using the age-structured model, optimal control measures were found that would reduce the total (and infected) rat population. Costs of the controls as well as the cost of human infection were included in the analysis.

We conclude that vertical and environmental transmission occurs in the wild, and that environmental transmission is the most important route for the maintenance of infection in Norway rats. To control wild Norway rats, combinations of controls are recommended but environmental control should also be investigated to reduce prevalence of infection in rats.

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Contents

Chapter 1

Introduction

1.1. Urban health

Urban health is defined by the World Health Organisation as the health risks associated with living in urban areas. Residents in urban areas have a higher risk of non-communicable diseases, injuries from accidents and crime, and acquiring infectious diseases (World Health Organisation, 2010). The burden of infectious diseases lies predominately with those urban residents living in slum sites.

Currently one third of residents in urban areas live in slums, and by 2030, every 6 out of 10 people will live in urban areas (World Health Organisation, 2010). Poor sanitation and water access lead to increased risk of water-borne diseases, mosquito-borne diseases and parasites (Sclar et al., 2005). Slum sites also provide the optimal habitat for wild animal reservoirs of human infection (Costa et al., 2014a) leading to an increased risk of zoonotic diseases. Disease burden in urban slums is underreported, as often, hospitals or other health sectors become aware of a chronic illness nearing the end of the infection (Riley et al., 2007). Urban slums provide optimum conditions for the transmission of leptospirosis; the next section covers leptospirosis in more detail.

1.2. Leptospirosis

1.2.1. Epidemiology

Leptospirosis is a zoonosis (de Faria et al., 2008) and is thought to be the most widespread zoonosis in the world. It is present on every continent, except Antarctica (Adler & de la Peña Moctezuma, 2010). There is a lack of recognition of leptospirosis for a number of reasons: the diagnosis is confirmed by laboratory tests which are not always available, it is often misdiagnosed as of some its symptoms are identical to other diseases, and when only acute symptoms are present the disease is not always reported (World Health Organisation, 2003). The World Health organisation has set up the Leptospirosis Burden Epidemiology Reference Group (LERG), the goal of which is to establish accurate estimates of disease burden.

Leptospirosis is caused by pathogenic spirochaete bacteria of the genus *Leptospira*, commonly called leptospires (World Health Organisation, 2003). The genus *Leptospira* has over 200 serovars. Humans can contract leptospire infections via direct contact with animal reservoirs or with water contaminated with their urine when leptospires enter open cuts or wounds (Figure 1.1) (Haake & Levett, 2015). Human to human transmission is very rare as humans do not shed a sufficient amount of leptospires to serve as reservoirs (World Health Organisation, 2003).

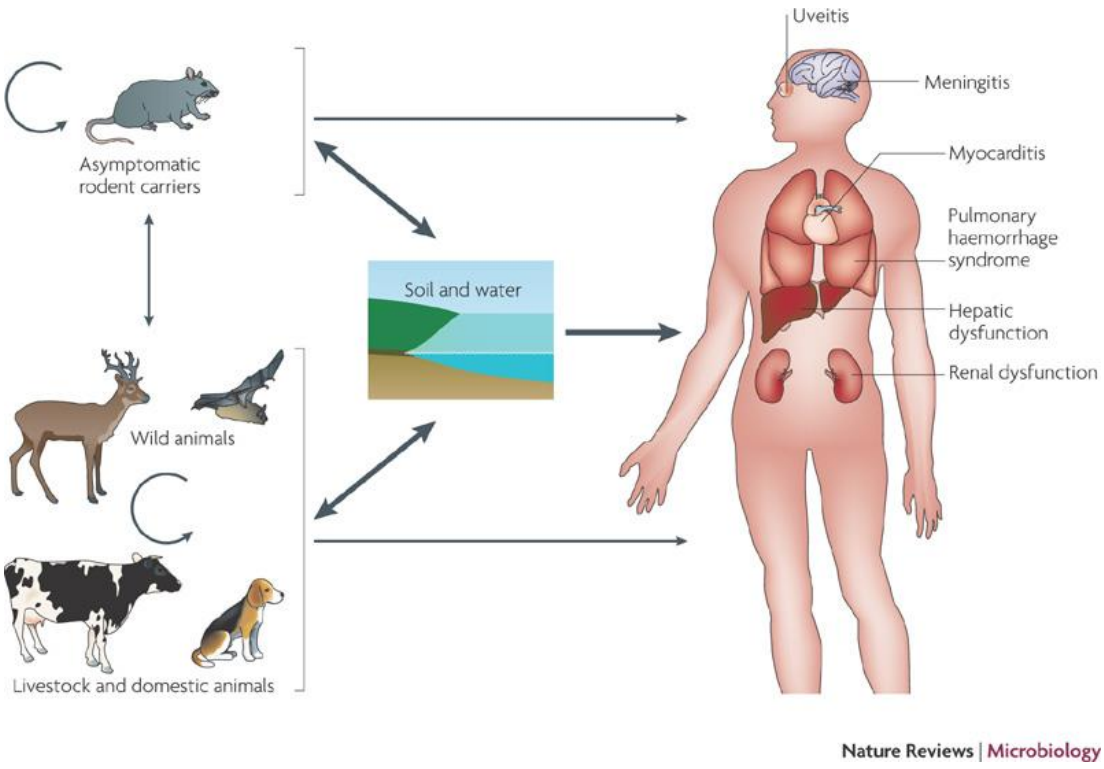


Figure 1.1: The transmission cycle of leptospirosis, taken from Ko et al. (2009).

Leptospirosis has many reservoirs. Here we define a reservoir species as one that has the ability to spillover infection to another species, either by asymptomatic lifelong infection or infection with disease. By this definition, most mammals can serve as reservoirs, and so different leptospirosis systems present themselves on every continent. Animals are usually asymptomatic when infected with a co-adapted strain, but when the strain is not co-adapted to the animal, the animal may suffer disease (Gay et al., 2014). Leptospires can survive in warm, moist soil and water for weeks to months (Bharti et al., 2003; Levett, 2001; Mwachui et al., 2015) hence it is often tropical and sub-tropical regions that are characterised by higher incidence of leptospirosis. Some serovars have been found to have shorter survival times in the environment (Cosson et al., 2014). Evidence has also been found of leptospires surviving in sea water (Grune Loffler et al., 2015).

Leptospire infection can result in asymptomatic, mild or severe disease. Symptoms of acute leptospirosis include fever, chills, headache, severe myalgia, redness of the eyes, anorexia and vomiting (Haake & Levett, 2015). Severe leptospirosis presents as Weil's disease; this can occur as a single illness or as the second phase of a biphasic illness and has a high fatality rate of 5-15%. Symptoms of severe disease include jaundice and renal failure, where mortality occurs through renal failure or pulmonary haemorrhage (bleeding from the lungs). There are some vaccines to prevent human leptospirosis but these are not widely available (Bharti et al., 2003).

1.2.2. Reservoirs of leptospirosis

1.2.2.1. Rodents

As discussed above, most mammals serve as reservoirs. Rats are a significant reservoir for leptospirosis: they have been found to carry the serovar Copenhageni (Costa et al., 2014a) which is associated with severe disease in humans (Ko et al., 1999). In many urban regions, rats are suspected to be a significant reservoir for human infection but the level of prevalence varies in different rat populations. In Tokyo, Japan, rats, cats and dogs were tested for leptospire infection, but only rats had a positive prevalence of 16% (n=127) (Koizumi et al., 2009). A similar level of prevalence was found in Vancouver, Canada of 11% (n=592) (Himsworth et al., 2013a). High prevalence of leptospirosis in rats has also been reported in the urban poor areas of Baltimore, Maryland, USA (65.3%, n=201) (Easterbrook et al., 2007). In an urban farmers' market in Medellin, Columbia, prevalence of infection in the rat population was 20% (Agudelo-Flórez et al., 2009). In Demark, the prevalence of leptospirosis in sewer rat populations is variable according to location, ranging

between 0 and 89% (Krøjgaard et al., 2009). In the UK, wild farm rats have been found to have a leptospirosis prevalence of 14% (Webster et al., 1995).

Rodents infected with leptospirosis have also been found in the tropics. In New Caledonia, rodent abundance and prevalence is higher during the hot and wet season (Perez et al., 2011). Infection levels in rodents were higher in the rain-fed fields of Cambodia in the wet season; also rodents had higher levels infection in forests than those living in houses (Ivanova et al., 2012). Rats are also thought to be important reservoirs in Mekong Delta of Vietnam (Loan et al., 2015), the Philippines (Villanueva et al., 2010) and Malaysia (Benacer et al, 2013).

Likewise, in Thailand multiple rodent reservoirs of leptospirosis have been reported (Wangroongsarb et al., 2002) and in Sante Fe, Argentina both new and old world rodents carry leptospire infection in urban, suburban and natural corridors (Vanasco et al., 2003). In France, leptospire infection has been found in coypus, muskrats and rats (Aviat et al., 2009; Michel et al., 2001). Mice, voles and shrews in Zurich's city parks, Switzerland, had a combined leptospirosis prevalence of 12.6% (Adler et al., 2002). In Terceira Island, the Azores, house mice and black rats were found to carry leptospirosis and house mice have a high prevalence of infection in Croatia (71.4%) (Turk et al., 2003).

1.2.2.2. Other targets

Rodent borne human infection arises because humans and rodents often share the same environment. It’s not surprising then, that there are systems in which rodents are the reservoir responsible for not just human infection, but also for other targets.

For example, in Trinidad, rodents are the main reservoir for canine leptospirosis (Suepaul et al., 2010). In Columbia, rats were responsible for an outbreak of leptospire infection in capuchin monkeys (Szonyi et al., 2011). There is also potentially rodent-bat transmission occurring in the tropics (Dietrich et al., 2015). Rats are also thought to be responsible for infecting race horses (Hamond et al., 2012) and livestock (dos Santos et al., 2012).

1.2.2.3. Livestock

Unlike rodent reservoirs, livestock can suffer acute or chronic leptospirosis (Suepaul et al., 2011), which can result in great economic loss due to reproductive problems (Hartskeerl et al., 2011). There is occupational risk to humans from livestock farming particularly in developing countries (Levett, 2001). Also, in New Zealand sheep leptospirosis in abattoirs provides an occupational health risk for meat workers (Dorjee et al., 2008). Similarly, butchers in Jamaica are at risk of contracting leptospires from cattle or pig (Brown et al., 2011). The impact of livestock leptospirosis is global. Incidence in some or all of cattle, buffaloes, sheep and goats has been reported in Thailand (Suwancharoen et al., 2013), Trinidad (Suepaul et al., 2011), Tanzania (Schoonman & Swai, 2010), Jamaica (Brown et al., 2011), Mexico (Segura-Correa et al., 2003), Brazil (Martins & Lilenbaum, 2013) and New Zealand (Dorjee et al., 2008).

The transmission of leptospirosis within livestock is thought to occur indirectly within the population, from different species including rodents and other livestock species. A study in Tanzania found that the degree of seropositivity in cattle increased with grazing and contact with other livestock species (Schoonman & Swai,

2010) suggesting that transmission can occur across species. Risk factors for goat infection in Minas Gerais, Brazil included the presence of rodents (rats and mice) and intensity of production (dos Santos et al., 2012). Boqvist et al. (2002) suggest that transmission of leptospirosis to sows in Mekong delta, Vietnam occurs indirectly from contaminated water food (potentially by rodents). Segura-Correa et al. (2003) found that the management practices of different regions was the only risk factor for increased risk of leptospire infection in cattle, suggesting that the environment must play an important role for livestock transmission.

Vaccination is used to prevent infection in cattle and pigs (Ellis, 2015). Other recommended preventative measures include closed herd policies and assessing the infection status of new animals (Ellis, 2015).

1.2.2.4. Other reservoirs

There are other reservoirs of leptospirosis similar to the rodent system. As in rodents, urbanisation has increased human-bat interactions as humans enter bat habitat and bats roost in artificial structures (Hayman et al., 2013). In the tropics and subtropics bats have been found to carry leptospire infection. The within population transmission routes are unknown but its hypothesized that bat roosting could facilitate contact with contaminated urine (Dietrich et al., 2015).

There are some systems which are more complex than the simple single reservoir system; on occasion multiple reservoir species are present. A recent study on African wildlife found leptospire infection present in mammals, birds and reptiles (Jobbins & Alexander, 2015). In the Peruvian Amazon basin region, rodents,

marsupials and bats have been found to carry leptospirosis (Bunnell et al., 2000). The transmission cycles between species can be complex. In New Caledonia, deer and pigs contract rodent-borne leptospirosis, whereas dogs were not reservoirs, but had pathogenic leptospires in the kidney, suggesting that they are part of the transmission cycle (Gay et al., 2014).

Leptospirosis also causes disease in some mammals, including dogs (Raghavan et al., 2012. Canine leptospirosis occurs in both temperate and tropical regions (Raghavan et al., 2012; Weekes et al., 1997). Risk factors in the US for canine leptospirosis include distance to water features, walking in rural environment, swimming in outdoor water and drinking outdoor water (Raghavan et al., 2012). Once infected, dogs can be treated with antibiotics to prevent shedding (Gay et al., 2014). Prevention of contact with water bodies would prevent infection but vaccination is thought to be the most efficient control measure (Raghavan et al., 2012; André-Fontaine, 2006).

Leptospirosis has been found to infect and on occasion cause disease in some marine mammals including sea lions (Gulland et al., 1996) northern elephant seals (Colegrove et al., 2005) and on one occasion a southern right whale (Grune Loffler et al., 2015). Leptospirosis is endemic in California sea lions but outbreaks also occur on a 4-5 year cycle where hundreds of animals die (Lloyd-Smith et al., 2007). The California sea lion presents an interesting case of an animal which serves as both an asymptomatic reservoir and an accidental host (Lloyd-Smith et al., 2007). The routes of transmission within California sea lion populations have not been confirmed.

1.2.3. A global zoonosis

Leptospirosis is a global zoonosis. The highest incidence of human infection occurs in the Caribbean and Latin America, the Indian subcontinent, Southeast Asia and Oceania (Pappas et al., 2008). However, there is little or no data in some developing countries, so the true global incidence is not known (Pappas et al., 2008). Risk of acquiring leptospire infection differs greatly in rural and urban areas of the tropics and temperate regions. In this subsection we discuss the incidence of leptospirosis in temperate regions (1.2.3.1) and then in the tropics (1.2.3.2).

1.2.3.1. Temperate regions

In most temperate countries leptospirosis is not common, but still presents the risk of fatality. For Europe, risk of infection is predominately from occupational or recreational exposure to contaminated water (Dupouey et al., 2014b). However, in the UK improvements in health and safety measures in the workplace have led to a decrease in occupational risk of leptospirosis and exposure now is more commonly from leisure activities (Forbes et al., 2012).

Rodents are thought to be the main reservoir for human infection in the UK, with human infection more often arising from indirect contact rather than direct contact. (Forbes et al., 2012). In Bulgaria, sources of human leptospirosis infection are mostly attributed to contact with contaminated water, pigs or rodents, either from recreational or occupational activities (Christova et al., 2003). In Germany, risk for human infection is predominately from agricultural risks in rural environments and travelling abroad (Jansen et al., 2005). New Zealand is ranked in the top ten for

incidence of leptospirosis globally (Pappas et al., 2008) where the disease is occupational for livestock farm workers, meat processing workers and forestry related workers (Thornley et al., 2002).

Human cases in the city of Baltimore, Maryland, US have been reported since the 1990's (Vinetz et al., 1996). Massive immigration in Israel has led to urbanisation and a shift from predominately rural cases of leptospirosis, to almost entirely urban (Kariv et al., 2001). Recently, there has been an increase of studies into leptospirosis risk in urban centres in European countries. A recent suspected rodent borne transmission has been reported in suburban France (Dupouey et al., 2014a). In Marseille, France leptospirosis cases were reported following a garbage strike. It has been hypothesized that the cause was an increased presence of infected rats due to the garbage in the streets (Socolovschi et al., 2011). Human risk of leptospirosis in Denmark arises from infected sewer rats entering homes and factories from defective sewers (Krøjgaard et al., 2009). In Germany, 12% of leptospirosis cases between 1962 and 2003 occurred in urban areas (Jansen et al., 2005). Leptospirosis is not considered an urban disease in Europe and so may be misdiagnosed by clinicians (Jansen et al., 2005) it is also thought that mild cases go unreported in rural areas of temperate countries (Forbes et al., 2012).

1.2.3.2. The tropics

Leptospirosis is more common in the tropics and sub-tropics than in temperate regions due to the longer survival of leptospires in higher temperatures and the increased likelihood of flooding. Flooding is a significant risk factor in the tropics as it occurs often (discussed in more detail in section 1.2.3.3); in the Philippines for

example typhoons and cyclones occur up to 20 times per year (Yanagihara et al., 2007).

In rural areas of tropical countries, poor drainage means that rural villages can easily become flooded (Victoriano et al., 2009; Kawaguchi et al., 2008). Infected livestock are thought to contribute to human infection in rural areas (Biggs et al., 2011). Leptospirosis is also an occupational risk in the rural tropics; those at risk include rice farmers who are exposed to contaminated water (Victoriano et al., 2009)

Urban slum residents in many parts of the world suffer leptospirosis risk. The effects of the floods are worsened in urban areas as garbage clogs drainage and deforestation means an absence of trees to absorb flood water (Yanagihara et al., 2007). Brazilian slums in Rio de Janeiro and Salvador have leptospirosis outbreaks attributed to rodents (Barcellos & Sabroza, 2001; Pereira & Andrade, 1988; Reis et al., 2008). Human leptospirosis cases have been recorded in the urban slums of India, in East Delhi and Mumbai (Kaur et al., 2003; Karande et al., 2002) where the presence of numerous rats and dogs has been noted, but not confirmed as reservoirs. In an urban slum market in Peru, *Leptospira* was found in gutter water and humans, suggesting a rodent reservoir was present (Ganoza et al., 2006). In Kenyan slum settlements, rats have been found to carry leptospires (Halliday et al., 2013). Urbanisation is expected to have a significant effect on leptospirosis outbreaks in Sub-Saharan Africa where leptospirosis is thought to be endemic but the true incidence is unknown as there is little data available (de Vries et al., 2014).

1.2.3.3. Leptospirosis, flooding and climate change

Leptospirosis outbreaks have occurred following typhoons (Taiwan and China), flooding (Thailand and India) (Kouadio et al., 2012) and cyclones (Fiji) (Lau et al., 2016). Flooding is the most commonly occurring natural disaster (Ahern et al., 2005) and climate change is expected to bring increasing rainfall, cyclone intensity and flooding to the tropics in particular (Lau et al., 2010). Urban slums, low-lying coastal areas and small island states are likely to have the greatest increase in leptospirosis incidence due to climate change, because they are most susceptible to flooding, have abundant reservoirs and in the case of slums have poor sanitation (Lau et al., 2010).

For leptospirosis, Lau et al. (2010) describe how climate changes will affect leptospirosis incidence. Flooding brings an increased chance of contact with contaminated water and an increase in temperature aids survival of leptospires. The reservoir-human interactions will change depending on the reservoir species. For rodents, resource availability may increase as a result of flooding but may decrease suitable habitat.

1.3. Leptospirosis in the urban slums of Salvador, Brazil

1.3.1. Pau da Lima, Salvador

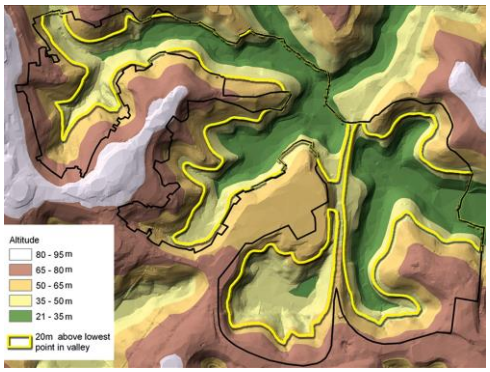
Salvador is the capital city of the state of Bahia, Brazil and is the third largest city in Brazil (Riley et al. 2007). Salvador has a seasonal climate with highest temperatures of between 26.7°C and 27.1°C occurring in the summer and lower temperatures of 23.8°C and 24.3°C and heavy rains occurring in the winter (June to September)

(Porter et al., 2015). A massive increase in the urban population from 58% to 80% of the total population (between 1970 and 2000) (da Mata et al., 2005) has led to the creation and expansion of urban slum settlements (Ko et al., 1999). These urban slums, as elsewhere in Brazil and in many parts of the world, are overcrowded and lack basic sanitation.

The Pau da Lima neighbourhood in Salvador is an urban slum community where a study site has been established, comprising of three valleys namely: valley 1, valley 2 and valley 4 (valley 3 was once used as a field site but due to concerns of safety, is no longer visited) (Figure 1.2a,b). Until the 1970s Pau da Lima was Atlantic rainforest, following the expansion of slum settlements the valleys in Pau da Lima now comprise of slum houses with patches of dense vegetation (Figure 1.2c). A census conducted by Reis et al. (2008) found that of 3,171 residents in Pau da Lima, 85% were squatters and the median household per capita income per day was US$ 1.30.

People in residence in Pau da Lima live in close proximity to the animal and environmental reservoirs of infection. The tropical climate of high temperatures and seasonal rainfall means that leptospirosis thrives in Salvador's slums. In the next sub-sections the studies into the outbreaks of leptospirosis in Salvador, and then in Pau da Lima that have been conducted are discussed.

a.



b.

c.

Figure 1.2: a) Aerial photo of valley 1, valley 2 and valley 4. b) Topographic map taken from Reis et al.(2008). c) Slum houses in valley 1 in Pau da Lima.

1.3.2. Annual epidemics

A notable outbreak of severe leptospirosis occurred in Salvador in 1996 when active surveillance at a state run hospital in Salvador between March 10 and November 2 1996 reported 326 case of leptospirosis (of which 59% we either laboratory confirmed or probable) (Ko et al., 1999). Misdiagnosis was common; a number of cases (42%) were misdiagnosed as having dengue fever. With a high case fatality

rate of 15% (50 cases), this outbreak was, at the time, the largest recorded case series for a leptospirosis outbreak.

The people with the highest risk of acquiring leptospirosis were slum residents and of the 193 confirmed or probable cases, 69.1% had had recent exposure to contaminated water and 80% had had recent exposure to rodents. Peaks in the number of cases occurred between 1 and 4 weeks after an increase in rainfall. At this time it was hypothesized that transmission occurred through contact with flood water that had been contaminated with rodent urine. Adult males were the most common demographic group to suffer leptospire infection.

1.3.3. Transmission routes investigated

Further investigation found that the role of the rodent reservoir and its interaction with the environment presented itself as an important component to the outbreaks of leptospirosis in Salvador's slums. During another outbreak at a state run hospital in Salvador between March and October 2000, 157 leptospirosis cases (101 laboratory confirmed cases) were recorded (Sarkar et al., 2002). Risk factors for leptospire infection were identified as residence close to an open sewer, sightings of rats close to the home, sightings of groups of five or more rats and exposure to contaminated environment via the workplace. All of the recorded cases were from slum residents.

A community-based survey of 3,171 slum residents from Pau da Lima, Salvador found an overall prevalence of *Leptospira* antibodies of 15.4% (Reis et al., 2008). Risk factors for infection related to the environment and rodents were identified

again, namely residence in areas prone to flooding and with refuge close by, sighting rats and the presence of chickens. Demographic risk factors were low income and black race. Reis et al. (2008) also investigated the spatial difference in risk. Cases cluster at the bottom of the valleys, where residents are mainly squatters living close to open sewers.

Transmission was further investigated at the household level by Maciel et al. (2008). Within slum communities there is significant household clustering of human leptospirosis cases. This may be because members of the same household are all exposed to the same risk factors close to the home (proximity to sewer for example). A more recent study found that the presence of Norway rat faeces, rodent burrows, access and water and un-plastered walls increased the risk of household *Leptospira* infection (Costa et al., 2014b), highlighting the importance of rats near the home for household infection.

Slums residents are constantly exposed to leptospirosis; hence re-infection of human leptospirosis occurs in Pau da Lima (Felzemburgh et al., 2014). Seasonal outbreaks occur in the rainy season. Increased flooding creates a higher risk of transmission via the environment. Risk was found to be not homogenous throughout the slum valleys, the proximity to sewers, and refuge and the presence of rats increases risk of household and individual transmission. Rats were thought to be responsible for the human infection, and so studies conducted in Pau da Lima shifted to focus on the rodent reservoir.

The first study into leptospirosis in Norway rats in Pau da Lima was conducted by de Faria et al. (2008). Animals were trapped close to the homes of confirmed severe

leptospirosis cases and tested for leptospire infection. More than 80% of the animals were infected with leptospirosis, and with the same serovar (Copenhageni) that had been isolated from human cases in previous outbreaks. A second more recent study found prevalence of infection in two rat populations (1998 and 2010) to be 80.3% (114/142 positive rats) and 63.1% (53/84 positive rats) respectively (Costa et al., 2014a). Rats appear to be the single reservoir responsible for the outbreaks of human leptospirosis in Pau da Lima, in the next section we present on overview of the Norway rat as a reservoir for leptospirosis.

1.4. Norway rats

1.4.1. Background

Norway rats (*Rattus norvegicus*) (Figure 1.3), also known as brown rats or sewer rats, are a widespread rodent species, found on every continent except Antarctica (Lund, 1994). They are a commensal rodent species (they are found with humans) (MacDonald & Fenn, 1994) due in part to them being opportunistic omnivores, they eat what becomes available to them (Bonnefoy et al., 2008).

Figure 1.3: Wild Norway rat. Photo credit: J.A. Panti-May.

They are a burrowing species, nesting in underground burrows (Figure 1.4) which are often large and complex (Calhoun, 1962). The lifecycle of Norway rats is fairly simple; pups are born into the nest where they are confined until weaning is completed. Once weaned, they leave the nest and begin to roam. They reach adulthood once they become sexually mature (Calhoun, 1962). Wild Norway rats have a short lifespan, on average they live less than a year, but the reasons for this are unknown (Feng & Himsworth, 2014).

Norway rat populations live in colonies which have social structure. Animals are deemed dominate or subordinate; both sexes exhibit social hierarchy (Calhoun, 1962; Ziporyn & McClintock, 1991). Dominance is associated with older age in Norway rats (Macdonald et al., 1995). Dominant rats have fewer wounds (Calhoun, 1962; Blanchard et al., 1995) and heavier weight (Barnett, 1958).

Rats living in urban areas have many distinct differences to their rural counterparts. Increase in the presence of rats is a direct effect of urbanisation. The increase in food availability and refuge provide habitat for wild urban rats (Gratz, 1999). Urban rats tend to grow quicker, reach sexual maturity at a younger age and live in higher densities compared to rural populations (Glass et al., 1989).Urban rats have a smaller home range than rural rats (Clapperton, 2006).

Wild Norway rats are difficult to control, in part because they are neophobic animals (fear unknown objects in familiar places) (Clapperton, 2006). Also, they have the capability to reproduce at fast rates (Bonnefoy et al., 2008) with up to 5 litters per year (Feng & Himsworth, 2014).

Rats are known to carry a wide range of bacterial infections, viruses and parasites. Many of these infections are also zoonotic: they can be transmitted either directly or indirectly to humans. Recent evidence of zoonotic infections of wild Norway rats includes Seoul hantavirus (Hinson et al., 2004). Bacterial zoonoses carried by rats include leptospirosis, Y*ersinia pestis*, *Rickettsia typhi*, *Bartonella* spp. and *Streptobacillus moniliformis* (Himsworth et al., 2013b). Zoonotic parasites include *Capillaria hepatica* (Ceruti et al., 2001), *Angiostrongylus cantonensis* (Himsworth et al., 2013b), *Toxoplasma gondii* (Lélu et al., 2010), *Calodium hepatica*, *Hymenolepis sp*. and *Laelaps echidninus* (Easterbrook et al., 2007).

Rats make efficient zoonotic reservoirs. With the exception of *Yersinia pestis*, there has been little evidence of symptoms in rats associated with infection of zoonotic diseases (Himsworth et al., 2013b). Incidence of rat-borne zoonoses has increased with changes in climate and urbanisation (Himsworth et al., 2013b). Hence rat-borne zoonoses are more common in developing countries and in urban areas (Himsworth et al., 2013b).

1.4.2. Norway rats in Pau da Lima, Salvador

Almost 100% of the rats trapped in Pau da Lima are Norway rats (Costa et al., 2014b). The true abundance of Norway rats in Pau da Lima is unknown but recent estimations show that on occasion, population sizes surpass 100 per 3330 m^2 (Pedra et al, *in preparation*). Rodent burrows are found in Pau da Lima (Figure 1.4) and rodent infestation has been detected in the majority of households (Costa et al., 2014b) human *Leptospira* infection case control study, 78% of case houses and 42% of control houses had rodent infestation).

Figure 1.4: Entrance to a Norway rat burrow in Pau da Lima.

Residents in Pau da Lima employ household control measures to reduce contact with rats. A study conducted by Navegantes de Araújo et al. (2013) found that around half of the slum resident participants (122/257) used some kind of rat poison at the home and 117/257 residents attempted to reduce rat access to the home. Chemical rodenticide is applied in Pau da Lima during outbreaks of human leptospirosis. Studies leading to an improved understanding of which households have an increased risk of infection are being conducted (Costa et al., 2014b) but the most effective rodent control is still unknown.

1.4.3. Leptospirosis in Norway rats

The within population dynamics of leptospire infection for Norway rats are not well understood. Norway rats are believed to be able to transmit leptospires for the entirety of their life without showing any symptoms of the disease (Bharti et al., 2003; Eliis, 2014). The presence of leptospires in the mammary gland and semen of rats provide biological evidence that vertical and sexual transmission may occur (unpublished work). Costa et al. (2015) found that Norway rats from Pau da Lima

had a very high *Leptospira* load in the urine of 6.1 x 10^6 per ml (range 2.2-9.4 x10^6). This high shedding rate of leptospires in the urine suggests that environmental transmission occurs.

In order to prevent outbreaks of human leptospirosis, the cycle of transmission must be broken. For leptospirosis, this means reducing contact with contaminated environment or reducing the shedding rate of the reservoir into the environment. Hence understanding infection dynamics within a zoonotic reservoir can aid in understanding how the infection is maintained, and then how it might be controlled.

1.5. Understanding wildlife infectious disease dynamics

Theoretical epidemiology allows us to develop theoretical frameworks of disease systems to make predictions of, and better understand, the infection dynamics of a system. These theoretical approaches have advanced understanding of infection in wildlife systems. Anderson & May (1979) were the first to use mathematical models to study more 'ecological' systems in which the size of the host population may vary and indeed be determined by mortality caused by the parasite. Models have been used alongside empirical data to determine functional forms for transmission routes (Begon et al., 1999), the role of indirect transmission (Almberg et al., 2011) and coinfection in wildlife systems (Fenton, 2008). See Joseph et al. (2013) for a fuller review of theoretical papers in disease ecology.

Developing a model framework for a wildlife disease system helps to identify the unknowns of a system (Smith et al., 2009). When control is of interest, models can

be developed to predict failed intervention strategies (Joseph et al., 2013). Recently, models have been used to make predictions about the effects of different control strategies in wildlife disease systems (Davidson et al., 2008; Wasserberg et al., 2009). However, wildlife infection dynamics are often difficult to fully parameterise due to the lack of sufficient data on demography, behaviour and transmission (Alexander et al., 2012). For zoonotic diseases in particular, mathematical models have aided understanding of a number of different reservoir-human systems (Lloyd-Smith et al., 2009). Also, for each system the complex animal-human interactions need to be understood in order to predict when human infection will occur (Alexander et al., 2012).

There is only one existing model for leptospire dynamics in a rodent population: the Holt et al. (2006) framework for leptospire infection in the African multimammate mouse. The framework is a susceptible-infected model with three age classes: juvenile, sub-adult and adult with three routes of within population transmission. Their analysis revealed that most important transmission route for affecting the prevalence of leptospirosis in rats was indirect (via the environment). In terms of control, they found that mortality rate was the most sensitive parameter for prevalence, number of rats and number of free-living leptospires. The parameter related to carrying capacity was less sensitive. In other words, killing rats, as opposed to habitat management, would a more effective control.

1.6. Aim

The Norway rat is the most widespread natural reservoir of leptospirosis and has been identified as the single reservoir responsible for outbreaks of human leptospirosis in Pau da Lima, Salvador. Therefore, the principal aim of this thesis is to further understand the maintenance of leptospire infection in the Norway rat in Brazilian slums using empirical analyses of field data from Pau da Lima, Salvador and mathematical models.

Moreover, this thesis aims to use this improved knowledge of within population dynamics to inform rodent management programs tailored to urban Norway rats. Finally, the conclusions we make about infection dynamics within Norway rat population in Pau da Lima can be compared to different climatic systems and other targets of infection (not just humans).

1.7. A note on data collection and collaboration

This project is part of a much larger collaborative project between the University of Liverpool (UK), Yale University (US) and the Fiocruz, Salvador (Brazil). All field data were collected and analysed in the laboratory and not by the author of this thesis. As part of the continuous control of leptospirosis in Pau da Lima, rats are trapped and removed from the three valleys: valley 1, valley 2 and valley 4 (Figure 1.5) by field teams in Fiocruz, Salvador and the Center for Control of Zoonoses. The field and lab team based in Fiocruz, Salvador record demographic information of the rats and perform laboratory analysis of samples at Fiocruz and Yale University.

Figure 1:5: Live capture of a Norway rat in Pau da Lima.

1.8. Chapter outlines

1.8.1. Chapter 2: Development of a model for leptospire dynamics in its reservoir host

This chapter is a short presentation of the development of a compartmental modelling framework for leptospire infection in the Norway rat. The content has been included to show the thought processes which led to the framework in chapter 3.

1.8.2. Chapter 3: A model for leptospire dynamics in its reservoir host

A modelling framework for leptospire dynamics (without age structure) and a full analytical analysis is presented. Global sensitivity analysis of the basic reproduction number is used to determine which transmission routes are most likely responsible

for the occurrence of endemic infection. Target reproduction numbers were found to aid in understanding the control measures for leptospire infection in rats.

1.8.3. Chapter 4: Identifying evidence of multiple transmission routes: leptospirosis in *Rattus norvegicus*

Chapter 4 is an empirical study of evidence for multiple transmission routes occurring in the wild. Established survival analysis methods are applied to leptospirosis prevalence data on rats to seek changes in risk over the lifetime of an animal.

1.8.4. Chapter 5: Inference for differential equations: estimating adult mortality rate and sub-adult maturation period

In this chapter a simple population dynamics model for Norway rats is presented. Using this framework, adult mortality rate and maturation period of sub-adults are estimated based existing values in the literature and on cross sectional data on the population structure of Norway rats.

1.8.5. Chapter 6: Optimal control measures for leptospire infection in the Norway rat

This chapter presents a pilot analysis of control measures for leptospirosis in rats based on an age-structured infection model. Optimal rodenticide and habitat management measures are found using optimal control theory.

1.8.6. Chapter 7: Discussion

The general discussion of the chapters is presented in chapter 7. The methods and results are discussed in a wider context and unanswered questions are presented.

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Chapter 2

Development of a model for leptospire dynamics in its reservoir host

2.1. Introduction

In this chapter the development of a mathematical model for leptospire infection in the Norway rat (*Rattus norvegicus*) is presented. Factors such as population demography and different routes of transmission will affect the dynamics of infection. By identifying which factors are responsible for driving infection in the rat population, possible interventions for human infection can also be found. A simple model may be relatively far removed from the complex reality of a field system, but it brings with it analytically tractability so that a full (global) analysis of the behaviour of the model can be performed. The aim here was to find the simplest possible model to describe leptospire dynamics in the rat population in a satisfactory (insightful) way. Simple, analytically tractable models can set a background of understanding of more complex and realistic but analytically intractable models. Additionally, the model for rat infection will feed into a model describing leptospire dynamics in the environment which will explicitly model risk of human infection.

Kermack & McKendrick (1927) introduced a modelling framework to investigate how epidemics behave in a population of fixed size. In this framework, hosts within

a population are considered to be in one of three disease states: susceptible, infected or recovered. May & Anderson (1979) initiated the study of ecological epidemiology by extending the Kermack & McKendrick (1927) model with the introduction of population demography into the host population. The May & Anderson (1979) model with demographic processes is not only a more realistic approach than that of Kermack & McKendrick (1927) but can also be used to examine how a disease can be self-sustained in a population, i.e. the dynamics of endemic infectious disease can be investigated (Keeling & Rohani, 2008). Anderson & May (1981) introduced free-living infective stages into host-parasite dynamics models. Their framework explicitly modelled the population size of the number of infective stages with the rate of infection of susceptible individuals being dependent on the number of infective stages. Anderson & May's (1981) model has been extended to include multiple hosts (Bowers & Begon 1991) and single or multiple hosts with host self-regulation (Bowers et al., 1993; Begon & Bowers, 1994).

There is only one previous mechanistic model for leptospirosis in rodents: that developed by Holt et al. (2006) for infection in African mice. More recently, there have been a number of models for human leptospire infection in Thailand which have acknowledged the importance of rodent borne transmission, but do not detail the mechanisms within the rodent population itself (Pongsuumpun et al., 2008; Khan et al., 2014; Pongsumpun, 2014; Pongsumpun, 2012; Kongnuy & Naowanich, 2012; Pimpunchat et al., 2013; Zaman et al., 2012; Triampo et al., 2007). Baca-Carrasco et al. (2015) developed a framework for human leptospire infection with simple within-reservoir dynamics for multiple animal reservoirs.

To identify the simplest model that can satisfactorily capture leptospire dynamics in rat populations in an urban slum setting, a number of different numerical experiments were performed to investigate whether proposed models were able to predict the level of prevalence as observed in the field. In the absence of true transmission parameters (but see subsequent chapters), values from the literature were used.

2.2. Proposed models

Compartmental models can be used to condense a complex system into its simplest form to investigate the dynamics of infection over time, occurrence of endemic behaviour and the implication of control efforts (Hethcote, 2000; Alexander et al., 2012). We propose deterministic compartmental models to describe leptospire dynamics in Norway rats to identify factors affecting infection dynamics at the population level. We assume that rat populations within each valley in Pau da Lima are closed: streets create barriers which rats are unlikely to cross to seek resources (Feng & Himsworth, 2014) and the valleys in Pau da Lima are separated by some form of street (see Figure 1.2a in chapter1) and so the proposed models represent the population within one valley.

In this section, three different compartmental models for leptospire dynamics in rat populations are presented. Proposed models were evaluated according to whether they could predict the observed prevalence of infection in field animals. The prevalence of *Leptospira* in rats in Salvador has been found to be between 60- 80% (Costa et al. 2014). In two sampling periods, 1998 and 2010, the prevalence was found to be 80.3% (114/142 positive rats) and 63.1% (53/84 positive rats)

respectively. Therefore, a model should be able to achieve prevalence in that range given realistic parameter values.

