Ecology of Cat-borne Parasitoses in Australia

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List of included publications:

Taggart, P. L., Stevenson, M. A., Firestone, S., McAllister, M. A., and Caraguel, C. G. B. (2019. [Chap.2]). Spatial analysis of a cat-borne disease reveals that soil pH and clay content are risk factors for sarcocystosis in sheep. *Frontiers in Veterinary Science*. DOI: 10.3389/fvets.2019.00127

Taggart, P. L., McAllister, M. M., Rutley, D., and Caraguel, C. G. B. (In Review. [Chap. 3]). Oesophageal sarcocystosis observed at slaughter provides a reliable and efficient proximate measure of *Toxoplasma gondii* seroprevalence in sheep. *Small Ruminant Research*.

Taggart, P. L., Fancourt, B. A., Peacock, D. E., Caraguel, C. G. B., and McAllister, M. M. (In Press. [Chap. 4]). Variation in *Toxoplasma gondii* seroprevalence: effects of site, sex, species and behaviour between insular and mainland macropods. *Wildlife Research*.

Taggart, P. L., Fancourt, B. A., Fabijan, J., Peacock, D. E., Speight, K. N., Caraguel, C. G. B., and McAllister, M. M. (In Press. [Chap. 6]). No evidence of *Toxoplasma gondii* exposure in South Australian Koalas (*Phascolartos cinereus*). *Journal of Parasitology*.

Taggart, P. L., Fancourt, B. A., Bengsen, A. J., Peacock, D. E., Hodgens, P., Read, J. L., McAllister, M. M., and Caraguel, C. G. B. (In Press. [Chap. 7]). Evidence of significantly higher island feral cat abundance compared to the adjacent mainland. *Wildlife Research*.

Taggart, P. L., Peacock, D. E., and Fancourt, B. A. (In Review. [Appendix 1]). Camera trap flash type does not influence feral cat behaviour. *Australian Mammalogy*.

Abstract:

Cat-borne parasitoses have substantial impacts on livestock, wildlife and human health worldwide. *Toxoplasma gondii*, *Sarcocystis gigantea* and *S. medusiformis* are all cat-borne parasites that share similar biology and ecology, and cause the diseases toxoplasmosis and macroscopic ovine sarcocystosis. I aimed to study the ecology of these cat-borne parasitoses to develop a better understanding of what ecological factors influenced their ability to cycle within an ecosystem. However, it was first necessary to find a study ecosystem where these parasitoses thrived.

Using abattoir surveillance data I mapped the occurrence of macroscopic ovine sarcocystosis in the skeletal muscles of sheep across South Australia. Sarcocystosis was highly clustered on Kangaroo Island compared to the South Australian mainland. Second, I investigated if *Toxoplasma* infection in sheep was associated with macroscopic ovine sarcocystosis to see if I could provide indirect evidence for the clustering of *Toxoplasma* infection in sheep on Kangaroo Island. *Toxoplasma* infection was highly prevalent in sheep on the island (56.8%) and was associated with macroscopic ovine sarcocystosis in the oesophagus, but not in skeletal muscles, at the animal- and farm-level. By surveying macropods on Kangaroo Island and the adjacent mainland, I showed that *Toxoplasma* infection was also higher in western grey kangaroos on the island (20.4%) than on the mainland (0%). This suggested that these parasitoses are well established and thrive on Kangaroo Island and that the island is an appropriate ecosystem in which to study the ecology of these cat-borne parasitoses.

Pushing my mapping analyses further, I identified environmental characteristics positively associated with higher densities of sarcocystosis affected locations. The occurrence of sarcocystosis increased at locations with low soil pH and high clay content. I then examined the seroprevalence of *Toxoplasma* in rodents (*Mus musculus* and *Rattus fuscipes*), brushtail possums (*Trichosurus vulpecula*) and koalas (*Phascolarctos cinereus*) to explore the impact of the ecology of these species on their risk of infection. *Toxoplasma* seroprevalence in all species was found to be negligible, suggesting that the intermediate host's lifespan, feeding ecology and niche influence the parasite's ability to cycle.

To investigate how much cat (*Felis catus*) abundance may explain the occurrence of these cat-borne parasitoses, I conducted a camera trap survey in both regions and estimated their

relative abundance using a simultaneous standardised approach. Cat abundance on the island was estimated to be over ten times higher than that on the adjacent mainland.

I suggest that high cat abundance is the primary reason for the high occurrence of cat-borne parasitoses in sheep and macropods on the island, although the ecology of the intermediate host likely influences the ability of the parasites to cycle in these populations. I recommend that the control of cats should be the most effective and acceptable intervention to control these two cat-borne parasitoses in ecosystems where they occur frequently.

Declaration:

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name, in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission in my name, for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint-award of this degree.

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Last, but not least, thank you to my amazing wife and family who have supported and encouraged me, provided me advice and assistance with all aspects of my PhD studies throughout the past three years.

Thesis structure:

This thesis is presented as a 'Thesis by Publication' and includes a combination of accepted and submitted manuscripts under review. Each chapter forms a separate scientific manuscript. Accordingly, some repetition between chapters exists in their introduction and or methods sections. For consistency I have standardised chapter formatting throughout this thesis. For details of the journal each manuscript has been submitted to and the current status of the manuscript in the publication process please refer to the 'Statement of Authorship' accompanying each chapter. All references to manuscripts/chapters of this thesis reflect the current status of that manuscript in the publication process (in press or in review).

Chapter 1: Introduction

The domestic cat (*Felis catus*) is a host to over 180 species of parasite (Taylor *et al.* 2016). However, within the ecological literature, three species feature more frequently than others due to their impacts on human, livestock and wildlife health; these parasites are *Toxoplasma gondii*, *Sarcocystis gigantea* and *Sarcocystis medusiformis* (Doherty *et al.* 2017). Infection with *Toxoplasma gondii* can cause the disease toxoplasmosis, and infection with *Sarcocystis gigantea* and *Sarcocystis medusiformis* can cause macroscopic ovine sarcocystosis.

