Predicting environmental risk of transmission of leptospirosis

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Gabriel Ghizzi Pedra

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Abstract

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Gabriel Ghizzi Pedra

Leptospirosis is a zoonotic disease distributed worldwide, caused by contact with the spirochete bacteria *Leptospira*. The bacteria are transmitted when animal and human reservoirs come into contact with an environment contaminated with the urine of an infected animal. The ecology of leptospirosis includes complex interactions between the environmental reservoir, the animal reservoir, human infection and the bacteria. Most of the knowledge built up about leptospirosis and human infection is related to medical epidemiology and the animal reservoir. In this thesis, some of the main issues related to the dynamics of *Leptospira* in the environment were explored and tools were developed to improve understanding of the dynamics of the bacteria in the environment.

In order to improve parameter estimation related to the dynamics of the bacteria in the environment, some major gaps were identified and techniques to fill those gaps improved. The first technique developed was to improve animal abundance estimation using removal methods. The improvement of the technique showed that animal abundance could be estimated more accurately and precisely while also being robust to intrinsic variation. This method will provide a more accurate estimation of the level of environmental contamination by rats.

Although models that estimate bacterial survival in the environment exist, no models specifically looked at the survivability of leptospires within the environment. Therefore, a survival model was developed that could estimate survival rates of leptospires in microcosms designed to replicate natural environments. The results provided very insightful results that can help planning the duration and frequency of an environmental intervention.

Water has been shown to be very relevant for the transmission of human leptospirosis, where rainfall has been associated with infections in endemic regions. The last two studies developed here explored different hydrological techniques in order to produce fine scale environmental risk maps which can be used in disease management.

The results obtained here demonstrated in particular the role of multidisciplinary research. Here, the research produced an improvement of the knowledge in different areas such as population ecology, microbiology, hydrology and public health.

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Chapter 1: Introduction

1.1 Infectious diseases and environment

The global population is growing fast. In the past 50 years, city-dwelling has increased exponentially and now more than 50% of the population are living in urban centres. Unfortunately, access to safe drinking water and sanitation has not followed the rapid urbanisation and the number of inadequate settlements has increased: 881 million people were living in urban slums in 2014 (Moreno *et al.*, 2016). In addition, 32% of the global population still lack adequate sanitation and it is estimated that half of the world's population will be living in tropical environments by 2050, which is the region with the highest incidence of infectious diseases on the globe (Guernier, Hochberg and Guégan, 2004; Hemingway, 2014).

As a consequence of population growth there has been an increase in the number of infectious diseases, mainly in urban areas. Pathogens are emerging or re-emerging, representing a threat to people's life and wellbeing. This might be caused by changes in the environment, such as the expansion of urban areas into natural environments, which increases exposure to pathogens and changes the dynamics of infectious diseases. Zika, Ebola, SARS and H1N1 are examples of emerging infectious diseases that have caused a significant impact due to change in the pathogen dynamics and environment in the last decade. In 2012, environmental factors could be identified as the cause of 23% of all deaths reported globally, mostly in low and middle-income countries hold most of it (Prüss-Ustün *et al.*, 2016).

The role of the environment in the dynamics of infectious disease transmission is important both because it may be involved in the life cycle of a pathogen and/or may be one of the routes of transmission. Annual variations in mean precipitation, and annual temperatures, have been shown to be predictors of pathogens species distributions based on a global analysis (Guernier, Hochberg and Guégan, 2004). The main taxa affected by these patterns have an 'external' stage, such as helminths, which requires a free-living stage to complete its life cycle. Besides explaining global distribution of the pathogens, rainfall and temperature have been reported as a risk factor for humans for many infectious diseases. Malaria, cholera, dengue and leptospirosis are a few of the examples of diseases that have reported this association.

Climate change predictions are showing that temperature and precipitation are rising globally and that tropical environments will expand towards temperate environments. This expansion can affect the distribution of the many infectious diseases that are related to temperature and rainfall and occur in the tropics. Therefore, together with rapid urbanisation and population growth, climate change will change the distribution of infectious diseases by increasing its distribution range towards temperate climates. Understanding the role of the environment in the transmission and life cycle of pathogens can be relevant to inform health authorities in the public management of resources to reduce transmission and improve people's life and wellbeing.

A disease that have been changing its epidemiology due to urbanisation in the last 50 years is leptospirosis. Leptospirosis used to affect mostly miners and rice plantation workers in rural areas. However, the rapid urbanisation and the growth of inadequate settlements have changed its epidemiology and, nowadays, it is associated with low-income communities and can be considered an occupational disease. The disease is worldwide in its distribution but the places with the highest

incidence are tropical countries. The next sections will provide an overview of leptospirosis, how the environment is involved in the life cycle of the pathogen, and in its disease transmission.

1.2 Leptospirosis

1.2.1 Epidemiology

Leptospirosis is a zoonotic disease distributed worldwide and caused by contact with the spirochete bacteria *Leptospira* of which there are over 200 serovars described. It was originally considered mostly a rural disease, but rapid urbanization and other environmental changes have led to changes in the dynamics of the bacteria. In 2003 the World Health Organization (WHO) classified leptospirosis as a neglected disease and found it to be more frequent in developing countries with poor sanitation conditions (WHO, 2003b). It is estimated that approximately 1,000,000 cases and 59,000 deaths are caused by disease around the world each year (Costa, Hagan, *et al.*, 2015).

Many animals are considered to be reservoir hosts in which the infection can be asymptomatic and pathogenic *Leptospira* are shed through the urine during the entire lifetime of the host (Babudieri, 1958; Thiermann, 1981; Faine *et al.*, 1999). Nearly all mammals can carry the bacteria (Thiermann, 1977; Bunnell *et al.*, 2000; Levett, 2001), but the natural reservoirs of bacteria threatening humans are often rodents (Faine *et al.*, 1999). The bacteria colonize the kidneys of its hosts and are eliminated in the urine from infected animals and can persist in the environment, potentially, for long periods (between one week and a few months) (Faine *et al.*, 1999; Trueba *et al.*, 2004b). Humans may be infected through direct contact with an infected animal or through an environment contaminated by urine of those animals.

Infection might occur through penetration of damaged skin or mucous surfaces (Faine *et al.*, 1999). There is no human to human infection as the concentration of the bacteria shed by humans is too low for them to be considered a reservoir (WHO, 2003b).

Infections in humans by *Leptospira* do not always result in clinical symptoms; it can be asymptomatic or the disease can be considered biphasic with an acute and/or a severe phase. Acute leptospirosis symptoms can include fever, severe headache, myalgia, nausea, vomiting, chills, malaise and conjunctival hyperaemia (Fraga *et al.*, 2015). In severe cases, the clinical symptoms can be jaundice, myocarditis, meningitis, renal failure, lethal pulmonary haemorrhage and multiorgan failure. Severe cases can be a result of a single illness or a second phase of the acute phase. Renal failure (Weil's disease) and pulmonary haemorrhage cause fatality in 30% and 50% of cases, respectively (Faine *et al.*, 1999; World Health Organization, 2003). Leptospirosis is often misdiagnosed due the similarity that acute symptoms have with other diseases, such as dengue and typhoid fever, which results in an underestimated number of cases (WHO, 2003b; Marchiori *et al.*, 2011; Fraga *et al.*, 2015). In addition, laboratory diagnose is complex, expensive and time consuming because it involves *Leptospira* culture, PCR techniques, IgM-ELISA and microscopic agglutination test (MAT) depending which infection phase patients are in (Faine *et al.*, 1999; Levett, 2001; Marchiori *et al.*, 2011; Fraga *et al.*, 2015).

There are varying patterns of human infection and those patterns depend on the ecological setting. In rural settings, the infections are associated with agricultural and livestock areas and there are peaks of transmission during rainy seasons (Faine *et al.*, 1999; Bharti *et al.*, 2003; McBride *et al.*, 2005). In urban areas the infections are associated with poor sanitation, overcrowding and poverty in developing countries (A I Ko *et al.*, 1999; Levett, 2001; Vanasco *et al.*, 2008). In developed

countries, outdoor recreational exposure and international travel have been associated with the infection (Lau *et al.*, 2010a).

1.2.2 Reservoir hosts

Rodents have been found to be the main reservoir of the bacteria despite the bacteria also occurring in other mammal species. In urban areas, rats carry the bacteria with varied prevalence. High prevalence of infected rats has been reported in Baltimore, USA for example, where 65% of rats sampled were infected with the bacteria (Easterbrook et al., 2007). In Salvador, Brazil, the prevalence was even higher with 80% of the animals infected (de Faria et al., 2008). On the other hand, only 11% of the animals were infected in Vancouver, Canada (Himsworth et al., 2013). Rats are the main source of human leptospirosis in urban areas. Most of the human cases come from serovars that is found in species of *Rattus* (Levett, 2001).

Other rodent species can also carry the bacteria and serve as a reservoir. For example house mice, voles, shrews, muskrats and coypus were also found to be infected (Michel *et al.*, 2001; Adler *et al.*, 2002; Turk *et al.*, 2003; Aviat *et al.*, 2009). Other mammals can also be a reservoir for leptospirosis, such as livestock animals and wild animals. In livestock farming, infected animals can be a risk factor for farmers and butchers in developing countries (Levett, 2001). Cattle, sheep, pigs and goats are farming animals that have been reported as carrying leptospirosis (Levett, 2001; Dorjee *et al.*, 2008; Brown *et al.*, 2011; Suepaul *et al.*, 2011; dos Santos *et al.*, 2012; Martins and Lilenbaum, 2013). Wildlife populations have also been reported as reservoirs. In Pantanal, Brazil, 40% of the animals of four wild mammals species were positive for the bacteria (Vieira *et al.*, 2016), whereas in Africa, birds and reptiles were found positive (Jobbins and Alexander, 2015). Finally, marsupials, bats and rodents have been found carrying the bacteria in the Amazon (Bunnell *et al.*, 2000).

Some animals are reservoir for one serovar and susceptible for others. For example, canine populations are reservoirs for the serovar *Canicola* but are very susceptible to others serovars (Goarant, 2016). Similarly, for cattle, the common serovar is *Hardjo* and for pigs it is *Australis*. These associations can be related to evolution and adaptation of the parasite which is advantageous to a successful establishment in the host population. Other associations are between rats and serogroup Icterohaemorrhagiae; and mice and serogup Ballum (Goarant, 2016). These associations have helped health authorities to identify the source of human leptospirosis in particular cases.

1.2.3 Environment

Pathogenic leptospires are not able to reproduce in the environment but are able to survive from days to months, as elaborated below. Animal reservoirs and humans can be infected if in contact with an environment contaminated with the urine of an infected animal. However, the role of the environment seems to be more relevant in human infections than in the animal reservoir. Minter *et al*. (2017) modelled the routes of transmission between animal reservoirs and identified that 17% of the youngest captured animals had leptospirosis. Their results indicate that transmission occurs before the animals leave their nests and suggest vertical transmission as one of the routes of transmission together with the environment transmission. In humans, the environment can be considered its primary source of infection where environmental variables are one of the main risk factors related to the disease (see section 1.2.3.2).

The search for the bacteria in the environment started when the epidemiology of the disease revealed that the infections were associated with a patient's occupation and were first described in soldiers, miners, and sewer worker and rice planters, all

in wet conditions (Faine, 1982; Katz, Manea and Sasaki, 1991; Faine *et al.*, 1999). Some epidemics were found in sugar cane cutters, rice harvesters and stable hands in cattle stables (Faine *et al.*, 1999). These findings boosted studies to search for the presence and distribution of the bacteria in the environment, such as water and soil samples, where the infections occurred.

Baker and Baker (1970) were one of the pioneers to demonstrate the pathogenicity of water and wet soil in transmitting leptospirosis. Firstly, they identified water and soil samples that were positive for pathogenic leptospires, then inoculated them in hamsters and observed the survival of the animals. Approximately 30% of the deaths could be attributed to leptospirosis and the estimated survival time was around nine days. On the other hand, Henry and Johnson (1978) isolated leptospires from water and soil samples, but they were from the Biflexa serogroup and they could not infect any of the experimental animals, supporting the believe that this serogroup is nonpathogenic. The following sections will explore how the environment is related with the bacteria itself in terms of persistence and occurrence; and how it is related to the transmission of human leptospirosis.

1.2.3.1 Leptospires in the environment

The search for the bacteria in the environment was inspired from epidemiological studies as previously mentioned. Here, a literature review was performed in 2016 and was intended to find and describe the occurrence of the bacteria in the environment. The literature review was performed using Web of Knowledge and the words used were: "*Leptospira*"; and "*Leptospira**Environment". The criteria of inclusion were studies where the presence of the bacteria was evaluated in environmental samples, such as water and soil. In total, 21 studies

were selected and information was collected in each case such as location, setting (urban or rural), year, species group (pathogenic or saprophytic), type of sample (rodent, soil or water), total number of samples, proportion of positive samples and method used (culture, PCR or qPCR). The results are presented in Table 1.1.

Table 1.1: Results of the presence of *Leptospira spp.* obtained from environmental studies. NA= not available.

Year	Area	Location	Country	Species	Rodents			Water			Soil				Method	Authors (year)
					Positive	Total	%	Positive	Total	%	Positive	Total	%	% moisture		
1973	rural	Illinois	USA	both	NA	NA	NA	56	101	55.4	NA	NA	NA	NA	culture	Tripathy & Hanson 1973
1978	natural reserve	lake	Minnesota (USA)	saprophytic	NA	NA	NA	83	126	65.9	15	20	75.0	6.8-86.5	culture	Henry & Johnson 1978
1978	natural reserve	stream	Minnesota (USA)	saprophytic	NA	NA	NA	30	30	100.0	NA	NA	NA	NA	culture	Henry & Johnson 1978
1978	natural reserve	bog	Minnesota (USA)	saprophytic	NA	NA	NA	2	35	5.7	15	34	44.1	27-75	culture	Henry & Johnson 1978
1978	natural reserve	spring water/soil	Minnesota (USA)	saprophytic	NA	NA	NA	8	28	28.6	22	37	59.5	25-82	culture	Henry & Johnson 1978
1994	NA	NA	China	pathogenic	NA	NA	NA	3	140	2.1	5	102	4.9	NA	culture	Yang *et al*. 1994
2006	urban	market area	Belem (Peru)	pathogenic	NA	NA	NA	53	78	67.9	NA	NA	NA	NA	qPCR	Ganoza *et al*. 2006
2006	urban	living area	Belem (Peru)	pathogenic	NA	NA	NA	38	114	33.3	NA	NA	NA	NA	qPCR	Ganoza *et al*. 2006
2006	rural	rural area	Padrecocha (Peru)	pathogenic	NA	NA	NA	60	236	25.4	NA	NA	NA	NA	qPCR	Ganoza *et al*. 2006
2009	rural	camps	Malasya	both	NA	NA	NA	15	144	10.4	15	145	10.3	NA	culture/PCR/MAT	Ridzlan *et al*. 2009
2009	urban	multiple cities	France	pathogenic	38	516	7.4	114	151	75.5	NA	NA	NA	NA	PCR	Aviat *et al*. 2009
2009	rural areas	Guilan province	Iran	both	NA	NA	NA	40	222	18.0	NA	NA	NA	NA	culture	Issazadeh *et al*. 2009
2010	urban	Rio de Janeiro	Brazil	both	NA	NA	NA	3	100	3.0	NA	NA	NA	NA	Multiplex/PCR	Vital-Brazil *et al*. 2010
2011	coastal streams	NA	Hawai	pathogenic	NA	NA	NA	87	88	98.9	NA	NA	NA	NA	qPCR	Viau & Boehn 2011
2013	urban	Metro Manila	Philipines	both	NA	NA	NA	8	39	20.5	NA	NA	NA	NA	culture	Saito *et al*. 2013
2013	rural	Nueva Ecija	Philipines	both	NA	NA	NA	13	18	72.2	3	3	100.0	NA	culture	Saito *et al*. 2013
2013	urban	Fukuoka	Japan	both	NA	NA	NA	10	16	62.5	3	12	25.0	NA	culture	Saito *et al*. 2013
2013	rice crop	Tonekabon	Iran	saprophytic	NA	NA	NA	35	67	52.2	16	36	44.4	NA	culture	Yassouri *et al*. 2013
2013	urban	multiple sites	Malasya	both	NA	NA	NA	28	121	23.1	7	30	23.3	NA	culture	Benacer *et al*. 2013
2013	pig farm	Monteria	Colombia	pathogenic	NA	NA	NA	2	54	3.7	NA	NA	NA	NA	cultue/PCR	Calderon *et al.* 2013
2014	coast	Leyte province	Philipines	pathogenic	NA	NA	NA	NA	NA	NA	11	23	47.8	NA	culture	Saito *et al*. 2014
2014	urban/flooding	Lublin	Poland	both	NA	NA	NA	2	40	5.0	0	40	0.0	NA	PCR	Wójcik-Fatla *et al*. 2014
2014	urban/non flooding	Lublin	Poland	both	NA	NA	NA	0	64	0.0	0	68	0.0	NA	PCR	Wójcik-Fatla *et al*. 2014
2014	rice crop	Tonekabon	Iran	pathogenic	NA	NA	NA	29	67	43.3	9	36	25.0	NA	culture	Yassouri *et al*. 2014
2014	rural village	Los Rios	Chile	pathogenic	NA	NA	NA	27	213	12.7	NA	NA	NA	NA	PCR	Muñoz-Zanzi *et al*. 2014
2014	farms	Los Rios	Chile	pathogenic	NA	NA	NA	50	357	14.0	NA	NA	NA	NA	PCR	Muñoz-Zanzi *et al*. 2014
2014	island	St kitts	Caribe	pathogenic	NA	NA	NA	8	44	18.2	NA	NA	NA	NA	qPCR	Rawlins *et al*. 2014
2015	urban	Sedayu district	Indonesia	pathogenic	6	31	19.4	13	32	40.6	13	36	36.1	NA	qPCR	Sumanta *et al.* 2015
2015	urban	Bantul district	Indonesia	pathogenic	10	36	27.8	29	52	55.8	38	61	62.3	NA	qPCR	Sumanta *et al.* 2015
2015	urban	Sewon district	Indonesia	pathogenic	9	32	28.1	9	35	25.7	13	53	24.5	NA	qPCR	Sumanta *et al.* 2015
2012	urban	Andaman Island	India	pathogenic	NA	NA	NA	11	113	9.7	NA	NA	NA	NA	PCR	Lall *et al*. 2016
2012	urban	Andaman Island	India	nonpathogenic	NA	NA	NA	50	113	44.2	NA	NA	NA	NA	PCR	Lall *et al*. 2016
2012	rural	Andaman Island	India	pathogenic	NA	NA	NA	9	133	6.8	NA	NA	NA	NA	PCR	Lall *et al*. 2016
2012	rural	Andaman Island	India	nonpathogenic	NA	NA	NA	58	133	43.6	NA	NA	NA	NA	PCR	Lall *et al*. 2016
2014	urban	Andaman Island	India	pathogenic	NA	NA	NA	15	56	26.8	NA	NA	NA	NA	PCR	Kumar *et al*. 2015
2014	rural	Andaman Island	India	pathogenic	NA	NA	NA	19	86	22.1	NA	NA	NA	NA	PCR	Kumar *et al*. 2015

The presence of *Leptospira* in the environment varies between locations, and consequently the proportion of positive samples varied in each study. It was observed that *Leptospira* are able to survive in alkaline soil, mud, swamps, streams and rivers, organs and tissues of live and dead animals or dilute milk (Faine *et al.*, 1999). Overall, water and soil samples were taken from streams, nature reserves, rice crops, farms, rural areas, urban areas, flooding and coastal areas. The locations varied from temperate countries such as the USA, France and Poland to tropical countries such as the Philippines, Indonesia and the Caribbean. Saprophytic and pathogenic bacteria were examined together or separately, and the technique used to detect the bacteria varied from culture methods to DNA amplification methods such as qPCR.

There was a gap of approximately 20 years from the first studies that evaluated the presence of the bacteria in water and sample soils until the next study was published. This could be a result of the development of more sophisticated techniques to detect *Leptospira* in the environment. Despite culture techniques being considered gold standard methods, they are expensive, time consuming and the media used are not specific for leptospirosis which increases the number of false negatives. When DNA techniques became more accessible, studies that explored the occurrence of leptospires in the environment started appearing again. The renewal of interest may also have happened because leptospirosis has changed its epidemiology. The incidence of human leptospirosis increased and WHO included leptospirosis as a neglected disease of general concern in 2003. This raised awareness of leptospirosis and studies started to explore and understand patterns of infection and how transmission occurs.

Tripathy and Hanson (1973) were the pioneers who isolated pathogenic bacteria from water samples in an agricultural center in Illinoi. They were followed by

Alexander *et al*. (1975) who isolated leptospires from surface water and wet soil in the Malaysian jungles. They found a variety of serovars that was consistent with the ones identified from soldiers who had leptospirosis at the time. Henry and Johnson (1978) found over 92% of positive water and soil samples in Minnesota when the average temperature was 25°C. In addition, the proportion of positive sample was higher for soil samples than water where the moisture content of the soil was greater than 65%.

In general, the proportion of positive water samples varied from 3% in an urban area of Rio de Janeiro, Brazil (Vital-Brazil *et al.*, 2010), to 67% and 75% in an urban area in Peru and France respectively (Ganoza *et al.*, 2006; Aviat *et al.*, 2009). In rural areas, this varied from 3.7% in a pig farm in Colombia (Calderón *et al.*, 2014) to 100% in a nature reserve in Minnesota (Henry and Johnson, 1978). The bacteria were also able to survive in seawaters. Saito *et al*. (2014) observed that the bacteria that comes from soil to the seawater, survived for four days in seawater whereas the bacteria inoculated straight in seawater survived for three days only. Their results suggest that the soil increased the chance of the bacteria surviving in seawater.

Fewer studies have evaluated the presence of the bacteria in the soil, and only one study looked at how the moisture of the soil is related with positive samples. The proportion of positive soil samples in urban areas varied from 0% in Poland (Wójcik-Fatla *et al.*, 2014) to 62% in Bantul district, Indonesia (Sumanta *et al.*, 2015) ,whereas, in rural areas, it varied from 10% in rural camps in Malaysia (Ridzlan *et al.*, 2010) to 75% in a stream in Minnesota (Henry and Johnson, 1978). Henry and Johnson (1978) were the only investigators who observed that soils with higher moisture had the highest proportion of positive samples.

Another factor that affects the survival of leptospires in the environment is pH. Okazaki and Ringen (1957) observed that the bacteria survived in the environment with pH ranging from 6 to 8.4. Similarly, Diesch *et al*. (1969) observed that the

bacteria survived in creek water at a pH ranging from 6.9 to 8.7. None of these studies has quantified the survival of the bacteria in the environment, and until now, the rate at which the cells died in the environment is unknown.

The results on the occurrence of pathogenic bacteria in soil and water samples indicates that these environments can be considered a reservoir for the bacteria. The association between outbreaks of leptospirosis and floods and/or heavy rain (see section 1.2.3.2) could plausibly be a result of the presence of the bacteria in the environment that increases exposure to the disease. Most of the research, if not all, has focused on finding the bacteria in different environments. However, understanding the survival and transportation of the bacteria in the environment can be crucial to understanding its distribution and how the environmental risk of transmission of leptospirosis can be estimated.

1.2.3.2 Environmental risk factors in human leptospirosis

Human leptospirosis was previously considered an occupational and rural disease, as mentioned, where the first description of cases came from farmers, soldiers, miners and rice plantations. Baker (1965) described a leptospirosis outbreak between soldiers in Malaysia and identified that most of the individuals had been to the forest a drunk water that might have been contaminated. In Britain, most of the cases reported between 1933-48 were farmers/fisher workers, coal miners, sewer workers and soldiers (Waitkins, 1986). Additionally to the importance of occupation, recreational activities were also related to occurrence of infection, whereby canoeists were reported having leptospirosis (Waitkins, 1986). Nowadays, human leptospirosis cases have increased and it is also frequently reported in urban environments. However, environmental risk factors are reported in both urban and rural environments. From a combination of epidemiological, cohort (transversal and

longitudinal) and case-control studies, risk factors have been identified and related with socioeconomics and environmental characteristics (A I Ko *et al.*, 1999; Barcellos and Sabroza, 2001a; Sarkar *et al.*, 2002; Maciel *et al.*, 2008; Reis *et al.*, 2008; Ko, Goarant and Picardeau, 2009; Oliveira *et al.*, 2009; Hagan *et al.*, 2016).

Lower levels of income and education are the most important socioeconomic characteristics affecting the infection (Barcellos and Sabroza, 2001a; Reis *et al.*, 2008; Oliveira *et al.*, 2009). Environmental risk factors varied and included variables such as contact with mud, elevation, flooding, open sewage and litter less than ten meters from residences. Such links have been found with asymptomatic infection and with severe cases of the disease (A I Ko *et al.*, 1999; Sarkar *et al.*, 2002; Reis *et al.*, 2008; Oliveira *et al.*, 2009; Hagan *et al.*, 2016).

Hagan *et al*. (2016), in a four year prospective study, identified that leptospirosis transmission in a urban slum in Salvador, Brazil, was associated with poverty, geography and climate. Illiteracy, the level of rat infestation, contact with mud, and elevation were all related with the infections throughout the four years follow-up. Distance to an open sewer and household income were related to primary and secondary infections in the same urban slum, suggesting that environmental setting and behaviour contributes to exposure to *Leptospira* (Felzemburgh *et al.*, 2014a). Similarly, in a urban slum in Rio de Janeiro, Brazil, the environmental risk factors associated with leptospirosis were solid waste accumulation and coverage, flood risk areas, proximity to sewer and proximity to rainwater drainage (Barcellos and Sabroza, 2001a; Sarkar *et al.*, 2002; Reis *et al.*, 2008). However, these environmental risk factors are imperfect proxies for the presence of the bacteria and do not represent the intensity of environmental contamination. Flooding and heavy rain are also associated with infections in endemic and non-endemic regions. They are responsible for outbreaks and for the seasonality on the number of cases observed in endemic regions. Ko *et al*. (1999) observed that rainfall was responsible

to an increase on the number of cases in Salvador, Brazil where the maximum number of infections occurred two weeks after the heaviest rainfall of the year. In Malaysia, leptospirosis is endemic, and flooding have been associated with human cases (Garba, Bahaman, Khairani Bejo, *et al.*, 2017). Studies have shown this relationship with between rainfall and leptospirosis cases in another endemic regions such as Thailand and tropical islands (Goarant *et al.*, 2009a; Desvars *et al.*, 2011a; Chadsuthi *et al.*, 2012a).

An outbreak in Philippines in 2009 occurred after a flooding event where 178 people died after a typhon caused a major flood in the city of Metro Manila (Amilasan *et al.*, 2012a). Similarly, unusual epidemics of leptospirosis and melioidosis occurred after a typhon reached Taiwan on the same year (Su, Chan and Chang, 2011). Furthermore, outbreaks of leptospirosis, also have been associated with rainfall in many other countries such as Australia (Smythe *et al.*, 2002), Brazil (Barcellos and Sabroza, 2001a; Blanco and Romero, 2015), France (Socolovschi *et al.*, 2011), Guyana (Dechet *et al.*, 2012), Hawaii (Gaynor *et al.*, 2007), Honduras (Naranjo *et al.*, 2008), India (Sehgal, Sugunan and Vijayachari, 2002; Jena, Mohanty and Devadasan, 2004; Pappachan, Sheela and Aravindan, 2004), Malaysia (Garba, Bahaman, Khairani-Bejo, *et al.*, 2017), Nicaragua (Zaki and Shieh, 1996; Schneider *et al.*, 2012), the Philippines (Amilasan *et al.*, 2012b; SUMI *et al.*, 2017), Thailand (Chadsuthi *et al.*, 2012b), United States (Stern *et al.*, 2010).

1.2.4 Geographical distribution of leptospirosis

Costa *et al*. (2015) estimated global morbidity and mortality of leptospirosis where the estimations shown that the regions with the highest incidence of the disease are Southeast Asia, Oceania and Caribbean (Figure1.1). Those areas also have the highest annual average rainfall on the globe (Fick and Hijmans, 2017) as

shown in Figure 1.2, from which it is clear that water is playing an important role in human infection – another reason for environmental studies indicating environmental levels of contamination are important both to understand and to predict the environmental risk of infection.

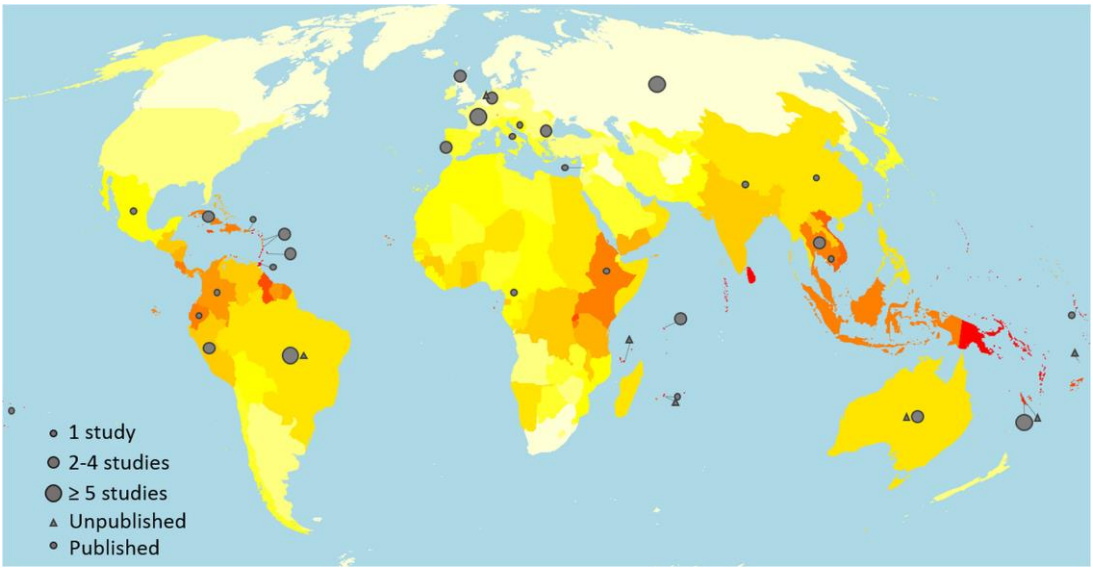

Figure 1.1: Figure extracted from Costa *et al*. (2015) showing the estimated morbidity of leptospirosis based on systematic literature review. The colour gradient represents number of cases per 100,000 population where white represent cases from 0 to 3, yellow from 7 to 10, orange from 20 to 25 and red over 100.

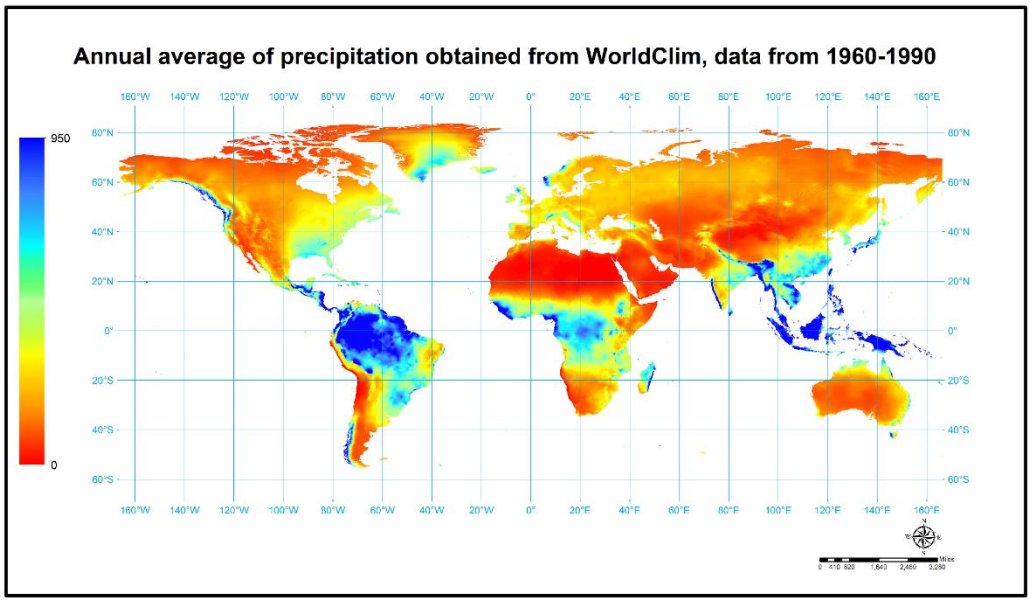

Figure 1.2: Annual average of precipitation (mm) data between 1960-1990 obtained from WorldClim repository - http://worldclim.org/version2 (Fick and Hijmans, 2017). Map author: Gabriel Ghizzi Pedra.