2.2.1 Model 1: Simplest model

2.2.1.1. Model 1: Framework

Our first proposed model is a system of differential equations (model 1, equations 2.1-2.3, Figure 2.1) which builds on the Holt et al. (2006) model for leptospire infection in African rodents. Here X represents the number of susceptibles, Y the number of infecteds, and L the number of free-living leptospires. Rats are either free from and susceptible to infection, or infected and infectious. There is no latent period of infection, and once infected, rats are infected for their entire lifetime.

$$\frac{dX}{dt} = b(X + (1 - \upsilon_1)Y) - \upsilon_2 \frac{XY}{X + Y} - \upsilon_3 XL - mX \tag{2.1}$$

$$\frac{dY}{dt} = b\upsilon_1 Y + \upsilon_2 \frac{XY}{X + Y} + \upsilon_3 XL - mY \tag{2.2}$$

$$\frac{dL}{dt} = \lambda Y - \mu L \tag{2.3}$$

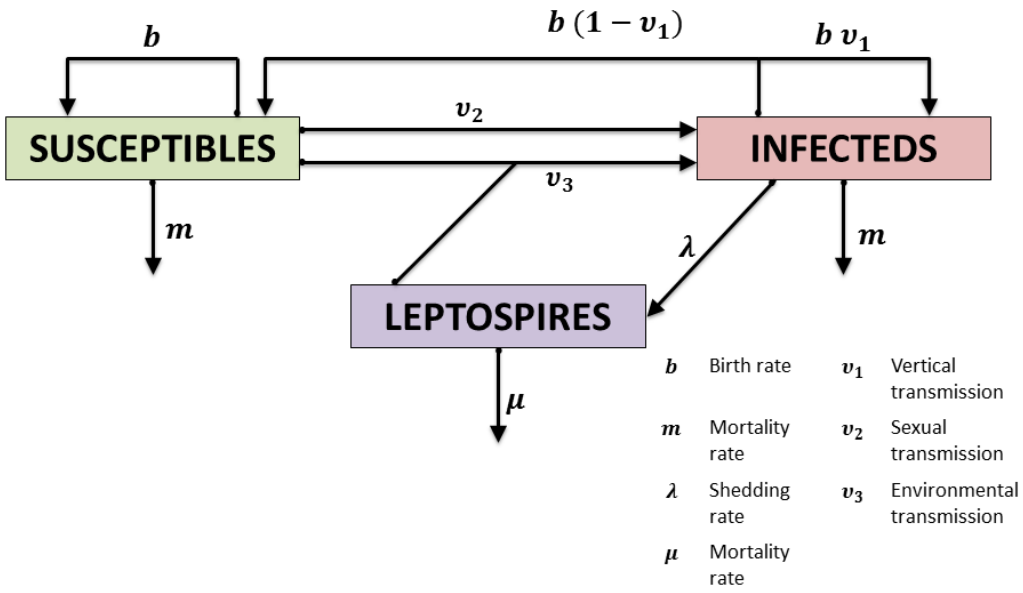

Figure 2.1: Flow diagram of model 1.

Susceptible (X) and infected (Y) rats give birth at a constant rate b through time. No evidence of seasonal birth rate has been found for the rats in Pau da Lima, the uniform temperature in Salvador may be responsible for this constant birth rate (Barnett & Bathard, 1953). There is assumed to be no infection-induced mortality; susceptibles and infecteds suffer mortality at the same rate (Bharti et al., 2003). Vertical transmission can occur via two routes: infected rats can give birth to infected offspring or rats can contract infection from suckling. It was assumed that both of these events happen instantaneously at birth and so the two routes were combined into one vertical transmission parameter υ_1.

Susceptible rats can move to the infected state via sexual transmission with coefficient υ_2, where the rate of sexual contacts is assumed to be unaffected by population size i.e. frequency dependent transmission (Begon et al., 2002). Susceptible rats can also contract infection environmentally, υ_3, where the risk of infection increases linearly with the number of leptospires.

Infected rats shed leptospires into the environment into a pool of transmissible leptospires (L) at a rate of λ per day per infected individual. In this state the leptospires present a risk of environmental transmission for the susceptible rats. Leptospires are lost through mortality at a constant rate per individual leptospire, μ.

2.2.1.2. Model 1: Model exploration

In the absence of estimates from the field site in Salvador, parameters were taken from the Holt et al. (2006) model (Table 2.1). The simulation of the simplest model (Figure 2.2) shows the number of susceptibles quickly decreasing and the number of infecteds continuing to increase. With the increase of infected rats, more leptospires are shed and so the number of free-living leptospires also increases.

Table 2.1: Parameter definitions and values used in simulation of model 1.

Parameter	Definition	Units	Value	Source/Comments
b	Per capita rat birth rate	Day^{-1}	0.12	Constant birth rate from Holt et al. (2006)
m	Rat mortality rate	Day^{-1}	0.012	Adapted from Holt et al. (2006)
υ_1	Proportion of pups infected from suckling and born infected	Day^{-1}	0.01	Holt et al. (2006)
υ_2	Transmission rate via sexual transmission	Day^{-1}	0.01	Holt et al. (2006)
υ_3	Transmission rate via the environment	Day^{-1}	0.00005	Adapted from Holt et al. (2006)
λ	Leptospires shed per day per infected individual	Day^{-1}	1000	Holt et al. (2006)
μ	Mortality rate of leptospires in the environment	Day^{-1}	0.1	Constant mortality rate (Holt et al., 2006)

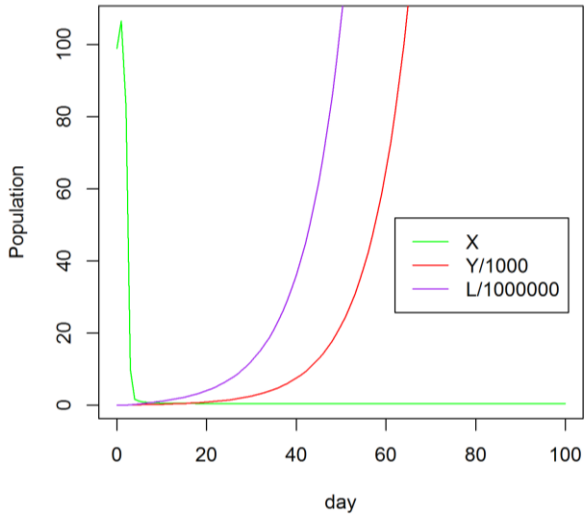

Figure 2.2: Predicted number of susceptible (green) and infected (red) rats, and abundance of leptospires in the environment (purple) through time (days) using model 1, from a single infected rat in a population of 100 individuals (see Table 1.1 for parameter values).

The prevalence of infection at the end of the simulation ($t = 100$ days) was approximately 99%, i.e. almost all animals are infected after a short time. This was an unrealistic value of prevalence. Perhaps more concerning was the absence of host self-regulation in the simulations. With no recovered class, infection-induced mortality, or indeed any cost of fitness to infected animals, infection should not alter the total numbers of rats. However, it is of interest to investigate whether population size affects the persistence and prevalence of infection. Therefore the second proposed model included self-regulation into the system for biological realism and also to investigate the effect of population size on prevalence.

2.2.2 Model 2: Carrying capacity and two states for free-living infective stages.

2.2.2.1. Model 2: Framework

Model 2 (Figure 2.3, equations 2.4-2.7) was developed by modifying model 1 in three ways. First it was acknowledged that animal populations reach a carrying capacity due to self-regulation (intraspecific competition) (Begon et al. 1992). Self-regulation was attached to the birth rate of susceptible and infected rats. The self-regulation term k was specified as function of the total number of rats, $(k-(X+Y))/k$. Given that regulation only applies to birth in the model the carrying capacity, K, is $K = k - k\left(\frac{m}{b}\right)$.

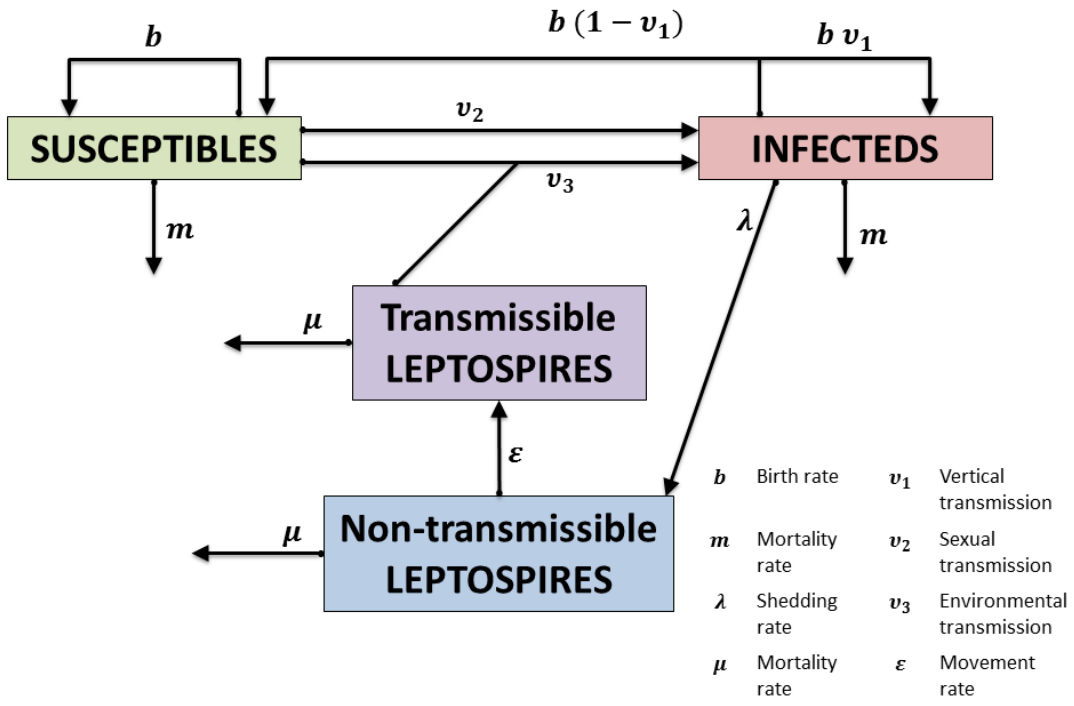

Figure 2.3: Flow diagram of model 2 with two states for the free-living leptospires, wastage of leptospires from the transmissible state and self-regulation incorporated into the model.

$$\frac{dX}{dt} = b(X + (1 - \upsilon_1)Y)\left(\frac{k - (X + Y)}{k}\right) - \upsilon_2 \frac{XY}{X + Y} - \upsilon_3 X L_T - mX \quad (2.4)$$

$$\frac{dY}{dt} = b\upsilon_1 Y\left(\frac{k - (X + Y)}{k}\right) + \upsilon_2 \frac{XY}{X + Y} + \upsilon_3 X L_T - mY \quad (2.5)$$

$$\frac{dL_{NT}}{dt} = \lambda Y - \mu L_{NT} - \varepsilon L_{NT} \quad (2.6)$$

$$\frac{dL_T}{dt} = \varepsilon L_{NT} - \mu L_T - \upsilon_3 \phi X L_T \quad (2.7)$$

Secondly, an additional state for the free-living leptospires was added. In model 2 leptospires are either transmissible free-living leptospires or non-transmissible free-living leptospires. This builds on work by Hochberg (1989) who was the first to introduce a framework where the pathogen population is divided into two states: transmissible and protected. Two states for leptospires were included because it was believed to be a more realistic structure of the pathogen population. It is plausible to assume that rats shed leptospires into a non-transmissible pool, perhaps deep in the soil, which can be translocated by flooding, for example, to new areas where other rats may pick the leptospires up. In model 2, infecteds shed leptospires into the non-transmissible state of leptospires at a rate of λ per day per infected individual. Leptospires in the non-transmissible state pose no risk of environmental transmission to rats. The free-living leptospires move into the transmissible leptospires state at a rate ε.

Finally, an additional wastage of leptospires was included. Leptospires are lost from the transmissible state upon being picked up by hosts at a net rate of $\upsilon_3 \phi X$ when infection via the environment takes place. Also, individual leptospires are lost at an rate via suffering mortality at a constant rate μ.

2.2.2.2. Model 2: Model exploration

With movement of leptospires from the non-transmissible state to the transmissible state set at ε=0.5, number of leptospires removed when infection takes place set at φ=10000 (Athanazio et al., 2008), and all other variables as in Table 2.1, a model simulation was run (Figure 2.4). The inclusion of the carrying capacity term successfully resulted in self-regulation of the total population size, but the prevalence was still too high (approximately 99%, the same as in model 1).

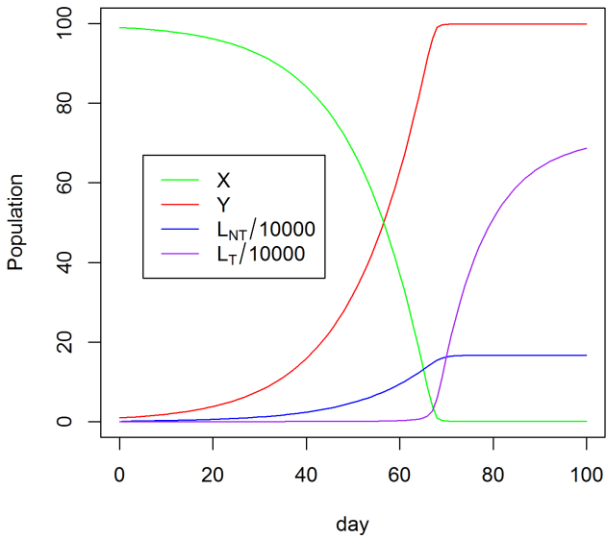

Figure 2.4: Predicted number of susceptible (green) and infected (red) rats, and transmissible leptospires (purple) and non-transmissible leptospires (blue), from a single infected rat in a population of 100 individuals (see Table 1.1 for parameter values).

To obtain a better understanding of why the prevalence was high, the equilibrium points, the points at which the values of X, Y, L_T and L_{NT} remain constant (i.e. $\frac{dX}{dt} = \frac{dY}{dt} = \frac{dL_T}{dt} = \frac{dL_{NT}}{dt} = 0$), were examined. There are two equilibrium states for this system, infection free and endemic disease. To focus on whether the high prevalence is due to the combination of parameter values the values of the endemic equilibrium, which we denote X^*, Y^*, ${L_T}^*$ and ${L_{NT}}^*$ were looked at in detail.

In order to stay within realistic parameter values, the parameter space shown in Table 2.2 was explored. The parameter ranges were obtained based on existing studies of Norway rats and from some preliminary results from Salvador. This space was sampled using Latin hyper cube sampling (LHS), as it ensures that the whole range of possible values are sampled by 'remembering' previous samples (McKay et al. 1979). In a random sampling scheme there is no guarantee that the entire parameter space will be sampled, as areas will be missed by chance. LHS ensures that the entire parameter space is sampled by dividing each parameter range into intervals of equal probability, and then samples of a parameter are taken once from each interval. These samples are then matched at random to provide the different combinations of parameters.

Using these parameters, the expected value of the endemic equilibrium (Y^*) and the prevalence of infection at the endemic steady state ($Y^*/(Y^*+X^*)$) were found using runsteady in the R package rootsolve (Soetaert & Herman, 2008) from LHS with 1000 random parameter sets (Latinhyper, R package FME, (Soetaert & Petzoldt, 2010)).

In a very small fraction (4/1000) of instances prevalence in the range 60-80% was observed. Hence the high prevalence is not intrinsic to the model, but dependent on parameter combinations. However, only a few, and hence arguably unlikely, parameter combinations result in the empirically observed prevalence (Costa et al. 2014).

2.2.3. Model 3: Altering the two states of free-living infective stages and updating parameter ranges

2.2.3.1. Model 3: Framework

Reconsidering the proposed framework, the two states for free-living leptospires in model 2 were arguably not specified as biologically realistic. Rats would be more likely to shed urine onto the surface, where the leptospires are transmissible, and here the leptospires would have a high mortality rate. If they survived for long enough, the leptospires would then move to a non-transmissible (sub-surface) state where the lifespan of leptospires is longer. Model 2 only allowed for movement of leptospires in one direction and assumed that leptospires in both states suffer mortality at the same rate. Model 3 allows for movement between the transmissible and non-transmissible states of leptospires and includes two different mortality rates of leptospires (model 3, equations 2.8-2.11, Figure 2.5).

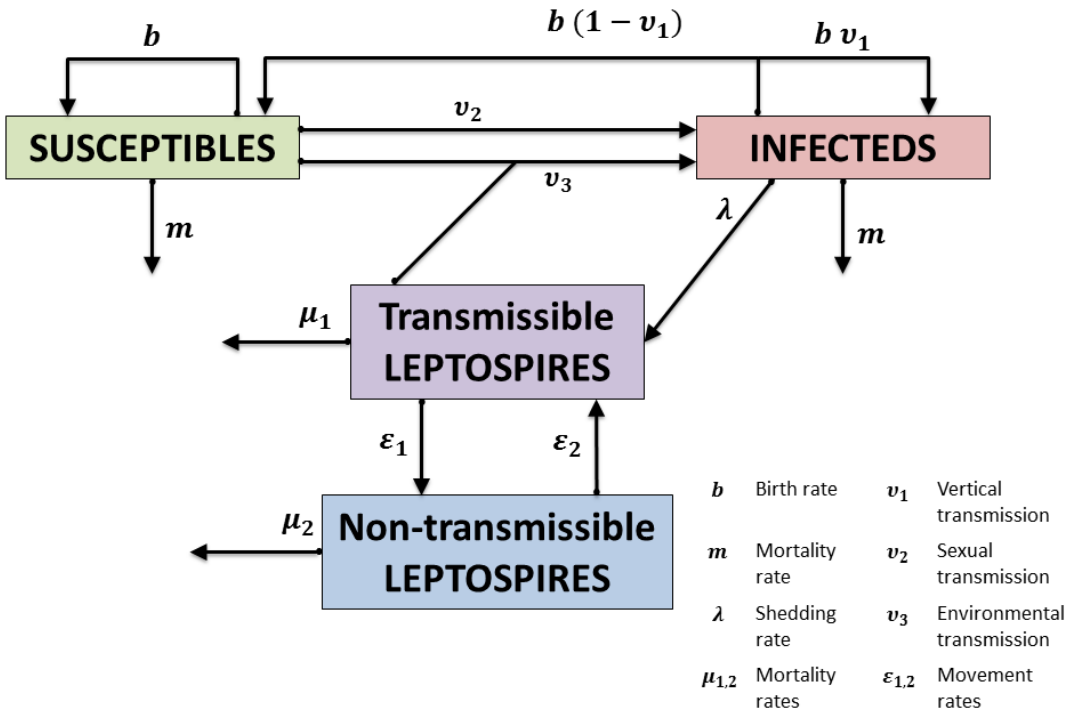

Figure 2.5: Flow diagram of model 3, with infected rats shedding into the transmissible state of leptospires and movement of leptospires between the two leptospire states.

$$\frac{dX}{dt} = b(X + (1 - v_1)Y)\left(\frac{k - (X + Y)}{k}\right) - v_2\frac{XY}{X + Y} - v_3 X L_T - mX \tag{2.8}$$

$$\frac{dY}{dt} = bv_1 Y\left(\frac{k - (X + Y)}{k}\right) + v_2\frac{XY}{X + Y} + v_3 X L_T - mY \tag{2.9}$$

$$\frac{dL_T}{dt} = \lambda Y + \varepsilon_2 L_{NT} - (\mu_1 + \varepsilon_1 + v_3\phi X)L_T \tag{2.10}$$

$$\frac{dL_{NT}}{dt} = \varepsilon_1 L_T - (\mu_2 + \varepsilon_2)L_{NT} \tag{2.11}$$

2.2.3.2. Model 3: Model exploration

It was also appropriate to consider adjustments in parameter estimates (Table 2.2). The most notable change would be the shedding rate of leptospires, since recent samples of animals obtained from the field were shedding a substantial amount more leptospires than proposed by Holt et al. (2006).

As with the previous model, the equilibrium states were examined. There were two equilibria in the feasible region – infection free and endemic infection. The values of the endemic equilibrium were calculated for 1000 sets of parameters simulated using Latin hyper cube sampling.

A wide range of endemic equilibria was observed but no prevalence lower than 99%. Since this model framework is biologically realistic, the reason for the high prevalence may be because there is no longer a 'delay' between leptospires being shed and being available for transmission. However, if a simulation of model (specified in section 2.2.2) is run but with the higher shedding rate, lower prevalence cannot be achieved.

All animals become infected very quickly; this may be due to sexual or environmental transmission. Therefore a Latin hypercube was created with 10^5 random samples of υ_2(sexual transmission) and υ_3(environmental transmission) with minimum values of 0 and maximum values of 0.5 and 0.00005 respectively. The remaining parameter ranges are as specified in Table 2.2. Out of the 10^5 random samples, one combination of υ_2 and υ_3 gave prevalence (61%) in the desired range (between 60-80%).

For this sample, the value for both transmission parameters was low, $\upsilon_2 < 0.01$ and $\upsilon_3 < 0.00000001$.

2.3. Discussion

The aim in the development of the model was to find the simplest possible model capable of predicting the prevalence of leptospire infection that had been observed in field animals. The first proposed model, model 1, was adopted from the only other existing model for leptospire infection in rodents by Holt et al. (2006). Model 2 was created by incorporating a carrying capacity in the rat population and two states for the free-living leptospires into model 1. The states for transmissible and non-transmissible leptospires were included to reflect how animals became infected environmentally. After altering the interactions between the two leptospire states in model 3, it was observed that the model was still incapable of predicting the prevalence observed in the field.

Rats become infected via environmental transmission because they are in the wrong place at the wrong time: near free-living leptospires. This phenomenon was incorporated into models 2 and 3 by including two states for the free-living pathogens. The low value of υ_3 may be explained if the transmission coefficient is interpreted as:

υ_3=(contact rate x probability of transmission)/average number of leptospires needed for infection

So the phenomenon of rats being in the wrong place at the wrong time is incorporated into υ_3, instead of having two states for free-living leptospires. This

observation suggests that the additional state for non-transmissible leptospires may be unnecessary. If transmission parameters are low enough then the simple model with carrying capacity could also be used to achieve the desired prevalence.

Another observation was that the wastage of leptospires (ϕ) would be a difficult value to quantify. When prediction is of interest for a specific system, a mathematical model should be fully parameterised using empirical data from that system (Keeling & Rohani, 2008). Dose response experiments conducted in the laboratory can provide some insight into the value of the wastage but often do not represent realistic routes or modes of transmission. The study conducted by Athanazio et al. (2008) found that 10^4 leptospires was the minimum inoculation required to establish renal colonization 28 days after infection in the Norway rat. Inoculation however is not representative of the transmission routes that occur in the wild. Also, given the large magnitude of the number of leptospires in the environment, the wastage of leptospires would have a negligible effect on the dynamics of the free-living leptospires. Hence the wastage term was not included in our final model.

The simplest possible model capable of describing leptospire dynamics in the reservoir host was desirable so that the factors responsible for persistence of infection could be identified. Here such a model has been sought by investigating whether various models were capable of predicting realistic values of prevalence. The results from simulations suggest that the simple model framework of susceptible and infected rats with self-regulation and one state for free-living

leptospires, will be capable of reflecting reality, and so this model was selected to explore analytically in chapter 3.

Table 2.2: Parameter definitions, values and minimum and maximum values for model 2 and 3.

		Model 2, adapted from Holt et al. (2006)			**Model 3**			
Parameter	**Definition**	**Value**	**Min**	**Max**	**Value**	**Min**	**Max**	**Source/Comments**
b	Per capita birth rate	0.12	0.05	0.15	0.1	0.05	0.15	Davis (1951) and estimates from Salvador (unpublished).
m	Mortality rate	0.012	0.013	0.04	0.02	0.013	0.04	Glass, Childs, Korch, & LeDuc (1988).
υ_1	Proportion of pups infected from suckling and born infected	0.01	0.001	0.25	0.2	0.001	0.25	Around 20% pups are infected (unpublished).
υ_2	Transmission co-efficient for sexual transmission	0.01	0.0001	1	0.5	0.001	0.1	One female will have contacts with many males, difficult to estimate.
υ_3	Transmission via the environment	0.00005	0.000000001	0.001	0.00005	0.000000001	0.001	-

Table 2.2 (continued): Parameter definitions, values and minimum and maximum values for model 2 and 3.

Parameter	Definition	Value	Min	Max	Value	Min	Max	Source/Comments
λ	Leptospires shed per day per infected individual	1000	100	10^5	10^5	100	10^7	Recent estimates from Salvador (unpublished).
μ	Mortality rate of leptospires in the environment	0.1	0.01	1	-	-	-	-
μ_1	Mortality rate of leptospires in the environment	-	-	-	0.2	0.001	1	Most leptospires die immediately when shed.
μ_2	Mortality rate of leptospires in the environment	-	-	-	0.04	0.001	1	In warm, moist conditions they can survive for months.
ε	Movement of leptospires	0.5	0.01	1	-	-	-	-
ε_1	Movement of leptospires	-	-	-	0.2	0.001	1	Higher movement from transmissible to non-transmissible.

Table 2.2 (continued): Parameter definitions, values and minimum and maximum values for model 2 and 3.

Parameter	Definition	Value	Min	Max	Value	Min	Max	Source/Comments
ε_2	Movement of leptospires	-	-	-	0.04	0.001	1	Slow movement back to surface.
ϕ	Wastage of leptospires	10^4	-	-	10^4	-	-	Taken from Athanazio et al. (2008)
K	Carrying capacity	100	-	-	100	-	-	Self regulation term $k = K/(1 - m/b)$.

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Chapter 3

A model for leptospire dynamics in its reservoir host

3.1. Introduction

Leptospirosis is a globally distributed zoonosis, but the majority of the disease burden lies in the poorest communities in tropical climates (Costa et al., 2015; Haake & Levett, 2015). Humans become infected with the bacteria (leptospires, of the genus *Leptospira*) either by direct contact with an animal reservoir or contact with environment (water or soil) that has been contaminated with animal urine. The Norway rat (*Rattus norvegicus*) has been identified as the most important reservoir for urban human leptospirosis infection (Haake & Levett, 2015). Alike many natural reservoirs, Norway rats can transmit leptospirosis for their entire life without presenting with disease (Bharti et al., 2003; Eliis, 2014). Without effective human vaccination (Bharti et al., 2003), prevention of infection is key to reducing the burden of disease. Understanding the dynamics of infection within the primary animal reservoir can inform intervention strategies to control the rat population and so contribute to reduction in the risk of human disease.

Urban slums are often overcrowded, lack basic sanitation and residents typically living in close proximity to animal reservoirs of infection (Ko et al., 1999). Pau da Lima, an urban slum in Salvador, Brazil register annual outbreaks of leptospirosis (Ko et al., 1999) where annual flooding events, associated with the rainy season,

wash contaminated soil and water into areas of potential human use. Until recently, most studies have centred on the study of human leptospirosis. These studies have identified that risk of leptospire infection in humans is associated with presence of rats (Costa et al., 2014b) and residence in areas prone to flooding (Felzemburgh et al., 2014; Reis et al., 2008). Given that the Norway rat thrives in urban areas (Gratz, 1999), it is not surprising that they are abundant in the slums of Salvador.

Leptospire infection in the rodent population in Salvador is believed to be endemic. Prevalence of infection is high, between 60-80% (Costa et al., 2014a) and currently there is no evidence of seasonality in the level of prevalence (unpublished work). Once infected the Norway rat can transmit leptospires for the entirety of its life without showing any symptoms of the disease (Bharti et al., 2003). The Norway rat is a common carrier of a highly virulent serovar Copenhageni (Hartskeerl et al., 2011; Vanasco et al., 2003) and it has been found in the rodents of Salvador's slums (Costa et al., 2014a; de Faria et al., 2008). Therefore it is of interest to understand what characteristics of leptospire infection in rats may be responsible for the maintenance of endemic infection.

Mathematical models can be utilised to describe and provide insights into infectious disease dynamics (Hethcote, 2000). Previous models to describe leptospire infection include the Holt et al. (2006) model for leptospire infection in African mice, rat to human infection models in Thailand (Pongsuumpun et al., 2008; Khan et al., 2014; Pongsumpun, 2014, 2012; Kongnuy & Naowanich, 2012; Pimpunchat et al., 2013; Zaman et al., 2012; Triampo et al., 2007) and a multiple reservoir to human model

(Baca-Carrasco et al., 2015). However these models lack empirical information to inform the model parameters.

Here, a model is presented to describe the dynamics of leptospire infections in urban slum Norway rats. It is related to the Holt et al. (2006) model, but is simpler as an age structure is not included. The proposed model comprises of three ordinary differential equations representing the numbers of susceptible rats, infected rats and number of free-living leptospires. Our model includes the important elements needed to describe the dynamics of infection while maintaining the considerable advantage of analytical tractability. Additionally, this simplicity means that the framework could be applied to other water-borne infections with multiple routes of infection.

The primary interest of this study was to quantify control efforts for reducing infection in the rat population. The basic reproduction number, R_0, is a useful analytical tool in mathematical epidemiology (Keeling & Rohani, 2008). If R_0 can be characterised for a particular system, then the parameters which enter the expression are the parameters which could be responsible for the spread of infection. Further, recent developments of the target reproduction number allow for controls to be targeted at sub-populations of the host population (Shuai et al., 2013). This chapter also presents empirically informed control measures that can be applied to leptospire infection in rats.

3.2. Model framework

The model (Figure 3.1) is described by a system of three ordinary differential equations representing the numbers of susceptible rats (X), infected rats (Y) and free-living leptospires in the environment (L) (equations 3.1-3.3).

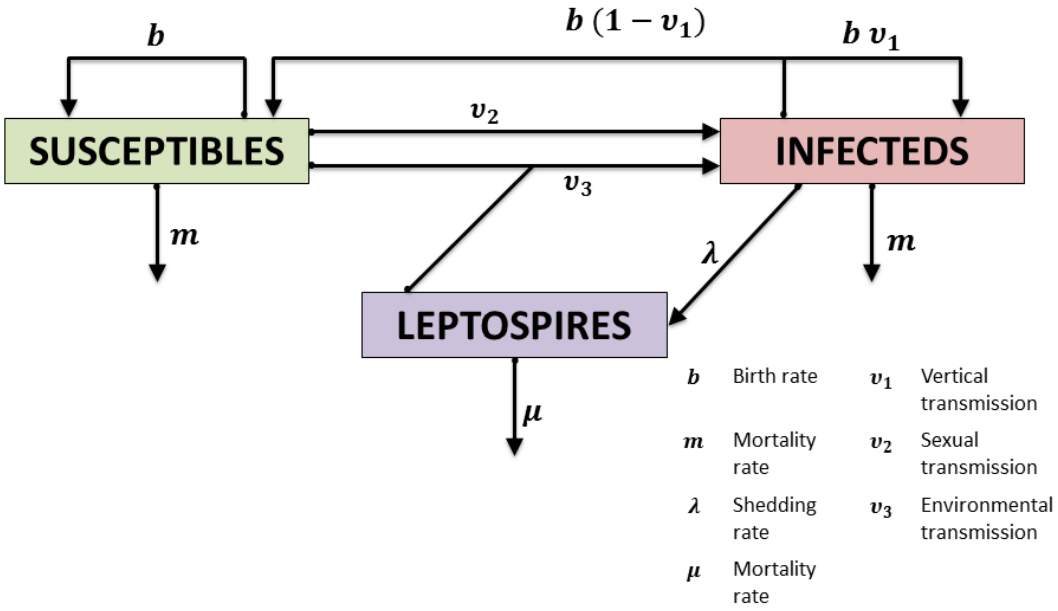

Figure 3.1: Flow diagram of the model with self-regulation.

$$\frac{dX}{dt} = b(X + (1 - v_1)Y)\left(\frac{k - (X + Y)}{k}\right) - v_2\frac{XY}{X + Y} - v_3XL - mX \tag{3.1}$$

$$\frac{d[illegible]}{dt} = bv_1Y\left(\frac{k - (X + Y)}{k}\right) + v_2\frac{XY}{X + Y} + v_3XL - mY \tag{3.2}$$

$$\frac{dL}{dt} = \lambda Y - \mu L \tag{3.3}$$

Rats are born at a constant rate b through time and a proportion (v_1) of infected rats will give rise to infected offspring. As in chapter 2, there is assumed to be no

time delay between acquiring infection and becoming infected, and once infected, rats are infected for their entire lifetime. Susceptible rats can become infected via direct transmission (assumed to be via sexual contact) (υ_2) or environmental transmission (υ_3). Sexual transmission is assumed to be frequency dependent (Begon et al., 2002); environmental transmission is assumed to be density dependent and hence described in equation 3.2 by $\upsilon_3 XL$; the rate of transmission linearly increases with the number of susceptibles and the number of free-living leptospires. Once infected, rats shed leptospires at a rate of λ per day. In the environment, leptospires die at a rate of μ per day. In the absence of evidence of disease, susceptible and infected rats suffer mortality at the same rate m. There is self-regulation in the system applied to the birth rate (which is zero when $X + Y = k$), where both susceptible and infected rats are considered to be competing for the same resources. Given that regulation only applies to birth in the model the carrying capacity, K, is found to be $K = k - k\left(\frac{m}{b}\right)$.

3.3. Basic reproduction number

The basic reproduction number R_0 gives 'the average number of secondary cases arising from an average primary case in an entirely susceptible population' (Keeling & Rohani, 2008). In this section the expression for R_0 in this system is presented. The value of R_0 indicates whether an infection can invade a population. If $R_0 > 1$ then the primary case gives rise to more than one infected/infectious individual, and so the infection can invade and then spread for as long as the reproduction number remains greater than one (Keeling & Rohani, 2008).

Due to the multiple routes of transmission, the expression for the reproduction number was found using the next generation matrix (NGM) method (Diekmann et al., 1990). The next generation matrix describes the secondary infections of the different population types in the system. The equations for the number of infected and the number of free-living leptospires describes new infections and so only those states are considered. We also acknowledge that from our assumptions regarding population growth, that the total population size H will always converge to the carrying capacity $K$and so we can remove the density dependent term attached to the birth rate, and have $b = m$.

As discussed in chapter 2, υ_3 must be very low in value in order for the model to predict values of prevalence that were observed in the field. However, dealing with parameter values so low in numerical analysis, such as parameter estimation, can be problematic. Therefore, we re-scale the free number of living leptospires to $L' = L/\lambda$, and the environmental transmission rate as $\upsilon_3' = \upsilon_3\lambda$ and write the model as described by equations 3.1-3.3 with $X = H - Y$ as,

$$\frac{dY}{dt} = b\upsilon_1 Y + \upsilon_2 \frac{(H-Y)Y}{H} + \upsilon_3'(H-Y)L' - mY \tag{3.4}$$

$$\frac{dL'}{dt} = Y - \mu L' \tag{3.5}$$

In this model, L' and υ_3' are the number of free living leptospires and the environmental transmission rate now expressed in shedding units. To find the basic reproduction number, first the terms responsible for new infections need to be distinguished from all other terms in the system. The matrix $\mathscr{F}$ comprises of these

'new infection terms' and the matrix $\mathscr{V}$ comprises of all other additions and removals from the two states. Taking the partial derivatives of the components of $\mathscr{F}$ and $\mathscr{V}$ with respect to Y and L' give matrices F and V respectively. The next generation matrix is defined as $F.V^{-1}$.

As discussed in Bani-Yaghoub et al. (2012) the choice of $\mathscr{F}$ and $\mathscr{V}$, with particular reference to treatment of the state variable for the free-living pathogens, will lead to different expressions for R_0. If it was believed that the free-living leptospires acted as a reservoir, then secondary free-living leptospires would be added to the state via shedding, and shedding would be placed in the $\mathscr{F}$ matrix. As an example, Lélu et al. (2010) modelled the risk of *Toxoplasma gondii* infection as arising directly from the environment, and so placed the shedding rate into the $\mathscr{F}$ matrix. In the present case, rats do acquire infection from the environmental reservoir but the rats are also responsible for maintaining the environmental reservoir. We considered two formulations of R_0. In the first case, we assumed that the free-living leptospires are an extension of the first infections in the system and place the shedding into the $\mathscr{V}$ matrix. The second case is when the environment is treated as the reservoir of infection, and so we placed shedding in the $\mathscr{F}$ matrix. We denote these two formulations using the definitions as in Bani-Yaghoub et al. (2012), namely transition, R_0^I and reservoir, R_0^{II}, respectively. The notable difference between the two formulations is best represented by flow diagrams of the next NGMs (Figure 3.2). The reservoir system has the additional movement from L' to Y, depicting the role of the free-living leptospires as a reservoir for infection.

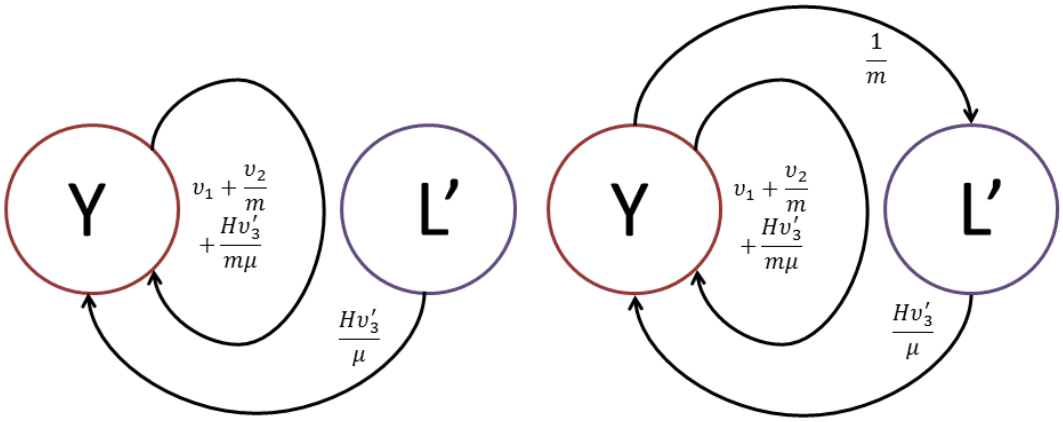

Figure 3.2: Diagrams depicting the next generation matrices NGM's for the when shedding is treated as an extension of the first infections in the system transition (left) and for when the environment is treated as a reservoir (right). The number of infected rats is denoted Y and the number of leptospires L.