1.1 Significance of toxoplasmosis and sarcocystosis in humans, livestock and wildlife

1.1.1 Toxoplasmosis impact

Infection with *T. gondii* can cause acute toxoplasmosis when the parasite is actively multiplying and disseminating within the host, or chronic toxoplasmosis when the parasite is dormant within the host. However, whilst approximately one third of the human population are infected with *T. gondii* (Saadatnia and Golkar 2012), in the majority of infections, no clinical signs are visible (latent infection). Acute toxoplasmosis in humans is documented to cause abortion, congenital defects, ocular lesions, and even death in immunosuppressed individuals (Saadatnia and Golkar 2012). It has also been reported to cause several other less well-known clinical diseases such as retinochoroiditis, hydrocephaly and seizures (Saadatnia and Golkar 2012). Chronic toxoplasmosis has been associated with altered human behaviour (Flegr 2007), mental illness (Torrey *et al.* 2012), slower reaction time (Havlíček *et al.* 2001) and a tendency for traffic accidents (Kocazeybek *et al.* 2009; Stepanova *et al.* 2017).

Documenting the full health and welfare impacts of human toxoplasmosis is challenging due to most infections being sub-clinical or due to the misdiagnosis of clinical cases (Dubey 2016b). Despite this, human toxoplasmosis is known to have substantial social and economic impacts (Suijkerbuijk *et al.* 2018). For example, health care costs associated with food-borne toxoplasmosis in the United States of America (USA) have been estimated at approximately $3 billion annually (Batz *et al.* 2012; Hoffmann *et al.* 2012). Similarly, annual health care costs of congenital toxoplasmosis in the USA and United Kingdom have been estimated at $0.4-8.8 million and $1.2-12 million respectively (Roberts *et al.* 1994). Chronic toxoplasmosis has additionally been hypothesised to have influenced cultural diversity via causing subtle but highly prevalent changes in human personalities at a global scale (Lafferty 2005).

In livestock, toxoplasmosis predominately impacts on sheep (*Ovis aries*) (Dubey 2016a). Similar to humans, pregnant ewes infected with *T. gondii* for the first time are at increased risk of pregnancy complications such as abortion, still birth, foetal resorption and post-birth lamb mortality (Dubey 2016a). Seroepidemiologic studies of *T. gondii* in sheep show large geographic variation worldwide (Dubey 2009). Accordingly, toxoplasmosis is considered an economically important disease of sheep in countries where sheep farming industries are large, such as New Zealand (Charleston 1994), Uruguay (Freyre *et al.* 1997), or Great Britain (Bennett *et al.* 1999).

Health impacts from toxoplasmosis in wildlife are reported to be more frequent and severe in Australian marsupials and new world monkeys, likely due to these taxa having evolved in the absence of *T. gondii* (Dubey 2016c). For example, infected Bennett's wallabies (*Macropus rufogriseus rufogriseus*) and Tasmanian pademelons (*Thylogale billardierii*) can suffer blindness and incoordination (Obendorf and Munday 1983), while tammar wallabies (*M. eugenii*) and eastern barred bandicoots (*Perameles gunni*) can experience acute mortality when experimentally infected (Bettiol *et al.* 2000; Lynch *et al.* 1993; Reddacliff *et al.* 1993). Acute toxoplasmosis may also cause mortality in common brushtail possums (*Trichosurus vulpecula*) (Eymann *et al.* 2006) and neurological abnormalities in western ringtail possums (*Pseudocheirus occidentalis*) (Parameswaran 2008). Chronic toxoplasmosis may cause eastern barred bandicoots to be more active during the day compared to non-infected animals (Bettiol *et al.* 2000). Gastrointestinal and respiratory problems following *T. gondii* infection have been reported in macropods, koalas (*Phascolarctos cinereus*), wombats (*Vombatus ursinus*), possums, dasyurids, numbats (*Myrmecobius fasciatus*), bandicoots, and bilbies (*Macrolis lagolis*) (Canfield *et al.* 1990). In addition to the impacts of toxoplasmosis on wildlife health and welfare, this disease likely has conservation and economic impacts on wildlife associated activities, such as tourism and conservation efforts.

1.1.2 Sarcocystosis impact

Sarcocystosis is very similar to toxoplasmosis. It is caused by *Sarcocystis spp.* parasites that are thought to infect most vertebrate species (Dubey *et al.* 2015a). Two *Sarcocystis spp.* in particular*, Sarcocystis gigantea* and *Sarcocystis medusiformis*, infect sheep as an

intermediate host exclusively. This condition is called macroscopic ovine sarcocystosis. Infection in sheep causes the development of macroscopic cysts in the musculature (Figure 1.1). Infected sheep do not show obvious clinical signs (i.e. no disease). Cysts are trimmed by slaughterhouse meat inspectors for aesthetic reasons, and in the case of highly infected carcasses, the whole carcass is condemned and not used for human consumption. Infected carcasses are trimmed and boned out to remove visible cysts and are sold as a lower quality meat product. Residual cysts, missed at the slaughterhouse, can additionally cause the rejection of meat shipments when found by overseas inspectors (McMahon 1978). Whilst some studies estimate this condition to have a substantial economic impact (Martínez-Navalón *et al.* 2012), its economic impact to the sheep industry relative to other conditions or diseases is debated.