Human leptospirosis does not only vary globally. Distributions of cases within a city or a country are also found to be geographically clustered. For example, at a city level, Gutiérrez & Martínez-Vega (2018) identified six clusters of incidence of leptospirosis in Colombia, South America, and identified that anomalous rainfall were associated with elevated number of cases. There was evidence that agriculture was a common factor in municipalities that have higher incidence of leptospirosis (García-Ramírez *et al.*, 2015). At a district level, an uneven distribution of leptospirosis cases was observed in New Caledonia (Goarant *et al.*, 2009a), Futuna, south pacific (Massenet *et al.*, 2015), Republic of Serbia (Svirčev *et al.*, 2009), Thailand (Suwanpakdee *et al.*, 2015), Siri Lanka (Robertson, Nelson and Stephen, 2012), Mexico (Sánchez-Montes *et al.*, 2015), American Samoa (Lau *et al.*, 2012), Nicaragua (Schneider *et al.*, 2012), China (Dhewantara *et al.*, 2018) and Trinidad (Vega-Corredor and Opadeyi, 2014). Even at a very small scale, geographical variation have been observed. In Brazil, Hagan *et al*. (2016) observed geographical variation on the risk of infection at a very small scale, within a low-income community in Salvador, Brazil.

All those studies have found association with environmental variables, such as rainfall and flooding, which also supports the idea that water is an important risk factor at many scales. Furthermore, Gracie *et al*. (2014) evaluated the effect of different risk factors on the incidence of leptospirosis at different scales. They found an association between the proportion of areas prone to flood and leptospirosis at a local scale, whereas the percentage of densely urbanized areas and the number of households in slums were associated at a regional level. The use of geographically distributed data helps to identify high risk areas that can further be used to target actions to control and prevent leptospirosis incidence.

1.2.5 Conceptual model of the bacteria in the environment

To understand the mechanisms of disease transmission, it is necessary to know the life cycle of the pathogen and the determinants of its occurrence. There is still a lack of information on the determinants of bacterial occurrence and transport in the environment. We have shown the role of the environment in human leptospirosis as well as in its reservoir host, but knowledge of what happen with the bacteria in the environment is still very basic and is growing. In this section, a conceptual model that represents the dynamics of the bacteria in the environment is proposed and this thesis will explore key compartments of this conceptual model.

Based on what has been shown here and further discussion with experts on leptospirosis, a conceptual model was developed. This conceptual model is a simplification of the complex interaction between the bacteria and the environment and it is shown in Figure 1.3.

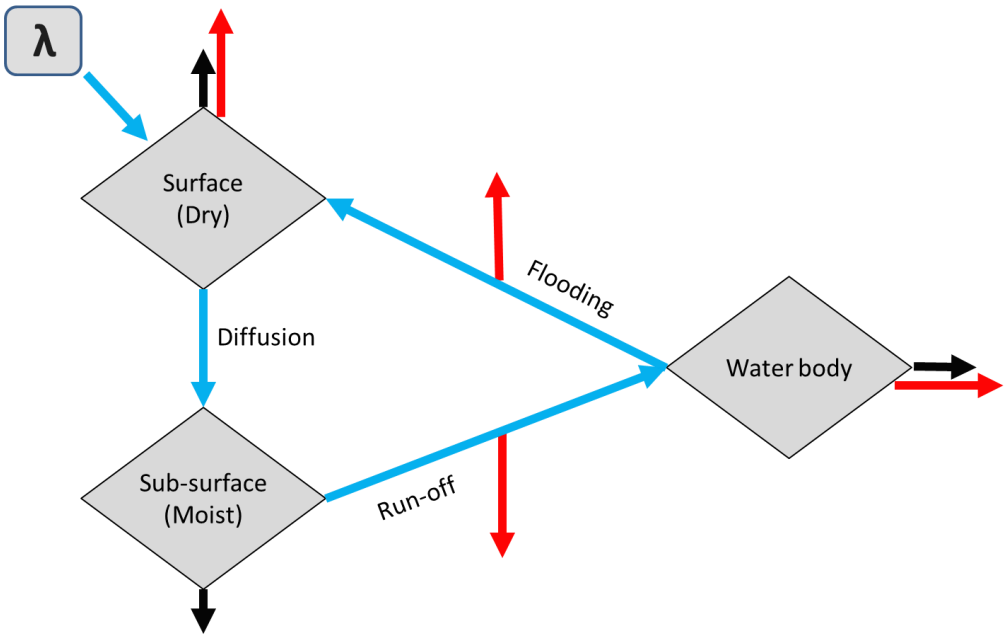

Figure 1.3: Conceptual model representing the dynamics of the bacteria in the environment. The boxes represent the location where the bacteria occur, called compartments. Blue arrows represent movements between compartments. Red and black arrows are the way the bacteria leave the system – by mortality (dark) or human infection (red). Lambda represents a reservoir of the bacteria external to the system, often a population of rats.

The bacteria get into the environment through shedding from an animal reservoir, represented by lambda. This quantity depends on the reservoir's abundance and the shedding rate, which will indicate the level of local contamination. Leptospirosis shedding rate varies depending on the animal reservoir. Rats, *Rattus norvegicus,* shed approximately 5.7×10^6 cells/ml of urine whereas large mammals such as cattle shed less, $1.7x10^5$ cells/ml (Barragan *et al.*, 2017). Costa *et al*. (2015) estimated that a population of 82 rats would shed daily more than 9.1×10^{10} leptospires. However, the population size of the reservoir often remains unknown and in studies that attempt to incorporate abundance, this has either been assumed to be the total number of animals trapped (Costa, Wunder, *et al.*, 2015) or relative abundance has been used (Himsworth *et al.*, 2013). These attempts do not represent absolute abundance of a population and therefore the level of contamination cannot be properly estimated. CHAPTER 2 of this thesis will demonstrate a new method to estimate absolute abundance, applicable especially to rats in urban environments, and show the difficulties of current techniques on estimating animal abundance.

The central assumption of the model in Figure 1.3 is that there are three environmental states where the bacteria is present: soil surface, soil sub-surface and water bodies. Saito *et al*. (2013), for example, observed *Leptospira* at 3cm depth after three drought days at a location that was a dried rain puddle previously. In addition, the same place was positive for *Leptospira* five months later. This observation indicates a role for the sub-surface as a reservoir of the bacteria in the environment, but there is still very little information about this environmental state.

The bacteria leave each state based on a survival rate or human infection represented by the black and red arrows, respectively, in the figure. Neither the quantitative nor the qualitative nature of the survival curve of the bacteria is known, and in a collaboration between the University of Liverpool and Yale University,

microcosms experiments were developed to estimate the survival rate of the bacteria in different environments such as spring water, soil, mud and sewage. The results of these experiments are shown on CHAPTER 3 where a statistical survival model was developed.

It is evident that water plays an important role in leptospirosis transmission, since most of the environmental risk factors are water related variables. There are peaks in the number of cases every year in endemic areas and they have been associated with rainfall. In addition, outbreaks are reported after extreme weather events, such as typhoons, El Niño events and heavy rainfall. Therefore, the mechanisms behind bacteria transportation between the compartments in the environment are driven by hydrology, where runoff carries the bacteria from soil to a water body and flooding will do the opposite (blue arrows). The survival of the bacteria is very short at the soil surface as the bacteria is exposed to ultraviolet radiation and dehydration. Hence, there is no movement from the surface to the water body and the bacteria pass to the sub-surface via diffusion.

Hydrology has not been explored extensively in leptospirosis studies. An investigation by Vega-Corredor & Opadeyi (2014) was the only study that looked at how hydrology can be associated with human leptospirosis. Their research was based in Trinidad and Tobago, two islands in the Caribbean region and they collected information on leptospirosis cases at a community level (~250 communities). It found that areas that are more likely to be flooded are the ones where more cases of leptospirosis were observed. CHAPTER 4 will develop methodologies, exploring how it is possible to use hydrology to create more informative maps related to bacteria contamination, and in CHAPTER 5 the association between these maps will be validated with surveillance data on leptospirosis in Salvador, Brazil.

1.3 How hydrology can help infectious disease dynamics

As a basic definition, hydrology is a discipline focused on the movement, distribution and quality of water. Some of the areas within Hydrology are interested in water flow, which can be looked at different scales. The process behind water flow can involve precipitation, topography, evapotranspiration, soil saturation and groundwater movement, all of which will influence the amount of water flowing or accumulating in certain areas. However, water flow will primarily depend on the rain that falls on the ground and the amount of that rain that becomes runoff. Two different classes of mathematical models have been developed to understand the water flow – lumped and distributed models. Lumped models work at a catchment level and do not include finer-scale spatial variation, whereas distributed models work at a fine scale and therefore rely on the spatial resolution of the data captured.

Waterborne diseases, as the name suggests, rely on water for pathogen transportation and/or infection. They are called waterborne because the main route of transmission occurs through water. Examples of waterborne diseases include the historical cholera that is estimated to have killed over one million people in Europe in the 19th century, typhoid fever, diarrheal diseases, schistosomiasis, dengue, cryptosporidiosis, SARS (Severe Acute Respiratory Syndrome) and leptospirosis.

Hydrological models have been integrated with dynamic modelling of waterborne diseases. The assumption behind the use of hydrology is that water can mobilize pathogens, hence, modelling water flow can indicate the routes of pathogen contamination and distribution. Medema & Schijven (2001) modelled the transportation of *Cryptosporidium* and *Giardia* in the Netherlands to identify the

origin of contamination. It was observed that most of *Cryptosporidium* contamination came from wastewater treatment plants, whereas, *Giardia* had most of contamination coming from untreated wastewater discharge and sewer overflow. Pathogen load were also addressed by Ferguson *et al*. (2007) and Mahajan *et al*. (2014) where their aim was to identify the sources of contamination.

Additionally, the assumption of pathogen transportation was incorporated by using river networks. Bertuzzo *et al*. (2008) integrated river pathways to model an outbreak of cholera in South Africa in 2000. They were able to show how the predictive power of the tool and how the river pathways played an important role in controlling the direction of the infections. Subsequently, Mari *et al*. (2012) showed that long-range human movement was able to explain the unexplained inter-catchment movement of the pathogen. For schistosomiasis, the hydrological transport was integrated in the vector compartment. Channel flows were key to predict infection intensity and in periods of absence of flow, the risk was significantly reduced (Remais, Liang and Spear, 2008). The ephemerality of rivers we also shown to be associated with schistosomiasis cases in West Africa (Perez-Saez *et al.*, 2017).

Hydrological models can also help predict abundance of vectors. Shaman *et al*. (2002) used a dynamic hydrological model to predict mosquitoes' abundance. They used a combination of historical meteorological data, topography, soil and vegetation information to produce wetness map of the surface. From that, they identified potential swamp and fresh water hence and predicted mosquitoes' abundance.

1.4 Aims

In summary, the ecology of leptospirosis includes complex interactions between the environmental reservoir, the animal reservoir, human infection and the bacteria. All those compartments have an important role to play in transmission, but most of the knowledge built up about leptospirosis and human infection is related to epidemiology and the animal reservoir. Therefore, the main aim of this thesis is to develop new tools related to the dynamics of the bacteria in the environment. It is hoped that the tools developed here will facilitate environmental research and contribute to understanding the main environmental drivers of human leptospirosis.

Furthermore, this thesis aims to enhance the application of hydrology in infectious disease dynamics by exploring hydrological techniques and associating results with disease outcomes. Understanding these relationships will help to produce fine scale environmental risk maps, which can be used in disease management.

1.5 Note on collaborative research and data collection

This project is part of a multidisciplinary project with three institutions involved, Oswaldo Cruz Foundation (Fiocruz, Brazil), University of Liverpool (UK) and Yale University (USA). The project is called “Eco-epidemiology of urban leptospirosis” and the main aim is to determine the main drivers of human infection. There are data collection in all compartments related to the infection, reservoir, humans and environment. Not all data used in this thesis was collected by the author. The data

collection on rodents were performed by a team, including the author, based in Salvador, Brazil, where Fiocruz was responsible for the data collection and quality control. Laboratory analysis from chapter 3 and 5 were performed at Yale University and the author was not involved.

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Chapter 2: A new approach to making multiple estimates of animal abundance using removal methods

2.1 Introduction

A key interest for ecologists is in estimating the population size of organisms. This is important for monitoring population dynamics over time and in managing ecosystems and making conservation decisions (Soulé, 1987; He and Gaston, 2000; Wilson *et al.*, 2004; Lyons *et al.*, 2008). Also, by following variation in the population size of an organism over time, it may be possible to obtain further demographic information such as survival and migration rates (Seber, 1986; Borchers, Buckland and Zucchini, 2002). However, population ecologists face difficulties in the process of estimating population size, including constraints on effort and costs, animal detection, and invalid assumptions made by estimation methods (Seber, 1986; Schwarz and Seber, 1999; MacKenzie and Manly, 2001).

The crucial issue in estimating animal abundance is the detectability of individual animals. Various statistical models have been developed to estimate the probability of detection in order to estimate the abundance of the population (Leslie and Davis, 1939; Moran, 1951; Zippin, 1958; Otis *et al.*, 1978; Seber, 1982; MacKenzie and Manly, 2001; Borchers, Buckland and Zucchini, 2002; Davis *et al.*, 2016). Capture-recapture methods are widely used in wildlife research, where individuals are tagged and then released into to the population for subsequent possible recapture (Schwarz and Seber, 1999). These methods do not impact on population size, but problems may occur with animals not retaining marks, or when capture and marking is associated with increased mortality. In addition, recapture rates need to be high

enough to generate reliable estimates (Schwarz and Seber, 1999) and the methods are typically costly in terms of time and effort.

A simpler alternative is to use methods that are based on removing individuals from the population (Seber, 1986; Borchers, Buckland and Zucchini, 2002; Dorazio, Jelks and Jordan, 2005; Dorazio *et al.*, 2008; St. Clair, Dunton and Giudice, 2012). If the same trap effort is used in each removal occasion, it is expected that the same proportion of individuals will be removed from the population (Schwarz and Seber, 1999). Hence, the sizes of samples and their rate of decrease can be used to infer the probability of capture and abundance of the population. But these methods are prone to error when the number of individuals caught in each occasion does not decrease consistently (Schwarz and Seber, 1999).

Monitoring abundance may be necessary in pest control or conservation, because of the need to know whether the population is declining. Also, monitoring invasive species before, during and after control may help to improve action plans to minimize the impact of that species (Blossey, 1999; Davis *et al.*, 2016). For these and other reasons, serial estimates of abundance may be required. Alternatively, it may be necessary to estimate the abundance of a species at different sites at the same time – for example in case-control population studies.

This paper focuses on an improvement to the removal method of estimating animal abundance to facilitate its use. The motivation of this study arose from a need to estimate the true population size of the rodent reservoir for human disease, *Rattus norvegicus*. For rats, there is difficulty of consistently observing a decrease over time in the number of rats captured per unit effort, as assumed by removal models. Note particularly that despite these difficulties, removal methods may be the only option when working in pest control or on a reservoir for infectious disease, where release of a captured animal into the population is typically not acceptable. In many cases, for example in infectious diseases studies, removing and killing

animals may also provide data for other parameters (transmission, pathology) as well as for the prevalence of an infection.

Making multiple related estimates of abundance may, however, provide a means of overcoming shortcomings in individual data sets in cases where the use of removal methods is unavoidable. These methods typically provide an estimate of abundance by considering probability of 'capture', that is, the probability that an animal will enter a trap (or whatever capture method is used). Abundance estimates are inaccurate or impossible to make if estimates of the probability of capture are inaccurate or impossible. Abundance is likely to vary from time to time and from place to place, in which case monitoring that variation is likely to be an objective of the study, but the probability of capture can be assumed in many cases to remain constant, since this will reflect intrinsic qualities of the study species, the habitat and the field methods. The method developed here, therefore, combines data from multiple similar sites in order to derive a consensus estimate of the probability of capture with the aim of overcoming shortcomings in individual small data sets – and then applies this probability to each site in turn to generate individual abundance estimates. This new method was applied to data collected as part of a study of the dynamics of leptospirosis in rats, *Rattus norvegicus* (Berkenhout 1769), in the tropical urban setting of Salvador, Brazil (Kajdacsi *et al.*, 2013; Costa *et al.*, 2014, 2016; Costa, Wunder, *et al.*, 2015; De Oliveira *et al.*, 2016; Hagan *et al.*, 2016; Panti-May *et al.*, 2016; Richardson *et al.*, 2016; Walker *et al.*, 2017).

2.2 Materials and methods

2.2.1 The Borchers et al. (2002) method

The removal method was first proposed by Leslie & Davis (1939) to estimate the absolute abundance of the black rat, *Rattus rattus,* in Sierra Leone. Moran (1951) and Zippin (1958) then improved the Leslie & Davis model using Maximum Likelihood Estimation (MLE) to estimate total abundance under explicitly declared assumptions, namely: (1) the population is closed (no migration into or out of the area of trapping); (2) the probability of an individual being caught is the same for all individuals; and (3) the probability of capture is the same for all trapping occasions of a survey. Borchers *et al.* (2002) subsequently included one more assumption, namely (4) captures are independent between occasions.

Two parameters are estimated by MLE using the notation in Borchers: total abundance (*N*) and probability of capture (*p*). The likelihood is based on the following joint probability distribution for the numbers, n_s, of animals removed on sampling occasions $s = 1, 2, \dots, S$:

$$L(n|p,N) = \prod_{s=1}^{S} \binom{N_S}{n_s} p^{n_s} (1-p)^{N_s - n_s}$$

eqn 1

where,

n = $(n_1, n_2, \dots, n_s)$

p = probability of capture;

$N_s = N_1 - \sum_{j<s}^{S} n_j$: population size immediately before sampling occasion ***s = 1,2,…,S.***

The model parameters are N and p, where $N = N_1$, the initial population size, is the parameter of interest. The likelihood of *N* and *p* is a product of the likelihood contributions from each removal occasion *s*. Estimation of *N* by maximizing this likelihood is referred to here as the individual method.

Figure 2.1 illustrates how the estimation procedure works; if the same effort is applied to remove individuals on each removal occasion, then the expected number of individuals removed at each occasion will decrease. The predicted cumulative number of removals (Figure 2.1) will reach an asymptote when most of the individuals have been removed and the estimated number of individuals at occasion one (N_1) will be the height of the curve. When the number of animals caught does not decrease over time, estimation becomes problematic because the set of admissible combinations of *N* and *p* becomes more complex. Underestimation can also arise when there is heterogeneity in the probability of capture between each removal occasion (Seber, 1982; Borchers, Buckland and Zucchini, 2002).

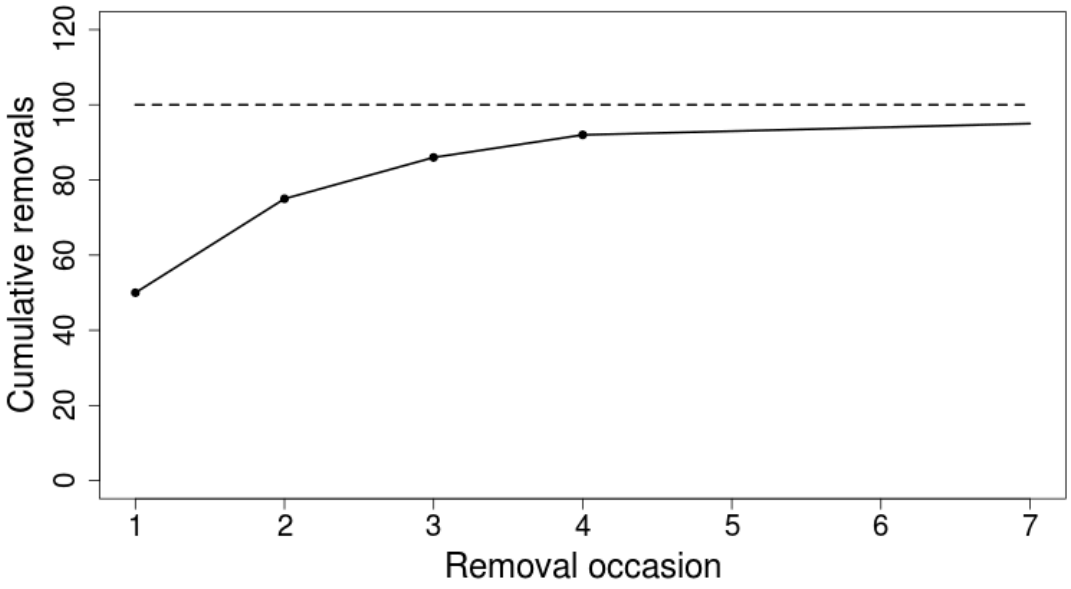

Figure 2.1: Cumulative removal used to estimate animal abundance. Dots are the observed data and solid line is the predicted cumulative curve. The height of the dashed represents the abundance estimated for the population.

2.2.2 Pooled method

In contrast to this individual method, we now propose a new, 'pooled method' in which we assume, additionally to (1) to (4) above, that: (5) all individuals have the same probability of being caught in any survey. The likelihood now is a joint probability distribution for the numbers, $n_{i,s}$, of animals removed on sampling occasions $s = 1, 2, \dots, S$ in each survey $i = 1, 2, \dots, I$:

$$L(\boldsymbol{n}|p, \boldsymbol{N}) = \prod_{i=1}^{I} \prod_{s=1}^{S} \binom{N_{i,s}}{n_{i,s}} p^{n_{i,s}} (1-p)^{N_{i,s}-n_{i,s}}$$

eqn 2

where,

n = $(n_{1,1}, n_{1,2}, \dots, n_{1,s}, n_{2,s}, \dots, n_{i,s})$

p = probability of capture;

$N_{i,s} = N_{i,1} - \sum_{j<s}^{S} n_{i,j}$: population size in each survey *i = 1,2,…,I* immediately before sampling occasion *s = 1,2,…,S*.

Now, the likelihood of *N* in each survey *i* is the product of the likelihood for each removal occasion *s* with a common probability of capture *p*. Note that if the number of surveys is one (*I*=1), we recover the individual method.

2.2.3 Extension to non-constant probability of capture

Heterogeneity in probability of capture can generate biased animal abundance estimates if it is not taken into account (Seber, 1982; Borchers, Buckland and Zucchini, 2002). Borchers *et al*. (2002) extended the individual method by allowing the probability of capture to vary between surveys. In their example, the probability of capture can be a function of the *catch-per-unit-effort* (CPUE), a relative measurement that reflects the effort used on each removal occasion *s*. Here, the

effort is assumed to have a linear effect on the log-odds of capture, so the larger the effort, the larger the probability of capture. Other functions can be assumed. For example, an exponential function could be used if the relationship with effort reaches a plateau corresponding to a proportion of "untrappable" animals.

Therefore, for the individual method, the probability of capture p_s is now a function of the effort (l_s) used in each removal occasion *s*:

$$p_s = {}^{1}/_{(1 + e^{-\alpha_s})}$$

eqn 3

$$\alpha_s = \beta_0 + \beta_1 * l_s$$

eqn 4

where the parameters β_0 and β_1 are to be estimated.

Similarly, the probabilities of capture p_{is} in the pooled method would depend on the effort ($l_{i,s}$) used in each removal occasion *s* of a survey *i*.

$$p_{i,s} = {}^{1}/_{(1 + e^{-\alpha_{i,s}})}$$

eqn 5

$$\alpha_{i,s} = \beta_0 + \beta_1 * l_{i,s}$$

eqn 6

2.2.4 Simulation and validation of the model

To examine the performance of the model, the trapping scheme of rats from Salvador, Brazil (Panti-May et al., 2016) was used as a basis for choosing appropriate population sizes and probabilities of capture. Thus, perfect and imperfect datasets (see below) with four consecutive occasions of trapping were simulated, grouped into sets of ten surveys that could be independent surveys varying in time and/or space but from within the same region. The characteristics of the simulated datasets were based on a previous study by Borchers *et al*. (2002) and on results of applying the individual method to the trapping data from Salvador.

Borchers *et al*. (2002) performed simulations to validate the individual method and concluded that accurate estimation depends on both the size of the population and the probability of capture. Their estimates were inaccurate with small populations ($N<250$) or with lower probabilities of capture ($p<0.3$). However, with $p>0.5$, the probability of capture fell within the 95% confidence interval of its estimate in 90% of the cases, and this did not change significantly when p was increased. When the individual method was applied to data from Salvador, the results typically led either to a bad performance of the algorithm or to large confidence intervals. The average estimated population size was 48 individuals with a mean probability of capture of 0.27.

Hence, for the simulations, the value for population size was rounded to 100 individuals, and two different types of 'imperfect' dataset were created. One, called 'imperfect removal' incorporated a reduction in the numbers of captured individuals in each removal occasion with a small but constant probability of capture in each survey ($p=0.24$). The other, called 'messy' included random variation around this same probability of capture, normally distributed, with standard deviation of 0.1. 'Perfect' datasets were also created with a probability of capture of 0.5. The simulated data were randomly sampled from a binomial distribution of size, N, equal to the population size minus the cumulative number of animals removed on any

previous occasions, sampled according to the probability of capture defined in each type of dataset.

Each of the three types of dataset, with five independent sites/surveys and four removal occasions per survey, was reproduced 2,000 times, producing 10,000 estimates in total per dataset. All analyses used the R software (R Core Team, 2014). See supporting information for a description of the R code (S2 Appendix).

Both methods, individual and pooled, were applied to the same sets of simulated data of the three types (perfect, imperfect and messy). The results from each method were first examined on the basis of the performance (satisfactory or otherwise) of the optimization algorithm, specifically checking whether the optimization process converged to a global maximum of the likelihood surface. Once performance in this regard had been checked, it was possible to evaluate which estimations had been successful by examining their accuracy, defined as whether the true value was within the confidence interval of the estimate. Also, precision of each accurate estimate was evaluated, defined as the width of its confidence interval.

2.2.5 Application of the pooled method to Salvador data

Estimates obtained by the individual and pooled methods were also compared for rat population size in Salvador, Brazil (Costa, Wunder, *et al.*, 2015; Panti-May *et al.*, 2016). Briefly, the sampled area was divided into three valleys of the Pau da Lima district, an urban slum community (Felzemburgh *et al.*, 2014b). Within each valley, three surveys of trapping (also called events) were performed, each over four consecutive days, with intervals of a month between them, so there are replicate estimates of abundance in both space and time. Trapping sites were chosen according to a stratified random scheme but, primarily because of the non-

compliance of households, some points were not trapped. Thus, the valleys had 23, 38 and 39 trapping points, respectively. Surveys were pooled by valley and a probability of capture for each valley was obtained. The three valleys are separated by more major thoroughfares, and genetic data have shown that from 70% to 90% of migrations occur within each valley. The streets appear to be serving as barriers to impede movement (Kajdacsi *et al.*, 2013; Richardson *et al.*, 2016). Hence, we assumed that each valley had a closed population of rats during the study period.

For both the individual and pooled methods, the rat abundance in each valley and event was estimated assuming first homogeneity then heterogeneity in the probability of capture, and models for the two cases were compared. In this case, sample effort was assumed to influence the probability of capture and was defined as the total number of traps minus 50% of traps that were closed (had been triggered) but contained no rats. This sample effort is a standard method that accounts for traps where rats had a limited chance of being trapped due to disturbance of some traps. A best model, with constant or non-constant probability of capture, was selected based on the best fit (log-likelihood) given its complexity (number of parameters) by using likelihood ratio tests (LRTs). LRTs use the fact that in comparing two nested models, if the simpler of the two models is correct, twice the difference in maximized log-likelihoods between the two follows a chi-squared distribution with degrees of freedom equal to the difference in the number of parameters (Neyman and Pearson, 1928a, 1928b; Wilks, 1938; Lewis, Butler and Gilbert, 2011).

In addition, standardized residuals were plotted against the fitted values to check for any systematic lack of fit of the model. For the individual method this was not possible as there were only four data points in each case.

2.2.6 Ethical statement

The ethical approval the field data was issued by the ethics committee for the use of animals from Oswaldo Cruz Foundation, Salvador, Brazil (protocol number 003/2012), which is in accordance to the guidelines of the American Society of Mammalogists for the use of wild mammals in research and the guidelines of the American Veterinary Medical Association for the euthanasia of animals. In addition, the protocol was also approved by Yale University's Institutional Animal Care and Use Committee (IACUC), protocol number: 2012-11498.

2.3 Results

For the simulated data, the optimization process to maximize the likelihood was very successful for perfect datasets using both methods (individual and pooled) with all optimizations converging. However, a difference in performance of the algorithm between the methods was apparent when the data were not perfect. Approximately, 33% and 18% of attempts to estimate abundance did not converge for the individual method in messy and imperfect datasets, respectively, whereas for the pooled method, the corresponding Figures were only 4% and 0.008%, respectively (Table 2.1).

Table 2.1: Summary of the performance of the models for each dataset produced.

	MESSY		IMPERFECT		PERFECT	
	Individual	Pooled	Individual	Pooled	Individual	Pooled
Number of sites	10000	10000	10000	10000	10000	10000

Flat or did not converge	3316 (33%)	380 (4%)	1797 (18%)	8 (0.008%)	0 (0%)	0 (0%)
Accurate	3655 (55%)	4880 (51%)	6943 (85%)	8056 (81%)	8203 (82%)	8151 (82%)

For those cases where estimation was possible, Figure 2.2 shows the distribution of estimated population sizes and probabilities of capture for each type of dataset using the two methods. For the perfect datasets, using the pooled method estimates ranged from 85 to 137 individuals, and 82% were accurate, i.e. the 95% confidence interval contained the true population size. For the individual method, the range of the values was between 85 and 125, and 82% were accurate. Regarding the distribution of probability of capture, the pooled method gave narrower range of values, than the individual method; estimates ranged between 0.30 and 0.60 and between 0.26 and 0.72, respectively.

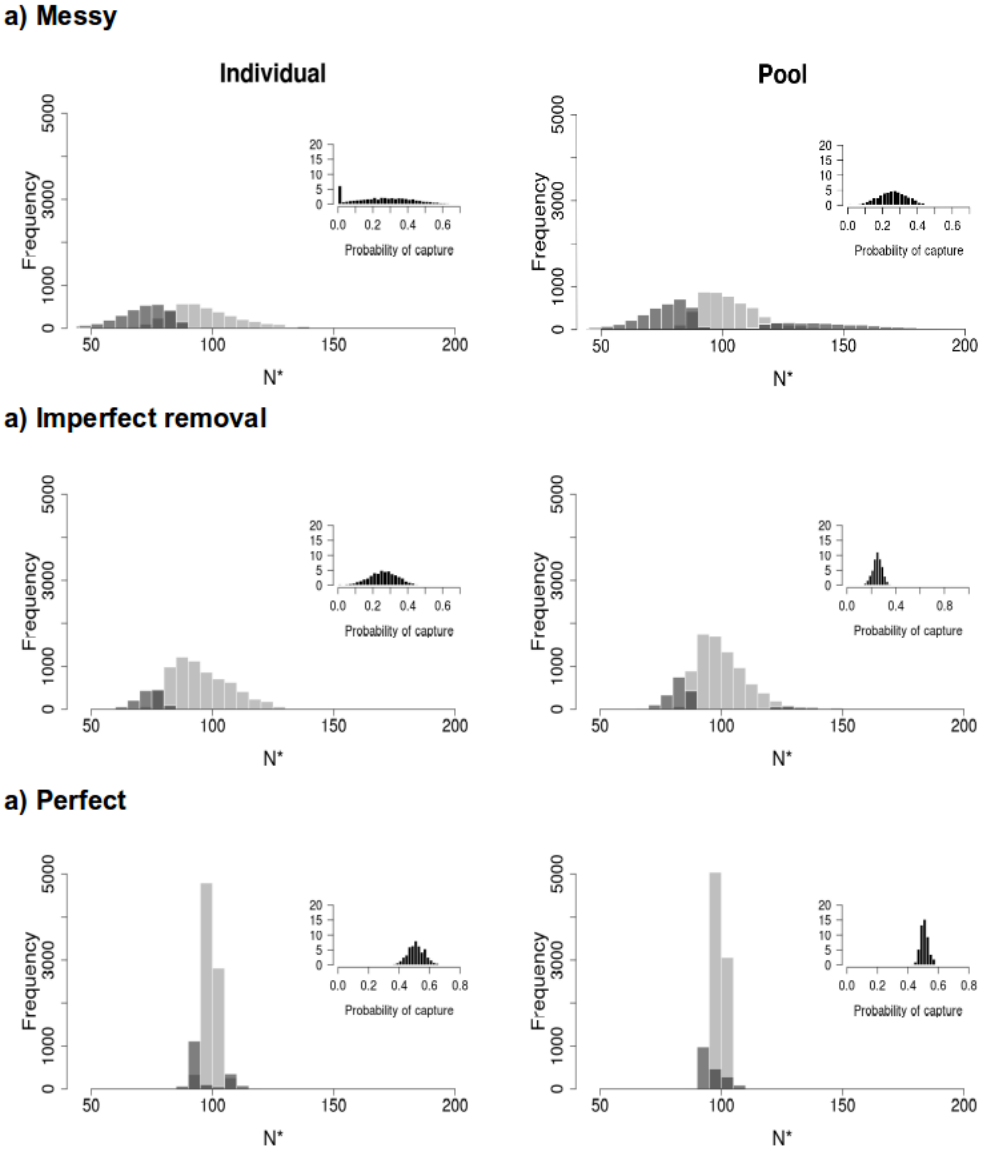

Figure 2.2: Distribution of the estimations using individual and pooled methods for each dataset (Messy, Imperfect removal and Perfect). Light grey bars represents accurate estimations whereas dark grey bars are innacurate. Small graphs inside are the distribution of the probability of capture for each method.