3.3.1. Transition, R_0^I

Assuming that the free-living leptospires are an extension of the first infections in the system, we place the shedding into the $\mathscr{V}$ matrix, as follows.

$$\mathscr{F}=\begin{bmatrix} bv_1 + \frac{v_2 Y(H-Y)}{H} + v_3'(H-Y)L' \\ 0 \end{bmatrix} \text{ and } \mathscr{V}=\begin{bmatrix} mY \\ \mu L' - Y \end{bmatrix}.$$

Then the partial derivatives of the components of $\mathscr{F}$ and $\mathscr{V}$ with respect to Y and L' give matrices F and V respectively.

$$\text{F}=\begin{bmatrix} bv_1 + \frac{v_2(H-Y)-v_2 Y}{H} - v_3'L' & v_3'(H-Y) \\ 0 & 0 \end{bmatrix} \text{ and V}=\begin{bmatrix} m & 0 \\ -1 & \mu \end{bmatrix}.$$

The NGM is $F.V^{-1}$ with $Y = Y^0 = 0, L' = L'^0 = 0$ (the infection free equilibrium, see section 3.4),

$$\text{NGM}=\begin{bmatrix} v_1 + \frac{v_2}{m} + \frac{Hv_3'}{m\mu} & \frac{Hv_3'}{\mu} \\ 0 & 0 \end{bmatrix}.$$

The spectral radius of the NGM evaluated at the infection free equilibrium then gives the basic reproduction number (Diekmann et al., 1990).

$$R_0^I = \upsilon_1 + \left(\frac{\upsilon_2}{m}\right) + \left(\frac{1}{m}.\frac{H\upsilon_3'}{\mu}\right) \tag{3.6}$$

$$= R_{\upsilon_1} + R_{\upsilon_2} + R_{\upsilon_3'} \tag{3.7}$$

The basic reproduction number is the sum of the individual reproduction numbers for the three different transmission routes: vertical (R_{υ_1}), sexual (R_{υ_2}) and environmental ($R_{\upsilon_3'}$).

The basic reproduction number for vertical transmission is simply υ_1, the proportion of offspring that are born infected. This is due to the system being at its carrying capacity. We have $b = m$ and so $b\upsilon_1/m$ becomes ν_1. In a system with only vertical transmission and a population with self-regulation at equilibrium, the offspring of an infected rat must all themselves be infected, or the infection cannot invade the population, since otherwise any infection will steadily decline.

For sexual transmission, the basic reproduction number is the rate at which sexual transmission occurs over the lifespan of an infected rat ($1/m$). The basic reproduction number for environmental transmission can be interpreted as the rate at which leptospires are shed λ (after re-scaling this is a rate of 1 per rat), over the lifespan of an infected rat ($1/m$), which will either infect new hosts ($H\upsilon_3'$) or die at rate μ.

3.3.2. Reservoir, R_0^{II}

In the second formulation, leptospires are added to the free-living leptospire state via shedding of infected rats, and so shedding is placed in the $\mathscr{F}$ matrix:

$$\mathscr{F}=\begin{bmatrix} b\upsilon_1 + \frac{\upsilon_2 Y(H-Y)}{H} + \upsilon_3'(H-Y)L' \\ Y \end{bmatrix} \text{ and } \mathscr{V}=\begin{bmatrix} mY \\ \mu L' \end{bmatrix}.$$

Again, the partial derivatives of the components of $\mathscr{F}$ and $\mathscr{V}$ with respect to Y and L' give matrices F and V respectively.

$$\text{F}=\begin{bmatrix} b\upsilon_1 + \frac{\upsilon_2(H-Y)-\upsilon_2 Y}{H} - \upsilon_3' L' & \upsilon_3'(H-Y) \\ 1 & 0 \end{bmatrix} \text{ and V}=\begin{bmatrix} m & 0 \\ 0 & \mu \end{bmatrix}.$$

The next generation matrix is defined as $F.V^{-1}$ with $Y = Y^0 = 0, L' = L'^0 = 0,$

$$\text{NGM}=\begin{bmatrix} \upsilon_1 + \frac{\upsilon_2}{m} & \frac{H\upsilon_3'}{\mu} \\ \frac{1}{m} & 0 \end{bmatrix}.$$

The second formulation of the basic reproduction number is then

$$R_0^{II} = \frac{1}{2}\left(R_{\upsilon_1} + R_{\upsilon_2} + \sqrt{4R_{\upsilon_3'} + (R_{\upsilon_1} + R_{\upsilon_2})^2} \right). \qquad (3.8)$$

Where R_{υ_1}, R_{υ_2} and $R_{\upsilon_3'}$ are as defined previously.

Clearly, for leptospire infection in rodents, assuming that the reservoir of leptospires contributes to infection risk is the correct biological assumption for the formulation of the basic reproduction number. The transition basic reproduction number has an additive form, meaning that there is no interaction between risk from the multiple transmission routes. The reservoir basic reproduction number is a more complicated expression than the transition formulation due to the additional interaction between the rats and the environment. In particular, the term non-linear term arises as the first infections in a susceptible system occur as a result of

the animals infected by vertical or sexual transmission shedding and providing additional risk from environmental transmission.

3.4. Local stability analysis

The (local) stability of an equilibrium point indicates whether, once perturbed, the system will return to the original equilibrium point (the point is stable) or diverge away to another equilibrium state (the point is unstable) (Soetaert & Herman, 2008). In models describing infection, it is of particular interest to know the conditions which lead to the infection free equilibrium point being unstable, allowing infection to invade the population, and also when the endemic infection equilibrium point is stable, allowing infection to persist. Here, expressions for the equilibrium states of the model are presented with corresponding stability analysis.

The equilibrium states of the model are the points at which the rate of change of numbers of susceptible rats, infected rats and free-living leptospires are zero. Expressions for the equilibrium states were found by setting each of the three equations to zero ($dY/dt = dL'/dt = dH/dt = 0$). The model as described by equations 3.4-3.6 has two equilibrium states: infection free and endemic infection, denoted Y^0, L'^0 and Y^*, L'^* respectively. Here $Y^0 = 0$, $L'^0 = 0$ and,

$$Y^* = \frac{H(R_0 - 1)}{(R_0 - \nu_1)} \tag{3.9}$$

$$L'^* = \frac{H(R_0 - 1)}{\mu(R_0 - \nu_1)} \tag{3.10}$$

The value of the endemic infection equilibrium is calculated using the analytical expression for R_0^I. It is worth noting that the endemic infection point is only biologically feasible only if $R_0 > 1$, so permitting positive abundances of infection.

An equilibrium point is stable if the sign of real part of all of the eigenvalues of the Jacobian matrix are negative, and unstable if the signs are positive (Keeling & Rohani, 2008). Firstly, the Jacobian was found for the system described by equations 3.4 and 3.5:

$$J = \begin{bmatrix} -m + bv_1 - L'v_3' + \frac{v_2(H-Y)}{H} - \frac{v_2 Y}{H} & v_3'(H-Y) \\ 1 & -\mu \end{bmatrix}.$$

For the infection free equilibrium, the characteristic polynomial of J could be written as:

$$cp(J) = A\Lambda^2 + B\Lambda + C$$

$$f(\Lambda) = A\Lambda^2 + B\Lambda + C$$

where,

$$A = 1$$

$$B = m\left(1 - (R_{v_1} + R_{v_2})\right) + \mu$$

$$C = m(1 - R_0).$$

The basic reproduction number R_0 is as defined in equation 3.6.

By Descartes' rule of signs, the number of sign changes between the coefficients A, $B$and C equals the maximum number of positive roots of the polynomial. Conversely, the number of sign changes of the coefficients in $f(-\Lambda)$ equals the

maximum number of negative roots. The Routh- Hurwitz criteria for stability are that the sign of coefficients of a (second-order) polynomial are positive. Finding the conditions of A, B and C which lead to negative or positive (or complex) roots will be equivalent to necessary conditions of stability. Table 3.1 shows a summary of the conditions for stability of the equilibrium points.

Table 3.1: Stability conditions for infection free and endemic infection equilibrium points.

	Condition	**Roots**	**Point**
Infection free	$R_0 < 1$	No positive roots, two negative roots	Stable
	$R_0 > 1$	One positive root and one negative root	Saddle
Endemic infection	$R_0 > 1$	No positive roots, two negative roots	Stable

The coefficients of $f(-\Lambda)$ for the infection free equilibrium were:

$$A = 1$$

$$B = -\left(m\left(1 - \left(R_{\upsilon_1} + R_{\upsilon_2}\right)\right) + \mu\right)$$

$$C = m(1 - R_0).$$

For the infection free equilibrium point, if $R_0 < 1$ then $R_{\upsilon_1} + R_{\upsilon_2} < 1 + \mu/m$, and so there will be no sign changes in $f(\Lambda)$, and two sign changes in $f(-\Lambda)$. There will be no positive roots, two negative roots, and no complex roots, and so the infection free equilibrium point is stable when $R_0 < 1$ by the Routh- Hurwitz criteria. For the characteristic polynomial of the infection free equilibrium, when $R_0 > 1$ there will be at most one sign change in $f(\Lambda)$ (and one positive root), and at most one sign change in $f(-\Lambda)$ (one negative root). Given that for a system of two differential

equations there will be two roots in total, there will be one positive and one negative (and no complex) roots if $R_0 > 1$. Hence the infection free equilibrium point is a saddle point when $R_0 > 1$. The two expressions for the basic reproduction number agree at the threshold, $R_0 = 1$ (see Appendix 1 for proof) so for the stability analysis, the expressions R_0^I and R_0^{II} are equivalent.

For the endemic infection equilibrium, the characteristic polynomial $f(\Lambda)$ can be written with,

$$A = 1$$

$$B = \frac{m\mu(mR_{\upsilon_3'}(1 - R_{\upsilon_1}) + (R_{\upsilon_2} + R_{\upsilon_3'})\mu + (H\upsilon_3' + \upsilon_2)(R_0 - 1))}{H\upsilon_3' + \upsilon_2\mu}$$

$$C = m\mu(R_0 - 1)\frac{H\upsilon_3'\mu + \upsilon_2\mu}{(H\upsilon_3' + \upsilon_2)}.$$

B will have a positive sign if $R_0 > 1$ (as $R_{\upsilon_1} \leq 1$) and C will have a positive sign if $R_0 > 1$. Similarly for $f(-\Lambda)$, there will be no sign changes if $R_0 > 1$. Hence there will be no sign changes, and no positive roots if $R_0 > 1$. The endemic infection equilibrium is stable if $R_0 > 1$ by the Routh- Hurwitz criteria. When $R_0 < 1$ the endemic infection equilibrium point is not biologically feasible (equation 3.10).

When $R_0 > 1$ the endemic infection equilibrium point is stable and the infection free equilibrium point is a saddle. For a saddle point, depending where a path is initiated the trajectory may diverge away from the point or approach it (Soetaert & Herman, 2008). Phase plots can be used to understand the paths which stay in the saddle points and those which diverge away. For the infection free saddle point, the only path which is within biologically realistic limits (numbers of rats and leptospires

are both positive) diverges away from the infection free point towards the endemic infection point (Figure 3.3, R_0^I =5.1). The trajectories initiated from the biologically realistic areas all converge to the stable endemic infection equilibrium point.

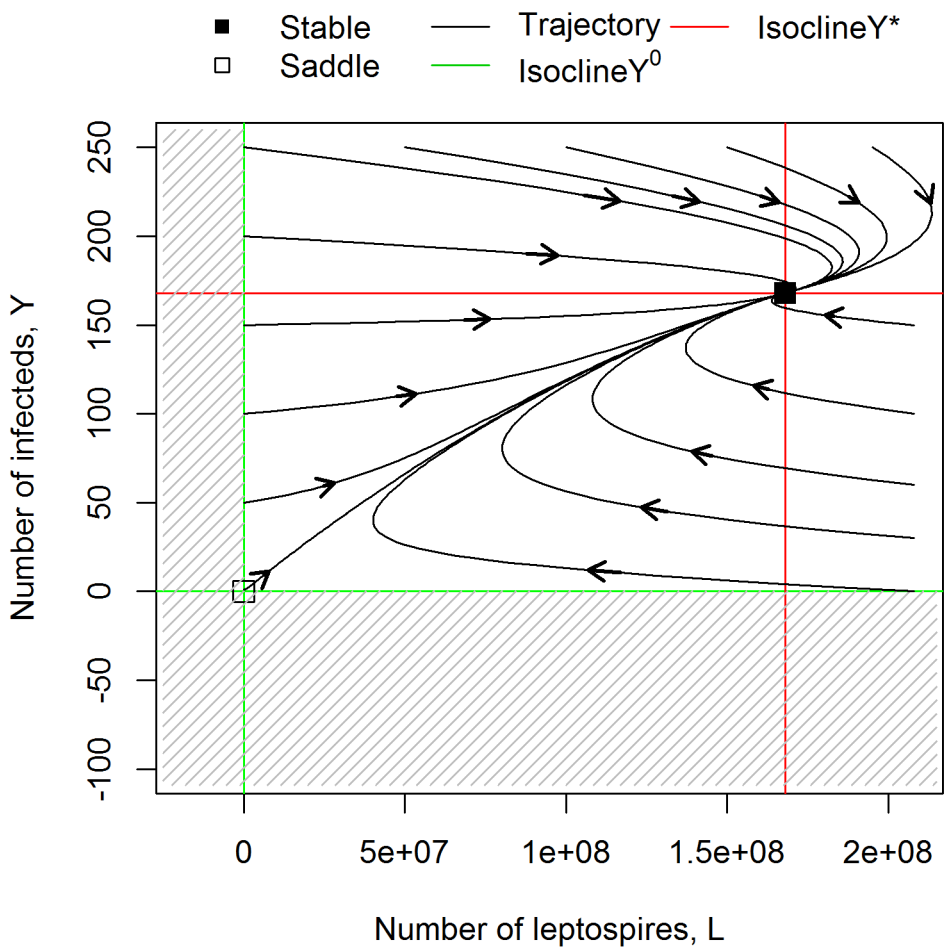

Figure 3.3: Phase plane of the model for $\boldsymbol{R_0^I}$=5.1. Greyed out sections indicate areas which contain biologically unrealistic values (negative population sizes). Isoclines indicate the values at which the rate of change of at least one of the variables is zero. The point at which the pairs of isoclines cross are the equilibrium points (Soetaert & Herman, 2008). Trajectories indicate the path that the model takes given different initial conditions.

3.5. Global sensitivity analysis of R_0

In determining the drivers of endemic infection, it is of interest to understand the importance of the different transmission routes. This translates into investigating the contribution of the components of R_0 (the overall basic reproduction numbers for the different transmission routes) leading to $R_0 > 1$.

The Sobol' (2001) method is a variance based sensitivity analysis. It calculates sensitivity 'indices' by dividing up the variance of the output of a function into fractions, to be attributed to the inputs. The first order indices (main effects) are the effects of the various parameters of a function. The total indices (total effects) measure the overall effect of a parameter, including all the variance caused by its interactions with other parameters. Here the main effect measures the effect of varying one component of R_0. The total effect is the main effect and the interaction effects where two components of R_0 are interacting, when their joint effect on the output is different from the sum of their individual effects. When the output is binary (whether $R_0 > 1$) the total effect is of most interest: is there a component which contributes most to the occurrence of endemic infection.

The Sobol' (2001) method requires as inputs parameter spaces on which to perform the sensitivity analysis. The parameter ranges specified in Table 3.1 were used in Latin hyper cube sampling (LHS) (Latinhyper, R package FME). LHS was used as it ensures that the entire parameter space is sampled; in a random sampling scheme some areas will be missed by chance (see chapter 2 for a fuller description of LHS). For the demographic variables, the birth rate was informed by field data (Panti-May

et al., 2016) and the mortality rate was obtained from studies in urban systems (Glass et al., 1988). Estimates of ranges for the remaining parameters were provided by the fieldwork team in Salvador, except for the rate of environmental transmission υ_3 (Table 3.2).

Table 3.2: Ranges of parameter values used in the sensitivity analysis of $\boldsymbol{R_0}$.

Parameter	Definition	Units	Range	Source/Comments
m	Rat mortality rate	Day^{-1}	0.007-0.024	A 'lifespan' of 20 to 6 weeks (Glass et al. 1988). Note $b = m$.
υ_1	Proportion of pups infected from suckling and born infected	Day^{-1}	0-0.25	Around 20% pups are infected (unpublished).
υ_2	Transmission rate via sexual transmission	Day^{-1}	0-0.01	Based on Holt et al. (2006).
υ_3'	Transmission rate via the environment	Day^{-1}	2.12x10^{-5}	Estimated in section 3.5.
μ	Mortality rate of leptospires in the environment	Day^{-1}	0.01-0.1	Long (approx. 100 days) or short lived (approx. 1 day).
H	Carrying capacity	Number of rats	200	The number of rats at carrying capacity.

The environmental transmission rate, υ_3, can be thought of as the product of the contact rate and the probability of transmission scaled by the average number of leptospires needed for transmission. The rate of infection from the environment is not an easily measured quantity, and so it is necessary to estimate a value for it in order to achieve a realistic output. Therefore, here the value is estimated

dependent on other parameters, but judgement of whether the output is realistic should be based on data obtained independently of those parameters.

Given the midpoint of the ranges for the birth rate (b), mortality rate (m), mortality rate of leptospires (μ) and transmission parameters set to zero (Table 3.2) values of $\upsilon_3{}'$ were found such that the model could achieve realistic prevalence. Specifically, the endemic equilibrium was calculated for given values of the environmental transmission rate υ_3, and the values were 'accepted' if the resulting prevalence of infection was projected to be in the range 60-80% (as found by Costa et al. (2014a)). This highest value accepted was 2.12×10^{-5}, which was used as the upper limit of the range for environmental transmission rate υ_3. The lower limit was zero.

The range of the basic reproduction number for vertical transmission generated by the parameter values in Table 3.2 (Table 3.3) does not include one, so vertical transmission alone cannot be responsible for the occurrence of endemic infection. The range for sexual transmission does include one, but the mean is 0.361 (Table 3.3), so for most of the parameter values, sexual transmission will not be solely responsible for endemic infection. For environmental transmission, the highest basic reproduction number observed was 5.458, but the mean was much lower (0.616, Table 3.3). Environmental transmission does have the potential to be solely responsible for endemic infection.

Table 3.3: Ranges of the basic reproduction numbers for each transmission route based on LHS used in sensitivity analysis.

Component	**Mean (Min, Max)**
Vertical transmission, R_{υ_1}	0.125 (0,0.25)
Sexual transmission, R_{υ_2}	0.361 (0, 1.393)
Environmental transmission, $R_{\upsilon_3'}$	0.616 (0, 5.458)
Transition, R_0^I	1.102 (0.01, 6.654)
Reservoir, R_0^{II}	1.102 (0.01, 3.105)

The mean values for R_0^I and R_0^{II} were both greater than one, which held for 45% of the calculated basic reproduction numbers of the 4x10^5 LHS samples. The range of R_0^I was much wider than for R_0^{II}. This is due to how R_{ν_3} enters each of the expressions: the relationship between $R_{\upsilon_3'}$ and R_0^I is linear, but for R_0^{II} the relationship is non-linear and so as $R_{\upsilon_3'}$ becomes larger, R_0^{II} increases at a slower rate.

Using the ranges as shown in Table 3.2, global sensitivity analysis of R_0 to its different components was performed using LHS and the scheme proposed by Saltelli (2002) (sobol2002, R package sensitivity) (Figure 3.4). The two formulations of the basic reproduction number agree at the threshold $R_0 > 1$, so it was only necessary to perform the sensitivity analysis on one formulation. If a main or total effect of a component is equal to one, the outcome depends only on that component. Conversely, if a main or total effect of a component is equal to zero, then the outcome does not depend on that component.

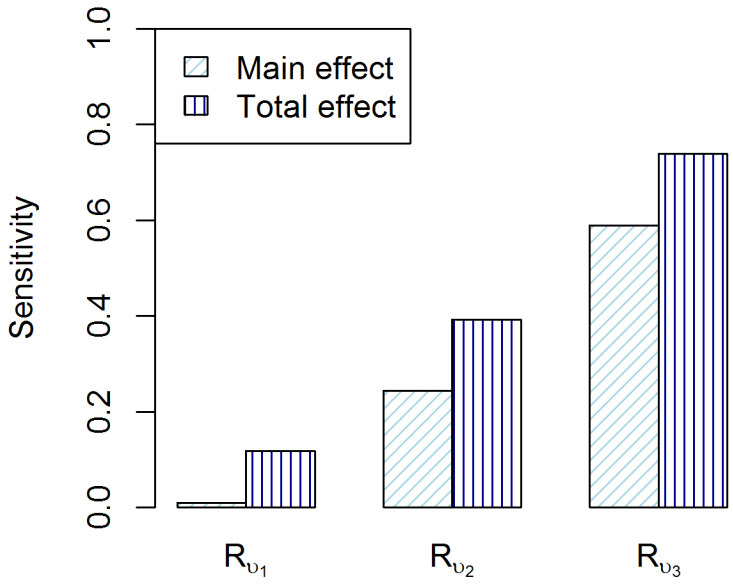

Figure 3.4 Main and total effect for the different components of $R_0 > 1$.

The main effect for R_{υ_1} was very low, suggesting that varying that component solely had little effect on going over the threshold $R_0 > 1$ (Figure 3.4). The component R_{υ_2} had a higher main effect, and $\boldsymbol{R}_{\boldsymbol{\upsilon_3'}}$ had the highest main effect. The same pattern holds for the total effect, but with R_{υ_1} having a relatively higher value than its main effect, meaning that an increased value of R_{υ_1} will have a greater effect in going over the threshold $R_0 > 1$ when the other components are taken into account. This result is logical based on the summary statistics of R_{υ_1} (Table 3.3). For our parameter ranges, R_{υ_1} could not be more than one, and so the only role it can play in the occurrence of endemic infection is in combination with the other transmission routes.

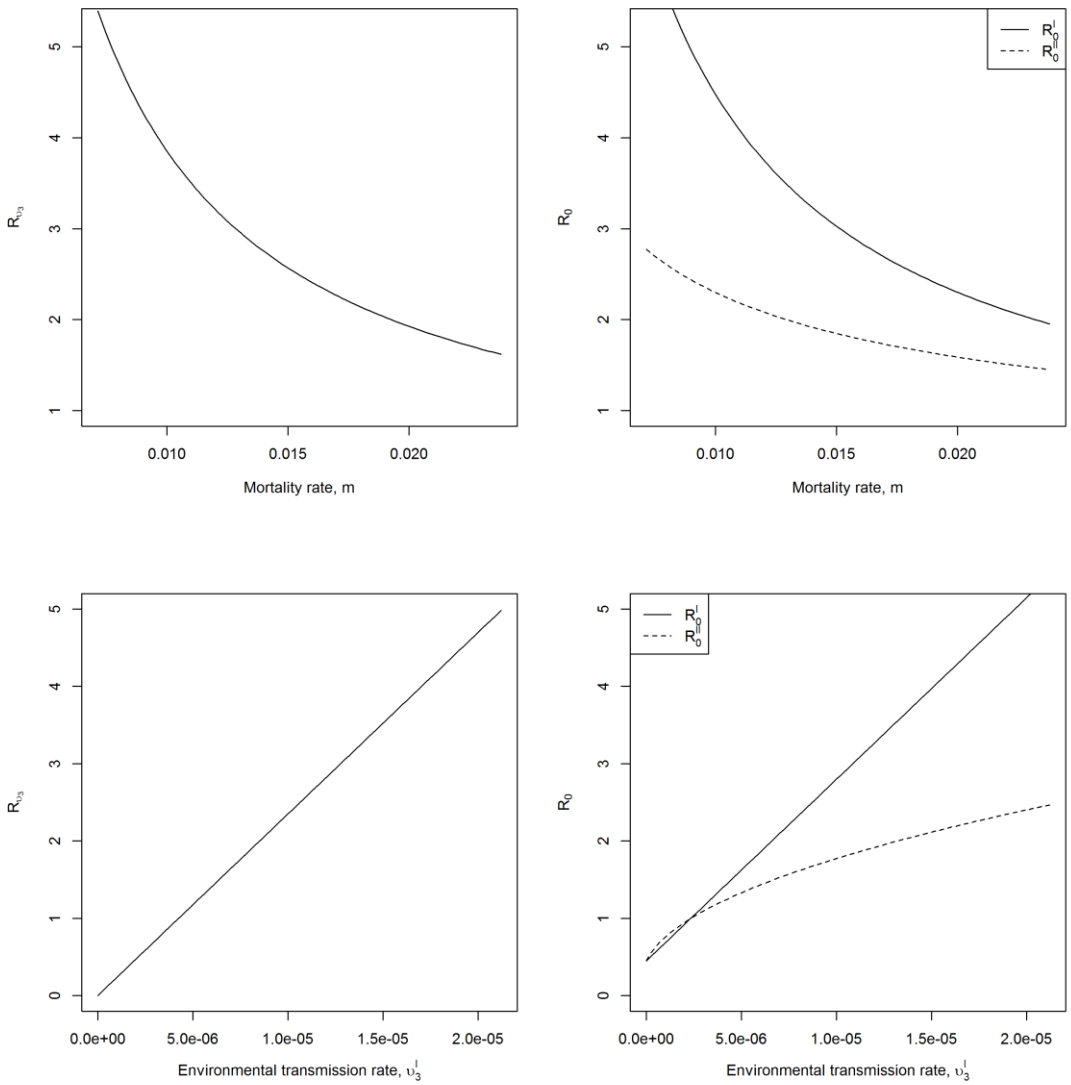

Figure 3.5: Changes in $\boldsymbol{R_{\upsilon_3}}, \boldsymbol{R_0^I}$ and $\boldsymbol{R_0^{II}}$ with respect to the parameters that enter $R_{\upsilon_3'}$.

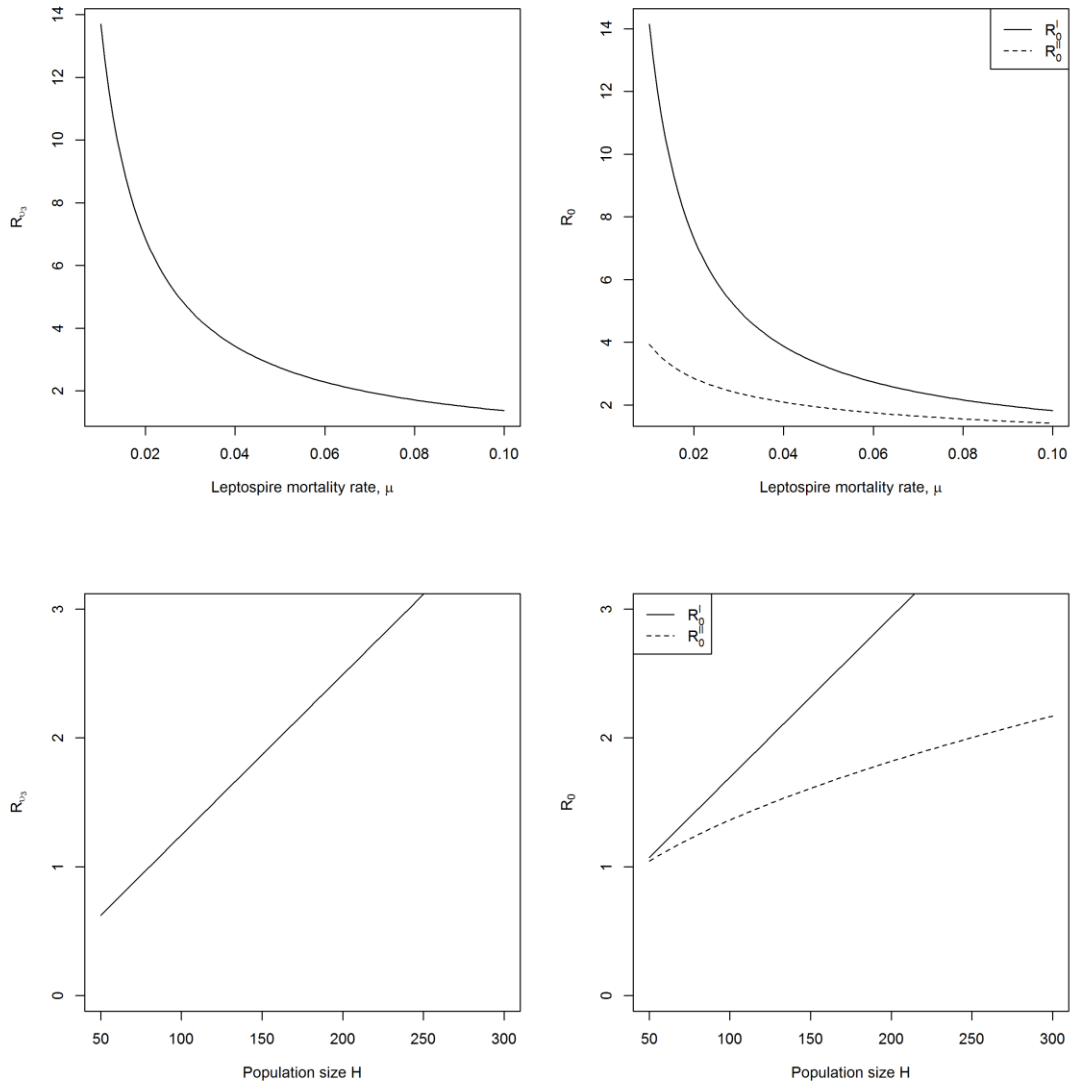

Figure 3.5 (continued): Changes in $\boldsymbol{R_{v_3}}$, $\boldsymbol{R_0^I}$ and $\boldsymbol{R_0^{II}}$ with respect to the parameters that enter $R_{v_3'}$.

For the sensitivity analysis of the magnitude of the two expressions for the basic reproduction number, preliminary results suggested that the magnitude depended almost entirely on $R_{\upsilon_3'}$ (both main and total effect were almost equal to one). The results are not presented here because accurate results were not achieved (main effect was not less than the total effect). This may be because the magnitude of the basic reproduction number is almost entirely dependent on $R_{\upsilon_3'}$. Instead, the changes in the magnitude of the two expressions of the basic reproduction were investigated in respect to changes in parameters which contribute to $R_{\upsilon_3'}$ (Figure 3.5).

When changes in a parameter value results in a non-linear decrease in $R_{\upsilon_3'}$, the same relationship is observed between changes in that parameter value and R_0^I and R_0^{II} (Figure 3.5). This is true for mortality rate of rats, m, and mortality rate of leptospires, μ. For changes in the value of environmental transmission rate, υ_3', and population size, H, there is a linear increase in $R_{\upsilon_3'}$ and R_0^I , but a non-linear increase in R_0^{II}. In general, the value of R_0^I is more than R_0^{II} except when environmental transmission rate, υ_3', and population size, H, are low in value. The greatest differences between the two numbers are observed when environmental transmission rate and population size are high or when mortality rate of rats or leptospires is low.

3.6. Target reproduction number

The basic reproduction number provides a threshold for the occurrence of endemic infection, which can be used to inform the implementation of disease control measures. In the control of any infectious disease there may be multiple control strategies available, which instead of targeting both the host and the environment, may target just one of the two, or even target one sub-population of either. The type reproduction number (Roberts & Heesterbeek, 2003) is an expression that provides a threshold for the occurrence of infection in the host population for different population types, e.g. the host population or the environment. If control measures for the environment were cheaper or easier to implement, a type reproduction number for the environment might be of more use than the basic reproduction number.

The target reproduction number introduced by Shuai et al. (2013) extends this approach even further. Target reproduction numbers provide a threshold value similar to the basic reproduction number and the type reproduction number, but where a sub-population within a population type is targeted in order to eradicate infection in the host population. The elements of the NGM describe the secondary infections of different population types and so these sub-populations, or targets, can be selected using the elements of the NGM.

We believe that the basic reproduction number (equation 3.9) found when the environment is treated as a reservoir is most representative of the field system at

hand. Therefore, we can find the target reproduction numbers using the reservoir NGM:

$$\text{NGM}=\begin{bmatrix} \upsilon_1 + \frac{\upsilon_2}{m} & \frac{H\upsilon_3'}{\mu} \\ \frac{1}{m} & 0 \end{bmatrix}.$$

The first row of the NGM describes the secondary infections, either by vertical and sexual transmission ($\upsilon_1 + \upsilon_2/m$) or environmental transmission ($H\upsilon_3'/\mu$). Secondary free-living leptospires are only generated by shedding (we do not included any kind of bacterial growth), and so the only entry in the second row is the 'shedding rate' multiplied by the lifetime of a rat ($1/m$).

Target sets were created by targeting different elements of the NGM (referred to as $(1,1), (1,2)$ and so on in Table 3.4). When the target set, $S = \{(1,1), (1,2)\}$, representing the host population, the target reproduction number is equal to the transition basic reproduction number (equation 3.8). When the target is set at $S = \{(1,1)\}$, the sexual and vertical transmission entry of the NGM, the target reproduction number only holds when $R_{\upsilon_3'} < 1$. Infection could be eradicated by controlling only sexual and vertical transmission only if environmental transmission would not otherwise sustain infection. The converse holds when the target set is $S = \{(1,2)\}$, the environmental transmission entry of the NGM. That is, infection could in principle be eradicated by only targeting environmental transmission, so long as vertical and direct transmission would not otherwise be responsible for occurrence of endemic infection.

Table 3.4: Target populations with corresponding control measure, target set and target reproduction number.

Target	Control	Target set	Target reproduction number
Host population	Remove rats	$S = \{(1,1),(1,2)\}$	$T_S = R_{v_1} + R_{v_2} + R_{v_3'}$
Control via sexual and vertical transmission only	Destroy burrows and remove adult rats	$S = \{(1,1)\}$	$T_S = \frac{\left(R_{v_1} + R_{v_2}\right)}{1 - R_{v_3'}}$
Control via environmental transmission only	Destroy burrows near water sources	$S = \{(1,2)\}$	$T_S = \frac{R_{v_3'}}{1 - \left(R_{v_1} + R_{v_2}\right)}$
Control via shedding	Improve drainage	$S = \{(2,1)\}$	$T_S = \frac{R_{v_3'}}{1 - \left(R_{v_1} + R_{v_2}\right)}$
Control via environmental transmission and shedding	Destroy burrows near water sources and improve drainage	$S = \{(1,2),(2,1)\}$	$T_S = \frac{1}{2}\left(R_{v_1} + R_{v_2} + \sqrt{4R_{v_3'} + (R_{v_1} + R_{v_2})^2}\right)$

The target reproduction number for the environment, target set $S = \{(2,1)\}$, is a function of the transmission routes. Infection will be eradicated if a proportion of target S entries greater than $p_s = 1 - 1/T_s$ can be removed (Shuai et al., 2013). In order to eradicate infection, and provided that $R_{v_1} + R_{v_2} < 1$, the free-living leptospire state must be reduced by,

$$p_{2,1} = 1 - \left(1 - \frac{\left(R_{v_1} + R_{v_2}\right)}{R_{v_3'}}\right).$$

Controlling leptospires in the environment can only result in eradicating infection in the rat population if vertical and sexual transmission would not otherwise sustain the occurrence of infection (if $R_{v_1} + R_{v_2} > 1$). The parameter ranges used in the Latin hyper cube samples (LHS) represent realistic values of the model parameters, so that any results based on the LHS should include all possible scenarios. Based on the LHS, we have obtained $R_{v_1} + R_{v_2} < 1$ in approximately 95% of the parameter sets. Given our uncertainty in the model parameters, it is likely that a control applied to the environment would reduce infection successfully. However it should be acknowledged that there are occasions where it could not. The target reproduction number for control via shedding is the same expressions as for control by environmental transmission. A measure to reduce leptospires in the environment would require the same reduction as a control measure to reduce contact between rats and leptospires.

Finally, if both environmental transmission and the shedding into the environment are the target set, the target reproduction number equals the reservoir basic

reproduction number (equation 3.9). Environmental control measures can be applied without the constraint of $R_{v_1} + R_{v_2} < 1$ if the threshold used is,

$$p_{(1,2),(2,1)} = 1 - \frac{1}{\frac{1}{2}\left(R_{v_1} + R_{v_2} + \sqrt{4R_{v_3'} + \left(R_{v_1} + R_{v_2}\right)^2}\right)}.$$

In order to compare the control efforts required for each of the different type reproduction numbers, the proportions $p_S = 1 - 1/T_S$ were calculated based on the LHS. The proportion $p_{1,1}$, target vertical and sexual transmission only, is constrained by $R_{v_3'} < 1$. Even when this constraint is held, the distribution of proportions is wide given our parameter ranges (Figure 3.6). Similarly, control via different environmental routes individually $p_{1,2} = p_{2,1}$ is constrained by $R_{v_1} + R_{v_2} < 1$. We observe a heavily skewed distribution with high valued proportions.

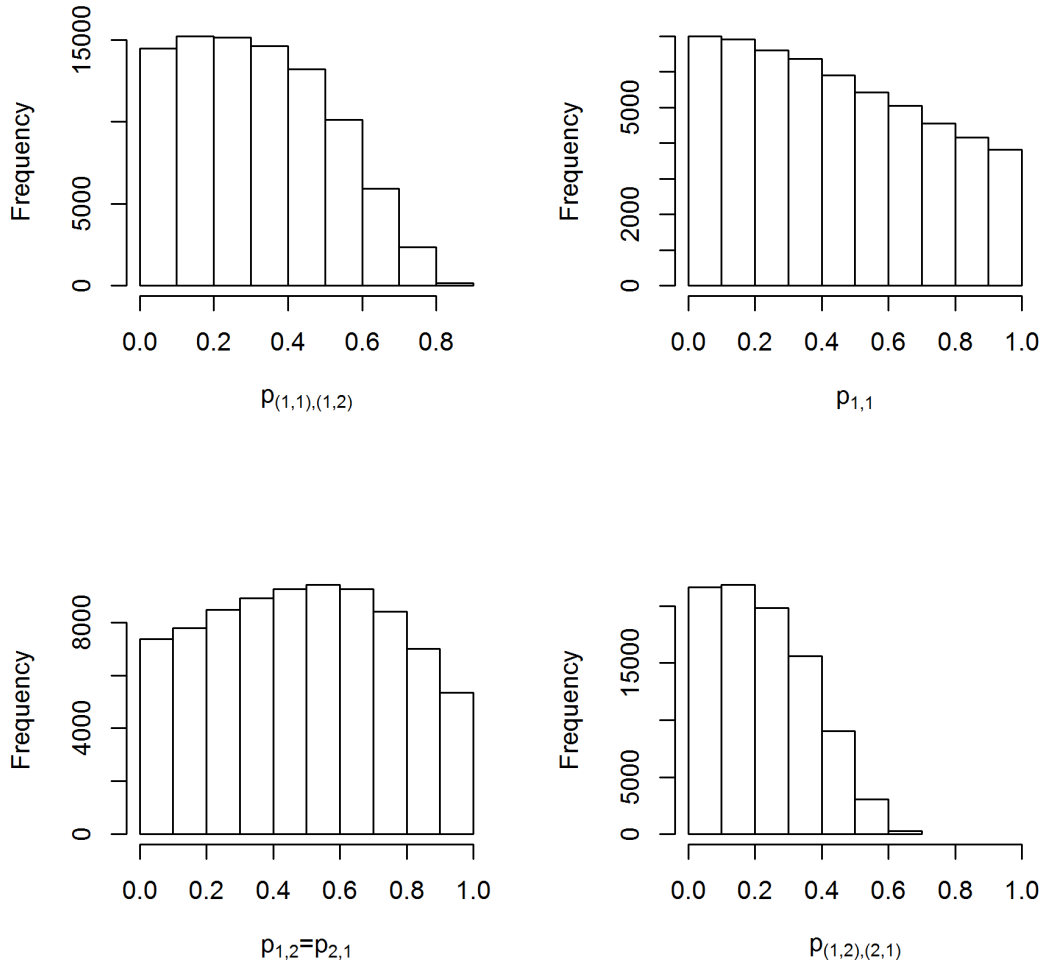

Figure 3.6: Proportions $p_S = 1 - 1/T_S$ calculated from the LHS for each type reproduction number (Table 3.4).

