Invasive animals and the Island Syndrome: parasites of feral cats and black rats from Western Australia and its offshore islands

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Author's Declaration

I declare that this thesis is my own account of my research and contains as its main content work that has not previously been submitted for a degree at any tertiary education institution.

Narelle Dybing

Statement of Contribution

The five experimental chapters in this thesis have been submitted and/or published as peer reviewed publications with multiple co-authors. Narelle Dybing was the first and corresponding author of these publications, and substantially involved in conceiving ideas and project design, sample collection and laboratory work, data analysis, and preparation and submission of manuscripts.

All publication co-authors have consented to their work being included in this thesis and have accepted this statement of contribution.

Abstract

Introduced animals impact ecosystems due to predation, competition and disease transmission. The effect of introduced infectious disease on wildlife populations is particularly pronounced on islands where parasite populations are characterised by increased intensity, infra-community richness and prevalence (the "Island Syndrome"). This thesis studied parasite and bacterial pathogens of conservation and zoonotic importance in feral cats from two islands (Christmas Island, Dirk Hartog Island) and one mainland location (southwest Western Australia), and in black rats from Christmas Island. The general hypothesis tested was that Island Syndrome increases the risk of transmission of parasitic and bacterial diseases introduced/harboured by cats and rats to wildlife and human communities.

To investigate the Island Syndrome, necropsies were performed on feral cats and black rats and the macro parasites identified were collected and quantified to ascertain parasite prevalence, infra-community richness and intensity. On Christmas Island, it was determined that 92% of feral cats and 84% of rats harboured helminth parasites with an infra-community richness of 0-6, and 0-7, species in cats and rats, respectively. A high intensity (number of individual parasites recovered per host) was observed for some parasite species. These findings demonstrated that three epidemiological characteristics (high prevalence, infra-community richness and intensity/abundance) conformed to the characteristics of the Island Syndrome. However, contrary to the Island Syndrome hypothesis, a high regional richness of parasites was observed on Christmas Island, with nine species of helminth recorded in cats and 10 species in rats). The parasite community characteristic observations were

repeated on Dirk Hartog Island, which also exhibited the same three characteristics of Island Syndrome (high prevalence, infra-community richness and intensity/abundance), but where no difference in regional richness was observed compared with the mainland environment. Specifically, the overall prevalence was significantly higher ($p \leq 0.01$) on Dirk Hartog Island (100%) compared to southwest WA (79.6%), as was mean infra-community richness ($p \leq 0.001$) (3.61±1.41 on Dirk Hartog Island and 1.57±1.29 from southwest WA). For those parasite species occurring on Dirk Hartog Island and in southwest WA, the prevalence and abundance was found to be significantly higher on Dirk Hartog Island than the southwest WA ($p \leq 0.019$ and $p \leq 0.003$, respectively). These findings suggest that not all facets proposed by the Island Syndrome hypothesis apply to all island environments, particularly for parasite communities harboured by invasive species.

Parasites of both zoonotic and conservation significance were detected in the cats and rats from both islands and from mainland Western Australia. Pathogenic bacteria of public health importance were identified; two species of *Bartonella* in rats (*Bartonella phoceensis* and an unidentified *Bartonella* species) on Christmas Island, two species *Bartonella* in cats (*B. henselae* and *B. koehlerae*) from southwest Western Australia, and *Leptospira interrogans* from both cats and rats on Christmas Island. The presence of *Trypanosoma* in cats and rats (from all three locations) and *Leishmania* (Christmas Island only) were investigated, with neither of these vector-borne protozoans identified at any of the locations.

In summary, this thesis presents new data pertaining to parasite community structures in two invasive mammalian pest species of global importance following their introduction to islands, and the potential relationship between their parasite

community structures and parasite biology, prevailing physiographic factors and faunal biology. The observations suggest that cats and rats are important in contributing to and maintaining artificially elevated parasite species' richness within both insular and mainland environments. The findings also highlight potential threats that invasive animals pose with respect to disease transmission to susceptible ecological communities, in particular insular ecosystems, as reservoir hosts for parasitic and bacterial organisms.

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Publications Arising from this Thesis

Journal publications

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Dybing, N. A., Jacobson, C., Irwin, P., Algar, D., Adams, P.J., 2017. Challenging the dogma of the 'Island Syndrome': A study of helminth parasites of feral cats and black rats on Christmas Island. Australasian Journal of Environmental Management. (Submitted: invited paper).

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Conference oral presentations

Dybing, N. A., C. Jacobson, P Irwin, D Algar, and P. J Adams. 2016. "What did the feral cat drag in? Feral cats, helminths and the Island Syndrome." Ecological Society of Australia, Fremantle, Western Australia.

Dybing, N.A., Jacobson, C., Irwin, P., Algar, D., Adams, P.J., 2015. *Leptospira* species identified in rodent and feline hosts from island and mainland Australia, Australasian Wildlife Management Society annual conference, Perth, Western Australia.

Dybing, N.A., Irwin, P., Jacobson, C., Algar, D., Adams, P.J., 2014. Introduced species and their parasites: a tale of two islands, Australasian Wildlife Management Society annual conference, Brisbane, Australia.

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Dybing, N.A., Jacobson, C., Irwin, P., Algar, D., Adams, P.J., 2012. Parasites of Feral Cats (*Felis catus*) and Black Rats (*Rattus rattus*) on Christmas Island, 22nd Annual Combined Biological Sciences Meeting, Perth, Western Australia.

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Dybing, N.A., Jacobson, C., Irwin, P., Algar, D., Adams, P.J., 2013. Poster: Feral Peril on Tropical Christmas Island, 62nd Annual Wildlife Disease Association (WDA) International Conference, Knoxville, Tennessee.

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A note on thesis layout

This thesis consists of chapters that have been prepared as stand-alone manuscripts for publication in journals. Three manuscripts have been accepted for publication (Chapters 4, 5 and 6). Chapters 2 and 3 are invited manuscripts for special issue journals. To maintain formatting consistency throughout the thesis, published chapters differ slightly from the published manuscripts.

The first two chapters focus on the macroscopic parasite community. The last three chapters focus on molecular detection of microscopic (specifically haemotropic and vector-borne) pathogens.

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List of Abbreviations

ANOVA	analysis of variance
BB	Boyup Brook
BCI	body condition index
BLAST	Basic Local Alignment Search Tool
CHI	Christmas Island
Co	Coorow
Cr	Cranbrook
Da	Darkan
DHI	Dirk Hartog Island
DNA	deoxy ribonucleic acid
dNTPs	deoxynucleotide triphosphates
Do	Dowerin
Du	Dumbleyung
Dw	Dwellingup
Es	Esperance
Fr	Frankland
GG	Gingin
GI	Gastro-intestinal
GI ICR	gastro-intestinal infra-community richness
HB	head-body length
ICR	infra-community richness
ITS	internal transcriber space
Ka	Katanning
La	Latham
LP	Leschenault peninsula
Ma	Mandurah
MB	Mt Barker
MgCl2	Magnesium chloride
MI	mean intensity
MR	Moore River
Ny	Nyabing
PCR	Polymerase chain reaction
Qu	Quairading
RI	range intensity
SE Asia	south east Asia

swWA	southwest Western Australia
TICR	total infra-community richness
UW	Upper Warren
VICR	visceral infra-community richness
WA	Western Australia
Wo	Woodanilling

Chapter 1 General Introduction

Invasive species are those which, having been either deliberately or accidentally introduced to a new environment, are able to establish self-sustaining populations which are subsequently difficult to control (West, 2008). Invasive species spread beyond their natural range, typically facilitated by anthropogenic activities, and their advantageous biological features lead to adverse impacts on extant communities, influencing native faunal species richness, diversity, abundance and interactions. Impacts of invasive species may be attributed to predation, competition for resources and disease transmission to native species (Colautti *et al.*, 2005; Daszak *et al.*, 2000).

1.1 Invasive animals and establishment success

Throughout human history, deliberate introductions of vertebrate animals have occurred for many reasons including for livestock, companion animals, sporting as well as biological control (Clout and Russell, 2008; Long, 2003). Unintentional introductions have also occurred, generally as stowaways on ships (e.g. rodents, birds), or on food stuffs and packaging material (e.g. reptiles) (Holdgate, 1967; Savidge, 1987). Island ecosystems are particularly susceptible to species invasion due typically to high levels of species endemism coupled with human trade and movement over numerous centuries. Ebenhard (1988) documented 644 reported mammalian introductions on islands alone due to anthropogenic dispersion, which represents 80% of all known animal introductions.

The enemy release hypothesis posits that the success of invasive animals in a new environment is due to their ability to out compete native species, readily adapt to

a range of environmental conditions and flourish in the absence of natural predators and disease (Colautti *et al.*, 2004; Mitchell and Power, 2003; Torchin *et al.*, 2003). Characteristics which favour the successful establishment of an invasive species include a high abundance in its original range, short generation times, polyphagous feeding habit, ability of fertilized females to colonise alone, an association with humans, and a high genetic variability (Ehrlich, 1989). Whilst many successful invasive species can survive in a wide variety of habitats, favourable climatic conditions, environmental features and fauna presence (prey availability and competition) will help determine the subsequent impacts and abundance on native flora and fauna (Ehrlich, 1989).

1.2 Vulnerability of island ecosystems

Vulnerability of island ecosystems, to the effects of invasive species, is exacerbated due to increased endemicity, lack of diversification, simplified trophic (or food) webs and relatively low species richness. The physical distance of oceanic islands from continental mainland environments limits the ability of fauna to reach and colonise island systems naturally (Reaser *et al.*, 2007). Long evolution in isolation from other species is linked to a loss of defences in insular fauna and leaves them particularly susceptible to impacts by introduced species (Elton, 1958; Mack *et al.*, 2000; Moors and Atkinson, 1984; Simberloff, 1986; van Aarde and Skinner, 1981), including a lack of resistance to disease or parasites introduced by invasive species themselves (D'Antonio and Dudley, 1995; Loope, 1986; Mueller-Dombois *et al.*, 1981). As such, potential consequences of an introduced disease or parasite becoming established in a novel environment can be highly significant, even catastrophic (e.g. reduced fecundity of native species, increased stress leading to lower vigour,

extirpation) (Courchamp *et al.*, 2003; D'Antonio and Dudley, 1995; Loope, 1986; Reaser *et al.*, 2007; Vitousek, 1988).

1.3 Invasive parasites and invasion success

Human encroachment into wildlife habitats and the anthropogenic introduction of invasive species into new environments enhance the potential for disease transmission to humans, wildlife and domestic animals (Averis *et al.*, 2009; Daszak *et al.*, 2000; Daszak *et al.*, 2001). Pathogens, including parasites and bacteria, are introduced when invasive species themselves are introduced to a new location. Introduced pathogens can contribute to the success of an invasive species by providing a competitive advantage particularly on islands with naïve host populations (Daszak *et al.*, 2001; Hudson and Greenman, 1998). There has been a long hypothesised link between invasive animal introductions and disease outbreaks in wildlife on islands (Cunningham, 1996; Daszak *et al.*, 2000; Daszak *et al.*, 2001; Gurevitch and Padilla, 2004; Harris, 2009). Most of these links are based primarily on anecdotal evidence and there is a lack of baseline disease data for islands.

During an introduction event, founding individuals rarely carry a full suite of parasites, therefore a lower parasite species richness is introduced compared to that of the original host population, termed the founder effect (Torchin *et al.*, 2003). Introduction of parasites into the new environment does not guarantee successful establishment of an invasive parasite population. In reality, for the successful establishment of new parasites to occur, prevailing conditions are required to be amenable to transmission and parasite cycling; however, all parasites have the potential to be invasive.

Three main factors contribute to the success of introduced parasites in a new environment; the biology of the parasite, the insular physiography, and free-living fauna. Invasive parasites need suitable conditions specific to themselves to successfully establish in the new environment. Attributes of successful parasite invaders include the capacity to adapt to conditions of the new external environment and new potential intermediate hosts, as well as having a simple rather than complex life cycle (Kisielewska, 1970; Mas-Coma and Feliu, 1984).

1.3.1 Parasite biology

Faunal and physiographical features work with or against a parasite's biology to determine the success of the parasite in the new environment (Mas-Coma and Feliu, 1984). For continued development and survival, parasites have evolved a specific sequence of required hosts and/or external environmental stages. Transmission strategies relate to parasite biology, including capacity to form environmentally resistant stages, utilisation of vectors and transmission to sequential hosts.

Characteristics of a given parasite's life cycle may help or hinder the chances of a successful establishment post-introduction to a new environment. Shorter, simpler life cycles have a greater probability of success, whereas longer, more complex life cycles will typically reduce the chance of a parasite establishing within a new environment. There are two main life cycle types; direct and indirect, both incorporating many different transmission strategies.

Direct life cycles are typically simpler (and often shorter) as the parasites do not require a secondary or alternative host to survive. Parasites with direct life cycles can be transmitted from host-host by direct contact, skin penetration, via droplets or by

environmental stages. Environmental stages include eggs, cysts or free living larvae, and may be capable of persisting for long periods (often months or years) in the environment. Some environmental stages require a minimum length of time within the environment to become infective to the next host. Thick protective walls of eggs confer ability to withstand extremes in physiographic conditions e.g. humidity, temperature and pH levels. For example, *Toxocara* spp. have a thick-walled, resistant egg that requires 2-3 weeks to embryonate and become infectious to the next host, after which they remain viable for approximately 18 months in the environment (Bowman *et al.*, 2002). Less commonly utilised strategies include trans-placental and trans-mammary transmission from the mother to offspring (e.g. *Strongyloides* spp.), or autoinfection within the same host (e.g. *Rodentolepis nana*) (Baker, 2008; Miller, 1981).

Indirect life cycles are more complex, and are assumed to have a decreased probability of survival due to their requirement for multiple hosts during development and maturation. This lifestyle is subsequently anticipated to have less chance of encountering suitable hosts in a new environment. Transmission routes commonly utilised in indirect life cycles include ingestion of an intermediate or paratenic host (juvenile parasite stage), or the use of a vector (usually an arthropod) which is required to infect (often through biting) the definitive host (adult parasite). Intermediate and paratenic hosts, as well as arthropod vectors, serve as a protective barrier from environmental extremes. However, indirect life cycles can also involve external environmental stages (e.g. *Fasciola hepatica* requires three weeks to embryonate and hatch in the physical environment). Generally, parasites that are host adapted i.e. they live and proliferate within a particular host species, are referred to as specialist

parasites. Alternatively, generalist parasites are capable of incorporating a variety of wildlife and/or domestic host species into their life cycle and have an increased probability of successful establishment and persistence (Agosta *et al.*, 2010; Altizer *et al.*, 2003a; Altizer *et al.*, 2003b; Hudson *et al.*, 2006; Mas-Coma and Feliu, 1984). Parasites capable of using both a direct or indirect life cycle (e.g. *Toxocara cati*), as well as generalist parasites, are assumed to have the greatest chance of survival in a new environment.

The method by which a parasite is introduced to a new environment can strongly influence the likelihood of establishment in a new environment. These include the abundance of parasites (or hosts) introduced at a given time, single introductions or repeated events as well as the season of introduction owing to the annual oscillations of some parasite species (Mas-Coma and Feliu, 1984). If the introduced form of the parasite is the stage infective to a host (compared to the adult stage) then there is a higher likelihood of establishment occurring sooner (Mas-Coma and Feliu, 1984). Additionally, a parasite's life cycle has the potential to alter on islands with the increased transmission probabilities, in part due to the higher density and rate of contact between hosts (Daniels *et al.*, 2013; Mas-Coma and Feliu, 1984). Capacity to acquire new hosts and adapt to the new conditions in the external environment will also determine parasite success. Competition between parasites within hosts can also limit the survival of parasite species (Holmes, 2002).

1.3.2 Insular physiography

Insular physiography refers to the environmental conditions, or physiographic, characters of insular environments. The physiography can include the climate of a region, vegetation type and density as well as land-use. These conditions can influence

and/or facilitate selection of parasitic fauna both directly (by acting on the free-living stages) or indirectly (by determining the quantitative and qualitative composition of free-living fauna which can act as alternative hosts). Environments in which the parasite colonises will favour different parasitic species. For example, tropical environments with extended wet seasons and year-round humidity can be conducive to the persistence of parasites with an external life cycle stage, particularly those that require an extended period of development in the external environment. Most terrestrial and marine life show an increase in diversity as you move towards the equator, as do metazoan parasite species (Rohde and Heap, 1998; Vignon *et al.*, 2009).

1.3.3 Free-living fauna and parasite success

The relationship between free-living fauna (i.e. hosts) and parasite success relates to the diversity and nature of insular host species available, as well as, the host in which the parasite was simultaneously introduced to the new environment. Host traits (i.e. age, sex, immunological status and body size) as well as the presence and abundance of hosts are significant drivers of parasite presence and diversity (Goater *et al.*, 2014; Hudson *et al.*, 2002; Richards *et al.*, 1995). Host sex can relate to differences in home ranges [e.g. male dingoes often have larger home ranges (Thomson, 1992)] or in some species the one individual is responsible for the training of young to hunt and/or scavenge [e.g. female queens responsible for training kittens (Burt *et al.*, 1980)] thereby increasing the likelihood of picking up transmission opportunities, and, consequently making them more susceptible to disease transmission. Sex hormones can also affect host immunity (Abu-Madi *et al.*, 2008; Festa-Bianchet, 1989; Grzybek *et al.*, 2015; Schalk and Forbes, 1997). Host age is considered one of the most important intrinsic factors in influencing the host parasite community (Kisielewska *et al.*, 1973;

Montgomery and Montgomery, 1988). Younger animals have not had the opportunity to encounter as many parasite species as adults. Alternatively, young hosts do not have a built-up immunity in which to defend against infection.

Additionally, interspecific host factors can also play a role in parasite presence and diversity. Body size also plays a role in parasite transmission. A larger host body size may be related to a higher nutrient intake (i.e. greater number of food items ingested), therefore greater body condition resulting in enhanced immunity against pathogen infection. Alternatively, a greater number of prey being ingested implies a greater number of parasites being opportunistically ingested. Alternatively, a larger body can also provide a greater number of niches in which parasites or pathogens can occupy within the host (Morand and Poulin, 1998).

Generally, host species richness is a determinant of parasite richness as many parasites require alternative hosts to complete their life cycle. A higher diversity and availability of intermediate host species within a landscape can reflect an increased diversity of parasite species (Kamiya *et al.*, 2014; Krasnov *et al.*, 2004; Thieltges *et al.*, 2011). This is particularly relevant to the richness of mammalian species (Dobson and May, 1986; Hegglin *et al.*, 2007). Parasites requiring multiple alternative hosts are limited by the host's home ranges where the definitive and intermediate hosts intersect (Hegglin *et al.*, 2007). Some parasites are reliant on the prey-predator interaction, therefore changes in the procurement of food by the definitive host may adversely affect the parasite success. The parasite transmission rate is presumably enhanced if an intermediate host is both abundant in the environment and is a frequent prey species for a final host (Hegglin *et al.*, 2007). If the required intermediate host has a low abundance or is absent, then the presence of this

particular parasite species would be limited, regardless of abundance of definitive host because survival is dependent on cycling through the intermediate host. Alternatively, the 'dilution effect' assumes that infection risk is associated with host diversity. This means that a high species diversity and richness can dilute or decrease the disease in the environment (Johnson and Thieltges, 2010; Keesing *et al.*, 2006; Ostfeld and Keesing, 2000; Schmidt and Ostfeld, 2001).

1.4 Effects of introduced pathogens - the trickle-down effect

By definition, parasitism is the relationship whereby one individual (the parasite) will cause harm to another individual (the host) by exploiting host resources to enhance the parasite's survival (Goater *et al.*, 2014). However, parasites can have both positive and negative effects on the host. The effects discussed below are considered at both an individual and a population level.

1.4.1 Host effects

Effects of a parasite on an individual can range from undetectable impact to evident harm to the host (Goater *et al.*, 2014). For example, the pathogenicity of helminths varies by species, with asymptomatic infections common for many species (e.g *Toxocara cati* in domestic cats), and particularly at low levels of infection (Bowman, 2000). Parasites may even have positive effects on their hosts [e.g. the removal of environmental toxins from the physical environment once ingested by a host (Sures, 2004)]. When host species are exposed to little (or no) parasite pressure, there is less of an investment in their immune system compared to an area with a high parasite pressure i.e. islands versus mainland environments (Goüy de Bellocq *et al.*, 2002). Instead of an investment in immune functions, energy and nutrient portioning

will be directed to other "features" (e.g. reproduction and growth). Consequently, once challenged by a foreign pathogen, after the absence of a previous challenge, the host has no built-up defences and may not be able to mount a suitable immunological response to the pathogen (Rachowicz *et al.*, 2005). Alternatively, previous infection from a pathogen can have a protective effect on the host in the face of additional pathogens (Steeves and Allen, 1990).

Generally, the effects of parasitism on hosts are negative. Parasites can affect host populations directly by reducing fecundity or survival (Heins *et al.*, 2004; Tompkins and Begon, 1999), or indirectly by altering host behaviour that increases susceptibility to predation that in turn facilitates parasite transmission (Holmes and Bethel, 1972; Schutgens *et al.*, 2015). Changes to host population density can affect competitive interactions or alter resource availability (D'Antonio and Dudley, 1995; Hudson *et al.*, 1992; Lafferty, 1999). An increased variety of animal species in the host's diet may be associated with better body condition and resilience to infection when associated with increased nutrient intake, but is also associated with increased risk of parasite transmission via ingestion of intermediate or paratenic hosts (Cheng, 1986).

1.4.2 Conservation implications

Invasive species can introduce a range of pathogens (including parasites, bacteria and viruses) that may threaten both native populations already in decline, as well as in flourishing communities (Altizer *et al.*, 2003a; Hochachka and Dhondt, 2000; Jensen *et al.*, 2002; Roelke-Parker *et al.*, 1996). Isolated, endemic populations that have not been previously exposed to a pathogen have very little acquired immunity to

infection, and introduced pathogens can have detrimental consequences in these naïve populations (McCallum and Dobson, 1995).

In reality, it is difficult to implicate disease as the causal factor in declines or extinctions in faunal populations. As such, there are few documented cases of parasite induced extinction (Hudson *et al.*, 2006). The first definitive, documented example of this occurring is the land snail (*Partula turgida*) that had become extinct in the wild due to introduced species and was persisting only as a single captive population but was extirpated by infection with a microsporidian parasite (Cunningham and Daszak, 1998; Hudson *et al.*, 2006).

An important aspect in control and management conservation programs is assessing the risks posed by introduced pathogens. An understanding of the epidemiology of parasites in both endemic and introduced animals is important in management programs or reintroduction programs. In particular, identification of risks posed by parasites to new populations can be exploited in developing programs that support successful reintroduction of species.

1.4.3 Zoonotic implications

Approximately 61% of human diseases are zoonotic, i.e. linked to wildlife and/or domestic animals (Taylor *et al.*, 2001). Global population growth resulting in increased urban expansion into wildlife habitats, and increased travel and trade are important factors in the altered geographic distribution of zoonotic diseases (Patz *et al.*, 2004). For example, the increasing risk of Lyme disease in the north-eastern United States has been attributed to a combination of factors, including forest fragmentation and urban sprawl resulting in increased human-wildlife interaction

(Schmidt and Ostfeld, 2001). Fauna that are attracted to peri-urban and urban habitats due to the abundance of food and presence of shelter can act as important reservoirs for zoonotic diseases (Mackenstedt *et al.*, 2015). Free movement of wildlife into human settlements, increasing human-wildlife interactions are especially problematic where zoonotic parasites occur and are readily transmitted to humans (Mackenstedt *et al.*, 2015; Myers *et al.*, 2000). In areas where biodiversity levels are decreasing, the geographic distributions of pathogens, including vector borne diseases, have expanded thereby increasing the rate of exposure of these diseases to humans (Allan *et al.*, 2009; Keesing *et al.*, 2006; LoGiudice *et al.*, 2008; Ostfeld *et al.*, 2006; Swaddle and Calos, 2008).

1.4.4 Domestic transmission

In addition to zoonotic transmission, encroachment of urban areas to native vegetation increases the risk of transmission of pathogens (including parasites) from wildlife to domestic animals due to the greater interface between domestic animals and wildlife. For example, red foxes (*Vulpes vulpes*) are capable of cycling and transmitting many of the same parasites as domestic dogs (*Canis lupus familiaris*). Urban areas where both domestic dogs and red foxes cohabit are at an increased risk of infection with parasites such as *Dirofilaria immitis* (Mackenstedt *et al.*, 2015). Additionally, wild birds roosting in urban bushland and backyards are capable of transmitting parasites to companion animals through shedding infective stages and/or being predated on. Additionally, companion animals allowed to roam or hunt are more likely to become infected via wildlife compared to confined animals.

1.5 The Island Syndrome

Combination of the founder effect, insular physiography, host fauna and parasite biology limits potential establishment success of parasites on islands, and is associated with a condition called the Island Syndrome (Mas-Coma and Feliu, 1984). The Island Syndrome stipulates that the island parasite local richness will be low (due to reduced potential for establishment success) but the parasite prevalence, infra-community richness and intensity will be high (due to increased host species densities) (Goüy de Bellocq *et al.*, 2002; Mas-Coma *et al.*, 1998, 2000; Pérez-Rodríguez *et al.*, 2013). The Island Syndrome arises due to the decreased area generally associated with islands, as well as a reduction in the number of potential host species and absolute number of host individuals. However, given the increased host density and close contact between hosts on islands, there are amplified transmission possibilities that occur, leading to an increased parasite prevalence, infra-community richness and intensity (Nieberding *et al.*, 2006).

In addition to the founder effect, the origin and frequency of host species introductions impact diversity and richness of parasites present in the new environment. Hosts originating from areas with high parasite richness and high infra-community richness are associated with the introduction of a greater range of parasite species compared to areas with low parasite species richness. The introduction of multiple host species to a new environment may introduce a different parasite assemblage with each introduction event. Parasite establishment in a new island environment can be sustained if the intermediate host is introduced at approximately the same time as the definitive host (Mas-Coma and Feliu, 1984).