For imperfect removal, the pooled method estimates of the true population size ranged from 66 to 154, with 81% accurate, whereas the individual method estimates ranged from 56 to 144 with 70% accurate. The probability of capture ranged from 1.70×10^{-5} to 0.53 for the individual method and 0.1 to 0.39 for the pooled method. For the messy datasets, the pooled method had a range of 8 to 221 with 49% accuracy, whereas for the individual method the range was from 5 to 156 individuals

with only 37% accuracy (Figure 2.2). Finally, the probability of capture ranged from 5.41 x 10^{-6} to 1 for the individual method and between 9.78 x 10^{-5} and 0.56 for the pooled method.

In terms of the precision of the accurate population size estimates, for the perfect dataset, the pooled method had a mean confidence interval width was 7.0 for the pooled method and 10.6 for the individual method. (Figure 2.3). A similar pattern is seen with the other two datasets, where imperfect removal had means of 35.0 and 89.9 for the pooled and individual methods (Figure 2.3), respectively, while for the messy dataset the corresponding values were 39.7 and 82.7 (Figure 2.3).

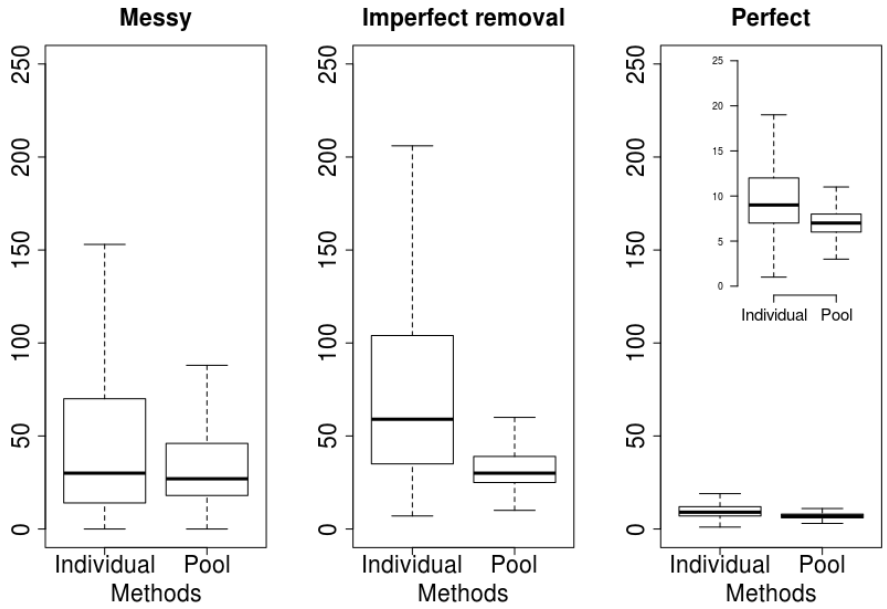

Figure 2.3: Boxplot of the size of the confidence intervals (precision) for the accurate estimations for all datasets. The small graph for perfect dataset is a zoom in of the same graph.

2.3.1 Salvador data

The trapping campaigns in Salvador caught 282 rats in three surveys covering the three valleys (hence nine campaigns overall). Some data had poor patterns of removal, in which the pattern of fewer animals being removed in successive days of trapping was not seen (Table 2.2). For the pooled method, abundance was

estimated in all cases, always with a bounded confidence interval and there was no pattern showing any lack of fit of the model when looking at the residual plots (Figure 2.1 in S1 Appendix). This was observed for three of the nine surveys for the individual method; in the others, no confidence interval could be estimated or the upper bound was infinite. None of the models with CPUE were selected as the best model (Table 2.1 in S1 Appendix).

Table 2.2: Results of capture data and the abundance estimated ($N_{OBSERVED}$) with individual and pooled method for Salvador data.

		VALLEY 4			VALLEY 1			VALLEY 2		
	Event	1	2	3	1	2	3	1	2	3
Capture data	Day 1	20	15	10	9	4	11	12	3	6
	Day 2	15	14	5	13	6	4	8	8	5
	Day 3	16	4	15	9	1	4	8	9	3
	Day 4	11	9	8	6	0	1	7	0	3
	$N_{OBSERVED}$[a]	62	42	38	37	11	20	35	20	17
Pooled method	$N_{ESTIMATED}$[b]	123	83	75	45	13	24	55	31	26
	CI_{lower}[c]	90	60	54	39	11	21	42	23	20
	CI_{upper}[d]	251	171	154	55	17	30	98	56	48
	p[e]		0.16			0.35			0.22	
Individual method	$N_{ESTIMATED}$[b]	113	58	10684	70	11	20	59	32	22
	CI_{lower}[c]	80	47	67	46	NA	NA	41	22	18
	CI_{upper}[d]	453	113	Inf	Inf	NA	NA	Inf	Inf	83

p[e]	0.18	0.27	0.00	0.17	0.58	0.57	0.20	0.21	0.30

[a] Total numbers of animals caught;

[b] Estimated animal abundance;

[c] Lower confidence interval;

[d] Upper confidence interval.

[e] Probability of capture

2.4 Discussion

We have proposed an improvement to the removal method for estimating abundance of a closed population, either assuming a constant probability of capture between surveys (*p*), or a varying probability of capture which can be described by a suitable non-linear regression model. We evaluated the performance of the model in three different situations: variation in *p* (messy datasets), a constant but relatively small *p* (imperfect removal), and a constant but higher *p* (perfect removal). These datasets were created to reflect a range of possible field scenarios. Such field studies can be divided into those that do and those that do not have an approximately constant *p* between surveys, and those where *p* is high (and numbers trapped generally decline between trapping occasions) and those where *p* is low (and numbers often fluctuate), creating four categories of studies in all. Of these, studies where *p* is variable but generally high are clearly more suited to the application of the original, individual method, because it avoids the additional assumption that *p* either is constant or can be assumed to vary according to a suitable non-linear regression model.

We found that even for 'perfect' data sets where the individual method is expected to perform well, the pooled method gave a marginal improvement precision, with a mean confidence interval width of 7.0 compared to 10.6 for the individual method. In contrast, when *p* was constant but low (imperfect removal), in

which case the individual method is known to perform poorly (Borchers, Buckland and Zucchini, 2002), the improvement in performance by using the pooled method was greater: almost 100% of estimations converged, compared with 81% for the individual method, and 81% were accurate compared with 69%. In addition, the mean confidence interval width was 35.0 compared with 89.9.

Finally, when *p* was variable (messy data sets), the improvement in performance by using the pooled method was even greater: 96% of estimations converged compared to 67%, 49% were accurate compared to 37%, and the mean confidence interval width was 39.7 compared to 82.7. Note, however, that these measures of performance must be considered together. For example, in this last case, the mean confidence interval of 82.7 applies only to the 37% of estimates which were accurate (many were relatively precise but inaccurate), and these metrics fail to take account of the 63% of cases where the estimation procedure did not converge. Similarly, the range of the estimates of probability of capture using the pooled method were narrower and closer to the true value in all three types of dataset. However, the bigger difference in their distribution is for the messy dataset, where the distribution of *p* using the pooled method narrowed significantly in comparison with individual method.

Overall, therefore, the simulations indicate that for wide range of scenarios the pooled method is likely to perform better than the individual method. On the other hand, in cases where the data sets are all well behaved (a steady fall over time in the numbers trapped) and there are reasons to be cautious in adopting a regression model for the probability of capture, it will still be preferable to use the individual method.

While analyzing field data on rodent populations in a Brazilian urban slum setting, we could not evaluate accuracy, since the true abundances are not known. Nonetheless, it is notable that several patterns apparent in the simulations are again

evident. With the pooled method, all campaigns had their abundance estimated, all confidence intervals were bounded, and the lower bound was always greater than (or in one case equal to) the number caught. In these senses, all estimates were informative. With the conventional individual method, on the other hand, this was true for only three of the nine campaigns, whereas in the others either the estimation procedure did not converge, or the upper confidence interval was infinite.

The number of sites/surveys to be pooled should be considered cautiously. If the data are too sparse (too much inconsistency in animals caught between sites/surveys), the algorithm might not find an admissible combination of *N* and *p*. In the present study, the data were originally produced by pooling ten sites/surveys, but 20% of the attempts to estimate the abundance failed to converge for messy data sets. Therefore, the data were rearranged and the number of pooled sites/surveys was reduced to five, such that the estimation procedure converged in 96% of the cases for messy data sets.

As noted above, the main issue with abundance estimation using the removal method is the difficulty in validating some of the assumptions of the model. Attempting to overcome this, several authors have assumed that there is variation in each removal occasion driven by the effort used to remove individuals from the population, basing estimates on catch-per-unit-effort (CPUE) or sampling effort (E) (Seber, 1982; Lancia *et al.*, 1996; Borchers, Buckland and Zucchini, 2002). The new method presented here incorporates variable sampling effort in the analysis, provided this can be modelled using available information, for example trapping effort or time of year, that might affect the probability of capture in particular contexts. This extends the applicability of the method to other situations where there are variations in catchability and the cause of these can be identified. For example, in bat surveys the effect of the moon on the detectability of the animals is well known (Morrison, 1978; Mello, Kalko and Silva, 2013; Saldaña-Vázquez and

Munguía-Rosas, 2013). The phase of the moon should therefore be considered as affecting the probability of capture in the abundance estimation of bats. However, for the Salvador data, there was no support for assuming heterogeneity in effort (Table 2.1 in S1 Appendix); a model with variable sampling effort did not significantly improve the fit to the data.

Overall, our results provide support for the use of the pooled method even in circumstances where the probability of capture *p* is high and the individual method would be expected to perform satisfactorily. On the other hand, if *p* is high but also variable for identifiable reasons (for example, different environments), then the individual method may be preferred. The main benefits of using the pooled model are likely to arise where *p* is low, and/or variation in *p* can be modelled using available covariate information. Our simulation results also suggest that the pooled method is more robust to stochastic variation in *p.* For our application, all pooled surveys took place in the same valley in Pau da Lima. As stated previously, much of the variation observed with real trap datasets like these may be random variation in the number of animals trapped rather than a reflection of underlying differences in the probability of capture. In such cases, the pooled method provides estimates of abundance when the individual method would be, at best, inefficient. Given that such data are often difficult and expensive to obtain, we believe that this improved method will often prove valuable.

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2.7 S1 APENDIX

Table 2.1: Results of the likelihood ratio test of a model with catch-per-unit-effort for each valley in Salvador, Brazil. In all cases the simple model was considered the best model to estimate the abundance of rats.

Valley	Model type	l(θ)	k	p
4	simple	-30.92	4	
	effort	-30.27	5	0.254
1	simple	-22.89	4	
	effort	-21.80	5	0.140
2	simple	-26.75	4	
	effort	-26.60	5	0.584

l(θ) is the maximum log-likelihood of the model; k is the number of parameters of the model; p is the p-value.

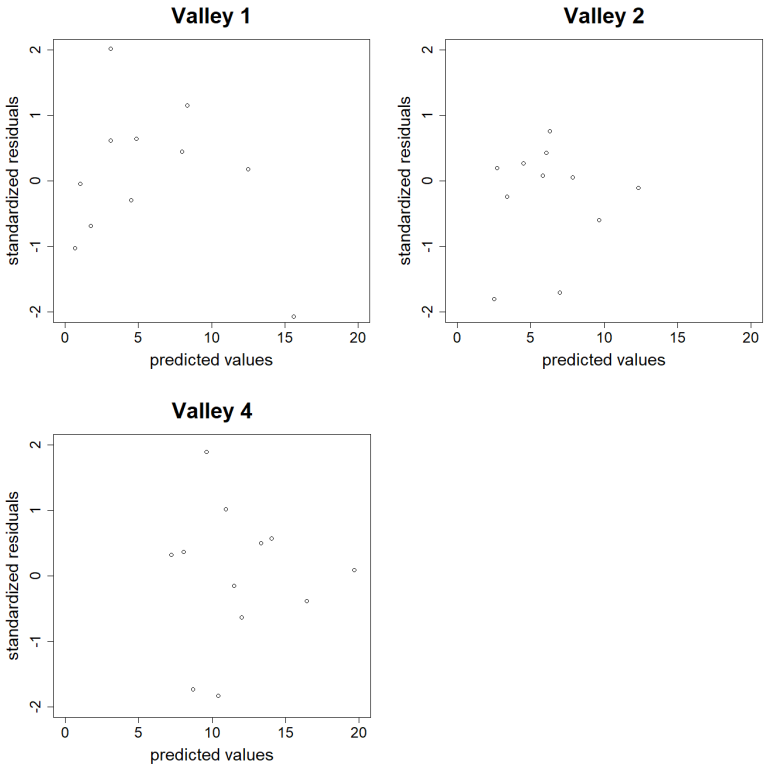

Figure 2.1: Standardized residuals of the estimations of rat abundance from three valleys in Pau da Lima, Salvador, Brazil. The estimations were produced using pooled method.

2.8 S2APPENDIX

Source animal abundance

Gabriel G. Pedra, Amanda Minter, Soledad Serrano, Arsinoe Pertile, Federico Costa, James Childs, Albert Ko, Peter Diggle & Mike Begon

Individual method

Likelihood function

Borchers et al. (2002) proposed a method to estimate the abundance on a site using the binomial distribution. The parameters of interest are the number of individuals on occasion one, N_1, the probability of capture (p). N_1 is an integer and p is bounded from zero to one. All animals are assumed to have the same probability of capture. The binomial log-likelihood function described in the manuscript to estimate those parameters is:

$$l(n|p,N) = \sum_{s=1}^{S} \log\left(\frac{(N_s)!}{n!(N_s-n_s)!}\right) + n_s \log p + (N_s - n_s)\log(1-p)$$

Where $N_s = N_1 - R_s$ and $R_s = \sum_{j<s}^{S} n_j$. S is the number of removal occasions and n and R are vectors of the number of animals caught and the cumulative number of removals in each occasion s respectively.

$$n = n_1, n_2, n_3 ... n_S$$

$$R = R_1, R_2, R_3 ... R_S$$

The model assuming the effect of probability of capture by effort (l_s) would replace p by:

$$p_s = \frac{1}{(1+e^{-\alpha_s})}$$

where:

$$\alpha_s = \beta_0 + \beta_1 * l_s$$

The model with effort will not be shown in the example but the code for the likelihood and the gradient function will be available bellow. R has a built in binomial function **dbinom** which we use to evaulate our likelihood function:

```
####  LOG-LIKELIHOOD FUNCTION  ####
loglik<-function(N1,alpha,n,R){
  N1<-round(N1,0)
  p<-1/(1+exp(-alpha))
sum(dbinom(n,size=(N1-R),prob=p,log=T))
}
####  LOG-LIKELIHOOD FUNCTION WITH EFFORT  ####
loglik<-function(N1,beta0,beta1,n,R,l){
  N1<-round(N1,0)
  loglik<-0
  for (j in 1:S){       #S is the number of surveys
    alpha<-beta0+beta1*l[j]
    p<-1/(1+exp(-alpha))
    loglik<-loglik+dbinom(n[j],size=(N1-R[j]),prob=p,log=T)
  }
```

```
  loglik
}
```

Optimization

We use the **optim()** function to estimate the parameters using maximimum likelihood. The algorithm to optimize the function was *Nelder-Mead*, which is robust and works well for non-differentiable functions (see `?optim`). The **optim()** function performs minimization but will maximize when the gradient function is negative.

```
####  GRADIENT FUNCTION  ####
fn<-function(par){
  N1<-par[1]
  alpha<-par[2]
  result<--loglik(N1,alpha,n,R)
  result
}
####  GRADIENT FUNCTION EFFORT MODEL  ####
fn<-function(par){
  N1<-par[1]
  beta0<-par[2]
  beta1<-par[3]
  result<--loglik(N1,beta0,beta1,n,R,l)
  result
}
```

Now, considering one of the sites of Salvador data (Valley one, event one), the number of animals caught and its cumulative removals are:

```
n<-c(20,15,16,11)
R<-c(0,20,35,51)
```

The optimization process is:

```
####  STARTING POINTS  ####
N<-150
p<-0.5
x<-log(p/(1-p))
par<-c(N,x)

####  OPTMIZATION  ####
fit1 <- optim(par,fn,hessian=F)
repeat{
  par<-fit1[[1]]
  fit1<-optim(par,fn,hessian=F) # repeat as necessary
  par1<-fit1[[1]]
  if(identical(round(par,4),round(par1,4))==TRUE)break
}
####  CHECK CONVERGENCE  ####
fit1$Convergence  #It has to be zero in order to successful convergence
```

And its abundance estimation ($\hat{N}$) and probability of capture ($\hat{p}$) are:

```
####  RESULTS  ####
res<-fit1[[1]]
Nhat<-round(res[1],0) #abundance estimated
phat<-1/(1+exp(-res[2])) #probability of capture estimated
```

Profile likelihood based confidence intervals

Standard methods of obtaining confidence intervals from normal distribution could not be applied. *optim()* is a continuous optimizer but N_1 is an integer, so `hessian` matrix is not appropriate in this case. Profile likelihood confidence intervals are based on the asymptotic chi-square distribution of the log likelihood ratio test statistic.

Considering our model with parameters N_1 and p, the parameter of interest is N_1 and p is the nuisance parameter. Given $l(N_1,p)$ is the log-likelihood function, the profile log-likelihood function of N_1 is:

$$l_l(N_1) = \max_p l(N_1, p)$$

$l_l(N_1)$ is the maximum of the likelihood function for each value of N_1.

The profile likelihood based confidence intervals states that a $100(1-\alpha)\%$ confidence interval for N_1 is the set of all values of N_{1_0} in which the null hypothesis, $H_0 : N_1 = N_{1_0}$, would not be rejected at an α level of significance. Therefore, the confidence interval for N_1 is:

$$N_1 : \left[2[l(\hat{N_1}, \hat{p}) - l(N_{1_0}, \hat{p_0})] < \chi^2_{1-\alpha}(1)\right]$$

Where $l(\hat{N_1}, \hat{p})$ is the maximum likelihood estimator (MLE) for the full model and $l(N_{1_0}, \hat{p_0})$ is the MLE for the reduced model considering $N_1 = N_{1_0}$. $\chi^2_{1-\alpha}(1)$ is the quantile for a χ^2 distribution with one degree of freedom.

A new likelihood function has to be built in order to maximize p for each candidate value of N_1 (which is a vector of fixed values *nvec*). Then taking the ratio difference between the profile function and the full model to, finally, check which one of the differences are considered same as the full model (based on the χ^2 distribution).

```
####  PROFILE LIKELIHOOD  ####

nvec <- (max(R)+n[4]):400   #vector of fixed values of N1, integer values

####  GRADIENT FUNCTION WITH JUST ONE UKNOWN PARAMETER, p  ####
fn1<-function(par){
  alpha<-par
  result<--loglik(N1,alpha,n,R)
  print(c(N1,result))
  result
}

####  PARAMETER p  ####
p<-0.5
par<-log(p/(1-p))

####  OPTIMAZATION  ####
LogL <- numeric(length(nvec))
for (i in 1:length(nvec)) {     #optimize 'p' for each value of N1
  N1<-nvec[i]
  LogL1 <- optim(par,fn1,method="BFGS")
  repeat{
    par1<-LogL1[[1]]
    LogL1<-optim(par1,fn1,method="BFGS") # repeat as necessary
    par2<-LogL1[[1]]
```

```
    if(identical(round(par1,4),round(par2,4))==TRUE)break
  }
  LogL[i]<-LogL1[[2]]
}

ratio<-2*(fit1[[2]]-LogL)  #ratio between logL(Nhat,phat)-logL(N,phat)

#### CONFIDENCE INTERVALS FOR N  ####
Nint<-nvec[which(ratio>=-1.92)]
if(max(nvec)==max(Nint)){  #if the maximum values are the same, it means a flat likelihood and superior
  cinf<-Nint[which.min(Nint)]
  csup<-"Inf"
}else{cinf<-Nint[which.min(Nint)];csup<-Nint[which.max(Nint)]}
```

Pool method

Likelihood function

Our proposed method assumed that the probability to catch an animal is the same in different surveys. Therefore, the surveys were pooled together and the model proposed by Borchers *et al.* (2002) was modified to add the new assumption to it. Then, the log-likelihood for the pool method is:

$$l(\mathbf{n}|p,\mathbf{N}) = \sum_{i=1}^{I}\sum_{s=1}^{S} \log\left(\frac{(N_{is})!}{n!(N_{is}-n_{is})!}\right) + n_{is}\log p + (N_{is}-n_{is})\log(1-p)$$

Where I is the number of surveys, $N_{is} = N_{i,1} - R_{is}$ and $R_{is} = \sum_{j<s}^{S} n_{ij}$. Now, n and R are matrices of I columns and S rows.

$$\mathrm{n} = \begin{bmatrix} n_{11} & n_{12} & \cdots & n_{1I} \\ n_{21} & n_{22} & \cdots & n_{2I} \\ \vdots & \vdots & \ddots & \vdots \\ n_{S1} & r_{S2} & \cdots & n_{SI} \end{bmatrix}$$

$$\mathrm{R} = \begin{bmatrix} R_{11} & R_{12} & \cdots & R_{1I} \\ R_{21} & R_{22} & \cdots & R_{2I} \\ \vdots & \vdots & \ddots & \vdots \\ R_{S1} & R_{S2} & \cdots & R_{SI} \end{bmatrix}$$

Again, the model with probability of capture as a function of the effort ($l_{i,s}$) would replace p by:

$$p_{i,s} = \frac{1}{(1+e^{-\alpha_{i,s}})}$$

where:

$$\alpha_{i,s} = \beta_0 + \beta_1 * l_{i,s}$$

And the simple model with no effect of effort using *dbinom* function is:

```
#### LOG-LIKELIHOOD FUNCTION  ####
loglik.pool<-function(N1,alpha,n,R){
  N1<-round(N1,0)   #N1 parameter to be estimated, rounded values
  p<-1/(1+exp(-alpha))   #probability of capture
  logL.pool<-0
  for (i in 1:I){      #for each ith site
    for (j in 1:S){    #for each jth removal occasion
      logL.pool<-logL.pool+dbinom(n[j,i],size=(N1[i]-R[j,i]),prob=p,log=T)
    }
  }
  logL.pool
}
#### LOG-LIKELIHOOD FUNCTION WITH EFFORT  ####
loglik.pool<-function(N1,beta0,beta1,n,R,l){
  N1<-round(N1,0)   #N1 parameter to be estimated, abundance of rats
  logL.pool<-0
  for (i in 1:I){      #for each ith site
    for (j in 1:S){    #for each jth removal occasion
      alpha<-beta0+beta1*l[j,i]
      p<-1/(1+exp(-alpha))   #probability of capture with CPUE
      logL.pool<-logL.pool+dbinom(n[j,i],size=(N1[i]-R[j,i]),prob=p,log=T)
    }
  }
  logL.pool
}
```

OPTIMIZATION

We use *optim()* function as previously described. The gradient function is:

```
####  GRADIENT FUNCTION  ####
fn.pool<-function(par){
  N1<-round(par[1:I],0)
  alpha<-par[I+1]
  result<--loglik.pool(N1,alpha,n,R)
  print(c(N1,result))
  result
}
####  GRADIENT FUNCTION EFFORT MODEL  ####
fn.pool<-function(par){
  N1<-round(par[1:I],0)
  beta0<-par[I+1]
  beta1<-par[I+2]
  result<--loglik.pool(N1,beta0,beta1,n,R,l)
  result
}
```

Now, using Salvador as an example, to estimate the Valley one using the simple model with no effort.

```
####  DATA  ####
n<-read.csv('n.csv',h=T)
R<-read.csv('R.csv',h=T)


I<-length(n[1,])  #NUMBER OF SITES
```

```
S<-length(n[,1])  #NUMBER OF REMOVAL OCCASIONS

####  INITIALS  ####
N<-rep(150,I)
p<-0.5
x<-log(p/(1-p))
par<-c(N,x)

####  OPTIMIZATION  ####
fit2 <- optim(par,fn.pool)
repeat{
  par<-fit2[[1]]
  fit2<-optim(par,fn.pool) # repeat as necessary, for convergence
  par1<-fit2[[1]]
  if(identical(round(par,4),round(par1,4))==TRUE)break
}

####  CHECK CONVERGENCE  ####
fit2$Convergence  #It has to be zero in order to successful convergence
```

Saving the results for each event on Valley one:

```
res<-fit2[[1]]
Nhat<-round(res[1:I],0) #abundance estimated
phat<-1/(1+exp(-res[(I+1)])) #probability of capture estimated
```

Profile likelihood based confidence intervals

We use the same process of obtain the profile likelihood as before, however, because the likelihood function has more parameters of interest, the process is a bit more complex, as for each paramter of interest a profile has to be created. Then, our code for the profile likelihood is:

```
nvec1<-min(R[S,])+n[S,which.min(R[S,])]:400   #defining the vector of fixed values to get the profile f
                                              #value of N1
logl<-matrix(NA,length(nvec1),I)   #saving the results

#####Running the profile likelihood for each kth parameter and each ith fixed value
#This function, define where the fixed value will be, as there are more parameter of interest
for (k in 1:I){    #kth site/ I=number of sites
  k1<-k
  if(k1<=(I-2)){
  fn<-function(par){
      N1[0:(k1-1)]<-par[0:(k1-1)]    #arranging parameters to be estimated
      N1[(k1+1):I]<-par[(k1):(I-1)]
      N1[k1]<-nvec1[i]              #adding the fixed values for the k parameter
      alpha<-par[I]
      result<--loglik.pool(N1,alpha,n,R)
      print(c(N1,result))
      result
  }}
  if(k1==(I-1)){
    fn<-function(par){
      N1[0:(k1-1)]<-par[0:(k1-1)]   #arranging parameters to be estimated
      N1[I]<-par[(I-1)]
      N1[k1]<-nvec1[i]              #adding the fixed values for the k parameter
```

```
      alpha<-par[I]
      result<--loglik.pool(N1,alpha,n,R)
      print(c(N1,result))
      result
    }}
  if(k1==I){
    fn<-function(par){
      N1[0:(k1-1)]<-par[0:(k1-1)]  #arranging parameters to be estimated
      N1[k]<-nvec1[i]              #adding the fixed values for the k parameter
      alpha<-par[I]
      result<--loglik.pool(N1,alpha,n,R)
      print(c(N1,result))
      result
    }}
    #After determined which N1 will be fixed, then opmize 'p' for each value
  for (i in 1:length(nvec1)){    #ith fxed values
    N1<-numeric(0)
    N<-rep(150,I-1)        #initial condition for parameters N
    p<-0.5
    x<-log(p/(1-p))        #probability of capture
    par<-c(N,x)
    fit<-try(optim(par,fn),silent=T)   #use try function because of the range of values which might be
    if('list' %in% class(fit)){
      repeat{       #repeat as necessary
        par<-as.numeric(fit[[1]])
        fit<-try(optim(par,fn),silent=T)
        testt<-as.numeric(fit[[1]])
        if (identical(round(par,4),round(testt,4))==TRUE) break
      }
      if('list' %in% class(fit)){
        logl[i,k]<-fit[[2]]    #saving results
      }else{logl[i,k]<-NA}
    }
  }
}


ratio<-2*(fit2[[2]]-logl)  #ratio between logL(Nhat,phat)-logL(N,phat)

##confidence based on profile
cinf<-matrix(NA,1,I)
csup<-matrix(NA,1,I)
for (i in 1:I){
  Nint<-nvec1[which(ratio[,i]>=-1.92)]
  if (length(Nint)!=0){
    if(max(nvec1)==max(Nint)){
      cinf[1,i]<-Nint[which.min(Nint)]
      csup[1,i]<-"Inf"
    }else{cinf[1,i]<-Nint[which.min(Nint)];csup[1,i]<-Nint[which.max(Nint)]}
  }}
```

Simulation code

Generating datasets

We generated three different datasets, but all with four consecutives removal occasions in each survey and the true value of the population of 100 individuals. Primarily, we pooled 10 surveys in each set of data to use pool method, so 1000 sets of 10 pooled surveys were generated for each type of dataset:

```
library(arrayhelpers)
messy<-function(N1,I,sim){  #N1-true value; I- number of surveys; sim- N of simulations generated
x<-1
nhat<-array(NA,dim=c(4,I,sim))
for (j in 1:sim){
  for (i in 1:I){
    n1<-rbinom(x,N1,rnorm(1,0.24,0.1))
    if(n1==0||is.na(n1)==TRUE){repeat{
      n1<-rbinom(x,N1,rnorm(1,0.24,0.1))
      if(n1!=0||is.na(n1)==FALSE)break
    }
    n2<-rbinom(x,(N1-n1),rnorm(1,0.24,0.1))
    n3<-rbinom(x,(N1-n1-n2),rnorm(1,0.24,0.1))
    n4<-rbinom(x,(N1-n1-n2-n3),rnorm(1,0.24,0.1))
    nhat[,i,j]<-c(n1,n2,n3,n4)
    }else{
      n2<-rbinom(x,(N1-n1),rnorm(1,0.24,0.1))
      n3<-rbinom(x,(N1-n1-n2),rnorm(1,0.24,0.1))
      n4<-rbinom(x,(N1-n1-n2-n3),rnorm(1,0.24,0.1))
      nhat[,i,j]<-c(n1,n2,n3,n4)
    }
  }}
  return(nhat)}

messy.data<-messy(100,10,1000)
```

However, we rearranged the data and pooled five sites in each set, hence the number os sets increased from 1000 to 2000.

```
dim(messy.data)<-c(4,5,2000)
```

For Imperfect removal:

```
imperfect<-function(N1,I,sim){  #N1-true value; I- number of surveys; sim- N of simulations generated
x<-1
nhat<-array(NA,dim=c(4,I,sim))
for (j in 1:sim){
  for (i in 1:I){
    n1<-rbinom(x,N1,0.24)
    if(n1==0||is.na(n1)==TRUE){repeat{
      n1<-rbinom(x,N1,0.24)
      if(n1!=0||is.na(n1)==FALSE)break
    }
    n2<-rbinom(x,(N1-n1),0.24)
    n3<-rbinom(x,(N1-n1-n2),0.24)
    n4<-rbinom(x,(N1-n1-n2-n3),0.24)
    nhat[,i,j]<-c(n1,n2,n3,n4)
    }else{
```

```
      n2<-rbinom(x,(N1-n1),0.24)
      n3<-rbinom(x,(N1-n1-n2),0.24)
      n4<-rbinom(x,(N1-n1-n2-n3),0.24)
      nhat[,i,j]<-c(n1,n2,n3,n4)
    }
  }}
  return(nhat)}

imperfect.data<-imperfect(100,10,1000)
```

Rearranging data:

```
dim(imperfect.data)<-c(4,5,2000)
```

For Perfect removal:

```
perfect<-function(N1,I,sim){  #N1-true value; I- number of surveys; sim- N of simulations generated
x<-1
nhat<-array(NA,dim=c(4,I,sim))
for (j in 1:sim){
  for (i in 1:I){
    n1<-rbinom(x,N1,0.5)
    if(n1==0||is.na(n1)==TRUE){repeat{
      n1<-rbinom(x,N1,0.5)
      if(n1!=0||is.na(n1)==FALSE)break
    }
    n2<-rbinom(x,(N1-n1),0.5)
    n3<-rbinom(x,(N1-n1-n2),0.5)
    n4<-rbinom(x,(N1-n1-n2-n3),0.5)
    nhat[,i,j]<-c(n1,n2,n3,n4)
    }else{
      n2<-rbinom(x,(N1-n1),0.5)
      n3<-rbinom(x,(N1-n1-n2),0.5)
      n4<-rbinom(x,(N1-n1-n2-n3),0.5)
      nhat[,i,j]<-c(n1,n2,n3,n4)
    }
  }}
  return(nhat)}

perfect.data<-perfect(100,10,1000)
```

Rearranging data:

```
dim(perfect.data)<-c(4,5,2000)
```

Simulation code

Use the source code for the simulation and run and save the results:

```
res.messy<-simu(messy.data)
res.imperfect<-simu(imperfect.data)
res.perfect<-simu(perfect.data)
```

Chapter 3: Survival analysis of *Leptospira spp*. in microcosms

3.1 Introduction

Leptospirosis is a disease caused by a spirochete bacterium of the genus *Leptospira*. It is a worldwide disease that affects over one million people each year, of which more than 50% occurs in poor and developing countries (Costa, Hagan, *et al.*, 2015). Its epidemiology has changed over the recent years in that it used to be considered a rural disease but now is more often seen as an occupational urban disease associated with poverty. All mammals can get infected, but the main reservoirs of the disease are rodents that shed the bacteria into the environment through urine with humans getting infected through the contact with an environment contaminated by this urine (Albert I Ko *et al.*, 1999).