When the target is either the entire host population ($p_{(1,1),(1,2)}$) or both environmental controls at the same time ($p_{(1,2),(2,1)}$), the distributions have a slight skew. The proportion for both environmental controls at the same time ($p_{(1,2),(2,1)}$) has on average the lowest valued proportions of all controls.

3.7. Spatial difference in risk

The slums in Salvador can be considered as three valleys, each with different patterns and incidences of human leptospirosis. To investigate whether there is a

corresponding difference in the prevalence of infection in the rat population, we performed a valley level analysis of the model. The model predicts that a population will either be infection free or have endemic infection (of a particular level in the population). It is of interest whether there is differential level of infection by valley and so we present the results based on the behaviour at endemic infection equilibrium only.

Mortality rate of rats, the transmission parameters and the mortality rate of leptospires are assumed not to vary by valley. The model parameters which may be considered to differ by valley were the shedding rate and population size. There is evidence to indicate that animals captured in valley 4 have a lower shedding rate than valley 1 and 2 (see Appendix 1 for detail) and so we employed a valley level shedding rate (Table 3.5). The population size for each valley was calculated by scaling abundance estimations (unpublished data) to the total trapping area.

Table 3.5: Valley level parameter values. Shedding rate values are mean (95% confidence interval) (Appendix 1), population size values are mean (lower, upper) of estimates.

Valley	**Population size (H)**	**Shedding rate (λ)**	**Environmental transmission (υ_3')**
1	52 (24, 96)	2×10^5 (8×10^4, 7×10^5)	8.4×10^{-5} (3.4×10^{-5}, 3.0×10^{-4})
2	63 (34,125)	9×10^4 (4×10^4, 2×10^5)	3.8×10^{-5} (1.7×10^{-5}, 8.4×10^{-5})
4	72 (32,127)	6×10^4 (3×10^4, 1×10^5)	2.5×10^{-5} (1.3×10^{-5} 4.2×10^{-5})

To estimate the rescaled rate of environmental transmission υ_3' we first note that $\upsilon_3' = \lambda\upsilon_3$. Then for valley 1, 2 and 4 we will have $\upsilon_{3,1}' = \lambda_1\upsilon_3$, $\upsilon_{3,2}' = \lambda_2\upsilon_3$ and $\upsilon_{3,4}' = \lambda_4\upsilon_3$ respectively where $\lambda_i$is the shedding rate of valley i. The rescaled

environmental transmission rates for the three valleys share a common 'baseline' υ_3. With the known prevalence and shedding rate for valley 1, we estimated the baseline level of environmental transmission υ_3. The baseline rate of environmental transmission, υ_3, was estimated for valley 1 by taking all other parameters aside from mean population size and shedding rate at their midpoint, then finding the value $\upsilon_3$which predicted the correct level of prevalence as observed in valley 1. This process was repeated until 1000 values of υ_3 has been accepted, final value of υ_3 was the mean of these 1000 values. Using this estimated baseline υ_3 the rescaled environmental transmission rate (υ_3') was calculated using the valley level shedding rate.

Using the midpoints of parameters in Table 3.2 and the mean, lower and upper values, the values of the two basic reproduction numbers and the prevalence at endemic equilibrium were calculated (Table 3.6). The changes in the basic reproduction numbers are due to changes in environmental transmission, as the valley level parameters are only related to environmental transmission. The upper limit for carrying capacity was highest in valley 2 and 4, resulting in a high upper limit of prevalence and reproduction number, though these numbers were smaller than the upper limit for valley 1 (Table 3.6). There is no consistent pattern in the measures of infection in the rat population, but there is also no consistent pattern in human incidence of infection (Table 3.7).

Table 3.6: Valley level basic reproduction numbers, prevalence, number of infecteds (Y^*) and leptospires (L^*) at endemic infection equilibrium using mean values (lower, upper) values.

Valley	R_0^I	R_0^{II}	Prevalence	Number of infecteds, Y^*	Number of leptospires, L'^*
1	5.60 (1.40, 33.75)	2.51 (1.22, 6.00)	0.84 (0.31, 0.97)	44 (8, 94)	794 (137, 1700)
2	3.26 (1.12, 12.84)	1.92 (1.07, 3.75)	0.72 (0.12, 0.93)	44 (4, 116)	825 (76, 2116)
4	2.59 (0.92, 6.74)	1.70 (0.95, 2.74)	0.64-(NA, 0.87)	46 (NA, 110)	844 (NA, 2004)

Table 3.7: Valley level incidence of human leptospirosis (Sacramento, in preparation).

	Valley 1	Valley 2	Valley 4
Time period	**Incidence/1000**	**Incidence/1000**	**Incidence/1000**
Feb-Jul,2013	59.74	64.46	11.64
Aug-Dec,2013/Jan, 2014	29.90	63.12	93.53
Feb-Jul, 2014	42.48	28.27	24.11
Aug-Dec, 2014/Jan,2015	23.16	61.04	49.57

There was substantial variation in incidence of human infection in valley 4 (Table 3.7). Rats were trapped over time periods close in time, but not exactly the time periods when the human incidences were recorded. Table 3.8 shows the infection measures for the rat population based on abundance measures from the first trapping event in the trapping time period given and shedding rates as in Table 3.5. The highest observed values for all infection measures were observed in the time periods May-August 2013 and October-December 2013. These dates were closest to the time period in which the highest incidence of human leptospirosis was observed in valley 4. Similarly, the lower observed infection measures for rats correspond to the decrease in incidence in valley 4 (Table 3.7, Table 3.8).

Table 3.8: Trapping period differences (for valley 4 only) in basic reproduction numbers, prevalence, number of infecteds (Y^*) and leptospires (L^*) at endemic infection equilibrium using mean values (lower, upper) values.

Trapping period	R_0^I	R_0^{II}	Prevalence	Number of infecteds, Y^*	Number of leptospires, L^*
May-Aug,2013	3.39 (1.52, 6.74)	1.95 (1.28, 2.74)	0.73 (0.37, 0.87)	72 (27, 110)	1318 (487, 2004)
Oct-Dec,2013	3.06 (1.44, 5.85)	1.86 (1.25, 2.56)	0.70 (0.34, 0.85)	62 (23, 92)	1124 (410, 1679)
Mar-Aug,2014	2.14 (0.97, 4.41)	1.54 (0.98, 2.23)	0.57 (NA, 0.80)	32 (NA, 64)	587 (NA, 1158)
Sep-Dec, 2014	1.73 (0.92, 3.12)	1.38 (0.95, 1.88)	0.45 (NA,0.71)	20 (NA, 38)	355 (NA, 695)

3.8. Discussion

The model framework presented here has been developed specifically to describe leptospire dynamics in *Rattus norvegicus* in urban habitats. The Holt et al. (2006) framework is the only existing model of leptospire infection in rodents. The most similar existing model to ours is that of Xiao et al. (2007) for *Salmonella* in livestock populations. The Holt et al. (2006) framework is an age structured model. Here we ignored the significance of age dependent transmission with the aim of finding the simplest model to be explored analytically and adding to existing host-pathogen models where stability analysis and behaviour at equilibrium have been presented. The Xiao et al. (2007) framework is an SIR model with three transmission routes: vertical, environmental and direct (density dependent). Our framework as presented above has a number of differences. Direct transmission assumed to occur via sexual contact and so is modelled as being frequency dependent. Further, there is no recovery class, an assumption that is appropriate for *Leptospira* carriage in Norway rats but not all systems (Bharti et al., 2003; Eliis, 2014). We also did not include any 'wastage' of bacteria (leptospires that are lost from the environment when picked up by animals), in contrast to Xiao et al. (2007). Results from laboratory dose response studies on *Leptospira* in Norway rats (Athanazio et al., 2008) suggest that the number of leptospires required for infection is likely to be negligible compared to the size of the total number of free-living leptospires. Therefore, we chose not to include a parameter to describe wastage in the model.

The parameter ranges were mostly obtained from the literature or were informed by recent field studies in Salvador. All these, therefore, have a firm empirical basis.

We obtained a range for the value of the environmental transmission rate υ_3' by performing an estimation procedure. There are previous examples of estimation of indirect transmission rate. For example, Mukandavire et al. (2011) used non-linear least squares estimation applied to cumulative number of infections data, and Tien et al. (2011) used pseudo-estimation by tuning parameter values to obtain a satisfactory fit to data. As an alternative to estimating parameters, some studies have used tests of the robustness of results when changing the value of an arbitrarily chosen parameter (Breban et al., 2009). However, values for indirect transmission rates (such as environmental transmission) are often unknown and so are assigned assumed values (Xiao et al., 2005) or are based on results from animal species other than the one of interest (Ivanek & Lahodny, 2015). In the absence of longitudinal data on infection dynamics in rats, we could not apply least squares or tuning methods based on obtaining a satisfactory fit to longitudinal data. There is no evidence that prevalence is seasonal, and so prevalence data from the field is considered a stable value. We tuned the value of the environmental transmission rate to prevalence data from the field (obtained independently of the empirical parameter value estimates) and to the behaviour of the model at endemic infection equilibrium.

In order to identify which factors may be responsible for the maintenance of endemic infection, stability analysis was performed on the equilibrium points of the model. Given the simplicity of the model, it was possible to find analytical expressions for the equilibrium points and to elaborate the stability conditions of these points. In particular, the stability of the equilibrium points was found to be

dependent on the threshold of the basic reproduction number being more or less than one. Two different expressions were found for the basic reproduction number, resulting from whether the environment was treated as reservoir as infection or not. In both expressions, the basic reproduction number was found to be a function of basic reproduction numbers for each of the three transmission routes in the model.

Global sensitivity analysis was performed on the basic reproduction number as a binary value as in Davis et al. (2010). The sensitivity analysis suggested that all transmission routes have the potential to play a role in the occurrence of endemic infection. Vertical transmission cannot be solely responsible for the occurrence of endemic infection (Table 3.3, Figure 3.4), but may contribute when accompanied by other transmission routes. Changes in the rate of sexual transmission will have a greater effect on the occurrence of endemic infection than vertical transmission, but changes in the rate of environmental transmission will have an even greater effect. Similar results were found by Xiao et al. (2007) who investigated the contribution of different transmission routes on the dynamics of infection in an unmanaged animal population. They concluded that vertical transmission had little effect on the model dynamics, whereas changes in direct and indirect transmission led to changes in the behaviour of the model at equilibrium.

The sensitivity results were based on parameter ranges that were deemed realistic for leptospire infection in rats in the slums based on our current knowledge of the system. In some cases, the biology behind the parameter value is well understood, whereas in others, the range was assigned based on studies on other reservoirs or

given a wide range to accommodate all possible scenarios. The value of the sexual transmission basic reproduction number can be affected both by the rate of sexual transmission and the average lifespan of a rat. Small variations in mortality rate by system are expected, but in general the mortality rate of rats in wild systems is high (Feng & Himsworth, 2014) and is thought not to differ much across different settings (Glass et al., 1989). The rate of sexual transmission here was adopted from Holt et al. (2006), as there are no existing quantitative studies on sexually transmitted leptospire infection in rats. Sexual transmission comprises of contact rate and probability of successful infection. We expect the contact rate of adult rats to remain constant, but how the probability of successful infection may change is unknown. The sensitivity results of sexual transmission could change if we had a better estimate for the rate of sexual transmission or the probability of successful leptospire infection.

The parameters related to environmental transmission were assigned wide ranges to accommodate for their associated uncertainty. Shedding rate for example, although based on observed data from animals in the slums included a wide range of values. It is not known whether animals shed at a consistent rate throughout their lifetime or if shedding rate decreases at any point. In the model, we assume that animals do shed at the same rate throughout their lifetime. The mortality of leptospires was also given a wide range of values, as the average lifespan of a leptospire could change depending on the type of environment. As we have discussed previously, the rate of environmental transmission was estimated. The rate here is not presented as a quantity which can be estimated from data on rat

and leptospire contact, but as a parameter which needs to be assigned a realistic value. Whereas the sensitivity results do suggest that the environmental transmission route is most important for a wide range of scenarios, if some parameters had a better biological basis and so a narrower parameter range, then the conclusions could change.

Aside from Bani-Yaghoub et al. (2012), there is only one other study which considers multiple forms of the basic reproduction number in reference to how the environment is treated. Ivanek & Lahodny (2015) found which of three of the basic reproduction numbers was most similar to the empirical basic reproduction number estimated from experimental data. We presented two expressions for the basic reproduction number, formulated by different treatments of the role of the environment. When the basic reproduction number of environmental transmission is zero, the two expressions are equal. Changes in the magnitude of the two different reproduction numbers were investigated for parameters related to environmental transmission. The greatest differences between the two basic reproduction numbers were observed when shedding rate, environmental transmission rate and population size at equilibrium were high, and when rats and leptospires were longer lived. Hence it is the role of the environment which leads to the appearance of different levels of control.

The reservoir basic reproduction number is better representative of the field system. In most wildlife infection systems, shedding of infectious particles will feed into a reservoir of infection. For this reason, the target reproduction numbers were found based on the reservoir next generation matrix. The two expressions were

directly related to the target reproduction numbers: the transition basic reproduction number was equal to the target reproduction number for the host population, and the reservoir basic reproduction number was equal to the target reproduction number for both environmental controls. The current control method applied in the slums is the removal of rats, but it is of interest to know whether targeted controls would require a lower level of effort or could even be applied successfully.

Controls for infection can only be considered when the required effort can be considered realistic or feasible in the given setting. The histograms in Figure 3.6 show the potential reductions of prevalence in rats required for the different target reproduction numbers based on the parameter ranges we believe represent all possible scenarios. Eradicating leptospirosis by targeting vertical and sexual transmission is not a viable option in the slums. Even when the constraint on the environmental transmission is met, which is unlikely to occur, there is no guarantee that effort will be low. Often the constraint on vertical and sexual transmission is met, but then proportions needed to control via environmental transmission only are too high to be considered feasible. The distribution of proportions that was on average lowest was for environmental controls, transmission and the reservoir. Applying environmental controls would be most difficult in terms of allocation of resources and organisation. The distribution of proportions for controlling the entire host population was similar in shape to the environmental controls. Though controlling via environmental transmission and the reservoir would on average require a smaller reduction than controlling the host population, removing rats is

easier to implement, and so we would recommend the removal of rats to control leptospirosis in the host population.

Human incidence of leptospirosis is expected to vary by season, as seasonal flooding increases risk of acquiring infection (Ko et al. 1999). The rats in Salvador do not have a seasonal birth rate, and hence we do not expect to see a seasonal risk of transmission directly from rats. Increased risk of transmission to humans from the rat population may come from spatial differences in abundance and shedding. There were no consistent differences in the prevalence in the rat populations of each valley. However, when abundance values were stratified by time period, the patterns observed in rat infection measures were similar to the patterns in human incidence of leptospirosis in one location. Although there is no seasonal reproduction of rats in Salvador, it seems that natural variation in rat population sizes may be important in predicting human infection risk.

Decisions regarding the best measures to control infection need to be based on numerical results and considerations of availability resources and ease of implementation. For controlling leptospire infection in the slums, applying the two environment controls was the best numerical result, but removal of rats is a control that would be easier to implement. The costs of these two controls differ greatly in terms of monetary terms and effort. Optimal control is investigated further by applying optimal control theory to an age structured model in chapter 6..

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Chapter 4

Identifying evidence of multiple transmission routes: leptospirosis in *Rattus norvegicus*

4.1 Introduction

There are often multiple potential routes of intraspecific transmission of pathogens within wildlife and other populations. Seeking evidence of these different transmission routes by experimental infection in a laboratory setting is difficult and often does not represent transmission as it would occur in the wild. In particular, for pathogens causing zoonotic diseases, knowing whether these transmission routes occur in practice, and their relative importance, may have implications for control measures to reduce infection prevalence in the reservoir host and ultimately prevent human infection (Lloyd-Smith et al., 2009). Inferring the relative importance of different potential transmission routes from field data may therefore be of both fundamental and practical interest. However, inferring routes of transmission from statistical associations is not straightforward. There is a need to consider multiple statistical models with different underlying assumptions to better understand associations between risk and reality.

Leptospirosis is a zoonosis (de Faria et al., 2008) caused by pathogenic bacteria of the genus *Leptospira*, commonly called leptospires (World Health Organization, 2003). Most mammals can serve as reservoirs, many becoming chronically infected

and shedding infectious leptospires in urine. Humans are incidentally infected (World Health Organization, 2003) and do not contribute to human-to-human transmission except in rare circumstances as in utero infection to the fetus or neonatal infection via milk (Shaked et al., 1993; Bolin & Koellner, 1988). The main routes of human infection are through contact with environmental sources that have been contaminated with animal urine or direct contact with animal reservoirs. There are some vaccines to prevent human leptospirosis but these are often not effective (Bharti et al., 2003).

Salvador, a coastal city in north-east Brazil, has experienced a recent population increase typical of other cities in Brazil, where intense migration swelled the urban population from 58% to 80% of the total population between 1970 and 2000 (da Mata et al., 2007) leading to the creation and expansion of urban slums (Ko et al., 1999). The prevalence of human leptospirosis in the slums of Salvador is high. A recent community-based survey of 3,171 slum residents from Pau da Lima, a community in Salvador found an overall prevalence of *Leptospira* antibodies of 15.4% (Reis et al., 2008).

Residents in the slums live in close proximity to the primary animal reservoir, the Norway rat (*Rattus norvegicus*) (Ko et al., 1999; Ganoza et al,. 2006; Costa et al., 2014) and environments contaminated with leptospires shed in rat urine (the environmental reservoir). Increased risk of exhibiting leptospire antibodies has been found to be associated with residence regions prone to flooding, with open sewers and accumulated refuse close by, and sightings of rats at the home (Reis et al., 2008; Sarkar et al., 2002). The prevalence of *Leptospira* carriage among rats in Pau

da Lima, Salvador has been found to range between 60- 80% (Costa et al., 2014). A previously described population of 82 rats from Salvador (Porter et al., 2015), stratified into three age classes, was estimated to shed 9.1×10^{10} leptospires per day with a mean density of 5.0×10^{10} leptospires per m^2 of soil around households (Costa et al., 2015). However, we do not currently understand the pathways of intra-specific transmission of leptospires in the rat reservoir, nor the patterns of persistence of leptospires in the environment.

For rats, there are multiple potential routes of leptospire transmission: 1. vertical and pseudo-vertical transmission, where rats are either born infected or acquire infection via suckling from infected mothers (we combine these as they may be impossible to distinguish in the field); 2. direct transmission, either by sexual contact or by some other direct mechanism; and 3. infection from exposure to environmental sources contaminated with bacteria. There is biological evidence that vertical and sexual transmission may occur, namely the presence of leptospires in the mammary gland and semen of rats (unpublished work). A high concentration of leptospires are shed in the urine (Costa et al., 2015) so we assume that environmental transmission occurs. However, whether these transmission routes successfully occur in the wild is unknown, and yet evidence of their occurrence and importance in the slums of Salvador is crucial for our understanding of leptospire dynamics overall.

We can address this by noting first that the multiple transmission routes are age dependent. When rats are born they are initially confined to the nest. Once weaned, they leave the nest and begin to roam, eventually becoming sexually

mature. Vertical and pseudo-vertical transmission both occur prior to weaning and we can therefore consider them to be reflected, together, in the proportion of animals infected once they first appear in the free-roaming population. Once animals reach sexual maturity they are at risk of direct transmission during sexual contact; and throughout an animal's free-roaming life it will be at risk of environmental and (non-sexual) direct transmission. The level of wounding is a risk factor for Hantavirus infection in wild rats (Hinson et al., 2004), for which the primary route of infection is direct (via biting). For leptospirosis, wounding has found to be associated with a higher *Leptospira* load in the urine and kidney (Costa et al., 2015) and leptospire infection in the kidney (Himsworth et al., 2013) in Norway rats. However, leptospire presence in saliva has not been tested (Costa et al., 2015). If we can age animals trapped from a natural population, and determine whether they are infected, whether they are sexually mature, and whether there is evidence of other activities conducive to direct transmission, then we can assess which combination of the different transmission routes best accounts for the age-profile of infection observed in the field.

Previous studies of wildlife disease have used age-prevalence data to infer evidence of transmission routes based on the force of infection (FOI), also known as the hazard of infection. The force of infection (FOI) is the 'the per capita rate at which susceptible hosts acquire infection' (McCallum et al., 2001) and can be represented algebraically based on a mathematical framework or in the case of data analysis, modelled as a survival distribution (Heisey et al., 2006). Caley & Ramsey (2001) investigated how leptospirosis in brush tail possums was transmitted by finding the

two algebraic expressions for the FOI based on whether transmission was either frequency or density dependent. Then the transmission coefficients were estimated using age –prevalence data. To investigate how *Bordetella bronchiseptica* is transmitted between rabbits, Long et al. (2010) created an *a priori* set of hypotheses related to possible routes of transmission. They utilised age-prevalence data in a survival model with piece-wise constant hazard, i.e. over fixed periods of time, risk of infection is assumed to be constant. A similar approach was taken by Caley & Hone (2012), who proposed different piece-wise hazard functions related to multiple combinations of the five possible transmission routes of *Mycobacterium bovis* in ferrets. We wish to answer a similar biological question to Long et al. (2010) and Caley & Hone (2012): which of the hypothesized transmission routes of leptospirosis are biologically significant (demonstrable) in our wild Norway rat populations. Our approach differs in that we do not make an *a priori* assumption about how risk of infection changes over time by specifying a piece-wise constant hazard. Instead, we employ a flexible survival distribution with demographic covariates to model the hazard of infection.

In studies where rats are trapped and removed, weight is often used as a proxy for age despite weight not having a linear relationship with age (Glass et al., 1989). This may hinder accurately relating age to prevalence of infection. The von Bertalanffy equation has been used effectively to convert weight to age for mammalian and, in particular, rodent populations (e.g. Burthe et al., 2010). Hence, we convert the observed weights to ages using this equation. Then we seek evidence of multiple transmission routes occurring in the wild, and their relative importance, in two

ways. First, we identify risk factors of infection from among demographic (age, sex etc.) and other variables (e.g. levels of bite wounds). Second, we use a survival analysis to estimate the risk of infection over time and seek evidence for differential risk among different sub-populations of rats. We present an extension to the practice of analysing age-prevalence data by considering the changes in cumulative risk of infection based on demographic variables related to age-dependent transmission routes. The analysis methods applied here could be applied to any system with multiple transmission routes.

4.2. Methods

4.2.1. Data Collection

Animals were trapped in Pau da Lima, Salvador over five collection periods (June-July 2012, May-August 2013, October-December 2013, March-August 2014, September-December 2014) during which demographic information was recorded (sex, weight, body length, reproductive status (scrotal testes for males and the occurrence of pregnancy, lactation or placental scars for females)) and urine and kidney samples were taken. For further details of the study sites and standard trapping protocols see (Costa et al., 2015). Wounding grade, previously identified as a risk factor for infection among Norway rats (Costa et al., 2015), was recorded using the criteria used by Glass et al. (1988). Infection was a binary variable, where animals are classified as infected or not according to qPCR diagnosis of their urine (Costa et al., 2015). For 17 of the total of 517 animals (3.29%), urine could not be collected and infection was determined by presence of leptospires on kidney qPCR, which has a correlation of R^2=0.78 with urine qPCR results (Costa et al., 2015).

4.2.2. Ageing field animals

In relating weight to age, we acknowledge that a proportion of the females trapped were pregnant and may have to have their weight adjusted downward. Hence, we test whether pregnant animals were on average heavier than non-pregnant females. So that we do not attribute to pregnancy a weight increase due to age, we include only animals that are at an age at which they have potential to be pregnant. For female rats, a perforate vagina is often used as an indicator for sexual maturity, but this does not always also indicate sexual activity (Calhoun, 1962). Hence, we include in our sample females that are either pregnant, lactating or have placental scars (total of 140 animals) and test whether for these, pregnancy leads to a higher weight. A linear model with weight as the response variable and pregnancy as the only explanatory variable was fitted using lm in R (R Core Team, 2015). Weights of pregnant females were subsequently adjusted by the point estimate of the coefficient for pregnancy in the linear model.

We then aged the animals that had been trapped and removed from the field site by using the recorded weight of the animals. The von Bertalanffy equation can be used to describe change in weight over time,

$$\text{weight} = a[1 - \exp\{-r(\text{age} - c)\}]$$

where a is the asymptote (the maximum weight), r is the constant growth rate and c is the age at which maximum growth occurs (Burthe et al., 2010).

Both male and female rats caught in the field had the same range of weights, and so we converted their weights to ages using one growth curve. The von Bertalanffy

curve was fitted to data deduced from the growth curve presented by Calhoun (1962) for the heaviest male animals in his sample (since the Salvador rats had weights comparable with these). The fitted von Bertalanffy curve had asymptote $a = 562$ days and estimated values for growth rate $r = 0.01337$ (grams per day) and point of inflection $c = 23$ days.

4.2.3. Prevalence analysis

With infection status as a binary response variable, we fit a generalised linear model using the bias reduction method developed by Firth (1993) with explanatory variables age, sexual maturity, sex and level of wounding using brglm in R (R core team, 2015; Kosmidis, 2007). There were 486 animals with records of all of these variables. The bias reduction method was used as there was complete or quasi-complete separation present during the generalised linear model fitting. For ease of statistical computation, we collapse the level of wounding (Glass et al., 1988) into three grades: 0 (absent), 1 (very light and light combined) and 2 (moderate and severe combined). A male is classified as sexually mature if it is scrotal and a female is sexually mature if it is pregnant, lactating or has placental scars. The level of prevalence was independent of collection time (χ^2 = 6.02, degrees of freedom = 4, $p = 0.20$) and so collection time was not included as an explanatory variable in the model selection process.

Model selection is often performed using a comparison of goodness of fit such as Aikake's Information Criterion (AIC) (Burnham & Anderson, 2003). However AIC is not an appropriate measure when the estimation procedure used is bias reduction (Kosmidis, 2007). Model selection was performed by backward selection; a full

model was specified and explanatory variables with the highest p-value were removed one at a time. The final model had all explanatory variables significant at a 5% level. The final model was then used to identify risk factors for acquiring infection and to estimate the risk of infection for animals that have just left the nest.

4.2.4. Survival analysis

Identifying risk factors associated with infection is useful, but such approaches do not take into account the fact that an infected animal could have acquired infection at any time from when they were born until the age they were captured. Also, while uninfected animals have not been infected up to the age they were captured, they could have become infected subsequently. We can impose a binomial regression on the distribution of time to first infection by treating the seroprevalence data as,

$$Y_i = \begin{cases} 0 & \Leftrightarrow T > t_i \\ 1 & \Leftrightarrow T \leq t_i \end{cases}$$

where T is the time of first infection, and t_i is the observed time (age at capture). In other words, animals with positive seroprevalence had their first infection either at the age at which they were captured or before. Those animals with negative seroprevalence are not currently infected, but may be infected in the future. The probability of not yet being infected can be modelled using the survival function,

$$P(Y_i = 0) = 1 - F(t_i)$$

where $F(t_i) = 1 - \exp(-(t_i/\phi)^{\kappa})$ with scale parameter ϕ and shape parameter κ, is the Weibull cdf. To investigate the effect of explanatory variables on risk of

infection, we can specify the scale parameter as log linear $\log(\phi) = X\boldsymbol{\beta}$. The shape parameter κ determines how quickly rats will become infected early in their lifetime. If $\kappa < 1$ then the risk of infection is higher earlier in the animal's lifetime; if $\kappa > 1$ then risk of infection is higher later in their lifetime; and if $\kappa = 1$ then there is constant risk of infection.

To estimate the coefficients of the explanatory variables and the shape parameter κ we can transform the response $P(Y_i = 0)$ such that the probability of being infected has a binomial distribution (see Appendix 2 for more details). To investigate the effect of multiple variables on the risk of infection and also whether risk is constant, we fitted a model with sex, maturity status and a binary wounding variable (absent/present) and then tested for significant interactions between the variables where the final model was found by backward selection.

4.3. Results

4.3.1. Ageing field animals

There was no significant effect of pregnancy on weight (linear model, estimate=20.81, $p = 0.159$). However, the weights of the pregnant females were still adjusted by taking away the point estimate, 20.81. The resulting weight and age distributions are shown in Figure 4.1.

The weight distributions of males and females had similar ranges and shapes which resulted in similar distributions of estimated ages. Most animals were less than 100 days old; a few animals were over 200 days old (10 animals in total).

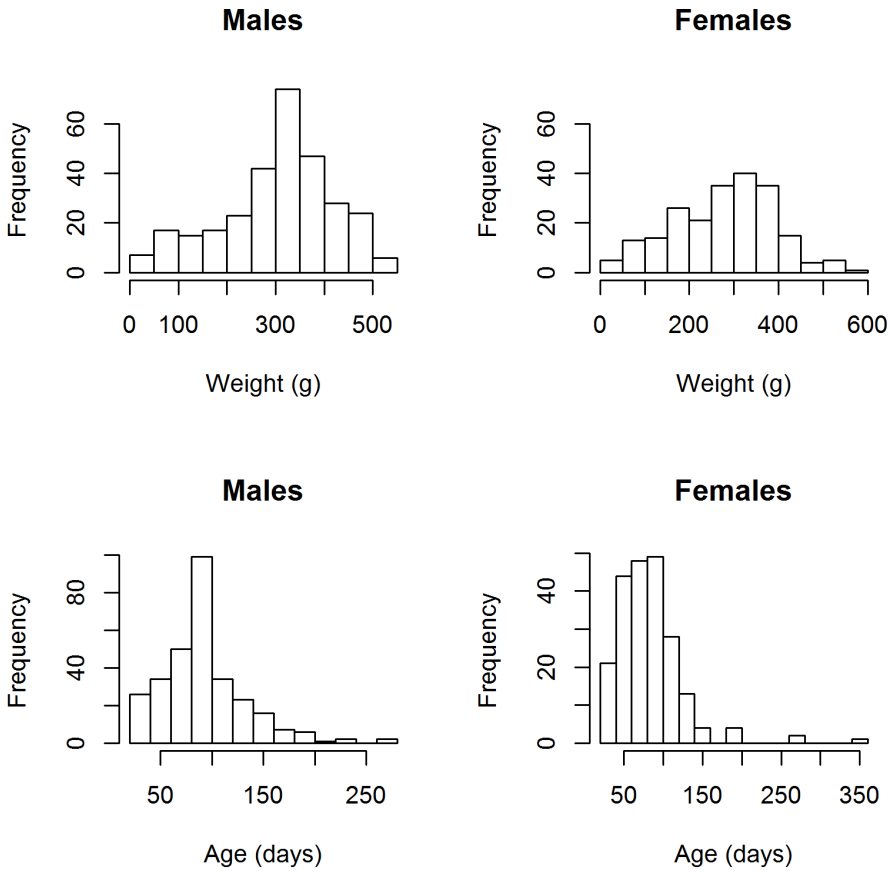

Figure 4.1: Histograms of the weights and estimated ages of male and female animals.

4.3.2. Prevalence analysis

The final model included age, wounding, sexual maturity and an interaction between sexual maturity and age (AIC 375.40). Risk of infection increases with age, level of wounding and being sexually mature (Table 4.1), but the risk of infection decreases for older animals with higher levels of wounding. For an animal that is 27 days old, has no wounding and is sexually immature, the probability of infection is 0.209 (0.124, 0.329).

Table 4.1: Summary of final model fit.

	Estimate	**Std.Error**	**z-value**	**Pr(>|z|)**
Intercept	-2.518	0.3525	-4.792	p<0.0001
Age	0.044	0.010	4.528	p<0.0001
Wounding 1	2.483	0.919	2.704	0.007
Wounding 2	6.510	1.585	4.108	p<0.0001
Mature	0.941	0.343	2.740	0.006
Age*Wounding 1	-0.032	0.012	-2.635	0.008
Age*Wounding 2	-0.057	0.014	-4.131	p<0.0001

The cumulative probability of infection with age is shown in Figure 4.2. For immature animals, increased level of wounding leads to increased risk of infection, with heavily wounded animals having close to 100% of chance of infection (Figure 4.2a) but sexual maturity leads to an increased risk of infection for animals without wounds (Figure 4.2b) When all animals are wounded, risk of infection is lower for lightly wounded sexually immature animals compared to heavily wounded immature animals and all wounded mature animals (Figure 4.2c).

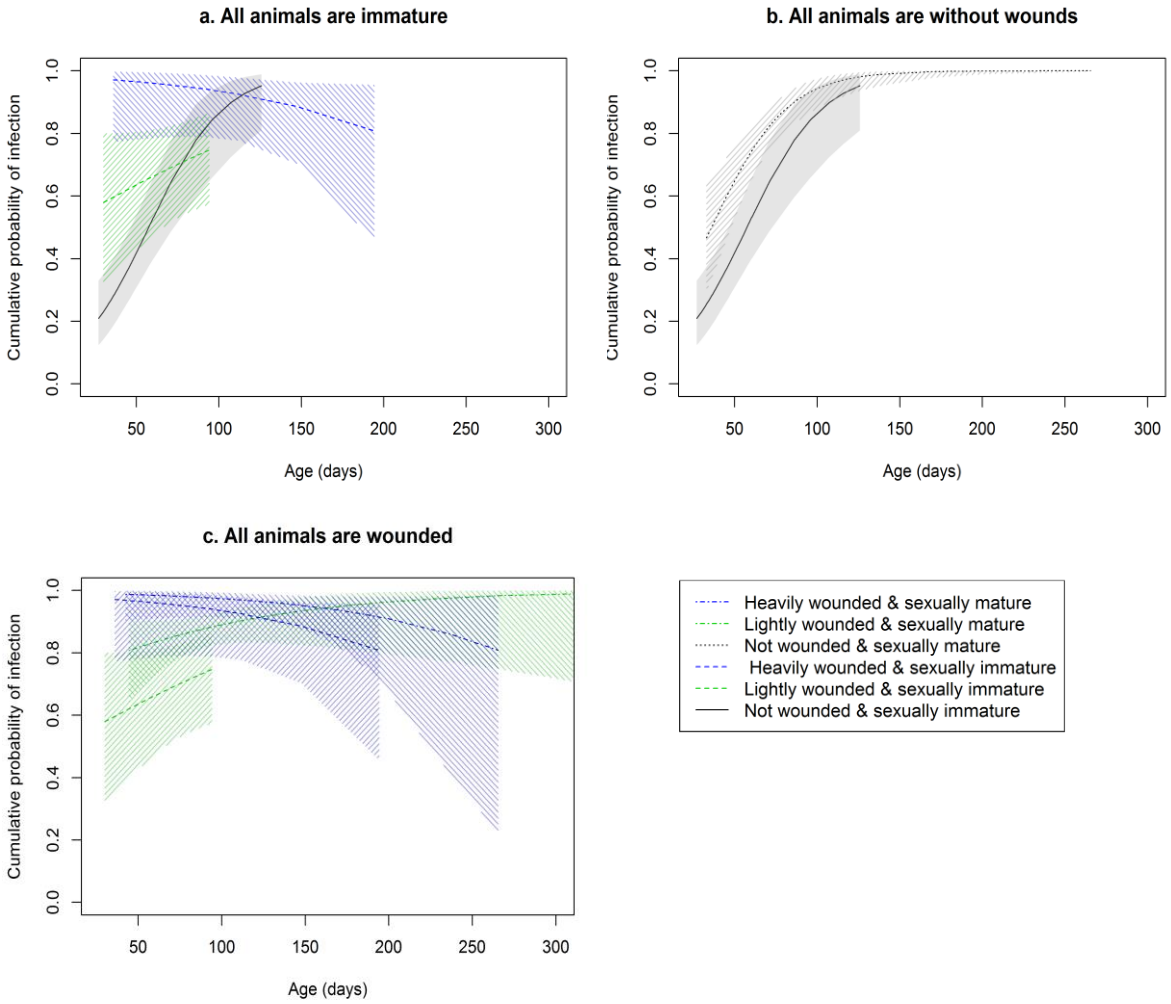

Figure 4.2: Cumulative distribution function of the Weibull distribution with parameters estimated from the prevalence model and 95% confidence intervals. (a) All animals are immature. (b) All animals are without wounds. (c) All animals are wounded.

4.3.3. Survival analysis

The final survival model included wounding, sexual maturity, sex and an interaction between wounding and sexual maturity (AIC 386.13) (Table 4.2). Having wounds, being sexually mature and being female increased the risk of infection. The estimate of the shape parameter κ was 0.81 (95% confidence interval 0.51, 1.28). Hence, there was not a significant change in risk of infection over time. The probability of leaving the nest with infection (an animal 27 days old, with no wounds, sexually

immature) for males was 0.232 (0.011, 0.453) and for females was 0.298 (0.090, 0.506).

Table 4.2: Summary of final survival model fit.

	Estimate	**Std. Error**	**z value**	**P(>|z|)**
(Intercept)	4.935	0.366	13.50	p<0.0001
Wounded	-1.228	0.553	2.219	0.026
Mature	-1.232	0.474	2.596	0.009
Sex (female)	-0.362	0.184	1.973	0.048
Wounded*Mature	1.123	0.532	2.112	0.035
Shape parameter, κ	0.813	95% CI (0.515, 1.283)		

The plots in Figure 4.3 show the cumulative distribution function of the Weibull distribution with parameters estimated from the survival model and standard errors calculated using the delta method (see Appendix 2 for more detail). As well as females having a consistently higher risk of infection than males, wounding clearly increased the risk of infection among immature animals (Figure 4.3a), whereas maturity increased the risk of infection among those without wounds (Figure 4.3b), however, there was there was no significant difference for those with wounds between mature and immature animals (Figure 4.3c).