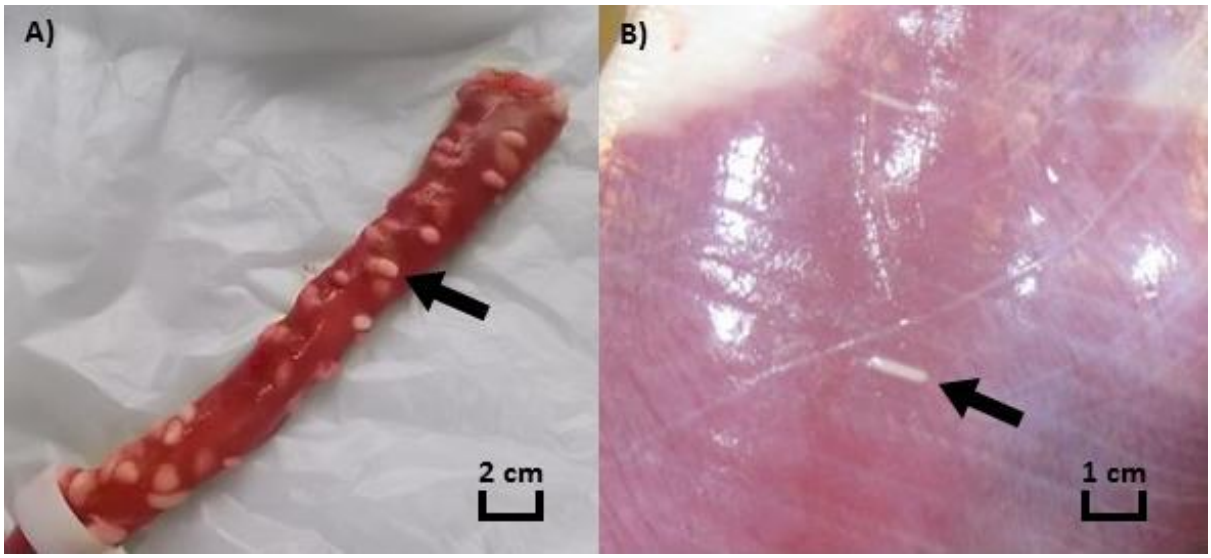

Figure 1.1: Macroscopic cysts found in the A) oesophagus, and B) diaphragm of sheep. Photos: Dr Elise Spark, Primary Industries and Regions South Australia.

1.2 Comparison of the natural history of *T. gondii*, *S. gigantea* and *S. medusiformis*

1.2.1 Discovery and phylogeny

Toxoplasma gondii was first reported in 1908 by Nicolle and Manceaux (1908) in a gundi (*Ctenodactylus gundi*) in Tunisia, a medium-size African rodent, and in the same year by Splendore (1908) in a rabbit (*Oryctolagus cuniculus*) in Brazil. In comparison, *S. gigantea* and *S. medusiformis* were first recognised as separate species in 1979 by Collins *et al.* (1979).

Toxoplasma gondii is the sole species within the genus *Toxoplasma*, which resides within the sub-family Toxoplasmatinae (Figure 1.2) (Sercundes *et al.* 2016). *Sarcocystis gigantea* and *S. medusiformis* are among approximately 200 species in the genus *Sarcocystis* (Dubey *et al.* 2015b) which resides within the sub-family Sarcocystinae. Both sub-families are sister groups and reside within the same family Sarcocystidae and phylum Apicomplexa (Figure 1.2) (Gjerde 2013).

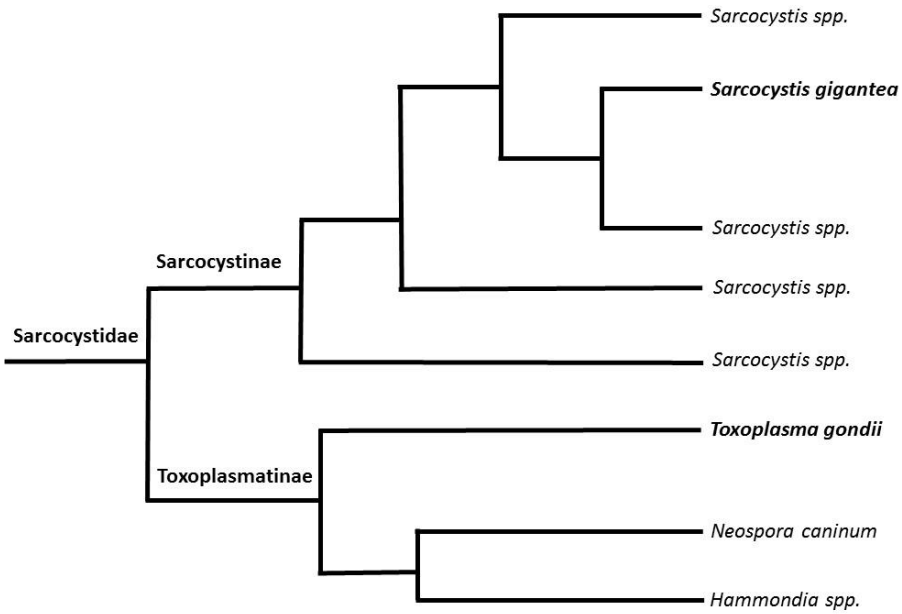

Figure 1.2: Phylogenetic tree inferred from the mitochondrial cytochrome oxidase subunit 1 gene, showing family Sarcocystidae, sub-families Toxoplasmatinae and Sarcocystinae, and species *Toxoplasma gondii* and *Sarcocystis gigantea* (Gjerde 2013). Note that *Sarcocystis medusiformis* has not yet been sequenced.

1.2.2 Lifecycle

Toxoplasma gondii, *S. gigantea* and *S. medusiformis* all have two-host predator-prey lifecycles (Dubey *et al.* 2015a; Dubey 2016c) (Figure 1.3). For *T. gondii,* any felid can act as a definitive host (Dubey 2016c), but for *S. gigantea* and *S. medusiformis* the domestic cat (*Felis catus*) is the only known definitive host (Collins *et al.* 1979). Following the ingestion of tissue cysts by the definitive host, organisms excyst as bradyzoites which penetrate the intestinal epithelium (Dubey *et al.* 2015a; Dubey 2016c). Within epithelial cells, bradyzoites transform into male and female gamonts which initiate the sexual reproduction and development into oocysts.

Toxoplasma oocyst shedding predominately occurs in naïve felids infected for the first time, but can also occur after subsequent re-infections (Zulpo *et al.* 2018). *Toxoplasma* oocysts are released into the intestinal lumen, and subsequently passed in faeces (Dubey 2016c). Similarly, *Sarcocystis spp*. oocysts form in the cat's intestine but their walls are thinner and often rupture, resulting in sporocysts being shed in faeces (Dubey *et al.* 2015a). For *S. gigantea* and *S. medusiformis*, it is currently thought that the shedding of oocysts in cat's faeces follows each infection or re-infection of the definitive host (Dubey *et al.* 2015a).