Once the bacteria are released into the environment, there are certain conditions that can affect their survival such as pH, salinity and water content. *Leptospira* can survive in the environment from hours to months. Saito *et al*. (2013), for example, observed the presence of the bacteria in a puddle for over five months and found that the bacteria survived in wet soil during dry days and appeared in the surface water after the rain. In water, experiments have shown that the bacteria can survive up to several months, whereas in the soil the longest lifespan reported was 193 days (Chang, Buckingham and Taylor, 1948; Smith and Self, 1955; Kirschner and Maguire, 1957; Okazaki and Ringen, 1957; Smith and Turner, 1961; Hellstrom and Marshall, 1978; Khairani-Bejo *et al.*, 2004; Saito *et al.*, 2013, 2014; Andre-Fontaine, Aviat and Thorin, 2015). Despite all the interest in understanding the survival conditions of the bacteria in the environment, all those studies used culture techniques or direct animal inoculation which is time consuming, insensitive and

prone to errors such as the overgrowth of autochthonous microbiota. In addition, those methods are qualitative and the quantitative survival of *Leptospira* remains unknown.

Determining how long a parasite and/or microbe can persist in their environment has an important role in studies of the dynamics of diseases, mainly because this can help to predict areas with high risk for infection and to determine the appropriate duration and frequency of an intervention. The determination of the process behind cell inactivation (death) started with Chick (1908) where the first model for survival curves was proposed. His work is based on the first order of a chemical reaction, which assume that population survival depends on the initial concentration and a rate of decay. The product of this reaction through time on a logarithm scale is a straight line and it is called a first order kinetic model.

Nowadays, it has been observed that the proportion in which cell die is not a linear function, and thus this process is not always driven by kinetic order (Peleg and Cole, 1998a). The kinetic order, for example, does not cover situations in which the cells are not affected equally by the environment and die at a different rate (Peleg and Cole, 1998a). Hence, different models have been developed to represent different curves which are widely used in food microbiology (Peleg and Cole, 1998b; Xiong *et al.*, 1999; Geeraerd, Herremans and Van Impe, 2000; Nevecherya *et al.*, 2005). Their main interest is to know how long it takes for a microbe population to decrease to zero under certain conditions.

There are four commonly observed types of survival curves (on semi-log plots): linear curves (Figure 3.1, curve A), curves with a shoulder (Figure 3.1, curve B), curves with a tail (biphasic curves) (Figure 3.1, curve C and D) and sigmoidal curves (Figure 3.1, curves E and F). In some systems, there is a time lag before the population starts to react to a certain condition and these are described by curves with a shoulder. In addition, some population have resistant cells, which have a

different decay rate, or residual cells that persist for longer. These are represented by biphasic curves. When both situations can happen, this is represented by a sigmoidal curve (Xiong *et al.*, 1999).

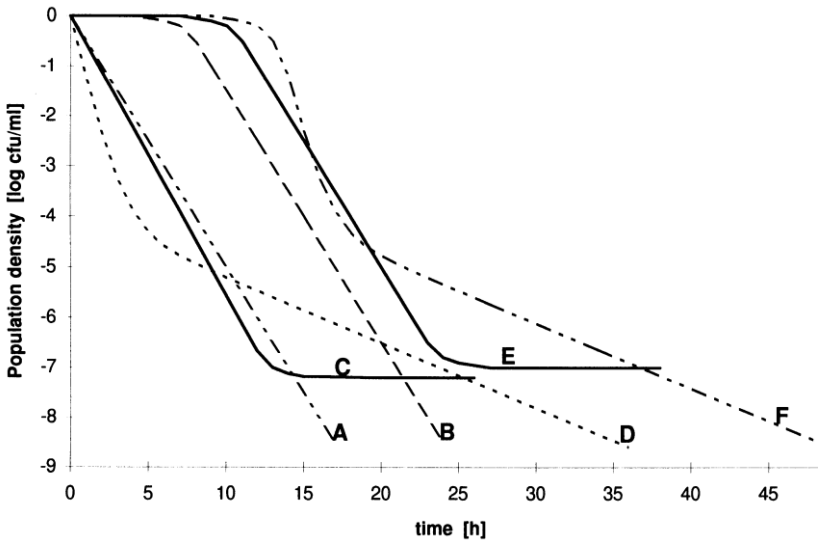

Figure 3.1: Graph extracted from Xiong et al. (1999) representing the different types of bacterial survival curves.

From each survival curve, a variety of models have been developed. For example, biphasic curves can be formed because the bacteria population can be divided into two distinctive groups, more and less resistant cells, and a model with two linear curves was adapted from the first order kinetic model. On the other hand, the biphasic shape can also occur because dead cells aggregate, creating a microclimate which increases the survival of the remaining cells, and there is a different model for this situation (see Xiong *et al*. (1999) for a review of the survival models). However, many models still rely on the assumption of knowing the shape of the survival curve and only the rate of decay is estimated. To overcome some of these issues, Weibull functions have been used, where the shape of the survival and the decay can be estimated together. Weibull survival functions can estimate most of the shapes in Figure 3.1 when combined with a long survival sub-population term but it does not consider D and F curves where there is a slow decay. Here in this chapter, the quantitative survival curve of the bacteria *Leptospira* will be

addressed by developing a survival model where the shape of the survival will be estimated together with its decay rate.

Understanding the survival of *Leptospira* in the environment is particularly important because this can provide insights into the role of the environment in driving both reservoir and human infection. It is well known from epidemiological studies that water and other environmental variables such as distance to sewers are important risk factors for leptospirosis (A I Ko *et al.*, 1999; Sarkar *et al.*, 2002; Reis *et al.*, 2008; Oliveira *et al.*, 2009; Lau *et al.*, 2016; Zhao *et al.*, 2016). Therefore, knowing the survival of *Leptospira* in the environment can indicate how long after shedding it is possible to find live bacteria, which are the ultimate risk factor for leptospirosis.

3.2 Materials and methods

3.2.1 Microcosms

To perform a survival experiment of *Leptospira*, microcosms were created where the bacteria was inoculated into different matrices and their concentration evaluated. Two species of *Leptospira* were used in the microcosms, *Leptospira interrogans* serovar Copenhageni strain Fiocruz L1-130 (Nascimento *et al.*, 2004) and *Leptospira biflexa* serovar Patoc strain Patoc1 (Babudieri, 1961). The former is a pathogenic bacteria isolated from an infected person in Salvador, Brazil. The latter is a non-pathogenic (saprophyte) isolated from the environment. A total of six different matrices, which came from soil and water samples, were created to inoculate the bacteria. Soil samples were a sandy loam soil (60% sand, 35% silt, 5% clay and 3.17% organic matter) collected in an urban slum in Salvador (Bahia, Brazil) and a loam soil (40% sand, 35% silt, 25% clay and 12.3% of organic matter) collected in

New Haven (Connecticut, US). To create mud conditions, soil moisture was increased to 35% and 45%, respectively. The water samples were a bottled spring water obtained from a local retailer, and sewage collected from the New Haven wastewater facility after use of a bar screen (large object filter) and grit removal. Finally, some of the soil and water samples were sterilized in order to see the role of the community of other microbes in the persistence of the bacteria in the environment.

Each microcosm was prepared by distributing either 40 g of soil or 40 mL of water or sewage in sterile Pyrex glass beakers. The surface of the microcosm was spiked by dispersing droplets of *Leptospira spp.* suspensions to achieve a concentration of 10^6 cells/g or mL and thoroughly mixed. After spiking, microcosms were thoroughly homogenized, sealed with plastic paraffin film to protect them from external inputs and prevent evaporation, and placed in a humid thermostatic chamber at 29°C under dark conditions. Samples of 1 g or 1 mL were withdrawn from each microcosm at 0, 1, 2, 4, 6, 7, 12, 16, 21 and 28 days, for a total of 10 sampling time points. A growth control was carried out using EMJH medium instead of the environmental matrix. All microcosms were conducted in three independent biological replicates for *L. interrogans* serovar Copenhageni and in two for *L. biflexa* serovar Patoc.

3.2.2 DNA extractions and bacteria quantification

The DNA extraction from soil samples and sewage were based on Power Soil™ DNA Isolation Kit (Mobio), with minor modifications. Spring water and EMJH samples were extracted using a bead beating method with CTAB and phenol/chloroform/isoamyl alcohol. For the PMA assays, spring water was extracted with the automated Maxwell® 16 Cell DNA Purification Kit (Promega).

Two techniques were used to quantify the bacteria concentration, qPCR and PMA-qPCR, representing the quantification of DNA and intact cells respectively.

qPCR: The quantification of DNA consists of running a standard curve on each plate which is used to transform quantification cycles (Cq) into concentrations (genome equivalents (GE)/reaction). In addition, non-template controls were randomly included in all rows of each plate to discard the presence of contaminating DNA. All negative controls were negative in all cases. For the description of the marker used, calibrators and inhibitors, please see Casanovas-Massana *et al*. (2018) and its supplementary material in the appendix.

PMA-qPCR: The ability of propidium monoazide (PMA) to selectively amplify DNA from membrane-intact *L. interrogans* cells in spring water and Brazilian soil was investigated in the original manuscript. This technique was used to quantify the concentration of intact cells as a more accurate way to consider only infective cells. See Casanovas-Massana *et al*. (2018) for a fuller description of the protocol used.

3.2.3 Statistical modelling

3.2.3.1 Model

Following Peleg and Cole (1998) and van Boekel (2002) we used a Weibull distribution to model the survival time, *T*, with the following survival function:

$$S(t;\phi,k) = P(T > t) = \exp\left(-\left({}^{t}/_{\phi}\right)^{k}\right) : t \geq 0 \quad (1)$$

The parameter k determines the shape of the survival curve, whilst ϕ defines how stretched the shape is; specifically, ϕ is the expectation (average value) of *T*, which following from equation 1 and if we consider a closed population of cells with

initial concentration μ_0 at time *t=0* and measure the concentration of surviving cells at a subsequent time *t*, the expected concentration at time *t* is $\mu_t = \mu_0 S(t; \phi, \kappa)$. However, in our experiments, we observed that a proportion of the cells appeared to survive well beyond the maximum follow-up time. We therefore extended the model to $\mu_t = \mu_0(\alpha + (1-\alpha)S(t; \phi, \kappa))$, where α is the proportion of long-term survivors.

We now consider a set of experiments, *i=1,..,r*, the *ith* of which is characterized by the values of a set of covariates x_i. In each experiment we measure the concentration at a sequence of times $t_j : j = 1,2, \dots, m$. Our model for the complete set of experiments becomes:

$$\mu_{ij} = \mu_0 * (\alpha_i + (1-\alpha_i) * \left(S(t_j; \phi_i, \kappa)\right) \tag{2}$$

In equation (2), the effects of the covariates on the values of ϕ and α were explored to determine if there were any differences in survival between species, treatment and method of quantification by specifying log-linear and logistic models for ϕ and α respectively, hence:

$$\phi_i = e^{x_i'\beta} \tag{3}$$

and

$$\alpha_i = {}^{1}/_{(1 + e^{-x_i'\gamma})} \tag{4}$$

where x_i is the design matrix for the explanatory variables and β and γ its coefficients related to ϕ and α, respectively.

Finally, we assume that observed concentrations Y_{ij} are independent and Normally distributed,

$$Y_{ij} = \mu_{ij} + Z_{ij} \quad i = 1, \dots, r; j = 1, \dots, m \tag{5}$$

where Z_{ij}, the observation level residuals, are Normally distributed $Z_{ij} \sim N(0, \tau^2)$ with variance τ^2.

3.2.3.2 Log-likelihood

The log-likelihood for the complete set of data contains contributions of two kinds: measured values y_{ij} and results recorded only as below-detection, representing values $y_{ij} < d$. Let $f(y; \mu, \tau^2)$ denote the probability density, and $F(y; \mu, \tau^2)$ the cumulative probability distribution, of the Normal distribution mean μ and variance τ^2. Then, the log-likelihood for the complete set of parameters $\theta = (\beta, \gamma, \mu_0, \tau^2)$ is:

$$l(\theta) = \sum_{i=1}^{r} \sum_{j=1}^{m} l_{ij}(\theta) \tag{6}$$

where:

for observations y_{ij} , $l_{ij}(\theta) = \log f(y; \mu_{ij}, \tau^2)$;

for observations $y_{ij} < d$, $l_{ij}(\theta) = \log F(d; \mu_{ij}, \tau^2)$;

The parameters were estimated by optimizing the log-likelihood function, using the *optim()* function in the R software (R Core Team 2014).

3.2.3.3 Confidence intervals

95% Confidence intervals for individual parameters (β, λ, μ_0, k) were calculated as

$$\hat{\theta} \pm 1.96SE(\hat{\theta}) \tag{7}$$

where SE denotes the square root of the variance of $\hat{\theta}$ as given by the information matrix. To calculate 95% confidence intervals for ϕ_i we calculate the variance of $\log(\phi)$ as $v = x_i' Var(\hat{\beta}) x_i$, calculate limits *a* and *b* as $x_i'\hat{\beta} \pm 1.96\sqrt{v}$, then transform *a* and *b* to give the confidence interval (e^a, e^b). Similarly, to calculate 95% confidence intervals for α_i, we calculate $v = x_i' Var(\hat{\gamma}) x_i$, calculate limits *a* and *b* as $x_i'\hat{\gamma} \pm 1.96\sqrt{v}$, then transform *a* and *b* to give the confidence interval $\left({}^{1}/_{(1+e^{-a})}, {}^{1}/_{(1+e^{-b})}\right)$.

3.2.3.4 Checking assumptions

A plot of standardized residuals against fitted values was inspected to check the fit of the model to the data. The plot (Figure 3.2) indicates a reasonably good fit in that the residuals do not deviate away from zero (more than two units) and do not show a clear trend in any direction.

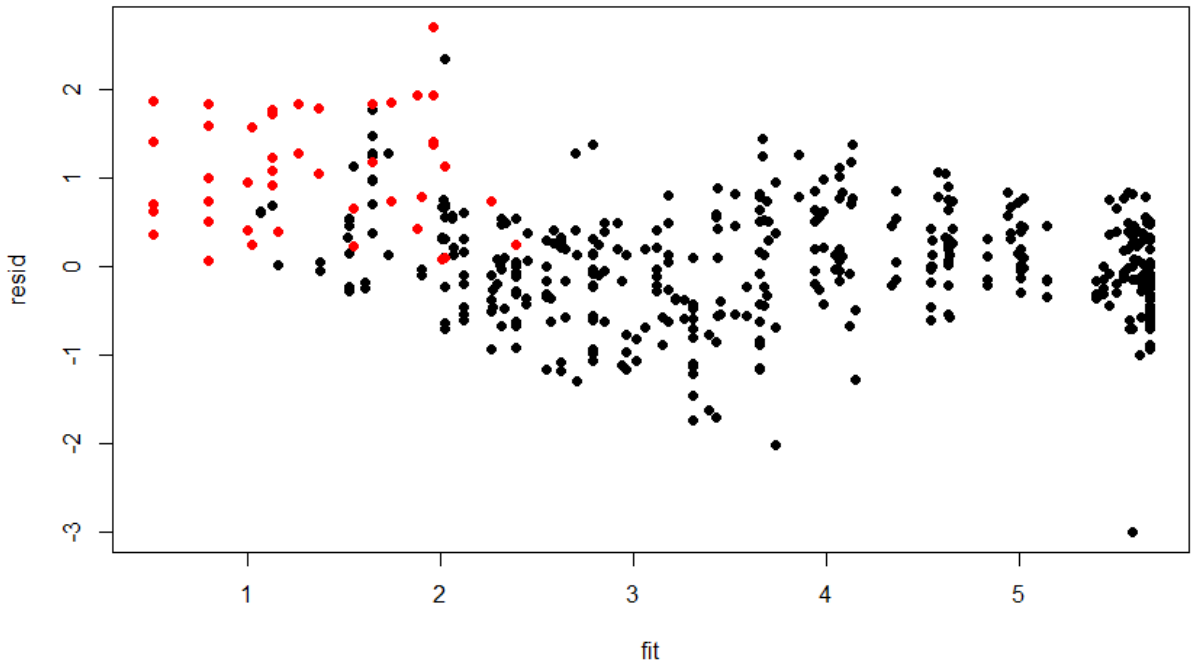

Figure 3.2: Standardized residuals vs fitted values. The red dots are the fitted values for below detection limit samples.

3.2.3.5 Model selection

The selection of the covariates in the model was based on a Likelihood ratio test (LRT), whereby twice the difference between the log-likelihoods of two nested models is compared with critical values of a chi-squared distribution with degree of freedom equal to the difference in the number of parameters in the two models. Firstly, the main effect of each covariate was tested against a null model with no covariates. Then, interactions were tested against the selected main-effects-only model.

3.3 Results

For the quantification of the survival of *Leptospira* in microcosms, there were initially four candidate covariates, species (*L. interrogans* and *L. biflexa*), medium (spring water, soil, mud and sewage), treatment (sterile and non-sterile) and quantification method (qPCR and PMA-qPCR). The model selection showed that treatment did not contribute significantly to the model fit. Hence, the final model only included species, medium and quantification method (Table 3.1).

Table 3.1: Candidate models with effects of the covariates on $\boldsymbol{\alpha}$ and $\boldsymbol{\phi}$ and Likelihood Ratio test (LR) showing the best model. The final model selected is highlighted in bold.

	Log Likelihood	**k***	**df****	**LR**
Null model	-739.74	5	-	-
Treatment	-738.08	7	2	0.19
Species	-736.08	7	2	0.03
Method	-716.83	7	2	<0.0001
Medium	-496.32	15	10	<0.0001
Medium	-496.32	15	-	-
Medium+species	-458.25	17	2	<0.0001
Medium+Method	-265.09	17	2	<0.0001
Medium+Method	-265.09	17	-	-
Medium+Method+Species	-201.46	19	2	<0.0001
Medium+Method+Medium*Method	-257.89	25	8	0.072
Medium+Method+Species	-201.46	19	-	-
Medium+Method+Species+Medium*Method	-196.59	27	8	0.28

* Number of parameters;

** Degrees of freedom is based in the difference between the number of parameters of each pair of comparison.

The shape of the survival curve (k) was 0.75±0.03 and the initial concentration (μ_0) was 5.673±0.041 log10 units, 2.3log10 units different from the concentration

spiked. The estimated rate of decay (ϕ) and the residual population (α) for each experiment are shown in Table 3.2.

Table 3.2: Modelled decay parameters (***ϕ*** and ***α***) and 95% confidence intervals of *L. interrogans* and *L. biflexa* markers in spring water, soil, mud and sewage microcosms. Estimates with intervals that overlap are not significantly different at the 95% significance level.

			φ	**LCI**	**UCI**	***α***	**LCI**	**UCI**
L. interrogans	qPCR	Spring Water	51.45	38.44	68.86	0.90	0.80	0.95
		Brazilian Soil	16.34	13.16	20.29	0.08	0.03	0.17
		Brazilian Mud	14.11	11.05	18.02	0.10	0.05	0.18
		US Soil	4.33	3.07	6.11	0.21	0.14	0.29
		US Mud	5.67	4.13	7.80	0.28	0.21	0.35
		Sewage	2.23	1.66	2.99	0.18	0.13	0.23
	PMA-qPCR	Brazilian Soil	8.20	7.43	9.05	0.00*	0.00	1.00
		Spring Water	25.82	22.46	29.69	0.00*	0.00	1.00
L. biflexa	qPCR	Spring Water	42.16	27.43	64.81	0.96	0.92	0.98
		Brazilian Soil	13.39	9.21	19.46	0.21	0.11	0.37
		Brazilian Mud	11.56	7.94	16.85	0.25	0.15	0.39
		US Soil	3.55	2.42	5.20	0.45	0.39	0.51
		US Mud	4.65	3.15	6.87	0.54	0.48	0.60
		Sewage	1.83	1.32	2.53	0.40	0.36	0.44

* Not significantly different from 0.

The concentration of markers (based on qPCR) for both *L. interrogans* and *L. biflexa* decreased in all the microcosms after spiking (Figure 3.3). No differences were observed between decay rates (***ϕ***) of *L. interrogans* and *L. biflexa* markers in

spring water or soil. In spring water, *Leptospira* markers presented an almost flat decay curve (ϕ = 51.45 and 42.16 for *L. interrogans* and *L. biflexa*, respectively) in which the DNA concentration had decreased by approximately 0.5 $\log_{10}$ units at the end of the experimental time. In addition, more than 90% of the cells survived beyond the time of the experiment in spring water (α = 0.9 and 0.96 for *L. interrogans* and *L. biflexa*, respectively). In contrast, the decay in soil microcosms was significantly faster than spring water (ϕ = 16.34 and 13.39, for *L. interrogans* and *L. biflexa*, respectively), with a rapid decrease during the first 8 days (Figure 3.3; Table 3.2).

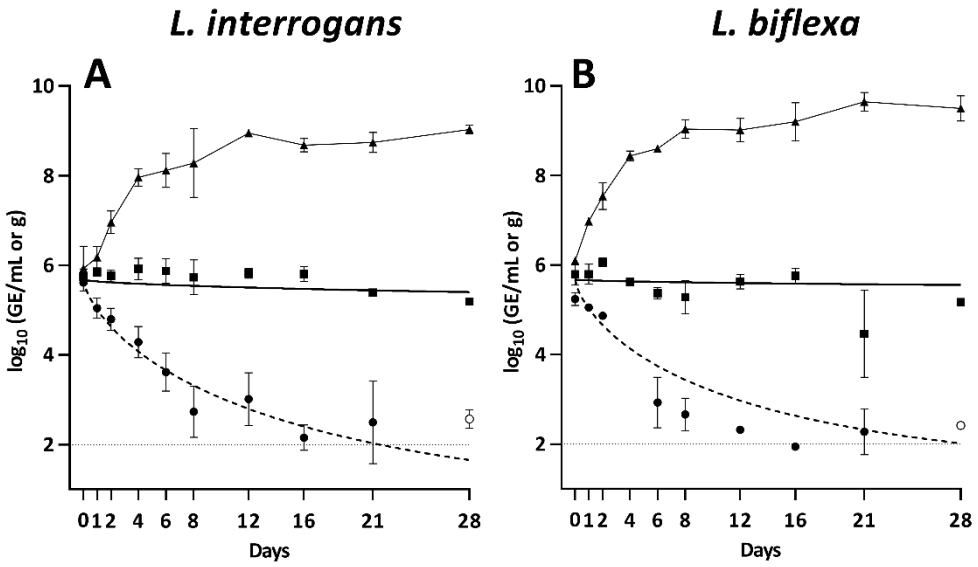

Figure 3.3: Fate of *L. interrogans* (A) and *L. biflexa* (B) markers measured by qPCR in microcosms of spring water (squares), soil (circles) and EMJH media (triangles). The solid line represents the modeled decay curve in spring water and the dashed line in soil. Open symbols represent data points for which at least one observation was below the limit of detection. Error bars indicate standard deviations. The horizontal dashed line indicates limit of detection in soil samples.

Looking at the survival curves and their estimated parameters for mud and soil, the decay parameters ϕ were not statistically different from each other (Figure 3.4;

Table 3.2). However, the decay rates (ϕ) of microcosms with Brazilian medium were significantly slower for *L. interrogans* and *L. biflexa* in comparison with US. Conversely, the proportion of long term survivors (α) was significantly higher for both species in US soil and mud than in Brazilian soil and mud, except for *L. interrogans* in Brazilian soil that showed no difference (Figure 3.4; Table 2). In addition, the proportion of survivors between the two species were different on US soil and mud microcosms, where the proportions were equal to 0.21 and 0.28 respectively for *L. interrogans* and, 0.45 and 0.54 for *L. biflexa* (Table 3.2).

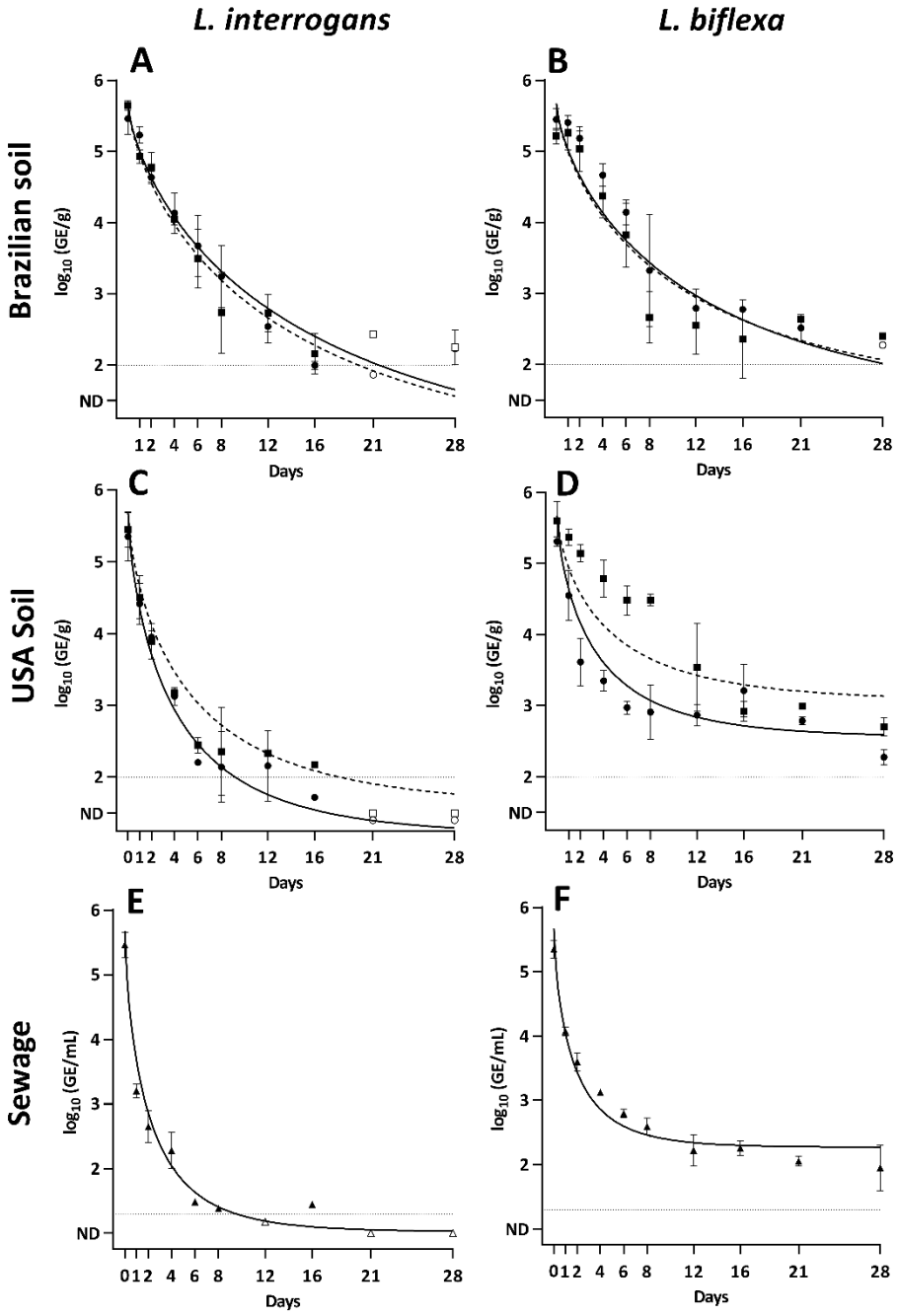

Figure 3.4: Persistence of *L. interrogans* and *L. biflexa* measured by qPCR in microcosms of Brazilian soil (A and B), US soil (D and E) and sewage (F and G). In soil microcosms, circles denote soil adjusted to field capacity and squares denote mud soils. Sewage samples are represented by triangles. The solid line represents the modeled decay curve in field capacity soil and the dashed line in mud soils. Open symbols represent data points for which at least one observation was below the limit of detection. Error bars indicate standard deviations. The horizontal dashed line indicates the limit of detection.

In sewage microcosms, *Leptospira* markers showed a rapid decay (ϕ = 2.23 and 1.83 for *L. interrogans* and *L. biflexa*, respectively), significantly faster than the

decays observed in other media (Figure 3.4E and 4F; Table 3.2). In addition, it was observed that *L. interrogans* markers could only be consistently quantified above the limit of detection for eight days (Figure 3.4E) as opposed to *L. biflexa,* which was detected until the end of the experiment (Figure 3.4F). This result is consistent with the estimated α which indicated that a larger proportion of *L. biflexa* markers persisted beyond the experimental time than *L. interrogans* (Table 3.2).

There was a bigger difference in the concentration of the bacteria depending on the method used in spring water and Brazilian soil microcosms, in that while the decay rate based on qPCR were very slow (ϕ = 51.45 and 16.34 respectively), the PMA-qPCR showed that most of the intact cells died very quickly with ϕ = 25.82 and 8.20 respectively (Figure 3.5; Table 3.2). There was no effect of treatment on the survival of the bacteria, so the curves shown on Figure 3.4 are the same for sterile and non-sterile.

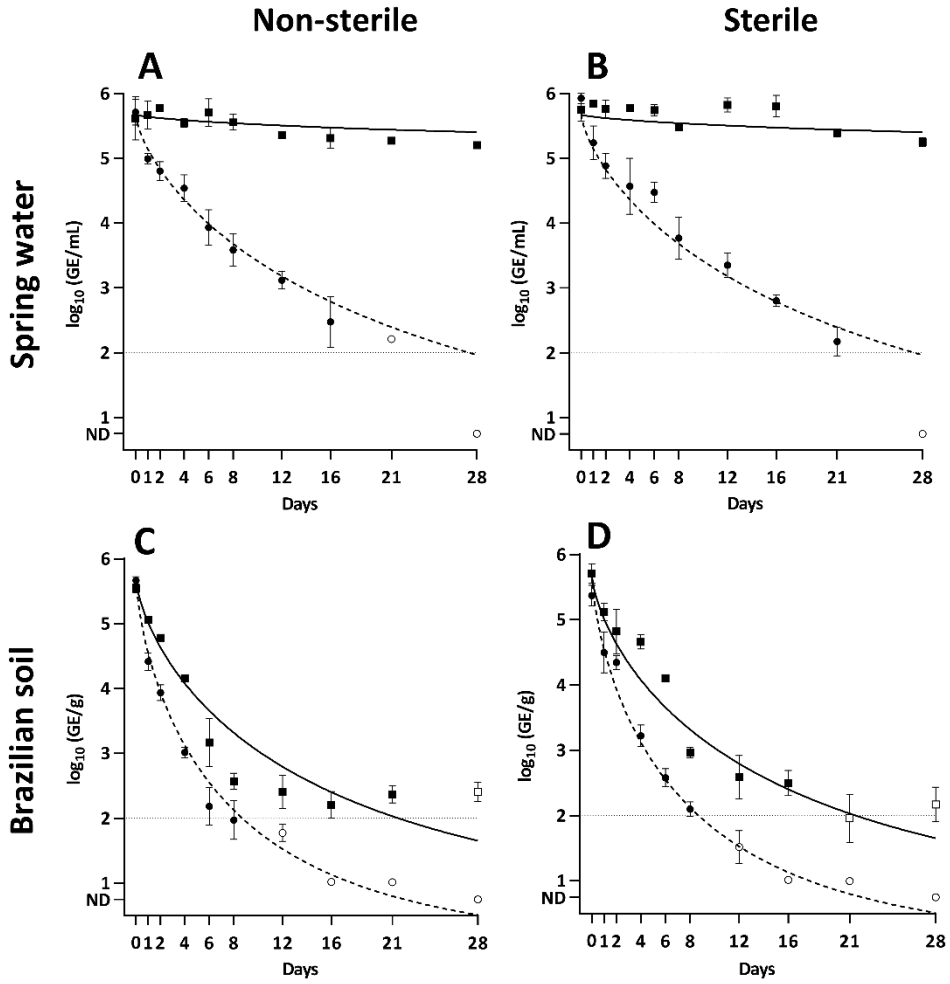

Figure 3.5: Persistence of *L. interrogans* measured by qPCR and PMA-qPCR in sterile and non-sterile microcosms. (A and B) Spring water. (C and D) Brazilian soil. Squares denote measurements by qPCR and circles by PMA-qPCR. The solid line represents the modeled curve for qPCR measurements and the dashed line for PMA-qPCR ones. Open symbols represent data points for which at least one observation was below the limit of detection. Error bars indicate standard deviations. The horizontal dashed line indicates the limit of detection.