1.6 Feral cats and black rats

This thesis concentrates on two global invasive species; feral cats (*Felis catus*) and black rats (*Rattus rattus*). Feral cats and black rats are widely recognised for having detrimental impacts worldwide. At least 80% of islands globally have been invaded by *Rattus* spp. and over 65 island groups have been invaded by cats (Atkinson, 1985, 1989). Both species are widely distributed across all continents and most offshore islands, and have been associated with the failure of several endangered species reintroduction programs (Courchamp *et al.*, 2003).

Black rats have been introduced as commensals globally, and have a close association with human and urban dwellings but can wander between natural and manmade structures. Black rats can act as reservoirs, carriers and vectors of parasitic disease in the wild and can maintain pathogen transmission cycles in a wide range of environments (Battersby, 2015; Meerburg *et al.*, 2009). They are renowned for their role in disease transmission to humans [e.g. the bubonic plague (Banks and Hughes, 2012; Meerburg *et al.*, 2009; Smith and Banks, 2014)], as well as to domestic animals and wildlife. Black rats may become infected by (and potentially cycle) the same parasites as native rodents (Wells *et al.*, 2014).

Similarly, cats have a close association with humans, having been introduced to a multitude of environments largely as companion animals but their escape from domestication has led to the establishment of feral populations (Coman, 1991; Courchamp *et al.*, 2003; Dickman, 1996b; Sing, 2015). Historically, cats have been considered a primary source of rodent control, and as such were introduced globally, including to many oceanic islands (Courchamp *et al.*, 2003). Feral cats are common reservoirs of pathogenic diseases including bacteria, helminth and protozoan parasites

(Gerhold, 2011; Henderson, 2009). One of the most renowned protozoan parasites, *Toxoplasma gondii,* can be transmitted from cats to both humans and wildlife (Dubey, 1994). Given that feral cats have an opportunistic diet and are capable of adapting and surviving in a wide range of environments, their potential for disease transmission is most likely amplified.

1.7 Study Areas

This research project focused on three study regions, all of which are impacted by invasive species introductions, evident as faunal population declines (due to predation and/or disease introductions), as well as disease transmission to humans. As such, all three study regions currently have management and eradication programs for invasive species and reintroduction or species recovery programs. Each of the study regions are discussed in more detail below, including invasive species history and current conservation status. Feral cats were collected from all three study regions; however, black rats were only collected from Christmas Island. The trapping programs used on Dirk Hartog Island and southwest Western Australia were only collecting feral cats, and it was not possible to organise the trapping and subsequent necropsy of rodents from these two regions within the time constraints of the experiment.

1.7.1 Christmas Island

Christmas Island (CHI) is a strategically important site for human and animal health surveillance for Australia due to the migration of people from regions in the world with many endemic zoonotic pathogens. Additionally, CHI is in close proximity to Indonesia and northern Australia. Northern Australia has rigorous surveillance for exotic pests and disease due to the increased risk of invasion by exotic disease (e.g.

rabies) from Indonesia, but surveillance is challenging as the area is minimally populated, the vastness of coastline and land, and the high cost associated with disease detection and surveillance. The tropical climate and rainforest environment present on CHI is conducive to the persistence of many pathogens of zoonotic and conservation importance due to suitable humidity and vegetation cover for external stages and potential hosts.

There are many species of endemic and migratory birds on CHI, many with recovery programs in place due to declining populations (Hall *et al.*, 2011; Johnstone and Darnell, 2004). Recovery programs are also in place for other fauna, including reptiles. Since the introduction of feral cats and black rats in 1899, four of five endemic mammal species originally present on the island have gone extinct, with the fifth mammal species, the CHI flying-fox (*Pteropus melanotus natalis*), under threat with numbers steadily decreasing (Hall *et al.*, 2011). Black rats have been implicated in the introduction of a protozoan parasite to the island, *Trypanosoma lewisi*, which is hypothesised to have caused the extinction of two native rodent species (bulldog rat; *Rattus nativitatus* and Maclear's rat; *R. macleari*) (Wyatt *et al.*, 2008). Due to the swift colonisation of CHI by cats and black rats, it is difficult to assess the susceptibility of the endemic fauna to introduced pathogens at the point of colonisation; however, understanding the parasite community in black rats and cats on CHI will help inform management plans through better recognition of the potential risk they pose to endemic wildlife and human communities.

1.7.2 Dirk Hartog Island

Dirk Hartog Island (DHI) was utilised as a pastoral lease from 1800 following European colonisation of Australia, but has been gazetted as a National Park under the

management of Department of Parks and Wildlife (formerly the Department of Environment and Conservation) since 2009. Dirk Hartog Island is located within the Shark Bay World Heritage Area and is the largest island off Western Australia's coast. Like CHI, DHI has experienced significant extirpation of its native species, with ten of 13 native mammal species lost (Burbidge, 2001; Burbidge and Manly, 2002), attributed to overgrazing and cat predation (black rats and foxes not being present on the island). The native mammals remaining on the island include the ash-grey mouse (*Pseudomys albocinereus*), sandy inland mouse, (*P. hermannsbergensis*) and the little long-tailed dunnart (*Sminthopsis dolichura*). Efforts are underway to restore the island's faunal assemblage, and the Department of Parks and Wildlife has implemented an eradication and management program aimed at removing feral cats and other introduced species and reintroducing previously occurring native species. Much of the fauna included in reintroduction programs may be susceptible to pathogens originally introduced by invasive species, even after eradication of the invasive species. Understanding the parasite community present in these invasive species is required for successful reintroduction of native fauna on this island.

1.7.3 Southwest Western Australia

Southwest WA (swWA) is recognised as a biodiversity hotspot, defined as an area that features unique concentrations of endemic species which are experiencing ecological pressures such as high rates of species extinction and/or habitat loss, typically due to habitat degradation and introduced predators (Myers *et al.*, 2000; Reid, 1998). Of the 77 indigenous mammals in the swWA eco region, almost one third of those are now extinct within the region (Gole, 2006). Eight species are globally extinct, and 15 are locally extinct but survive elsewhere in Australia. Land clearing and

habitat fragmentation within this region for agricultural purposes has led to many native landscapes within swWA effectively being islands of habitat surrounded by a "sea of agriculture" (Burbidge and McKenzie, 1989; Hobbs, 2001). Native species within swWA are under threat from land degradation and loss of habitat (Gole, 2006; Hobbs, 2001), but also from predation pressure, competition and disease transmission. In particular, within recent years in swWA, woylie (*Bettongia penicillata*) population declines have been attributed to stress and disease transmission (Botero *et al.*, 2013).

1.8 Research methods

Studies investigating parasite epidemiology in invasive species have typically presented either restricted methodology (i.e. restricted to specific samples such as faeces or blood, or restricted to a limited number of parasite species) or restricted results even though they have conducted full necropsies (i.e. no infra-community richness, or no abundance/intensity data). Research aimed at identifying a broader, more accurate representation of the parasite community structure is improved by presenting entire necropsy data i.e. prevalence, richness, infra-community richness and intensity of parasite populations, in this case in feral cats and black rats. Some parasites found within visceral organs may not be evident using gross pathology and intensity may be difficult to determine (e.g. *Aelurostrongylus abstrusus* from feline lungs). Methodology based on necropsy and tissue samples offers advantages in identifying immature and adult pathogens, as opposed to stages that are intermittently present such as eggs that are intermittently shed in faeces, or organisms periodically circulating in the blood.

Molecular techniques are increasingly being used to complement (or replace) morphological techniques for specimen identification. Molecular techniques such as

polymerase chain reaction (PCR) can be performed on tissue or faecal samples, allowing identification to species or strain, as well as for detection of protozoan or bacterial pathogens in hosts that may or may not be showing symptomatic disease.

Studies described in this thesis utilised multiple techniques to gain a broad picture of the parasite and bacterial pathogen community in two important invasive species. Necropsies were conducted on both feral cats and black rats, including an external inspection for ectoparasites, examination of all visceral organs (including the gastrointestinal tract) for macro-parasites, and collection of tissue samples from visceral organs for molecular (PCR) analyses for protozoan parasites and bacteria. Each chapter discusses the relevant methodology utilised for each experiment.

1.9 Aims, objectives and significance of research

Understanding the parasite communities in invasive species populations is critical as introduced pathogens may have important conservation, agricultural and zoonotic repercussions. Understanding the baseline pathogen epidemiological data will inform disease risk posed by feral cats and black rats to wildlife, domestic animals and humans. This will allow us to infer native and non-native species that may be beneficially impacted by eradication and management of these invasive species based on disease risk.

This thesis describes a multifaceted approach to investigate the pathogen community in feral cat and black rats in three study locations (CHI, DHI and swWA). The general hypotheses tested were:

- Rat helminth populations on CHI and cat helminth populations on both tropical CHI and arid DHI will exhibit characteristics of the Island Syndrome compared to swWA;

- Rats and cats represent a disease risk to wildlife and human communities on CHI, DHI and swWA.

The overall aims of this thesis were to:

- Determine whether the community ecology of cat and rat helminth parasites exhibit characteristics consistent with the island syndrome;
- Identify risks associated with cat and rat parasites for conservation and public health management in three study locations;
- To propose explanations to the patterns in parasite communities in these hosts and locations;
- To determine if the prevalence of bacterial and protozoan pathogens in feral cats were higher in island environments.

To investigate the overall aims, the first part of this thesis focuses on helminths and the Island Syndrome (Chapter 2 and 3) and the second part focuses on the presence of protozoan and bacterial pathogens in our three study locations (Chapters 4, 5 and 6).

Chapter 2 Challenging the dogma of the 'Island Syndrome': A study of helminth parasites of feral cats and black rats on Christmas Island

2.1 Preface

This chapter is to be published as an invited paper in a special edition of the *Australasian Journal of Environmental Management* on "Wildlife Management on Inhabited Islands":

Dybing, N.A., Jacobson, C., Irwin, P., Algar, D., Adams, P.J., In Press 2017. Challenging the dogma of the 'Island Syndrome': A study of helminth parasites of feral cats and black rats on Christmas Island. Australasian Journal of Environmental Management.

This chapter identifies the helminth parasite community structure of feral cats and black rats from Christmas Island. The parasite community in the two invasive species were analysed to determine if aspects of the Island Syndrome were met. This is an island with a high number of faunal declines and extinctions, and has a current feral cat and black rat management and eradication program. This chapter describes interesting patterns in regards to the Island Syndrome in both feral cat and black rat parasite communities.

2.2 Abstract

Many island ecosystems are exposed to ecological threats as a result of invasive species and the parasites they harbour. Parasites are capable of impacting endemic island populations whether they are stable populations or ones already in decline. The 'Island Syndrome' hypothesis proposes that richness and diversity of introduced parasites differ to mainland populations with lower parasite species diversity on islands due to the founder effect. To examine the role of 'Island Syndrome' and impacts for faunal and human communities on a tropical island, helminth parasites were identified from feral cats (*Felis catus*) (n=66) and black rats (*Rattus rattus*) (n=101) on Christmas Island. Sixty-one (92%) cats and 85 (84%) rats harboured one or more helminth species with total infra-community richness ranging 0-6 species in cats and 0-7 species in rats, including species of zoonotic significance (*Angiostrongylus cantonensis*, *Toxocara cati*, *Ancylostoma braziliense, Taenia taeniaeformis*, *Moniliformis moniliformis* and *Hymenolepis nana*). High parasite prevalence and total infra-community richness was expected in island populations, however high parasite richness in cats and rats on Christmas Island was counter to the 'Island Syndrome'. These results suggest that introduced cats and rats may be responsible for maintaining an increased parasitological threat to fauna and human communities in certain ecosystems.

2.3 Introduction

The introduction of cats (*Felis catus*) and rats (*Rattus* spp.) to islands around the world has had deleterious impacts on endemic terrestrial vertebrates and breeding bird populations (Bonnaud *et al.*, 2010; Dickman and Watts, 2008; Global Invasive Species Database, 2015; Long, 2003; Ratcliffe *et al.*, 2010). Both cats and rats have

been responsible for driving numerous extinctions of endemic species on islands. Predation by feral cats currently threatens many species listed as critically endangered in both insular and mainland environments (Medina *et al.*, 2011; Nogales *et al.*, 2013). In addition, cats act as reservoir hosts for a number of diseases with implications for wildlife and human health (Adams *et al.*, 2008; Denny and Dickman, 2010; Dickman, 1996a, b; Gerhold, 2011; Gerhold and Jessup, 2013; Medina *et al.*, 2011). Black rats also pose threats to wildlife by predation and competition, as well as indirect effects including hyper-predation and as disease vectors (Banks and Hughes, 2012). Disease impacts of these two invasive species are exacerbated by close associations with humans, livestock and domestic pets, and both cats and rats may act as a transmission link between animal populations and humans (Banks and Hughes, 2012; Meerburg *et al.*, 2009).

Isolated wildlife populations, such as those occurring on islands, typically tolerate a stable coexistence with endemic diseases, but species introductions can cause disequilibrium and subsequently promote disease emergence (Kelly *et al.*, 2009; Rachowicz *et al.*, 2005). As a result, endemic island species are at risk of extinction by introduced parasites and diseases (McCallum and Dobson, 1995; Milberg and Tyrberg, 1993; van Riper *et al.*, 1986; van Riper *et al.*, 2002). Pathogens have been implicated in many studies as a threat to host populations (Altizer *et al.*, 2003a; Hochachka and Dhondt, 2000; Jensen *et al.*, 2002; Roelke-Parker *et al.*, 1996). Habitats with limited geographical range, including those frequently associated with islands, have been associated with elevated rates of host exposure to parasites due to higher parasite and host densities resulting in increased contact between hosts and parasites (Lindenfors *et al.*, 2007). However, island communities typically have a low parasite species

richness due to the founder effect; insular organisms typically originate from a small number of migrants harbouring a subset of common parasite species (Miquel *et al.*, 1996; Morand and Guégan, 2000).

2.3.1 Island Syndrome

When introduced into a new environment, some parasite species will become established, and even flourish, despite the often limited diversity of intermediate hosts present. However, not all introduced parasites are able to become established post-colonization (Dobson and May, 1986). A number of factors influence the likelihood of successful establishment and persistence of a parasite in an island environment including the presence and richness of hosts (i.e. intermediate, paratenic and definitive), host density and behaviour, parasite biology, quantity and frequency of introduction and physiographic characteristics of the new environment (e.g. climatic conditions). A favourable combination of these factors leads to a greater probability of successful introduction and establishment (Mas-Coma and Feliu, 1984). These factors also typically lead to characteristically low parasite local richness and diversity, high parasite prevalence, high intensity and high infra-community richness (ICR) in island environments, and in combination, are referred to as the 'Island Syndrome' (Fromont *et al.*, 2001; Goüy de Bellocq *et al.*, 2002; Goüy de Bellocq *et al.*, 2003; Mas-Coma and Feliu, 1984).

2.3.2 Cats and rats on Christmas Island

Christmas Island (CHI) is an Australian Territory, covering 135 km^2, located in the Indian Ocean (10˚25'S, 105˚40'E) approximately 360 km south of the Indonesian capital, Jakarta. In accordance with the island's equatorial climate it experiences distinct wet and dry seasons, year-round high humidity, and the temperature varies

minimally with a mean daily temperature of 27^{o}C (Bureau of Meteorology, 2012). Introduction of cats and black rats (*Rattus rattus*) to CHI occurred in the late 19th Century at the time of European colonisation and both species established self-sustaining populations soon thereafter (Tidemann *et al.*, 1994). Initially, cats were concentrated around settlement and mining areas where they had access to discarded human food (Tidemann, 1989; Tidemann *et al.*, 1994). With the expansion of introduced black rats across the island, feral cats became more widespread (Tidemann 1989). Prior to the commencement of a recent control program, there was an abundant domestic and stray cat population within the residential, commercial and light industrial areas, and feral cats in the National Park and remaining vegetated areas.

Christmas Island originally harboured five endemic mammal species, but is now largely depauperate of native species due, at least in part, to the introduction of these pests (Algar and Johnston, 2010; MacPhee and Flemming, 1999; Wyatt *et al.*, 2008). Two native rodent species, *Rattus macleari* (Maclear's rat) and *R. nativitatis* (Bulldog rat), became extinct within 25 years of the introduction of cats and black rats to the island (MacPhee and Flemming, 1999; Wyatt *et al.*, 2008). It has been hypothesised that these naïve rodents may have succumbed to infection by *Trypanosoma lewisi* introduced with the black rats (Wyatt *et al.*, 2008). However, recent parasitological investigation of both rats and cats on the island failed to detect any persistence of *T. lewisi* in either of these hosts (Dybing *et al.*, 2016b). Aside from cats and black rats, the only remaining mammalian species is the CHI flying fox (*Pteropus melanotus natalis*). House mice (*Mus musculus*) have been previously reported in the settlement (Gibson-Hill, 1947) and are included in the CHI Biodiversity Conservation plan (2014);

however, numbers and distribution are currently unknown and no confirmed observations have been made in recent times (David Algar and Dion Maple pers. comm. 2015).

Many of the remaining endemic species on CHI (i.e. birds and reptiles) are the focus of ongoing recovery programs, in part due to the ongoing impacts of cats and rats (Beeton *et al.*, 2010). In 1995 a Natural Resource Management program was initiated with a focus on pest management which, through extensive community engagement, included the planning and development of a Companion Animal Local Bill to prevent the ownership and importation of new cats and dogs onto the island, control programs for feral cats, and a veterinary service for free de-sexing of pet cats and euthanasia of unwanted cats. In 1999, following the departure of key staff from the island this program was not maintained and control of cats and cat ownership lapsed (Paul Meek pers. comm.). As a result, the environmental and social impacts of cats on CHI continued and became an increasing concern to island land management agencies and locals.

Eradication of cats from an island is generally difficult; if the island is inhabited and cats are a domestic pet, there is an additional level of complexity. When the human population is multi-cultural this level of complexity can be magnified, especially if cats have religious or cultural significance. Christmas Island, inhabited by a population consisting of Malay, Chinese and European residents, presents such a case. On CHI, the Malay community is primarily Muslim, and as such have a strong religious and spiritual connection to felines that stemmed from the prophet Muhammad. This connection appears to hold felines above other animals and generates a level of respect and thankfulness for its existence (Engels, 2015; Freeman *et al.*, 2011). The

Chinese connection to cats is less straight-forward. Although there were tales of ancient cats performing good deeds, there are also negative stories that made the Chinese fear the cat. It appears the cat is cared for in the culture but not enshrined (Turner and Bateson, 2004). The European community's attitude towards cats is also variable, ranging from dislike to a fondness and pet ownership (D. Algar pers. comm). Community attitudes to cat control on CHI have evolved over time with encouragement and support by land management agencies and community leaders. More recently, through a program that has included dissemination of information (through the local media, education and public seminars by various researchers and land managers), the need for cat control on the island has been highlighted and has maintained and fostered support and enthusiasm by the local community. Residents have become aware of the threat that cat predation poses to native species on the island and also the danger of diseases carried by feral and stray cats, such as Toxoplasmosis (Adams *et al.*, 2008), that can affect the wellbeing of wildlife and can also cause serious human health complications. The presence of numerous stray cats in residential areas has also enforced the need for control; with residents complaining of cats caterwauling, fighting, urinating, defecating and raiding refuse bins around houses.

To mitigate the problems associated with cats, the CHI Cat Management Plan was developed in conjunction with key land management agencies (including Parks Australia, Shire of CHI, CHI Phosphates and Regional Services & Infrastructure), interest groups (including Island Care CHI) and the CHI community (Algar and Johnston, 2010). This collaborative and inclusive approach ensured widespread support by various organizations and was enthusiastically embraced by the public, building a foundation

upon which feral/stray cat eradication could be achieved. Initially, local cat management laws were revised to limit domestic and stray/feral cat impacts on native fauna, promoting responsible cat ownership, compliance and enforcement of cat management laws. From 2010, amended local legislation required all domestic cats to be neutered, micro-chipped and registered with the Shire (Algar *et al.*, 2011). From 2011, all stray (unregistered) cats were removed from the residential, commercial and light industrial zones of CHI, including cats at the Immigration Detention Centre, with more than 600 stray/feral cats either trapped and euthanased or destroyed via a baiting campaign (Algar *et al.*, 2014). In addition to the cat control, methods to effectively bait rats were concurrently developed and applied to provide strategic control within the settled areas on the island. From 2015, the cat eradication programme was extended to include the National Park, mine leases and unallocated crown land using baiting, trapping and opportunistic shooting.

In developing the feral cat management program, numerous knowledge gaps were identified in relation to invasive species on CHI (Algar and Johnston, 2010). In particular, the unknown disease status of exotic flora and fauna was acknowledged as a high priority, particularly in light of the effect on native wildlife and public health in island ecosystems.

2.4 Methods

An examination of the parasitic helminth communities from cats and rats on CHI was undertaken as part of the pest animal management programmes to determine if cats and rats were harbouring parasites of conservation and public health concern. This represented a unique opportunity to investigate if the 'Island Syndrome' was applicable to the parasite communities in cats and rats on CHI.

Feral cats and black rats were trapped using Sheffield wire cages: large (200 x 200 x 550mm) cages for cats, and medium (160 x 160 x 450mm) cages for rats. Trapped cats were anaesthetised with 10mg/kg of tiletamine/zolazepam (Zoletil, Virbac, NSW 2214, Australia) administered intramuscularly before sex and body weights were recorded. Cats were subsequently euthanased by intracardiac injection of 150mg/kg of pentobarbital (Lethobarb, Virbac, NSW 2214, Australia) and immediately placed in individual plastic bags. The full trapping and euthanasia protocol is described in Algar *et al.* (2014). Trapped rats were euthanized via cervical dislocation and placed into individual bags. Cat cadavers were necropsied either while fresh (n=6) or stored at -20^{o}C (n=60) before thawing overnight (6-16 h) in their bags at room temperature (Figure 2.1). Rats were necropsied either fresh (n=47), stored in the freezer (n=1) or refrozen (n=53) (refreezing of carcasses was necessary due to a parallel dietary study (Hayes, 2011). Once the gastrointestinal (GI) tract was removed from rats used in the dietary study, the contents were sieved and kept in the freezer for parasite analysis.

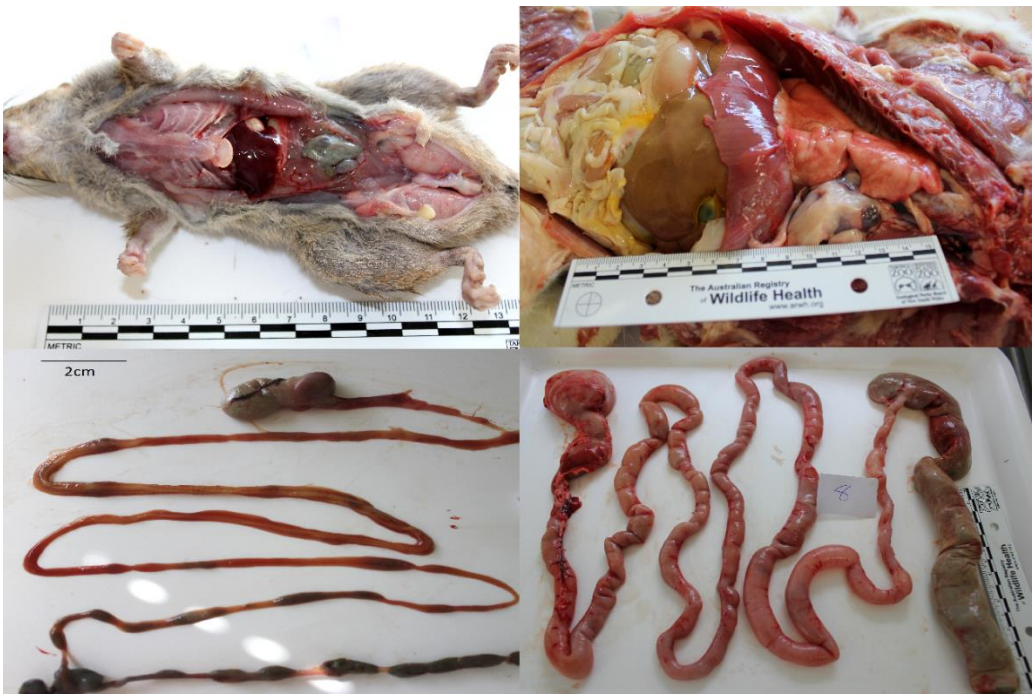

Figure 2.1: Necropsy and GI tract examination of cats and rats.

Head length (cats only), tail length (rats only) and head-body (HB) lengths (cats and rats) were measured, and a body condition score (BCI) was calculated for each animal (=weight/HB length) (Rodríguez and Carbonell, 1998; Vervaeke *et al.*, 2005). Tail length was measured in rats to confirm *R. rattus* identification (Menkhorst and Knight, 2001). Visceral organs of cats and rats (lungs, heart, spleen, kidney, lymph node, liver, tongue and brain) were examined visually and with a dissecting microscope for parasites. Tissue samples of these organs (as well as diaphragm for skeletal muscle) were collected and stored in both 70% ethanol and 10% neutral buffered formalin for later analysis. The GI tract, including stomach, small and large intestines, was removed and refrozen for later examination for parasites and for dietary analysis (Hayes, 2011). The GI tracts were thawed prior to analysis (up to 5 hours), incised and opened longitudinally, and examined using a dissecting microscope. GI contents were sifted and teased apart using soft forceps to uncover helminth parasites (Dybing *et al.*, 2013).