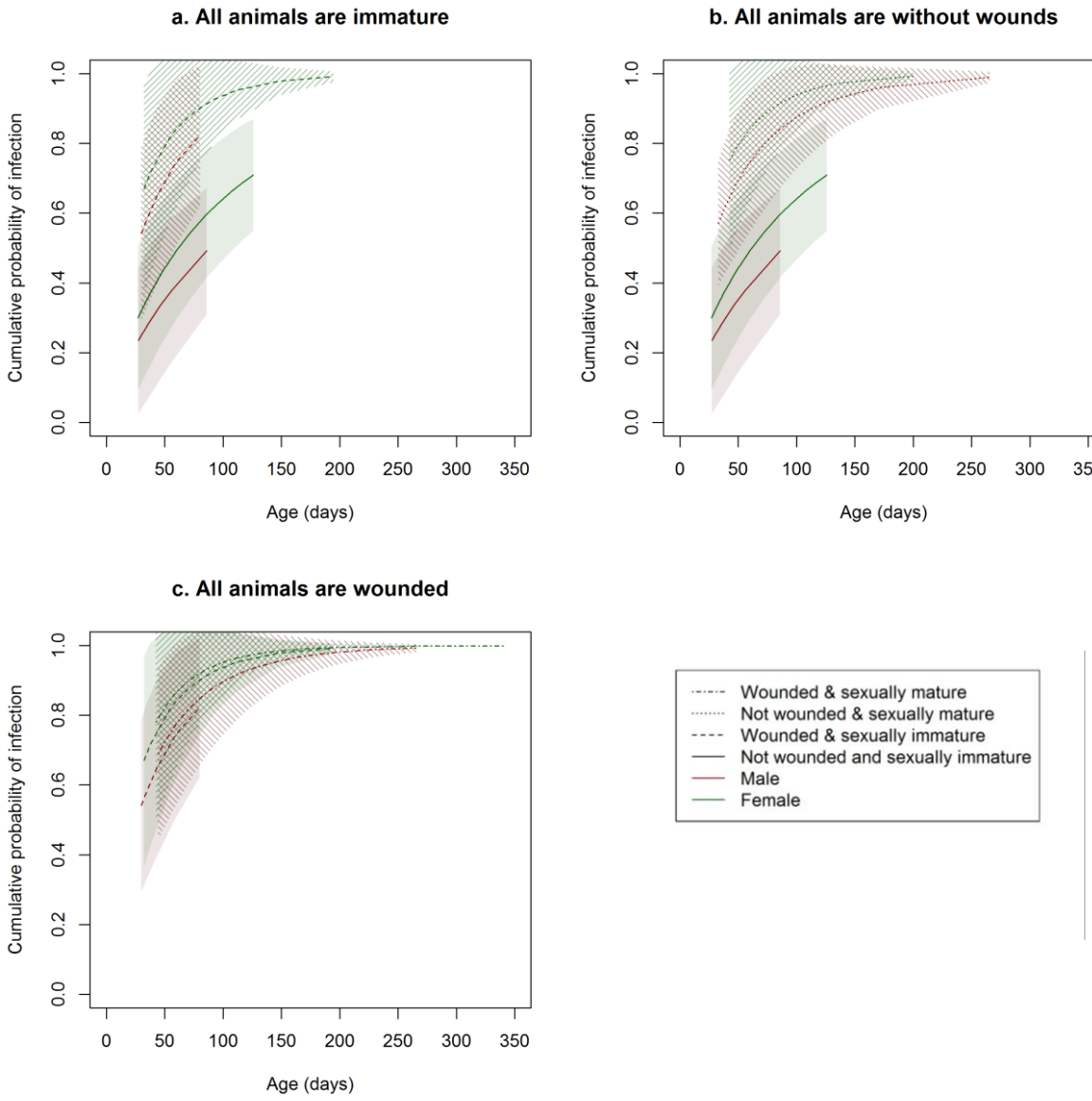

Figure 4.3: Cumulative distribution function of the Weibull distribution with parameters estimated from the survival model and 95% confidence intervals with standard errors calculated using the delta method. (a) All animals are immature. (b) All animals are without wounds. (c) All animals are wounded.

4.4. Discussion

Evidence of transmission routes occurring successfully and significantly in the field are more informative than experimental approaches, which, at best, can only represent the potential for transmission, not actual transmission. For leptospirosis, as for other zoonoses, control of the primary reservoir can in turn prevent

transmission to humans (Ashford et al., 1998; Zhang et al., 2010). Within the Norway rat population there are multiple potential routes of leptospire infection (elaborated below). We have sought evidence of these different transmission routes using prevalence data representing the infectious status of wild Norway rats.

Animals were aged using their observed weights and parameters estimated from the wild Norway rat growth curve from Calhoun (1962). We found no significant effect of pregnancy on the weight for female sexually mature rats. Porter et al. (2015) also found no difference in weight or body size for pregnant vs. non-pregnant females caught in Salvador. Despite this non-significant result, we adjusted the weight of pregnant females to compensate for an effect we could not capture in the linear model. We determined the weight difference by considering female animals with indicators of current, or previous pregnancy. However, our adjustment does not take into account that older animals may be less likely to be pregnant, which could lead to a biased estimate. Norway rats are often aged by their weight into distinct classes (Costa et al., 2014), but weight does not form a linear relationship with age (Calhoun, 1962) and so animals could be misclassified into these categories. By creating a continuous measure of age, we were able to investigate changes in risk over the lifespan of an animal.

One hypothesized transmission route of leptospire infection is vertical transmission. We do not capture animals confined to the nest, so it is not possible to distinguish true vertical from pseudo-vertical transmission (e.g. suckling), or from transmission from mother to pups in the nest. However, in terms of infection risk for humans, it

is more important to know simply whether animals leave the nest infected. The youngest animal we observed was 27 days old. This corresponds closely with the findings of Galef (1981) and Thiels et al. (1988) that milk transfer/production by mothers ceased by 27 and 30 days postpartum, respectively. Hence a 27 day old animal can be taken to be one that has not been exposed to any of the other transmission routes. Given the inclusion of sex in the final survival model, there were two predicted intervals for vertical transmission (risk of infection when an animal had just left the nest). The predicted risk for vertical transmission using the survival model was similar in value to the predicted risk based on the prevalence model, but with a wider interval. The calculated probability of infection based on the survival model or prevalence model was more than 0, with relatively narrow confidence intervals, strongly suggesting that a proportion of animals leave the nest infected. Our unpublished work has detected leptospires in the mammary gland, and an absence of infection in foetuses of 7 infected, pregnant mothers, but further work is required to determine what accounts for this proportion that are infected on weaning.

For free-roaming rats, the challenge for this study is to translate observed variations in risk with age, maturity, sex and wounding into an assessment of the relative roles of direct and environmental transmission, and within the former, of sexual and other forms of direct transmission. During the period in which animals have left the nest but are not yet sexually mature, there was a risk of infection. Wounding has been suggested to be one important risk factor for acquiring infection by Costa et al. (2015). From the cumulative distribution plot for sexually immature animals,

there is a significantly increased risk for those with wounds, though we cannot determine the route responsible from this difference. This may be true direct transmission (via biting for example), an increased risk created by a different behaviour of those animals most likely to be wounded, or an increased risk for animals with wounds of environmental transmission from exposure to leptospires in the environment. Himsworth et al. (2014) discuss the difficulties in distinguishing between these possibilities in urban systems.

Aggression has been found to be the primary transmission route among Norway rats of Seoul hantavirus (Hinson et al., 2004), which is present in saliva. Studies by Glass et al. (1988) and Himsworth et al. (2013) found an increased risk of acquiring leptospire infection among wounded rats, though increased wounding may be a characteristic of either dominant or subordinate rats. For dominant rats, increased wounding could be a result of more contacts, but the converse may also be true: subordinate rats have more wounding due to more unsuccessful fights (Himsworth et al. 2013). Calhoun (1962) hypothesised that high ranking males will have fewer wounds, as they are less frequently wounded in combat and there are field data suggesting that this pattern is present among both sexes (Glass et al., 1988). This result was also found by Blanchard et al. (1995), where among lab reared rats put into colonies, the animals deemed subordinate had more wounds than the dominants. We did not see an effect of wounding on mature rats. This may be due to the fact that most animals are infected by adulthood, and so wounding is no longer a risk factor. However, we would expect a difference in risk by wounding level at sexual maturity if older higher ranking animals were less likely to be

wounded and hence infected.We know of no evidence of leptospires in rat saliva, and leptospires are shed at high concentrations in the urine (Costa et al., 2015). Hence, we suggest that it is likely that wounding increases the risk of infection by increased exposure to environmental sources, either behaviourally or, perhaps more likely, by direct exposure through the wounds, as opposed to there being direct transmission during the act of wounding.

For adult, mature rats, we looked for evidence of a risk of infection beyond that from environmental transmission and wounding. In the prevalence analysis, the effect of sexual maturity was significant having adjusted for age and level of wounding. The cumulative distribution plot for animals without wounds (Figure 4.2b, 4.3b) also suggested that there was some additional risk for sexually mature animals. In Figure 4.2c there is a difference in risk for lightly wounded immature animals compared to heavily wounded immature animals and wounded mature animals. In the survival model we did not see this effect, the final model included wounding was as a binary variable and so the effect of lightly wounded and heavily wounded may have been combined. If sexual transmission occurred at an epidemiologically significant rate, we would expect to see a difference between wounded mature and immature animals occurring in the survival model predictions. Sexual transmission may occur therefore, and our unpublished evidence of leptospires in semen supports this, but, we propose, not at an epidemiologically significant rate.

Further, females had a higher risk of infection in the final survival model analysis. The cumulative distribution plots all showed that females had a higher risk of infection than males but only earlier in life. Given that there were no interactions between sex and the other variables in the model, there is no evidence to suggest that the additional risk is from a one-way sexual transmission route or a differential effect of wounding on the sexes. Instead, the additional risk for females may come from some behavioural or physiological difference between the sexes that is apparent from the early life stages.

Previous studies have used the force of infection to understand how transmission occurs in wild populations (Long et al., 2010; Caley & Hone, 2012). The notable difference in our study is that changes in risk were identified based on demographic variables instead of specified functions of hazard (piece-wise or step functions for example). In wildlife systems, there is not a distinct time threshold for when animals reach different phases of their life cycle. For example, rats can become sexually mature over a range of ages, and so it would be inappropriate to use a step function to model change in risk for sexually maturity. Non-linear functions of hazard could be used to represent these processes, but the use of covariates accounts for the variation of demographic processes in wild animals.

This study has illustrated methods to identify evidence of multiple transmission routes from prevalence data obtained from the field. Despite the prevalence model having a lower AIC, we believe that the survival model is a better predictor of risk as it can change non-linearly to due the formulation of the Weibull cumulative distribution function.The vertical, direct and environmental routes are shared not

only with other infections of rodents but with infections of other species too. Hence the approaches presented here can be directly applied to other wildlife systems where there are multiple routes of transmission which are age dependent. In the present case, we have found support for including both vertical and environmental transmission in the age structured mathematical model of rat leptospirosis described in chapter 6, but not for the inclusion of direct transmission.

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Chapter 5

Inference for differential equations: estimating adult mortality rate and sub-adult maturation period

5.1. Introduction

Ideally, mathematical models of infectious disease dynamics should be developed such that that they may be fully parameterised (Keeling & Rohani, 2008). Parameters can be obtained from literature, or estimated from field data, or field data may be used to improve or refine existing estimates from the literature. Some parameters in such models are system specific, and so should ideally be estimated using data from that system. In field studies, however, the quality of data can be limited by restrictions on collection. Also, on occasion, values obtained from the literature may provide an adequate estimate of the true system-specific value. Efforts to estimate these parameters from field data could be wasted when the values from the literature already exist.

Pau da Lima is a slum community site in the city of Salvador, Brazil where annual outbreaks of leptospirosis occur (Ko et al., 1999). Previous studies have indicated that higher risk of acquiring leptospirosis is associated with the presence of rats (natural reservoirs of infection) at the home (Reis et al., 2008). As part of ongoing studies into the dynamics of infection in this natural reservoir, rats are trapped and removed during multiple trapping campaigns with a view to constructing a dynamic

mathematical model describing infection in rats. In order to fully parameterise that model, estimates for demographic parameters of the rats need to be obtained.

When dealing with animals that are reservoirs for human infection, the ethical option is to trap and remove animals. In these cases, cross sectional data are obtained at multiple time points, with each animal therefore only contributing once to the data set. Hence, it cannot be assumed that the same population is being sampled at each time point as animals are removed. Classic matrix population models, and more recent approaches such as integral projection models, require longitudinal data in order to estimate parameters of interest (Leslie, 1945; Rees & Ellner, 2009). From removal data it is possible to infer demographic information such as birth rates (see Emlen & Davis (1948)). It is not possible however to calculate mortality rates from removal data.

Estimates for brown or Norway rat (*Rattus norvegicus*) mortality rate could be obtained from the literature based on other urban rat systems. In a mark-recapture experiment carried out in Baltimore, Maryland, Glass et al. (1989) obtained estimates of lifetime lengths by finding the median length of time to the last recorded capture of an animal. The results of these experiments will be affected by local factors but give some information on the lifespan of wild Norway rats. But the causes of mortality of rats are not well understood (Feng & Himsworth, 2014), and so by opting to use mortality rates estimated in other systems, we ignore the differences in mortality that could arise by differences in habitat. Note, moreover, that by estimating a mortality rate based on data from the urban slums, no

assumptions need to be made about whether the patterns of mortality in the slums are more similar to a rural or urban system.

Due to the ethical nature of working with wildlife reservoirs of infection, we have only removal data. Though we cannot treat removal data as indicators of Norway rat population sizes, we can assume that the proportions of animals trapped in each age class is representative of the proportions that would be observed at the true population size. Here, therefore, a mathematical model comprising of a system of differential equations describing the life cycle of a rat is presented. Rats are born, mature into sub-adults and then mature into sexually mature animals. From our mathematical model framework we infer a functional relationship between adult mortality and maturation rate of sub-adults. Field data obtained in Pau da Lima are used to supply proportion of sub-adults to adults present in the population. We estimated the ratio of the adult mortality to the maturation rate of sub-adults into adults for the captured rats from Pau da Lima. These estimates are then compared to values of adult mortality or maturation rate existing in other systems to infer demographic parameters for Norway rats in Pau da Lima.

5.2. Materials and Methods

5.2.1. Data collection and population structure

Animals were trapped over four different collection campaigns (May-August 2013, October-December 2013, March-August 2014, September-December 2014) in three different locations of Pau da Lima, Salvador, Brazil (valley 1, valley 2 and valley 4) during which demographic information was recorded (sex, weight, body length and

reproductive status: scrotal testes as an indicator of maturity for males, and the occurrence of pregnancy (and number of embryos if pregnant), lactation or placental scars for females). There is no evidence to suggest that reproduction of rats is seasonal in Pau da Lima, nor of clear seasonal patterns of abundance (Panti-May et al., 2016). The number of sub-adults, W(non-sexually mature) and adults, A (sexually mature) can be found using the indicators of sexual maturity. The data for the four campaigns in the three locations are shown in Table 5.1 (Panti-May et al., 2016).

Table 5.1: Estimated population structure based on four campaigns of trapping for valley 1, 2 and 4.

Campaign number (dates)	**Valley**	**Sub-adults (W)**	**Adults (A)**
1 (May-Aug 2013)	1	18	45
	2	20	49
	4	39	93
2 (Oct-Dec 2013)	1	16	23
	2	26	48
	4	28	63
3 (March-Aug 2014)	1	10	37
	2	13	53
	4	21	52
4 (Sep-Dec 2014)	1	5	26
	2	8	39
	4	17	33

5.2.2. Population model

A mechanistic model was formulated to describe the population dynamics of a rat population. The model consists of a system of three ordinary differential equations representing the numbers of juvenile rats (J), sub-adult rats (W) and adult rats (A) (Figure 5.1, equations 5.1-5.3). Juvenile rats are those animals that are born and confined to the nest. Once weaned, they leave the nest and become sub-adults, finally becoming adults when they reach sexual maturity (Calhoun, 1962). The total number of free-ranging rats is hence given by $W + A$.

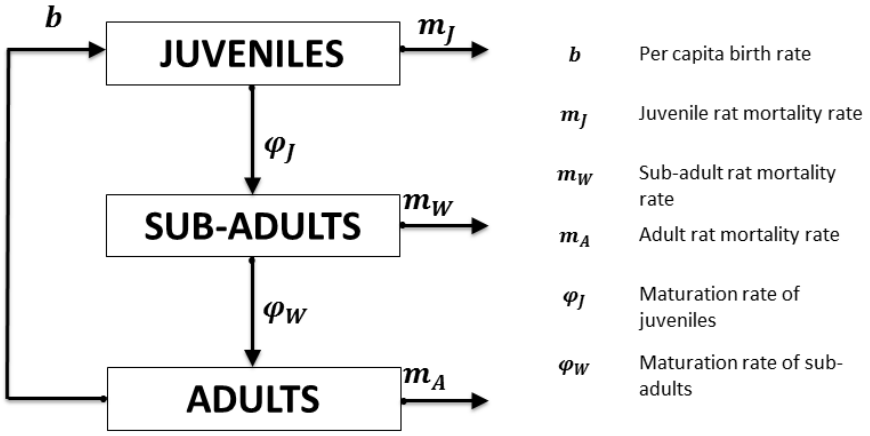

Figure 5.1: Flow diagram of the population model: animals are classed as juvenile, sub-adult or adult. Self-regulation is included in the framework.

$$\frac{dJ}{dt} = b\,A - \varphi_J J - m_J J \tag{5.1}$$

$$\frac{dW}{dt} = \varphi_J J - \varphi_W W - m_W\,W \tag{5.2}$$

$$\frac{dA}{dt} = \varphi_W W - m_A\,A \tag{5.3}$$

Juvenile rats can move into the sub-adult class by maturation at a rate φ_J or they can suffer mortality in the nest at rate m_J. Sub-adults become sexually mature, and move to the adult class at a rate φ_W. Sub-adults and adults are assumed to suffer mortality at the different rates, m_W and m_A respectively. Adult rats could be assumed to give birth at a constant rate b through time, we assume that the population is at is carrying capacity.

At equation 5.3, when the model is at equilibrium, we have,

$$\frac{dA}{dt} = 0$$

$$\varphi_W W^* - m_A A^* = 0$$

$$\frac{W^*}{A^*} = \frac{m_A}{\varphi_W}$$

Using the data in Table 5.1 we can estimate the ratio W^*/A^* using a generalised linear model (GLM) with a binomial distribution and logit link function. To test whether the ratio is different for location or time collected, separate models are fitted with valley (valley 1, valley 2 or valley 4) as a factor and campaign (1,2,3,4) as a factor.

Given that $W^*/A^* = m_A/\varphi_W$, we can calculate the adult mortality rate or maturation period based on values in the literature for the ratio of sub-adult to adult in Pau da Lima. There are no studies existing in the literature which have estimated both the mortality rate and maturation rates for a single Norway rat system. In order to make comparisons of our estimated ratio of m_A/φ_W to values in the literature, we calculate the predicted adult mortality rate based on assuming

the maturation rates observed in other systems. Then the converse, we calculate the maturation rates based on the adult mortality rates observed in other systems.

There are few studies on the demographics of wild urban Norway rats. We include one study on the mortality rate of urban Norway rats by Glass et al. (1989). Glass et al. (1989) estimated the median survival time of adult rats after first capture in urban areas of Baltimore city was 8 weeks, and in parkland (rural) areas was 7 weeks. Spencer & Davis (1950) found that 50% Hawaiian wild rural Norway rats had a lifespan of 42 days.

For the maturation rate of sub-adults into sub-adults we include two studies. Calhoun (1962) found wild rural Norway female rats exhibiting reproductive behaviour after around 74 days. Clark & Price (1981) performed maturation studies on captive reared wild Norway rats and found the mean age at which males were sexually mature was 64.6 days and females after 55.7 days. Assuming that the average animal will spend 27 days in the nest, then it would take a subsequent 45 days to reach sexual maturity in the Calhoun (1962) system and between 28.7 and 37.6 days for the Clark & Price (1981) system.

5.3. Results

The ratio of sub-adults to adults was independent of valley (deviance= 3.2058, degrees of freedom = 2, p=0.2013) but was not independent of campaign (deviance=16.877, degrees of freedom=3, p< 0.001). The GLM model fit with campaign as a factor is shown in Table 5.2 and Figure 5.2.

Table 5.2: GLM model fit with campaign as a factor.

	Estimate	Std. Error	z value	Pr(>|z|)
(Intercept)	-0.3567	0.1486	-2.400	p<0.05
Campaign 2	0.4463	0.2280	1.957	0.0503
Campaign 3	-0.4441	0.2345	-1.894	0.0583
Campaign 4	-0.4616	0.2648	-1.743	0.0813

There was no consistent seasonal pattern in the ratios with campaign number, though there was a decrease in the ratios for the later campaigns. The highest ratio was observed in campaign number 2 (Table 5.2, Figure 5.2).

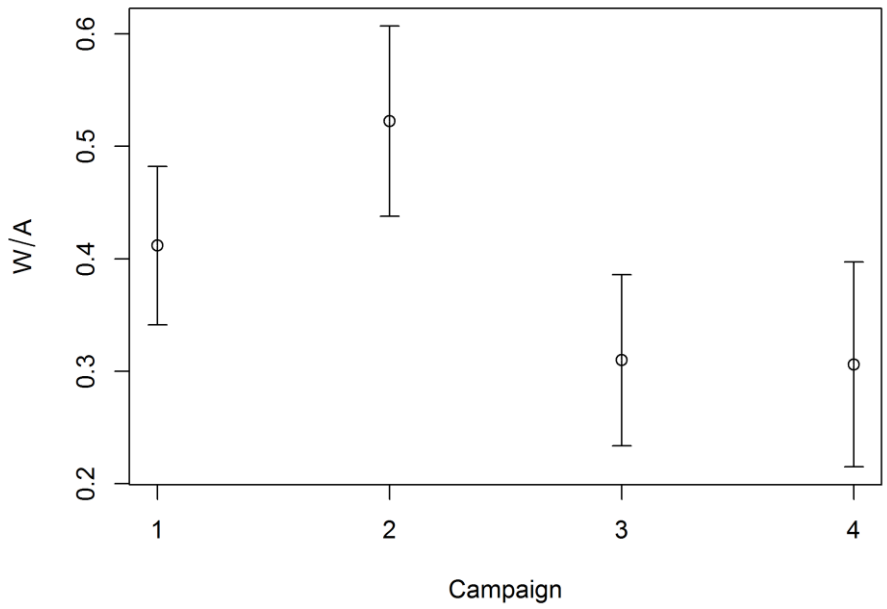

Figure 5.2: Predicted ratio of sub-adults to adults W/A for campaigns 1, 2, 3 and 4.

The predicted lifespans ($1/m_A$) and maturation period ($1/\varphi_W$) based on values from the literature are shown in Figure 5.3. The longest predicted lifespans were based on the maturation period from Calhoun (1962). There is some overlap of the lifespans based on the maturation periods from Clark & Price (1981).

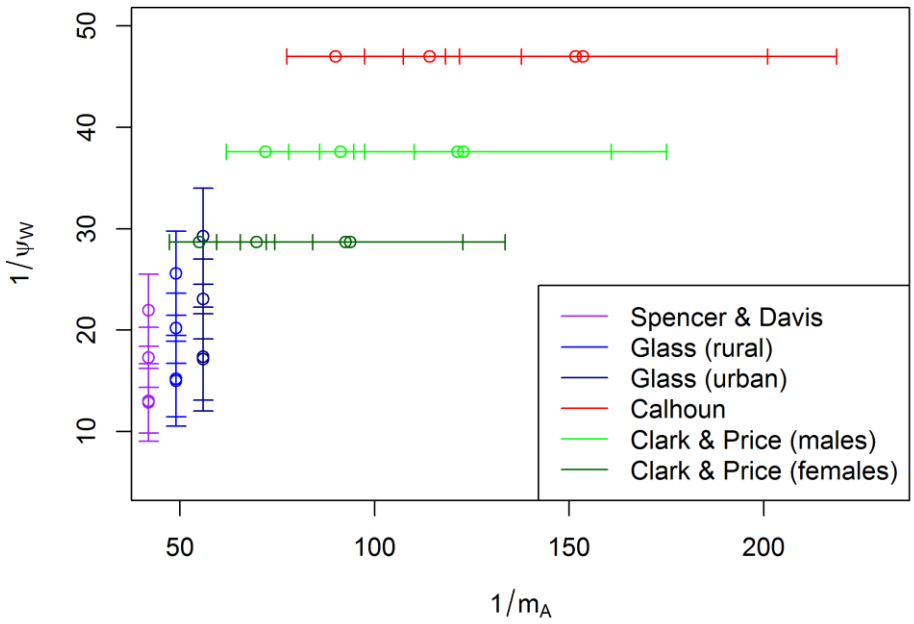

Figure 5.3: Predicted adult lifespans against maturation rates based on various literature sources. Coloured bars indicate the literature source.

Based on the lifespans from Glass et al. (1988) and Spencer & Davis (1950), the calculated maturation periods are relatively short (<35 days). The predicted maturation rates from Glass et al. (1988) overlap with the predicted lifespans for female rats from Clark & Price (1981).

5.4. Discussion

Mathematical models used to predict infection dynamics should be fully parameterised for the system at hand. When dealing with wildlife species, particularly those which are reservoirs of human infection, the ethical options is to trap and remove animals during data collection. The resulting data does not fully represent true population size. However, we can infer information from the observed proportions in different age classes. Utilising a mathematical model to describe the population dynamics of wild Norway rats, the proportion data was used to estimate the ratio of sub-adults to adults. From this ratio, values of the lifespan of adult rats and the maturation period were predicted using existing values in the literature. The approach here could also be implemented for any other population dynamics model coupled with proportion data.

The proportion data was collected in different valleys and at different time points. We did not observe significant effects of valley on the ratio of sub-adults to adults. The three valleys in Pau da Lima have some differences in their overall size and structure of housing, but we expect the same level of resources for rats. Hence it was not surprising to observe the same rat population structure in each valley.

The ratio of sub-adults to adults was different for the four different trapping campaigns. The highest value was observed in second campaign, which was during the summer months Brazil. The birth rate of rats and hence the population size is not seasonal in Pau da Lima (Panti-May et al., 2016) and so a significant effect of trapping campaign was not expected. The observed differences over time may be

due to natural fluctuations in population structure. However, following campaign 2 the ratio decreases in campaign 3 and remains at that level for campaign 4. This pattern could suggest there is an effect of trapping on population structure.

In campaign 2, there was a higher ratio of sub-adults to adults compared to the previous campaign. This effect could be due to the removal of adult animals in campaign 1. The sub-adults in campaign 2 then mature into adults, but those adults that have been removed in campaign 1 did not produce any offspring leading to fewer sub-adults in campaign 3 and a lower ratio of sub-adults to adults. However, the time between campaign 1 and 3 was close to a year, it is unrealistic to assume that the maturation period and adult lifespans were comparable to this time scale.

From the predicted ratios of sub-adults to adults in different campaigns, we calculated either the lifespan of an adult rat or the maturation period of sub-adults assuming that one of the parameter values is known. Assuming the maturation period from Calhoun (1962), the lifespan of adult rats would be between 100 and 200 days. These predicted lifespans are comparable to the Davis (1948) rural system. Davis (1948) found that 5% of wild rats live for a year in an initial population size of 100 at a rural farm (though this does not include pre-weaned animals and external factors affecting mortality risk, including children shooting rats for sport). Clark & Price (1981) estimated the time to sexual maturity for captive reared wild Norway rats. The values differed for sex, leading to different predicted lifespans for males and females. The predicted lifespans were shorter than those predicted using the Calhoun (1962) maturation value.

Maturation periods were calculated based on the lifespans estimated in Glass et al. (1989) and Spencer & Davis (1950). The predicted maturation periods were short in value. Glass et al. (1989) found that sexual maturity was size rather than age dependent, and so maturation rate into adulthood is expected to be short if there are ample resources for growth.

There was little agreement between maturation periods and lifespans estimated from values in the literature. But this was to be expected, as the systems had a number of differences. The only agreement between estimated lifespans and maturation period was between the estimated maturation period of urban rats in Glass et al. (1989) and the estimated lifespan of female captive reared wild Norway rats in (Clark & Price, 1981).

Urban Norway rats have short lifespans (Feng & Himsworth, 2014) and so we can assume that the lifespans from Glass et al. (1989) and Spencer & Davis (1950) are representative of the rats in Pau da Lima. The urban system in Glass et al. (1989) is most comparable to the system in Pau da Lima. Given the observed agreement with Clark & Price (1981), we can assume that maturation period calculated for female rats is most representative of animals in Pau da Lima. Clark & Price (1981) found differences in the time to sexual maturity for males and females, leading to different predicted lifespans for sex. Though it is reasonable to assume that there will be sex difference for maturation period for the rats in Pau da Lima as well, the model does not distnguish sex effects and so only one maturation rate will be used.

In chapter 4, ages were calculated for animals that had been trapped in the field. However, we do not know whether to what extent these observed ages represent

the true lifespan or maturation period. The mean observed age was approximately 88 days, which without time spent in the nest (juvenile maturation period estimated to be 29-37 days) gives an age outside the nest of 52-59 days. This value is similar to the lifespans taken from Glass et al. (1989) and the predicted lifespan for rats in the Clark & Price (1981) system. In the age distribution, half of the animals (both sexes combined) were mature by 98 days old we assume that 98 days old is representative of the average time to adulthood, this would give a maturation period of 71 days which is notably longer than the Clark & Price (1981) value of 55.7 days for female rats and the predicted maturation period for Glass et al. (1989) . The ages of trapped animals are simular in value to of estimated adult lifespans, but the age distribution of sexually mature animals may not be directly representative of the maturation period.

In summary, we have been able to calculate demographic parameters from various systems in the literature by using a simple analysis of proportion data coupled with a population dynamics model. We conclude our best estimates for the adult mortality rate and maturation period for the rats in Pau da Lima are the adult lifespan from Glass et al. (1989) and the predicted maturation period. Further analysis is required to determine how the ages of animals trapped in the field relates to adult lifespan and maturation period.

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Chapter 6

Optimal control measures for leptospire infection in the Norway rat

6.1. Introduction

Norway rats, *Rattus norvegicus*, are the natural reservoir of leptospirosis in Pau da Lima, an urban slum community of Salvador (Reis et al., 2008). The high concentration of leptospires shed by the rats (Costa et al., 2015), an apparent lifetime of infection (Bharti et al., 2003) and the high prevalence of infection the rat population (Costa et al., 2014) mean that Norway rats are an effective reservoir. Humans acquire leptospire infection with direct contact with the rodent reservoir, or more commonly, contact with the environment contaminated with animal urine. Given that the rat population are responsible for the maintenance of human risk of leptospirosis, control of the rat population should reduce human infection. Though environmental controls would directly reduce risk of infection for humans, they are in practice much more difficult to implement and maintain, and more costly than rodent control. Control of zoonotic diseases has previously been achieved by the removal of zoonotic reservoirs to prevent human risk of infection of Hantavirus (Zhang et al. 2010) and visceral leishmaniasis (Ashford et al., 1998). Therefore, we investigate rodent control to reduce risk of human leptospirosis.

Norway rats, and populations with density dependent regulation in general, have been shown to recover quickly after population decrease by rodenticide (Shilova & Tchabovsky, 2009). In a study by Emlen et al. (1948) populations of wild Norway rats that were reduced by between 50 and 90 per cent recovered at constant rates between 2% and 6% of their original size each month. A study by Barnett & Bathard (1953) showed that a sewer rat population that was reduced to 10% of its original size had reached its original size within 6 months. Though these studies illustrate that rodenticide often is only effective in reducing rat populations as a temporary measure, rodenticide has been used to eradicate rats, for example, from Clambell Island, New Zealand (McClelland, 2011).

It is expected that Norway rats will recover from population decreases via in situ survival with reproduction (Hein & Jacob, 2015) as opposed to migration. Hence another potentially effective control measure is habitat management. Reducing the complexity of the habitat can reduce survival by decreasing suitable habitat for nesting and increasing rat predation (Lambert et al., 2008; Buckle, 2013). The carrying capacity of a rat population can also be reduced by restricting access to food and refuges (Adrichem et al., 2013). In Pau da Lima, reducing access to food could be achieved by removal of garbage and reduced access to houses. Also, available refuges could be reduced by clearing larger pieces of garbage and dense vegetation.

Mathematical models can be used to test the effectiveness of control measures in an infected population (Hethcote, 2000). An age-structured model for leptospire infection in the Norway rat population will be presented, informed by empirical

analysis (chapter 4 and 5). We extend this model to include two rodent control measures: rodenticide and habitat management. Using data from previous rodenticide campaigns, we use the age structured model to illustrate the predicted effect of rodenticide control on the dynamics of infection and population size of the Norway rat population.

When considering control measures, time dependent effects cannot be ignored. It is always of interest to minimise costs at the same time as reducing infection rates and constant application of controls may be wasteful. For example, it would be unnecessary to continuously vaccinate a population at the same rate through time for 100 days, when the vaccination threshold has been met after day 50. Hence, we present a framework to plan time dependent control measures for rodent control using optimal control theory. Optimal control theory seeks the optimum time-dependent controls while taking into account both the cost of the control measures and (in this case) the cost of an infected rat.

In particular, we present five different control scenarios to illustrate the how the optimal framework can be used to plan rodent control programmes. Rodenticide, habitat management, or some combination of the two can be employed to control wild rodents. The effectiveness of either rodenticide or habitat management to reduce rat population sizes in an urban slum setting is not well understood, though we know that logistically, it will always be easier to implement one control measure at a time. Also, it is not known whether application of just one control measure is sufficient to reduce rat population sizes. Therefore, the five scenarios were: application of only rodenticide, only habitat management, both controls are applied

simultaneously, rodenticide is applied, followed by habitat management, and habitat management first, then rodenticide is applied afterwards. We present the predicted effect of the optimal controls on the total population size, infected population size and free-living leptospire population.

6.2. Methods

6.2.1. An age structured model for leptospire infection in *Rattus Norvegicus*

The age structured model is a system of 7 differential equations representing the number of juveniles (J), sub-adults (W) and adults (A) with subscript X and Y indicating susceptible and infected respectively (Figure 6.1, equations 6.1-6.7).

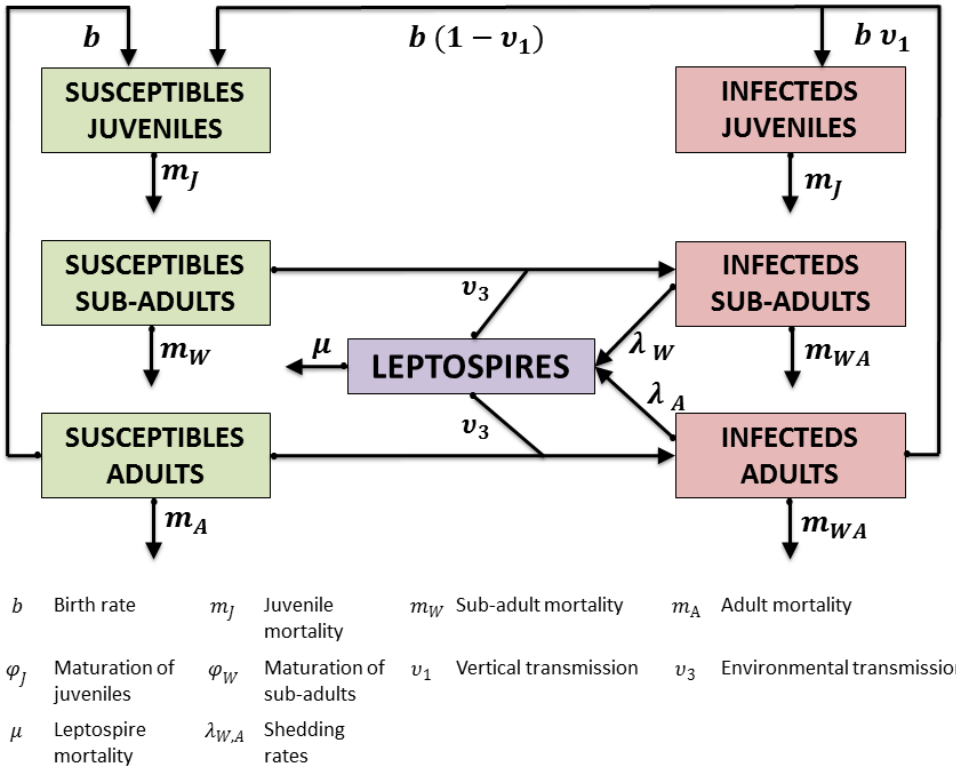

Figure 6.2: Flow diagram of the age structured model with self-regulation incorporated.

$$\frac{dJ_X}{dt} = b\,(A_X + (1-\upsilon_1)A_Y)\left(\frac{k-(W+A)}{k}\right) - \varphi_J J_X - m_J J_X \tag{6.1}$$

$$\frac{dJ_Y}{dt} = bA_Y\upsilon_1\left(\frac{k-(W+A)}{k}\right) - \varphi_J J_Y - m_J J_Y \tag{6.2}$$

$$\frac{dW_X}{dt} = \varphi_J J_X - \upsilon_3 W_X L - \varphi_W W_X - m_W W_X \tag{6.3}$$

$$\frac{dW_Y}{dt} = \varphi_J J_Y + \upsilon_3 W_X L - \varphi_W W_Y - m_W W_Y \tag{6.4}$$

$$\frac{dA_X}{dt} = \varphi_W W_X - \upsilon_3 A_X L - m_A A_X \tag{6.5}$$

$$\frac{dA_Y}{dt} = \varphi_W W_Y + \upsilon_3 A_X L - m_A A_Y \tag{6.6}$$

$$\frac{dL}{dt} = \lambda_W W_Y + \lambda_A A_Y - \mu L \tag{6.7}$$

Rats are born at a constant rate b throughout the year where all offspring of susceptible adults (A_X) are born susceptible and infected adults (A_Y) will give birth to a proportion (υ_1) of infected offspring. There is self-regulation in the system where sub-adults and adults are competing for resources ($W + A = (W_X + W_Y) + (A_X + A_Y)$). Juveniles suffer in nest mortality at rate m_J.

Juveniles (J) mature into sub-adults at a rate φ_J. Sub-adults can become infected via contact with the environment (υ_3). Sub-adults suffer mortality at rate m_W. Sub-adults then mature into adults at a rate φ_W where they are then at risk of

environmental transmission (at the same rate υ_3 as the sub-adults). Adults suffer mortality at rate m_A.

Sub-adults and adults both shed into the state for the free-living leptospires at different rates (λ_W, λ_A). Infected juveniles may shed but if they do it will be in the nest, not into the environment as we have defined it here. Here leptospires suffer mortality at a rate μ. The inclusion of self-regulation introduces a 'carrying capacity' to the population, given by $K = k(b\varphi_J\varphi_W - (m_J + \varphi_J)(m_W + \varphi_W)m_A)/(b\varphi_J\varphi_W))$.