Intermediate hosts are infected by consuming food, water or soil contaminated with oocysts/sporocysts respectively (Aramini *et al.* 1999; Hill and Dubey 2002; Obendorf and Munday 1987). All mammals and birds can be an intermediate host of *T. gondii*, but sheep are the only known intermediate host of *S. gigantea* and *S. medusiformis*. Within the gastrointestinal tract of the intermediate host, sporozoites excyst from oocysts/sporocysts and penetrate the intestinal epithelium. Sporozoites of all three parasites invade epithelial cells and transform into tachyzoites that rapidly multiply asexually. *Toxoplasma* tachyzoites cause cell lysis, releasing more tachyzoites to infect other epithelial cells and other cell types at proximity. The parasites then disseminate to the rest of the host organism via blood and lymph (Uzal *et al.* 2016).

In immunocompetent individuals, the activity of tachyzoites is suppressed by the immune system. This triggers the transformation of tachyzoites into bradyzoites and the development of tissue cysts. *Toxoplasma gondii* forms microscopic intracellular cysts in muscle and neurological tissues (Hutchison *et al.* 1971), in contrast to *S. gigantea* and *S. medusiformis* which form macroscopic intracellular cysts only within muscle tissues (Munday and Obendorf 1984; Obendorf and Munday 1987). Cysts remain dormant in the tissues of the intermediate host for life, but may undergo recrudescence, for example if the host becomes immunosuppressed (Ferguson *et al.* 1989). The lifecycle is complete when the definitive host consumes tissue cysts from an intermediate host.

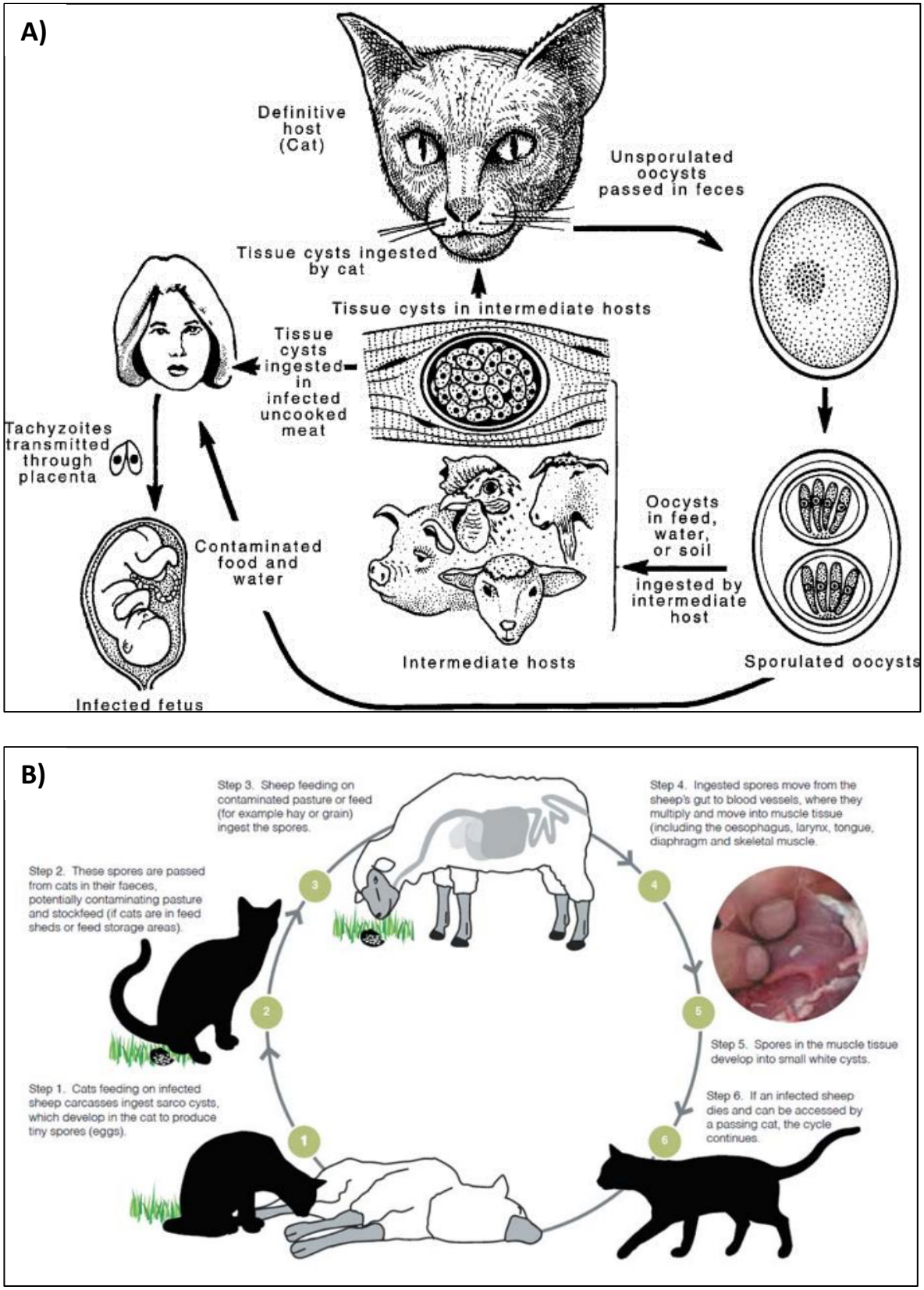

Figure 1.3: Lifecycle of *Toxoplasma gondii* (A) (source Dubey (2016c)) and *Sarcocystis gigantea* and *Sarcocystis medusiformis* (B) (source Sheep Connect Tasmania 2013).