3.4 Discussion

Here in this chapter, the survival of *Leptospira* was described in a fully quantitative way for the first time using microcosms experiments. Despite being a laboratory-based experiment, the conditions simulated a real situation, warm weather, and the experiments were standardized to make the different sets of

results comparable. Hence, the same initial concentrations of the bacteria, volume of the medium and temperature were used in all experiments. The role of different medium and species was mainly addressed, but also the method used to quantify the bacteria concentration (qPCR and PMA-qPCR) and the role of autochthonous microbial communities were explored. The quantification method was designed to discriminate between live and dead cells as a proxy for infective cells, whereas, sterile and non-sterile microcosms were created to explore the role of the autochthonous microbial communities in the survival of the bacteria. The first observation was lack of net growth of the bacteria in all microcosms which makes the environment not a habitat but a temporary carrier of the pathogenic bacteria.

The results from these experiments showed that the survival curves, for the two species of *Leptospira*, are biphasic with a tail, where the concentration decreased very quickly within the first couple of weeks then slowed down, and a proportion of individuals survived beyond the time of the experiment. The shape parameter of the Weibull function was lower than one (kappa=0.78), which means that the hazard decreased with time. Despite the mechanisms behind this long survival not being explored here, the formation of biofilms and cell aggregation in water have been shown and could potentially decrease hazard by creating a microenvironment and protecting the cells form dying (Trueba *et al.*, 2004a; Ristow *et al.*, 2008). Alternatively, for other species different processes have been proposed such as population regulation via quorum-sensing, predation and nutrient limitation (Tanaka *et al.*, 1999; Easton *et al.*, 2005).

Looking at the rates in which the cells are dying, it was observed that species of *Leptospira* survive differently depending on the environment. The concentration of the bacteria went down to the limit of the detection of the technique. However, these results are consistent with what is observed in the soil and sewage from an urban slum in Brazil, where the Brazilian soil was collected. Soil and sewage samples

have concentrations of pathogenic bacteria fluctuating around the limit of detection, depending on the location and the time of the day they were sampled (Casanovas-Massana, Costa, *et al.*, 2018).

Two different techniques to detect the bacteria into the environment was used, qPCR and PMA-qPCR which the former only detect DNA and the later detects intact cells. The results for spring water shown a big difference on the decay estimated by qPCR and PMA-qPCR where live cells and dying quicker but the DNA is remaining intact. When looking to the differences in the soil microcosm, qPCR is overestimating the decay but the shape is the same. Despite PMA-qPCR be more close to represent alive cells, this technique is more labor intensive and expensive to use whereas qPCR have more resources available. qPCR can still be recommended to use in soil samples but in spring water samples, it is not a good technique to capture the survival of *Leptospira*.

Studies on the epidemiology of leptospirosis have shown that water is involved in human infection. For example, rainfall, flooding and sewage are widely seen as risk factors for infection (Reis *et al.*, 2008; Oliveira *et al.*, 2009; Desvars *et al.*, 2011b; Felzemburgh *et al.*, 2014b; Zhao *et al.*, 2016). However, the results from the microcosms demonstrate that the survival of the bacteria in the sewage is very short, where more than a half of the population had died off within two days. This result might indicate that the infections could occur from either bacteria recently shed into environment from rats, from the runoff that washes off the soil contaminated, or a combination of both.

A key element of the model developed here was the use of samples below the detection limit, where previous studies have either removed samples below the detection limit from their data analysis or collected survival data only until they reach their detection limit (Kaden *et al.*, 2018; Li *et al.*, 2018). This can be crucial for survival analysis as a sample below the detection limit indicates the least observable

concentration at a time *t*. Here, the likelihood function of a sample that is below the detection limit was based on the cumulative probability function, where the probability of that sample being below or equal to the detection limit is maximized given the parameter values. Hence, this feature in the model makes the use of all data available. This added power provides further support for the parameter estimations.

Two species of *Leptospira* were used in the experiments, a saprophyte and a pathogenic species, with the expectation that the free-living species would either not decrease in abundance or decrease much more slowly than pathogenic species. However, the results observed here showed that *L. biflex* died at a very similar rate to *L. interrogans*. The decay rate of *L. biflexa* was slightly lower than *L. interrogans*, but the main difference was the proportion of long-term survivors, where a higher proportion of individuals survived beyond the time of the experiment for *L. biflexa* (Table 2). The saprophytic species used here was isolated from the environment decades ago, which could be one of the reasons for the fast decay, as the individuals might have lost their ability to survive in the environment. Alternatively, it could be that the environment's carrying capacity is low, around the detection limit, and the usual concentration of the bacteria is lower than the one used in the microcosm. The concentration defined here, 10^7 cells/g or ml, was designed to mimic the concentration of pathogenic bacteria that the rats shed into the environment, which might be different for the usual concentration of saprophytic species. Therefore, the concentration of *L. biflexa* might be decreasing until it reaches the environment's carrying capacity, where they would remain at that concentration, and here it has been shown as a higher proportion of long-term survivors.

This is the first evidence of the survival of the bacteria performed in a way that allowed key hypotheses to be tested. Microcosms were shown to be a useful

technique to represent the environment, although different settings could be explored such as adding variation in temperature, as the temperature is not the same throughout the day in the environment. Also, the long-term survivors are around the limit of the detection of the technique, which could be improved in order to observe lower concentrations values. However, even with some limitations it was possible to observe that the bacteria survive at different rates in the environment, most of the individuals dying within the first couple of weeks but with a proportion of individuals that can persist in the environment for longer. These results can generate further hypotheses regarding the life cycle of *Leptospira* species and the adaptive value of long-term survival, and can also be integrated into species distribution models to better predict and identify areas with high risk of transmission of leptospirosis.

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Chapter 4: Hydrology and its implications to waterborne diseases

4.1 Introduction

The Millennium Development Goals, signed in 2000 by member countries of the United Nations, aimed to increase accessibility to safe drinking water and basic sanitation. Although all countries have increased their access to water and sanitation, many parts of the world, especially the least developed countries, still had not reached the target in 2015. Nearly, 663 million people still do not have access to improved drinking water sources and 2.4 billion people still lack basic sanitation (WHO, 2015).

There are many possible consequences for a population of a lack of sanitation and/or access to safe drinking water sources, but one great concern is waterborne diseases, defined as any disease that is transmitted through water such as cholera, typhoid fever, leptospirosis and diarrheal diseases. The impact of waterborne diseases is enormous, causing significant numbers of deaths (5 million per year), having a big impact on the economy (for example, U$$950 million per year in US) and on people's life and wellbeing (Collier *et al.*, 2012).

In 2004, unsafe water, sanitation and hygiene were responsible for 4% of annual deaths and 5.7% of the health burden world-wide (Disability-Adjusted Life in Years) (Prüss *et al.*, 2002). The main cause of waterborne diseases is faecal contamination, and diarrheal diseases are the most common type of disease reported (Ozioma Forstinus *et al.*, 2016). For diarrheal diseases, 88% of the cases are attributable to unsafe water, sanitation and hygiene (WHO, 2003a). Many efforts have been made to understand the main drivers of infection of waterborne diseases, where in most cases rainfall and flood is a very common risk factor related to the

outbreaks (Curriero *et al.*, 2001; Auld, MacIver and Klaassen, 2004; Pappachan, Sheela and Aravindan, 2004; Gaynor *et al.*, 2007; Lau *et al.*, 2010a; Yang *et al.*, 2012; Cann *et al.*, 2013; Garba, Bahaman, Khairani-Bejo, *et al.*, 2017; SUMI *et al.*, 2017).

In Pau da Lima, a community of Salvador, Brazil, our group have been conducting research to understand the determinants of leptospirosis, a disease caused there by the spirochete bacterium, *Leptospira iterrogans*, by looking at the dynamics of transmission within its host (rat) population, human infection and environmental contamination by the bacteria. Three main factors have led us to explore deeply the effects of the environment on the dynamics of infection. First, there are peaks in number of cases every rainy season (Albert I Ko *et al.*, 1999). Second, 80% of the rats trapped are infected with the bacteria, which indicates a high level environmental contamination (Costa *et al.*, 2014). And third, most of the cases observed occur in the bottom of valleys, where flooding might be frequent (unpublished data). This observation leads to a hypothesis that rainfall might wash out the bacteria in the ground causing its mobilization and increasing the risk of infection in downstream areas.

One approach to test this hypothesis is to track where water flows, considering that water can mobilize microbes from one place to another. This can be from upslope to downslope or from the surface to underground, or vice versa. The results of such mobilization can, for example, increase the concentration of pathogens in areas that receive upslope, contaminated water, and therefore increase the risk of infection in those areas.

The transportation of pathogens in the environment is enhanced by their capability to attach to sediments, which can be weak or strong (Berry and Hagedorn, 1991; Jamieson *et al.*, 2004). Subsequently, the strength of the water flow can detach pathogens from the sediment or mobilize the sediment, hence causing their

transportation. *Cryptosporidium* sp. and *Escherichia coli* are the main microbial models widely studied as an indicator of faecal contamination and waterborne diseases (cryptosporidiosis and diarrheal diseases). Their fate and transportation in different types of soil (for example, soils high in clay content) have contributed to the development of mechanistic models to describe watershed contamination (S. E. Walker *et al.*, 1990; Edwards *et al.*, 1997; Medema and Schijven, 2001; Atwill *et al.*, 2002; Tian *et al.*, 2002; Crowther *et al.*, 2003; Davies *et al.*, 2004; Jamieson *et al.*, 2004).

Ferguson *et al*. (2007), for example, developed a process-based model to predict pathogen contamination of a drinking water catchment in Sydney, Australia. Their model has a hydrological compartment as well as microbiological information, which allows them to identify which sub-catchments contribute most to the pathogen loadings 'downstream'. Similarly, Mahajan *et al*. (2014) developed a dynamic model to quantify pathogen load from sewage overflow to assess microbial risk assessment downstream.

In addition, Mari *et al*. (2012) and Bertuzzo *et al*. (2008) have included hydrological transport of the pathogen into their dynamic epidemiological models to understand the spread of cholera during an epidemic in 2000 in South Africa. Their results support the hypothesis that a cholera epidemic relies on human mobility and the spread through an environmental matrix defined by river corridors (upstream to downstream rivers). Although the hydrological transport of the pathogen was assumed, no transport between catchments was considered.

These models have demonstrated the usefulness of incorporating hydrological information into a dynamic model to understand disease transmission. However, their frameworks rely on high quality information/resolution in which microbial, rainfall and demographic data is collected. Using topographic analysis by itself can be an alternative to provide a cheap but informative risk map for waterborne

diseases as the main outputs of the analysis are based on elevation information mainly.

For example, Vega-Corredor & Opadeyi (2014) used topographic analysis to observe associations with cases of leptospirosis in the West Indies, where their covariates were: a topographic wetness index (TWI – see below), river density, soil permeability and average rainfall. They observed a direct link between leptospirosis cases and areas that are more likely to be flooded (TWI) only, a measurement based on flow and slope. In addition, Herrera *et al*. (2017) have shown that upstream tree cover is associated with lower risk for diarrheal diseases in children. Those results are examples of how basic topography information can provide valuable insights regarding the risk of infection.

Since water can be seen an important risk factor for many waterborne disease, the question arises: Are places where we observe more cases of waterborne diseases the ones that are more likely to be flooded? Or are they likely to receive more upstream water flowing past them? Answering these questions can be crucial in understanding the dynamics of infection and in planning intervention control and surveillance.

Distance to rivers, river density and TWI are derived from topographic data, and have been used in epidemiology. However, other information can be extracted from topographic data. Water flow, for example, can be indicative of where the water passed through and might carry more pathogens hence increasing risk of infection. In addition, demographic data can also be related with waterborne diseases, as human population can be sources of direct or indirect water contamination in some cases and this have not been considered previously.

Depending on the characteristics of the disease, different hydrological maps can be used to understand the determinants of transmission. For example, the main

cause of diarrheal diseases is water contamination. Hence, including human density to evaluate upstream water contamination combined with the likelihood of flooding might be crucial for the risk of transmission. On the other hand, zoonotic diseases with a widespread wildlife reservoir might have their transmission associated with runoff as the reservoir might be assumed to be distributed throughout the environment. In addition, long-term flooding areas might indicate a higher infection dose in comparison with areas where the water flowing past because more pathogens could be coming in and not getting carried out. Therefore, the exploration of other measurements than TWI, distance to river and river density can provide additional insights of the dynamics of the pathogens in the environment and its transmission. Hence, the focus of this chapter is to develop tools that can be used to assess the risk for waterborne diseases based on different hydrological measurements using topographical data combined with population data.

4.2 Material and methods

One of the main aims of topographic analysis is to identify river basins, catchments, mountains and ridge patterns by using location and altitude points. The analysis is performed with a map, frequently called a Digital Elevation Model (DEM), containing X and Y coordinates and Z elevation. Therefore, based on the simple assumption that if all the rainfall that reaches the ground becomes runoff, and there are no barriers, the water will flow to the lowest elevation point (Figure 4.1). This basic hydrological assumption can generate very informative maps of water flow and flow accumulation that can be translated into a flood risk map, for example.

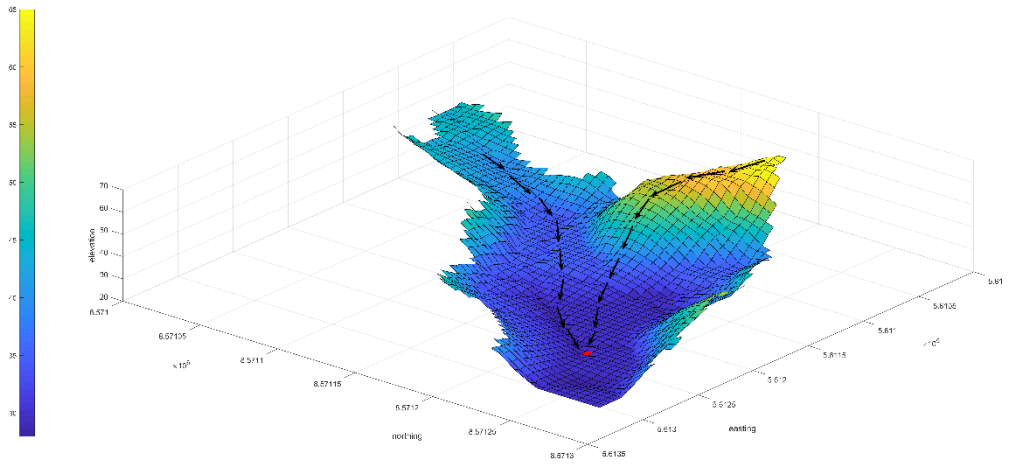


Figure 4.1- 3D graph demonstrating the basic hydrological process of water flowing from upslope to downslope, the red square represent the lowest point in the map and the arrows an example of the water flow. This graph is called a Digital Elevation Model (DEM).

Here, a series of hydrological measurements derived from a DEM and population information are developed so that they can be translated into maps of risk for waterborne diseases: flow accumulation, permanent and temporary rivers, water contamination, water rurality and a topographic wetness index.

Initially, to generate a DEM, a contour map of the elevation data of the city of Salvador with five meters resolution was used, kindly provided by the Urban Planning Department of Salvador, CONDER (BAHIA). The map was then transformed into a gridded map of the same resolution (DEM) using linear interpolation in MATLAB. However, as the elevation map does not account for houses or barriers, the street map of the city was added into the DEM by subtracting one meter in elevation wherever there are streets. This reflects the fact that water is more likely to flow to the streets than to an adjacent house, and is supported by the general observations of runoff during periods of rain, as the streets are impermeable.

Thus, from a DEM and population information from the area, the next sections describe how each map was produced. Figure 4.2 shows a visual representation of the work flow during the productions of the maps.

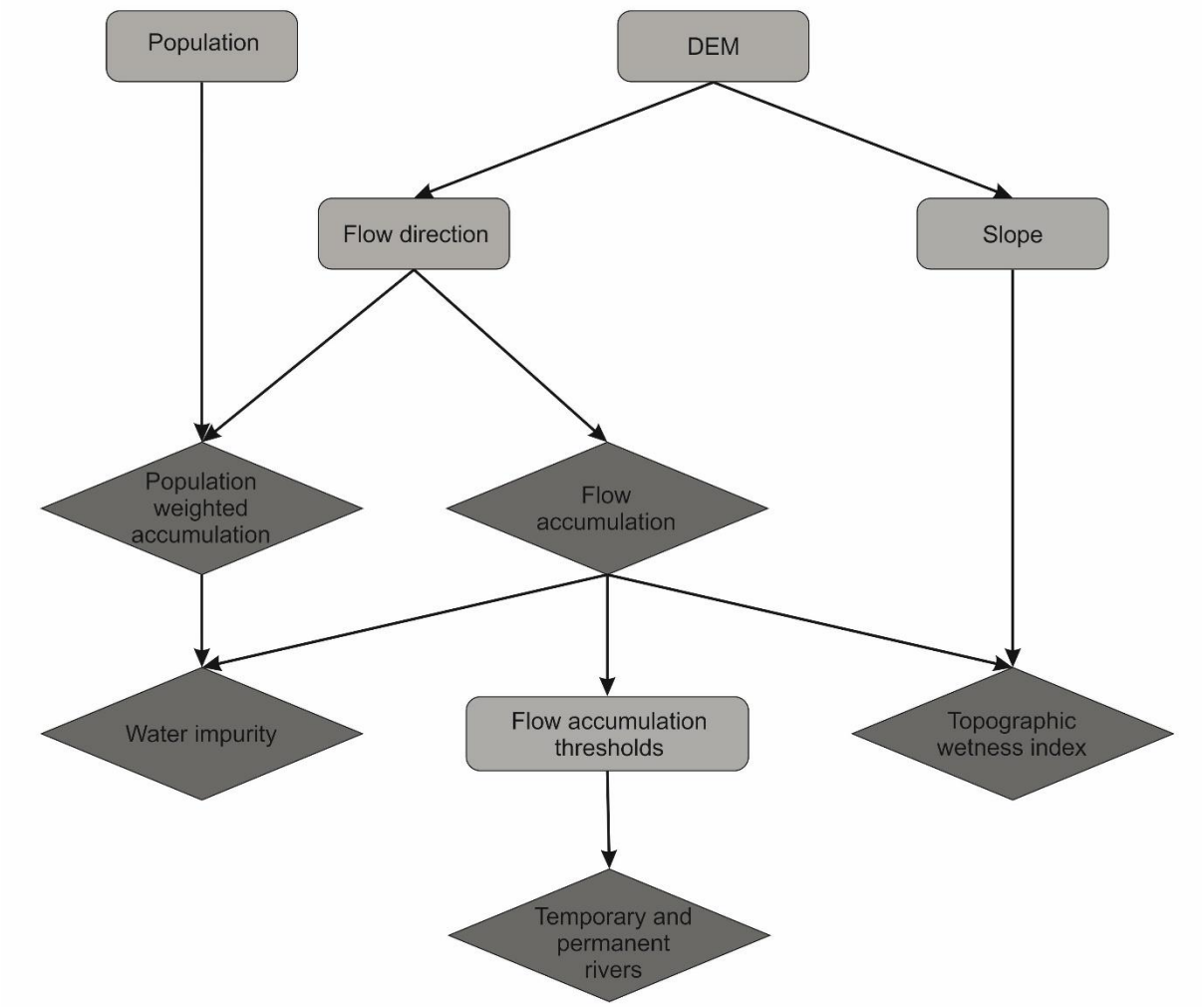


Figure 4.2: Work flow diagram of the layers used to obtain each map and how each layer contributed to the production of another. Light grey boxes represent informative layers necessary to produce the basic maps, here represented by rhombuses. Note that some maps were also used as an informative layer to produce other maps, and the directional arrows represent these contributions.

4.2.1 Flow accumulation

Flow accumulation refers to one of the fundamental procedures in obtaining hydrological features from a DEM such as channels, watersheds or ridges (Jenson and Domingue, 1988). The method calculates a flow accumulation value for every

cell in the DEM, that is, for every cell, the number of upslope cells that ultimately flow into it (O'Callaghan and Mark, 1984).

The procedure uses a flow direction map in which, based on elevation, every cell is assigned a direction of the flow. In this case, we use an approach developed by Tarboton (1997), where it is assumed the water can flow to more than one downslope neighbour, referred to as multidirectional flow. Hence, the flow from one cell to another depends on the proportion of upslope cells that flows to it.

Each cell is squared and has eight neighbours (diagonals were also included) with the exception of the border cells. Values of zero indicate that the cell does not receive any flow and correspond to being part of a ridge. A function called 'Upslope Area functions' (version 1.3.0.1), developed by Steven L. Eddins was used and is freely available in the repository MathWorks (https://uk.mathworks.com/).

In addition, the same procedure is used to identify river basin and catchments based on a defined threshold. This threshold is defined by the minimum number of cells flowing into a target cell, and is related to permanent rivers and streams. But this threshold can also be related to temporary rivers and runoff if a lower value is defined. The value of the threshold is a gradient going from temporary (lower value) to permanent rivers (higher value). Here, three thresholds were selected, 50, 500 and 5000, and every cell that had a number of cells flowing into it smaller than the threshold was assigned a value of zero.

4.2.2 Population weighted accumulation

Population density can affect the dynamics of infection of waterborne diseases in many different ways. The effect can be associated with higher infection rates due to more contact rates between susceptible and infected individuals, but also by

contaminating the environment with pathogens. In the case of leptospirosis, for example, the bacteria are shed into the environment in the urine of a reservoir host, Norway rats (*Rattus norvegicus*), the abundance of which is correlated with where people are, as a result of easy access to resources such as food and shelter (Boisier *et al.*, 1997; Guan *et al.*, 2009). Thus, when the rain falls, the water that flows through highly populated areas is expected to have a higher concentration of the pathogen. For that reason, areas that receive this water will have a greater risk of infection. Therefore, the risk seen in a cell would be a combination of the amount of water the cell receives but also the population density of the areas through which water flows before reaching the cell.

Furthermore, for example for gastro-intestinal illness, places that lack sanitation would have a bigger impact in the dynamics of infection. Medema & Schijven (2001) observed that 80% of *Giardia* discharged in the Netherlands was from untreated wastewater discharge and sewer overflows, whereas, for *Cryptosporidium*, the contamination came from wastewater treatment plants. Hence, considering population density alone may not be the best representation of the risk. Therefore, population weighted maps were developed that included two types of population information: population numbers and the number of people without sanitation. The approach used for flow accumulation was used again, but the number of cells flowing to a target cell was not counted. Rather, the cumulative number of people (total or without sanitation) in every upslope cell that the water would have passed through if the rain had become runoff was counted.

The population data was obtained from the census of 2010 performed by the *Instituto Brasileiro de Geografia e Estatistica (IBGE)*. The city of Salvador was divided into census districts where the total number of residents, the total number of houses, the number of houses without sanitation and other variables were collected. This data was rescaled to the size of the DEM, 5 meters pixel size, assuming

homogenous distribution of the population inside each census district. Therefore, number of people (total or without sanitation) per square meter (m^2) was multiplied by the area of the pixel, $25m^2$ to give final values per cell.

4.2.3 Water rurality

Another way of assessing risks for waterborne diseases is to focus on the concentration of the pathogen in the water. Flow accumulation maps carry no information of how clean or dirty the water is, whereas Population weighted maps can be a proxy for the absolute level (amount) of water contamination. Hence, population weighted and flow accumulation maps can be seen as two extreme points of a gradient and the ratio between them gives an insight into where most of the water is coming from.

4.2.4 Topographic Wetness Index (TWI)

The Topographic wetness index (TWI) is the main output of a mechanistic model, TOPMODEL, developed to understand theoretical and practical forecasting in which hydrological processes are perceived (Beven and Kirkby, 1979; Beven, 1997). The main difference from other maps described here is that it focuses on flood risk (ultimate destination of water) rather than the water flowing into (and possibly through) a cell. Specifically it takes into account the slope of the cell (β) and the area of a catchment (a) in order to produce a flood risk map.

$$TWI = \log\left({}^{a}/_{\tan\beta}\right) \quad (1)$$

TWI states that areas with the same value would have the same hydrological response to rainfall and hence are hydrologically similar. Higher values indicate that an area/cell is more suitable for flooding and are caused by lower slopes (flat areas) and/or convergence points of long slopes (Beven and Kirkby, 1979; Beven, 1997).

4.3 Model output

A dummy data set was created to exemplify how each map is produced. Then, in the following section, the maps will be produced using real data from Salvador city. Hence, given a digital elevation model (DEM) where each value is the elevation information of a pixel,

$$DEM = \begin{matrix} 2 & 1 & 0 \\ 4 & 3 & 2 \\ 6 & 5 & 4 \end{matrix}$$

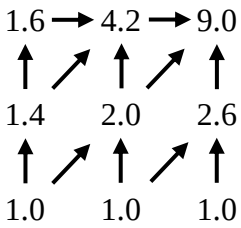
and a population map (P),

$$P = \begin{matrix} 0 & 4 & 3 \\ 0 & 0 & 0 \\ 6 & 0 & 0 \end{matrix}$$

Tarboton (1997) uses the elevation of the centre of the pixels to calculate slope and flow direction. The method uses facets of a triangle to calculate the slope of all eight neighbours cells and determine the steepest downward slope between

the eight planar triangular facets. Eight triangular facets are drawn from the centre of the pixel to all its neighbours. Each of these facets will have a downslope vector going outward from the centre and having an angle that falls within or outside of 45 degrees. The steepest vector is determined when the angle of the downslope vector lie within the 45 degrees at the centre of the facet and this is assign the direction of the flow. Once the steepest facet is identified, the proportion of flow to each slope can be obtained based on equations 1-5 of Tarboton (1997) method. The flow accumulation map (F) will produce a map showing the cumulative number of pixels where the water is coming from assuming that all pixels received water, starting from high to low elevated areas. The dark arrows indicate which pixels are receiving the flow and where the flow is going to.

F =



Thus, for example, the value of 2.0 in the central cell is arrived at by adding 1.0 (the cell itself) to 0.6 from the bottom-left cell and 0.4 from the centre-left cell, and so on. Note that the water can flow to more than one pixel given their elevation, so a proportion of one pixel will flow to more than one downstream pixel if their elevation is similar or equal. This is a feature of the model created by Tarboton (1997). In this example, the pixel in the centre is receiving its water from itself and the two pixels in the bottom line of the first two columns.

The flow accumulation threshold (T) can be applied by first defining a threshold value (in this example we arbitrary define a value of 1.5) and everything that is bigger than that value will be assigned a value of one, or otherwise zero. This map

displays a network of connected areas through which the water passes. The bigger the threshold defined, the less complex is the network and the more it will represent more permanent rivers/streams.

$$T = \begin{matrix} 1 & 1 & 1 \\ 0 & 1 & 1 \\ 0 & 0 & 0 \end{matrix}$$

Similar to the flow accumulation map, the population weighted map (PW) will produce a map showing the cumulative number of people that the water would have passed through. This map shows that even when there are no people living in the pixel, the pixel still receives water from upslope populated areas. In this model, any type of population information can be included. In the case of Salvador city, for example (below), population density and people without sanitation were used to produce two population weighted maps.

$$PW = \begin{matrix} 1.0 & 8.0 & 13 \\ 2.5 & 3.5 & 0 \\ 6.0 & 0 & 0 \end{matrix}$$

Now that Flow accumulation and the population weighted maps have been generated, the water rurality map (W) can be produced based in the ratio between them. The range of this measurement varies from zero to infinite, where zero means that the water did not pass through any populated pixels before it reached the target pixel, which happens with non-populated ridges areas, for example.

$$W = \begin{matrix} 0.6 & 1.9 & 1.4 \\ 1.7 & 1.8 & 0.0 \\ 6.0 & 0.0 & 0.0 \end{matrix}$$

The last map produced is the topographic wetness index (TWI), which from a DEM, a slope can be generated, and TWI produced based on Equation 4.1. This map indicates areas that are more likely to be flooded than others, higher values.

$$TWI = \begin{matrix} 7.7 & 8.7 & 13 \\ 6.8 & 7.1 & 7.5 \\ 6.5 & 6.5 & 6.6 \end{matrix}$$

Taking the second row and first column for demonstration, the flow accumulation represents the catchment and is firstly transformed in area by multiplying its values by the size of the pixel, five meters in this case ($1.4 * 5 = 7.05$). Then the TWI is the natural logarithm of the division between catchment area and the tangent of the slope in radiants: $\log\left({}^{7.05}/_{0.0079}\right) = 6.8$.

4.4 Application to Salvador city

A total of eight different sets of maps were generated as previously described, using elevation data from Salvador, Brazil. To demonstrate the differences within and between every map, at a range of scales, maps were generated in each case for the whole of Salvador and also for a section of a neighbourhood of the city,

named Piraja. Figure 4.3 shows the digital elevation model itself for the whole city and for Piraja, however, the other maps will be presented at three different scales, going from local scale (Piraja), medium scale (Piraja and its region) to a city level map.

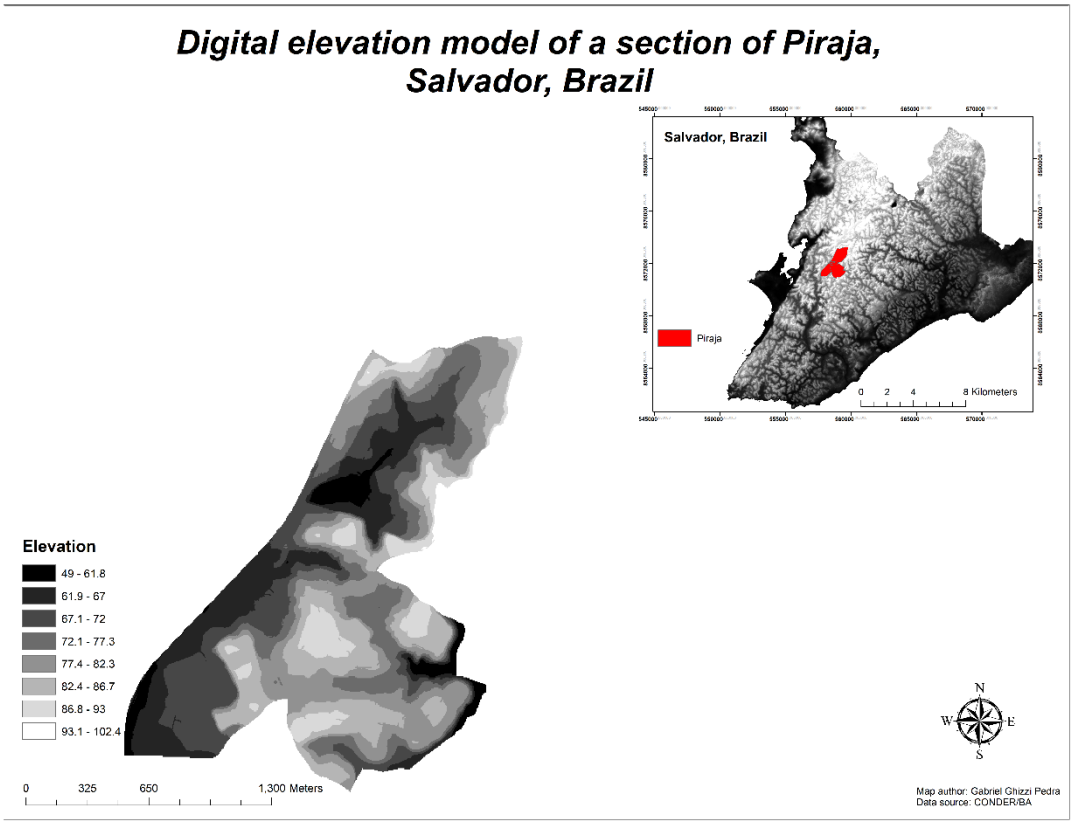


Figure 4.3: Digital elevation model of Salvador, Brazil used to demonstrate the difference between risk maps. A community inside Salvador named Piraja (red area on top right map) was selected to illustrate the output of the model at a local scale.

The DEM of Salvador shows a city composed of many valleys in the whole area, which makes the flow accumulation look heterogeneous, with higher and lower flow accumulation areas, throughout the city (Figure 4.4). There is an extended area with higher flow accumulation values going from middle to north in the flow accumulation map for Piraja (Figure 4.4). In addition, the inclusion of streets into the DEM makes the flow go to the streets in many cases, which can be seen to the south of Piraja on Figure 4.4.