Parameter values were informed directly from field data or estimated based on field data (Table 6.1). Most of the parameters are the central measures of the posterior distributions found in chapter 5. The birth rate is obtained from field data (Panti-May et al., 2016). The rate of vertical transmission was found in chapter 4 and the rate of environmental transmission has been 'estimated' using the same procedure as in chapter 3. The estimation procedure was repeated in the age-structured model as there were updated values for shedding rate. The prevalence in the model predictions was calculated based on sub-adults and adults only as only these animals were used to calculate combined prevalence in Costa et al. (2014).

Table 6.1: Parameter definitions and values for the age-structured model.

Parameter	Definition	Units	Value	Source/Comments
b	Per capita birth rate	Day^{-1}	0.285	Estimated from field data (Panti-May et al., 2016).
m_J	Juvenile rat mortality rate	Day^{-1}	0.125	High juvenile mortality.
m_W	Sub-adult rat mortality rate	Day^{-1}	0.013	Average lifespan is 125 days, most animals survive to mature into adults.
m_A	Adult rat mortality rate	Day^{-1}	0.015	Average lifespan 66 days.
φ_J	Maturation rate of juveniles	Day^{-1}	0.03	Average time spent in the nest 27 days (see chapter 4).
φ_W	Maturation rate of sub-adults	Day^{-1}	0.029	Average time to sexual maturity outside the nest is 50 days.
υ_1	Proportion of pups infected from suckling and born infected	Day^{-1}	0.2	Probability of infection at 27 days is 0.2 (see chapter 4).
υ_3	Transmission via the environment	Day^{-1}	4.7x10^{-14}	Estimated as in chapter 3 using combined prevalence.
$\lambda_{W,A}$	Leptospires shed per day per infected sub-adult, adult.	Day^{-1}	1.6x10^{7}, 8.1x10^{8}	Estimated from the media geq of urine (unpublished).
μ	Mortality rate of leptospires in the environment	Day^{-1}	0.05	Lifespan of 20 days, informed by recent experiments (unpublished).
K	Carrying capacity	Number of rats	75	Based on abundance estimates from field data.

Analytical expressions for the equilibrium solutions do not exist. A numerical exploration of the model performed using a combination of transmission coefficients (rate of both routes set to zero, one to zero etc.) leads to three distinct outcomes: infection free, endemic infection in the sub-adult and adult age categories and endemic infection in all age categories.

The model was run using the parameter values as specified in Table 6.1 (Figure 6.2). A low prevalence is observed in the juveniles as the only transmission route is vertical which has a low value. Prevalence in the sub-adult population reaches 53% and in the adult population reaches 85%. In the free roaming population (sub-adults and adults combined) the prevalence was 74%. In an independent data set, the prevalence in the sub-adult population was 48% (n=94) and in the adult population 88% (n=410).

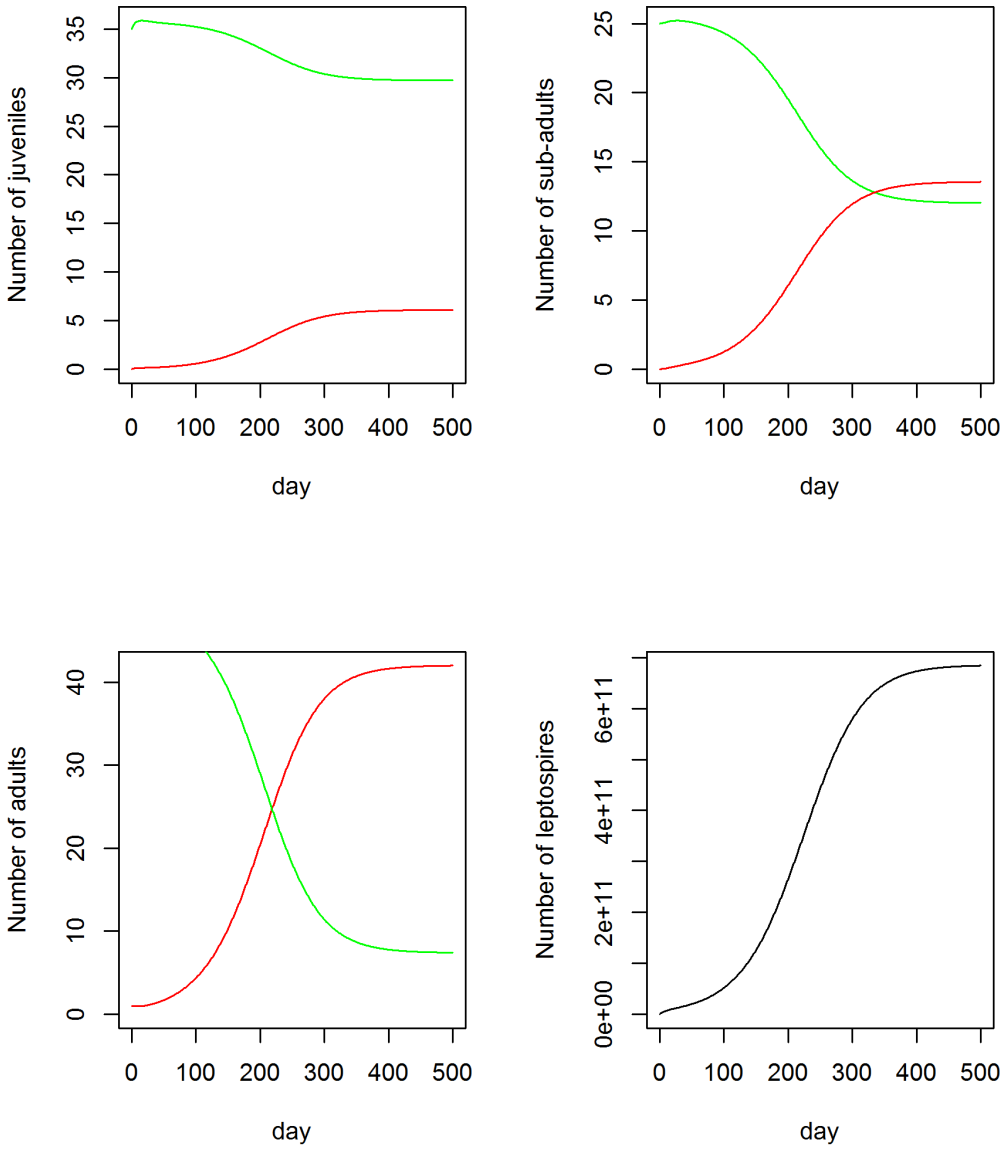

Figure 6.2: Predicted number of susceptible (green) and infected (red) juveniles, sub-adults and adults and leptospires (black) with initial conditions $J_X(0) = 35,\ J_Y(0) = 0, W_X(0) = 25,\ W_Y(0) = 0,\ A_X(0) = 49,\ A_Y(0) = 1$ (see Table 6.1 for parameter values).

6.2.2. Rodent control measures

We considered two possible control measures to reduce the number of infected rats: rodenticide and habitat management. It is worth noting that both of these control measures will target all rats, not just those that are infected. Habitat management can be implemented after a rodenticide program, the aim being to prevent the population from recovering.

Rodenticide is incorporated into the age structured model by assuming that a proportional number of susceptible and infected, sub-adults and adults are removed according to the total target percentage, τ and the probability that a rat contacts the rodenticide, p (equations 6.8-6.14). Rodenticide is placed outside houses and so animals that are confined to the nest (juveniles) will not be affected. We included the second control, habitat management, by reducing the birth rate by a proportion $(1-u)$

$$\frac{dJ_X}{dt} = b\big(1-u(t)\big)(A_X + (1-v_1)A_Y)\left(\frac{k-(W+A)}{k}\right) - \varphi_J J_X - m_J J_X \tag{6.8}$$

$$\frac{dJ_Y}{dt} = b\big(1-u(t)\big)A_Y v_1\left(\frac{k-(W+A)}{k}\right) - \varphi_J J_Y - m_J J_Y \tag{6.9}$$

$$\frac{dW_X}{dt} = \varphi_J J_X - v_3 W_X L - \varphi_W W_X - m_{WA} W_X - p\tau(t) W_X \frac{W_X}{W_X + W_Y + A_X + A_Y} \tag{6.10}$$

(6.11)

$$\frac{dW_Y}{dt} = \varphi_J J_Y + \upsilon_3 W_X L - \varphi_W W_Y - m_{WA} W_Y - p\tau(t) W_Y \frac{W_Y}{W_X + W_Y + A_X + A_Y}$$

(6.12)

$$\frac{dA_X}{dt} = \varphi_W W_X - \upsilon_3 A_X L - m_{WA} A_X - p\tau(t) A_X \frac{A_X}{W_X + W_Y + A_X + A_Y}$$

(6.13)

$$\frac{dA_Y}{dt} = \varphi_W W_Y + \upsilon_3 A_X L - m_{WA} A_Y - p\tau(t) A_Y \frac{A_Y}{W_X + W_Y + A_X + A_Y}$$

(6.14)

$$\frac{dL}{dt} = \lambda_W W_Y + \lambda_A A_Y - \mu L$$

6.2.3. Previous rodenticide campaigns

Previous rodenticide campaigns have been carried out in Pau da Lima by the CCZ. However, trapping counts have shown that the population of rats recovers after these campaigns. We wish to us the age-structured model predict the effect of these previous campaigns. Figure 6.3 shows the amount of rodenticide applied in valley 1 and valley 4 over one of those campaigns.

The probability of contact was calculated from data of previous rodenticide campaigns (unpublished data). Rodenticide was placed outside houses by employees of CCZ, when these houses were revisited it was recorded whether a total or partial block of rodenticide had been consumed. At a valley level, we calculated the percentage of total or partially consumed rodenticide blocks on the second visit to the houses, this was 20% of the rodenticide blocks, and so we assigned $p = 0.2$.

To run the mathematical model with the previous rodenticide campaign values we converted the amount of rodenticide applied at each time point (Figure 6.3) to the proportion of the population (τ) targeted at each time point. Rodenticide in the slums is placed in blocks. If we assume that one block of rodenticide will kill one rat, then for an arbitrary number of blocks, say 10 blocks of rodenticide we assume will kill 10 rats. Given that rodenticide is specified in the mathematical model as a target proportion, not numbers of rats killed, we can convert 10 rats killed by rodenticide to the target proportion as $10/K$, where K is the carrying capacity of the free-roaming rats. In general, if we denote the amount of rodenticide R then,

$$\tau(t) = R(t)/\,K.$$

To convert the rodenticide applied in Figure 6.3 the amount of rodenticide applied at each day was divided by the assumed carrying capacity at that time ($K = 75$). If the amount of rodenticide applied was greater than 75 then a value of 1 was given to τ (the maximum value τ can take is 1). Also, the habitat management parameter u was set to zero for a rodenticide-only scenario. The model was run with all other parameters values as in Table 6.1.

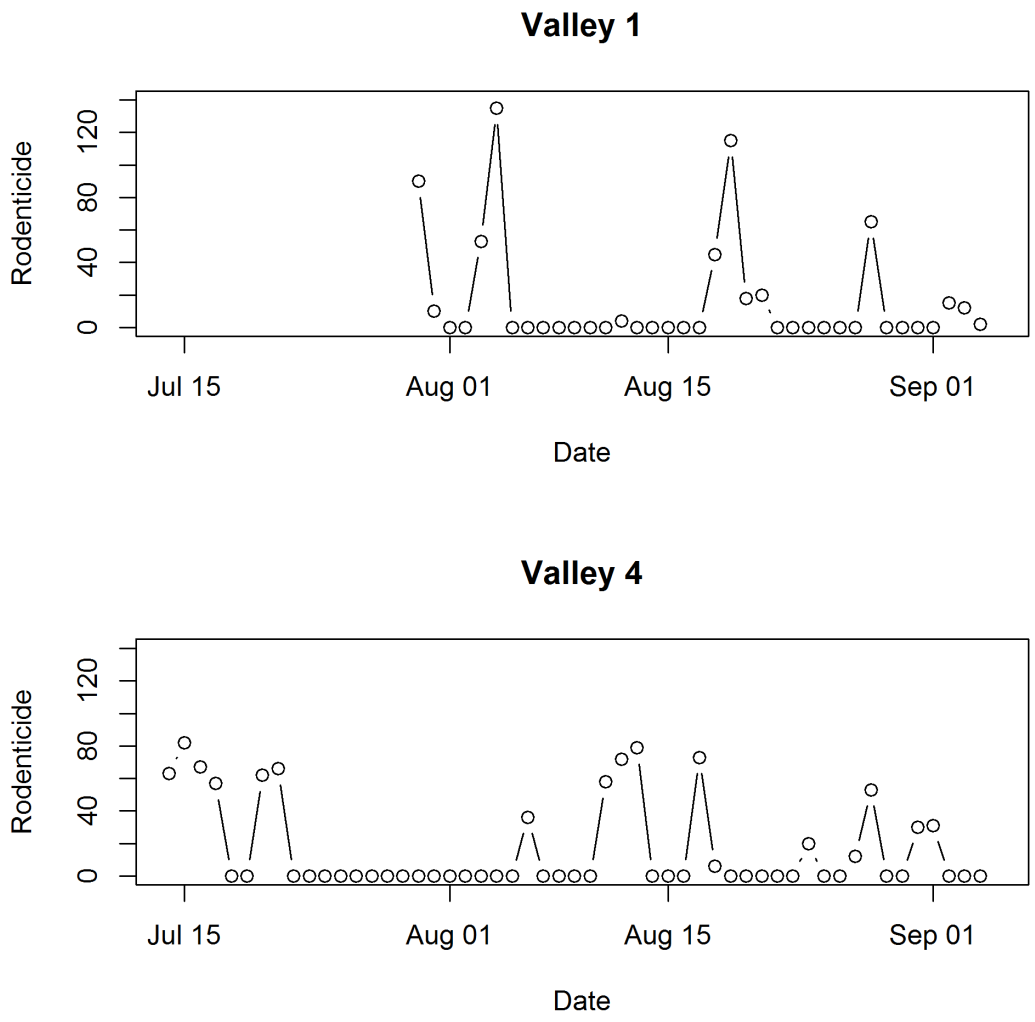

Figure 6.3: The number of rodenticide blocks applied over time in valley 1 and valley 4.

6.2.4. Optimal control

A constant application of control would be wasteful, for example, when a reduction in prevalence can be achieved by a decreasing amount of control over time. For rat management in particular, effects of an intervention programme need to be monitored constantly, with control measures adapting to changes in populations and environment (Traweger et al., 2006). Given these restrictions on resources and time, it is of interest to find the optimal amount of control to be placed in an intervention programme.

The control measures in the age-structured model with control (equations 6.8-6.14) target all rats (susceptible and infected). Though not all rats are born infected, they can in principle be infected at any point in their lifetime and so we wish to investigate the effect of reducing all rats on risk of human infection. We employ optimal control theory to find the optimal time-dependent controls to reduce the population size of rats. Optimal control theory can be used to find the optimum amount of control given restrictions on cost, the maximum amount of control and the length of the intervention programme (Sharomi & Malik, 2015). In the following sections details of the optimal control problem are presented, for those unfamilar with optimal control, see Appendix 3 for a brief introduction and Sharomi & Malik (2015) for examples in epidemiology.

The optimal control scheme is found by minimizing the objective functional. We aimed to reduce the total number of rats $H(t) = J_X(t) + J_Y(t) + W_X(t) + W_Y(t) + A_X(t) + A_Y(t)$) while simultaneously minimising the control efforts used. Hence the objective functional includes the total number of rats and two controls,

$$J(u,\tau) = \int_{t_0}^{t_f} c_1 H(t) + \frac{c_2}{2} u(t)^2 + \frac{c_3}{2} \tau(t)^2 dt \quad (6.15)$$

where c_1, c_2 and c_3 are the costs which transform the integral to monetary value (in this case Brazilian Real (R$)) over the time period $[t_0, t_f]$ (Table 6.4).

The 'cost' c_1 associated with a rat of any age class or infection status can be thought to be equivalent to the cost of human infection, assuming that any rat has the potential to infect a human in its lifetime. The relationship between number of rats and risk of human infection is not well understood, and so we assume a linear relationship between the 'cost' of a rat and the number of rats. We included quadratic terms for the control measures to account for the non-linear costs at high levels of control (Figure 6.5) (Posny et al., 2015; Miller Neilan et al., 2010; Malik et al., 2016).

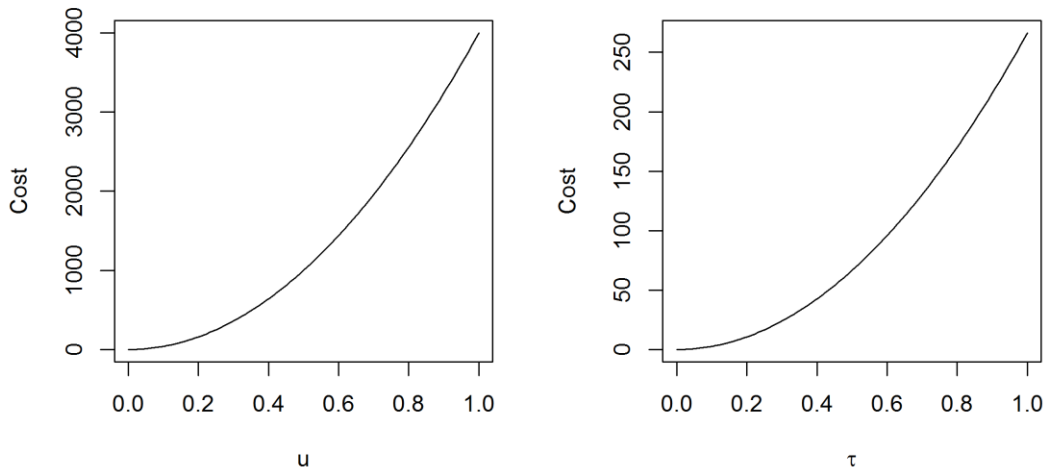

Figure 6.5: Cost functions for habitat management (u) and rodenticide (τ).

We assign arbitrary values to the costs c_1, c_2 and c_3 within reasonable orders of magnitude, with the additional assumption that rodenticide control will be cheaper than habitat management (Table 6.4).

Table 6.4: Value of fixed costs (in R$) of an infected rat and the control measures.

Parameter	Value
$\boldsymbol{c_1}$	R$ 1300 per rat
$\boldsymbol{c_2}$	R$ 8000 per (percent reduction)^2
$\boldsymbol{c_3}$	R$ 532.50 per (target percent)^2

6.2.4.1. Optimal control problem

We apply Pontryagin's Maximum Principle to find the optimal control (Lenhart, S. and Workman, 2007). We wish to identify optimal controls for the time period $[t_0, t_f]$. The control set is,

$$\Gamma = \{(u(t), \tau(t)) | 0 \leq u(t) \leq u_{\max}, 0 \leq \tau(t) \leq \tau_{\max}\}$$

which is closed and convex by definition. The objective function and its integrand are both convex and an upper bound of the state variables exists. Finally, the age-structured model is linear in the control variables and so an optimal solution exists (Posny et al., 2015).

The Hamiltonian and the adjoint equations of the system are supplied in Appendix 3. The optimal controls $\tilde{u}(t)$ and $\tilde{\tau}(t)$ are found by solving $\frac{\partial \mathcal{H}}{\partial u} = 0$ and $\frac{\partial \mathcal{H}}{\partial \tau} = 0$ (Appendix 3). Then the optimal control at time t is characterised as,

$$u^*(t) = \min(\max(0, \tilde{u}(t)), u_{\max})) \tag{6.16}$$

$$\tau^*(t) = \min(\max(0, \tilde{\tau}(t)), \tau_{\max})) \,. \tag{6.17}$$

In this analysis, we allow the entire population to be targeted and for the birth rate to be reduced to zero i.e. $u_{\max} = \tau_{\max} = 1$.

The optimal controls were found for each of the five different scenarios for an intervention programme of 365 days (t_f). Firstly, the age-structured model with control is solved forward in time using initial values for the control measures. Then the adjoint equations are solved backward in time using the solutions of the age-structured model. The values of the control measures are then updated using equations 6.18 and 6.19. This process is repeated until the control measures have converged. The convergence criterion used was that the values from subsequent iterations were the same to 5 decimal places.

For the sequential controls we apply the same algorithm as in Malik et al. (2016). The process as above is applied to the first control in the sequence for the time interval [0,t_s], where t_s is the 'switch' time. Then, using the final time values of the state solutions as initial values for the second optimal control is found for the time interval $[t_s + 1, t_f]$.

Given the optimal controls for the five different scenarios, the age structured model with control (equations 6.8-6.14) was run for 2000 days with the optimal controls to investigate the effect of these controls on infection dynamics.

6.3. Results

Rodenticide targets all free roaming animals, both susceptible and infected. Hence the model predicts that numbers will fall in the susceptible and infected populations (Figure 6.4). The numbers of juveniles falls because of the reduction in the number of sexually mature (adult) animals. As the number of free roaming infected animals decreases, so does the number of free-living leptospires in the environment. In valley 4, where rodenticide was applied over a longer time period, the infected sub-adult and adult population is reduced to half of its original size.

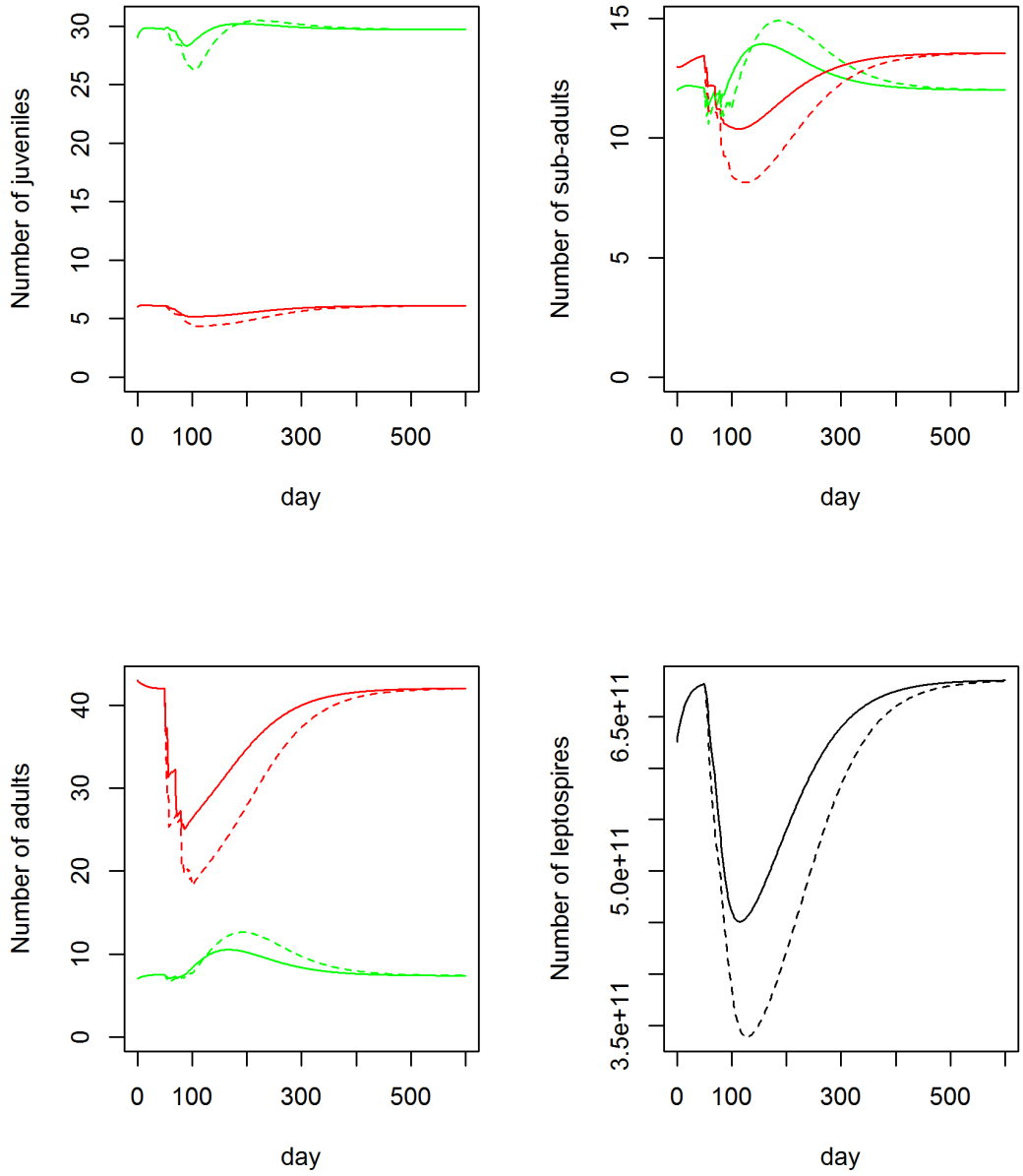

Figure 6:4:The effect of rodenticide applied to the predicted number of susceptible (green) and infected (red) juveniles, sub-adults and adults and leptospires (black) with initial conditions $J_X(0) = 35,\ J_Y(0) = 0, W_X(0) = 25,\ W_Y(0) = 0,\ A_X(0) = 49,\ A_Y(0) = 1$ (see Table 6.1 for parameter values). Solid lines are valley 1, dashed lines are valley 4.

In valley 4, too, the number of susceptible animals increases to a larger value post rodenticide than in valley 1. After approximately 450 days, population sizes in each age and infected class converge to the same values. The number of infected animals

in each age class returns to their numbers pre-rodenticide campaign. The model predicts that the rodenticide was effective in reducing the population size, but the reduction was not great, especially in the valley 1 case. As expected, the population recovered in a relatively short amount of time.

The optimal controls for the five scenarios are shown in Figure 6.6 and Figure 6.7. When both of the controls are applied simultaneously, the maximum amount is applied for a shorter length of time than when the controls are applied individually (Figure 6.6). When the habitat management control is implemented alone (Figure 6.6b), the maximum control should be implemented for almost the entire intervention period.

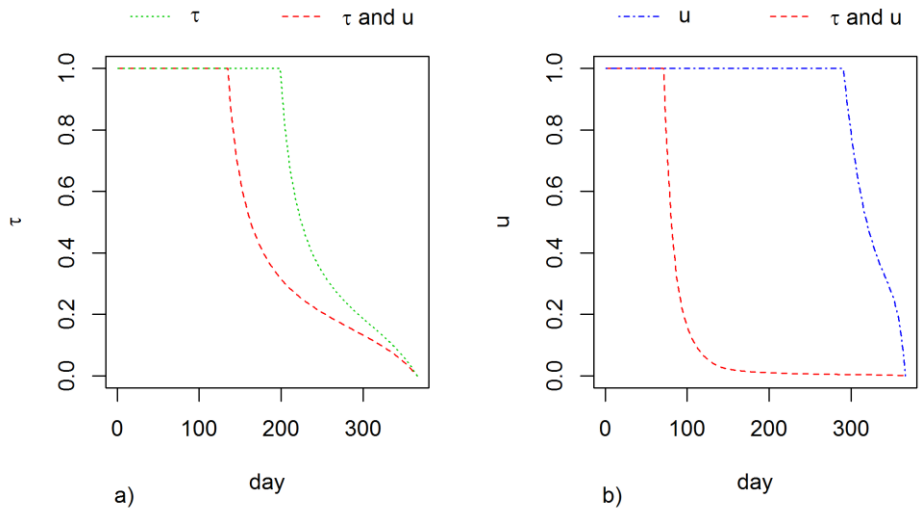

Figure 6.6: The optimal target percentage (τ) (a) and habitat management (u) (b) under the scenarios: both controls implemented (red dashed line), just rodenticide (green dotted line) and just habitat management (blue dash-dotted line).

When rodenticide is applied followed by habitat management (Figure 6.7a) rodenticide is applied at its maximum amount for almost the entire 182 days. Habitat management is then only applied at a low level (around 0.2). For the other

sequential control scenario, habitat management followed by rodenticide, habitat management is applied at its maximum amount for the entire 182 days followed by rodenticide also being applied at a high level for most of the 182 days (Figure 6.7b).

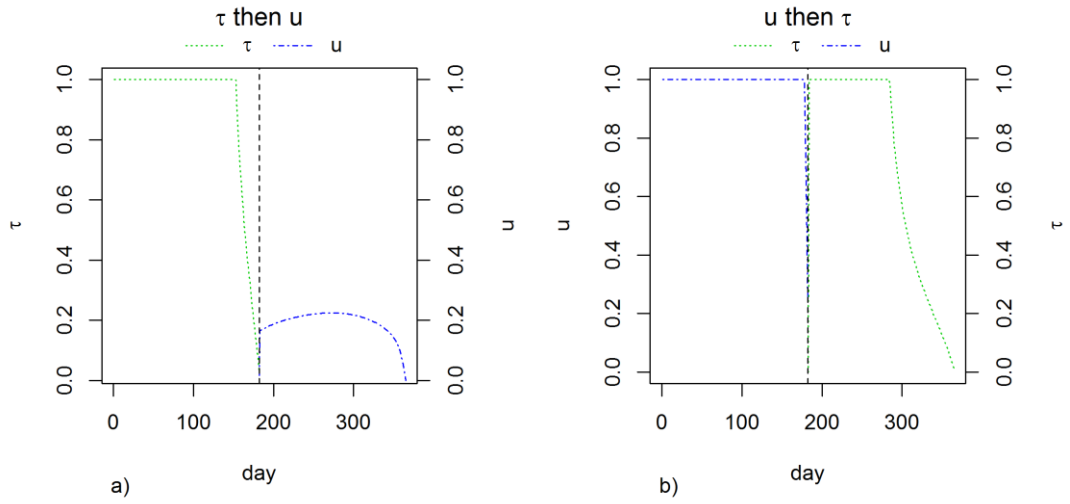

Figure 6.7: The optimal target percentage (τ) and habitat management (u) under the scenarios: rodenticide followed by habitat management (a) and habitat management followed by rodenticide (b). Rodenticide (green dotted line) and habitat management (blue dash-dotted line).

Turning to effects on total population size, infected population size and leptospire population size (Figure 6.8, Figure 6.9, and Figure 6.10), the combination of both controls applied simultaneously had the greatest immediate effect. Applying rodenticide alone had the same effect at the beginning of the intervention programme as rodenticide followed by habitat management. Likewise, habitat management alone had similar effects up until half way thought the intervention programme (from day 182) as did habitat management followed by rodenticide.

Towards the end of the intervention programme, however, greater differences were suggested between all the control scenarios. With rodenticide and habitat management applied simultaneously, it took longest to return to pre-intervention

levels of infection. The second longest time to return to pre-intervention levels of infection was achieved with rodenticide only. Most notably, an intervention programme with habitat management alone would result in a return to pre-intervention prevalence levels much earlier than if rodenticide alone was applied. All control scenarios eventually returned to the pre-intervention total population sizes and infected population sizes.

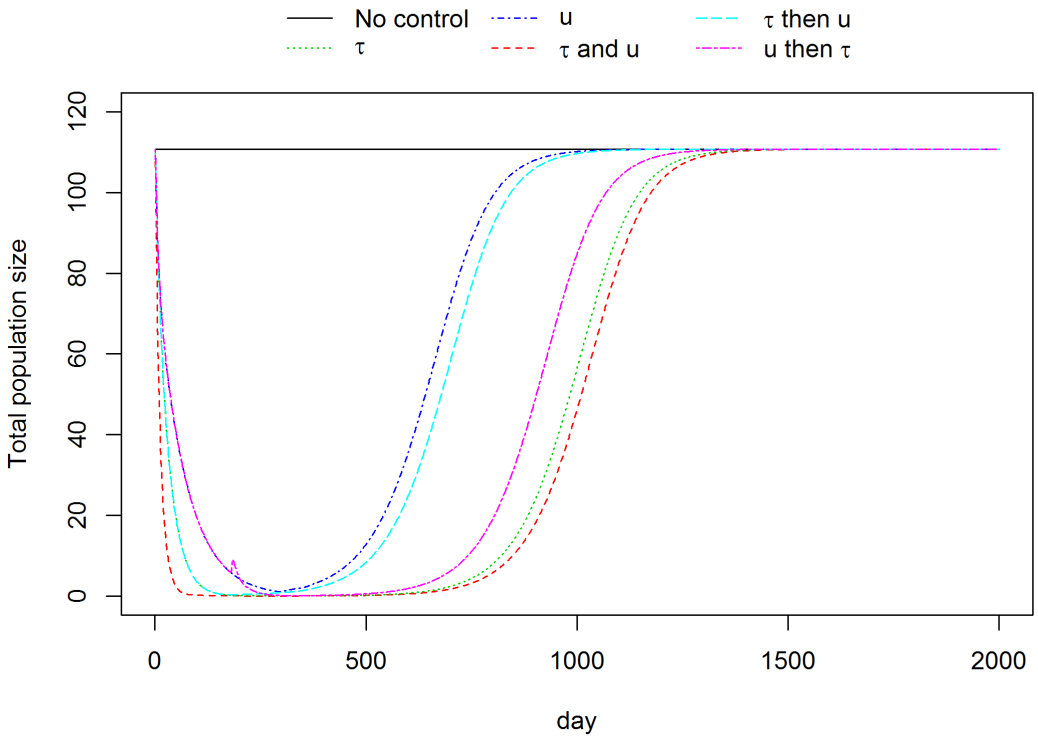

Figure 6.8: Changes in the total population size over time as predicted by the age structured model with optimal control measures in Figures 6.6 and 6.7.

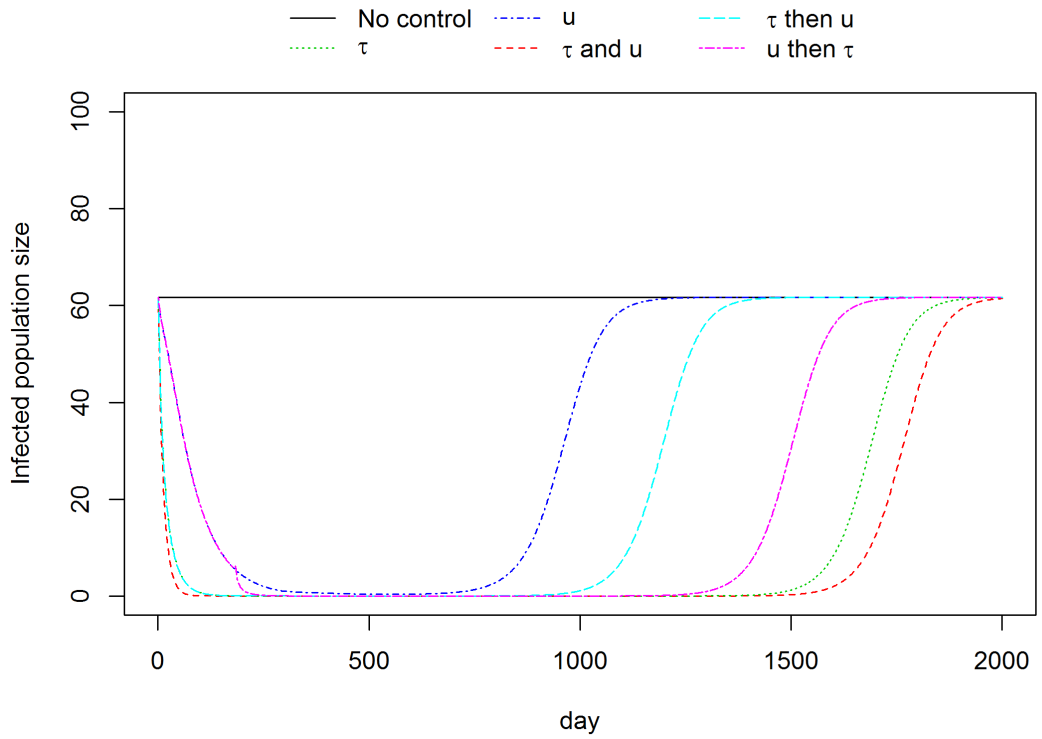

Figure 6.9: Changes in the infected population size over time as predicted by the age structured model with optimal control measures in Figures 6.6 and 6.7.

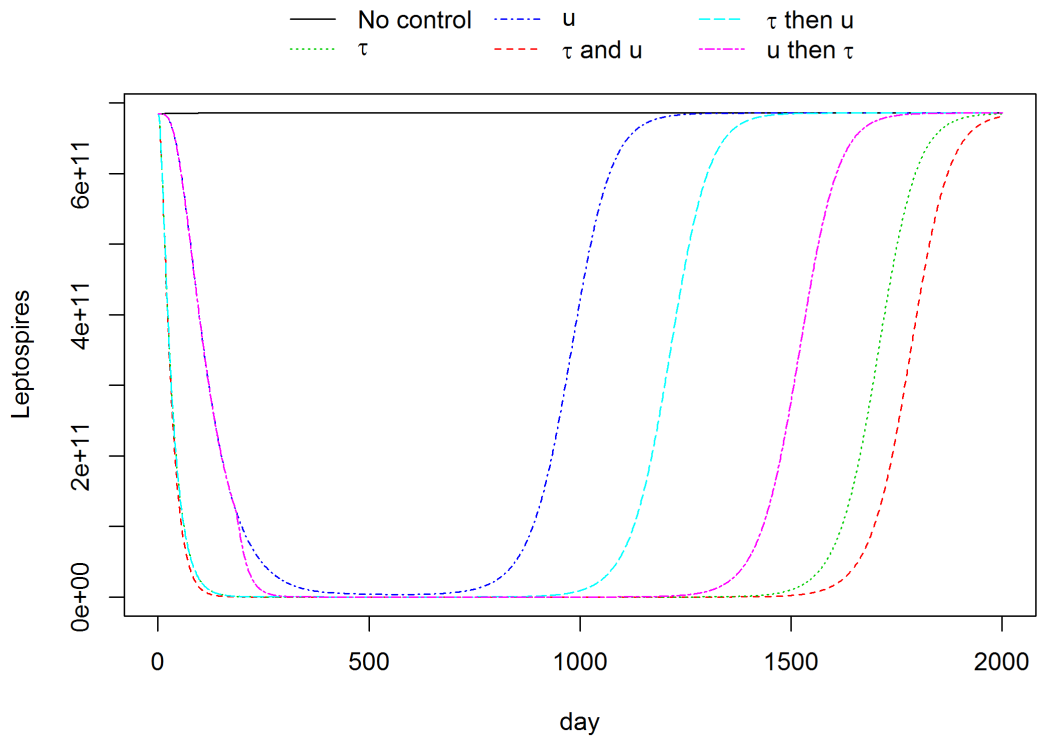

Figure 6.10: Changes in the number of free-living leptospires over time as predicted by the age structured model with optimal control measures in Figures 6.6 and 6.7.