1.2.3 Transmission

Toxoplasma gondii is transmitted to felids most efficiently following the consumption of bradyzoites in infected tissues (Dubey 2001), although infection may also occur following the

consumption of oocysts (Dubey 1996). In contrast, the transmission of *S. gigantea* and *S. medusiformis* to the domestic cat is only known to occur via the consumption of bradyzoites in infected tissues; the consumption of sporocysts by cats will not cause infection (Dubey *et al.* 2015a). The transmission of *T. gondii* to intermediate hosts occurs by four main routes;

1) the faecal-oral route through the consumption of oocysts (described above);
2) transplacental/vertical transmission (Parameswaran *et al.* 2009);
3) horizontal transmission between intermediate hosts following the consumption of bradyzoites - when an infected intermediate host is predated or scavenged by a naïve intermediate host (Boyer *et al.* 2011; Hill *et al.* 2011); and
4) sexual transmission via bradyzoites in sperm (Dass *et al.* 2011).

Sarcocystis gigantea and *S. medusiformis* are only known to be transmitted to intermediate hosts (sheep) by routes 1) and 2) (Moré *et al.* 2009).

As multiple routes of parasite transmission to intermediate hosts exist for *T. gondii*, *S. gigantea* and *S. medusiformis*, it is important to assess the relative importance of these transmission routes to best control and prevent future occurrences. In humans, it is estimated that 63-78% of infections originate from the consumption of oocysts in contaminated food, water or soil on unwashed hands or vegetables (route 1 above) (Boyer *et al.* 2011; Hill *et al.* 2011). The frequency and importance of the sexual transmission of *T. gondii* in all intermediate hosts remains largely unknown (Abdulai-Saiku *et al.* 2017). However, for the majority of the intermediate hosts of *T. gondii*, *S. gigantea* and *S. medusiformis* the major route of transmission can be speculated based on the feeding ecology of the intermediate host. For example, grazing herbivores would be expected to have a high risk of exposure to *T. gondii* oocysts on pasture, a low risk of transplacental transmission (Parameswaran *et al.* 2009), no risk of transmission via carnivorism, and a low risk of sexual transmission (depending on the frequency of intercourse). Similarly, as sheep are the only intermediate host of *S. gigantea* and *S. medusiformis*, sheep are solely herbivorous, and *Sarcocystis spp*. are rarely transmitted vertically (Moré *et al.* 2009), the consumption of sporocysts in contaminated pasture, water or soil would be the major route of *S. gigantea* and *S. medusiformis* transmission to sheep.

1.2.4 Mechanisms of disease

Toxoplasma gondii infection can cause both acute and chronic disease. Acute toxoplasmosis results from the rapid multiplication of tachyzoites within intermediate host cells (when parasite is active), causing cell lysis, organ failure due to tissue necrosis and associated inflammation, and sometimes death. Chronic toxoplasmosis occurs following the immunosuppression of tachyzoites and the formation of intracellular cysts containing bradyzoites (when parasite is dormant). The mechanism responsible for mild health impairments during the chronic stage of disease is not well-known (Vyas 2015).

Neither *S. gigantea* nor *S. medusiformis* are generally considered to be virulent to the definitive (*Felis catus*) or intermediate host (sheep) (Dubey *et al.* 1986). The primary concern regarding these two parasites is their negative impacts on meat aesthetics and value due to the formation of visible sarcocysts within the musculature of infected animals (Australian and New Zealand Food Regulation Ministerial Council. Food Regulation Standing Committee 2007).

1.3 Determinant factors in the sustainability of cat-borne parasitoses

Accepting that the faecal-oral route is the predominant transmission pathway, the ecology of *T. gondii*, *S. gigantea* and *S. medusiformis* include three essential and interacting 'ecological components' that influence the sustainability of these parasites within an ecosystem – the definitive host (#1), the environment where the oocysts/sporocysts persist (#2), and the intermediate host (#3) (Figure 1.4). Accordingly, the degree of overlap of one component with the others strongly influences the ability of the parasite to be sustained and thrive within an ecosystem, i.e. the frequency with which the parasite's lifecycle is completed.

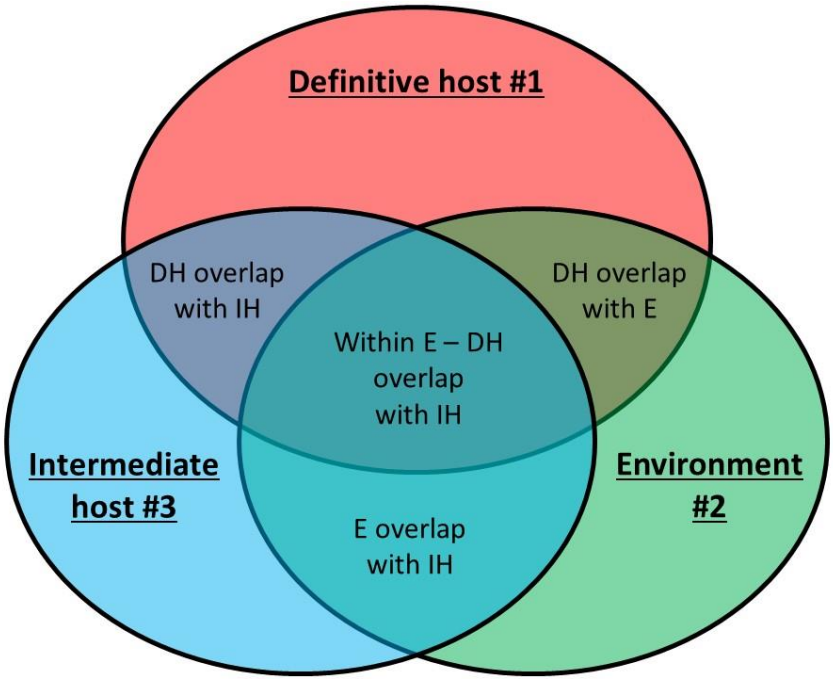

Figure 1.4: Schematic representation of the interaction between the three essential ecological components (#1, #2, #3) of the lifecycles of *Toxoplasma gondii*, *Sarcocystis gigantea* and *S. medusiformis*. DH: definitive host; E: environment; IH: intermediate host.