Cestodes were preserved in 10% neutral buffered formalin, all other helminths isolated from visceral organs and/or GI tracts were preserved in 70% ethanol. Parasites were identified morphologically using the relevant keys and references (Amin, 1987; Baker, 2008; Bowman *et al.*, 2002; Costa *et al.*, 2003; Soulsby, 1982). Nematodes were cleared in lactophenol to facilitate identification. Hookworms were identified if possible by morphology (Biocca, 1951); species were confirmed by PCR performed on a random subsample according to the protocol by Smout *et al.* (2013). For this, hookworm DNA was extracted from 5-10 worms/cat according to the manufacturer's instructions (Qiagen, Maryland, USA). The intensity of infection by cestodes was determined by the number of scoleces recovered from the intestine. The dispersed nature of helminth parasites located within host viscera precluded accurate counts and therefore only minimum intensity was estimated and used in the analysis. Intensities of an unknown Spirurid from rat lungs and *Aelurostrongylus abstrusus* within cat lungs were not estimated. The parasite community within hosts was determined using definitions described in Table 2.1.

Table 2.1: Parasite community ecology terminology used in this paper. Definitions derived from Bush *et al.* (1997).

Term	Definition
Diversity	Composition of a community in terms of the number of species present
Local richness	Number of parasite species in a particular population of the host
Infra- community richness (ICR)	Number of parasite species in one individual
Gastro-intestinal ICR (GIICR)	Number of parasite species in the GI tract in one individual host
Visceral ICR (VICR)	Number of parasite species in the visceral organs of one individual host
Total ICR (TICR)	Sum of GI and Visceral ICR
Intensity	Number of individuals of a particular parasite species in a single host
Range intensity (RI)	Minimum- maximum intensity of a particular parasite species in a population of a given host species
Mean Intensity (MI)	Average intensity of a parasite species in a population of a given host species

All statistical analyses were performed in STATISTICA (StatSoft Inc., 2010). Only parasite species that occurred in five or more feline or rodent hosts were included in statistical analyses. The effect of parasitism on body condition was examined through separate mixed model ANOVAs with a) parasite species presence and overall parasite presence as fixed categorical factors, b) total infra-community richness (TICR), visceral infra-community richness (VICR) and GI infra-community richness (GIICR) as fixed continuous covariates and c) parasite species intensities as fixed continuous covariates. The host's sex was included as a fixed categorical factor in each instance. The effect of sex on common parasite species intensities, TICR, VICR and GIICR were examined through separate one-way ANOVAs. Contingency tables were constructed for presence/absence of the parasite species as well as overall parasite presence to examine the relationship of host sex and parasite presence and Fisher's exact test, followed by a Bonferroni correction (where applicable) were used to determine significance. The Chao2 richness estimator was calculated using the equation in Poulin (1998) to identify the true species richness and establish if more samples would have increased the observed species richness.

2.5 Results

2.5.1 Cats and rats used in helminth analyses

Sixty-six cats (30 male and 36 female) and 101 black rats (47 males, 53 females and one not recorded) were sampled from the Settlement area during the management and eradication program. The body condition index ranged from 0.030-0.103 (kg/cm) in cats, and 0.002-0.012 (kg/cm) in rats. Head-body length was positively correlated with weight in both cats ($y=4.472x+34.20$; $R^2=0.682$) and rats ($y=52.294x+10.186$; $R^2=0.862$).

2.5.2 Parasite identification

Sixty-one cats (92%) and 85 rats (84%) harboured one or more helminth parasites (Table 2.2). Overall, 16 different helminth parasites were represented, with a local richness of nine identified in cats (representing three Phyla; Nematoda, Platyhelminthes, and Acanthocephala) and 11 identified in rats (three Phyla; Nematoda, Platyhelminthes, and Acanthocephala) (Table 2.2). Chao2 estimates the richness of feral cats on CHI as 10 species and black rats as 11 species indicating all of the species were most likely identified in rats, however, more samples would be needed to identify the estimated true species richness in cats on the island. Parasites found most commonly in cats were *Ancylostoma braziliense*, *Toxocara cati, Taenia taeniaeformis* and *Joyeuxiella pasqualei*. The four most common helminths in black rats were *Rictularia* spp., *Mastophorus muris*, *Syphacia muris* and *Taenia taeniaeformis* (larval stage). Figures 2.2 and 2.3 show some of the parasites found in black rats and feral cats from the gastrointestinal tract and visceral organs respectively. Overall parasite prevalences varied from 1.5-78.8% in cats and from 1.0-46.5% in black rats (Table 2.2).

Table 2.2: Helminths recovered from cats (n=66) and rats (n=101) on Christmas Island. Results in bold indicate parasites included in statistical analyses.

	Felis catus (n=66)					*Rattus rattus* (n=101)								
	N	P (%)	MI[g]	±SD	RI[h]	N	P (%)	MI[g]	±SD	RI[h]	Life cycle[a]	Location in host[b]	Z/C[c]	New geographic location
NEMATODA														
Ancylostoma braziliense	**52**	**78.79**	**98.08**	**151.96**	**1-676**	-	-	-	-	-	D	S, SI	Z	N
Rictularia spp.	4	6.06	1	0	1-1	**47**	**46.53**	**4.23**	**4.97**	**1-20**	I	S, SI		Y[f]
Mastophorus muris	-	-	-	-	-	**43**	**42.6**	**6.47**	**7.07**	**1-40**	I	S	C	Y
Toxocara cati	**37**	**56.06**	**11.14**	**15.19**	**1-74**	-	-	-	-	-	IDT	S, SI	ZC	N
Syphacia muris	-	-	-	-	-	**32**	**31.68**	**17.09**	**27.05**	**1-116**	D	SI, LI, C		Y
Angiostrongylus cantonensis	-	-	-	-	-	**10**	**9.9**	**18.1**	**24.69**	**1-76**	I	Lu	ZC	N
Strongyloides ratti [d]	-	-	-	-	-	**9**	**8.91**	**-**	**-**	**-**	I	SI		Y
Physaloptera spp.	3	4.55	2	1	1-3	**7**	**6.93**	**4.14**	**3.13**	**1-9**	I	SI	C	Y[f]
Aelurostrongylus abstrusus [d]	2	3.03	-	-	-	-	-	-	-	-	I	Lu		N
Heterakis spumosa	-	-	-	-	-	2	1.98	2.5	0.71	2-3	I	SI	C	Y
Unknown Spirurid [d]	-	-	-	-	-	4	3.96	-	-	-	?	Lu	?	Y
CESTODA														
Taenia taeniaeformis[e]	**40**	**60.6**	**5.03**	**4.83**	**1-20**	**29**	**28.71**	**1.17**	**0.76**	**1-5**	I	S, SI (Lv)	ZC	N
Joyeuxiella pasqualei	**31**	**46.97**	**85.42**	**147.19**	**1-561**	-	-	-	-	-	I	S, SI	C	Y
Hymenolepis nana	-	-	-	-	-	3	2.97	7.33	10.97	1-20	IDA	SI	Z	Y
TREMATODA														
Platynosomum concinnum	**7**	**10.61**	**421.86**	**304.94**	**34-1000**	-	-	-	-	-	I	Bd	C	Y
ACANTHOCEPHALA														
Moniliformis moniliformis	1	1.52	1	0	1-1	**15**	**14.85**	**2.8**	**11.56**	**1-45**	I	SI	ZC	N

[a] I= indirect life cycle, D= direct life cycle, T= transmammary, A= autoinfection

[b] S=stomach, SI= small intestine, LI= large intestine, Lu=lung, Lv=liver, Bd= bile duct, C=caecum

[c] Z=zoonotic significance, C= conservation potential

[d] Intensity not recorded

[e] larval stage in liver of Black rat

[f] new geographic location for species in rat only

[g] Mean Intensity

[h] Range of Intensity

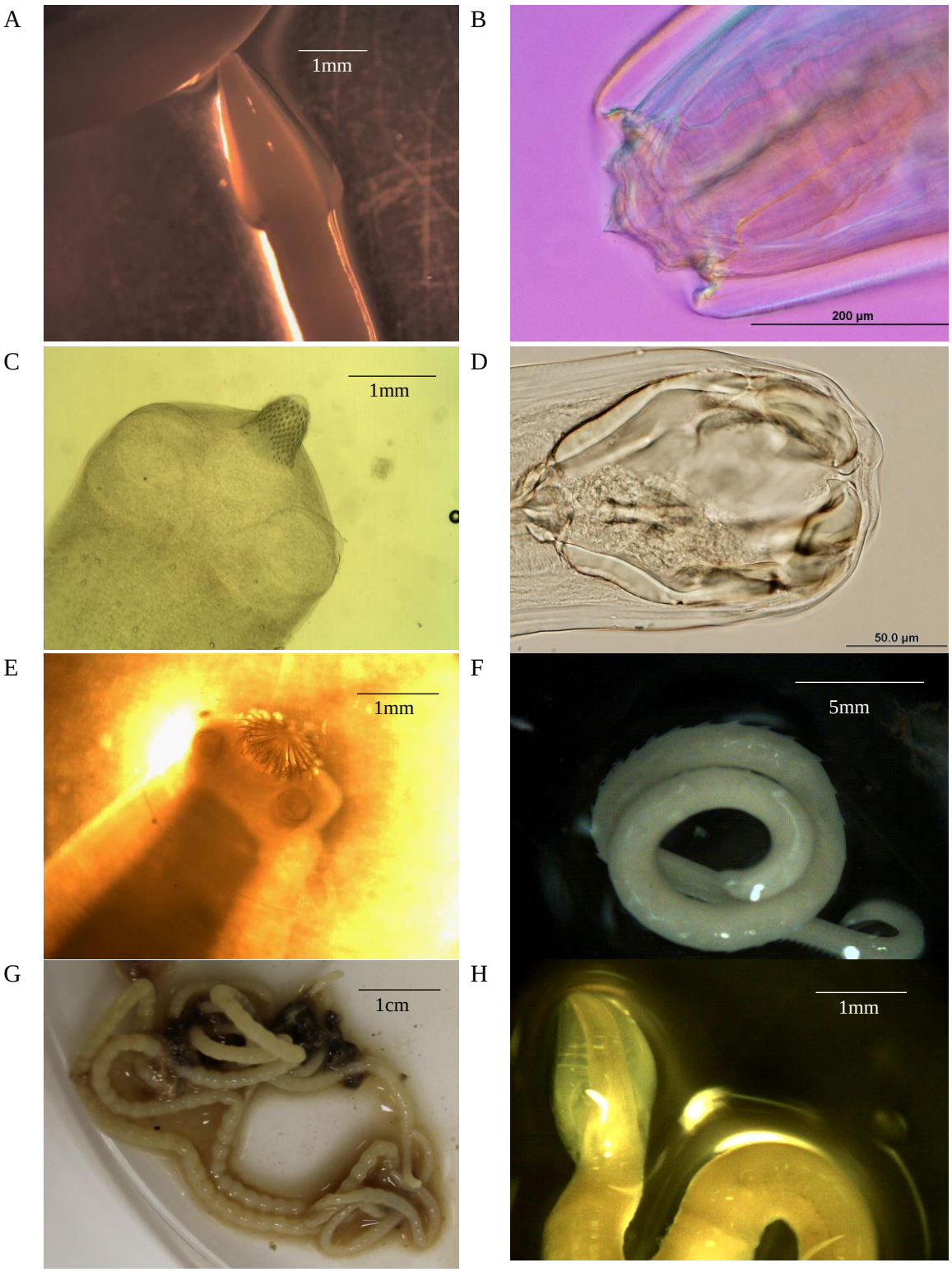

Figure 2.2: Some of the GI helminth parasites identified from feral cats (A-E) and black rats (F-H) on Christmas Island. A) Anterior end of *Toxocara cati* showing distinct cervical alae, B) Anterior end of *Physaloptera* spp., C) *Joyeuxiella pasqualei* scolex, D) Buccal capsule (anterior end) of *Ancylostoma braziliense*, E) Adult *Taenia taeniaeformis* scolex, F) Adult *Rictularia* spp., G) *Moniliformis moniliformis* adult worms, H) Posterior end of *Physaloptera* spp.

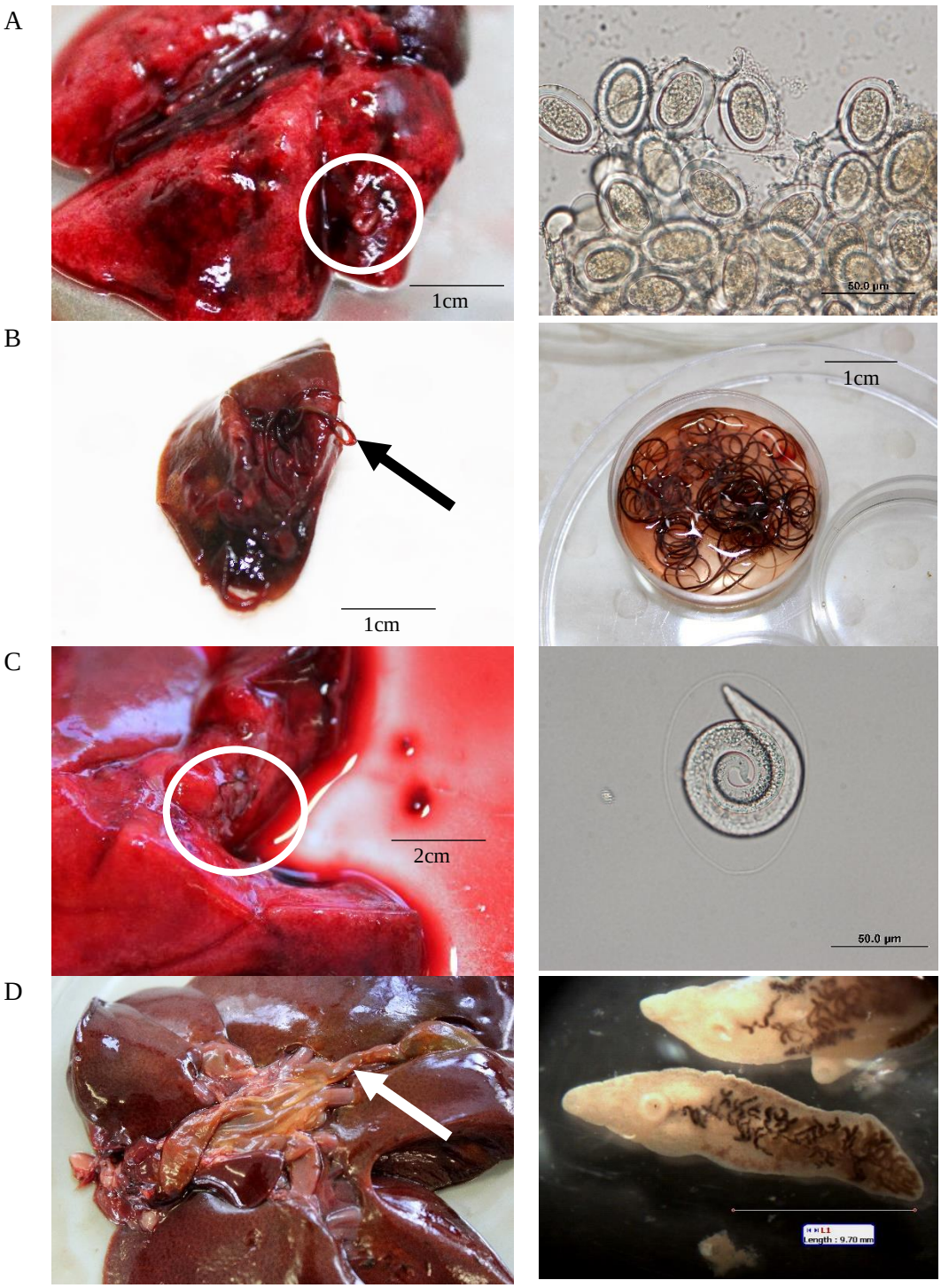

Figure 2.3: Helminth parasites (*in situ*, adults and eggs) from visceral organs of black rats and feral cats on CHI. A) Unknown Spirurid species (circled) from lung of black rat; eggs of unknown Spirurid species. B) *Angiostrongylus cantonensis* in lung of black rat; adult worms removed from black rat lung. C) Lung from a feral cat harbouring *Aelurostrongylus abstrusus* (circled); egg containing larvae of *A. abstrusus*. D) liver of feral cat infected with the bile duct fluke (*Platynosonum concinnum*) exhibiting dilated bile ducts (arrow); adult *P. concinnum*.

Some parasites could not be identified due to degradation or damage or the presence of juvenile forms preventing accurate identification and were recorded as either unknown nematode spp. or unknown *Acanthocephalan* spp. Degraded specimens were potentially artefact from food and were not included in the statistical analysis. As specimens of *Rictularia* spp. and *Physaloptera* spp. found in both cats and rats were morphologically distinct, it was assumed that they harboured host-specific species. The potentially novel unknown Spirurid found in the lung of four rats was not able to be definitively identified but was morphologically distinct from *A. cantonensis*.

Total infra- community richness (TICR) was 2.71±1.47 (mean ± standard deviation) in cats and 2.04 ±1.41 in rats, ranging between zero and six species for cats and zero and seven species for rats (Figure 2.4). Visceral infra- community richness (VICR) was 0.14±0.39 in cats and 0.44±0.67 in rats, ranging from zero to two species in cats and zero to three species in rats (Figure 2.4). Gastrointestinal infra- community richness (GICR) ranged from zero to six in both cats and rats with an average of 2.65±1.45 and 1.56±1.08 respectively.

2.5.3 Associations of helminths with host body condition index and sex

No variables included in the analyses were found to have a relationship with body condition in cats, though TICR and overall parasite presence had a positive relationship with body condition in rats (Table 2.3). Host sex appeared to have an effect on the parasite community in cats: females were more likely to have an increased TICR and VICR than males, and females had a higher intensity of *P. concinnum* (Table 2.3). There was an effect of sex on intensity of *T. taeniaeformis* in rats with a higher intensity found in males.

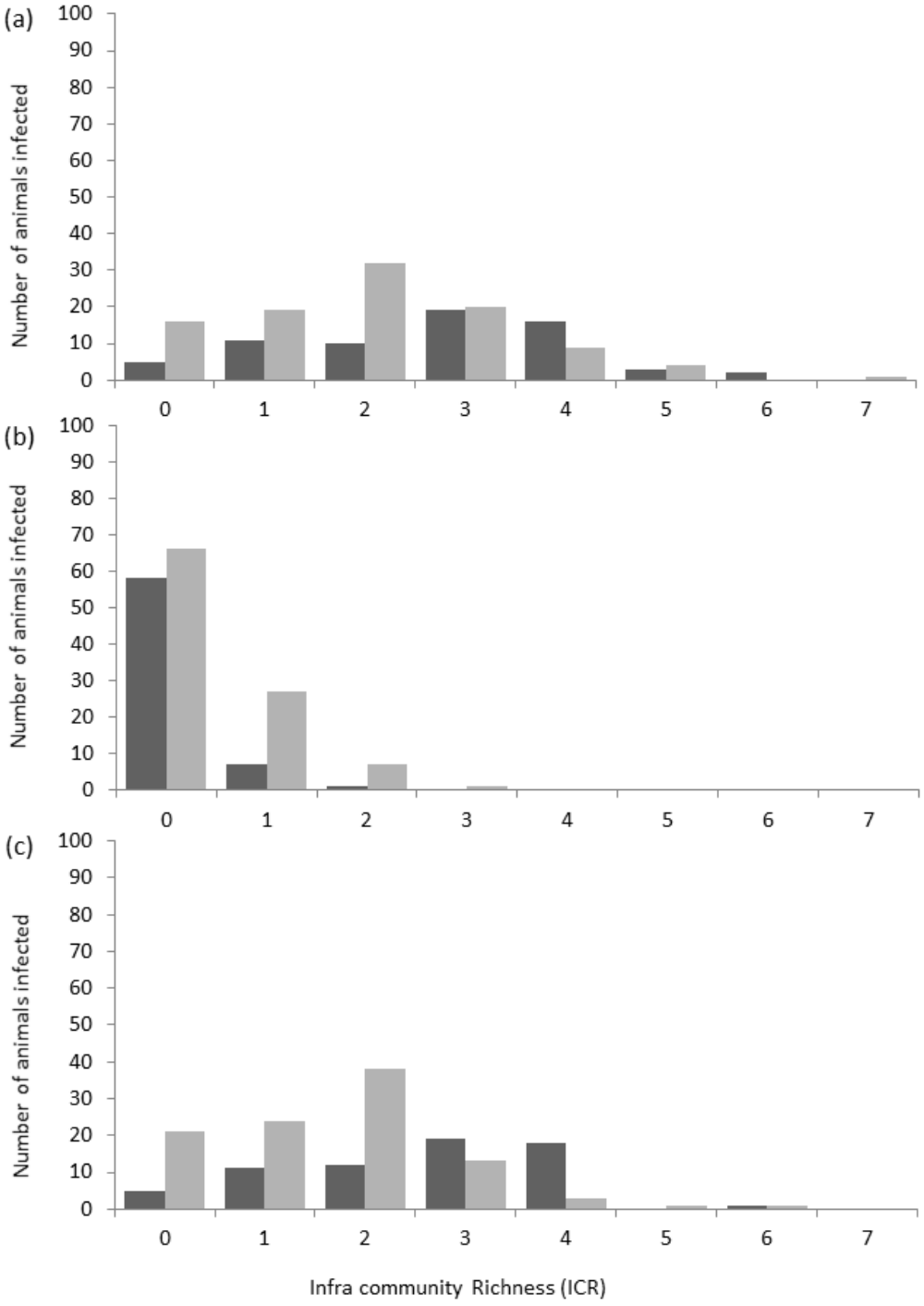

Figure 2.4: Infra- community richness of cats (n=66) and rats (n=101) on Christmas Island. Cats = dark grey, rats = light grey. a) Total infra- community richness; b) Visceral infra- community richness; c) Gastrointestinal infra- community richness.

Table 2.3: Associations between parasite community ecology with host body condition and sex

	Cat				Rat			
	Body condition		Sex		Body condition		Sex	
Overall presence	F=0.140, p=0.710[A]		p=0.652[B]		**F=4.188, p=0.044[A]**		p=0.587[B]	
TICR	F=0.192, p=0.663[A]		**F=4.592, p=0.036[C]**		**F=6.981, p=0.010[A]**		F=1.163, p=0.283[C]	
VICR	F=0.350, p=0.557[A]		**F=4.066, p=0.048[C]**		F=0.022, p=0.884[A]		F=2.132, p=0.147[C]	
GI ICR	F=0.253, p=0.617[A]		F=3.033, p=0.086[C]		F=0.140, p=0.710[A]		F=0.210, p=0.648[C]	
	Presence[A]	Intensity[A]	Presence[B]	Intensity[C]	Presence[A]	Intensity[A]	Presence[B]	Intensity[C]
NEMATODA								
Ancylostoma brazilliense	F=2.681, p=0.107	F=2.604, p=0.112	p=0.768	F=0.816, p=0.37				
Rictularia spp.					F=0.297, p=0.587	F=1.673, p=0.199	p=0.843	F=0.634, p=0.428
Mastophorus muris					F=3.495, p=0.065	F=2.691, p=0.105	p=0.843	F=1.778, p=0.185
Toxocara cati	F=1.750, p=0.191	F=2.406, p=0.127	p=1.000	F=1.011, p=0.318				
Syphacia muris					F=2.647, p=0.108	F=1.009, p=0.318	p=1.000	F=0.106, p=0.746
Angiostrongylus cantonensis					F=0.733, p=0.395	F=0.494, p=0.484	p=0.186	F=0.994, p=0.321
Strongyloides ratti					F=0.039, p=0.844		p=1.000	
Physaloptera spp.					F=0.703, p=0.404	F=0.275, p=0.601	p=0.766	F=0.130, p=0.720
CESTODA								
Taenia taeniaeformis	F=0.553, p=0.460	F=0.073, p=0.836	p=0.133	F=0.785, p=0.379	F=1.097, p=0.298	F=3.584, p=0.062	p=0.047**	**F=3.932, p=0.050**
Joyeuxiella pasqualei	F=1.364, p=0.248	F=0.875, p=0.353	p=0.332	F=1.760, p=0.189				
TREMATODA								
Platynosomum concinnum	F=3.782, p=0.057	F=0.005, p=0.941	p=0.013*	**F=4.360, p=0.041**				
ACANTHOCEPHALA								
Moniliformis moniliformis					F=3.330, p=0.071	F=1.634, p=0.205	p=0.585	F=2.616, p=0.109

[A]Mixed model ANOVA

[B]Contingency tables/fishers exact

[C]One-way ANOVA

*sex vs presence with bonferonni correction p value needs to be <0.01, therefore not significant

**sex vs presence with bonferonni correction p value needs to be <0.006, therefore not significant

2.6 Discussion

2.6.1 The 'Island Syndrome' and Christmas Island

Interestingly, this study revealed a parasite ecology that does not exhibit all the characteristics typically associated with an island population. Cats and rats on CHI were found to have a high prevalence (92% and 84% respectively) and high TICR (0-6 and 0-7 respectively) of helminth parasites, consistent with the hypothesis that insular populations experience elevated parasite prevalence and abundance (Arneberg *et al.*, 1998). However, the high local richness observed (nine species in cats and 11 species in rats) on CHI was consistent with previous reports of helminth species infecting cat or rat populations from island or mainland locations. Previous reports for local richness for cats include 11 species (n=46) on Kangaroo Island (O'Callaghan *et al.*, 2005), eight species (n=86) on King Island (Gregory and Munday, 1976), 13 species (n=13) in Dubai (Schuster *et al.*, 2009), eight species (n=58) from Majorca Island, Spain (Millán and Casanova, 2009) and eight species (n=425) in Malaysia (Mohd Zain and Sahimin, 2010). Similarly, reported helminth local richness for black rats include nine species (n=7) in Taiwan (Tung *et al.*, 2008), eight species (n=12) in the Philippines (Claveria *et al.*, 2005), 11 species (n=40) in Italy (Milazzo *et al.*, 2010) and four species (n=25) in the California channel islands (Smith and Carpenter, 2006). As both cats and rats are introduced species on CHI, it was assumed that the parasite species richness would be low compared to the mainlands in which the founding populations originated from (Europe and Asia) due to the founder effect (Arneberg *et al.*, 1998). The elevated richness observed may be due to the physiographic characteristics of the island and parasite biology, as well as the location of the island. Recent analysis by Spencer *et al.* (2015) suggests that cats on CHI have multiple countries of origin (i.e. Europe and Asia).