The cumulative costs associated with each of the optimal control scenarios are shown in Figure 6.11. The control scenario with the highest associated costs was habitat management, the second highest was the sequential control of habitat management followed by rodenticide. Applying rodenticide only or rodenticide followed by habitat management had very similar costs and applying both control simultaneously had slightly higher costs.

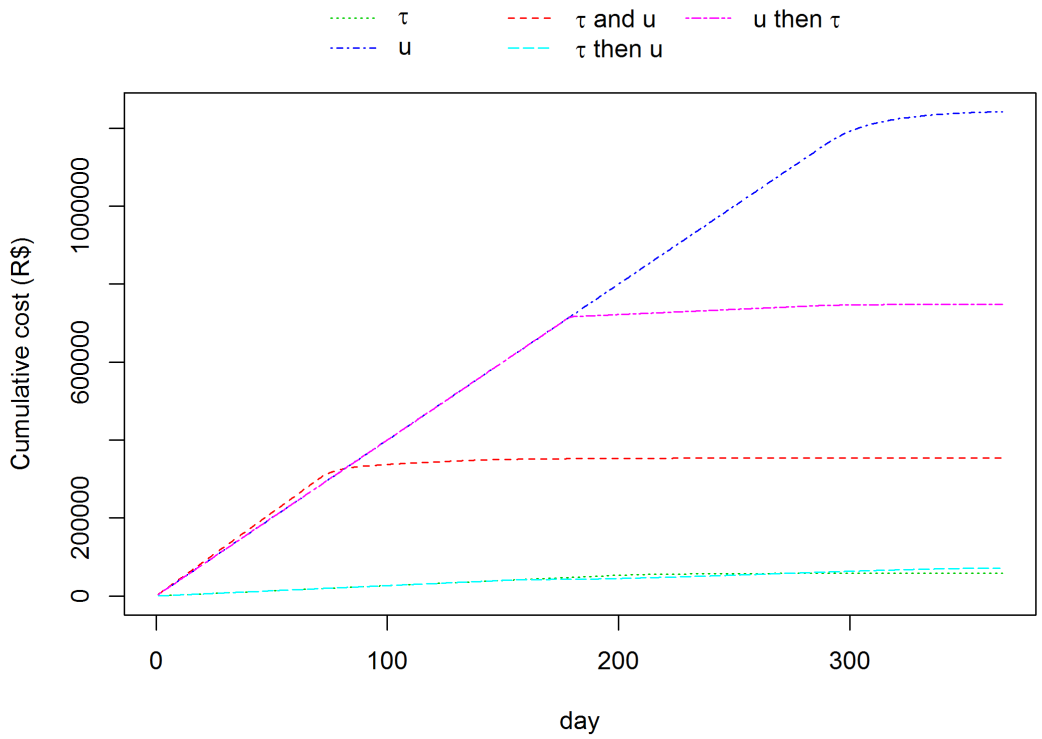

Figure 6.11: Cumulative costs associated with each of the scenarios.

6.4. Discussion

Human zoonotic infections can be prevented by reducing potential contact with the reservoir or controlling the reservoir itself. We have presented a framework to find control measures to reduce the population size of the natural reservoir of leptospirosis in Pau da Lima, the Norway rat. Optimal control theory has been used

recently for zoonotic diseases. Biswas (2015) found optimal controls for human Nipah fever but for controls specifically targeting humans only. Abdullahi et al. (2015) sought to reduce *Plasmodium knowlesi* malaria infection in humans and macaques by the quarantine of infected humans, culling of infected macaques and spraying mosquitoes with insecticide. Optimal controls have been found with the aim of controlling animal populations, namely agricultural pests (Ghosh & Bhattacharya, 2010; Kar et al., 2012; Gubbins et al., 2008; Bhattacharyya & Bhattacharya, 2007). Optimal control theory has not previously been applied to control rodent populations.

The optimal controls presented in this framework were a part of a pilot analysis of control measures for Norway rats. The optimal controls were based on an age-structured model for leptospire infection in Norway rats. The model was able to predict the prevalence levels in the sub-adult and adult population well. A valley level comparison should also be performed to further validate the model framework for predicting prevalence. Failed interventions can be used to validate a mathematical modelling frameworks (Joseph et al., 2013). The age-structured model with control measures predicted that current rodenticide campaigns employed in Pau da Lima would not be sufficient for long term rodent control. The recovery of rat populations in Pau da Lima after the rodenticide campaign is currently being investigated; the results of which can be used to validate the age-structured model with control measures.

In the scenarios that were tested application of rodenticide, either solely or in combination with habitat management, has an immediate effect on infected

population sizes. Control by habitat management alone led to a slower reduction. This is because habitat management reduces the birth rate which in turn reduces the population size, whereas rodenticide reduces the population size immediately. This same result was found by Holt et al. (2006) who recommended trapping mice instead of changing suitability of environment to reduce human risk of leptospirosis because of the immediate effect trapping had on population size and prevalence. In terms of a longer term effect, control by habitat management alone led to a return to pre-intervention campaign infection levels the quickest out of all possible control scenarios. The combined controls, applied either simultaneously or sequentially, had a slower return to pre-intervention infection levels. But this slower return did not also correspond with a quicker immediate effect on the infection levels.

The 'best' control is one that is cost effective. Given the arbitrary control costs used in this analysis, the most costly control scenario was also the least effective: applying habitat management only. Applying rodenticide followed by habitat management and rodenticide only had comparable costs but applying rodenticide only was a more effective control. Habitat management followed by rodenticide was the second longest lasting control but was also the second most expensive control scenario. Applying both controls simultaneously had the greatest immediate effect and also had a cost towards the lower end. However, the costs of the two controls have yet to be fully explored.

The rodenticide control cost was assumed to be lower in value than the habitat management, as the latter control has never been applied in Pau da Lima so initial costs are presumed to be costly. The habitat management cost will change with

more insight into how the control could be implemented in Pau da Lima, with this change in cost substantial differences in the optimal controls are also to be expected. Given that habitat management is yet to be implemented in Pau da Lima, multiple types of intervention should be proposed and costed to fully explore the effectiveness of habitat management.

Leptospirosis transmission between rats can occur at any point in their lifetime. Hence all rats in a population have the potential to become infected and in turn infect a human. It was for this reason that the 'cost' of a rat was given the cost of human infection. In this analysis, with the absence any knowledge on the relationship between risk of infection and number of rats, we assumed a linear relationship between cost and number of rats. In general, humans acquire leptospirosis infection via the environment, not directly from rats, hence the relationship between risk of infection and number of rats will be difficult to determine. To accommodate for this uncertainty, multiple forms of non-linear relationships should be implemented and the difference in optimal controls scrutinised.

For the rodenticide control, there are several extensions to the age-structured model which should be considered. Firstly, we did not include probability of success in the rodenticide control; if an animal contacts rodenticide then death is certain. Nakagawa et al. (2015) found a mortality rate of 83% when Norway rats consumed bromadiolone rodenticide. Mlynarèíková et al. (1999) found that Norway rats had 100% mortality 8 days after consumption of bromadiolone rodenticide. The inclusion of probability of success given contact would have accommodated for the

few animals that would contact rodenticide and survive, but this small discrepancy did not justify the inclusion of an extra parameter in the model.

Secondly, the control by rodenticide may also be overestimated if the contact of rodenticide of rats is not random. Norway rats are neophobic animals; they fear unknown objects in familiar places (Clapperton, 2006). This behaviour is noted to be a particular barrier to the success of rodenticide campaigns (Clapperton, 2006; Feng & Himsworth, 2014). Those neophobic animals in principle could never be removed via rodenticide. There are a number of ways to adapt rodenticide programmes to neophobic animals (permanent bait stations for example (Clapperton, 2006)) which should be carefully considered when trying to implement results alike those presented in this study.

Thirdly, we assumed that juveniles would not be targeted by rodenticide in an intervention programme, i.e. no rodenticide placed in burrows. It is not certain though whether there would be indirect effects of rodenticide on the survival of litters. Norway rats adopt communal nursing behaviour which leads better survival of abandoned young (Butler & Whelan., 1994; Meaney & Stewart, 1981). Hence those animals still confined to the nest whose parents have been killed via rodenticide are likely to survive if population sizes are large enough. If the population size becomes low enough, this nursing behaviour cannot occur (Hein & Jacob, 2015), and it is expected that those animals in the nest will die as a result of a rodenticide campaign. This population size-dependent behaviour has not been included in the modelling framework which could lead to an underestimation of the effectiveness of rodenticide control. A pulse removal of juveniles from the

population would not have the same effect as reducing the birth rate, but instead have an instantaneous effect on infection dynamics (as illustrated by rodenticide vs. habitat management control).

The formulation of habitat management in the age-structured model could also be extended. The current formulation of the habitat management control reduces the birth rate of all rats in the model. Transforming the value of the parameter in the control model to controls to be implemented in the field is not straightforward. Habitat management reduces the survival and increases the level predation by reducing refuges (Lambert et al., 2008; Buckle, 2013). Lambert et al. (2008) recommend that the home range of the rat should be clear from vegetation and refuge in order to successfully reduce rat population sizes by habitat management (in rural farm or urban areas). In Pau da Lima, clearing garbage will reduce access to food and in some cases refuge also. The amount of reduction which needs to take place in order to reduce the birth rate by a set amount needs to be informed by pilot field studies.

However, habitat management includes reduction of access to food to reduce carrying capacity (Adrichem et al., 2013). The habitat management control in this analysis can be thought of as a semi-permanent control, which reduces the birth rate but not the carrying capacity. In another scenario, habitat management could be a permanent change to the slums. In this case, the control would reduce the carrying capacity permanently and the formulation of the model framework would need to reflect this effect.

For either rodenticide or habitat management, our model does not include a time lag effect of control application. Though death can be assumed to be certain, death does not occur instantly upon contact with rodenticide (Mlynarèíková et al., 1999). This time lag may also apply to habitat management (Williams, 2007). Removal of garbage will have an effect on the birth rate of animals, but this effect would not occur instantly. The control by rodenticide may be interpreted so that controls must be applied say 8 days (Mlynarèíková et al., 1999) prior to as the model predicts. But the time lag of habitat management is not known and so this back calculation cannot be made. Also, if time lags were different for the two controls, and specified in the model as such, then the optimal controls may change.

For Pau da Lima, application of sequential controls would be logistically easier to implement in the field. Ward et al. (2009) recommend habitat management with minimum use of rodenticide to prevent animals becoming resistant to rodenticide. Traweger et al. (2006) advocate the use of integrated pest management, where the aim is to reduce the carrying capacity of rat populations using a combination of control measures for a longer lasting success in control. The switch time used here was just half way through the intervention programme (182 days). The optimal switch time should be further explored to investigate more subtle differences in the use of sequential controls.

The model predictions illustrate the rat population sizes decreasing to close to zero, as our age-structured model is continuous, fractions of rats can be predicted. The numbers predicted by our model can be thought of density of rats in an area. However, there is an argument to use stochastic version of the age-structured

model, in order to have the biological realism of discrete population sizes and also to include the stochastic uncertainty in population recovery post control.

Here optimal controls were found based on a mathematical model parameterised using field data. Uncertainties in the model parameter values relating to both demography and transmission have not been accounted for here and so future analysis will include sampling a full parameter space of costs, model parameters and switch times. In future analyses, an extension should be made so that optimal control measures are found for populations based on the entire valley, and not just the trapped population. Animals are trapped predominately at the bottom of the valley, and so the population sizes are not representative of the entire valley. Rodent control programmes, especially in the case of the control of zoonotic reservoirs, need to be adapted to the system at hand (Traweger et al., 2006). Optimal control theory has been applied to seek controls for human leptospirosis before, but focusing purely on control measures within the human population (covering cuts, personal hygiene etc.) (Sadiq et al., 2014; Khan et al., 2014). We have presented the first illustration of optimal control theory for rodent control based on a mathematical model fully informed and parameterised from field data of the system at hand.

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Chapter 7

Discussion

Leptospirosis has a global incidence, but the level of infection varies by country, climate and reservoir. In temperate and tropical regions, the Norway rat is a significant reservoir for human and animal leptospirosis (Bharti et al., 2003). The aim of this thesis was to understand the within population transmission dynamics of leptospirosis in the Norway rat in the urban slums of Salvador, Brazil to better understand transmission to humans and how to control wild Norway rats to reduce that risk. To explore this aim, a combination of mathematical modelling and empirical analyses has been used; all of which have a basis that could be applied to other leptospirosis systems.

This chapter is structured as follows. First we discuss the significance our results related to the infection dynamics within the Norway rat population (7.1) and validating model parameter estimates (7.2), and in 7.3 we discuss the implications that our results have for control of leptospire infection in Norway rats. In section 7.4, applications to other leptospirosis systems are discussed and in 7.5 our results are related back to the context of urban health. Finally, conclusions are made and future work is highlighted in section 7.6.

7.1. Infection dynamics

7.1.1. Environmental transmission

To understand the infection dynamics within the Norway rat population a theoretical approach was taken first. In chapter 3 a simple model describing leptospire dynamics in a Norway rat population was used to investigate how infection was maintained in the rat population. Global sensitivity analysis of the basic reproduction number threshold suggested that environmental transmission was the most important route for the occurrence of endemic infection. Other work has found similar results in other multiple transmission systems. Xiao et al. (2007) found that changes in direct and indirect transmission of *Salmonella* in animal populations led to changes in the behaviour of the model at equilibrium, whereas vertical transmission did not. Similarly, in a model for leptospire dynamics for the common African rodent, Holt et al. (2006) found that changes in environmental transmission rate had a greater effect on the number of free-living leptospires and prevalence of leptospirosis in the rodents than the other transmission routes (sexual and vertical).

Despite the importance of environmental transmission being supported by other modelling studies with multiple transmission routes, it should not be ignored that it was the only parameter which was estimated. By treating all other parameters as fixed and known, the environmental transmission rate was estimated according to whether model predictions of prevalence where within the range found in field animals. Model validation is an important step in the development of a

mathematical framework (Restif et al., 2012). The values of the basic reproduction number, R_0, that were obtained in part validate the model framework, as they were realistic. The global sensitivity analysis was used for finding which transmission route was most important in the occurrence of endemic infection, but it also directs us to which parameters we should have most certainty in. The analysis of the transmission routes was based on R_0, and not the level of prevalence used to estimate the environmental transmission rate so that our results were independent of the estimation procedure.

An extension to the model framework proposed in chapter 3 would be to consider cases where direct transmission can be used to represent environmental transmission. In some circumstances when pathogen survival is low (and an individual may recover from infection), direct transmission can represent environmental transmission in a mathematical framework (Breban, 2013). Day et al. (1997) were unable to achieve experimental infection of *Leptospira* in brushtail possums exposed to contaminated cages or grass, and Caley & Ramsey (2001) found that density dependent was the more appropriate term to describe natural infection in brushtail possums with leptospirosis in a field experiment. For many reservoirs of leptospirosis this may not apply, as animals do not recover from infection. But in the cases of brushtail possums where *Leptospira* is used as biological control, it may be that direct transmission is suitable for modelling infection dynamics.

7.1.2. Transmission in the wild

Multiple transmission routes of leptospirosis within rat populations have been hypothesised to occur on the basis of direct biological evidence, but for control of wild zoonotic reservoirs, it is important to know which transmission routes occur in the wild and at what rate. The results of chapter 4 strongly suggested that a proportion of animals leave the nest with infection, providing evidence for vertical transmission. Evidence in favour of environmental transmission was also found. There was no evidence to suggest that direct transmission occurred at a significant rate. These results complement the results of chapter 3, where environmental transmission was found to be the most important route for contributing to endemic infection in the rat population.

Few studies have been performed with the aim of identifying evidence of transmission routes in wild zoonotic reservoirs. However, Breban et al. (2009) identified evidence for an environmental transmission route of avian influenza through an empirically informed mathematical model. With environmental transmission in the model, they were able to explain observed periodicity of epidemics and infection was able to persist in small communities. VanderWaal et al. (2014) sought to identify evidence for direct or indirect transmission of *E.coli* within giraffe population by finding which individuals share the same genetic subtype of *E.coli* and comparing transmission networks to networks of social interaction and networks of shared space. They concluded that the transmission network was closely matched by the social network, but that this could represent indirect transmission occurring at the same time as well as direct transmission. These results

highlight how evidence of transmission routes can be found by assuming a mathematical framework. However, we wished to find evidence of transmission routes in the wild independently of our modelling framework, so that the results could validate our model.

Empirical evidence for transmission routes occurring in wild populations has been found based on prevalence data, information which is often collected during studies of wildlife systems and extends previous work based on age-prevalence profiles (Long et al., 2010; Caley & Hone, 2012). Given that the transmission routes of leptospirosis in Norway rats are age dependent (see chapter 4), finding evidence of transmission routes occurring in the wild can inform approaches to control infected rat populations. For example, if rats were not becoming infected vertically, then rats would not be leaving the nest with infection and so there would be no cause to target nests.

The results of chapter 4 are significant in that they illustrate the incorrect assumptions regarding transmission in chapter 3. Direct transmission (sexual) was included in the simple model framework in chapter 3 and all results based on this framework assume direct transmission takes place. In chapter 4 we concluded that direct transmission may occur, but not at a high enough rate to justify inclusion in a model framework. The results of chapter 3 are of course still relevant for another system or reservoir species with multiple transmission routes, but highlight the importance of data driven modelling frameworks.

7.2. Validating parameter estimates

Models need to be parameterised and validated by data for a given system to ensure the model predictions are robust (Lloyd-Smith et al., 2009). For systems which have few existing models in the literature, new models are often presented without parameterisation by data. There is only one existing model for within rodent population leptospire dynamics (Holt et al., 2006) and so many of the parameters in the framework of chapter 3 are informed by the work of Holt et al. (2006).

An age structured model was proposed and is presented in chapter 6. Before any kind of analysis was performed with this age-structured model we set out to confirm the parameter values. The process of confirming the validity of parameter estimates, either from the literature or field data, is essential for parameterising wildlife infection dynamic models (Cooch et al., 2010). In this thesis (where the aim is to find control measures for rats in Pau da Lima based on mathematical modelling) the model needs to reflect the population dynamics of Norway rats in urban slums.

In chapter 5 a population dynamics model for slum Norway rats was presented alongside a analysis of sub-adult to adult proportion data. We concluded that the lifespan found in Glass et al. (1989) and the matruration period based on that lifespan was most representative of the animals in Pau da Lima. In terms of model validation, this process ensured that the adult morality rate and maturation of sub-

adults parameter values obtained from the literature or field data were capable of predicting population dynamics of the rats in Pau da Lima.

7.3. Age-structured model and implications for control

In chapter 6 rodent control measures which targeted the host population were explored using an age structured infection dynamics model. This model was informed by the initial simple model presented in chapter 3, results of the empirical analysis in chapter 4 and the demographic parameter values found in chapter 5. The final parameterised age-structured model was able to predict the level of prevalence in the sub-adult and adult population well. Further validation of the models capability to predict infection levels should include comparing model predictions to valley level prevalence. An improved understanding of the population sizes of Norway rats in Pau da Lima can be achieved either from extensions of current removal methods to estimate abundance (Pedra et al., *in preparation*) or via the use of tracking plates to detect the untrappable rats (Hacker & Minter et al., 2016). Also, information on pre and post abundance levels from previous rodenticide campaigns should be utilised to ensure that the model is capable of predicting failed rodenticide campaigns.

We investigated control measures which would reduce the total rat population size using the age-structured model (chapter 6) and target reproduction numbers (chapter 3). Optimal control theory applied in chapter 6 showed that the combination of rodenticide and habitat management has the potential to be an effective control of rodent population size and of leptospires in the environment.

Empirically informed target reproduction numbers in chapter 3 illustrated that control via the environment would be an effect control to reduce infection in the rat population. However, the logistical and resource efforts for environmental and rodent control are not equal. The effect of rodent control programmes on not only rat population sizes, but also the incidence of human leptospirosis needs to be evaluated.

There are multiple ways to control zoonotic infection: target the host, target the pathogen or reduce the contact between the host and pathogen (Blancou et al., 2009). Rats are a pest species, and so targeting the host is often the preferred method of control. However, the aim of controlling rodents is to prevent human leptospirosis by reducing the number or concentration of leptospires in the environment. Hence a control which reduces the number of free-living leptospires should be investigated for the purpose of reducing prevalence of leptospirosis in the rat population, but not the size of the rat population itself.

For urban slum rats, host lifespan is longer than leptospire survival so removing rats is the optimal control but in other systems (where leptospires are longer lived) removing animals would not be the best control. When pathogen survival is longer than the host lifespan, the pathogens that the host sheds persist after the host has died. In this circumstance removing animals would not be sufficient to control infection as new infections will arise from the environment reservoir (Almberg et al., 2011). When the reservoir of leptospirosis is not a pest species or the interest is in animals which suffer leptospirosis associated disease, reducing the host population size is not an appropriate control. The effect of environmental control,

reducing the free-living bacteria state, should also be explored as a possible control for leptospirosis in other zoonotic reservoirs and animals with disease.

The next stage for understanding human risk of leptospirosis in Pau da Lima will be to relate the age-structured model for rats to a mathematical model for leptospire dynamics in the environment. Seasonal changes in climate, particularly rainfall, lead to increased risk of leptospirosis for residents of Pau da Lima. The age-structured model for infection in rat populations will feed into a mathematical model for the dynamics of leptospire in the environment which incorporates run-off and rainfall. Then the effect of environmental control on rat and human risk can also be investigated simultaneously.

7.4. Application to other leptospirosis systems

This thesis has focused on infection dynamics of leptospirosis in urban slum Norway rat populations. The urban slum system in which rats are living in close proximity to humans is common to many other systems beyond rats and humans. The Norway rat is has been acknowledged as the reservoir for leptospirosis in both temperate and tropical countries (Adler de la Peña Moctezuma, 2010).

Therefore, we expect findings of this thesis to be applicable to other tropical urban systems. For example, the prevalence of leptospire infection in urban rat populations in Malaysia is 70% (Benacer et al., 2013). The urban sites, mostly markets, have refuge and leftovers providing resources for rats. The climate is similar to that in Salvador, hot and humid all year round, with increased rainfall in the monsoon season.

There are also urban temperate systems which have many similarities with the urban slums system in Salvador. In Baltimore, Maryland US, rats have different body metrics to Salvador's rats, but are demographically similar with the same rate of pregnancy and size at sexual maturity (Porter et al., 2015). As in Salvador, high numbers of rats are trapped in the areas in Baltimore that have poor housing and sanitation (Porter et al., 2015; Easterbrook et al., 2005). *Leptospira* carriage is similar in Salvador's rats (between 63.1% (n=84) and 80.3% (n=142)) (Costa et al., 2014) and Baltimore's rats (65.3%, n=201) (Easterbrook et al., 2007). As in Salvador, no evidence of seasonal prevalence of leptospirosis has been found for rats trapped in Baltimore (Easterbrook et al., 2007). Any expected difference in prevalence of leptospirosis in rats between the systems may relate to climate and environment.

Baltimore has a seasonal climate with average winter temperatures of 3.9°C (Porter et al., 2015). In the Faroese Islands, where the mean temperature is 6.5°C, it was concluded that it is too cold for rural Norway rats to get leptospirosis (Jensen & Magnussen, 2016). However, high prevalence of leptospirosis has been observed in urban rat populations in temperature regions (Himsworth et al., 2013; Krøjgaard et al., 2009). A comparison of evidence of transmission routes between the Salvador and Baltimore systems would provide insight into how climate affects the within population infection dynamics in urban rat populations.

It has been well established that rodents in urban systems have different demographic characteristics to their rural counterparts (McGuire et al., 2006). Hence the results presented in this thesis might differ slightly once accommodations have been made for rural systems. Rural rats have a longer

lifespan than urban rats (Davis, 1948), and Holt et al. (2006) found that mortality rate was the most sensitive parameter in relation to changes in rodent numbers, prevalence and leptospire numbers. Hence changes in the lifespan of rats would change the results based on urban rats.

Leptospire infection is often endemic in maintenance reservoirs (Levett, 2001) and so the methods used to understand the within population infection dynamics of leptospirosis in rodents could be applied to other systems. The approach to identify transmission routes can be applied to any reservoir when changes of behaviour over time bring with them new risks of infection. The key is to understand how the life cycle of an animal and relates to infection risk.

As an example, for livestock transmission is thought to occur both within populations and between species (either other livestock species or rodents) (dos Santos et al., 2012; Schoonman & Swai, 2010; Boqvist et al. 2002). Constant risk of infection over the lifetime of a livestock single species farm would indicate risk comes from the environment or from another species such as rodents. When livestock is moved and contacts other livestock, patterns of infection would indicate whether infection occurred from other livestock species. For mixed species livestock farms, as is common in many countries, distinguishing between within and between species transmission based on prevalence data alone would be difficult and could require data on contact patterns. For livestock, contact structure is dictated by farm practices and hence transmission risk is not homogenous (Craft, 2015). In general, for systems that comprise multiple reservoirs and/or multiple serovars, identifying whom infected who requires data beyond only prevalence.

7.5. Urban health

Residence in urban areas gives rise to particular health risks associated with lifestyle and living conditions (World Health Organisation, 2010b). For urban slum dwellers, poor living conditions lead to an increased risk of infectious disease. With increasing urbanisation, urban health is set to become an increasing problem (Prasad et al., 2016).

Urban slum dwellers need improved sanitation and housing to improve health (World Health Organisation, 2010a; Eisenstein, 2016). Successes have been achieved in Ahmedabad, India, for example, where an upgrading of a slum led to reduced risk of waterborne and mosquito related illnesses (Butala et al., 2010). Indeed, the World Health Organisation runs the Healthy Cities project in multiple regions of the world with the aim of improving urban health (World Health Organisation, 2010a). One of the UN Sustainable Development Goals is to 'Make cities and human settlements inclusive, safe, resilient and sustainable' with the sub-goal of 'by 2030, ensure access for all to adequate, safe and affordable housing and basic services, and upgrade slums' (UN, 2015). However, upgrading slums is difficult to achieve in practice. There is often lack of commitment from residents as well as government bodies (Sheuya, 2008; World Health Organisation, 2010a). Hence for zoonotic diseases, understanding how infection is maintained in the reservoir provides an alternative method of control to upgrading slums.

Characteristics of urban slums provide good conditions for transmission of leptospirosis. For example, poor drainage and refuge provide optimum

environments for the survival of leptospires and habitat for reservoirs such as Norway rats. Urbanisation together with climate change is therefore expected to increase the global incidence of leptospirosis (Lau et al., 2010). Pau da Lima is just one example of urban slums found globally. In different parts of Asia the percentage of urban residents living in slums varies between 25-30% and in sub-Saharan Africa over 60% of urban residents live in slums (World Health Organisation, 2010a). While urban slum communities persist, studies into the animal reservoir of human infection, such as that described in this thesis, will, hopefully, provide insight into reducing the burden of infectious disease.

7.6. Conclusions

Using a combination of mathematical modelling and statistical analyses, we sought to better understand the within population transmission dynamics of leptospirosis in Norway rats. Environmental transmission is an important route of infection for Norway rats, as evidenced by the theoretical result (chapter 3) and empirical analysis (chapter 4). The analysis in chapter 6 provides insights into rodent control, using modelling approaches and intervention data.

The next steps for better understanding and also modelling infection dynamics in Norway rats rely on data. One critical assumption of the models presented in this framework is that once infected, rats are infected for their entire lifetime, and throughout their lifetime rats shed leptospires at a constant rate. Whereas it is accepted that rats when serving as reservoirs are infected for their lifetime (Bharti et al., 2003; Ellis, 2015), it is unknown whether they shed fewer leptospires as time

since infection increases. Given that contact with contaminated environment is an important route for human infection, an informed shedding rate of infected rats is needed to predict risk to humans. There are data available on the *Leptospira* load in the urine of Salvador's rats (Costa et al., 2015); it would be of interest to use these data to test the hypothesis that shedding rate remains constant over lifetime.

The evidence of multiple transmission routes was found using data on leptospires present in the urine of captured rats, i.e. chronically infected animals. Presence of leptospires in blood and internal organs (though not the kidney) indicate a recent infection, less than 10 days (Ellis, 2015). Recently, data have been collected from the field on the status of liver infection for a few animals captured in Pau da Lima which could be used to identify recent infection. Also, those animals positive for urine and liver could provide evidence for reinfection which should be coupled with a shedding rate analysis.

Transmission and population dynamics within the zoonotic reservoir is just one component of a much larger framework to investigate emergence of zoonoses (Wood et al., 2012). Annual outbreaks of human leptospirosis occur amongst the residents of the urban slums of Salvador. Through living in a shared environment, humans acquire leptospire infection from water sources the contaminated with rodent urine. The work presented in this thesis aids in understanding the infection dynamics and control of wild Norway rats.

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Appendix 1

Proof of the statement $R_0^I = 1 \iff R_0^{II} = 1$

If $R_0^I = 1$ then $R_{v_1} + R_{v_2} + R_{v_3} = 1$, therefore we can write $R_{v_1} + R_{v_2} = 1 - R_{v_3}$.

Substituting this expression into R_0^{II} gives,

$$R_0^{II} = \frac{1}{2}\left(1 - R_{v_3} + \sqrt{4R_{v_3} + \left(1 - R_{v_3}\right)^2}\right)$$

$$= \frac{1}{2}\left(1 - R_{v_3} + \sqrt{\left(1 + R_{v_3}\right)^2}\right)$$

$$= 1.$$

Conversely, if $R_0^{II} = 1$ then $\frac{1}{2}\left(R_{v_1} + R_{v_2} + \sqrt{4R_{v_3} + (R_{v_1} + R_{v_2})^2}\right) = 1$, and we can write $R_{v_3} = \frac{\left(2 - \left(R_{v_1} + R_{v_2}\right)\right)^2 - (R_{v_1} + R_{v_2})^2}{4}$. Substituting this expression into R_0^I gives,

$$R_0^I = R_{v_1} + R_{v_2} + \frac{\left(2 - \left(R_{v_1} + R_{v_2}\right)\right)^2 - \left(R_{v_1} + R_{v_2}\right)^2}{4}$$

$$= R_{v_1} + R_{v_2} + \frac{\left(4 - 4\left(R_{v_1} + R_{v_2}\right)\right)}{4}$$

$$= 1.$$

■

Calculating valley level shedding rate

For shedding rate, data were available of the results of urine qPCR of 362 infected animals and the valley in which they were trapped. The log of the media geq was approximately normally distributed so a linear model was used to test if there was a difference in level of log of the media geq by valley.

There was no difference in mean level of log media geq between valley 1 and valley 2 (p=0.1805), and between valley 2 and valley 4 (difference=0.4931, std. error=0.5764, p=0.393). Valley 4 had a lower mean level of log media geq than valley 1 (p=0.0347).

Table 1: Summary of log linear model fit of shedding data.

	Estimate	**Std. Error**	**t value**	**Pr(>|t|)**
(Intercept)	12.34	0.54	23.07	<2e-16
Valley 2	-0.91	0.68	-1.34	0.18
Valley 4	-1.41	0.66	-2.12	0.03

Table 2: The predicted mean with 95% confidence interval back transformed from the log scale (to 1 sf).

Valley	Mean (95 % confidence interval)
1	2×10^5 (8×10^4, 7×10^5)
2	9×10^4 (4×10^4, 2×10^5)
4	6×10^4 (3×10^4, 1×10^5)

Appendix 2

Survival model

If the probability of not yet being infected is modelled using the survival function, then,

$$P(Y_i = 0) = 1 - F(t_i)$$

$$\log\big(P(Y_i = 0)\big) = \log\left(\exp(-\left(\frac{t_i}{\phi}\right)^{\kappa}\right)$$

$$-\log\big(P(Y_i = 0)\big) = \left(\frac{t_i}{\phi}\right)^{\kappa}$$

$$\log\big(-\log\big(P(Y_i = 0)\big)\big) = \kappa\log(t_i) - \kappa\log(\phi)$$

if we chose to model the scale parameter as log linear, then $\log(\phi) = X\boldsymbol{\beta}$ and so,

$$\log\big(-\log\big(P(Y_i = 0)\big)\big) = \kappa\log(t_i) - \kappa X\boldsymbol{\beta}.$$

Then we can estimate coefficients $\boldsymbol{\beta}$ by maximising the likelihood function,

$$L(\beta|x_i) = \prod_{i=1}^{n} p_i^{y_i}(1 - p_i)^{1-y_i}$$

where $p_i$is the probability of already being infected, with

$p_i = 1 - \exp(-\exp(\kappa\log(t_i) - \kappa X\boldsymbol{\beta}))$.

The delta method (Oehlert, 1992) was used to find the standard errors of the Weibull cumulative distribution function (cdf) $F(t, X; \kappa, \boldsymbol{\beta}) = 1 - \exp(-(t/\phi)^{\kappa})$. The variance matrix of the Weibull cdf is,

$$Var\big(F(t, X; \kappa, \boldsymbol{\beta})\big) \approx \nabla F(t, X; \kappa, \boldsymbol{\beta})^T . Cov(X) . \nabla F(t, X; \kappa, \boldsymbol{\beta}).$$

Where $\nabla F(t, X; \kappa, \boldsymbol{\beta})$ is the vector of partial derivatives of $F(t, X; \kappa, \boldsymbol{\beta})$ with respect to the model parameters and $Cov(t, X)$ is the covariance matrix. The covariance matrix was estimated by numerical approximation of the hessian matrix.

Rewrite the cdf as $F(t, X; \kappa, \boldsymbol{\beta}) = 1 - \exp\left(-\left(t\exp(-\eta)\right)^{\kappa}\right)$ with $\eta = X\boldsymbol{\beta}$. In the final model $\eta = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_4 x_1 x_2$. As the shape parameter is strictly positive, we specify the shape parameter as $\kappa = \exp(\kappa^*)$, hence our covariance matrix is for the parameter κ^*. We must calculate the standard errors of $F(t, X; \kappa, \boldsymbol{\beta})$ with respect to κ^*. The partial derivatives were,

$$\frac{\partial F(t,X;\kappa,\boldsymbol{\beta})}{\partial \kappa^*} = \log\lambda \,.\, \lambda^{\exp(\kappa^*)} \exp\left(-\lambda^{\exp(\kappa^*)}\right) \exp(\kappa^*) \quad \text{where} \quad \lambda = t\exp(-\eta)$$

$$\frac{\partial F(t, X; \kappa, \boldsymbol{\beta})}{\partial \beta_0} = \kappa\, t^{\kappa} \exp(-\eta\kappa) \exp\left(-t^{\kappa} \exp(-\eta\kappa)\right)$$

$$\frac{\partial F(t, X; \kappa, \boldsymbol{\beta})}{\partial \beta_1} = \kappa x_1\, t^{\kappa} \exp(-\eta\kappa) \exp\left(-t^{\kappa} \exp(-\eta\kappa)\right)$$

$$\frac{\partial F(t, X; \kappa, \boldsymbol{\beta})}{\partial \beta_2} = \kappa x_2\, t^{\kappa} \exp(-\eta\kappa) \exp\left(-t^{\kappa} \exp(-\eta\kappa)\right)$$

$$\frac{\partial F(t, X; \kappa, \boldsymbol{\beta})}{\partial \beta_3} = \kappa x_3\, t^{\kappa} \exp(-\eta\kappa) \exp\left(-t^{\kappa} \exp(-\eta\kappa)\right)$$

$$\frac{\partial F(t, X; \kappa, \boldsymbol{\beta})}{\partial \beta_4} = \kappa x_4\, t^{\kappa} \exp(-\eta\kappa) \exp\left(-t^{\kappa} \exp(-\eta\kappa)\right)$$

Then the standard errors of $F(t, X; \kappa, \boldsymbol{\beta})$are obtained by taking the square root of $Var\left(F(t, X; \kappa, \boldsymbol{\beta})\right)$.

Appendix 3

Optimal control theory

Consider a system with state variable $x(t)$ with a time dependent control $u(t)$. The objective functional J contains the problem which we wish to minimise, usually a function of the control and the state variable. To find the optimal control we apply Pontryagin's Maximum Principle.

Pontryagin's Maximum Principle (Sharomi & Malik, 2015). If $u^*(t)$ and $x^*(t)$ are optimal for the problem

$$\max_u J[x(t), u(t)], \text{ where } J[x(t), u(t)] = \max_u \int_{t_0}^{t_f} f(t, x(t), u(t))\, dt\,,$$

subject to $\begin{cases} \frac{dx}{dt} = g\big(t, x(t), u(t)\big) \\ x(t_0) = x_0, \end{cases}$

then there exists a piecewise differentiable adjoint variable $\lambda(t)$ such that

$$\mathcal{H}(t, x^*(t), u(t), \lambda(t)) \leq \mathcal{H}\big(t, x^*(t), u^*(t), \lambda(t)\big)$$

for all controls u at each time t, where the Hamiltonian$\mathcal{H}$ is given by

$$\mathcal{H}\big(t, x(t), u(t), \lambda(t)\big) = f(t, x(t), u(t)) + \lambda(t) g\big(t, x(t), u(t)\big)$$

and

$$\begin{cases} \lambda'(t) = -\dfrac{\partial \mathcal{H}\big(t, x^*(t), u^*(t), \lambda(t)\big)}{\partial x} \\ \lambda\big(t_f\big) = 0. \end{cases}$$

The theorem finds optimal controls by maximising the Hamiltonian with respect to u at u^*. If we wish to minimise the Hamiltonian, then by the Arrow Sufficiency Theorem (Sharomi & Malik, 2015) the Hamiltonian must convex with respect to the

state variables. The method is easily extended for multiple state variables $(x_1(t), x_2(t))$ and controls $(u_1(t), u_2(t))$ by introducing an adjoint equation for each state variable and adding $\sum_{i=1}^{n} \lambda_i(t) g_i\big(t, x(t), u(t)\big)$ to the Hamiltonian.