The degree of overlapping between the definitive host and the environment is primarily influenced by definitive host specific factors. First, the density of definitive host should be positively correlated with an increase in parasite shedding and contamination of the environment. Definitive host defecation behaviour, such as burying or not burying faeces, may also influence overlap with the environment or the probability that oocysts/sporocysts can contribute to parasite transmission.

The definitive and intermediate hosts have to share the same environment, not necessarily simultaneous, for a naive intermediate host to become infected. Similarly, both hosts have to share the same environment simultaneously for a definitive host to be able to consume bradyzoites in an infected intermediate host and become infected. Therefore, parasite transmission should be reduced if the definitive and intermediate host niches are completely or partially separated in space. The separation of niches in space could occur due to the definitive and intermediate host species occupying allopatric distributions or when they occupy sympatric distributions that are separated vertically, for example due to one host being terrestrial and the other arboreal.

A combination of characteristics of the environment and intermediate host factors should influence the degree of overlap between the environment and the intermediate host. For example, intermediate host behaviours, such as grazing, may favour *T. gondii* transmission, and environmental characteristics should alter the survival of *T. gondii* oocysts and *S. gigantea*/*S. medusiformis* sporocysts. Under favourable conditions, *T. gondii* oocysts can last for at least 18 months in the environment (Frenkel *et al.* 1975; Yilmaz and Hopkins 1972) and *Sarcocystis spp*. sporocysts can last for at least 9 months (McKenna and Charleston 1994). Oocysts/sporocysts of all three parasites are resistant to freezing (Fayer and Johnson 1975; Frenkel and Dubey 1973; Leek 1986), and are killed by severe and extended desiccation (Dubey 2016c). All parasites are also killed almost immediately at approximately 60 ^{o}C (Dubey 1998; Dubey *et al.* 2015a) and their survival decreases with increasing exposure to ultraviolet radiation (Dumetre *et al.* 2008). Therefore, any environmental factors that affect the time to desiccation (e.g. ambient humidity) or exposure to ultraviolet radiation (e.g. vegetation cover) should impact the overlap between the environment and the intermediate host.

Both definitive and intermediate host factors should influence the overlap between the two hosts' to close the *T. gondii* lifecycle. *Toxoplasma gondii* transmission to the definitive host is more likely to occur where felids preferentially consume species that are at higher risk of infection. As the risk of *T. gondii* infection cumulates with age (Jones *et al.* 2001; Van der Puije *et al.* 2000), intermediate hosts with longer lifespans are more likely to be infected and to transmit the infection to the definitive host. Similarly, according to the size range of species that given definitive hosts could possibly predate and consume, intermediate host size should influence the likelihood of transmission between the intermediate and definitive hosts.

In contrast to what might be initially expected, a high *T. gondii* exposure in felids should have little direct influence on its transmission as cats generally only shed *T. gondii* once following their initial infection, therefore seropositive cats will no longer shed the parasite into the environment. Similarly, the density of intermediate hosts should have little influence on the transmission of all three parasites. In theory, intermediate host abundance

should only favour parasite transmission within an ecosystem if intermediate hosts are a limited food resource for definitive hosts.

I aimed to investigate the ecological factors impacting *T. gondii*, *S. gigantea* and *S. medusiformis* occurrence to better explain the relative importance of each of these factors. This information is essential to the management of the impacts of these parasitoses on humans, livestock and wildlife. Through studying the lifecycles of all three parasites, I predicted definitive host abundance, environmental characteristics, definitive and intermediate host niche separation, intermediate host lifespan and definitive host feeding behaviour to all be key factors contributing to whether these parasites thrived or not.

1.4 The study site: Kangaroo Island, South Australia

To better understand the ecology of *T. gondii*, *S. gigantea* and *S. medusiformis* it is necessary to study an ecosystems where these parasites thrive. Ideally the characteristics of an ecosystem in which they are highly prevalent should be compared to the characteristics of an ecosystem in which these parasites cycle less successfully.

A small body of evidence exists suggesting a higher occurrence of cat-borne parasitoses in cats and sheep on Kangaroo Island relative to the adjacent Australian mainland (Fancourt and Jackson 2014; O'Donoghue *et al.* 1987; Primary Industries and Regions South Australia 2019). Kangaroo Island is located only 13.5 km off the coastline and is Australia's third largest island, with a total area of 4,405 km^2 (Figure 1.5). In 1987, O'Donoghue *et al.* (1987) estimated *T. gondii* seroprevalence in sheep on Kangaroo Island (range 17% - 33%) to be approximately double that on the South Australian mainland across multiple different tests (range 4% - 30%). Recent evidence also suggests a higher prevalence of macroscopic sarcocystosis in sheep on Kangaroo Island (10.1%) relative to the South Australian mainland (0.08%) (Primary Industries and Regions South Australia 2019). This high prevalence of cat-borne parasitoses on Kangaroo Island is of concern to the island's animal production industry, but also to human and wildlife health. Agriculture and tourism are the two main industries on Kangaroo Island (Tourism Optimisation Management Model Kangaroo Island Comittee 2018). The island's agricultural industry supports approximately 300 farms that

focus on livestock production, particularly sheep, and wool growing. The seroprevalence of *T. gondii* in cats on Kangaroo Island was also shown to be high (multiple tests used, range 87% - 89%) (O'Callaghan and Moore 1986), and is reported to be the second highest cat seroprevalence known in Australia, after *T. gondii* seroprevalence in cats on Christmas Island (96%) (Fancourt and Jackson 2014). This suggests that this pattern of higher occurrence of cat-borne parasitosis on Kangaroo Island, might not be restricted to sheep.