Repeated introductions of both these host species to the island from multiple locations have most likely occurred since European colonisation and this could explain the greater diversity of parasite species observed in these hosts on the island (Mas-Coma and Feliu, 1984).

The subsequent persistence of these parasite species may also be explained, in part, by the predator-prey relationship between cats and rats. The introduction of a prey species (rats) along with the establishment of a predator (cats) is likely to facilitate the transmission and persistence of parasites that utilise both species as hosts, e.g. the high level of *T. taeniaeformis* detected in both cats and rats in the study (Figure 2.5). Furthermore, higher species richness is generally expected in tropical locations such as CHI in relation to more temperate environments; as is reflected in the general increase in species diversity closer to the equator (Rohde and Heap, 1998; Vignon *et al.*, 2009). The absence of seasonality in tropical regions can also facilitate high levels of parasite intensities being maintained throughout the year (Møller, 1998).

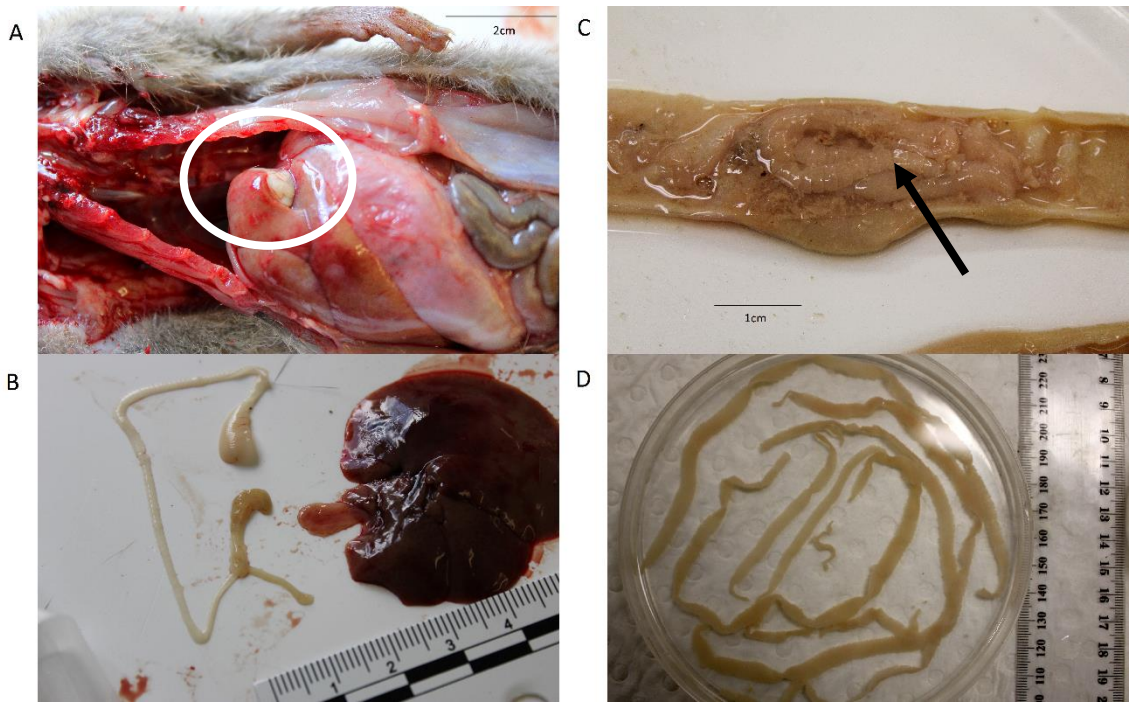

Figure 2.5: *Taenia taeniaeformis* isolated from both black rats (A-B) and feral cats (C-D) on CHI. The black rat acts as an intermediate host with the cat becoming the definitive host after ingesting an infected rat. A) Rodent cyst containing strobilocerci of *Taenia* in liver; B) The strobilocerci extracted from the liver cyst; C) section of cat small intestine with adult *Taenia*; D) Adult *Taenia* from feral cat intestine.

The successful establishment of a parasite in a new location is in part determined by the presence of suitable environmental and climatic conditions to facilitate transmission as well as the presence of potential intermediate or paratenic hosts. It is generally assumed that island environments will support a higher proportion of parasites with a direct life cycle (due to simpler requirements) and a high proportion of generalist parasites (parasites that lack host specificity for either definitive and/or intermediate hosts) (Dobson and May, 1986; Mas-Coma and Feliu, 1984; Vignon *et al.*, 2009). In this study 27 % of parasites utilise direct life cycles and while they don't require additional hosts appropriate environmental and/or climatic conditions are required (Stromberg, 1997). However, the majority (87%) of parasites identified in this study utilise an indirect life cycle (Table 2.2), some of which are

considered to be generalist parasites meaning they are able to use a range of intermediate hosts (Agosta *et al.*, 2010; Archie and Ezenwa, 2011) (Table 2.4). Parasites with an indirect life cycle presumably face the greatest challenge for persistence as they have a lower probability of finding appropriate intermediate or paratenic hosts (Dobson and May, 1986; Goüy de Bellocq *et al.*, 2003; Vignon *et al.*, 2009), yet maintenance of these parasites in a new island environment can be sustained if the intermediate host of these parasites is introduced concurrently (Mas-Coma and Feliu, 1984). Indeed, four parasite species detected in cats on CHI incorporate rodents as either intermediate or paratenic hosts (Table 2.4).

Table 2.4: Potential intermediate and paratenic hosts for some of the parasites species observed in cats and rats.

	Potential host	Parasites
Intermediate hosts	Invertebrates (including cockroaches, beetles, snails and fleas)	*M. muris, M. moniliformis, Physaloptera, Rictularia, R. nana, P. concinnum, A. cantonensis, A. abstrusus*
	Rodents	*T. taeniaeformis*
	Reptiles	*P. concinnum, J. pasqualei*
Paratenic hosts	Invertebrates (including Beetles, earthworms, snails and crabs)	*Physaloptera, T. cati, A. cantonensis*
	Rodents	*A. abstrusus, A. cantonensis, T. cati*
	Birds	*A. abstrusus, T. cati, Heterakis*
	Mammals	*Physaloptera*
	Reptiles	*Physaloptera, A. cantonensis, A. abstrusus*

The near simultaneous introduction of both cats and rats, together with their corresponding parasites, coupled with the high likelihood of multiple incursions of both species following introduction, may explain the higher than expected parasite richness observed on CHI. In addition, despite the restricted number of mammalian and reptile species on the island, there is a high diversity of avian (42 recorded species)

and invertebrate species which may potentially act as intermediate and paratenic hosts for these parasites (Anonymous, 2014). In restricted environments such as islands, introduced parasites have been reported to switch host species, usually involving a host that represents a similar resource as the original host (Agosta *et al.*, 2010). An example of this is the hypothesised introduction of *Trypanosoma lewisi* by black rats to CHI leading to the infection of, and subsequent extinction of, two native rodent species (Wyatt *et al.*, 2008); although a recent study by Dybing *et al.* (2016b) failed to find evidence of infection in the black rat population. Whilst the present study did not explore the involvement of native species in parasite transmission on CHI, host switching may have potentially contributed to the persistence and increased parasite richness observed.

With the decrease in endemic populations on CHI being attributed, at least in part, to predation by introduced species, it is interesting to note the potential for these native animals (including reptiles) to also act as intermediate hosts for a number of identified parasites. Little is known about the effect of some of these parasites on native fauna, although parasitism of prey animals has been reported to lead to an increased susceptibility to predation by cats and rats (Phillips, 2012). A number of studies have identified a disproportionately high number of infected prey being taken by predators (Holmes and Bethel, 1972; Hudson *et al.*, 1992). This raises the issue of whether infection of endemic species with parasites introduced by cats and/or rats may have contributed to the subsequent decline in native species abundance and diversity observed on CHI.

This study also examined the host-parasite relationship itself. Female cats were more likely to have greater overall and visceral infra-community richness than males,

as well as exhibiting a higher intensity of *P. concinnum* (Table 2.3). This may be related to queens training litters to hunt for a variety of prey species (potential intermediate hosts), resulting in a greater exposure to an increased diversity of parasites (Abu-Madi *et al.*, 2010; Abu-Madi *et al.*, 2008; Burt *et al.*, 1980; Engbaek *et al.*, 1984). An increased susceptibility to pathogens in female cats has been reported previously (Festa-Bianchet, 1989; Grzybek *et al.*, 2015; Schalk and Forbes, 1997). In contrast, increased intensity of the tapeworm *T. taeniaeformis* was observed in male rats compared to females. A study by Low *et al.* (2013) determined that female rats maintained smaller home ranges compared to males in urban areas of CHI, suggesting that males could possibly have a greater likelihood of encountering embryonated eggs of *T. taeniaeformis* due to their larger foraging range leading to increased levels of exposure.

2.6.2 Effects of parasites on conservation and public health

As mentioned previously, different parasites employ transmission strategies which aim to optimise their proliferation in their host communities. Wildlife and/or humans that are not typically involved in a parasite life cycle may still acquire the infective stage even though it is not capable of replicating or surviving in this host (i.e. dead-end host). As such, misdirected migration of helminth parasites within dead-end hosts can lead to varying health implications, ranging from a rash or minor irritation to more serious complications and in extreme cases even death. Of the parasites identified in this study, 37% have zoonotic (transmissible to humans) potential; those with the most clinical significance and/or incidence of human infection globally include *Angiostrongylus cantonensis*, *Ancylostoma braziliense*, *Moniliformis moniliformis* and *Toxocara cati*. Nine species identified in this study have been identified as parasites of

conservation potential (Table 2.2). This category encompasses species with known conservation significance (can impact endemic species either as individuals or at a population level) e.g. *Angiostrongylus cantonensis* in mammals, as well as parasites with unknown effects on wildlife or those that can utilise multiple species as intermediate or paratenic hosts e.g. *T. cati*.

Angiostrongylus cantonensis (rat lungworm) commonly infects rats in tropical and subtropical environments and has been previously reported on CHI (Hall *et al.*, 2011). In the current study, it was detected in approximately 10% of rats examined. The larvae of *A. cantonensis* commonly cycle through snails and slugs and the ingestion of these hosts or slime containing the L3 larvae (i.e. on fruits or vegetables) is infective to a wide range of both vertebrate and invertebrate hosts (Spratt, 2015). Infection with this parasite can occasionally cause chronic, disabling disease, even death (Pien and Pien, 1999). Recent investigations into *A. cantonensis* in Australia has identified this parasite as being associated with disease in black and grey-headed flying foxes (*Pteropus alecto* and *Pteropus poliocephalus*) (Barrett *et al.*, 2002; Reddacliff *et al.*, 1999), and as contributing to the decline of tawny frogmouths (*Podargus strigoides*) (Monks *et al.*, 2005). As such, *A. cantonensis* may have implications for the declining flying fox (*Pteropus melanotus natalis*) population on CHI therefore the potential for spread of this parasite in remaining flying foxes warrants further investigation.

2.6.3 Public health risks

Whilst the impact of parasites on populations of intermediate or incidental hosts is typically difficult to quantify, the negative aspects of parasitic infection and transmission to human communities is widely appreciated. The risk posed by zoonotic parasites to the human population may well be exacerbated by the geographically

isolated nature of the community which may limit the level of health care available as well as the likelihood of accurate diagnosis (Sheorey and Bradbury, 2016). These conditions are likely to result in increased rates of morbidity, or even mortality, associated with zoonotic parasitoses due to the low likelihood of patients obtaining appropriate or adequate treatment.

High, localised densities of free ranging hosts (i.e. cats and rats) coupled with a restricted geographic extent, typical of insular environments, is also likely to increase exposure rates to infectious parasitic stages of high risk cohorts of the population i.e. children, pregnant women and immunocompromised persons. Of particular importance, is the manner in which inhabitants interact with potential hosts; this was especially true of cats on CHI. Whilst there was a large number of domestic/stray/feral cats co-existing within areas of human habitation on CHI, there was very little individual ownership associated with any of the cats, rather that they were free ranging cats that 'belonged to the community'. Whilst supplementary feeding of these 'community cats' was undertaken by a portion of the population, these cats maintained a free ranging and scavenging existence within the community and were not subject to any veterinary care or intervention. These conditions are highly likely to exacerbate the zoonotic risk represented by these cats to the human population on the island. As such, education and awareness programs targeting the zoonotic risk associated with wild/stray animal populations, such as those on CHI, is a key step toward encouraging the adoption of improved hygiene and mitigation strategies to reduce infection risk at the population level.

2.6.4 Challenges of parasites on islands

The introduction of parasites and diseases by invasive animals increases the level of complexity of initiating effective conservation and/or management programs. Not only do you need to take into account the direct predatory effects of invasive animals, but also the indirect disease transmission risk to endemic fauna populations. In small geographic areas, such as islands, there is an increased likelihood of transmission of parasites from host to host, as well as spill-over into human populations on inhabited islands (Dobson, 1988). These issues are further compounded by accidental or deliberate anthropogenic introductions of invasive species on inhabited islands which are capable of transmitting parasites (e.g. cats typically introduced as pets, rats whose introduction is typically facilitated by human activities). Generalist parasites (no/low host specificity) and parasites with complex life cycles (more hosts) are more difficult to manage as they may be utilising a larger number of hosts in the new environment. Despite managing one host in the parasite's life cycle i.e. black rats, other hosts may still be perpetuating parasites in the environment i.e. birds or reptiles. For example, for islands which host a large diversity of migratory birds (i.e. CHI), there is potential for these birds to continually introduce parasites, whether they are acting as intermediate hosts or as biological or mechanical vectors. These varied mechanisms of parasite dispersal/introduction require due consideration in any conservation or management strategy, but can be of particular importance when considering island environments.

2.7 Conclusions

This study highlights the commonly overlooked impact of introducing the concomitant parasites of invasive species on islands. The introduction of a single invasive species can have far reaching negative impacts on island and mainland communities alike; however, the introduction of multiple species, and their parasites, can compound these impacts significantly. The co-introduction of rats along with cats can augment the establishment of cats, including their associated parasites that utilise rodents as intermediate hosts. The pattern of parasite richness observed on CHI (in both feral cats and black rats) was not significantly different to that observed on other mainland and island communities, and this challenges the notion of islands exhibiting a lower local richness. Factors involved in determining the establishment and diversity of parasites on islands include the frequency of host introduction, species co-introductions and host origin as well as the climate and environment of the island, the fauna present on the island that are capable of acting as alternate hosts for introduced parasites as well as the type of parasites introduced. Understanding the ecological role of introduced species and the parasites they harbour is a crucial step towards improving the ability to mitigate their threats to endemic fauna and ecosystems, particularly insular populations which are increasingly susceptible to extinction.

Chapter 3 Helminths of feral cats from Dirk Hartog Island and south west Western Australia

3.1 Preface

This chapter is to be published as an invited paper in a special edition of *Austral Ecology* journal that will include papers presented as part of the disease symposium at the Ecological Society of Australasia annual conference;

Dybing, N.A., Jacobson, C., Irwin, P., Algar, D., Adams, P.J., In Review. What did the feral cat drag in? Feral cats, helminths and the Island Syndrome. Austral Ecology.

After examining the parasite community of two invasive species on Christmas Island (CHI), it was determined that parasite species richness was unexpectedly high on CHI and therefore not all facets of the Island Syndrome occur on all islands (Chapter 2). Chapter 3 describes characteristics of parasite communities on an island (Dirk Hartog Island) and mainland (southwest Western Australia) in relatively close proximity to each other to observe if the same patterns were observed on this island as they were on CHI.

3.2 Introduction

Understanding the characteristics of parasite populations and the risk posed by parasites potentially transmitted from invasive animal hosts to humans and wildlife communities is important in determining management plans for islands. The "Island Syndrome" refers to a hypothetical framework of parasite ecology on islands that is typically characterised by a combination of low parasite local richness and diversity, high parasite prevalence, and high intensity and high infra-community richness relative to mainland populations (Fromont *et al.*, 2001; Goüy de Bellocq *et al.*, 2002; Goüy de Bellocq *et al.*, 2003; Mas-Coma and Feliu, 1984). Dybing *et al.* (2017a) identified that not all facets of Island Syndrome were evident in helminth parasites from cats and rats on Christmas Island (CHI). Specifically, the species richness of cat and rat helminth parasites on CHI was not different to that reported for mainland populations, contrary to what is predicted for the Island Syndrome. High parasite species richness on islands is relevant where those parasites can impact public health, animal health or conservation programmes. Understanding the role of invasive animals as reservoirs for these parasites can help improve public health and conservation management programmes.

It was speculated that the tropical environment on CHI may have contributed to the observation that cat parasites on the island did not fulfil all aspects of the Island Syndrome (Dybing *et al.*, 2017a). This chapter examines the influence of the Island Syndrome for helminth parasites of cats on another Australian island with very different environmental attributes, to determine whether physiographic differences could be contributing to the Island Syndrome discrepancy. Like CHI, Dirk Hartog Island (DHI) is under intense ecological pressure from invasive species, whilst ecotourism is

an increasingly important industry for the island. However, DHI has a semi-arid climate, has a wider degree of seasonal variation, fewer human inhabitants and a different faunal composition compared with CHI (Table 3.1). Dirk Hartog Island has conservation significance as a national park and refuge for native species with plans for fauna re-introduction programmes to be implemented in the future. Therefore, understanding the risks posed by the parasite community in the remaining invasive predators is important for informing the management plans for the island.

Table 3.1: Physiographic and ecological characteristics of Dirk Hartog Island (DHI) and Christmas Island (CHI)

	DHI	CHI
Area	620km^2	135km^2
Population size (n)	2	~2 000
Climate type	Semi-arid	Tropical
Average annual temp	26.5ºC	27ºC
Average rainfall (mm/annum)	228	2,129
Host species diversity		
Birds (n)	81	>100
Reptiles (n)	48	13
Mammals (n)	5	3
Amphibian (n)	1	0

This study aimed to compare helminth parasite populations of feral cats on DHI with the nearby mainland of Western Australia (WA) and determine whether the parasite populations on DHI exhibit characteristics consistent with Island Syndrome. The hypothesis tested was that cat helminth parasites on DHI will exhibit lower parasite local richness and diversity, higher parasite prevalence, higher intensity and higher infra-community richness relative to helminths of feral cats from mainland WA.

3.3 Methods

3.3.1 Study locations

Samples for this study were collected from DHI and from several locations on the mainland in southwest Western Australia (swWA) (Figure 3.1). Dirk Hartog Island is a semi-arid, inshore island (25°50'S 113°05'E) located 1km off the Western Australian coast. It is characterised by an average annual rainfall of 228mm predominantly falling during May-June (Bureau of Meteorology, 2012).

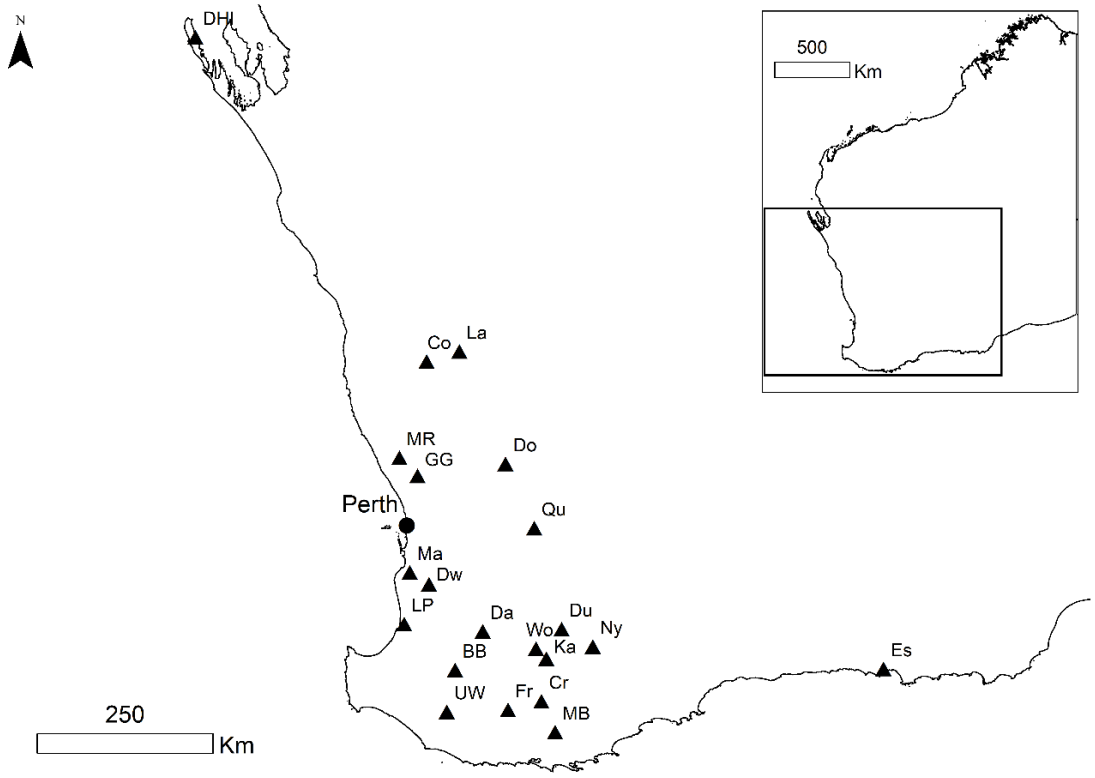

Figure 3.1: Helminth sample locations. BB- Boyup Brook; Co- Coorow; Cr-Cranbrook; Da- Darkan; DHI- Dirk Hartog Island; Do- Dowerin; Du- Dumbleyung; Dw- Dwellingup; Es- Esperance; Fr- Frankland; GG- Gingin; Ka- Katanning; La- Latham; LP- Leschenault Peninsula; Ma- Mandurah; MR- Moore River; MB- Mount Barker; Ny- Nyabing; Qu- Quairading; UW- Upper Warren; Wo- Woodanilling.

Southwest WA is a large ecoregion, located south of a line from Geraldton ($28^0$46'28" S, $114^0$36'32" E) to Esperance ($33^0$51'40" S, $121^0$33'31" E). The climate is predominately Mediterranean (hot dry summers, cool wet winters). Gingin ($31^0$19'16" S, $115^0$59'45" E) is a regional town located 92km north of the Perth metropolitan area and is part of the agricultural region of swWA. Samples collected from the townships of Mooliabeenee, Bindoon, and Mogumber surrounding Gingin (within a 20km radius of each other) will hereon be referred to collectively as Gingin (GG).

3.3.2 Sample collection

Feral cat cadavers were sourced from DHI as part of the DHI National Park Ecological Restoration Project conducted by Department of Parks and Wildlife in March 2012 (n=9) and March/April 2013 (n=14). Feral cat cadavers from swWA were sourced predominantly from an annual coordinated culling program (Red Card for Rabbits and Foxes) during two weekends (in February and March) in 2010 and 2012 from 23 locations (n=71). Additional samples were sourced in 2012 from private culling operations throughout the year (n=23) and during an introduced predator control and monitoring program 2012/13 (n=6) with the Department of Parks and Wildlife in the Upper Warren region ($34^0$22'8" S, $116^0$16'52" E) (Balban and Boyicup).

Collection methods and use of cadavers were approved by Department of Parks and Wildlife (DEC AEC 2009/35 and 2012/41) and Murdoch University Animal Ethics Committee (W2266/09; animal cadaver notification dates 27th March and 12th September 2012).

3.3.3 Carcass examination and parasite collection

Individual cat cadavers were uniquely identified immediately following culling were placed in plastic bags which were sealed and subsequently stored at -20°C for transport to Murdoch University. Cadavers were defrosted at room temperature (~7h) prior to necropsy. Cats were grouped into age categories according to weight; kitten (weight <1.5kg) or adult (weight >1.5kg). Visceral organs (lungs, heart, spleen, kidney, lymph node, liver, tongue and brain) were examined visually with the aid of a dissecting microscope for parasites. Tissue samples from these organs, as well as diaphragm for skeletal muscle, were collected and stored in both 70% ethanol and 10% neutral buffered formalin. The gastro-intestinal tract (GI), including stomach, small and large intestines, was removed and refrozen for subsequent examination for parasites and for dietary analysis. The GI tracts were then thawed (up to 5 h), incised and opened longitudinally, and examined using a dissecting microscope. Gastro-intestinal contents were sifted and teased apart using soft forceps to uncover helminth parasites (Dybing *et al.*, 2013) and dietary items.

3.3.4 Dietary analysis

Dietary analysis was conducted on 23 cats from DHI and 61 cats from swWA between 2012 and 2013. The diet of 14 cats from DHI (2013) was analysed alongside another project (Deller *et al.*, 2015); these data were pooled with the remainder of the cats from 2012. Food items were sorted and identified visually and by dissecting microscope and smaller items were washed through 1mm sieves. Items were then categorised into five main food groups (bird, reptile, amphibian, invertebrate and mammals). Mammals were identified to species where possible. The percentage occurrence of each food group was calculated for each region (DHI and swWA). Food

items were identified by presence of the whole body, limbs, skeletons or smaller fragments. Birds were considered present if feathers, beaks, wings or claws were recovered. Reptiles were identified by the presence of scales and mammals were distinguishable by presence of hairs.

3.3.5 Parasite identification

Parasites were retrieved from the necropsy tissues; cestodes were preserved in 10% neutral buffered formalin and all other helminths were preserved in 70% ethanol. Nematodes were cleared in lactophenol to facilitate identification. Parasites were identified morphologically using relevant keys and references (Amin, 1987; Baker, 2008; Bowman *et al.*, 2002; Costa *et al.*, 2003; Mawson, 1968; Soulsby, 1982). All hookworms were identified by buccal capsule morphology with species confirmation performed by PCR on a random subsample (Biocca, 1951; Smout *et al.*, 2013) as follows: DNA was extracted from 5-10 worms/cat and PCR performed according to the protocol described by Smout et al. (2013). Stomach nodules were dissected carefully for the presence of buried helminths.