Details of optimal control problem

For the objective functional in equation 6.15 and state variables in equations 6.8-6.14 the Hamiltonian, $\mathcal{H}$ is given by,

$$
\begin{aligned}
\mathcal{H} &= c_1 H(t) + \frac{c_2}{2}\tau(t)^2 + \frac{c_3}{2}u(t)^2 \\
&+\lambda_{J_X}\left[b(1-u(t))\,(A_X + (1-\upsilon_1)A_Y)\left(\frac{k-(W+A)}{k}\right) - \varphi_J J_X - m_J J_X\right] \\
&+\lambda_{J_Y}\left[b(1-u(t))A_Y\upsilon_1\left(\frac{k-(W+A)}{k}\right) - \varphi_J J_Y - m_J J_Y\right] \\
&+\lambda_{W_X}\left[\varphi_J J_X - \upsilon_3 W_X L - \varphi_W W_X - m_W W_X - p\tau(t)W_X\frac{W_X}{W_X+W_Y+A_X+A_Y}\right] \\
&+\lambda_{W_Y}\left[\varphi_J J_Y + \upsilon_3 W_X L - \varphi_W W_Y - m_W W_Y - p\tau(t)\frac{W_Y}{W_X+W_Y+A_X+A_Y}\right] \\
&+\lambda_{A_X}\left[\varphi_W W_X - \upsilon_3 A_X L - m_A A_X - p\tau(t)A_X\frac{A_X}{W_X+W_Y+A_X+A_Y}\right] \\
&+\lambda_{A_Y}\left[\varphi_W W_Y + \upsilon_3 A_X L - m_A A_Y - p\tau(t)A_Y\frac{A_Y}{W_X+W_Y+A_X+A_Y}\right] \\
&+\lambda_L[\lambda_W W_Y + \lambda_A A_Y - \mu L].
\end{aligned}
\tag{1}
$$

The adjoint equations satisfy $\frac{d\lambda_{J_X}}{dt} = -\frac{\partial \mathcal{H}}{\partial J_X}, \ldots, \frac{d\lambda_L}{dt} = -\frac{\partial \mathcal{H}}{\partial L}$ with final time conditions $\lambda_{J_X}(t_f) = 0, \lambda_{J_Y}(t_f) = 0, \lambda_{W_X}(t_f) = 0, \lambda_{W_Y}(t_f) = 0, \lambda_{A_X}(t_f) = 0, \lambda_{A_Y}(t_f) = 0, \lambda_L(t_f) = 0$. The adjoint equations are,

$$\frac{d\lambda_{J_X}}{dt} = -c_1 + \lambda_{J_X}(\varphi_J + m_J) - \lambda_{W_X}\varphi_J \tag{2}$$

$$\frac{d\lambda_{J_Y}}{dt} = -c_1 + \lambda_{J_Y}(\varphi_J + m_J) - \lambda_{W_Y}\varphi_J \tag{3}$$

$$\begin{aligned}\frac{d\lambda_{W_X}}{dt} = {} & -c_1 + \lambda_{J_X}\frac{\big(b(1-u)(A_X + (1-v_1)A_Y)\big)}{k} \\ & + \lambda_{J_Y}\frac{b(1-u)A_Y v_1}{k} \\ & + \lambda_{W_X}\left(v_3 L + \varphi_W + m_W + p\tau\frac{W_X(W_X + 2(W_Y + A_X + A_Y))}{(W_X + W_Y + A_X + A_Y)^2}\right) \\ & - \lambda_{W_Y}\left(v_3 L + p\tau W_Y\frac{W_Y}{(W_X + W_Y + A_X + A_Y)^2}\right) \\ & - \lambda_{A_X}\left(\varphi_W + p\tau A_X\frac{A_X}{(W_X + W_Y + A_X + A_Y)^2}\right) \\ & - \lambda_{A_Y} p\tau A_Y\frac{A_Y}{(W_X + W_Y + A_X + A_Y)^2}\end{aligned} \tag{4}$$

$$\begin{aligned}\frac{d\lambda_{W_Y}}{dt} = {} & -c_1 + \lambda_{J_X}\frac{\big(b(1-u)(A_X + (1-v_1)A_Y)\big)}{k} \\ & + \lambda_{J_Y}\frac{b(1-u)A_Y v_1}{k} - \lambda_{W_X} p\tau W_X\frac{W_X}{(W_X + W_Y + A_X + A_Y)^2} \\ & + \lambda_{W_Y}\left(\varphi_W + m_W + p\tau\frac{W_Y(W_Y + 2(W_X + A_X + A_Y))}{(W_X + W_Y + A_X + A_Y)^2}\right) \\ & - \lambda_{A_X}\, p\tau A_X\frac{A_X}{(W_X + W_Y + A_X + A_Y)^2} \\ & - \lambda_{A_Y}\left(\varphi_W + p\tau A_Y\frac{A_Y}{(W_X + W_Y + A_X + A_Y)^2}\right) - \lambda_L\lambda_W\end{aligned} \tag{5}$$

$$\begin{aligned}\frac{d\lambda_{A_X}}{dt} = {} & -c_1 + \lambda_{J_X}\left(\frac{b(1-u)(2A_X - k - A_Y(-2 + v_1) + W_X + W_Y)}{k}\right) \\ & + \lambda_{J_Y}\left(\frac{b(1-u)A_Y v_1}{k}\right) - \lambda_{W_X} p\tau W_X\frac{W_X}{(W_X + W_Y + A_X + A_Y)^2}\end{aligned} \tag{6}$$

$$-\lambda_{W_Y} p\tau W_Y \frac{W_Y}{(W_X + W_Y + A_X + A_Y)^2}$$

$$+\lambda_{A_X}\left(v_3 L + m_A + p\alpha \frac{A_X\big(A_X + 2(W_X + W_Y + A_Y)\big)}{(W_X + W_Y + A_X + A_Y)^2}\right)$$

$$-\lambda_{A_Y}\left(v_3 L + p\tau A_Y \frac{A_Y}{(W_X + W_Y + A_X + A_Y)^2}\right)$$

$$\frac{d\lambda_{A_Y}}{dt} = -c_1 \tag{7}$$

$$+\lambda_{J_X} \frac{\Big(b(1-u)\big(-(A_X(-2+v_1) - (-1+v_1)(-k + 2A_Y + W_X + W_Y)\big)\Big)}{k}$$

$$+\lambda_{J_Y} \frac{bv_1(1-u)(-k + (A_X + 2A_Y + W_X + W_Y)}{k}$$

$$-\lambda_{W_X} p\tau W_X \frac{W_X}{(W_X + W_Y + A_X + A_Y)^2}$$

$$-\lambda_{W_Y} p\tau W_Y \frac{W_Y}{(W_X + W_Y + A_X + A_Y)^2}$$

$$-\lambda_{A_X}\, p\tau A_X \frac{A_X}{(W_X + W_Y + A_X + A_Y)^2}$$

$$+\lambda_{A_Y}\left(m_A + p\tau \frac{A_Y\big(A_Y + 2(W_X + W_Y + A_X)\big)}{(W_X + W_Y + A_X + A_Y)^2}\right) - \lambda_L \lambda_A$$

$$\frac{d\lambda_L}{dt} = \lambda_{W_X} v_3 W_X - \lambda_{W_Y} v_3 W_X + \lambda_{A_X} v_3 A_X - \lambda_{A_Y} v_3 A_X + \lambda_L \mu \tag{8}$$

The characterisations of the optimal controls in equations 6.16 and 6.17 are based on,

$$\frac{\partial \mathcal{H}}{\partial u} = c_3 u(t) - \lambda_{J_X} b(A_X + (1 - v_1)A_Y)\left(\frac{k - (W + A)}{k}\right) \tag{9}$$
$$-\lambda_{J_Y} bA_Y v_1 \left(\frac{k - (W + A)}{k}\right)$$

$$\frac{\partial \mathcal{H}}{\partial \tau} = c_2 \tau(t) - \lambda_{W_X} pW_X \frac{W_X}{W_X + W_Y + A_X + A_Y} \tag{10}$$

$$-\lambda_{W_Y} p W_Y \frac{W_Y}{W_X + W_Y + A_X + A_Y}$$

$$-\lambda_{A_X} p A_X \frac{A_X}{W_X + W_Y + A_X + A_Y}$$

$$-\lambda_{A_Y} p A_Y \frac{A_Y}{W_X + W_Y + A_X + A_Y}.$$

Appendix 4

Urban Ecosyst
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A comparative assessment of track plates to quantify fine scale variations in the relative abundance of Norway rats in urban slums

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Abstract Norway rats (*Rattus norvegicus*) living in urban environments are a critical public health and economic problem, particularly in urban slums where residents are at a higher risk for rat borne diseases, yet convenient methods to quantitatively assess population sizes are lacking. We evaluated track plates as a method to determine rat distribution and relative abundance in a complex urban slum environment by correlating the presence and intensity of rat-specific marks on track plates with findings from rat infestation surveys and trapping of rats to population exhaustion. To integrate the zero-inflated track plate data we developed a two-component mixture model with one binary and one censored continuous component. Track plate mark-intensity was highly correlated with signs of rodent infestation (all coefficients between 0.61 and 0.79 and all *p*-values < 0.05). Moreover, the mean level of pre-trapping rat-mark intensity on plates was significantly associated with the number of rats captured subsequently (Odds ratio1.38;

Kathryn P. Hacker and Amanda Minter contributed equally to this work.

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95 % CI 1.19–1.61) and declined significantly following trapping (Odds ratio 0.86; 95 % CI 0.78–0.95). Track plates provided robust proxy measurements of rat abundance and distribution and detected rat presence even when populations appeared 'trapped out'. Tracking plates are relatively easy and inexpensive methods that can be used to intensively sample settings such as urban slums, where traditional trapping or mark-recapture studies are impossible to implement, and therefore the results can inform and assess the impact of targeted urban rodent control campaigns.

Keywords Indirect abundance · *Rattus norvegicus* · Track plates · Urban slum · Zero-inflated · Zoonotic diseases

Introduction

Rodents are a major public health and economic concern in urban environments and are capable of harboring a variety of helminthic, viral, and bacterial zoonotic pathogens (Costa et al. 2014a, 2015; Davis et al. 2005; Himsworth et al. 2013b; Krojgaard et al. 2009; Mills and Childs 1998). The rapid growth of slum settlements globally has created new and expanding habitats for rats, most notably the Norway rat, *Rattus norvegicus*, and has increased the burden of some rat-borne diseases in these vulnerable areas (Cavia et al. 2009; Himsworth et al. 2013b; Mills and Childs 1998). Norway rats thrive in peri-urban and urban environments and are therefore of particular importance for slum communities. For example, the Norway rat is a major reservoir host for *Leptospira* spp. that causes leptospirosis in the urban slum setting (Barocchi et al. 2001; Costa et al. 2014a, b, 2015; Ko et al. 1999, 2009). In Brazil alone, this zoonotic disease is responsible for more than 10,000 cases reported annually (Brazil 2005; Maskey et al. 2006) and causes seasonal outbreaks in slum settlements (Flannery et al. 2001; Ko et al. 1999; Sarkar et al. 2002).

Despite the importance of urban rats, little is known about their distribution, abundance and demography, especially in tropical urban settings, and the contribution of these factors to zoonotic disease transmission. Epidemiological studies in Brazil found that leptospirosis is associated with rodent infestation in peri-domicilary areas within slum (*favela*) communities (Maciel et al. 2008; Reis et al. 2008b; Sarkar et al. 2002). Residents of these areas who reported seeing five or more rodents in their household environment were at significantly higher risk of leptospirosis (Reis et al. 2008a; Sarkar et al. 2002), as were residents residing in un-plastered homes where rat feces and burrows were present (Costa et al. 2014b). Although ecological factors associated with the rat population likely play a major role in pathogen maintenance and prevalence of leptospiral infection (Costa et al. 2014a, b), the dynamics of rat abundance across space and time are not well-delineated, which in turn has hampered identification of effective rodent control strategies in complex urban settings.

A critical barrier in assessing the role of urban rat population dynamics on the transmission of leptospires, or any other rat-borne pathogen, is the lack of reliable measures of rodent abundance. Based on rat trapping results, the size of populations can be estimated (Brown 1969; Mills et al. 1995; Sheppe 1967); however, trapping of animals to obtain these metrics is time, cost, and labor intensive (Emlen et al. 1949). Absolute rat abundance, the exact enumeration of all individuals in a given area, is rarely possible (Davis 1953; Davis et al. 1948). The few estimates available depend on capture-

mark-recapture methods [e.g. (Glass et al. 1988)]. However trap shyness and trap habituation can alter rat behavior and confound the accurate interpretation of abundance obtained from these studies (Emlen et al. 1949; Leslie and Davis 1939). Other indices of relative abundance, such as catch per unit effort or trap success, have frequently been used as indices of rat abundance (Glass et al. 1988; Himsworth et al. 2013a; Lord et al. 1971; Villafane et al. 2013), but results from these studies are subject to the same limitations imposed by variability in rat behavior and can generate inconsistent results (McKelvey and Pearson 2001). Additionally, fine scale sampling at spatial resolutions of <10 m between placements of non-kill traps requires a large number of often expensive traps, and trap loss when sampling within high-density urban environments is a major concern. As alternatives, a number of proxies of abundance have been used, that can give estimates of relative abundance, that is, estimates that are related to absolute abundance by an unknown multiplier, but can be used for comparative purposes.

Simpler and more easily applied methodologies (such as systematic surveys for signs of rodent infestation and use of track plates) have been recommended for urban areas to evaluate the effectiveness of control programs (CDC 2006). Surveys of signs of rodent infestation, such as rodent runs, feces, and burrows, are widely used (de Masi et al. 2009; Glass et al. 1997; Lambropoulos et al. 1999). However, these require systematic training of personnel, and the quality and consistency of results are subject to variation due to the reliance on independent surveyors and their level of experience (Allen and Engeman 2014; Lord 1983; Whisson et al. 2005). Additionally, attempts to associate rodent-survey results with measures of absolute abundance have failed to obtain conclusive results (Lord 1983). Track plates, where rodent tracks and markings are recorded on plates covered in ink or powder, can provide measures of rodent mark intensity and relative abundance and present an attractive alternative to trapping and surveying techniques (Connors et al. 2005; Lord 1983; Lord et al. 1971; Quy et al. 1994; Sheppe 1965).

Track plate methods have been used successfully to quantify reductions in Norway rat mark intensity following a trapping campaign on farms (Quy et al. 1994), and to monitor seasonal abundance of rats in one urban area (Promkerd et al. 2008). However, previous studies using track plates reported only rodent paw print marks (Brown 1969; Connors et al. 2005; Glennon et al. 2002; Nams and Gillis 2003; Quy et al. 1993, 1994) and evaluated changes in rat populations at large spatial scales, which limited the applicability of these findings to local population dynamics and rodent control. Methodological limitations in quantification (i.e. accurately identifying rat signs and censoring unspecific signs) and in analysis of the data have precluded track plate applications to monitoring rat population changes at finer geographical scales. Particularly, tailored statistical analyses are necessary not only to accommodate zero-inflated data, a common output of these studies, but also to account for varying rodent mark intensity over short distances (Himsworth et al. 2014).

As a first step towards densely sampling rat abundance throughout an urban slum, we developed a track plate method and statistical framework to predict both the probability of rat presence and rat intensity as a proxy for rat abundance in an urban slum community in Salvador, Brazil. Our model captured the variability of rodent relative abundance throughout an urban slum community and identified hot spots of rodent infestation. The results of this study can be used to improve targeted rodent control interventions and further elucidate Norway rat population dynamics within urban slums at fine spatial resolution.

Methods and preliminary results

Results obtained from initial studies designed simply to refine methodologies are reported in the Online Resources and noted in the Methods sections below, rather than being reported in the Results.

Study area

The city of Salvador, with 2.7 million inhabitants, is located on the northeast coast of Brazil (12° 55′ 34″ southern latitude and 38° 31′ 12″ western longitude) and is the third largest city in the country. Salvador has a subtropical climate and temperatures are relatively constant across the year. During the wet season from April-July heavy rainfall is common (mean 272.2 mm/mo) while during the relatively dry season from September - December rainfall is much reduced (mean 124.2 mm/mo).

This study was performed in the slum community of Pau da Lima, which has been described in detail previously (Reis et al. 2008a, b). Briefly, the area comprises a series of valleys and has a high human population density. Community members are mainly squatters (88 %) with low level of education (66 % did not finish primary school) and low income (mean per capita daily household income, US$ 2.60) (Riley et al. 2007). Lack of structural planning (Fig. 1a) and the lack of basic sanitation (e.g. open sewers) and trash collection are characteristics of this community (Fig. 1b).

Assessing the types of rat markings on track plates

Given the lack of previous comparative studies, three types of weather resistant track plate methods were evaluated for use in urban slum environments: lampblack and methyl alcohol (Sherpherd and Greaves 1984), equal parts of black ink and canola oil (Lord et al. 1971), and a graphite mixture (Connors et al. 2005). The lampblack and ink solutions were applied to 0.2 m by 0.2 m polyvinyl floor tiles using a paint roller; the graphite solution was applied to an acetate sheet. We assessed the time each mixture took to dry and the homogeneity of the paint coverage on the tiles or acetate sheets. We evaluated the mixtures' ability to resist water by pouring 100 mL of water over the dried plates as well as during different levels of rainfall when set outdoors. The lampblack plates performed best in the prevailing conditions. These plates dried more rapidly (<5 min) compared to ink/oil and graphite (15 min and 24hs, respectively). Additionally, lampblack application produced a uniform, homogeneous dark surface cover, which generated clearly defined rat-specific marks.

As previous studies documented only rodent paw prints and rats leave additional specific marks, such as tail slides and scratches (CDC 2006), we assessed our ability to detect multiple types of marks left by rats in slum areas. In a single household with known high levels of rat infestation, eight sausage-baited tomahawk traps were left overnight with two track plates placed inside the traps and a single track plate placed at the entrance of each trap. The traps were either 'exposed' or 'shielded' from rats. Four traps were baited and wired open (exposed), two traps were set to catch rats (exposed), and two traps were baited, but wired closed (shielded). Eighty three percent (10/12) of all track plates in the baited and wired open group showed evidence of rat marks, and plates within the two triggered traps (traps not wired open) were marked and successfully captured Norway rats. The shielded track plates remained unmarked. Based on this trial, we identified three specific types of rat marks: rat paw prints

Fig. 1 The densely populated urban slum dwellers of Pau da Lima in Salvador, Brazil, reside in a heterogeneous landscape demarcated by steep slopes and valleys (panel 1**a**). Track plates were placed near the valley floor where open sewers (1**b**), poorly constructed houses and limited sanitation services provide abundant resources for Norway rats (1**c**). Rat signs, including paw prints, tail slides and scratching are readily visible on a track plate (1**d**) and were scored for activity/abundance metrics

(Fig. 1c and d, Online Resource Fig. 1a), tail marks (Fig. 1c and d, Online Resource Fig. 1b) and rat scratches (Online Resource Fig. 1c), and subsequently, all track plates with these marks were considered positive for rat markings.

Scoring track plates and sampling design

Prior to assessing the effects of rat removal on track plate mark intensity, we developed methods to score track plates in field conditions. We used a binary variable (presence/absence of rat marks on a plate) and a continuous variable (the intensity of marks on plates) to score track plates. A total of 100 track plates were placed in 10 houses (10 track plates per house). Five of the houses had high rodent infestation and 5 had low levels of rodent infestation. Track plates were placed for three nights in houses with low rat infestation or four nights in houses

with high rat infestation in areas likely to be frequented by rats (along rat runs, against walls, and near food or water resources).

Track plates were collected the morning after placement, identified by location site, and photographed. After imposing a 5x5 grid over the photographs, two independent reviewers assessed the presence of marks on the plate and scored each grid for rat-specific marks, providing 25 data points per plate. Initially, training was performed among three reviewers to ensure accurate identification of rat signs and non-specific markings. When discrepancies were found (greater than 3 score difference between reviewers) both track readers reviewed the plates and reached a consensus, which improved the concordance of future scoring. The level of agreement between the results obtained by the two trained reviewers was assessed using the kappa method (Landis and Koch 1977) and agreement was excellent (kappa = 0.90). We additionally assessed the correlation between the two scorers ($r^2 = 0.75$, $p < 0.0001$) and found very high agreement. Marks other than those of rats (chickens, dogs, opossums and unknown blowing debris) were observed on the plates, Plates were censored when >70 % of the area either within the grid or the plate was unreadable for specific rat-marks (ie the lampblack ink was completely wiped off, and no marks were distinguishable) (Online Resource Fig 2).

To evaluate the number of track plates and the number of nights required to accurately capture variability in rat activity between slum households, we varied the number of tracking boards and number of days placed. Boards were placed for 1, 3, 4, 5, and 7 days, and we placed plates in groups of 3, 5, 7, and 10 boards per house. The general aim was to select a combination of number of nights and number of track plates that provided an acceptably low standard deviation across the plates, while taking into consideration the effort for data collection. The methodology and results of this are summarized in Online Resource Figure 3. Based on these results, and to allow field teams to examine fine scale rodent activity per house and still cover large areas in a time- and resource-efficient manner, we opted to place 5 track plates per house for 3 days.

Track plates as a proxy for the number of rats captured and their association with rodent infestation

We examined the relationship between the number of rats captured in a trapping campaign designed to 'exhaust' the population (remove the local rat population to estimate population size by the catch per unit method: CPUE (Efford 2004)) and the track plate scores around households before and after that campaign. We used data from previous rat studies to inform our sampling scheme and identify areas where rats were previously captured to calibrate the model (Costa et al. 2014a; Porter et al. 2015). Initially, 40 sites (locations at least 15 m apart) within the study area were selected as potential trapping sites by spatial randomization incorporating spatial heterogeneity throughout Pau da Lima, and of these, 10 sites were chosen with the highest rat-capture success from previous sampling. Within each site, three randomly selected households (a triad; total of 30 households) were identified, creating an effective sampling area of 450 m.

Five plates were placed within15m of each household for three days during each of four tracking board periods: periods 1 and 2 before the trapping period (days 1–3 and 20–22, respectively) and periods 3 and 4 after trapping (days 34–36 and 61–63), giving a potential total of 1200 track-plate nights (Fig. 2). Results were not significantly different between periods 1 and 2, so results were pooled for analyses. Following the pre-trapping period two households of a triad were selected as "trapped households" ($n = 20$), where both track plates

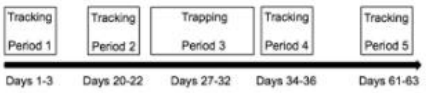

Fig. 2 Timeline of the track plate and trapping experiment conducted in Pau da Lima

and traps were placed. In the third house at each site ($n = 10$) only track plates were set. These "nearby" houses enabled us to observe the impact of the trapping in the immediate neighborhood. Track plates were evaluated each morning and replaced whenever marks were present. Rodent trapping was performed at trapped households by setting two tomahawk traps per house for 6 days (days 27–32, a total of 240 trap-nights; Fig. 2) and the number of rats caught per household was recorded. Each morning, traps containing rats were removed and new traps were set. No pre-baiting of the traps was utilized.

The CPUE was computed across the 6 days of trapping to evaluate whether we exhausted the rat population within the study area (CPUE reached an asymptote). The cumulative CPUE was calculated by dividing the cumulative number of captured rats by the cumulative sampling effort. The sampling effort each day was computed as the number of traps containing rats or not sprung plus half the number of traps that were found to be sprung but empty (Borchers et al. 2002).

Simultaneously with tracking period 1, an interview with the head of the household and a visual survey of rodent signs were performed. During the interview, data on the number of rat sightings at night during the last month were collected (Reis et al. 2008b). The visual survey was performed by a trained team from the Zoonosis Control Center (Salvador Municipal Secretary of Health), using a form previously adapted from the Centers for Disease Control for urban settings (CDC 2006), to record peri-domestic rat signs (rat burrows, trails and feces). Details of this form were previously described in Costa et al. 2014a, 2014b, Table S1. All individuals who participated in rodent surveys were considered experts in rodenticide campaigns and had extensive experience in performing infestation surveys. Rat burrows were identified as fist sized holes with signs of rodent activity (typically with defined trails leading from the entrances and no rubbish or cobwebs present at entrance). Trails were identified as well worn areas at least 10 cm wide, typically leading from burrows or other foraging sites. Feces were identified according to CDC recommendations, and when possible the species were identified as *Rattus norvegicus* or *Rattus rattus* (CDC 2006). We evaluated the association between track plate scores and other signs of rodent infestation using a correlation matrix for a subset of 13 households where trapping and extensive rodent surveys took place. Additionally, since the variables were evaluated on different scales, we performed a Principal Components Analyses (PCA) using the correlation matrix rather than the covariance matrix.

Development of a statistical method to analyze track plate data

The distribution of grid-score data was zero-inflated and heavily right-skewed (Fig. 3) and we were not able to apply a linear modeling approach incorporating random effects as previously described (Whisson et al. 2005). As previously described methodologies limit the use of spiked zero data to binomial analysis (Welsh et al. 1996), we developed a mixed model that separately assessed the probability of the presence of rats (dichotomous) and intensity levels (continuous) of rat markings (for full details of the model see Online Resource 1). In brief, our model considered the probability of the presence or absence of a rat (θ and $1-\theta$ in Online

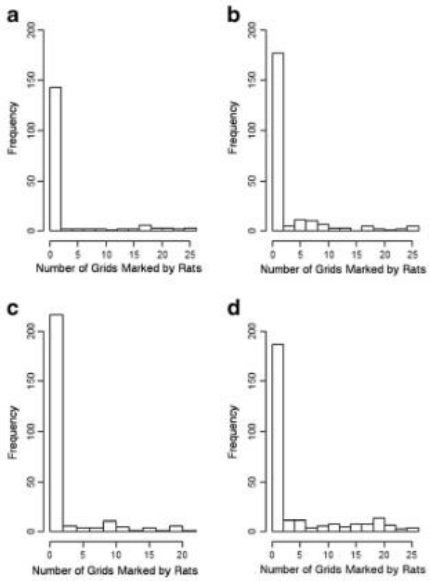

Fig. 3 Histograms of track plate mark intensity, recorded as a score out of 25 (number of grids potentially marked by rats) for each of the four sampling periods; **a**) details the distribution of track plate intensity for tracking period 1, **b**) details the distribution of track plate intensity for tracking period 2, **c**) details the distribution of the track plate intensity for period 4, and d) details the distribution of the track plate intensity for period 5. The frequencies across the time periods are unequal due to missing data outlined in the results

Resource 1) and a measure of the intensity of rat markings (λ; the proportion of the plate with marked cells) as an interval-censored underlying continuous measure. For example, a plate with 5 out of 25 marked cells (observed proportion 0.20) was assigned to an underlying continuous score binned to the interval of 0.18 to 0.22. The model also weighted observations by the number of censored cells, so that a plate with 25 readable grids would be assigned a higher weight than a plate scored out of only 20 grids where 5 grids were censored. The variation in the underlying continuous score was considered to have a gamma distribution as it proved to be sufficiently flexible to fit our data, but, in principle, any continuous distribution could be used.

Lamda (λ) and θ were modeled as logistic and log-linear regressions, respectively. Maximum likelihood methods were used to estimate the effect on each of total trap counts at the house level and observation period. As the number of track plates (and hence the number of track-plate nights of data) varied to some degree, a model was developed taking into account site as a ten-level factor (see above), trap count per house, and an interaction between the trap count and the observation period (a three-level factor: periods 1 and 2 combined, 4, and 5). The presence or absence of rats and levels of rat intensity were assumed to be measures of the same underlying phenomenon, and so the same parameterization was used for both. The analysis was implemented using R: an R package *zig* is available from the authors to perform parameter estimation using the zero inflated gamma model.

Results

Association between tracking plates, rodent surveys, household rat-sightings, and trapping

Track plate scores were highly correlated with the number of rats captured (0.77, $p = 0.002$) and visual signs of rodent infestation (correlation 0.83, $p < 0.001$ with any rat sign) and showed strong associations with independent measures of the presence of feces, burrows, and trails (all coefficients between 0.61 and 0.79 and all p-values < 0.05; see Online Resource 2 Table 1). The highest association between plates and rat signs were the presence of rat trails ($r = 0.79$, $p = 0.001$). Track plate scores were less strongly correlated with rat sightings ($r = 0.38$, $p = 0.2$). However, rat sightings did not correlate well with any signs of rodent infestation or number of rats captured (r between 0.18 and 0.38 and all p-values > 0.05).

In the PCA that evaluated the association of track plate scores, signs of rodent infestation, and trapping results in period 1 of the experiment, the first principal component explained 60 % of the variation. All loadings in this component had the same sign. All variables contributed similarly to the variation (loadings between 0.40 and 0.49) except for the variable of reported rat sightings (loading of 0.23), indicating that track plate scores were positively associated with all other indicators of rodent infestation, but the association was weakest with reported rat sightings (Online Resource 2 Table 2). There were no substantial trends in the remaining components, which accounted for the remaining variation (40 %).

Track plates as a proxy for the number of rats captured

By the end of the trapping period, a total of 18 rats were captured in 10 out of the 20 houses sampled. The CPUE approached an asymptote by day 4, indicating that the rat population was trapped out (Fig. 4a). Overall, we collected information from 88.5 % of track plates. The remaining plates were lost or removed by residents. Also, during period 1, the field data collected from the third day were lost for technical reasons. Rat mark intensity assessed by track plates during the experiment is summarized in Fig. 4b.

Prior to rat trapping (Periods 1 & 2; 522 track nights), 38 % of the houses with track plates had rat markings and the mean level of rat mark intensity was 0.144. Further, both trapped and nearby houses had similar levels of rat mark intensity (0.142 and 0.147). Indeed, throughout, the track board mark intensity in both trapped and nearby houses followed similar patterns (Fig. 4). Using our modeling approach, we compared the track plate metrics for the tracking periods 3 and 4 to the combined tracking periods 1 and 2 in both trapped and nearby houses (Online resource Table 3). In houses where trapping took place, the mean level of rat mark intensity (0.795 CI 0.627, 1.009) and the presence/absence of rats (0.712 CI 0.478, 1.062) decreased immediately following trapping (Online resource Table 3), as it did for the intensity of rat marks in nearby houses (0.769 CI 0.551- 1.073). However, four weeks after trapping, the presence/absence of rats increased significantly in both trapped (1.783 CI1.229, 2.586) and nearby houses (1.863 CI 1.144, 3.035) (Online resource Table 3).

When assessing the association between rats captured and track plate intensity pre- and post-trapping, for the pre-trapping periods, the mean level of rat mark intensity increased by a factor of 1.38 (95 % CI 1.19-1.61), for each rat captured during the subsequent trapping session (Table 1). The relative odds of rats being present on tracking boards for the same time period was 2.54 times higher (95 % CI 1.93-3.33) for each unit increase in the number of rats captured. The estimate for

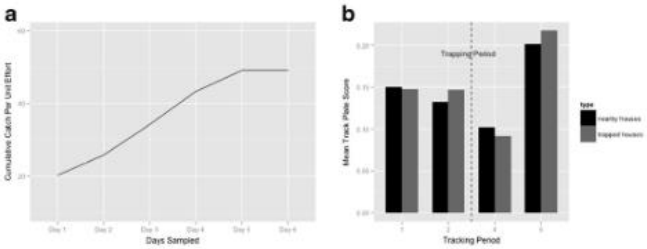

Fig. 4 Figures of rats captured at the two-trapped households (both traps and plates set: panel 2A) and the mean track plate scores for the trapped and the nearby households (only plates) households are similar, indicating that the presence of traps did not influence subsequent rat activity/abundance. **a**) Data for rats trapped during period 3 (excluded for clarity from panel **b**), after baseline track plate sampling (periods 1 & 2: Fig. 2b), show an asymptotic curve based on the Catch Per Unit Effort function, suggesting the population was exhausted. The mean level of rat activity declined significantly immediately after trapping (period 4) but rebounded 4 weeks after the trapping (period 5) for both experimental and control groups

the interaction between the number of rats captured and post-trapping rat mark intensity (period 4) was below one (Factor 0.86, CI 0.78-095), indicating that track plate mark intensity decreased after trapping (Table 1). Four weeks after trapping (period 5) we observed a rapid increase in track plate intensity following the exhaustive trapping (see Fig. 4b). At period 5 (4 weeks after trapping) there were no significant interaction between the number of rats captured and the intensity of rat marks or rat presence.

Discussion

Previous track plate studies monitoring rodents have primarily been conducted in undisturbed (Brown 1969; Connors et al. 2005; Drennan et al. 1998; Glennon et al. 2002; Nams and Gillis 2003; Sheppe 1965, 1967; Taylor and Raphael 1988) or rural environments (Lord 1983; Lord et al. 1971; Quy et al. 1993). Studies in Mexico (Lord 1983) and Lao PDR (Promkerd et al. 2008) at the municipal and village level, respectively, are rare examples using track plates in urban settings. However, no studies have focused on urban slums or evaluated the use of track

Table 1 Association between rats captured and track plate intensity pre- and post-trapping

Time period	Association between number of rats captured and track plate metrics	
	Mean level of rat mark intensity factor (95 % CI)	Presence/absence of rat marks relative odds (95 % CI)
Immediately before trapping	1.38 (1.19, 1.61)	2.54 (1.93, 3.33)
Immediately after trapping	0.86 (0.78, 0.95)	0.96 (0.75, 1.23)
Four weeks after trapping	0.98 (0.88, 1.08)	1.18 (0.91, 1.52)

The table describes the two-component model fit summarized by parameter estimates and 95 % confidence intervals. The model measures rat activity as a continuous measure (mean level of rat mark intensity) and the presence/absence of rats (odds of rat marks being present) as a binomial measure

plates at the household level, though these areas are critical for evaluating an individual resident's risk for acquiring a rat-borne disease, such as leptospirosis, as demonstrated by numerous studies in our Pau de Lima study site in Salvador (Kajdacsi et al. 2013; Ko et al. 1999; Reis et al. 2008b). Track plates can be easily distributed throughout urban habitats in areas where it would be impossible to place traps allowing for broad spatial coverage. Scores were not only strongly correlated with proxies for rodent abundance (rodent signs and trapping) at the household level, but also were sensitive enough to differentiate between relative levels of abundance before and after trapping. Track plates also detected the presence of rats even when the CPUE curve suggested that the rat population was trapped out. Furthermore, this methodology, although targeted at Norway rat-borne leptospirosis, provides a means for the study of other rat-borne pathogens in rural and urban settings, which include Seoul virus, hantavirus, plague, bartonellosis, and toxoplasmosis present in Norway rats (Childs et al. 1995; Costa et al. 2014a).

As noted above, abundance estimates obtained through trapping do not account for variability in rat behavior, such as neo-phobia and trap avoidance, although our nearby sites suggest that trapping did not influence rat activity at household triads. Both the intensity of rat marks and the binary index of presence/absence of specific rat markings on track plates were associated with the number of rats trapped. These findings are consistent with previous studies performed in other environmental settings (Brown 1969; Dickman 1986; Drennan et al. 1998; Glennon et al. 2002; Lord et al. 1971; Promkerd et al. 2008; Quy et al. 1993, 1994) and support the use of this methodology in urban slums. Furthermore, as noted above, track plates detected the presence of rats even when the rat population appeared to have been trapped out. The finding that rat mark intensity scores rebounded four weeks after trapping to levels comparable or higher than pre-trap values clearly indicates that a considerable rat population remained after apparent 'exhaustive' trapping efforts, an effect that is consistent in temperate cities (Lambropoulos et al. 1999; Leslie and Davis 1939).

Track plate methods are appropriate for evaluating the effectiveness of integrated pest-management program and the success of rodent control programs using targeted placement of rodenticides (Engeman and Witmer 2000), even if only presence/absence data are scored. Municipal zoonotic control centers, such as the CCZ in Salvador, implement rodent control for the prevention of leptospirosis and may target interventions at groups of households in and surrounding that residence at which a case of leptospirosis has been identified. These efforts have been associated with reductions in the level of rodent infestation as evaluated by rodent signs (de Masi et al. 2009), but the extent to which rat populations have been controlled and the period of control and rate of population recovery has not been assessed.

In particular, re-infestation or the "boomerang effect", whereby rodents quickly recolonize intervention areas is not well understood (de Masi et al. 2009; Smith 1963). This effect has been documented in both rural and urban studies and is critical to the timing and implementation of rodent control strategies (de Masi et al. 2009; Smith 1963). Genetic studies have demonstrated that both uncontrolled remnants of the pre-existing rat population and immigration from other locations have a role in population recovery of *R. norvegicus* in urban areas where rodent control has been implemented (Gardner-Santana et al. 2009; Kajdacsi et al. 2013). Herein, we ascertained that immediately post-trapping there was a decrease in track plate scores, but just four weeks after trapping, the scores had returned to previous levels, suggesting rapid recolonization of the study areas. Track plates may therefore provide an easily applied metric to evaluate the effectiveness of rodent control interventions and recolonization events, particularly in difficult to sample urban environments.

Analyzing data sets with zero-inflated data, such as the track plate data, has been problematic in the fields of ecology (Welsh et al. 1996; Wenger and Freeman 2008), epidemiology (Conceicao et al. 2013) and other areas of research (Lambert 1992). It is not desirable to solve these problems by discarding information from data with a mixed distribution when many resources have been used to collect experimental data. To address this challenge, we developed a model based on the two main features of the data: the spike at zero and the smoothly-varying distribution over positive values. Using our model, we quantified the two phenomena of interest – the presence or absence of rats in the urban slums of Salvador and the level of rat intensity –without forcing the data into an inappropriate parametric framework. This framework enabled us to prioritize the biological features of interest, namely the presence of rats and the intensity of rats within a given area. Rather than using other regression-based techniques, this model, tailored to the biological outcomes of presence and abundance, enabled us to utilize all data generated from the track plates and monitor abundance throughout a complex urban setting.

One concern with track plate studies is their ability to accurately assess the abundance of rodents and not simply activity defined as the movement patterns of an individual rat – a two-fold difference in abundance and a two-fold difference in activity with the same abundance would not be distinguishable with track plates. For example, an increase in track plate scores post-trapping may be due to an increase in foraging behavior of un-trapped rodents, owing to the lessening of social constraints. However, the high correlation found between signs of rodent infestation and track plate scores suggests that track plates are measuring the degree of infestation and therefore the relative abundance of rodent populations. Similarly, while track plates are measuring some combination of activity and abundance (Allen and Engeman 2014), the significant association found between track plate scores and number of rats captured indicates that track plates are measuring a metric of abundance and not solely rat activity. This finding is consistent with other studies that found positive associations with rat abundance and track plate scores (Drennan et al. 1998; Glennon et al. 2002; Lord 1983; Quy et al. 1993).

We observed a decrease in rat intensity in nearby households where no trapping occurred (following the same pattern as trapped households). In fact, rat mark intensity in nearby houses followed the same trends as trapped households throughout the study period. In urban environments, rats can move longer distances (Kajdacsi et al. 2013); therefore it is likely that rats caught in trapped households were also circulating in nearby households, causing the reduction in those houses. Track plate data from locations distant to trapped and nearby sites were not used in this experiment, which limited options for a direct control group. In future efforts, such data will be collected. However, our findings were robust across the triads of three houses and indicate that household-based intervention methods, aimed at reducing zoonotic pathogen transmission to humans, can be better informed by this simple methodology that can be used alone, in conjunction with rodent survey methods, and as a complement to traditional rat trapping-abundance measures. Given these characteristics, our methodology will facilitate studies that aim to provide a better understanding of the ecology of urban rodents and the risk of spill-over of zoonotic infection from these reservoir hosts, and inform evidence-based rodent control campaigns within vulnerable urban areas.

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