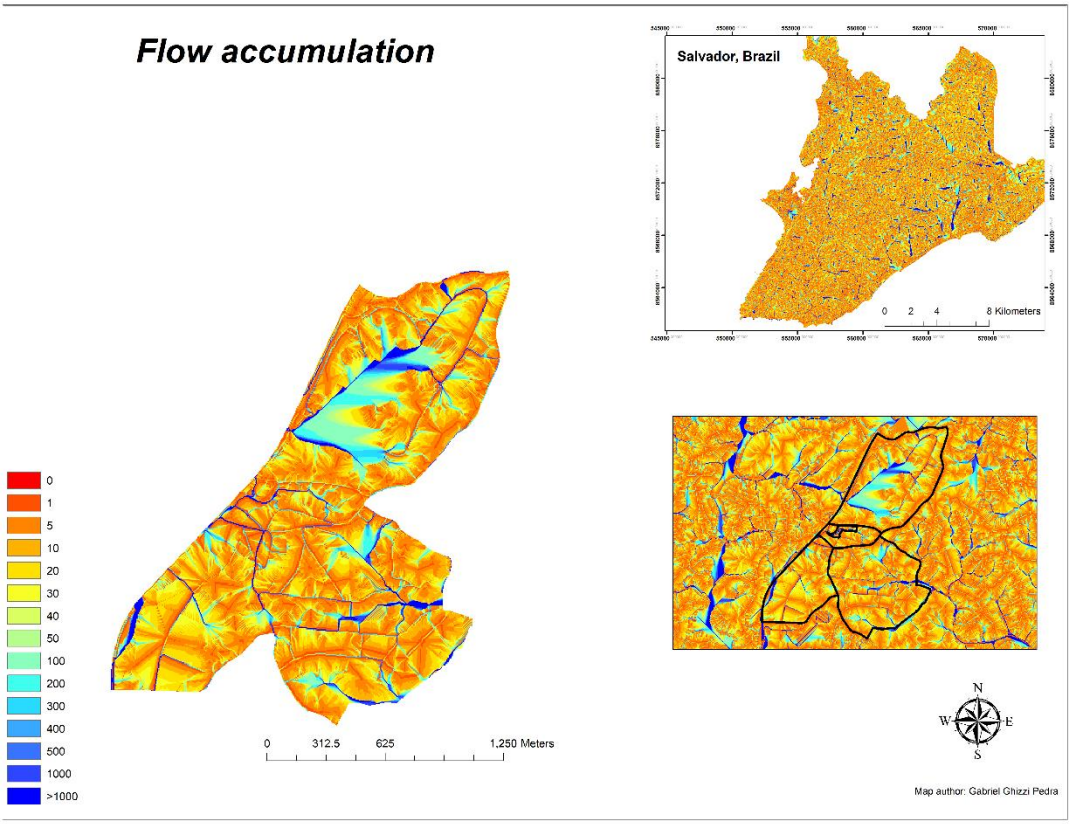


Figure 4.4: Flow accumulation risk map, which represents water flow. The map in the centre is the flow accumulation of a neighbourhood named Piraja. The bottom right is a medium scale map showing Piraja and its region and top right map is the flow accumulation for the whole city.

For the flow accumulation thresholds (Figures 4.5-4.7), the maps displayed a connected network of runoff/rivers throughout the space. When the threshold is higher, equal to 1000, fewer networks can be seen. For example, two more extended paths are shown in the north and southwest of the map for Piraja in Figure 4.5. Then, if the threshold decreases to 500, more paths are connected to the main path (Figure 4.6) and if the threshold decreases even more, to 50, there are more short paths revealing a very complex network (Figure 4.7).

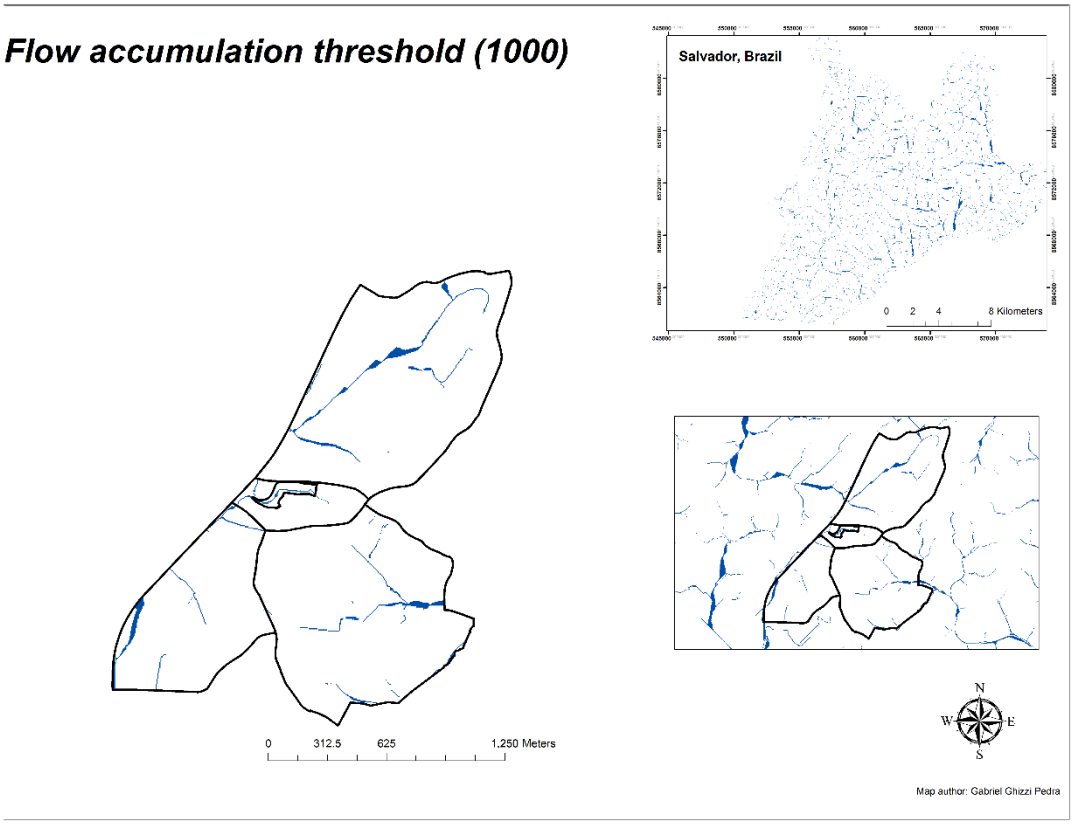


Figure 4.5: Flow accumulation threshold obtained from the Flow accumulation map, the threshold defined is 1000. The map in the centre is the flow accumulation threshold of a neighbourhood named Piraja. The bottom right is a medium scale map showing Piraja and its region and top right map is the flow accumulation threshold for the whole city.

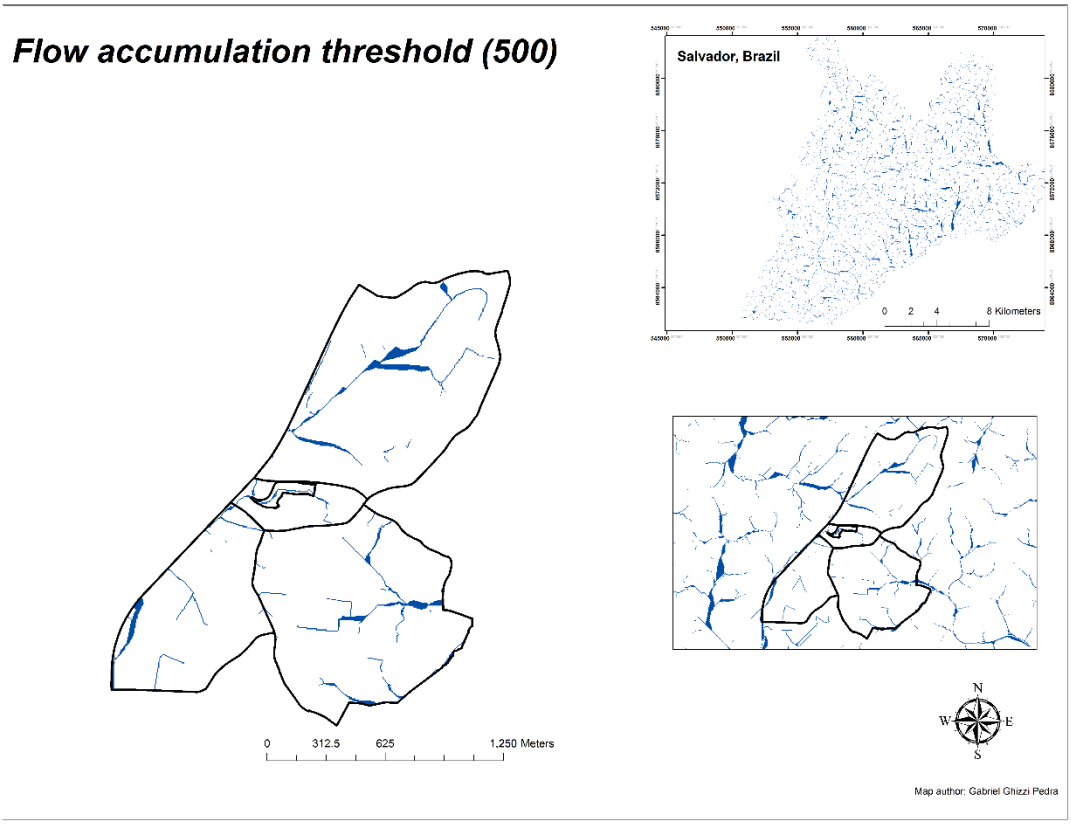


Figure 4.6: Flow accumulation threshold obtained from the Flow accumulation map, the threshold defined is 500. The map in the centre is the flow accumulation threshold of a neighbourhood named Piraja. The bottom right is a medium scale map showing Piraja and its region and top right map is the flow accumulation threshold for the whole city.

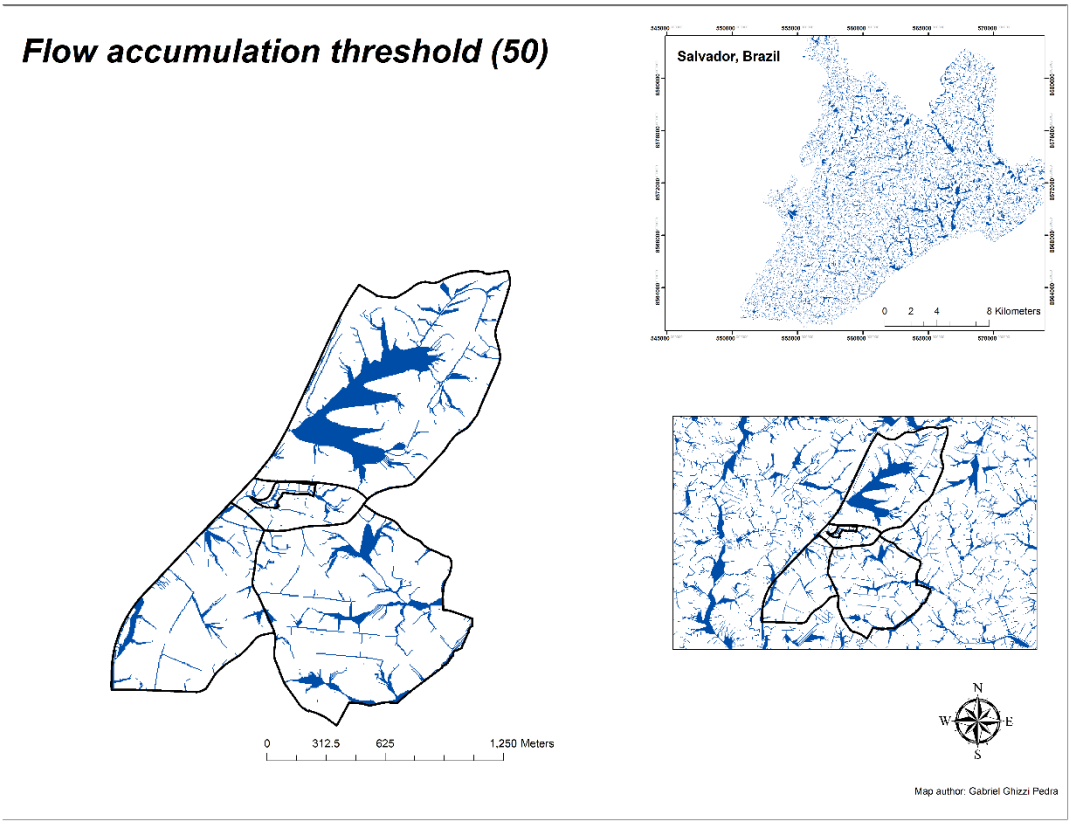


Figure 4.7: Flow accumulation threshold obtained from the Flow accumulation map, the threshold defined is 50. The map in the centre is the flow accumulation threshold of a neighbourhood named Piraja. The bottom right is a medium scale map showing Piraja and its region and top right map is the flow accumulation threshold for the whole city.

The population weighted accumulation show a map with some patches of homogenous low risk at the city level, which is associated with low density areas (red and orange colours in Figure 4.8). However, looking at Piraja (local scale) in Figure 4.8, it is possible to see that in those homogeneous areas, there are a few lines associated with higher risk - for example a line path going from the centre to the north of Piraja. This is an indication that the risk is coming from upslope dense populated areas. Similarly, when the density of people without sanitation is considered, the map shows a pattern associated with higher density areas of people without sanitation, which in this case is concentrated at the north bound of the city (top right map in Figure 4.9). In addition, at a local scale, the map show areas with heterogeneous risk such as the southeast but also homogeneous areas, at the north, with a line of higher risk locations (Figure 4.9).

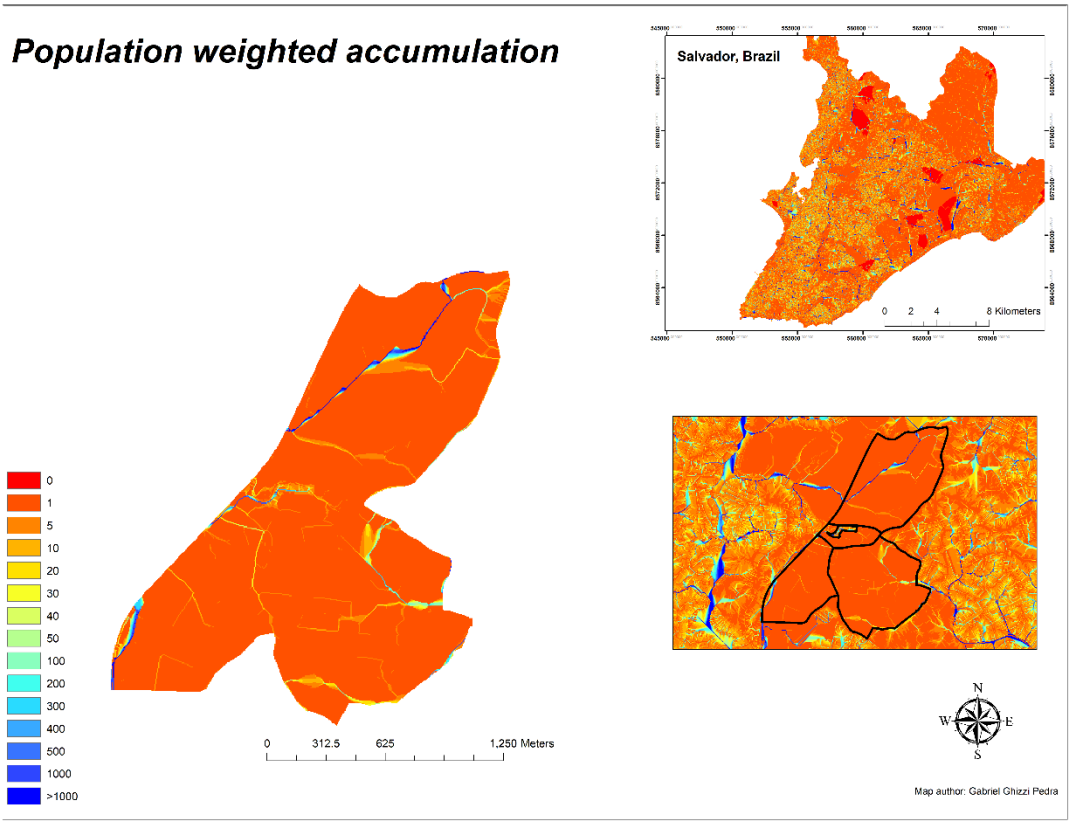


Figure 4.8: Population weighted accumulation map showing the cumulative number of people where the water would have flown through. The map in the centre is the population weighted accumulation of a neighbourhood named Piraja. The bottom right is a medium scale map showing Piraja and its region and top right map is the population weighted accumulation for the whole city.

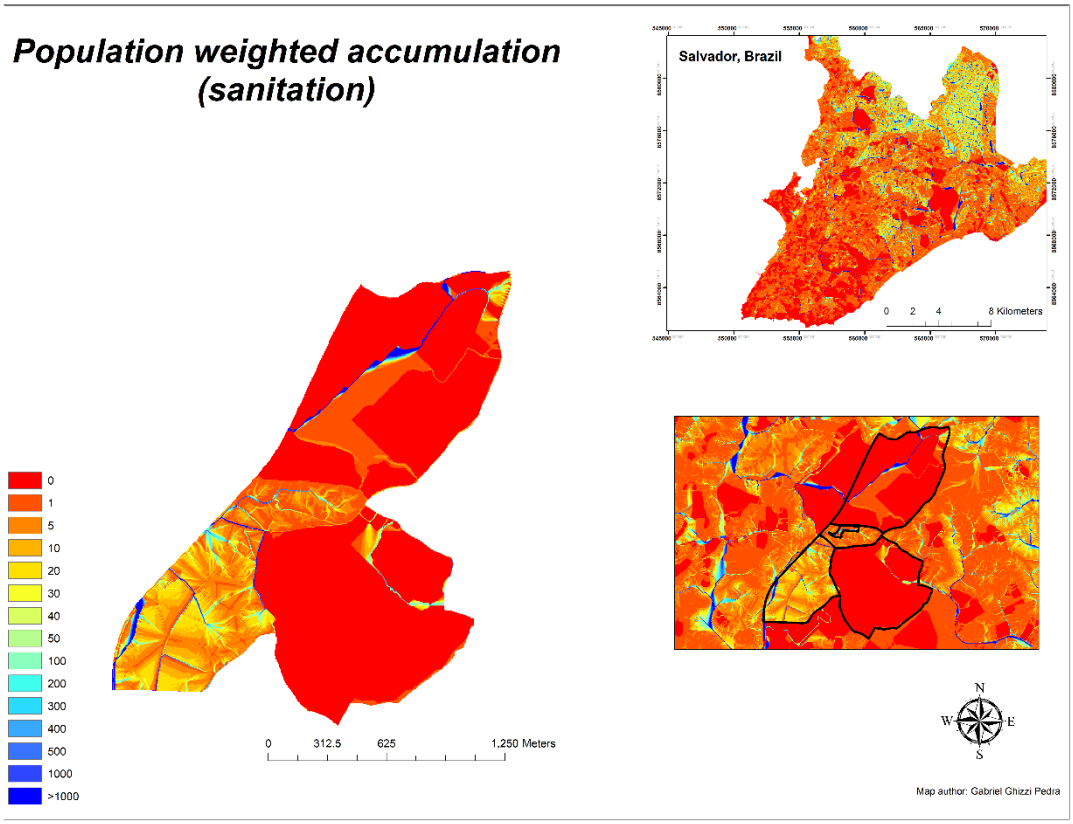


Figure 4.9: Population weighted accumulation map where is only considering the population density of people without sanitation. The map in the centre is the population weighted accumulation of a neighbourhood named Piraja. The bottom right is a medium scale map showing Piraja and its region and top right map is the population weighted accumulation for the whole city.

The water rurality map (Figure 4.10) represents how diluted the water is, where the red colours represent a higher concentration of pathogens because most of the water flowing to the pixel is coming from risky areas. The map in Figure 4.10 shows an area at the centre of Piraja where the risk is very high and surrounded by low risk. Finally, for the flooding risk map, measure by the TWI, the risk is very heterogeneous and is evidently focused on streets in many cases, which it is possible to see in the straight lines of higher values in the map (Figure 4.11).

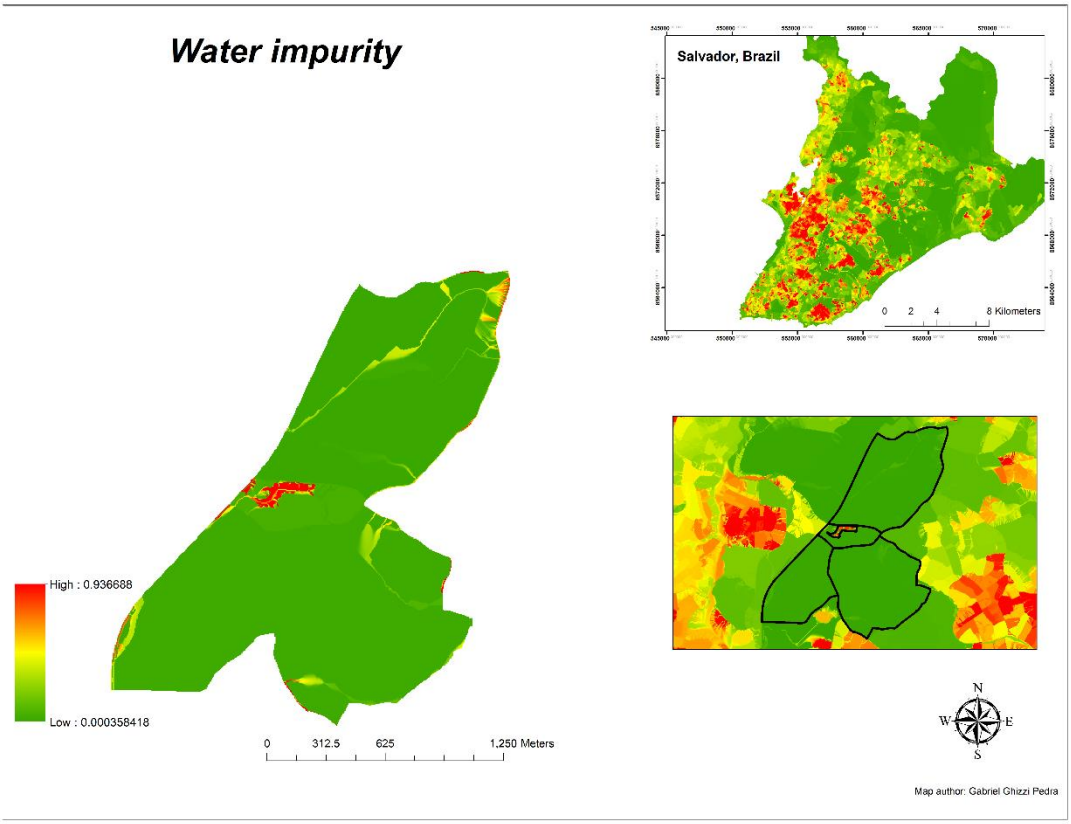


Figure 4.10: Water rurality map obtained from the ratio between population weighted accumulation and flow accumulation. The map in the centre is the water rurality of a neighbourhood named Piraja. The bottom right is a medium scale map showing Piraja and its region and top right map is the water rurality for the whole city.

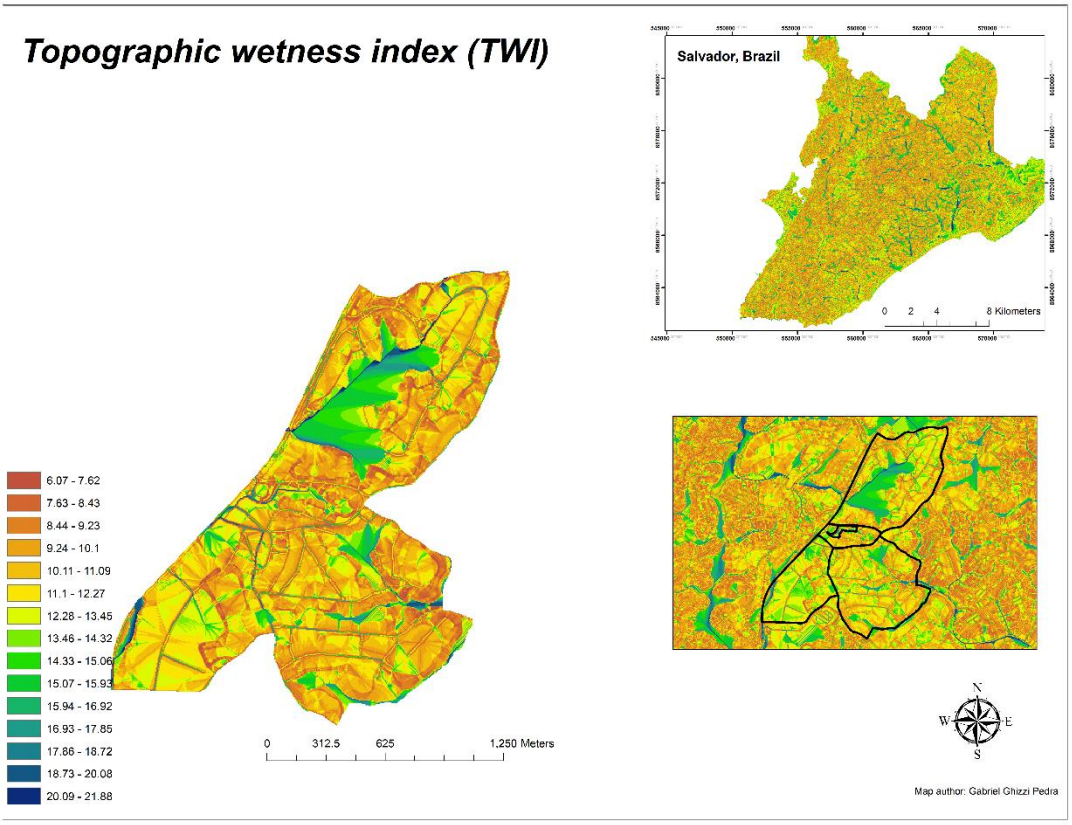


Figure 4.11: Topographic wetness index (TWI) map, which is a flooding risk map. The map in the centre is the TWI of a neighbourhood named Piraja. The bottom right is a medium scale map showing Piraja and its region and top right map is the TWI for the whole city.

4.5 Discussion

This chapter aimed to develop a range of topographical analyses that can be used as measures of risk for waterborne diseases in public health. Even though the direct association with waterborne diseases has not been addressed here, the results should help to produce risk maps that can be used to understand hydrological drivers of waterborne disease outcomes (see Chapter 3). The contrasts between the results of each measurement demonstrate how complementary each map can be in estimating the risk, and hence their importance.

The role of the environment in disease transmission has been increasingly addressed recently and has been shown to be relevant in disease transmission. Studies have either incorporated the environment in dynamic models of

transmission or have looked at how those variables are associated with diseases outcomes. In dynamic modelling, hydrological transport of pathogens or rainfall-runoff models have been integrated with epidemiological dynamic models (Bertuzzo *et al.*, 2007, 2008; Remais, Liang and Spear, 2008; Mari *et al.*, 2012; Collender *et al.*, 2016; Rinaldo *et al.*, 2017). For cholera, for example, the transport of pathogens is assumed to happen through river networks and infection depends on the contact rate between a susceptible individual and the river network (Bertuzzo *et al.*, 2008; Mari *et al.*, 2012), whereas for schistosomiasis, the hydrological transport of the pathogen occurs through the transport of the intermediate host (snail) which is aquatic and gets carried out by runoff (Remais, Liang and Spear, 2008; Perez-Saez *et al.*, 2016). In addition, direct associations between flooding risk (TWI), river density and leptospirosis have been found (Vega-Corredor & Opadeyi 2014). In these different ways, the incorporation of the environment has helped explain disease infection patterns. Here, our results can provide additional environmental characteristics that have not been addressed yet but could improve our understanding of the role of the environment in the dynamics of waterborne diseases.

Flow accumulation thresholds provided a network map that can represent, depending on the threshold defined, a network of temporary and/or permanent rivers. Those maps carry information of where the possible runoff or river routes are, which can be a risk for waterborne diseases due to the water mobilization of pathogens. Complementary to flow accumulation threshold maps, flow accumulation by itself carries information on how long the water travels before it reach a pixel, which is indicative of the catchment area feeding the pixel. In that case, flow accumulation can be hypothesized that the longer the water travels, more pathogen is carrying, and hence, the risk of infection is higher.

On the other hand, while flow accumulation shows areas that receive more flow than others, most of its flow can be coming from non/less populated area. That is the difference between flow accumulation and population weighted maps. When population data are incorporated, the data generated show the cumulative number of people that the water might have passed through which can be a proxy for water contamination or rat's distribution in the case of leptospirosis. Despite using the same principle (flow direction), maps produced by flow accumulation and population weighted accumulation generated different patterns. Population weighted accumulation showed high risk even in some non-populated areas because those areas are receiving water from upslope-populated areas. In addition, the risk map changed significantly when a map with sanitation information was produced, but, there are higher risk areas in places with sanitation, indicating that the risk is mostly coming from upslope areas that does not have sanitation.

The combination of hydrology and population data was inspired based on the leptospirosis study in Salvador, Brazil. The issue came from the difficulty to obtain high resolution data on rat's distribution throughout the city. Alternatively, population data can be seen as a proxy for the distribution of Norway rats as its occurrence can be associated with human density (Boisier *et al.*, 1997; Guan *et al.*, 2009). However, the use of population data go beyond that, *Cryptosporidium*, for example, is a pathogen that are eliminated in the environment through human (and other mammals) faeces, therefore, considering people without sanitation can be seen as a risk for local and downstream contamination (Fayer, Speer and Dubey, 1997). Furthermore, Katz et al (2006) observed that the reason of prolonged propagation of *Giardia* during an outbreak in Boston was caused by person-to-person transmission, indicating that population density can play a role in the transmission of giardiasis.

The topographic wetness index (TWI) has been used in epidemiology as noted previously. The inclusion of this measurement in this chapter demonstrates how TWI

by itself does not catch all the hydrological variation in the environment such as the water that flows through populated places. However, TWI combines flow accumulation and slope of each cell in order to have a proxy of how likely it is for a cell to be flooded. While flow accumulation may show large differences between neighbouring cells, perhaps because some of them are receiving water from long distance, the correction by the slope indicates if the water will be retained in a cell or will just pass through. Therefore, even if a cell is receiving water from a short distance (low flow), they might have the same likelihood of flooding as a cell that has high flow, simply because of differences in slope.

A big contrast within water rurality map was observed, where the results showed a patchier pattern with areas being either ‘dirty’ or ‘clean’. The reason for this contrast is a combination of the population distribution throughout the city and the water flow. The north area of the city is less populated, with most of the flow in those areas coming from low or non-populated places, whereas in the other parts of the city the opposite is the case. The population information used here were based on district census, which varies with area and has been rescaled to the same scale of the DEM. A better resolution of population density would result in a smoother transition between ‘dirty’ and ‘clean’.

The maps produced here are considered proxies of water flow and do not represent areas that will produce runoff or will be flooded every time it rains. To predict runoff in the environment with high accuracy, it is necessary to collect a set of many other factors such as high resolution rainfall data and soil permeability, which makes the model very complex and expensive. Using topographic analysis can be much cheaper and could be seen as an initial step in understanding not only the dynamics of disease transmission in the environment but also the movement and distribution of pathogens.

Therefore, the results shown her, demonstrate the usefulness of different measurements of risk that can be applied to a range of waterborne diseases. These features are important in terms of risk perception, which therefore changes depending on the approach that has been used, or the approaches can be complimentary. Use of these maps may help organizations and governments to understand patterns of infection and derive actions to reduce transmission.

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Chapter 5: 14 years of leptospirosis surveillance in Salvador city, Brazil: spatial distribution of leptospirosis cases and its relationship with hydrology

5.1 Introduction

From 1990, the world urban population had approximately doubled its size by 2015, going from 2.3 billion to 4 billion people living in urban areas. Most of this change came from low income countries in Asia, Africa and Latin America. Despite many efforts to reduce the number of urban slum settlements, in developing countries 880 million people lived in urban slums (low income settings) in 2015, which represents a substantial increase in comparison with 690 million in 1990 (Moreno et al., 2016). This drastic change in human demographics changed the epidemiology of human leptospirosis, a disease caused by spirochete bacteria (Leptospira spp.). The transmission of the disease involves complex interactions between the animal reservoir, human demographics and the environment, where the infection can occur through direct or indirect contact with urine of an infected animal or water contaminated with urine.