3.3.6 Statistical analysis

The terms prevalence, regional richness, abundance and infra-community richness of parasites were used based on definitions described by Bush *et al.* (1997) (refer to Table 2.1). Prevalence and 95% confidence intervals were determined using Jeffrey's method (Brown *et al.*, 2001). The abundance of cestodes was determined by the number of scoleces recovered from GI tracts. Cats with stomach nodules present, regardless of the presence of individual helminths, were considered positive for *Cylicospirura seurati* with an abundance of one unless multiple worms were recovered (Pence *et al.*, 1978).

Given the large geographic area of swWA and the large distances between collection locations, helminth population characteristics for cats collected from the GG area were compared with remainder of the swWA to determine if there was evidence of significant within-region variation in helminth population characteristics. Chi-squared analysis was employed to compare regions with a) overall parasite prevalence and b) individual parasite prevalence. Abundance of each parasite was compared between regions using a generalized linear model with a Poisson distribution. A univariate general linear model was used to compare the ICR between regions. Parasite prevalence, abundance and ICR was calculated using IBM SPSS Statistics version 21 (IBM). Statistical analyses were not performed on parasite species found in <5 individuals.

Community diversity statistics were analysed using PAST software package (Hammer *et al.*, 2001). An ANOSIM was conducted to compare the variation in species abundance and composition between the two study regions. A SIMPER was then conducted to determine which parasites contributed primarily to the difference between the regions. The Simpson's diversity index was calculated for both regions; it measures biodiversity in a community which considers the number of species present and relative abundance. A Chao2 species richness estimate was performed using the formula in Poulin (1998) to estimate true species richness for both DHI and swWA.

3.4 Results

3.4.1 Host demographics

One hundred and eight cats were sampled from swWA (including 32 from GG, the largest number sampled from a single location) and 23 cats from DHI (Table 3.2).

Table 3.2: Origin, sex and age categories of feral cats examined (n=131) from Western Australia

			Sex		Age	
		Total	F	M	Adult	Kitten
swWA*	all	108	58	50	82	26
2010	year 1	47	25	22	33	14
2012	year 2	56	31	25	45	11
2013	year 3	5	2	3	4	1
GG	*all*	*32*	*20*	*12*	*28*	*4*
2010	*year 1*	*2*	*1*	*1*	*2*	*0*
2012	*year 2*	*30*	*19*	*11*	*26*	*4*
DHI	all	23	5	18	22	1
2012	year 2	9	2	7	8	1
2013	year 3	14	3	11	14	0

*inclusive of cats from Gingin

3.4.2 Dietary analysis

Four food categories were found in the GI tract of cats from DHI and five food categories were found from swWA (Table 3.3). The primary food item categories found in cat GI contents on DHI were birds and reptiles and from swWA arthropods and mammals.

Table 3.3: Occurrence (% cats sampled) of food items in gastro-intestinal tract of cats from DHI and swWA.

		DHI (n=23)	swWA (n=61)
Bird		78%	21%
Reptile		78%	11%
Arthropods		57%	72%
Amphibian		-	3%
Mammal - overall		57%	85%
	mouse	57%	72%
	rat	-	13%
	rabbit	-	5%
	sheep	-	3%
	cow	-	2%

3.4.3 Parasite prevalence and diversity

Gastro-intestinal parasites from four phyla (Nematoda, Cestoda, Acanthocephala and Arthropoda) were recovered from cat GI tracts (Table 3.4). No helminths were recovered from visceral organs. Prevalences over 20% were observed for *T. taeniaeformis*, *O. pomatostomi*, *S. erinaceieuropaei* and *T. cati* from swWA and *A. tubaeforme*, *O. pomatostomi*, Physalopterids and *C. seurati* from DHI (Table 3.4). The potential fauna capable of acting as intermediate or paratenic hosts for the parasites species identified in both study areas is reported in Table 3.5.

Regional richness was similar for GG (9 species) and the remainder of swWA (10 species). All helminth species identified were recovered from cats from both GG and the remainder of swWA, with the single exception of *C. seurati* (not recovered from GG). Infra-community richness ranged from 0-4 species per cat from GG and 0-6 species per cat from remainder of swWA, but mean ICR did not differ ($p=0.269$) between GG (1.75±0.22) and remainder of swWA (1.45±1.32). The overall prevalence also did not differ ($p=0.29$) between the two locations (GG= 87.5%; remainder swWA = 76.3%). As there were no differences in helminth population characteristics between GG and remainder of swWA, helminth populations for GG and swWA (remainder) were considered to be uniform and were combined for subsequent analyses.

Table 3.4: Helminths recovered from feral cats from Dirk Hartog Island (DHI) and southwest Western Australia (swWA) as well as their corresponding zoonotic and conservation significance. Chi squared analysis and generalized linear models with a Poisson distribution indicate difference in prevalence and abundance between locations respectively. Values of statistical significance (P<0.05) are included in the table.

Parasite	Prevalence % (95% confidence interval)			Abundance Mean ± standard error (range)			Conservation	Zoonosis	Life cycle[c]
	DHI (n=23)	swWA (n=108)*	P value[a]	DHI (n=23)	swWA (n=108)*	P value[b]			I/D
NEMATODA									
Ancylostoma tubaeforme	100 (85, 100)	12.0 (7,20)	<0.001	88.26±11.11 (5-199)	1.64±1.41 (0-149)	<0.001	*		I/D
Physaloptera spp.	60.9 (39, 80)	2.8 (0.6, 8)	<0.001	4.35±1.25 (0-22)	0.23±0.15 (0-15)	<0.001			I
Cyathospirura seurati	43.5 (23, 66)	1.9 (0.2, 7)	<0.001	5.26±3.05 (0-64)	0.78±0.68 (0-71)	<0.001	*		I
Cylicospirura felineus	26.1 (10, 48)	7.4 (3, 14)	0.019	0.65±0.31 (0-4)	0.51±0.33 (0-29)	NS			I
Toxocara cati	17.4 (5, 39)	21.3 (14, 30)	NS	2.87±1.86 (0-32)	1.48±0.55 (0-49)	<0.001	*	*	I/D
Rictularia spp.	8.7 (1, 28)	0 (0, 3)	-	0.70±0.65 (0-15)	0.00±0.00	-			I
Toxascaris leonina	0 (0, 15)	3.7 (1, 9)	-	0.00±0.00	0.04±0.02 (0-1)	-			I/D
CESTODA									
Taenia taeniaeformis	4.4 (0, 22)	48.2 (38, 58)	0.017	0.04±0.04 (0-1)	4.47±1.04 (0-57)	<0.001		*	I
Spirometra erinaceieuropaei	0 (0, 15)	21.3 (14, 30)	0.013	0.00±0.00	2.77±1.11 (0-91)	<0.001	*	*	I
Dipylidium caninum	0 (0, 15)	7.4 (3, 14)	NS	0.00±0.00	0.54±0.30 (0-26)	<0.001		*	I
ACANTHOCEPHALA									
Oncicola pomatostomi	91.3 (72, 99)	25.0 (17, 34)	<0.001	133.52±38.88 (0-718)	6.00±1.94 (0-139)	<0.001			I
CRUSTACEA									
Linguatula serrata[d]	13.0 (3, 34)	0 (0, 3)	-	0.57±0.478 (1-11)	0.00±0.00	-		*	I

* includes Gingin

NS: not significant P>0.100

[a] chi squared analysis used to compare prevalence between location

[b] independent 2 sample t-test

[c] I=indirect, D= direct

[d] Adult *Linguatula serrata* found in large intestine of cats

Table 3.5: Host-parasite relationships for parasites identified on DHI and swWA.

	Potential hosts	Parasites
Intermediate hosts	Invertebrates (including cockroaches, beetles, snails, fleas, copepod)	*Physaloptera, Rictularia, S. erinaceieuropaei, C. felineus, C. seurati, D. caninum*
	Rodents	*T. taeniaeformis*
	Livestock (cows and sheep)	*L. serrata*
	Birds	*O. pomatostomi*
	Rabbits	*T. taeniaeformis*
Paratenic hosts	Invertebrates (including beetles, earthworms, snails and crabs)	*Physaloptera, T. cati*
	Rodents	*T. cati, A. tubaeforme, T. leonina*
	Birds	*S. erinaceieuropaei, C. felineus, A. tubaeforme*
	Mammals	*Physaloptera, S. erinaceieuropaei, C. felineus*
	Reptiles	*Physaloptera, C. seurati, S. erinaceieuropaei, C. felineus*

3.4.4 The Island Syndrome

Parasite population characteristics for DHI and swWA are shown in Table 3.6. Observed overall richness was similar, only differing by one species between island and mainland (Table 3.6). Chao2 estimates the species richness of DHI as 10 species identical to swWA, indicating that the true species richness has been reached in the swWA with a sufficient number of hosts sampled, however more samples are needed to reach the estimated true species richness on DHI (Table 3.6). However, composition of parasite species varied between locations with 5/12 species identified from only one location, and 5/12 species with differences in abundance between locations (Table 3.4). The Simpson's diversity index indicates the diversity of the two communities were significantly different (Table 2.6). The ANOSIM showed that community structure between DHI and swWA was significantly different ($p<0.001$; $R=0.164$). The SIMPER demonstrated that two parasites (*Ancylostoma tubaeforme* and *Oncicola*

pomatostomi) contributed the most to this difference with a cumulative difference of >85%. Fifty-eight percent of parasite species identified were recovered at both locations, although the prevalence and abundance varied between locations (Table 3.4). Both overall prevalence and ICR were higher on DHI than swWA (Table 3.6). Three species (two from both locations, one from swWA only) of the 12 recovered were capable of utilising a direct life cycle (with capacity to use paratenic hosts for transmission).

Table 3.6: Island Syndrome factors swWA vs. DHI. A Chi-squared analysis was used for overall parasite prevalence and generalized linear model with a Poisson distribution was used to compare ICR.

Factor	DHI	swWA	p-value	Consistency with Island Syndrome?
Simpson's diversity index	0.54	0.20	<0.001	-
Observed (*Chao2*) species richness	9(*10*)	10(*10*)	-	No
Mean ICR	3.61±1.41	1.57±1.29	<0.001	Yes
Overall helminth prevalence	100%	79.60%	0.01	Yes

ICR: Infra-community richness

3.5 Discussion

This study compared cat helminth parasite populations on DHI with the mainland and showed that parasite communities on DHI only partially fulfilled the characteristics of Island Syndrome, consistent with similar observations from CHI described and discussed by Dybing *et al.* (2017a). Prevalence, mean ICR and abundance were higher for DHI compared to swWA, which is in line with the Island Syndrome. However, the local richness was not significantly different to the mainland, contrary to predictions of Island Syndrome, suggesting that this characteristic is not reliably exhibited by cat parasite populations on islands.

The similarity between local richness between DHI and swWA was surprising. Typically, low richness is expected on islands compared to a close mainland from which the founder population originated. Low richness on islands is generally attributed to a combination of factors including; the founder effect, restriction in the number of suitable hosts present on an island, and differing environmental or climatic conditions to which the parasites and hosts originate (Abdelkrim *et al.*, 2005; Mas-Coma and Feliu, 1984; Nieberding *et al.*, 2006). Previous reports of feral cat helminth parasites in Australian populations found similarly high overall richness; nine species in Victoria and New South Wales (n=327) (Coman *et al.*, 1981); five species in Tasmania (n=39) (Milstein and Goldsmid, 1997); nine species in New South Wales (n=146) (Ryan, 1976); 11 species on Kangaroo Island (n=46) (O'Callaghan *et al.*, 2005). After correcting for sampling effort, similar richness (10 species) were identified for both DHI and swWA. The Chao2 estimator was used to correct observed species richness, and has been used in other studies (Gompper *et al.*, 2003; Ishtiaq *et al.*, 2010; Morand *et al.*, 2000), although Poulin (1998) suggested the bootstrap estimator may be more appropriate to extrapolate species richness data. The similar local richness observed on DHI compared with swWA could be explained by multiple introduction events of the now feral cats onto DHI from swWA (Koch *et al.*, 2014), potentially contributing to the introduction of additional parasite species. Alternatively, the original introduced cat population could have been harbouring an already high infra-community richness, thus introducing a large number of parasite species to DHI at the time of colonisation. Although the probability of finding the required intermediate hosts and/or paratenic hosts in a new region is low, DHI appears to support suitable host species for a number of parasite species with indirect life cycles (Table 3.4 and 3.5). Additionally, some host species (including intermediate and/or paratenic hosts) are present both on DHI and in

swWA, therefore some parasites species may have been introduced to DHI by species other than cats.

Given the DHI cats originated from swWA (Koch *et al.*, 2014), it would be assumed that most, if not all, of the parasite species present on DHI would be present on swWA. Only five (of 12) species of parasites were recovered from a single location (Table 3.4). The assumed absence of these parasites in particular regions most likely reflects differences in availability of potential alternative hosts or vectors, as well as differences in environmental characteristics that determine parasite survival. For example, the absence of *Spirometra erinaceieuropaei* and *Dipylidium caninum* from DHI reflects the likely unsuitable conditions on the island for these parasites to persist, whether it be the absence of standing freshwater to accommodate the first intermediate host (freshwater copepod) for *S. erinaceieuropaei*, or the absence of the cat flea (*Ctenocephalides felis felis*) on DHI (Dybing *et al.*, 2016a), required for the *D. caninum* life cycle. *Rictularia* spp. and *L. serrata* were identified only from DHI and at low prevalences (8.7% and 13% respectively). The presence of *L. serrata* on DHI was not entirely surprising as livestock species are typical intermediate hosts, and historically the island has been a pastoral lease for sheep (*Ovis aries*) and goats (*Capra aegagrus hircus*) with the last remaining individuals being eradicated from the island presently. However, cats from swWA were sourced predominantly from the agricultural region with a high livestock presence. In addition, the presence of carrion including cattle (*Bos taurus*) and sheep in cat stomachs (3%) from swWA suggests exposure to suitable *L. serrata* intermediate hosts. It is possible that the original founding cat populations of DHI have originated from areas where *L. serrata* was present. For example, the original cat population arriving on DHI have most likely

originated from a region other than the swWA, or alternatively, it is possible that the study failed to identify *L. serrata* in cats from swWA due to low prevalence or variable temporal or spatial distribution across the swWA region. Future sampling should be conducted closer to the study island to identify whether any parasites were potentially missed.

Whilst most (7/12) of the helminths identified in this study were detected in cats from both DHI and swWA, the prevalence and abundance varied. This is most likely a reflection of variation in the faunal composition and diet of the cats from these locations. Whilst the presence of food items in the GI tract of cats can only show the most recent meals, it also indicates the species at risk of predation by feral cats as well as the species capable of acting as intermediate and paratenic hosts of feline parasites. The significantly higher presence of *Physaloptera* spp., *C. seurati* and *C. felineus* detected in cats from DHI may be related to the greater frequency of reptiles in cat diet on DHI (78% of cats) compared to swWA (11% of cats). Similarly, the increased prevalence and abundance of *O. pomatostomi* may relate to the higher presence of birds in cat diet (78% of cats) on DHI compared with swWA (21% of cats). The combination of the smaller area of DHI compared to swWA and the increased cat density on the island could account for the increased prevalence and intensity of *A. tubaeforme* on DHI compared to swWA. The increased host density and restricted geographic size of DHI consequently leads to closer contact between individuals resulting in amplified parasite transmission possibilities. Conversely, *T. taeniaeformis* and *S. erinaceieuropaei* had higher prevalence and abundance in swWA compared with DHI. Both mice and rats act as the main intermediate host for *T. taeniaeformis,* although rabbits can also act as an intermediate host. Of these, only *Mus musculus* is

present on DHI. In swWA, multiple species of rodents (including native species), and rabbits are present, therefore there are greater number of *T. taeniaeformis* transmission routes present in swWA compared to DHI, resulting in the increased presence and abundance observed for this location.

This study highlights the importance of environmental and climatic factors in the successful establishment of parasites on islands. The Island Syndrome suggests there will be a lower parasite local richness on islands compared to mainland regions. However, this study indicates that given the right conditions (i.e. suitable intermediate hosts, presence of fresh water bodies and suitable vegetation) a high parasite richness is attainable on islands. This observation was consistent for semi-arid DHI, as well as tropical CHI (Chapter 2). Therefore, it cannot necessarily be assumed that introduced animals are not contributing to a high diversity of parasite species in a specific region just because it is an island.

Given DHI's close proximity (<1.5km) to the mainland, there is a high probability of future invasion from invasive animals (e.g. rats arriving in cargo containers). These potential future invasions could similarly represent new suites of parasite introductions which could adversely affect native wildlife and public health as well as become incorporated into the cat population. Therefore, invasive animal management on islands is critical not only due to their predatory or competitive impacts, but also due to the impacts of introducing their associated parasites. Given that parasite community structures differ between islands just as much as it does between islands and mainland environments, management practices for these unique communities should consequently be assessed on an island by island basis.

Chapter 4 *Bartonella* species identified in rodent and feline hosts from island and mainland Western Australia

4.1 Preface

The following three chapters each explore the presence of different haemotropic and vector-borne pathogens from all three study sites and potential factors contributing towards the distribution of each pathogen.

Chapter 4 has been published in *Vector-borne and Zoonotic Diseases*:

Dybing, N.A., Jacobson, C., Irwin, P., Algar, D., Adams, P.J., 2016. *Bartonella* Species Identified in Rodent and Feline Hosts from Island and Mainland Western Australia. Vector- borne and Zoonotic Diseases 16, 238-244.

Chapter 4 examines the presence of *Bartonella* species and potential ectoparasite vectors for feral cats from the three study locations (Christmas Island, Dirk Hartog Island and southwest Western Australia), and for black rats on CHI. *Bartonella* is a vector borne, haemotropic bacterium that is the causative agent of human disease (including cat scratch fever and urban trench fever) on every continent (including Australia). As such the exact distribution and prevalence needs to be understood.

4.2 Abstract

Bacteria of the genus *Bartonella* have been described in multiple mammalian hosts with many species capable of causing disease in humans. Cats and various species of rats have been reported to play a role as vertebrate hosts to a number of *Bartonella* spp. This study aimed to identify *Bartonella* spp. in Western Australia, Dirk Hartog Island and Christmas Island, and to investigate the presence of potential arthropod vectors. Feral cats were collected from Christmas Island (n=35), Dirk Hartog Island (n=23) and southwest Western Australia (n=58), and black rats were collected from Christmas Island (n=48). Individuals were necropsied, ectoparasites were collected by external examination of carcasses, and splenic tissue was collected for PCR analysis to detect *Bartonella* DNA. *Bartonella henselae* DNA was detected from two cats and *Bartonella koehlerae* DNA from one cat in southwest WA, but *Bartonella* DNA was not identified in cats on Dirk Hartog Island or Christmas Island. *Bartonella phoceensis* (28/48=58.3%) and a novel *Bartonella* genotype (8/48=16.7%) based on the ITS region were detected in the spleens of black rats on Christmas Island. Detection of *Bartonella* spp. in each location corresponded to the presence of ectoparasites. Cats from southwest WA harboured four species of flea including *Ctenocephalides felis*, and black rats on Christmas Island were infested with multiple species of ectoparasites including mites, fleas and lice. Conversely, cats on Dirk Hartog and Christmas Island were free of ectoparasites. This study has identified the DNA of *Bartonella* species from island and mainland southwest Western Australia with some (*B. henselae* and *B. koehlerae*) of known zoonotic importance. This study further extends the geographical range for the pathogenic *B. koehlerae*. The association of *Bartonella* with

ectoparasites is unsurprising, but little is known about the specific vector competence of the ectoparasites identified in this study.

4.3 Introduction

Bartonella species are fastidious, intracellular, gram negative bacteria belonging to a group of emerging and re-emerging zoonotic bacterial pathogens (Chomel *et al.*, 2009; Tsai *et al.*, 2011b). Vector ranges, climatic conditions, and the presence of suitable reservoir hosts all appear to play a role in the prevalence of *Bartonella* species around the world. Approximately 26 species and subspecies of *Bartonella* have been identified with around half of these recognised as being capable of infecting humans (Bouhsira *et al.*, 2013; Chomel *et al.*, 2009).

Bartonella infection has been described in a large number of mammalian species including livestock, domestic pets and wildlife (Saisongkorh *et al.*, 2009). However, the geographical distribution and epidemiology of many *Bartonella* species, including the vectors involved in their transmission, are not fully understood. The infection and replication of *Bartonella* within arthropod vectors has only been shown to definitively occur in three species *B. quintana* (phlebotomine sand flies), *B. henselae* (fleas) and B*. schoenbuchensis* (lice) (Chomel *et al.*, 2009), although epidemiological research supports the role of ticks and lice as competent vectors (Billeter *et al.*, 2008). Reported prevalence of *Bartonella* in cats (*Felis catus*) vary markedly between populations and geographical locations, presumably related to rate of infestation with vectors such as *Ctenocephalides felis* (Assarasakorn *et al.*, 2012; Guptill, 2012).

In Australia, *Bartonella* species have been isolated from a wide range of mammalian species including domestic cats (*B. henselae*) (Flexman *et al.*, 1995) and a

number of rodent species (*B. coopersplainsensis*, *B. rattiaustraliensis* and *B. queenslandensis*) (Saisongkorh *et al.*, 2009). Newly described species of *Bartonella* have also been identified in native mammals in southwest Western Australia (swWA) (Kaewmongkol *et al.*, 2011a; Kaewmongkol *et al.*, 2011b; Kaewmongkol *et al.*, 2011c; Kaewmongkol *et al.*, 2011d).

Domestic cats represent an important reservoir in the life cycle of at least three *Bartonella* species (*B. henselae*, *B. clarridgeiae* and *B. koehlerae*), and feral and stray cats are more likely to be bacteraemic than domesticated individuals (Chomel *et al.*, 2006). Importantly, at least four *Bartonella* species isolated from cats (*B. henselae*, *B. clarridgeiae*, *B. koehlerae* and *B. quintana*) and seven species from rodents (*B. grahamii*, *B. elizabethae*, *B. vinsonii* subsp. *arupensis, B. volans, B. tribocorum, B. rattimassiliensis* and *B. washoensis*) are confirmed or suspected human pathogens (Castle *et al.*, 2004; Chomel and Kasten, 2010; Ellis *et al.*, 1999; Kosoy, 2010; Saisongkorh *et al.*, 2009). It has been hypothesised that all *Bartonella* species have the potential to cause human bartonellosis (Gil *et al.*, 2010; Lin *et al.*, 2010).

This study aimed to identify the presence of *Bartonella* species in swWA and two offshore islands with close wildlife/human interface and/or high conservation priorities; Christmas Island (CHI) and Dirk Hartog Island (DHI), and to investigate the potential importance of feral cats and black rats (*Rattus rattus*) as reservoir hosts for *Bartonella* species.

4.4 Methods

4.4.1 Study locations

Samples were collected from three geographically and climatically distinct locations; mainland swWA, CHI, and DHI (Figure 4.1). The swWA is a large ecoregion, located south of a line from Geraldton (28°46'28"S 114°36'32"E) to Esperance (33°51'40"S 121°33'31"E). Samples were collected from 12 different locations within swWA encompassing urban landuse, agricultural farmland, Mallee and Jarrah forest, and plainlands. This region has a Mediterranean climate characterised by a hot, dry summers and cool, wet winters.

Christmas Island is an Australian Territory, located in the Indian Ocean (10° 29' S, 105° 38' E) approximately 360 km south of the Indonesian capital, Jakarta. The island has an equatorial climate with distinct wet and dry seasons, and year-round high humidity. A large proportion of the island is dense tropical rainforest National Park (~70%) and mining lease area (~20%) with the remainder being settled land.

Dirk Hartog Island (25°50'S 113°05'E) is an inshore island located to the west of Shark Bay. The vegetation is sparse with low open shrubland and sand dunes. The island experiences a semi-arid climate region. This island was previously under pastoral lease but has now been returned to National Park status with an emphasis on ecotourism.