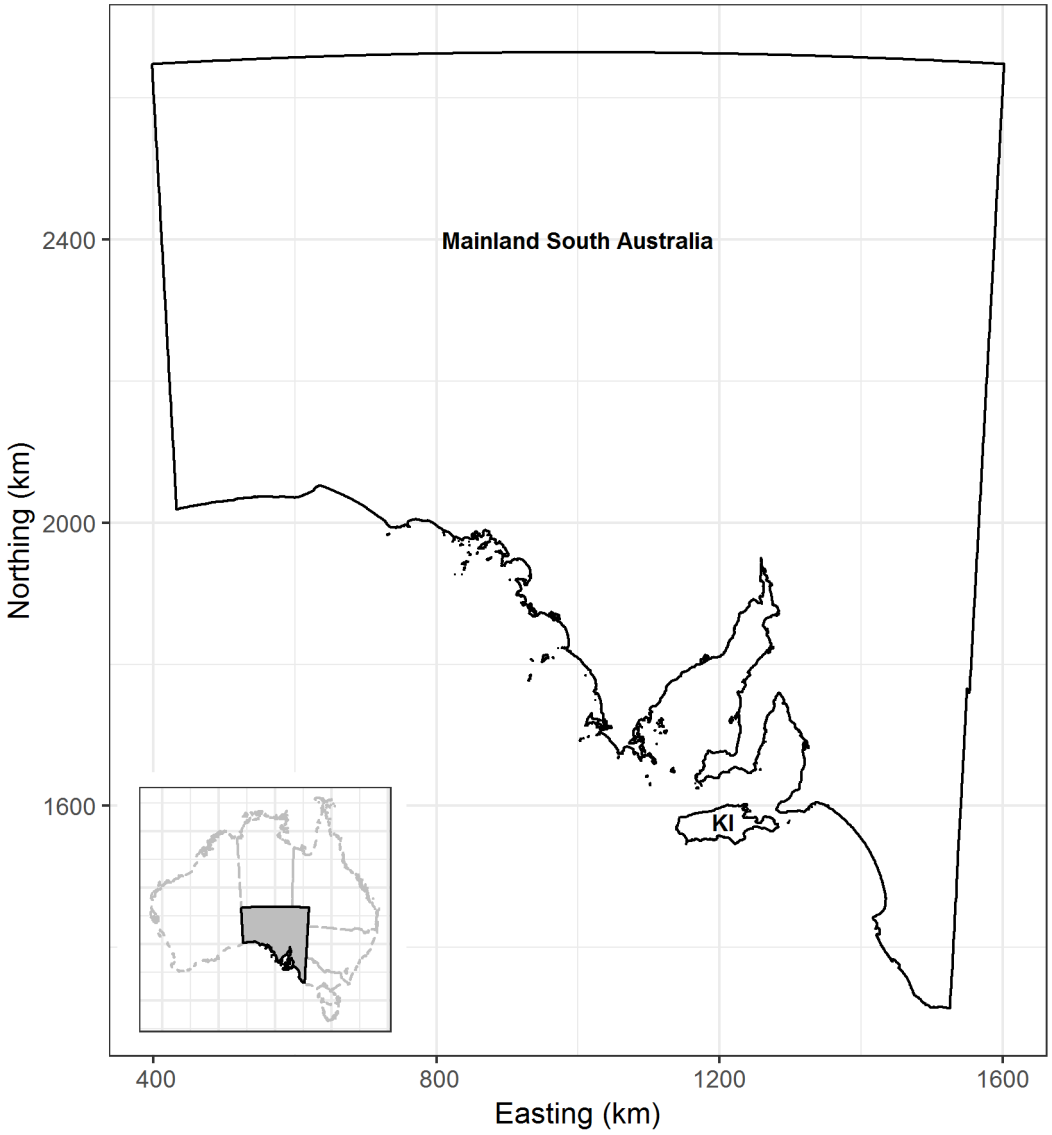

Figure 1.5: Location of Kangaroo Island (KI) and the South Australian mainland relative to Australia.

Kangaroo Island supports approximately 4,700 residents (Australian Bureau of Statistics 2017) and is one of South Australia's most visited tourist destinations, receiving over 225,000 visitors annually (Tourism Optimisation Management Model Kangaroo Island

Comittee 2018). Consequently, the high seroprevalence of *T. gondii* in multiple animal species on Kangaroo Island is of public health concern for residents and visitors due to the many possible clinical manifestations of acute and chronic toxoplasmosis in people (Dubey 2016b).

Kangaroo Island is also of high conservation value and well-known for its abundant wildlife. The island supports many threatened and endemic species that could be expected to suffer from toxoplasmosis. About half of the island retains native vegetation, predominately low *Eucalyptus* spp. woodlands, with mixed crop and pasture comprising the remainder (Willoughby *et al.* 2001). This abundance of remaining native vegetation likely contributes to Kangaroo Island's value as a refuge to a number of threatened native wildlife species that are now extinct or have suffered severe population declines on the mainland. For example, populations of the threatened southern brown bandicoot (*Isoodon obesulus obesulus*) (Paull 1995) (Figure 1.6), Australian sealion (*Neophoca cinerea*) (Gales *et al.* 1994), pygmy copperhead snake (*Austrelaps labialis*) (Read and Bedford 1991), and bush-stone curlew (*Burhinus grallarius*) (Gates and Paton 2005) are all known to be more secure on the island relative to the mainland, or other South Australian islands.

Figure 1.6: Populations of the threatened southern brown bandicoot (*Isoodon obesulus obesulus*) are more secure on Kangaroo Island compared to the adjacent South Australian mainland. Image captured on camera trap on Fleurieu Peninsula, mainland South Australia. Photo: Dr Elisa Sparrow, Natural Resources Fleurieu Willunga Basin.

The apparent higher occurrence of cat-borne parasitoses in cats and sheep on Kangaroo Island suggests a higher risk of infection and therefore a higher risk of health, welfare, economic and conservation impacts to humans, livestock and wildlife on the island. This preliminary evidence suggests that the island and adjacent mainland may be ideal study regions within which to study the ecology of cat-borne parasitoses and develop a better understanding of what factors influence whether these parasites thrive within an ecosystem, or not. Specifically, I was interested in:

1) confirming that Kangaroo Island is a good candidate region within which to study the ecology of *T. gondii*, *S. gigantea* and *S. medusiformis*, and;
2) investigating what factors influence the ability of cat-borne parasitoses to thrive within an ecosystem.