Historically, human leptospirosis mostly occurred in rural settings such as rice plantations, mining and livestock farms (Faine, 1982; Waitkins, 1986; Katz, Manea and Sasaki, 1991; Faine et al., 1999). However, nowadays, human leptospirosis is considered an occupational disease in tropical countries occurring, mainly, in low income including urban settings (Albert I Ko *et al.*, 1999; Felzemburgh *et al.*, 2014a; Costa, Hagan, *et al.*, 2015; Torgerson *et al.*, 2015). The risk factors associated with the infections are related to socioeconomics and environmental characteristics such

as lower levels of income and distance to an open sewer (Albert I Ko *et al.*, 1999; Barcellos and Sabroza, 2001a; Sarkar *et al.*, 2002; Maciel *et al.*, 2008; Reis *et al.*, 2008; Ko, Goarant and Picardeau, 2009; Oliveira *et al.*, 2009). In addition, outbreaks of the disease have been observed after heavy rainfall and flooding, and also, peaks in the number of cases have been observed during rainy seasons in endemic areas (Albert I Ko *et al.*, 1999; Barcellos and Sabroza, 2001b; Smythe *et al.*, 2002; Jena, Mohanty and Devadasan, 2004; Gaynor *et al.*, 2007; Desvars *et al.*, 2011b; Blanco and Romero, 2015; Gutiérrez and Martínez-Vega, 2018). For example, Ko et al (1999) observed an association between an outbreak that occurred in Salvador, Brazil two weeks after heavy rainfall and flooding in 1996. Similarly, many other studies reported leptospirosis outbreaks after heavy rainfall and flooding such as Malaysia in 2000 and 2010, Guyana 2005, New Caledonia in 2008, Philippines in 2009, Fiji in 2012 and many others (see Lau et al (2010b)).

These findings, which did not in themselves aim to look the spatial distribution of the cases, led to explorations of the spatial variation of human leptospirosis (i.e. at the community level) and its association with environmental variables. Schneider et al. (2012), for example, identified that municipalities closer to the Pacific ocean in Nicaragua have more cases of leptospirosis. Average precipitation over the previous two months, maximum precipitation and the rural proportion of the population were the main drivers that explained the spatial distribution of cases. In American Samoa, the distribution of cases was associated with factors at the environmental household-level as well as individual factor-levels, and four spatial clusters were identified (Lau et al., 2012). Occupation, knowledge of leptospirosis and gender were individual factors associated with cases, whereas the environmental factors were vegetation and soil type. In China, the use of spatial techniques combined with time (spatiotemporal analysis) not only helped to observe a decline in the number of

cases from 2005-2015 at county level but also observed a decreased disease burden in endemic areas (Dhewantara et al., 2018).

Vega-Corredor & Opadeyi (2014), in a study of human leptospirosis cases between communities in Trinidad, used a Geographic Information System (GIS) approach in order to generate water-related environmental variables such as rainfall, proportion of soil free drainage, proportion of imperfect/impeded drainage soil, river density and the topographic wetness index (TWI). Firstly, their study observed evidence of spatial variation in cases between communities. Secondly, rainfall, imperfect/impeded drainage soil and TWI were associated with those cases, where rainfall and imperfect/impeded drainage soil had a stronger association with cases in the south, and TWI had higher odds ratios in the north of the island. Their spatial scale looked at cases at a county level, which gave them a large areal coverage but low resolution.

The addition of spatial data at a regional level in human leptospirosis studies in Trinidad confirms that the environment has an important role to play in the spatial variation of cases. This was only possible due to the use of spatial statistics and GIS techniques, which allowed associations with disease outcomes to be explored, given the evaluation of spatial dependency. Although rainfall has been demonstrated to be associated with leptospirosis cases world-wide, with the exception of the study of Vega-Corredor & Opadeyi (2014), the exploration of other hydrological variables has not been addressed previously. Human leptospirosis can be considered a waterborne disease, and so other water-related variables can be associated with disease outcomes, as shown by Vega-Corredor & Opadeyi (2014). Chapter Four developed a set of different hydrological variables based on topographical and population data, which have been shown to be complementary to each other. These hydrological variables are designed to track the path of the rainfall once it has reached the ground. Looking for an association with those variables can be very

informative about the mechanisms of transmission of the disease. Therefore, this chapter will evaluate the spatial distribution of human leptospirosis cases in Salvador, Brazil and make inferences about how those cases might be associated with the hydrology of the city.

5.2 Material and methods

5.2.1 Study area

The study was carried out in Salvador, a city located in the northeast of Brazil, which is the third biggest city in the country. Salvador is divided into approximately 4000 census districts used by the Brazilian Institute of Geography and Statistics (IBGE) to perform census counts and collect other demographic information on the population. The latest census performed in Brazil is from 2010 and will be used here to generate population counts for each district.

Human leptospirosis is endemic in Salvador with seasonal peaks every rainy season. In 1996, the city had an outbreak reported later by Ko *et al* (1999) who evaluated the epidemiology of the outbreak and helped to implement protocols for surveillance of the disease. Until 2010, all the suspected cases of human leptospirosis were designated to Couto Maia hospital, an infectious disease state hospital, for treatment and confirmatory diagnosis. After 2010, the protocol for the diagnosis of leptospirosis changed, which changed the coverage of the surveillance, whereby more hospitals could receive suspected cases and perform laboratorial diagnoses.

5.2.2 Data collection and case definition

Since the outbreak in 1996, the Oswaldo Cruz Foundation (Fiocruz) has been conducting active population-based surveillance in Salvador. This is a collaboration together with the state and city's secretaries of health. The surveillance is conducted in the reference hospital for infectious disease diagnosis and treatment, Couto Maia Hospital, where a prospective analysis is performed on each patient admitted into the hospital. The clinical definition of severe human leptospirosis is: acute undifferentiated fever associated with either bleeding, acute renal failure, jaundice, or acute liver injury with transaminases <1,000 U/L (Nabity *et al.*, 2012).

Each individual who fulfilled the criteria for a clinical suspicion of severe leptospirosis was asked for their consent to participate in the study. Once consent was given, the patients were submitted to a questionnaire to collect demographic information, symptoms, length of hospital stay and outcome (discharged). The protocol was approved by the hospital, the Oswaldo Cruz Foundation, the Brazilian National Commission for Ethics in Research and Yale University. All individuals admitted with clinical leptospirosis between 1996 and 2010 were included in the study. The data were grouped by census district and this will be the scale of the analysis carried out here.

5.2.3 Hydrological data

The hydrological data used in this chapter were produced based on a topographical (elevation) map layer obtained from CONDER, the urban planning department of Salvador. This map had squared pixels of 5 meters resolution containing the elevation information and was used to produce a set of four different hydrological maps. The main assumption is that once water has reached the ground, it will become runoff and will flow to the lowest point in the surface. Flow accumulation, population weighted accumulation (PWA), water rurality and

topographic wetness index (TWI) are the maps used here (see Chapter 4). Flow accumulation represents how far the water have travelled through the surface until reaches a pixel, in other words, it is the cumulative number of pixels that the water has flown through. Similarly, PWA represents the number of people the water would have passed through which can be used as a proxy for water contamination. PWA was generated with a combination of demographic data obtained from 2010 population census and was re-scaled to the same resolution as the topographical map. Water rurality is the ratio between flow accumulation and PWA and can represent a spectrum of water contamination (more rural or urbanised areas). The TWI has been used previously by Vega-Corredor & Opadeyi (2014) and evaluates the likelihood of a pixel being flooded given its flow accumulation and the slope. The higher the value the more likely is a pixel to be flooded. All the maps were produced using MATLAB. For an in depth description of the methods please see the previous chapter.

There is a difference in the resolution between hydrological maps and the recorded number of leptospirosis cases. The former were obtained with very high resolution (hydrological information every five meters), whereas the latter was the observation of cases at district level. The hydrological data were grouped inside the areas of each district and the 90^{th} quantile of the range of values was obtained. The decision to use quantiles rather than averages was made with the intention of reflecting the spectrum of the hydrological values that represent a risk for leptospirosis. The histogram of the values in each district is very right skewed, with large numbers of small values. Hence, averages would not capture the true distribution of values at district level.

5.3 Statistical analysis

Statistical analysis was performed in three steps: development of a non-spatial model, seeking evidence of spatial variation in the data, and development of a geostatistical model. The non-spatial model was developed to select covariates to further evaluate if there was spatial variation in the predictions. Once the evidence of spatial variation was shown, the geostatistical model was developed.

5.3.1 Non-spatial model

Let Y_i denote the total number of human leptospirosis cases and P_i the population of each *ith* census district of Salvador. The generalised linear mixed effects model assumes that Y_i are conditionally independent observations of the random effect Z_i. Hence, Y_i are Poisson distributed with expectation e^{η_i}

$$Y_i|Z_i \sim Poisson\ (\ e^{\eta_i}\ )$$

where,

$$\eta_i = \log P_i + X\beta + Z_i + e_i$$

P_i is the offset for the population; β is a vector of the coefficients for the explanatory variables; X is the design matrix containing the variables for the fixed effects; Z_i is a vector of unobservable random effects which follows a multivariate normal distribution centered at zero with variance D, where D is diagonal with entry of the unknown vector of random effects; e_i is the residual variance assumed to be normally distributed $e_i \sim N(0, \sigma^2)$. The analysis was performed using R software, function *glmer()* from the package *lme4*.

There are four explanatory (hydrological) variables which were selected via nested model selection - flow accumulation, PWA, water rurality and TWI. The mixed effects model used the district census as a random effect and the

hydrological variables as fixed effects. The first step of the model selection is to compare univariate models against a null model. This comparison was ranked from the most significantly different to the least from the null model. The models which were not significantly different from the null model were removed from the next steps because this indicates that they are not associated with human leptospirosis cases. The model with the highest rank was considered a baseline and the remaining variables were added individually as candidate new models. The same process was then used in comparing the baseline model with the new candidate models. If the candidate model was significant different from the baseline, then it was ranked and the same process repeated until no more variables could be added into the model.

5.3.2 Empirical variogram

Empirical variograms are used to describe the degree of spatial dependency in a set of data, hence demonstrating whether closer locations are more similar than far locations. A semivariogram of each paired location was calculated and its distance recorded. If there is evidence of spatial dependency, the semivariogram values would be smaller for points closer to each other, and the values should increase until reaching, in most of the cases, an asymptote.

There are three components of the empirical variogram: the nugget, sill and range. The nugget represents the variation which is not explained by spatial location, in other words, it is the semivariogram evaluated right after a zero meter distance. The sill is where the semivariogram values reaches an asymptote. Finally, the range is where the semivariogram values reaches 95% of the sill. Practically, the nugget will give an insight as to whether there is unexplained variance that space does not capture, and the range is the distance interval over which all the observations are spatially dependent.

Another feature to be considered when exploring spatial dependency with empirical semivariograms is the direction of the dependency. Some diseases can spread more in one direction than another and this represents an uneven spread (for example, airborne diseases downwind of a source). Here, for leptospirosis studies, it is assumed to be evenly spread in all directions, which is called an isotropic process. In this case, the semivariogram obtained from a point *a* to *b* is the same as the semivariogram from point *b* to *a*. The last feature is that there is no temporal dependency from the data observed, which are assumed to be stationary. Therefore, the variogram for each unit distance u is:

$$v(u) = \tau^2 + \sigma^2 * \big(1 - p(u)\big)$$

where, u is a vector of the Euclidean distances between locations x and x' that was used to evaluate the variogram; τ^2 is the nugget effect; σ^2 is the variance of the spatial process and $p(u)$ is the spatial correlation function. An exponential correlation function was used as $p(u)$ and is a special case of the Matern correlation function where the smoothness parameter is fixed at $\kappa = 0.5$ (see Giorgi & Diggle (2017) for the Matern correlation function). The validity of the correlation function was explored through Monte Carlo simulations which evaluate if the empirical variogram is different from what would be expected if there was no spatial dependency.

The analysis was performed using R software. The predicted random effects of the non-spatial model were extracted using the function *ranef()* from the package *lme4* and the empirical variogram was generated using the package *PrevMap* and function *spat.corr.diagnostic*().

5.3.3 Geostatistical model

A geostatistical model assumes that Y_i are realisations of a continuous space such that Y_i are dependent observations of an unobserved spatial stochastic process $S(x_i)$. Therefore, the total number of cases Y_i given the location $x_i: i = 1, \dots, n$ is Poisson distributed:

$$Y_i|S(x_i) \sim Poisson\left(e^{\eta(x_i)}\right) for\ i = 1, \dots, n$$

where,

$$\eta(x_i) = \log P_i + d(x_i)'\beta + S(x_i) + Z_i$$

P_i is the population of each district; $d(x_i)$ is a vector of explanatory variables associated with location *i*; β is a vector with the coefficients for each explanatory variables; $S(x_i)$ is the structured random effect that accounts for the spatial dependency which is an isotropic and stationary Gaussian process with mean zero, variance σ^2 and correlation function $\rho(x, x') = Corr[S(x), S(x')]$ where x and x' are two distinct locations. Z_i are the unstructured random effects that account for the variance that is not explained by the space and is normally distributed with mean zero and variance τ^2.

The spatial process is assumed to be stationary and isotropic, hence $Corr[S(x), S(x')] = \rho(\|x - x'\|)$, where $\| . \|$ indicates Euclidean distance. A Matern correlation function $\rho(u; \phi, \kappa)$ was used with a smoothness parameter fixed at $\kappa = 0.5$ and ϕ being the scale of the spatial correlation. See Giorgi & Diggle (2017) for a description of the Matern correlation function and the method of estimating the spatial model. The routine is an example of Monte Carlo Maximum Likelihood (MCML) which uses an importance sampling in order to approximate the likelihood function and estimate the parameters from the model. All the statistical analysis

were performed using the R software, function *poisson.log.MCML()* from the package *PrevMap*.

5.3.4 Model validation

The correlation function was validated using a variogram algorithm to simulate data and compare with the results observed. This process was obtained from that described in Macharia *et al*. (2018) which was developed in the *PrevMap* package. The process consists of simulating data under the fitted model, computing a variogram and obtaining a 95% envelop of the empirical variogram. If the estimated variogram falls within the 95% envelope, indicates that there is no further spatial variation and the adopted spatial correlation function was considered sufficient.

5.3.5 Identification of areas with high risk of leptospirosis transmission

From the geostatistical model, a target T can be defined for predictive inference. Here, the interest is to identify areas that have more cases of leptospirosis than expected. It is assumed that those areas would have a higher risk of leptospirosis transmission. The baseline incidence of leptospirosis in Salvador will be estimated by α, which is the intercept of the linear predictor. The predicted incidence of leptospirosis for each location and its confidence interval can be given by $\hat{y}\ (\hat{y}_{0.25}; \hat{y}_{97.5})$. Areas with high risk of leptospirosis would be the ones where the lower confidence interval is greater than the baseline incidence. This indicates that those areas have an incidence rate higher than expected.

$$T_i = \{x: (\hat{y}_{0.25}(x_i) - \alpha) > 0\}\ i = 1, \dots, n;$$

5.3.6 Risk of having at least one case of human leptospirosis

From the predicted incidence of leptospirosis obtained in the geostatistical model, another great interest for public health management can be extracted, namely, finding the risk of a census district having at least on case of leptospirosis. This can be defined as the probability of Y_i being bigger than zero, hence, the target can be defined as:

$$T_i = P\left(Y_i > 0 \middle| S(x_i)\right) = 1 - e^{-e^{\hat{\eta}(x_i)}} \; i = 1, \dots, n;$$

5.4 Results

In total, 2168 individuals were admitted to Couto Maia Hospital with clinical leptospirosis between 1996 and 2010. However, there were some cases where explanatory variables were absent. The total number of cases used in the regression analysis was 2010 and the total number of districts was 3412. The average number of cases per district was 0.59±1.15 (SD) where the maximum number of cases reported was 13. Approximately, 31% (N=1060) of the districts had cases reported. Figure 5.1 shows the distribution of cases in each district of Salvador, Brazil, where most of the districts with the highest numbers of cases are located in the northwest of the city. Figure 5.2 shows the distribution of the aggregated values of the covariates in each district census of Salvador city. Flow accumulation and TWI had higher values in the north of the city whereas PWA and water rurality had lower values.

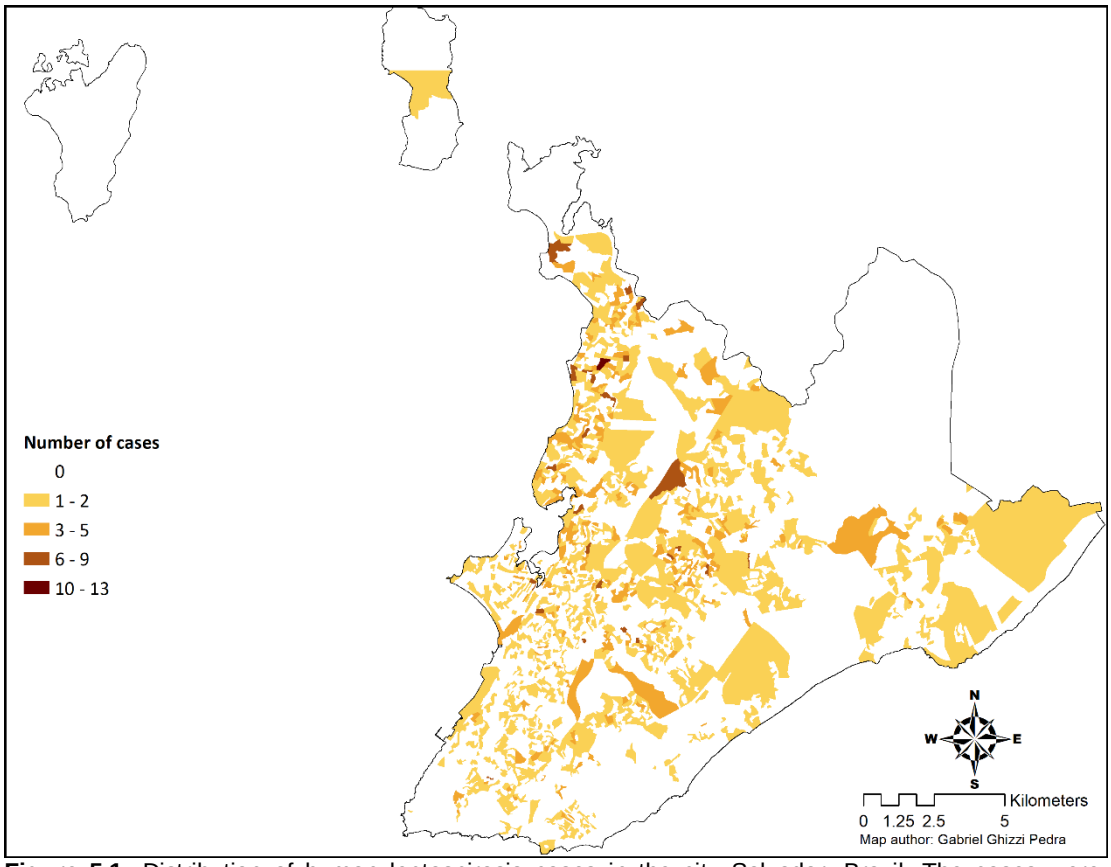

Figure 5.1: Distribution of human leptospirosis cases in the city Salvador, Brazil. The cases were obtained from active surveillance in Couto Maia Hospital from 1996-2010.

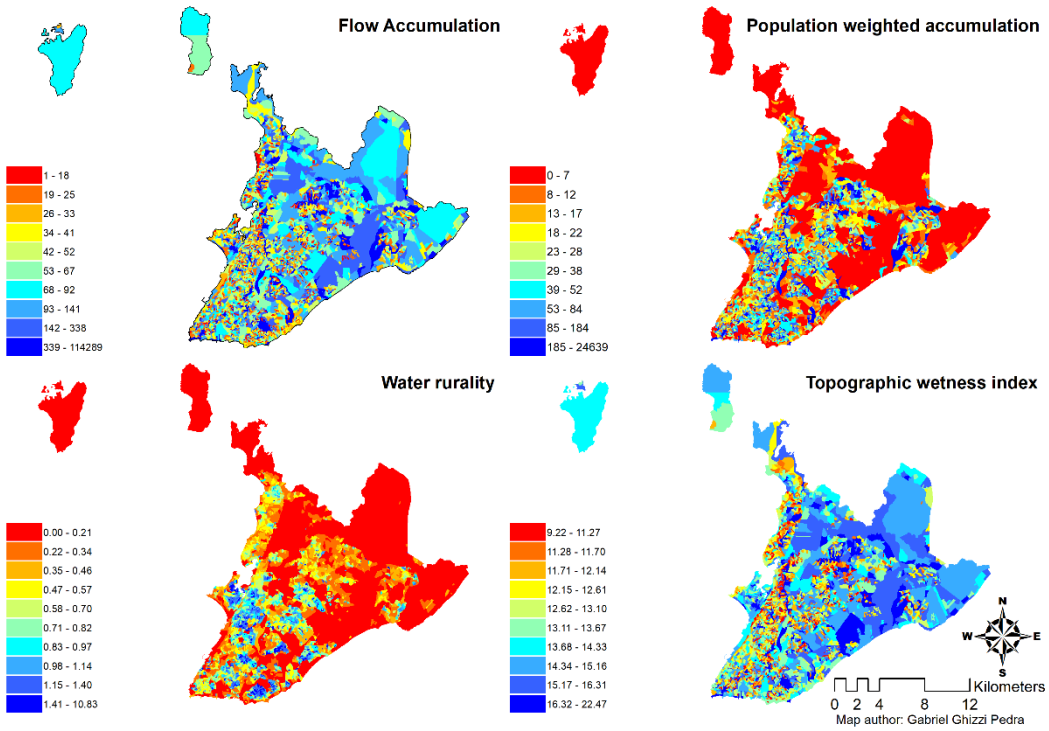

Figure 5.2: Hydrological explanatory variables of Salvador, Brazil used in the analysis. The values were aggregated from each census district where the 90th quantile was obtained.

The model selection results indicate that only water rurality was associated with leptospirosis cases in Salvador, Brazil (Table 5.1). The other covariates were not significantly different from the null model and were not used in the rest of the analysis. The empirical variogram of the predicted random effects demonstrated that there was spatial dependency in the predictions of the mixed effects model, which supports the use of spatial analysis (Figure 5.3).

Table 5.1: Results of the model selection base on likelihood ratio test where all the models were compared against the null model. (k is the difference on the number of parameters; LogLik is the log likelihood of the model)

	LogLik	k	p-value
Null model	-3482.62	-	-
Population weighted accumulation	-3482.46	1	0.58
Flow accumulation	-3482.22	1	0.37
Topographic wetness index	-3482.27	1	0.40
Water rurality	-3474.75	1	0.00007

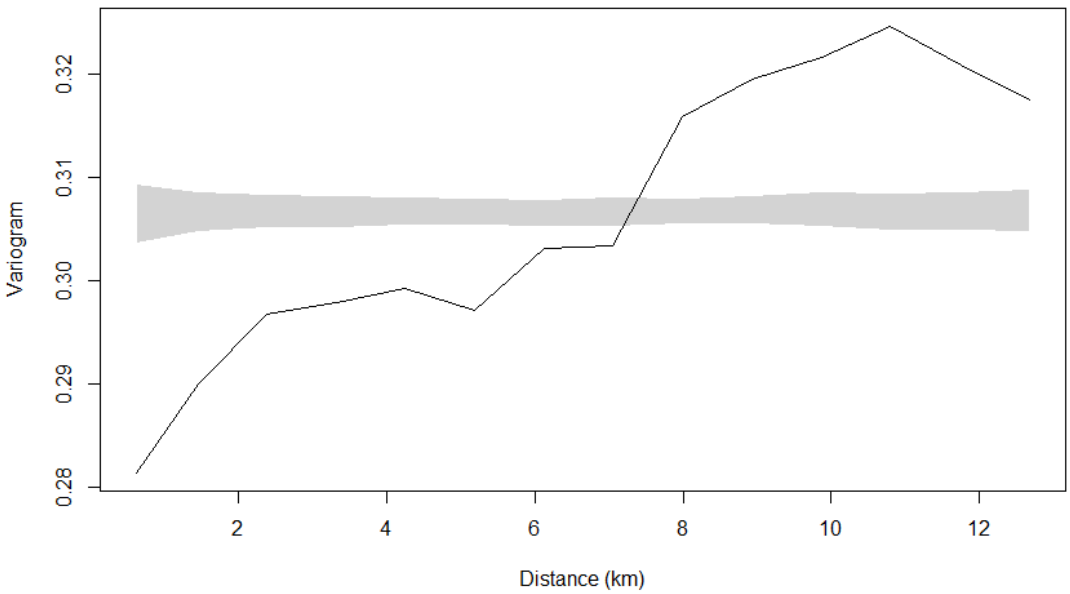

Figure 5.3: Empirical variogram of the predicted random effects from the best model selected. The grey area in the graph indicates the interval where the variogram is not different from what is expected by chance.

The correlation function of the spatial model was validated by simulation and Figure 5.4 show the envelope (grey shade) and the empirical variogram estimated (dark lines). The empirical variogram lay inside the envelope and indicates that there is no spatial dependency in the model and the correlation function used was appropriate. The practical range for an exponential function is three times the scale of the spatial dependency estimated which is approximately 12km. The model estimated two types of variance that are not explained by the linear predictor, the spatial structured variance (σ^2) and the unstructured variance (τ^2) (Table 5.2).

Table 5.2: Estimated coefficients of the geostatistical model. The first two rows are the predictors presented as odd ratio whereas σ^2 is the variance of the Gaussian process, ϕ is the scale of the spatial correlation and τ^2 is the variance of the nugget effect. (CI= Confidence interval)

	Estimates	Lower CI	Upper CI
Intercept	0.0002	0.0001	0.0006
Water rurality	0.7973	0.6964	0.9128
σ^2	1.0456	0.4122	2.6528
ϕ	4.2599	1.4617	12.4148
τ^2	1.0167	0.1592	6.4924

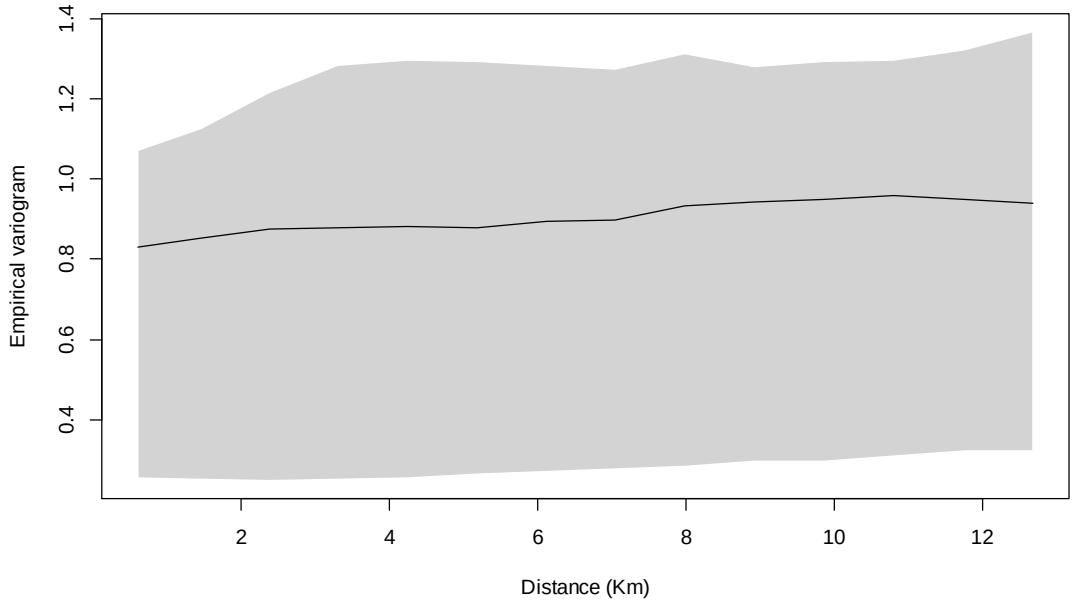

Figure 5.4: Validation of the empirical variogram estimated in the spatial model. Grey shade represent the 95% interval of the variogram and dark line is the variogram estimated.

Water rurality was the only covariate selected in the model selection process and the results of the regression shown that water rurality is negatively associated with human leptospirosis cases (Table 5.2). The predicted incidence per 10,000 people and 95% confidence interval are shown in Figure 5.5, Figure 5.6 and Figure 5.7. The northwest and centre of Salvador city are the regions where the predicted incidence per 10,000 people (Figure 5.5) and the risk of having at least one case of leptospirosis (Figure 5.8) are higher. However, areas where there were more cases than expected extended from the northwest, passing through the west and a few places in the south of the city (Figure 5.9). The standardized residual plot can be seen in Figure 5.10.

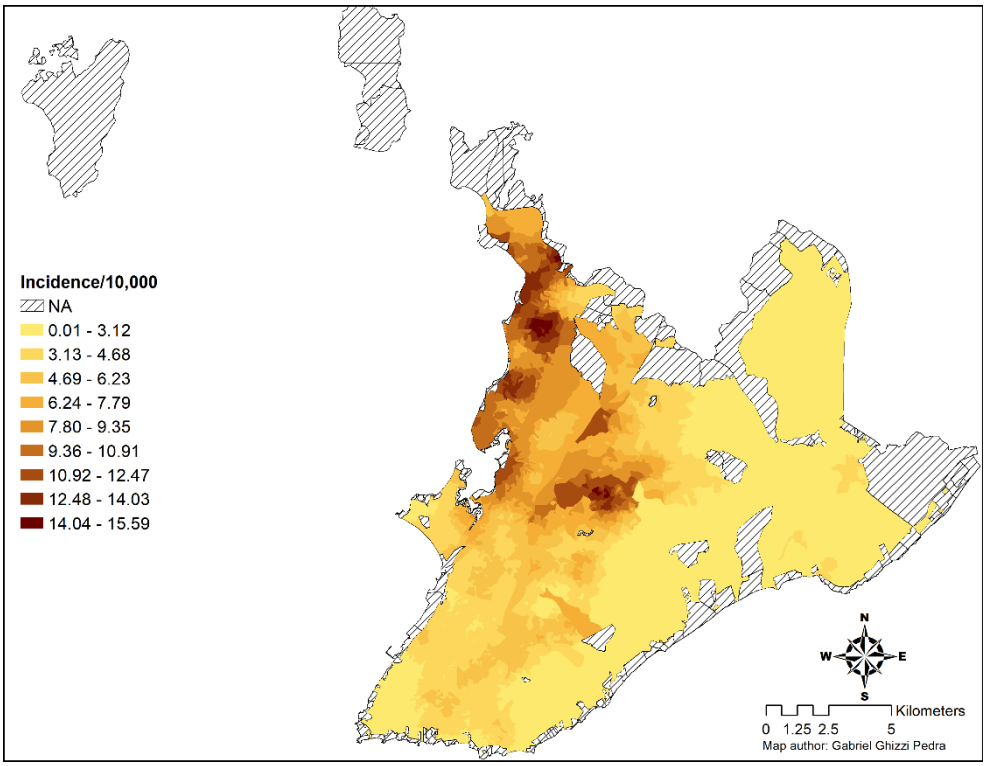

Figure 5.5: Predicted leptospirosis incidence per 10,000 population in Salvador, Brazil obtained from the geostatistical model.

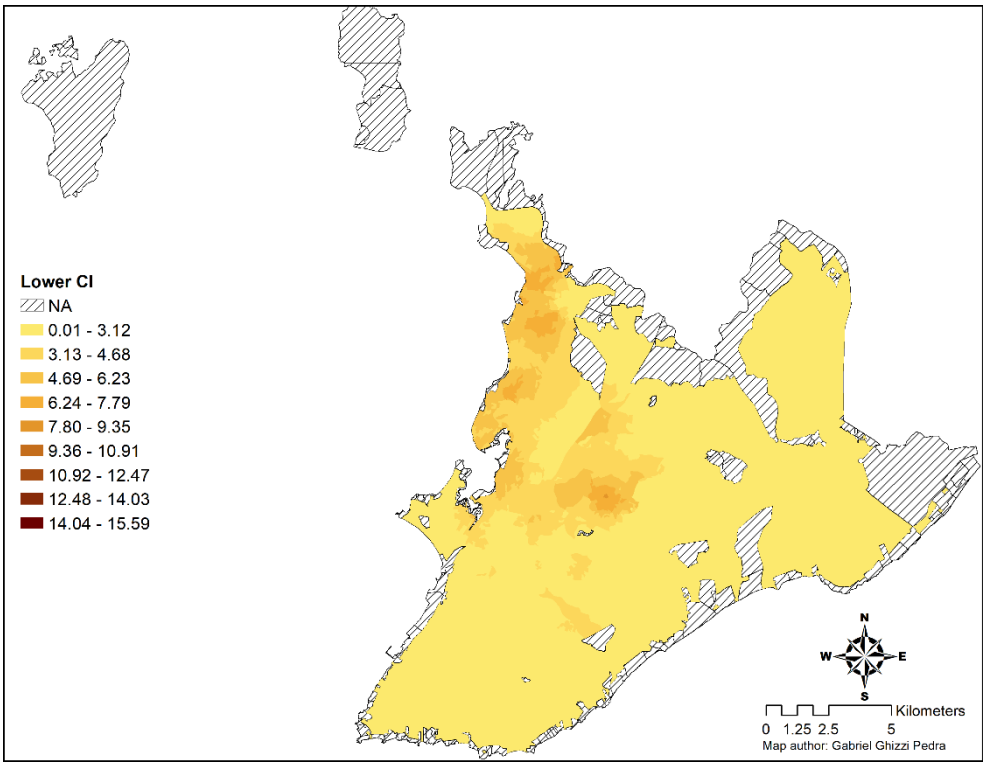

Figure 5.6: Lower confidence interval of the predicted leptospirosis incidence per 10,000 population of Salvador, Brazil.