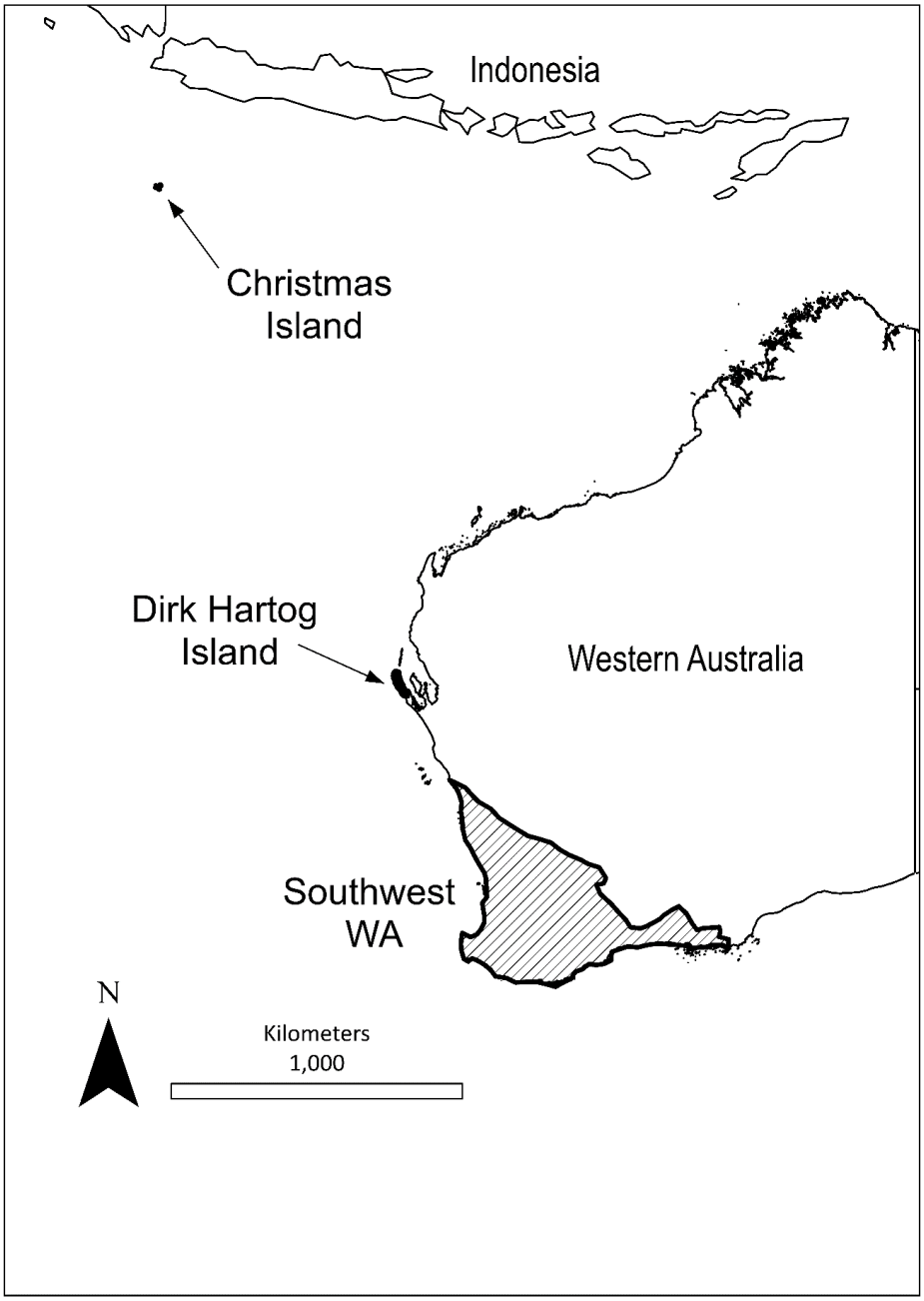

Figure 4.1: Map showing the geographical distribution of the three study sites sampled in this study; Christmas Island, Dirk Hartog Island and southwest Western Australia.

4.4.2 Sample collection and measurements

Cat cadavers (n=116) were collected from CHI (n=35), DHI (n=23) and swWA (n=58). Cats from CHI and DHI were sourced from Department of Parks and Wildlife management programs and cats from swWA were obtained during community-

coordinated culling programs from 12 locations. Rats (n=48) were collected from CHI concurrently with the cats. Weight was recorded for all carcasses and used to determine age category for cats (kitten ≤ 1.5 kg and adult cat > 1.5kg) according to the method previously described by Algar et al (2003). It was not possible to obtain accurate age estimates or determine age category for rats.

All cadavers were placed straight away into individually sealed plastic body bags then stored at -20^{o} C. Spleen tissue was collected at necropsy and preserved in 70% ethanol.

4.4.3 Ectoparasite identification

An external examination was conducted on all carcasses for the presence of ectoparasites before necropsy and the body bag was closely examined for ectoparasites that may have fallen off the carcass. Ectoparasites were identified to genus and species using morphological techniques and keys (Fritz and Pratt, 1947; Roberts, 1970; Voss, 1966).

4.4.4 DNA extraction

DNA was extracted from rat and cat spleens using Qiagen spin columns, tissue procedure, according to the manufacturer's instructions (Qiagen, Maryland, USA). Negative controls were used in the PCRs with the inclusion of PCR water instead of genomic DNA.

4.4.5 PCR conditions

Primers specific to the 16S-23S internal transcribed space (ITS) of *Bartonella* species were used to screen samples, 438s (5'- GGT TTT CCG GTT TAT CCC GGA GGG C-3') and 1100as (5'-GAA CCG ACG ACC CCC TGC TTG CAA AGC A-3') from Beard et al

(2011). PCRs were performed in an optimised 25 µl reaction volume containing 1X PCR Buffer [Fisher Biotech], 2.0 mM $MgCl_2$, 0.2 mM dNTPs, 0.02 U/µL Taq polymerase [Fisher Biotech], 1 µl template DNA, 6 µl of cresol red and 1 µM of each primer. The reactions were run under the following conditions: 1 denaturing cycle at 95° C for 2 min followed by 55 cycles at 94° C for 15 s, 66° C for 15 s and 72° C for 18 s, before a final extension cycle at 72° C for 30 sec. Amplified DNA fragments were visualised on a 1.5% agarose gel by electrophoresis.

4.4.6 DNA purification and sequencing

The tip elution method was used for extracting and purifying PCR products (Yang *et al.*, 2013). Positive bands were sliced from the gel and the fragment placed in a 100 µl filter tip (with the tip cut off) within a 1.5 ml Eppendorf tube. This tube was then spun at 20,000 x *g* for 5 min. The filter tip was discarded and the eluent retained for sequencing.

The purified DNA was sequenced using an ABI prism Terminator Cycle Sequencing kit (Applied Biosystems, Foster City, California, USA) according to manufacturer's instructions on an Applied Biosystems 3730 DNA Analyser. Sequencing results were compared against available sequences in GenBank using BLAST search. Multiple-sequence alignments were constructed using additional isolates from GenBank. Distance trees were constructed using MEGA version 6 (Tamura *et al.*, 2013). Genetic distances were calculated in MEGA using the Kimura 2 parameter model.

4.4.7 Statistical analysis

Prevalence was expressed as proportion (%) of animals infested (ectoparasites) or positive for *Bartonella* (PCR). Ectoparasite overall prevalence was expressed as proportion (%) of animals positive for at least one ectoparasite. The 95% confidence intervals for overall prevalence were calculated using Jeffrey's method (Brown *et al.*, 2001).

Statistical analyses were performed using the software IBM SPSS Statistics version 21 (IBM). Relationships between categorical host factors (sex, ectoparasite recovery, lice recovery, mite recovery, flea recovery and tick recovery) and the presence of rat *Bartonella* species were analysed using Pearson Chi square and Fisher's exact test. Statistical analyses were not performed for prevalence of *Bartonella* and host factors in cats due to a low prevalence.

4.5 Results

4.5.1 Ectoparasites

The details of the ectoparasites recovered from rats and cats are shown in Table 4.1. Black rats were infested with zero to three ectoparasite species with 85% of rats infested with at least one species. Of the 41 rats infested with ectoparasites, 18 were parasitised by one species, 19 by two species and four by three species. The cats from swWA were parasitised with up to two species of ectoparasites per host with 55% of cats infested with at least one species. Of the 32 cats infested with ectoparasites, 26 were infested with one ectoparasite species and six with two. No ectoparasites were found on cats from CHI and only lice were recovered from a single cat on DHI (Table 4.1).

Table 4.1: Ectoparasite infestation (n infested with % in parentheses and 95% confidence interval for overall prevalence only) of cats from three geographical regions and rats from Christmas Island.

		swWA	DHI	CHI	
		F. catus (n=58)	*F. catus* (n=23)	*F. catus* (n=35)	*R. rattus* (n=48)
Mites		0	0	0	35(73%)
Lice	*Hoplopleura pacifica*	0	0	0	21(36.2%)
	Polyplax spinulosa	0	0	0	8(16.7%)
	Felicola subrostratus	0	1(4.3%)	0	0
Fleas	*Ctenocephalides felis felis*	12(20.7%)	0	0	0
	C. canis	1(1.7%)	0	0	0
	Echidnophaga sp.	9(15.5%)	0	0	0
	Leptopsylla segnis	1(1.7%)	0	0	0
	Xenopsylla cheopis	0	0	0	2(4.17%)
Ticks	*Amblyomma* sp.	15(25.9%)	0	0	0
Overall prevalence		**32(55%)**	**1(4.3%)**	**0(0%)**	**41(85.4%)**
95% Confidence interval		**41.5, 68.3**	**0.1, 21.9**	**0, 10**	**72.2, 93.9**

4.5.2 Bartonella species and prevalence

The prevalence of *Bartonella* in rat and cat spleen samples are shown in Table 4.2. *Bartonella* DNA was identified in spleen tissue from 36 rats from CHI, and two kittens (*B. henselae* and *B. koehlerae*) and one adult (*B. henselae*) from swWA (Table 4.2).

Table 4.2: *Bartonella* species (n positive with % in parentheses and 95% confidence interval for overall prevalence only) in cats and rats from three geographical regions.

	swWA	DHI	CHI	
	F. catus (n=58)	*F. catus* (n=23)	*F. catus* (n=35)	*R. rattus* (n=48)
B. henselae	2(3.4%)	0	0	0
B. koehlerae	1(1.7%)	0	0	0
B. phoceensis	0	0	0	28(58.3%)
Unknown *Bartonella* sp A	0	0	0	8(16.7%)
Overall prevalence	**3(5.2%)**	**0(0%)**	**0(0%)**	**36(75.0%)**
95% Confidence interval	**1.5, 13.2**	**0, 14.8**	**0, 10**	**61.5, 85.5**

swWA - south west Western Australia; DHI - Dirk Hartog Island; CHI: Christmas Island

Overall, four species of *Bartonella* were identified by sequencing from two sites (CHI and swWA), specifically *B. henselae*, *B. koehlerae*, *B. phoceensis* and an unknown *Bartonella* sp. (Table 4.2). Blast results showed a 99% similarity with *B. phoceensis*, 99% similarity with *B. koehlerae* and 100% similarity with *B. henselae*. The unknown *Bartonella* sp. A had 91% similarity to *Bartonella* sp. SE-Bart-D previously reported by Loftis *et al.* (2006), from a *Rattus norvegicus* flea (*Xenopsylla cheopis*) in Egypt. The sequences identified in the present study have been deposited in GenBank under the following accession numbers; KU170606 and KU240393-KU240430. These sequences were then constructed into a dendogram along with sequences of known rat and cat *Bartonella* species obtained from GenBank (Figure 4.2). Of the 28 rats that *B. phoceensis* was detected in, only six were used in the phylogenetic reconstruction.

No association of *Bartonella* infection with host sex or recovery of ectoparasites was identified in rats.

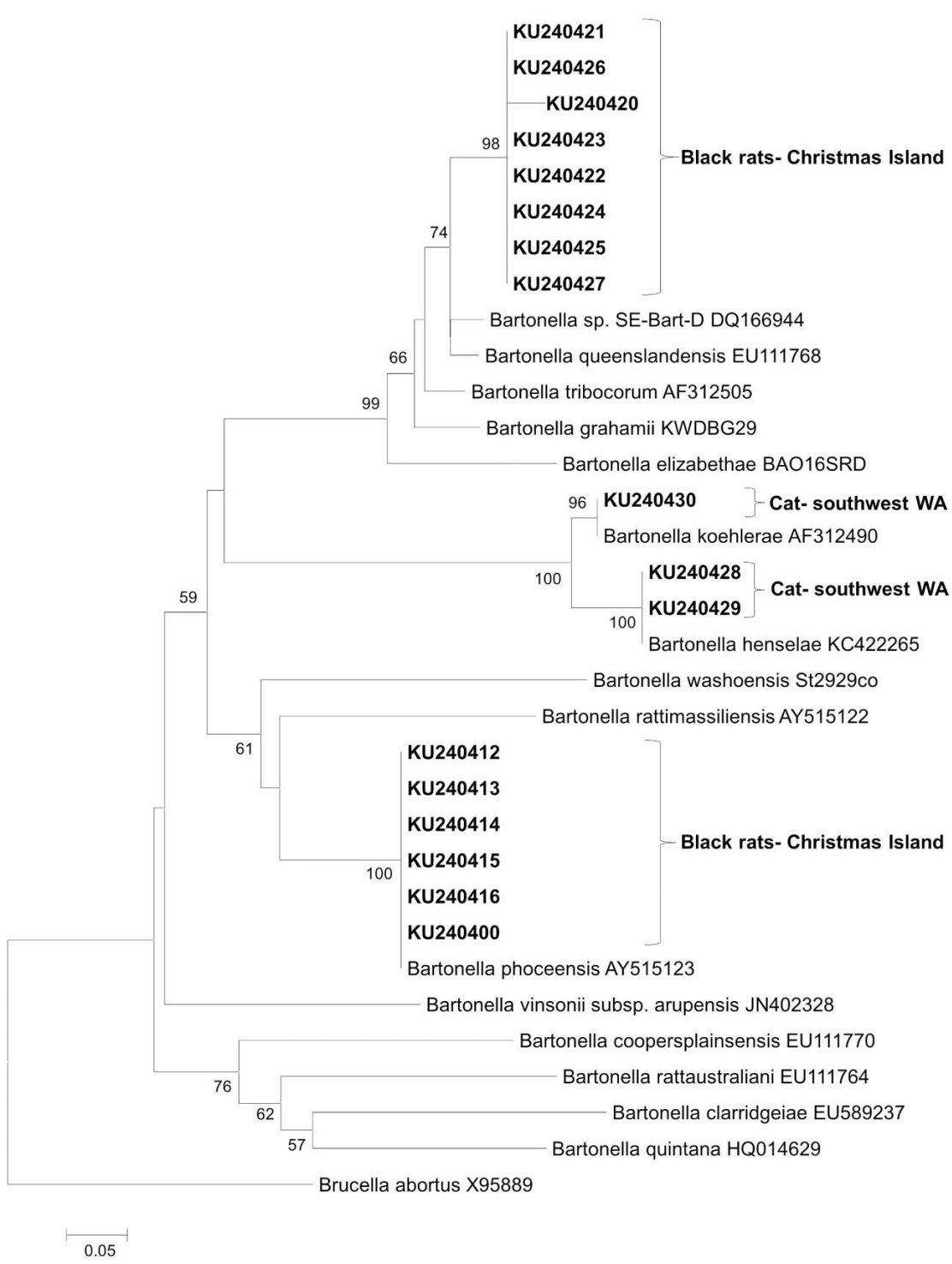

Figure 4.2: Phylogenetic relationship of *Bartonella* species detected in this study inferred by distance analysis of 16s-23s ITS sequences (indicated in bold). Percentage support (>50%) from 1000 pseudoreplicates from neighbour-joining analyses using bootstrapping is indicated at the left of the supported node.

4.6 Discussion

This study has provided new information about *Bartonella* infections in Australia. In general, members of the genus *Bartonella* are recognised as important emerging pathogens on a global scale. However, the occurrence and distribution of *Bartonella* species in rats, in particular, has not been well studied in Australia, and there are no data pertaining to these bacteria in feral species in island communities off the coast of Australia (Barrs *et al.*, 2010; Branley *et al.*, 1996; Dillon *et al.*, 2002; Fournier *et al.*, 2002).

Both species of *Bartonella* identified in cats from swWA in this study (*B. henselae* and *B. koehlerae*) have been reported previously in cats overseas (Branley *et al.*, 1996; Droz *et al.*, 1999; Fournier *et al.*, 2002; Maruyama *et al.*, 2001). *Bartonella henselae* has been identified in humans and felids in Australia and is considered the leading cause of cat scratch disease and zoonotic Bartonellosis worldwide (Barrs *et al.*, 2010; Branley *et al.*, 1996; Dillon *et al.*, 2002; Fournier *et al.*, 2002; Kaewmongkol *et al.*, 2011b; Saisongkorh *et al.*, 2009).

Identification of *B. koehlerae* was unexpected, and is the first report of this species from a southern hemisphere country. *Bartonella koehlerae* has been identified in small numbers of cats (seven in total) from California, France, Israel and Thailand (Assarasakorn *et al.*, 2012; Boulouis *et al.*, 2005; Fleischman *et al.*, 2015), from rodent fleas in Afghanistan (Marié *et al.*, 2006), feral pigs in North Carolina (Beard *et al.*, 2011) and from a dog in Israel (Ohad *et al.*, 2010). The identification of *B. koehlerae* in a cat from swWA therefore expands the known geographical distribution of this zoonotic pathogen. The epidemiology of *B. koehlerae*, including potential reservoirs and mode of transmission, are not well described. Of note is the wide diversity of symptoms that

have been attributed to *B. koehlerae* in humans including fatigue, insomnia, memory loss, decreased tactile sensation, hallucinations and endocarditis (Breitschwerdt *et al.*, 2010).

Interestingly, *Bartonella* species were identified in rats but not cats from CHI. Rodents are recognised as reservoirs for zoonotic species (Saisongkorh *et al.*, 2009; Tsai *et al.*, 2010) and the close relationship between rodents and humans globally highlights the importance of rats as a source of zoonotic infection (Castle *et al.*, 2004). *Bartonella*, including *B. phoceensis,* have been previously reported in rodents in Asia, including Thailand and Indonesia (Billeter *et al.*, 2008; Tsai *et al.*, 2010). Given the close geographical proximity of CHI to SE Asia, the finding of *B. phoceensis* in rats may be explained by rats arriving at the island over the years on ships from nearby Indonesia. Since *Bartonella phoceensis* has been identified in multiple rodent species, this highlights a potential risk for its spread into Australian rodent populations if it was to be introduced onto mainland Australia. Furthermore, identification of a potentially novel *Bartonella* species (Unknown *Bartonella* spp A) in rats from CHI adds to the diversity of *Bartonella* species currently described. In order to determine if this isolate represents a novel species, further molecular phylogenetic study would need to be conducted using more than one gene locus (La Scola *et al.*, 2003; Lin *et al.*, 2010).

Bartonellosis is a vector-borne disease (Gundi *et al.*, 2004) and is commonly reported from tropical environments (Chomel *et al.*, 2006), like CHI. The distribution of suitable arthropod vectors most likely explains the *Bartonella* prevalences observed in this study. Our detection of *Bartonella* species in rats but not cats on CHI was supported by our observation that cats on the island did not appear to have any ectoparasites. The reason for this is unclear – the cats were placed into plastic bags

that were sealed immediately after euthanasia. This procedure is expected to trap any ectoparasites for later identification, as was the case on DHI and in swWA. Lice infestations were identified in rats from CHI, including *Hoplopleura pacifica*, an ectoparasite for which transmission of *B. phoceensis* has been previously reported in various small mammal species (Billeter *et al.*, 2008; Reeves *et al.*, 2006; Tsai *et al.*, 2010). Dissimilar to the situation on DHI and CHI, flea and tick infestations were common in cats from swWA where *B. henselae* and *B. koehlerae* were identified at low prevalence. Transmission by the cat flea (*Ctenocephalides felis*) has previously been reported for *B. henselae* and it is suspected as a vector of *B. koehlerae* (Chomel *et al.*, 2009; Chomel *et al.*, 2006).

Despite over twenty years of research, the modes of transmission are still not well understood for many *Bartonella* species. Fleas appear to play a significant role in the transmission of multiple *Bartonella* species and ticks have been suggested as competent vectors (Tsai *et al.*, 2011a). Whilst it is possible for cat fleas to carry *Bartonella* species among cats, the vector competency has only been established for *B. henselae* (Guptill, 2012). One of the challenges in describing the epidemiology of *Bartonella* with respect to transmission is differentiating the presence of *Bartonella* spp. DNA due to a previous blood meal, rather than implying vector competence in those arthropods. Almost all the ectoparasites identified in this study have previously been associated with *Bartonella* species, including *B. tribocorum*, *B. elizabethae*, *B. queenslandensis*, *B. rochalimae*, *B. tamiae, B. rattimassiliensis*, *B. phoceensis*, *B. henselae* and *B. koehlerae* (Tsai *et al.*, 2011a). The diverse nature of ectoparasite species recovered from rats and cats in this study suggests further research into vector competency of these ectoparasites is required.

4.7 Conclusion

This study identified *Bartonella* species in cats from mainland swWA and black rats from CHI, including those with pathogenic and zoonotic potential. Additionally, we report *B. koehlerae* in Australia for the first time. The findings suggest that rodents as well as cats can be mammalian reservoirs for these vector-borne infections, and highlight the need for preventive measures with regard to public health and conservation management.

Chapter 5 Ghosts of Christmas past?: absence of trypanosomes in invasive animals from Christmas Island and Western Australia.

5.1 Preface

This chapter has been published in *Parasitology Open:*

Dybing, N.A., Jacobson, C., Irwin, P., Algar, D., Adams, P.J., 2016. Ghosts of Christmas past?: absence of trypanosomes in feral cats and black rats from Christmas Island and Western Australia. Parasitology Open 2, e4 (5 pages).

Chapter 5 describes the prevalence of *Trypanosoma* species and *Leishmania* species in the three study locations. The hypothesised introduction of *Trypanosoma lewisi* to Christmas Island by black rats has been reported as being responsible for the extinction of two endemic rodent species on the island. A recent study by Hall et al. (2011) identified an amastigote in a single blood smear from a black rat on Christmas Island suggesting the presence of either *Trypanosoma* or *Leishmania* species. As such, we investigated the occurrence of these two parasites in black rats and feral cats on Christmas Island, as well as from Dirk Hartog Island and southwest WA.

5.2 Abstract

Trypanosomes and *Leishmania* are vector-borne parasites associated with high morbidity and mortality. *Trypanosoma lewisi*, putatively introduced with black rats and fleas, has been implicated in the extinction of two native rodents on Christmas Island and native trypanosomes are hypothesized to have caused decline in Australian marsupial populations on the mainland. This study investigated the distribution and prevalence of *Trypanosoma* spp. and *Leishmania* spp. in two introduced pests (cats and black rats) for three Australian locations. Molecular screening (PCR) on spleen tissue was performed on cats from Christmas Island (n=35), Dirk Hartog Island (n=23) and southwest Western Australia (n=58), and black rats from Christmas Island only (n=46). Despite the continued presence of the intermediate and mechanical hosts of *T. lewisi*, there was no evidence of trypanosome or *Leishmania* infection in cats or rats from Christmas Island. Trypanosomes were not identified in cats from Dirk Hartog Island or southwest Western Australia. These findings suggest *T. lewisi* is no longer present on Christmas Island and endemic *Trypanosoma* spp. do not infect cats or rats in these locations.

5.3 Introduction

Trypanosomes and *Leishmania* spp. are vector-borne parasites associated with severe disease in both animal and human hosts. The global distribution of *Trypanosoma* spp. and *Leishmania* spp. is heavily reliant on the presence of both reservoir hosts and competent vectors. Worldwide, cats (*Felis catus*) are reported to become infected by at least six *Trypanosoma* spp.; *T. brucei*, *T. congolense*, *T. gambiense*, *T. cruzi*, *T. evansi* and *T. rangeli* (Bowman *et al.*, 2002). Likewise, 44 *Trypanosoma* spp. have been shown to infect rodents (Hoare, 1972; Milocco *et al.*,

2013; Pumhom *et al.*, 2015). *Leishmania* spp. are also `zoonotic protozoan parasites (closely related both morphologically and genetically to *Trypanosoma*) that are usually transmitted by biting phlebotomine sand flies and are widely distributed through both tropical and temperate regions of the world. Leishmaniasis is also associated with high levels of morbidity and mortality (Gramiccia and Gradoni, 2005; Peacock, 2010; Svobodová *et al.*, 2003). Over 40 mammal species are known to harbour *Leishmania* spp., including cats and rats, with the black, or 'ship' rat (*Rattus rattus*), increasingly recognised as an important natural reservoir in *Leishmania* transmission (Oliveira *et al.*, 2005; Quinnell and Courtenay, 2009; Sherry *et al.*, 2011). In Australia to date, eight novel *Trypanosoma* spp. have been identified in native wildlife (Paparini *et al.*, 2011; Thompson *et al.*, 2013; Thompson *et al.*, 2014), and leishmaniasis has been reported in macropods in the Northern Territory (Rose *et al.*, 2004) and is thought to be transmitted by biting midges (Dougall *et al.*, 2011). It is not known whether these endemic parasites can infect feline or rodent hosts.

On Christmas Island (CHI), the extinction of two native rat species has been attributed to infection with *Trypanosoma lewisi*, thought to have been introduced with black rats during incursions by sea-faring traders during the late 19th Century (Andrews, 1909; Durham, 1908). The observations of these early researchers appears to have been supported recently by Wyatt et al. (2008) who detected *T. lewisi* DNA in skin samples from two out of six (33.3%) museum specimens of the now-extinct Maclear's rats (*R. macleari*), and one out of six (16.7%) black rats collected by Durham at the time of European colonisation of the island (Wyatt *et al.*, 2008). More recently, speculation that trypanosomes were still present on CHI was based on the observational finding of *Trypanosoma*/*Leishmania*-like organisms in a blood smear of a

rat on the island (Hall *et al.*, 2011). Additionally, *Trypanosoma* spp. infections have been implicated in the precipitous decline of the woylie (*Bettongia penicillata*) on mainland Western Australia (swWA) (Averis *et al.*, 2009; Smith *et al.*, 2008), although the distribution and prevalence of *Trypanosoma* and *Leishmania* species in Western Australia (WA) is not well described.

As part of a larger research study into the effects of introduced species on wildlife in Australia and its islands, tissue samples from feral cats and black rats living on CHI, Dirk Hartog Island (DHI) and in swWA were examined for the presence of *Trypanosoma* and *Leishmania* DNA. These locations represent areas of importance for wildlife conservation as well as for public health.

5.4 Methods

5.4.1 Study locations

Samples were collected from three geographically and climatically distinct locations; from CHI, swWA, and DHI. Christmas Island is an Australian Territory, located in the Indian Ocean (10° 29’ S, 105° 38’ E) approximately 360km south of the Indonesian capital, Jakarta, with a tropical climate. The swWA is a large ecoregion, located south of a line from Geraldton (28°46’28”S 114°36’32”E) to Esperance (33°51’40”S 121°33’31”E) with a Mediterranean climate. Dirk Hartog Island is an arid inshore island (25°50’S 113°05’E) located to the west of Shark Bay off the WA coast.

5.4.2 Sample collection

Cat cadavers were collected from CHI (n=35; 8 fresh and 27 frozen), DHI (n=23; all frozen) and swWA (n=58; all frozen). Cats from CHI and DHI were sourced from Department of Parks and Wildlife management programs and cats from swWA were

obtained during community-coordinated culling programs from 12 locations. Rats (n=48; 23 fresh and 25 frozen) were collected from CHI concurrently with the cats. Spleen samples were collected at necropsy and preserved in 70% ethanol.