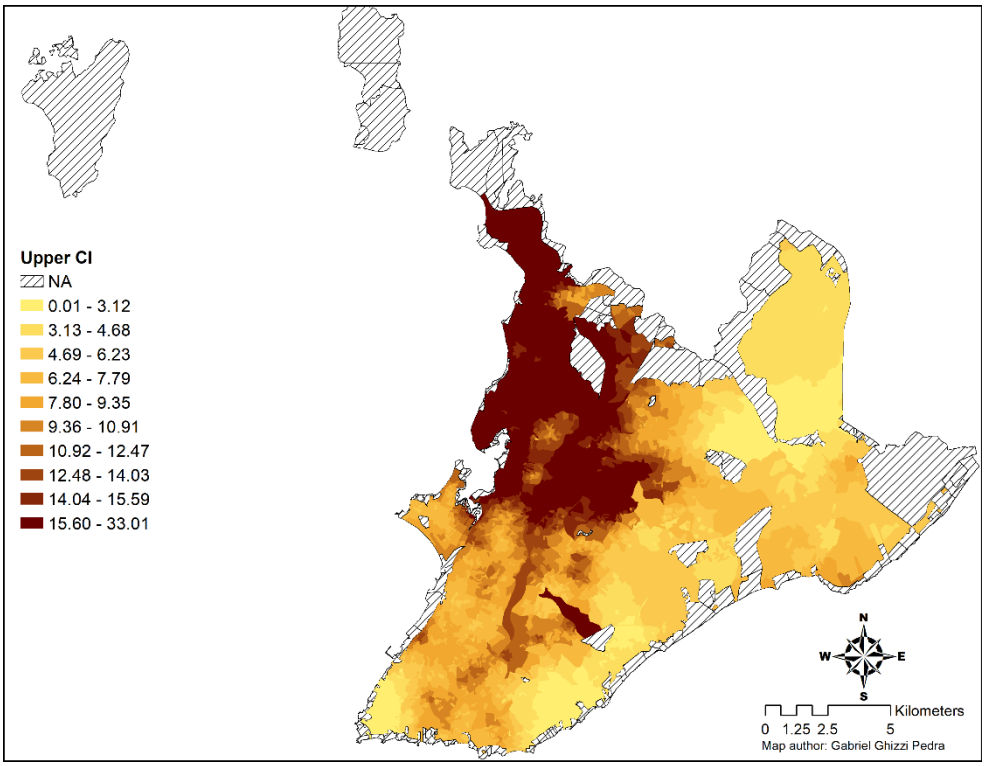

Figure 5.7: Upper confidence interval of the predicted leptospirosis incidence per 10,000 population in Salvador, Brazil.

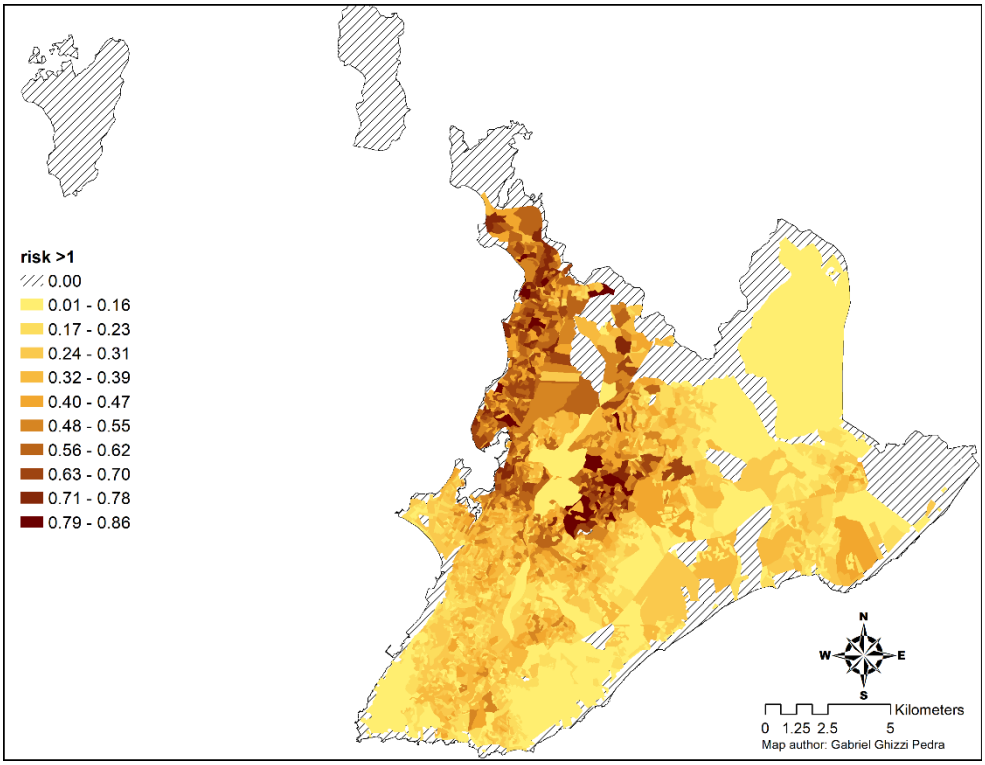

Figure 5.8: Risk of having one case or more of human leptospirosis in each census district of Salvador, Brazil.

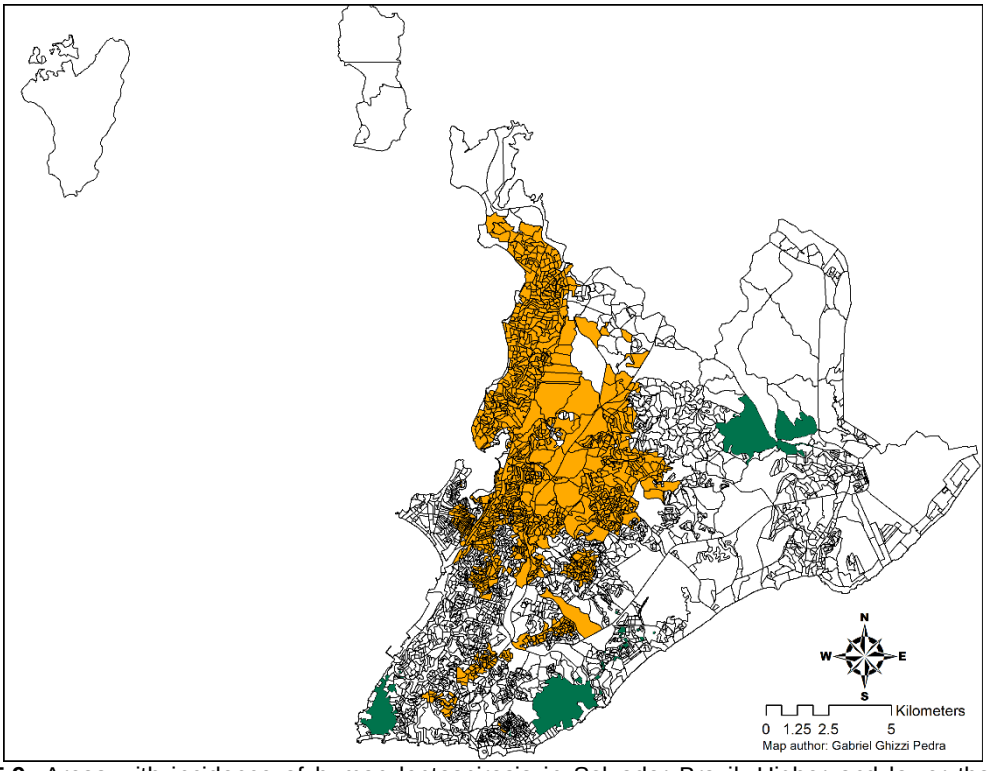

Figure 5.9: Areas with incidence of human leptospirosis in Salvador Brazil. Higher and lower than expected are shown in the colours orange and green, respectively.

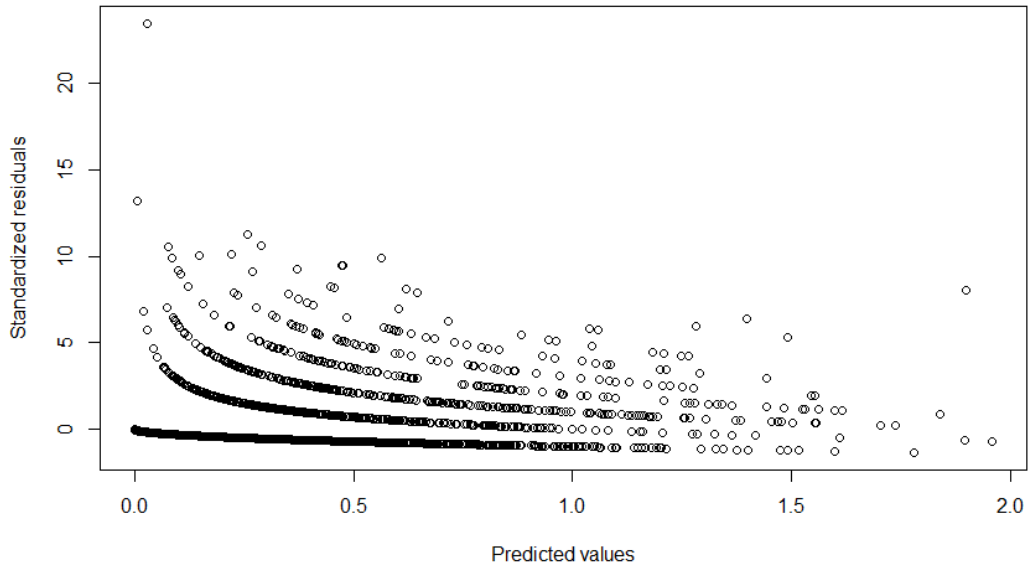

Figure 5.10: Standardized residual plot.

5.5 Discussion

This chapter aimed to explore the association between human leptospirosis and hydrological variables by looking at the total number of cases per district census and extreme (ninetieth percentile) hydrological values. A set of new hydrological variables was developed that are complementary to each other and can be used as measures of environmental risk for waterborne diseases such as leptospirosis. Before going into the results it is perhaps useful to reiterate the meaning of each hydrological variable and the motivation behind the use of extreme values in the statistical analysis. These explanations will help to understand how each hydrological variable can be translated into risk and how these risks can be associated with leptospirosis infections. There were four hydrological variables extracted from a Digital Elevation Model - flow accumulation, population weighted accumulation (PWA), water rurality and the topographic wetness index (TWI).

Flow accumulation can be considered as the area of a catchment because it summarises all the upslope cells that water flows through until it reaches a target cell. For that reason, it is possible to say that flow accumulation gives information on how far the runoff water has flown over the surface, but also how much water has been ‘collected’ since the same amount of water originally fell on each cell. In the context of waterborne diseases, it can be considered as a way of describing the abundance of pathogen reaching a cell by assuming simply that more water implies more pathogen. Similarly, PWA can be thought of as either an area or an amount, but in this case weighted by how populated areas are. Hence, the assumption might be that water that flows through populated areas carries more pathogens because more populated areas have more human waste and garbage and more rats. Alternatively, we might propose that water that flows through populated areas

carries *fewer* pathogens because in more populated areas water tends to flow over paved surfaces and has less opportunity to acquire pathogens. Furthermore, if a ratio between those two measurements is taken, it is possible to scale the origin of the water arriving in a targeted cell, independent of quantity. This ratio is given by 'water rurality' and higher values indicate the water is coming from more populated areas whereas lower values indicate water is coming from non-populated areas. Finally, TWI is a commonly used as a proxy for flood risk as it takes to account the catchment area (flow accumulation) and the slope. Higher values are indicative of a higher likelihood of flooding. In the present context, therefore, it is assumed that the higher is the likelihood of flooding, the larger the number of pathogens that will accumulate in a target cell (rather than simply passing through).

The hydrological variables were calculated at a very fine scale, five meters resolution. On the other hand, human leptospirosis cases were taken at a district level. To match the scale of the observed disease cases, the hydrological data had to be aggregated at the district level. The first exploration on the data showed that the distribution of the hydrological values for all variables were very right skewed, showing a lot of small values and a very few higher values. These results were expected at that scale due to the topography of the city. The city is not flat but is composed of many valleys (Figure 5.11). This characteristic means that each district has a very high variation of values and few very large values in the bottom of the valleys (canals), which is where there will be more cells flowing to target cells, consequently will also have a higher likelihood of flooding. These canals will connect areas but most of the values are very small and will be coming from the top of the ridges within each district. Averaging the hydrological values per district would therefore not identify those areas that it is assumed have higher risk. Therefore, in order to test the assumption that higher values represents a risk for transmission of

leptospirosis it was decided to use the 90th quantile from the distribution of hydrological values in each district.

Figure 5.11: Photo of a valley in the community of Pau da Lima in Salvador, Brazil. This photo demonstrate a common topography of the city which is composed of many valeys like in the photo.

The results from the model selection have shown that only water rurality is associated with human leptospirosis at the district level. However even considering the district level as a random effect, there was spatial dependency in the data. These results are consistent with other studies that have evaluated the distribution of human leptospirosis. For example, it has been shown that the distribution of

human leptospirosis cases is clustered in American Samoa (Lau *et al.*, 2012), New Caledonia (Goarant *et al.*, 2009b), Mexico (Sánchez-Montes *et al.*, 2015) and many other regions.

Water rurality was found to be negatively associated with the number of human leptospirosis cases in each district. This result indicates that water coming from less populated areas has a higher risk of causing leptospirosis infections. Gracie *et al*. (2014) found that the proportion of urban usage in areas was negatively correlated with leptospirosis incidence in Rio de Janeiro, Brazil. However, they did not find any evidence of spatial autocorrelation and used simple correlation functions. Similarly, Schneider *et al*. (2012) found a positive correlation between the proportion of rural population in an area and leptospirosis. However their results were based at the country level, a bigger scale than this analysis and the analysis performed by Gracie *et al*. (2014). Despite the differences in scale, these results might suggest that human leptospirosis is still a rural disease but present in urban areas. There are two possible reasons for these associations in urban areas. Firstly, more populated areas have more impermeable surfaces just because they will have more houses and streets, but leptospires have not been found in those surfaces and its survival in such exposed locations is likely to be very low. Therefore, water passing through populated areas is passing over impermeable surfaces and is less likely to carry any bacteria. On the other hand, less populated areas will have more impermeable surfaces such as exposed soil and the water will pass through the soil surface and will be more likely to carry the bacteria downslope. Furthermore, it has been observed that many unpopulated areas serve as locations for informal rubbish deposition for the community where rats have been associated with rubbish (Santos *et al.*, 2017). These areas can therefore be a source for environmental contamination and the water flowing through those areas might mobilise the bacteria carrying it downstream. Therefore, an important next step would be to explore how

soil permeability and/or a measurement of rubbish management is associated with infections between districts in Salvador.

The results from this analysis are very encouraging and have demonstrated that hydrology needed to be more explored in infectious disease research. Studies that have included hydrological variables in their analyses have used flooding risk as the main approach or have simply used rainfall (Vega-Corredor and Opadeyi, 2014; Suwanpakdee *et al.*, 2015; Gutiérrez and Martínez-Vega, 2018). Gutiérrez and Martínez-Vega (2018) looked at anomalies in rainfall and found evidence of association between rainfall anomalies and leptospirosis cases at a city level in Colombia. Suwanpakdee *et al*. (2015) analysed how flooding risk, measured by satellite imagery, is associated with leptospirosis cases also at a city level in Thailand. They found that flooding was less important than expected and suggested that agriculture and farming could explain more the pattern of leptospirosis infection found in Thailand. Conversely, Vega-Corredor and Opadeyi (2014) looked at the role of a set of hydrological variables and found that flooding risk were associated with leptospirosis infections in Trinidad at the city level. Here, it was demonstrated that water rurality was associated with risk of leptospirosis infection but at a district level. Hydrological variables seem to be associated with leptospirosis at many scales, but at the individual level, this association have not been explored yet.

Additionally, given the nature of how hydrology was assessed in this thesis (using topographic analysis based on elevation), the association with water rurality could be given by the correlation between water rurality and elevation. However, the Pearson correlation between those two variables were checked and it was observed very little correlation (r= -0.11). This correlation result gives more support that the association between leptospirosis cases and water rurality it is not caused by a colinearity between elevation and water rurality. The analysis on this chapter focused on evaluating purely hydrological, however, elevation should be considered

as an environmental variable in further analysis in order to quantify the environmental risk factors of leptospirosis.

Despite the predictions' confidence intervals showing there to be high variation around the predictions of the model, it was nonetheless possible to explore how the risk varies spatially. If the intention is to predict the number of cases in a target district, this would be very imprecise as it was possible to see the variation in the maps of the confidence interval (Figure 5.6 and Figure 5.7). This imprecision might be an feature of main cities in developing countries where inequality is very high and there are abrupt change in the living conditions between neighbouring communities. This feature can cause noise in the predictions. Additionally, human leptospirosis is not a very common disease, which results in lower case counts and hence in higher variance as the mean and variance of a poisson distribution are the same. However, the geostatistical model used here accounts for unexplained variation, both spatially structured and unstructured. Thus, despite the imprecision of the estimation at a target point, the spatial variation of the risk can still be explored and has shown parts of the city where the risk is higher than in others, such as the northwest of the city.

This analysis was performed at the district level, which was able to show an association between human leptospirosis and hydrology. Another issue of high interest is to explore how the disease cases are associated with hydrology at a more local scale. Exploring this association could show the importance that hydrology plays in the dynamics of infection. Pau da Lima is a community of Salvador where our research group has been investigating the dynamics of human leptosirosis involving epidemiological, ecological and environmental approaches. From a longitudinal cohort followed from 2003-2004, it was found that the environmental risk factors associated with transmission of assymptomatic cases were contact with mud, household elevation and rat infestation (Hagan *et al.*, 2016). They found that

the spatial distribution of risk in the community was varied and localized. Incorporating the hydrological variables into the system might direct and help to identify areas of high risk of transmission.

Attention might be needed in northwest Salvador, where the risk was identified to be high, in order to reduce infection rates and improve people's health and wellbeing. Despite the model not considering individual characteristics and any social aspects of the disease, it was possible to observe an association between hydrology and leptospirosis infections at the district level. Combining hydrological variables alongside socioeconomic characteristics of the individuals might help to improve the model fit and identify risk more accurately, but the challenge would be combine both types of data. Additionally, looking at the associations at the local scale can potentially give more insights on the role of the environment in the dynamics of leptospirosis infection.

5.6 References

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Chapter 6: General discussion

Leptospirosis has been shown to be a complex disease with many interacting factors influencing it that can be grouped into socioeconomic, ecological and environmental. Socioeconomic factors are related more to the individual such as illiteracy, income and gender. Ecological factors can be related to individual risk factors such as rat infestation or to the reservoir risk factors such as rat contact and contamination. Finally, environmental factors are more complex and can be related to the individual, the reservoir or the environment itself. Contact with mud, distance to the sewer, elevation and flooding are examples of environmental risk factors. They all seem to interact with each other, which produces very diverse outcomes with different associations that will depend on the scale and region of the observations. The introduction to this thesis showed that there is lack of understanding of the dynamics of the bacteria in the environment. Thus, this thesis tried to explore some key issues that can be very relevant to understand the dynamics of the bacteria in the environment and how this can be associated with human leptospirosis.

A conceptual model was developed based on what is known in the literature but there were several assumptions that were made. The first assumption of the model is that the bacteria enter into the system via shedding from its reservoir, which are Norway rats in the case of Salvador and many other urban centres. The second assumption is that there are three compartments where the bacteria can be found: water bodies, soil surface and sub-surface. The next assumption is that hydrology drives the transportation of the bacteria between compartments where runoff mobilises the bacteria from the soil to water bodies and flooding mobilises in the other direction. The last assumption is that the bacteria leave each compartment with a survival rate and/or through human infection. Issues found in addressing each

part of the model directed the development of this thesis and will be addressed in the following paragraphs.

The first issue arose from estimating abundance of the animal reservoir, Norway rats, which is essential to understand the level of contamination/shedding by leptospires in the environment. Standard methods to estimate animal abundance are based on capture-recapture methods where captured animals are marked and released, and the absolute abundance is estimated based on recording historical capture-recapture observations (Borchers, Buckland and Zucchini, 2002). However, Norway rats are invasive species that carry a significant number of zoonotic diseases, and releasing those animals is not appropriate, as they are a risk for people's health. Alternatively, animal abundance can be estimated if animals are removed and a decay in the number of animals captured per day is observed. The issue found in this method is that the assumption of a reduction in the number of animals captured is crucial and thus the model does not allow for intrinsic variation in trapping history that does not necessarily reflect variation in the abundance. In fact, the new proposed method, developed in Chapter Two, was shown to be more robust regarding those variations and the animal abundance could be estimated more precisely. The application of this method goes beyond its use in urban rats; it could be applied in any situation where removal methods are appropriate such as monitoring invasive species in wild.

Following animal abundance, another key issue found is the survival of leptospires in the environment. Once the bacteria are released into the environment, it is known that there is a set of factors that are related to its survival such as temperature, pH and soil moisture. Evidence has shown that the bacteria can be found in soil sub-surface for months but, before the analysis produced in Chapter Three, there was no evidence of the survival of the bacteria being quantitatively estimated. The first feature of the model developed in Chapter Three is to allow the

user to make inferences regarding the shape of the survival curve, which will be informative as to whether the hazard is constant through time. Secondly, the model can quantify the decay rate of the bacteria and make inferences regarding whether the decay rate is different depending on the environment. Finally, the last feature of the model is the ability to quantify the proportion of individuals that survive beyond the time of the experiment. The results from the model show that leptospires in microcosms have a survival shape with a tail, which means that most of the individuals died within the first two weeks of the experiment, but there is a small proportion of individuals who survived for longer. The bacteria have different decay rates in soil and sewage microcosms, which indicates that soil seemed to be more suitable for the survival of the bacteria. Despite the results being based on microcosms where there are controlled conditions, it is believed that this can be a good approximation of how the bacteria can survive in the environment. This information can help to coordinate the time and duration of an environmental intervention control.

The next two chapter of this thesis, Chapters Four and Five, were related to hydrology. The conceptual model assumed that hydrology is the main driver of leptospirosis in the environment and that human infection can occur from contact with the environment. However, the role of how hydrology is related to human infection is not well explored, mainly because hydrological modelling can be complex and requires high resolution of empirical data. In Chapter Four, hydrology was explored in a way that minimises the amount of data necessary to produce hydrological risk maps. Based on the assumption that if all rain that falls become runoff and there are no barriers, the water will flow into the lowest point. Hence, a set of hydrological measurements could be explored and used as environmental risk factors for leptospirosis or other waterborne diseases. The maps produced have shown to be complementary to each other such that the risk they describe depends

on the measurement. Those maps can be further explored and additional information can be included such as soil permeability, which will then influence the amount of water that reaches an area.

The hydrological measurements were applied to leptospirosis cases in Salvador, Brazil. The aim of Chapter Five was to investigate and validate the measurements used as risk factors for waterborne diseases. From four hydrological measurements (flow accumulation, population weighted accumulation, water rurality and topographic wetness index), only one variable was associated with human leptospirosis in Salvador. Water rurality was negatively associated with the number of cases in the districts of Salvador. This result indicates that if water is flowing through non-populated areas, the risk of infection seems to be higher than from populated regions. Considering that leptospirosis was originally described as a rural disease, it seems fair to say that leptospirosis in Salvador presents patterns of a rural disease but in an urban environment.

6.1 The value of methodological advances

Although this thesis is in many respects methodological, the results obtained here have many implications that will improve the understanding of the dynamics of the bacteria in the environment. Additionally, the results can direct the development of a mathematical model of the bacteria in the environment as well as parameter estimation. The conceptual model can be updated to incorporate the results based on this thesis. With the new abundance method, animal abundance will be estimated more precisely and accurately which could improve the parameter estimation related to the input of the bacteria into the environment. The survival model indicated that the survival of the bacteria varies between compartments, such

as soil and water, hence, a different survival parameter should be considered in each compartment.

New hydrological variables were produced that can represent different risks not only for leptospirosis, but also for waterborne diseases in general. Hydrological transport was used in the dynamic modelling of cholera where the movement of the pathogen would occur through a catchment network (Bertuzzo *et al.*, 2007; Mari *et al.*, 2012). The hydrological variables could be used instead of catchment network, mainly because they would provide richer information on water flow. The variables produced in Chapter Four could be applied to develop a dynamic model for the bacteria in the environment, which could go even further and a spatially explicitly model could be developed. The hydrological variables could help map bacteria concentration, however, the amount of bacteria that is mobilised by runoff should be considered as well as bacteria diffusion in the soil. Thus, an experiment could be designed to provide empirical evidence of the bacteria transportation via hydrology, runoff and flooding. Once proven and quantified, the information could be combined with hydrological variables and the dynamic model could be developed further.

Leptospirosis in Salvador is associated with water rurality, a new hydrological variable that measures if most of the water is flowing through areas that are considered rural. Those results can be incorporated into the dynamic modelling of human infection, where an exposure compartment could be included. A series of mathematical models for leptospirosis have been developed. Baca-Carrasco *et al*.(2015) developed a mathematical model for human and animal leptospirosis where there is a compartment related to the bacteria in the environment. However, their model only assumed that the bacteria enter via an infected animal or human and have an average survival time. Other models for human leptospirosis transmission have been developed. For example, Ismail *et al* (2017) had an exposure compartment in their dynamic modelling but only looked at a standard

susceptible-infected-recovered (SIR) model. Triampo *et al*. (2008), Pimpunchat *et al*. (2013) and Moustafa (2014) are other examples of studies of dynamic modelling of leptospirosis. However, none of those studies have used empirical data; they all performed analytical analysis to explore the behaviour of the infection in their system of equations. Here, the evidence of a hydrological factor being associated with leptospirosis cases in Salvador has shown that hydrological risk maps are important in studying infections. Similarly, Vega-Corredor & Opadeyi (2014) found that flood risk was associated to human leptospirosis. Despite there being only few studies that have linked hydrology to leptospirosis, we know that water-related variables such as rainfall, flooding and distance to sewage are risk factors that are found to be associated with leptospirosis cases (Albert I Ko *et al.*, 1999; Barcellos and Sabroza, 2001b; Sarkar *et al.*, 2002; Reis *et al.*, 2008; Goarant *et al.*, 2009b; Amilasan *et al.*, 2012b; Garba, Bahaman, Khairani Bejo, *et al.*, 2017). Therefore, a future direction in the development of a dynamic modelling of human leptospirosis would be to include an exposure compartment associated with hydrology.

Discussions around climate change are increasing as its effect on infectious disease dynamics is becoming more evident. Extreme weather events are increasing and epidemic events of leptospirosis have been reported worldwide (Lau *et al.*, 2010a). The association between heavy rainfall and flooding have been reported in many countries such as Brazil, France and Trinidad and Tobago (Albert I Ko *et al.*, 1999; Kupek, de Sousa Santos Faversani and de Souza Philippi, 2000; Baranton and Postic, 2006; Mohan *et al.*, 2009). There is an urgent need to understand how climate change will affect the spread of infectious diseases such as leptospirosis. Understanding the role of the environment in the dynamics of the bacteria might help to understand how climate change will affect leptospirosis transmission.

6.2 Post script: Two years of work in the community of Pau da Lima and how the results of this thesis can help the community

Before applying for my PhD scholarship, I moved to Salvador, Brazil to start working as a field technician in the project where Prof Mike Begon and Prof Peter Diggle were involved. The project was called “Eco-epidemiology of urban leptospirosis” and was developed in a low-income community of Salvador, called Pau da Lima. The project is a multidisciplinary one that aims to understand all facets (reservoir host, environment and disease) of human infection by *Leptospira*. There were three main types of field work to be done: serosurvey, rodent trapping and environmental sampling, though there was no overlap or real integration between these. My involvement in the project went through all facets, starting in the human infection theme, collecting samples for the serosurvey, then rodent trapping, and then planning environmental sampling. My time in the community helped significantly in the development of my PhD thesis.It elucidated the complexity of leptospirosis and how each compartment of the disease plays an important role in the infection.

Salvador city is located on the northeast cost of Brazil. Pau da Lima is located in the centre west of Salvador. Pau da Lima, like many regions in Salvador, is composed of many valleys where the bottoms of the valleys were the poorest areas we observed. Additionally, there were open sewers present. The study was based in three valleys of Pau da Lima where there were protocols for collecting each type of data. We always worked in pairs and had a small area to cover each day. During the serosurvey, we walked into the houses, asked for consent to collect blood samples and had a socioeconomic and environmental questionnaire to ask.

We collected information regarding their knowledge about leptospirosis, and their risk perception, and collected a set of environmental characteristics from the house and surrounding areas. In the environmental questionnaire, we identified if the house had a sewer closer than 20 meters, and if there were rat signs, burrows, and a continues to the present day, found that elevation and household income were risk factors for leptospirosis infection. For that reason, the study area in Pau da Lima was changed to be focused on the bottom of the valleys.

During my work in the field, we observed how the rats and people lived very close to each other, especially when they were living in the bottom of the valley. There were rat signs in the surrounding areas of many houses and near the open sewers. Rats were seen almost every day in those areas. During the interviews of the households, some residents commented that they used illegal poison to try and remove those animals from the surroundings of their houses but many of their pets died too. The Centre for Zoonosis Control (CCZ), a public organization that manage zoonotic diseases, define many areas in the city as endemic for leptospirosis and apply rodent control in order to exterminate (or at least control) the rodents. Pau da Lima was one such area. However, the rodent population seemed to be very abundant and widespread, indicating that any effect of the control measures seemed temporary and that rats are still present in the community. From this observation and the results from Costa *et al* (2014) that 80% of the rats were infected with leptospires, it can be assumed that the level of environmental contamination is widespread throughout the community.

Alongside the rats' presence, during the rainy season we observed pathways used by the households to connect to the main street becoming 'rivers' of water flowing down the valleys. The runoff washed through the soil and carried out many things down to the bottom of the valley, especially items of rubbish that were on the ground due lack of litter collection or overflows from collection points. Nevertheless,

the runoff did not stop members of the households from going out, and people were seen walking up the valley to go to the main street, probably to go to work or to buy groceries. Additionally, the bottoms of the valleys were flooded and sometimes the flood could reach the houses if a heavy rainfall event occurred. In order to counteract these flood events in the bottoms of the valleys, the community managed the open sewers and removed any barrier that could block the water flow such as rubbish and plastic bags. They also tried to create barriers to avoid the water going inside their houses. However, they did not have any protection to work in those open sewers and some households documented that they had leptospirosis. Given the results from Ko *et al* (1996) for Salvador, and many other authors around the world that observed seasonality in the infection during rainy season, we thought that hydrology can be important in the transmission of leptospirosis and should be further explored.

Given the widespread presence of rats in the community and aspects of human behaviour described above, it seems that the level of exposure to leptospires in the environment is very high. The results of the studies described in this thesis could assist communities endemic for leptospirosis in many aspects alongside directing new research questions to be addressed. For example, the survival analysis of leptospires indicate that after rainfall, where bacteria have been mobilized into the soil or the sewage, the risk of infection could be considered higher right after rainfall and decreases as the survival of the bacteria decreases with time. However, this is a hypothesis that has not been tested yet. Nonetheless, assisting and informing the population to avoid going out right after rainfall and educating them in the use of protection could make a difference and reduce infections. Ideally, it would be very informative to know what level of rainfall would be considered dangerous for transmission of leptospirosis and create a transmission alert system for high transmissibility periods.

The results linking leptospirosis cases with hydrological variables indicated that at the district level, water that passes through less populated areas (here called rural areas) were associated with higher number of cases. From a public health perspective, identifying areas of higher and lower risk of transmission is very informative in terms of management interventions and controls of the disease. One of the main challenges in this thesis was the difficulty on linking observations made at a local scale with data observed at a higher scale. However, even with those issues to overcome, the results obtained supported our observations in Pau da Lima. The bottoms of the valleys were areas with lower densities of people and were the poorest areas. Many animals were present in the houses in the bottoms of the valleys, such as farm animals (chicken, pigs and cows) as well as pets (cats and dogs). They provide accessible food for rats, which can increase the contact with rats or a contaminated environment, hence, increase infection risk. Additionally, those 'empty' areas were used for rubbish deposition and as places to deposit construction materials, which also provide shelter and nest for rats. Therefore, the results captured some of the features observed at a local scale even when the inference was based at a regional scale (district level). These results can be taken to the CCZ who could update their assessment of endemic areas and intervention protocols can be reassessed.

Thus, the results of this thesis will be taken to public health stakeholders to propose a collaboration where academic research can be directly applied, and the needs of public health management could also be met.

6.3 References

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