5.4.3 DNA extraction

DNA was extracted from cat and rat spleen tissue using the Qiagen spin columns for blood and tissue kit according to the manufacturer's instructions (Qiagen, USA). Negative controls were used in the PCRs with the inclusion of PCR grade water in place of genomic DNA.

5.4.4 PCR conditions - Trypanosoma

The nested PCR protocol from Botero et al. (2013) was employed with generic *Trypanosoma* primers of the 18S region which have been previously described (Maslov *et al.*, 1996; McInnes *et al.*, 2011). External primers used were SLF (5'- GCT TGT TTC AAG GAC TTA GC-3') and S762 (5'- GAC TTT TGC TTC CTC TAA TG-3') and internal primers were S823F (5'- CGA ACA ACT GCC CTA TAC GC-3') and S662R (5'- GAC TAC AAT GGT CTC TAA TC-3'). Cultured *Trypanosoma cruzi* and *Trypanosoma lewisi* were used as positive controls.

5.4.5 PCR conditions – Leishmania

Subsamples of spleens were tested for *Leishmania* spp. from CHI samples only, feral cats (n=10) and black rats (n=45), with genus specific primers adapted from Schonian (2003). Primers were from the internal transcriber region (ITS1), OL1853 (5'-CTG GAT CAT TTT CCG ATG-3') and OL1854 (5'- TGA TAC CAC TTA TCG CAC TT-3').

A touchdown PCR was performed on all samples using 3µL of DNA (at 5ng/µl) in an 11.5µL reaction. The reaction contained 1X Buffer, 3.0mM MgCl, 0.5mM dNTPs,

0.05M Betaine, 0.05µL Taq/Taq Gold and 10µM of each primer. PCR cycling conditions were optimized under the following conditions: 1 denaturation cycle at 94°C for 5min (Taq)/10min (Taq Gold) followed by 94°C for 20s, 63-56°C for 60s using 0.5°C/cycle increments and 72°C for 60s. This was then followed by 20 cycles of 94°C at 20s, 56°C for 60s, 72°C for 60s and a final extension of 72°C for 5 min (Blackwell lab, Australia). Positive controls included DNA extracted from *Leishmania major*, *L. braziliensis*, *L. tropica*, *L. donovani* and *L. australiensis*. All PCR products were run on a 1.5% agarose gel at 120V for 1 hour for visualisation.

5.4.6 Statistical analysis

Confidence interval values were calculated using the exact binomial methods (Graat *et al.*, 1997).

5.5 Results

No *Trypanosoma* spp. were detected by PCR in any of the spleen samples (Table 5.1). Positive controls for *T. cruzi* and *T. lewisi* amplified at the correct product size and no amplification was detected within the negative control. Similarly, no *Leishmania* DNA was detected in either cat or rat spleen samples (Table 5.1). All *Leishmania* positive controls produced amplified products at their corresponding sizes whilst all negative controls did not produce amplification.

Table 5.1: Prevalence (%) and 95% confidence interval for *Trypanosoma* and *Leishmania* in cats from three geographical regions and rats from Christmas Island.

	swWA	DHI	CHI	
	F. catus	*F. catus*	*F. catus*	*R. rattus*
Trypanosoma spp.				
Samples (n)	58	23	35	48
Positive samples (n)	0	0	0	0
Prevalence 95% confidence interval (%)	0.0, 6.2	0.0, 14.8	0.0,10.0	0.0, 7.4
Leishmania spp.				
Samples (n)	-	-	10	43
Positive samples (n)	-	-	0	0
Prevalence 95% confidence interval (%)	-	-	0.0, 30.8	0.0, 8.3

swWA - south west Western Australia; DHI - Dirk Hartog Island; CHI: Christmas Island

5.6 Discussion

This study was conceived when the opportunity arose to sample cats and rats on CHI after recent observations on pathology specimens suggested that *Trypanosoma* spp. may persist in this confined habitat where historical records had putatively incriminated trypanosomiasis for the extinction of two native rodents. We were also interested to investigate whether *Trypanosoma* DNA could be detected in the blood of introduced animals in regions where trypanosomiasis appears to be endemic in native marsupials upon which they undoubtedly prey. However, we found no evidence of infection with *Trypanosoma* spp. in feral cats from three geographical locations or black rats on CHI. Additionally, there was also no evidence of infection by *Leishmania* spp. in feral cats or black rats on CHI. These results suggest that cats and rats are not currently acting as reservoirs for trypanosomes or *Leishmania* in these locations, and questions whether *Trypanosoma* infection persists in any form on CHI.

The challenge presenting molecular research when pathogen DNA is not detected in any samples is to justify that target DNA was not missed due to inhibitors and/or suitability of the tissue for that particular work. We are confident that the samples used in this study yield viable DNA as these same samples were screened successfully for the presence of other blood-borne pathogens by molecular methods [e.g. *Bartonella* species; Dybing *et al.* (2016a)], thus ruling out the presence of inhibitors.

Regarding the suitability of tissues used, the spleen is regarded as a reliable location for detecting numerous different blood-borne pathogens, including *Trypanosoma*, for reasons including; the spleen is the blood filtering organ so pathogens circulating in the blood would be expected to be present (Mebius and Kraal, 2005); sampling from the spleen was more sensitive than blood for detecting vector and blood-borne pathogens including trypanosome and ehrlichial infections (Albright and Albright, 1991; Harrus *et al.*, 2004), and; the primers used in this study have been previously demonstrated/proven to detect trypanosomes in both blood and tissue samples (Botero *et al.*, 2013; McInnes *et al.*, 2011). The nested *Trypanosoma* PCR primers used in this study provide a highly sensitive methodology for detecting low DNA concentrations in tissues (McClatchey, 2002). Very low levels of DNA are highly likely to amplify when using nested PCR primers, compared to conventional single step PCR, as two primer pairs are required to amplify a target sequence and there is a decrease in non-specific banding (McClatchey, 2002; Pardo and Pérez-Villareal, 2004). Although the sensitivity of these primers has not been calculated for tissue, it has been calculated for blood (Dunlop *et al.*, 2014). Given that Dunlop et al. (2014) calculated that use of these primers and amplification conditions in blood with >74

parasites/0.3ml had a detection sensitivity of >80%, it is extremely likely that even low levels of parasitaemia would be detected in spleen samples.

Whilst the possibility of missing the presence of either *Trypanosoma* or *Leishmania* occurring at very low levels of parasitaemia cannot be discounted, we do feel confident that the PCR protocol used would detect even low levels of parasitaemia in our samples. For the scope of this paper though, quantification of parasitaemia (intensity) was not considered as important as the presence/absence of *Trypanosoma* or *Leishmania* species.

Unfortunately serum samples were not available for rats or cats in this study; serological testing may have provided additional information regarding exposure (or otherwise) of the hosts to these parasites and should be considered for future investigations.

Whilst cat and rat spleen samples were sourced from a focal area on CHI, specifically from around the town site, this was representative of the initial sampling area utilised by Durham (1908). Additionally, the relatively small size of this island (135km^2) suggests that *Trypanosoma* and *Leishmania* would have expected to have been identified in our samples if they were circulating within the rat and cat populations. Assuming a prevalence of at least 10%, the probability of missing a positive animal is 0.006 (0.6%) based on the sample size of 48 rats used in this study.

The only samples tested by Wyatt *et al.* (2008) from before black rat introductions to CHI were from the bulldog rat (*R. nativitatis*; n=3), with PCR failing to detect any trypanosomes. Therefore it is not possible to confirm the presence or absence of *T. lewisi* on CHI prior to black rat introduction (Wyatt *et al.*, 2008).

However, if *T. lewisi* was endemic on CHI in the native Maclear's and bulldog rats before black rat introduction, it could be hypothesized that it was similarly lost along with the demise of the native rats.

Based on the findings of this study, the link between black rats as the source of the *Trypanosoma* spp. on CHI is tenuous considering the hypothesised host (black rats), intermediate host [*Xenopsylla cheopis*; Hoare (1972)] and a mechanical vector [*Polyplax spinulosa*; Khachoian and Arakelian (1978)] are still present on the island (Dybing *et al.*, 2016a), whilst our efforts to detect the presence of any *Trypanosoma* spp. was not successful. If *T. lewisi* introduced with black rats has resulted in, or at least contributed to, the extinction of two endemic rodent species, it would not be unreasonable to expect *T. lewisi* to still be present in these hosts today.

Whilst rodent (rat and mice) trypanosomes have been studied, there is still a paucity of information in Australia. Exotic trypanosomes found in native rodents include *Trypanosoma lewisi* from *Rattus fuscipes*, the bush rat (25%); a species genetically similar to *T. lewisi* in *Pseudomys albocinereus*, the ash-grey mouse (50%); and an unknown *Trypanosoma* spp. in *Pseudomys fieldi*, the Shark Bay mouse (16.7%) (Averis *et al.*, 2009). Introduced rodents infected with *T. lewisi* in Australia include the house mouse (*Mus musculus*), brown rat (*Rattus norvegicus*) and the black rat (*Rattus rattus*) (Mackerras, 1959). Several novel *Trypanosoma* spp. have been identified in a range of Australian native wildlife species (Austen *et al.*, 2009; Averis *et al.*, 2009; Paparini *et al.*, 2011; Smith *et al.*, 2008). Indeed, *Trypanosoma* spp. have been implicated in the demise of marsupial populations in swWA (Averis *et al.*, 2009; Smith *et al.*, 2008).

In Australia, there is an absence of research into trypanosomes from cats however, natural and experimental infections of multiple species of *Trypanosoma* have been found in felines from other countries including; *T. brucei*, *T. cruzi*, *T. evansi* and *T. rangeli*. Experimental infections in cats have also been reported for *T. congolense* and *T. gambiense*. *Trypanosoma cruzi* is the most widely reported species occurring in cats with most cases originating from Latin America and prevalences ranging from 2.9%-63.6% (Mott *et al.*, 1978; Wisnivesky-Colli *et al.*, 1985; Zeledón *et al.*, 1975). However, many indigenous Australian mammal species are thought to be susceptible to infection by exotic *Trypanosoma* species if they were to establish in Australia (Thompson, 2013). The absence of trypanosome infection in cats sampled from swWA and DHI suggests that cats are not a major reservoir species in these areas and therefore may not contribute to trypanosome persistence in the environment.

Domestic cats and black rats have been identified as sources of infection of the pathogenic zoonosis caused by *Leishmania infantum* (Maia and Campino, 2011; Poli *et al.*, 2002; Quinnell and Courtenay, 2009). At the time of this study a significant proportion of the CHI population was transitory and originated from diverse geographic locations, including many areas where *Leishmania* is endemic. However, we found no evidence of feral cats or black rats acting as reservoirs for *Leishmania* spp. on CHI. The absence of feline *Leishmania* and *Trypanosoma* species may be explained by the absence of appropriate intermediate hosts (tsetse flies, phlebotomines and triatomid bugs) in these regions.

5.7 Conclusions

This study found no evidence of *Trypanosoma* spp. infection in cats from three geographically distinct locations or black rats from CHI. Of particular interest is the

absence of trypanosomes from black rats on CHI given that *T. lewisi* has previously been reported as occurring in rats and other rodent hosts on the island, and has been hypothesised as contributing to the extinction of two endemic rodent species. Previous studies have implicated *Trypanosoma* spp. in fauna decline; however, in the case of CHI, the findings of this study do not readily support this hypothesis. This study also found no evidence of feral cats or black rats harbouring *Leishmania* spp. on CHI.

Chapter 6 *Leptospira* species in feral cats and black rats from Western Australia and Christmas Island

6.1 Preface

This manuscript has been accepted for publication in the journal, *Vector-borne and Zoonotic Diseases*:

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The role of invasive animals (feral cats and black rats) in the maintenance of *Leptospira* was explored in the three study environments following the confirmation of a human autochthonous case of leptospirosis on Christmas Island. Chapter 6 not only explores the presence of this notifiable disease but also investigates other potential maintenance pathways in the environment.

6.2 Abstract

Leptospirosis is a neglected, re-emerging bacterial disease with both zoonotic and conservation implications. Rats and livestock are considered the usual sources of human infection, but all mammalian species are capable of carrying *Leptospira* spp. and transmitting pathogenic leptospires in their urine, and uncertainty remains about the ecology and transmission dynamics of *Leptospira* in different regions. In light of a recent case of human leptospirosis on tropical Christmas Island, this study aimed to investigate the role of introduced animals (feral cats and black rats) as carriers of pathogenic *Leptospira* spp. on Christmas Island and to compare this with two different climatic regions of Western Australia (one island and one mainland). Kidney samples were collected from black rats (n=68) and feral cats (n=59) from Christmas Island, as well as feral cats from Dirk Hartog Island (n=23) and southwest Western Australia (n=59). Molecular (PCR) screening detected pathogenic leptospires in 42.4% (95% Confidence Interval 29.6, 55.9) of cats and 2.9% (0.4, 10.2) of rats from Christmas Island. Sequencing of cat and rat positive samples from Christmas Island showed 100% similarity for *L. interrogans*. Pathogenic leptospires were not detected in cats from Dirk Hartog Island or southwest Western Australia. These findings were consistent with previous reports of higher *Leptospira* spp. prevalence in tropical regions compared with arid and temperate regions. Despite the abundance of black rats on Christmas Island, feral cats appear to be the more important reservoir species for the persistence of pathogenic *L. interrogans* on the island. This research highlights the importance of disease surveillance and feral animal management to effectively control potential disease transmission.

6.3 Introduction

Leptospirosis is a re-emerging zoonosis of global importance. Recently classified as a neglected tropical disease (Hartskeerl *et al.*, 2011; WHO, 2003), it is one of the most widespread zoonoses (Desvars *et al.*, 2011; Krøjgaard *et al.*, 2009; Pappas *et al.*, 2008; Zavitsanou and Babatsikou, 2008), occurring on every continent with the exception of Antarctica (Adler and de la Peña Moctezuma, 2010). Symptoms of leptospirosis in people range from non-specific influenza-like illness to multi-organ failure, with 1.7 million cases of the disease reported annually worldwide (Chiriboga *et al.*, 2015; Hartskeerl *et al.*, 2011; Krøjgaard *et al.*, 2009), however due to the non-specific symptoms, many cases of leptospirosis go undiagnosed and therefore the true incidence is expected to be higher (Azócar-Aedo *et al.*, 2014; Lau *et al.*, 2010; Meerburg *et al.*, 2009).

Twenty species of *Leptospira* have been described in three clusters (saprophytic, pathogenic, and intermediate pathogenic) with more than 250 known serovars (Hartmann *et al.*, 2013; Ko *et al.*, 2009; Zavitsanou and Babatsikou, 2008). Globally, rodents and domestic mammals including cattle (*Bos taurus*), pigs (*Sus scrofa*) and dogs (*Canis lupus familiaris*) are considered the most important reservoir hosts for this bacterium with respect to zoonotic potential; however, many mammal species are capable of acting as hosts (Faine *et al.*, 1999; WHO, 2003). Reptiles and amphibians are also capable of transmitting leptospires (Calle *et al.*, 2001; Everard *et al.*, 1990; Everard *et al.*, 1988; Everard *et al.*, 1983; Gravekamp *et al.*, 1991), as are migratory birds, carrying contaminated soil on their legs (Faine *et al.*, 1999; Guerra, 2009). Within Australia, *Leptospira* spp. have been detected in domestic animals and native mammal species (Cox *et al.*, 2005; Dickeson and Love, 1993; Milner *et al.*, 1981;

Perolat *et al.*, 1998). Although the role of rats in the transmission of leptospires is well recognised, the role of cats in the transmission of *Leptospira* spp. in different environments is not well described, yet is of interest given the close association between cats and humans (Jamshidi *et al.*, 2009).

Approximately 64% of human leptospirosis cases reported in Australia between 2000–2015 originated in Queensland, typically within the tropics (Australian Government Department of Health, 2015). Increased reports of leptospirosis in tropical regions reflects the favourable conditions which support transmission indirectly via contact with contaminated soil and/or water (Desvars *et al.*, 2011; Hartskeerl *et al.*, 2011; Lau *et al.*, 2010; Ricaldi and Vinetz, 2006). However, *Leptospira* transmission cycles can persist in a wide range of environmental conditions and the diverse range of potential host and reservoir species means that leptospirosis poses both a public and animal health issue.

A confirmed, autochthonous case of leptospirosis in a person on Christmas Island (Dr Julie Graham, pers. comm.) prompted further investigation into the role of invasive animals as carriers of *Leptospira* as part of a larger project concerning these species conducted on Christmas Island (CHI), Dirk Hartog Island (DHI) and southwest Western Australia (swWA). The three locations have distinct climatic characteristics, but all have conservation significance due to recent declines in native fauna. Only one of five originally endemic mammal species remain on CHI, where there are no livestock present (other than feral chickens) and two invasive mammal species (cats and black rats). Three of 13 endemic mammal species remain on DHI along with the introduced house mouse (*Mus musculus*). At the time of this study, there were only a small number of sheep and goats remaining on DHI. Reptiles (both endemic and invasive)

and birds (endemic, migratory, and invasive) are present on both CHI and DHI. Southwest Western Australia includes agricultural, urban and forested areas with a wide diversity of endemic and introduced reptiles, amphibians, birds and mammals (including cats and black rats). The aim of this study was to explore the role of two invasive animals (cats and black rats) as carriers of pathogenic *Leptospira* spp. in three locations with a close wildlife and/or human interface.

6.4 Methods

6.4.1 Study locations

Samples were collected from three geographically and climatically distinct locations; CHI, DHI, and mainland swWA (Figure 6.1). Christmas Island is an Australian Territory located in the Indian Ocean (10° 29’ S, 105° 38’ E) approximately 360 km south of the Indonesian capital Jakarta and experiences a tropical climate. Dirk Hartog Island is a large arid inshore island (25°50’ S, 113°05’ E) located to the west of Shark Bay off the Western Australian (WA) coast. The swWA is a large ecoregion, located south of a line from Geraldton (28^{o} 46’ 28” S, 114^{o} 36’ 32” E) to Esperance (33^{o} 51 ’40” S 121^{o} 33’ 31” E) with a predominately Mediterranean climate (hot dry summers, cool wet winters).

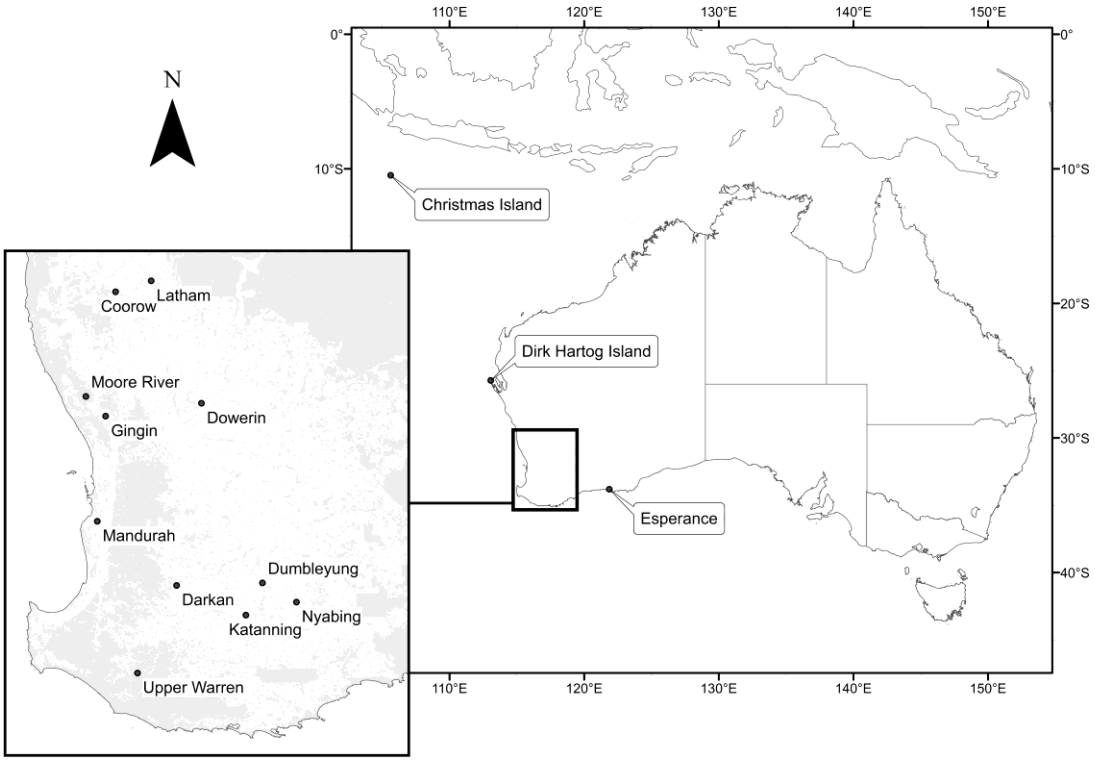

Figure 6.1: *Leptospira* sampling locations.

6.4.2 Sample collection

Cat cadavers were collected from CHI (n=59) in 2011, DHI (n=22) from 2012-13 and swWA (n=59) from 2012-13; black rats (n=68) were also collected concurrently from CHI. Cats from CHI and DHI were sourced from the Department of Parks and Wildlife management programs and cats from swWA were shot during community-coordinated culling programs from 12 locations (Red Card for Rabbits and Foxes). Trapping protocols from CHI and DHI have been described previously by Algar *et al.* (2014) and Deller *et al.* (2015), respectively.

All cadavers were placed individually in sealed plastic body bags after death and stored at -20^{o} C until necropsy was conducted, although some samples from swWA had slight delays in bagging and freezing (up to 7 hours). Animals were frozen up to 2

weeks before necropsy. Head-body (HB) lengths were measured and weights recorded for each animal to calculate a body condition index (BCI=weight/HB length) (Rodríguez and Carbonell, 1998; Vervaeke *et al.*, 2005). Kidney tissue was collected at necropsy and preserved in 70% ethanol.

6.4.3 DNA extraction and PCR conditions

DNA was extracted from kidney tissue of cats and rats using the Qiagen spin columns for blood and tissue kit according to the manufacturer's instructions (Qiagen, USA). A nested PCR *Leptospira* protocol to identify and differentiate between the presence of pathogenic (615bp product), and saprophytic/intermediate (316bp product) *Leptospira* spp. from the 23S rDNA region was conducted according to Kositanont et al. (2007). External primers used were LepF1 (5'-GTTACCAAGCACAAGATTAG-3') and LepR1 (5'-TAGTCCCGATTACATTTTC-3'). Internal forward primers used were PU1 (5'-TATCAGAGCCTTTTAATGG-3') and SU1 (5'-TTTAGGGTTAGCGTGGTA-3') and the reverse primer was again LepR1. A 50 µl reaction mixture was employed to amplify 5 µl of template DNA with 5 µl of 10xPCR buffer, 8 µl of 25 mmol/L $MgCl_2$, 1 µl of 20pmol of ea primer, 200 µmol/L of ea dNTP and 1U of Taq polymerase. Thermocycling conditions include an initial denaturation step of 96^{o}C for 5 minutes then 35 cycles consisting of denaturation at 94^{o}C for 1 minute, annealing at 50^{o}C for 50 seconds and extension at 72^{o}C for 1 minute (the extension time increased by 3 seconds for each cycle). The final elongation step was 72^{o}C for 7 minutes.

Leptospira interrogans serovar Pomona (WHO Collaborating Centre for Reference and Research on Leptospirosis, Queensland) was used as a positive control

and PCR-grade water was used as a negative control. Amplified DNA fragments were visualised on a 1.5% agarose gel by electrophoresis.

6.4.4 DNA purification and sequencing

Sequencing of PCR positive samples was conducted to confirm the presence of pathogenic species. PCR products were purified using the tip elution method described in Yang *et al.* (2013). The purified DNA was then sequenced using an ABI prism Terminator Cycle Sequencing kit (Applied Biosystems, Foster City, California, USA) according to manufacturer's instructions on an Applied Biosystems 3730 DNA Analyser. Sequencing results were compared against available sequences in GenBank using BLAST search (http://blast.ncbi.nah.gov/Blast.cgi).

6.4.5 Statistical analysis

Prevalence confidence intervals were calculated using the exact binomial method (Graat *et al.*, 1997). Cats were categorised for age based on weight (kitten <1.0kg; juvenile 1-2.4kg; adult >2.5kg) as previously described by (Algar *et al.*, 2014). All cats in this study were either juvenile or adult. Statistical analyses were performed using the software SPSS Statistics version 21 (IBM). Associations between the presence of *Leptospira* spp. in cats and sex (male or female; one degree of freedom) was analysed with Pearson Chi square two-sided test as all cells had an expected count greater than five. Association between the presence of *Leptospira* spp. in cats and age category (juvenile or adult; one degree of freedom) was analysed using Fisher's two-sided exact test as two cells had an expected count less than five. Association between body condition index and the presence of *Leptospira* spp. in cats was analysed using a univariate general linear model, with body condition index as the dependent variable and sex, age, and presence of *Leptospira* spp. included as fixed

factors. Statistical analyses were not performed on rodent *Leptospira* due to the low prevalence.

6.5 Results

Kidney tissue samples were PCR positive for *Leptospira* spp. for feral cats (42.4%; 95% CI 29.6-55.9) and black rats (2.9%; 0.4-10.2) on CHI, but not for cats in swWA or DHI (Table 6.1). No saprophytic/intermediate *Leptospira* spp. were detected.

Table 6.1: *Leptospira* spp. detected in cats from three geographical regions and rats from CHI.

	swWA	DHI	CHI	
	F. catus	*F. catus*	*F. catus*	*R. rattus*
Leptospira spp.				
Samples (n)	59	22	59	68
Positive samples (n)	0	0	25	2
Prevalence	0%	0%	42.4%	2.9%
95% confidence interval	0.0, 6.1	0.0, 15.4	29.6, 55.9	0.4, 10.2

Sequencing of *Leptospira* –positive samples from cats (n=24) and rats (n=2) showed 100% similarity for *L. interrogans.* All samples equally aligned with seven pathogenic serovars; Hardjo (CP013147 and CP012603), Manilae (CP011934 and CP011931), Bratislava (CP011410), Linhai (CP006723), Copenhageni (NR_076199 and AE016826), Canicola (X14249) and Lei (NR_076199, CP001221 and AE010300). Despite aligning with these seven serovars, sequencing was not able to discriminate the actual infective serovar or whether animals were harbouring one or a combination of serovars. Sequences identified have been deposited in GenBank under the following accession numbers; KU991650-KU991655 and KY230168-KY230186.

No significant associations between host sex (Pearson chi-square 2-sided test, p=0.346) or age category (Fisher's exact test, p=1.000) with presence of pathogenic *Leptospira* spp. were identified in feral cats from CHI. There was no association between presence of pathogenic *Leptospira* spp. and BCI (univariate general linear model *Leptospira* main effect p=0.843).

6.6 Discussion

To the best of the authors' knowledge, this is the first report of *Leptospira* spp. in introduced animals on CHI. The identification of *L. interrogans* in feral cats and black rats on CHI (a tropical environment) is further supported by a recently reported autochthonous case in a human working on the island (Dr Julie Graham, pers comm.). In contrast, *Leptospira* spp. were not identified in cats from DHI (arid environment) or swWA (a temperate/Mediterranean environment). This was consistent with the notion that environmental factors influence *Leptospira* spp. prevalence, and therefore the public and veterinary health risks associated with pathogenic *Leptospira* spp. transmission from feral animals. Although swWA and DHI have a greater number of potential mammalian reservoir host species present, it is likely that the prevailing climatic conditions in arid (DHI) and temperate (swWA) environments are less conducive for the survival and transmission of *Leptospira* spp. compared with tropical CHI. Leptospires are excreted via infected urine; a higher temperature and humidity in tropical environments is favourable for the longer persistence of leptospires in the environment (Bharti *et al.*, 2003; Hartskeerl and Terpstra, 1996; Hartskeerl *et al.*, 2011). This is reflected in the higher case notification rate for (human) leptospirosis for (tropical) north Queensland, compared to the remainder of Australia (Pappas *et al.*, 2008).

This study utilised PCR to detect the presence of *Leptospira* spp. DNA within host kidney tissue. Serological tests have been traditionally used to investigate the level of exposure within a population, however diagnostic sensitivity can be low as some leptospire serovars are shed in urine by animals with low to no detectable serological titre levels (Shophet, 1979). Utilisation of molecular screening techniques can increase sensitivity (Schreier *et al.*, 2013) and molecular (PCR) analyses using kidney tissue is considered an effective screening tool because leptospires can clear from all organs aside from renal tubules in susceptible hosts (Athanazio *et al.*, 2008). Furthermore, there is evidence that leptospires do not colonise the kidneys of non-carrier species (Cox *et al.*, 2005; Hartskeerl and Terpstra, 1996). Therefore, if cats were an incidental host or non-carrier species, minimal *Leptospira* spp. detection would be expected in kidney samples (Faine *et al.*, 1999; Hartskeerl and Terpstra, 1996).

The source of infection in the 2013 human case of leptospirosis on CHI was not confirmed. The patient was a fly-in-fly-out employee on the island (e.g. lived and worked on CHI for a period of time and then returned to his hometown for a number of days of rest). The infection was presumed to have been locally acquired as the patient had been on CHI for 28 days prior to the onset of symptoms. Indirect transmission via contaminated water (waterfalls used for recreation) was considered most likely based on the patient's history (Dr Julie Graham, pers. comm.). An increased risk for human leptospirosis has been associated with higher rates of contact with reservoir hosts in rural areas (Ghneim *et al.*, 2007; Hartmann *et al.*, 2013). Lau *et al.* (2010) reported a higher risk of *Leptospira* transmission on islands where multiple risk factors coexist in a smaller (restricted) area (e.g. favourable climatic conditions, poor sanitation, stagnant water and/or abundance of reservoir hosts). Human-human

transmission of *Leptospira* spp. has been demonstrated, but is considered rare (Adler and de la Peña Moctezuma, 2010).

Rats have been generally considered the most common reservoir host for *Leptospira* spp. However, this study unexpectedly revealed a higher prevalence in cats than rats on CHI. Cats were introduced to CHI at settlement in 1888 (Tidemann, 1994) and feral populations established soon thereafter. Black rats were thought to have been introduced accidentally from the SS Hindustan in the late 19th century (Andrews, 1900). Feline leptospirosis is likely under-diagnosed or under-reported as clinical presentations are highly variable, if present at all (Azócar-Aedo *et al.*, 2014). Cats in this study were feral and had not received any veterinary care. Cats are presumed to become infected by ingesting infected reservoir hosts, and predator-prey transmission between cats and rats is thought to be an important transmission route (Hartmann *et al.*, 2013). However, the *Leptospira* prevalence in black rats on CHI was low (2.9%), and only one other alternative mammalian prey species (flying fox; *Pteropus melanotis natalis*) is present on CHI. It is not known whether the flying fox population on CHI is infected or susceptible to *Leptospira* infection (Cox *et al.*, 2005). Pathogenic *Leptospira* spp. have been reported in house mice (*Mus musculus*) (Vanasco *et al.*, 2003), but there are conflicting reports about the presence of these species on CHI. The most recent CHI Biodiversity Conservation plan (2014) accounts for house mice on the island, however, numbers and distribution are currently unknown and no confirmed observations have been made recently (David Algar and Dion Maple, pers. comm.). Furthermore, a concurrent dietary study of feral cats conducted on CHI did not identify either house mice or flying foxes in their gastrointestinal tracts, while rats represented 27% of their diet (Hayes, 2011). It is possible that rats infected with *Leptospira* are

more susceptible to predation by cats thus leaving a majority of uninfected rats in the population. This suggests that the cat-rat transmission route cannot be excluded on CHI, however, an alternative transmission pathway may also exist on the island. Both horizontal and vertical transmission routes have been reported, and *L. interrogans* infection in cats on CHI may be attributed to inhalation, invasion through skin abrasions and mucous membranes, or via the genital tract (Hartskeerl and Terpstra, 1996; Hartskeerl *et al.*, 2011; WHO, 2003). Transmission via aquatic environments is unlikely due to cats' natural aversion to water and contaminated soils represent a more likely transmission route. With a higher population density and higher rates of contact between hosts on islands, there would be an inevitable higher rate of exposure and direct transmission of leptospires between feral cats.

Apart from climatic conditions favouring survival of leptospires in tropical environments, the higher prevalence observed on CHI compared with DHI and swWA could be related to abundance of suitable mammalian reservoir host species. This lack of diversity may contribute to the loss of the 'dilution effect', (i.e. a reduced number of competent reservoir hosts that can 'absorb' pathogens within the environment resulting in increased prevalence and disease risk) (Allan *et al.*, 2003; Mills, 2006; Wynwood *et al.*, 2014). Increased species diversity and richness may regulate the abundance of competent reservoir hosts, which in turn reduces the probability of the pathogen encountering a susceptible host (Keesing *et al.*, 2006).

It is widely accepted that *Leptospira* spp. are transmitted exclusively by mammals; however, *Leptospira* spirochaetes have previously been identified in the kidneys of birds (Everard *et al.*, 1985; Jobbins and Alexander, 2015). This begs the questions as to the role of birds in the transmission (indirect and/or direct) of

Leptospira. Direct transmission from birds to cats could be possible on CHI given that birds accounted for approximately 30% of feral cat diet (predominantly feral chickens) in one study (Hayes, 2011). Indirect transmission by birds is also possible through the shedding of leptospires and subsequent contamination of soil and water in the environment. However, the role of birds in the transmission of leptospirosis is largely unknown and further research is required to investigate this.

Apart from public health risks, the presence of pathogenic *Leptospira* spp. in both cats and rats on CHI (albeit small risk from rats) suggests a potential disease risk to the remaining flying fox population. It is currently unknown if the flying fox population on CHI harbours *Leptospira* spp., or, if they do, whether transmission occurs between the flying foxes and feral cats. Previous research suggests that some serovars may not impact flying fox populations negatively (Cox *et al.*, 2005), but flying foxes can be infected as accidental or incidental hosts that may be associated with clinical disease and can be fatal (Faine *et al.*, 1999; Hartmann *et al.*, 2013; WHO, 2003).

In conclusion, this study identified pathogenic *Leptospira* (*L. interrogans)* in cats and rats on tropical CHI, but not cats from the arid DHI or Mediterranean/temperate swWA. Although cats have not previously been considered important carriers or reservoir hosts of *Leptospira* spp. and leptospirosis is not usually considered in the differential diagnosis of feline disease (Arbour *et al.*, 2012; Dickeson and Love, 1993; Lilenbaum *et al.*, 2004), this study suggests feral cats play a role in disseminating leptospires within the tropical environment on CHI. Ongoing monitoring of *Leptospira* spp. in feral cats (as well as native and invasive fauna) will further improve our understanding of the public and veterinary health risks.

Chapter 7 General Discussion

7.1 Background

Invasive animals can have serious consequences on the environment into which they are introduced due to predation, competition and disease introduction. This thesis explores the role of two invasive mammal species; *Felis catus* and *Rattus rattus*, in the introduction of parasites onto two islands (Christmas Island and Dirk Hartog Island) and within a mainland region (southwest Western Australia). The results presented here challenge a previously articulated phenomenon referred to as 'the Island Syndrome'. The Island Syndrome stipulates that compared to nearby mainland, an island will, with respect to parasites, have lower local richness but will have increased infra-community richness, parasite prevalence and parasite intensity (refer to Table 2.1 for definitions). The two invasive mammalian species studied here are of public health importance given their close association with both urban and rural environments, and their ability to transmit parasites of zoonotic and conservation significance. All three study locations (CHI, DHI and swWA) have experienced current and historically significant native faunal declines and are therefore of conservation significance. An understanding of the potential disease risks associated with invasive species is considered to be critical in developing and ensuring successful management plans for islands.

This is the first study of its type to focus on parasites of invasive feral mammals on Australian offshore islands. Altering the distribution and density of species, whether by natural or anthropogenic means, would subsequently lead to changes in

disease distribution, and with this comes changed potential risks of disease transmission to humans as well as to domestic and native animals in previously unaffected regions.

This research was facilitated by governmental management and eradication programs at each study location. All three study sites reported in this thesis are of high conservation and tourism significance, with proactive native species recovery programs and invasive species management programs. A total of 197 feral cats and 101 rats were examined using a full suite of parasitological techniques including; gross observation at necropsy, microscopic evaluation of tissues, and the selective application of molecular tools. This broad combination of diagnostic techniques allowed a comprehensive assessment of the endo- and ectoparasite communities in these two invasive mammals.

The helminth parasite community structure was considered in light of the Island Syndrome (Chapters 2 and 3). Chapter 4 and 6 identified pathogens that may have zoonotic implications (*Bartonella* and *Leptospira* species). *Trypanosoma* and *Leishmania* species were not identified in any of the study regions (Chapter 5).

7.2 Does the helminth parasite community of feral cats and black rats exhibit characteristics of 'the Island Syndrome'?

Chapters 2 and 3 explored the helminth communities of feral cats in three geographically distinct locations (CHI, DHI and swWA), and additionally, of black rats on CHI, to observe if their parasite communities adhered to the theoretical expectations of the Island Syndrome. It was found that three of the four factors were

consistent with the Island Syndrome (high prevalence, infra-community richness and intensity), however a high parasite local richness was contrary to the predictions of the Island Syndrome. Black rats and feral cats were found to be contributing a high parasite richness to both island and mainland environments. This suggested that a low local richness does not always occur in parasite populations on islands and this parameter should not be incorporated into the theoretical framework of the Island Syndrome when predicting characteristics of helminth parasite communities, particularly when examining invasive fauna.

This thesis has identified features including parasite biology and host factors (such as the population origin and host richness), as well as physiographic factors (such as climate, and environmental features) that may account for this parasite community structure. These are outlined in more detail in section 1.3. These features are anticipated to aid in the establishment and persistence of parasites and therefore will help to influence the helminth faunal assemblage in a new environment.

7.3 Pathogens of potential risk to conservation and public health

Chapters 4, 5 and 6 investigated the presence of pathogens in cats from the three study regions and rats from CHI. Investigation of these specific pathogens was warranted due either to pre-existing concerns over the implications of these organisms for conservation in the region, or to reports of autochthonous related human clinical disease, prompting the evaluation of the role feral cats and black rats may play in transmission. The presence of *Trypanosoma* and *Leishmania* spp. was explored in Chapter 5 however, these parasites were not identified at any of our study regions (Dybing *et al.*, 2016b). The absence of these parasites was interesting as *T. lewisi* is

hypothesised to have played in the decline of two native rodent species on CHI. Given the current presence of both the definitive host (*Rattus rattus*) and intermediate host (*Ctenocephalides felis*) on CHI, it was previously assumed that trypanosomes would still be present on the island, suggesting that *T. lewisi* was endemic at the time of decline of the native rodents.

Chapter 4 described the identification of *B. phoceensis* and an unknown *Bartonella* sp. in black rats on CHI, and two zoonotic species (*B. henselae* and *B. koehlerae*) in feral cats from swWA (Dybing *et al.*, 2016a). This is the first report of *B. koehlerae* in Australia. Rats on CHI and cats from swWA harboured ectoparasites known to transmit the *Bartonella* identified in each region, however no *Bartonella* species were identified in cats on DHI or CHI. The absence of the same haemotropic pathogens on DHI was not surprising as ectoparasites were not recovered from any of the feral cats sampled.

Chapter 6 investigated the presence of *Leptospira* spp. and identified *L. interrogans* in both feral cats and black rats on CHI, but not from the other locations (Dybing *et al.*, 2017b). Unexpectedly and contrary to previous reports in the literature, a higher proportion of cats were infected with *Leptospira* compared to black rats, which is the usual host of *L. interrogans*. Given the lower prevalence of these spirochaetes in black rats on CHI, an alternate transmission route may explain the high prevalence in the cats, for example birds may be acting as maintenance or incidental hosts.

Collectively, the research presented in this thesis suggests that cats and rats are capable of transmitting, and are potentially important sources of, pathogens of conservation and public health importance, even those not normally associated with

these species (e.g. *Leptospira* spp. in cats, *Bartonella* spp. in rats). These results also describe the distribution of these pathogens in the study environments, and show that not all pathogen species are present in all regions. More research needs to be conducted into ectoparasite and maintenance host distributions in order to understand disease dynamics and ecology, particularly in island environments. Additionally, this research confirms the importance of environmental factors on the occurrence of parasites and pathogens. These factors include the presence of suitable ectoparasites or reservoir hosts and the location of origin of the introduced species (refer to section 1.3 for more information). However, given suitable conditions, these invasive species are capable of transmitting pathogens of importance in an increased number of locations.

7.4 Factors that may influence the parasite and pathogen community

There are multiple factors that must play a role in determining the presence (or absence) of the parasites and pathogens identified in this research, as well as to sustain high parasite prevalence, intensity, and the local- and infra-community richness in the environment. Although some aspects have been considered in previous chapters, specific factors are discussed further below. All of these features need to be considered when invasive species management programs are proposed to reduce the potential transmission risks to endemic/native species.

7.4.1 Fauna and parasite biology

The number and composition of parasite species found in a region is, in part, determined by the geographical origin of the introduced host species. Spencer *et al.* (2015) reported that the feral cats on CHI originally arrived from both Europe and Asia.

In contrast, the cats from swWA originated predominantly from Europe (Spencer *et al.*, 2015), since this is where most settlers to the region came from in the 18th and 19th centuries, most likely through multiple introduction events. The DHI cat population was introduced from mainland WA during two introduction events with multiple migrations within each (Koch *et al.*, 2014). It is hypothesised that each introduction event from different geographical regions is associated with the introduction of a distinct composition of parasites, and therefore host species introduced from multiple origins will likely contribute a greater richness, or more diverse assemblage, to a new region than if sourced only from a single origin.

Not only did the CHI helminth community have a high species richness (like DHI and swWA), but the helminth and haemotropic pathogen species assemblages differed from the other two regions in this study. Different species occurred on CHI compared to DHI and swWA. Interestingly, this supports the notion of multiple instances of introductions of cats and rats to the island from a diversity of geographical origins, on cargo ships etc., since the beginnings of merchant trade several Centuries ago (Andrews, 1900), and with ongoing multiple, accidental introductions expected to have occurred since then.

The feral cat parasite helminth assemblage on DHI was very similar to that found in swWA. Cats on DHI were predominantly introduced from swWA, therefore it would be expected that the helminth assemblage would be similar in both locations. The haemotropic pathogens however differed between these two locations, with none being found on DHI, yet two *Bartonella* species (*B. henselae* and *B. koehlerae*) were found in swWA. Any deviation in the occurrence or assemblage would also be due to

additional factors relating to parasite biology, free-living hosts/vectors and physiographic characters (Mas-Coma and Feliu, 1984; Poulin and Mouillot, 2003).

Chapters 2 and 3 identified a high proportion of parasites with indirect life cycles on the two islands (CHI and DHI). It is hypothesised that this is due to parasites with an indirect life cycle being less likely to find the required host in the new environment, thus host richness is typically lower on islands (referred to earlier as a component of the Island Syndrome). The subsequent persistence of some parasite species could be explained, in part, by the simultaneous introduction and establishment of a prey species along with a predator (in this case rats and cats respectively). This predator-prey relationship was likely to facilitate the cycling and persistence of parasites that utilise both species as hosts e.g. the high level of *T. taeniaeformis* detected in both cats and rats on CHI. Many of the parasites found in this study utilise various rodents as alternative hosts (refer to chapters 2 and 3), and whilst black rat parasites were not assessed from either DHI or swWA in this study, introduced rodents are present in both regions (albeit no black rats on DHI). This therefore suggests that introduced rodents were likely aiding parasite transmission for those parasites that utilise rodents as intermediate or paratenic hosts.

High parasite richness was observed on both DHI and CHI compared to nearby mainland environments (swWA), even though the two islands are depauperate of mammalian species due to local or global extinctions of native and endemic species. Within a region, parasite richness is generally associated with mammalian richness (Bordes and Morand, 2008; Krasnov *et al.*, 2004). Whilst rats/rodents are commonly implicated in feline parasite life cycles (Tables 2.4 and 3.5), many of the parasites identified in this study utilise hosts from other classes, such as invertebrates, birds and

reptiles. As the ingestion of alternative hosts is the preferred transmission strategy of the parasites detected in this study, it would be assumed that the parasite community in all regions should relate to the diet of feral cats which in turn relates to the availability and presence of particular hosts. This research found that the diet of the feral cats in the three study regions was reflective of the parasites and pathogens detected. For example, a higher proportion of cats on DHI (78%) had consumed birds in their diet than those on swWA (21%), corresponding with a higher proportion of cats infected with *Oncicola pomatostomi*, a parasite in birds (91% and 25% respectively) (refer to Chapter 3). Additionally, in all three locations, invertebrates accounted for a higher proportion of the diet (57% DHI, 72% swWA and 64% CHI) with many of the parasites identified utilising invertebrates as either intermediate or paratenic hosts (7 species DHI and swWA, and 9 species CHI). In conclusion, the presence of free-living hosts in the regions was an important determinant of the parasites that were able to persist.

7.4.2 Physiographic features

The three study sites investigated represent climatically distinct regions; tropical CHI, semi-arid DHI and Mediterranean swWA. Insular physiographic characteristics (i.e. climate) of the island will influence the persistence of parasite species both indirectly and directly. Physiographic conditions can directly affect the parasite (i.e. by their effect on the survival of external life cycle stages), or indirectly by determining the diversity of free-living fauna. Environmental life cycle stages of a parasite e.g. free-living larvae of *Ancylostoma* spp. may be vulnerable to extreme conditions, therefore unique variables are required for its survival (Stromberg, 1997). Although all three regions have different climate types, the cat helminth richness was

identical for both islands (9 species) and was similar to mainland swWA (10 species). This suggests that climate, alone, may not be a good determinant of parasite richness however, the community structure and parasite assemblage will differ between climate types as different parasites strive in different conditions.

The observations arising from this research also suggests that the presence and prevalence of some parasite species relates to the micro environment. Features like the vegetation cover and the presence of water bodies enhance the chances of survival of particular parasite species (Stromberg, 1997). A denser vegetation aids the survival of parasites with a free-living larval stage by protecting against the extremes of UV and moisture e.g. *Ancylostoma* spp. (Dybing *et al.*, 2013; Stromberg, 1997). Some parasites require the presence of fresh water which may facilitate survival of a particular stage of the life cycle (e.g. *Spirometra erinaceieuropaei* requires a freshwater copepod as its first intermediate host) (Bowman *et al.*, 2002). Christmas Island is predominantly covered by rainforest, with dense vegetation cover and year-round high humidity and rainfall. In contrast, vegetation on DHI is dense shrub land and rocky outcrops in a drier environment. Samples collected from swWA were predominantly from the wheatbelt region where vegetation cover is variable due to clearing for farming and agricultural activities (aside from remnant bushland/corridors). However, there were some areas of forested national parks. This indicates that variable vegetation cover may contribute to the varying parasite assemblages in these locations.

7.5 Impacts of introduced pathogens and the subsequent implications in invasive species management

The parasites and bacterial pathogens identified in this study of feral cats and black rats have the potential to be transmitted to domestic and native animals, as well as humans. To optimise management and conservation program outcomes and public health initiatives, a deeper understanding of the distribution and ecology of introduced pathogens is needed, as well as the role feral cats and black rats play in their transmission and the potential risk they pose to endemic species and human health.

7.5.1 Conservation implications

Surveillance for pathogens in invasive and endemic species has come increasingly into focus in recent years. This is not simply because of the high rate of faunal extinctions globally, but more specifically because of the hypothesised roles played by the introduction of parasites on the decline and extinction of native fauna. For example, the introduction of trypanosomes onto CHI by black rats is thought to have decimated the native rodents, and in Hawaii the spread of avian malaria (Atkinson *et al.*, 1995; Dybing *et al.*, 2016b; van Riper *et al.*, 1986; Wyatt *et al.*, 2008). These examples have highlighted the importance of parasites in conservation. The emergence of global human epidemics has also facilitated the need to pinpoint the course of infection and methods in which to reduce the frequency and occurrence of future epidemics.

Some of the impacts of introduced pathogens in naïve populations include decreased fecundity and survivorship of the new hosts, and some parasites alter the behaviour of its host making them more susceptible to predation. However, the

specific impacts of the identified pathogens on endemic species in these regions are unknown. Some of the parasites identified are generalists, or have the potential to act as generalists in new environments, and can utilise many different paratenic or intermediate hosts, refer to Tables 2.4 and 3.5 (Agosta *et al.*, 2010). In these new environments, the parasites need to find hosts capable of harbouring or transmission which is where issues can arise as endemic species have no prior defence against foreign pathogens (D'Antonio and Dudley, 1995; Daszak *et al.*, 2000; Loope, 1986; Mueller-Dombois *et al.*, 1981).

The most notable parasite with conservation significance recovered on CHI was *Angiostrongylus cantonensis*, found in black rats, which is capable of infecting any mammal species (and has also recently been identified in birds (Alicata, 1966; Barrett *et al.*, 2002; Monks *et al.*, 2005; Pien and Pien, 1999). Given the high pathogenicity and ability to infect a large diversity of fauna, further research is needed to find out the potential impact this parasite may be imposing on the CHI ecosystem, and more specifically in its role in the declining CHI flying fox population (*Pteropus natalis*). Both the definitive host (black rats) and intermediate host (snails e.g. the African giant snail; *Achatina fulica*) are invasive species on the island and therefore control of both hosts is required to control the parasite and its subsequent impacts. Many of the other helminths identified in this study have previously been reported in the native fauna of Australia e.g. *Spirometra erinaceieuropaei* (Berger *et al.*, 2009; Dickman, 1996a; Oakwood and Spratt, 2000; Whittington *et al.*, 1992). The concern is that these pathogens may be able to impact vulnerable host populations.

Invasive species control strategies and conservation programs are expensive and may be inefficient if there is little information on what parasites are being

introduced with the invasive species hosts. Therefore, understanding and continually surveying the parasite communities in invasive species is crucial. To mitigate the disease implications imposed by invasive animals, the life cycle of their associated parasites needs to be halted. This is done by removing the introduced, required host from the environment, in this case feral cats or black rats.

7.5.2 Zoonotic implications

Lastly, this research highlighted several parasites that are capable of zoonotic transmission. Zoonotic pathogens identified in this study included A*. cantonensis, Leptospira interrogans*, *Bartonella henselae* and *B. koehlerae*. Two of the haemotropic pathogens investigated in this study have been previously associated with human disease in these those locations (*Bartonella* spp. and *Leptospira* spp.). Both feral cats and black rats can roam freely around urban dwellings thereby increasing the risk of pathogen transmission to humans. This study has identified *B. koehlerae* for the first time in the southern hemisphere, a finding that indicates the need to continually survey feral cats for the presence of zoonotic pathogens in order to discern the distribution within Australia.

7.6 Final conclusions, recommendations and future directions

In addition to identifying parasites with conservation and zoonotic significance, this study has further expanded some of the pathogens recognised geographical distributions. New geographical distributions can be applied for the helminth parasite fauna from DHI and CHI primarily due to the dearth of previous parasitology research on invasive species in these regions. Given the growing threat of climate change, the

potential distribution of both pathogens and their ecto-parasite vectors will alter and continual disease surveillance is crucial.

The findings of this research contribute to our understanding of the impacts of feral animals on islands and will inform decisions when reintroduction programs are planned. Future research could assess the parasite community of the endemic fauna species and the effects these introduced parasites may be posing on this population of animals and on public health.

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