1.5 Thesis aims

To confirm that Kangaroo Island is a good candidate study region within which to study the ecology of *T. gondii*, *S. gigantea* and *S. medusiformis*, I aimed to:

1) confirm that the prevalence of *T. gondii* and macroscopic sarcocystosis is higher in sheep on Kangaroo Island compared to the adjacent South Australian mainland, and;
2) investigate if the seroprevalence of *T. gondii* is also higher in wildlife on the island.

After identifying an appropriate study site within which to study the ecology of *T. gondii*, *S. gigantea* and *S. medusiformis*, I aimed to:

3) investigate what factors influence the ability of these cat-borne parasitoses to thrive within an ecosystem by comparing Kangaroo Island to other regions in South Australia where these parasitoses are less prevalent.

Chapter 2 addresses aims #1 and #3 by mapping the prevalence of macroscopic sarcocystosis in sheep across South Australia and investigating environmental and climatic variables that may be associated with the presence of this parasitosis. Chapter 3 addresses aim #1 by indirectly confirming that *T. gondii* seroprevalence in sheep is also higher on Kangaroo Island compared to the adjacent South Australian mainland through exploring potential associations between *T. gondii* infection and macroscopic ovine sarcocystosis.

Chapters 4, 5, and 6 address aims #2 by investigating if *T. gondii* seroprevalence is higher in several species of wildlife with specific characteristics on Kangaroo Island relative to the adjacent South Australian mainland, and aim #3 by comparing seroprevalence between species. Chapter 4 compares *T. gondii* seroprevalence in macropods on Kangaroo Island and the adjacent mainland, to investigate if the same pattern of higher parasite occurrence on the island is evident in wildlife species with a similar life history to sheep (both large grazing mammals). Compared to macropods, chapter 5 assesses *T. gondii* seroprevalence in smaller species of wildlife with shorter lifespans on Kangaroo Island and the adjacent mainland, to investigate how characteristics of the intermediate host influence the prevalence of *T. gondii* in their population. Chapter 6 compares *T. gondii* seroprevalence in koalas which have a similar lifespan to macropods, but occupy a niche vertically separated from cats, to investigate how spatial separation of the definitive and intermediate host can influence *T. gondii* seroprevalence in intermediate host populations.

Chapter 7 solely addresses aim #3 by comparing feral cat abundance between Kangaroo Island and the adjacent mainland to investigate if and how strongly definitive host abundance is associated with any observed difference in the prevalence of cat-borne parasitoses between these two regions. In chapter 8, I summarise my findings and highlight factors that may influence whether cat-borne parasitoses thrive within an ecosystem or not, how cat-borne parasitoses could be managed, and the benefits management will bring to humans, livestock and wildlife.

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Chapter 2: Spatial analysis of a cat-borne disease reveals that soil pH and clay content are a risk factors for sarcocystosis in sheep

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Contribution to the Paper	Formulated experimental study design. Formatted, edited, analysed and interpreted data. Wrote and revised manuscript.		
Overall percentage (%)	80		
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i. the candidate's stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate in include the publication in the thesis; and

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Spatial analysis of a cat-borne disease reveals that soil pH and clay content are risk factors for sarcocystosis in sheep

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2.1 Abstract:

Cat-borne parasites and their associated diseases have substantial impacts on human, livestock and wildlife health worldwide. Despite this, large and detailed datasets that allow researchers to study broad-scale trends in the ecology of cat-borne diseases are either difficult to obtain or non-existent. One condition that is easily detected at slaughter is macroscopic sarcocystosis, a cat-borne parasitosis of sheep (*Ovis aries*). We conducted a cross-sectional study to describe the geographic distribution of sarcocystosis in sheep throughout South Australia and investigate ecosystem characteristics associated with the presence of disease. Data were obtained from two slaughterhouses which processed 3,865,608 sheep from 4,204 farms across 385,468 km^2 of South Australia's land mass for the period 2007-2017. A Poisson point process model was developed to quantify environmental characteristics associated with higher densities of sarcocystosis-positive farms. Sarcocystosis was highly clustered on a large island off of the Australian coast and the density of sarcocystosis-positive farms increased in areas of low soil pH (intensity ratio: 0.86, 95 % CI: 0.78, 0.95) and high clay content. We hypothesise that region was confounded by, and predominately acted as a proxy for, cat density. Our results have broader implications

regarding the health, welfare, economic and conservation impacts of other cat-borne parasitosis, such as toxoplasmosis.

2.2 Introduction:

Feral and domestic cats (*Felis catus*) harbour a range of infectious diseases that impact on the health and welfare of human and animal hosts (Robertson 2008). However, despite their importance, the majority of studies on cat-borne diseases are of limited scale, focusing on relatively small populations and relatively small numbers of locations. Studies describing or investigating the ecology of cat-borne diseases at a landscape scale are scarce.

In agricultural areas, cats transmit infections to food animals. Food animals are systematically inspected pre- and post-slaughter for clinical conditions to control and ensure food quality and safety. When inspection findings are centrally recorded, they can provide insights into the frequency and distribution of food animal diseases across wide geographical areas, including infections of feline origin. One of these conditions, sarcocystosis, can affect meat aesthetics and quality, but does not threaten consumer health (i.e. it is not zoonotic). Sarcocystosis is caused by a protozoan parasite in the genus *Sarcocystis*. *Sarcocystis spp*. generally have a two-host predator-prey lifecycle, where the carnivorous definitive host predates on an intermediate host (Dubey *et al.* 2015). In the intermediate host, the parasites develop into cysts (termed sarcocysts) within the skeletal musculature, that vary in size depending on the *Sarcocystis spp*. Large sarcocysts that are visible to the naked eye are described as 'macroscopic', whereas those not visible to the naked eye are described as 'microscopic'.

Macroscopic sarcocystosis in sheep (*Ovis aries*) (intermediate host) is caused by two parasites, *S. gigantea* and *S. medusiformis*. The sexual reproduction of these two parasites occurs in the digestive tract of the domestic cat, after which sporocysts are shed in the faeces (Collins *et al.* 1979; Dubey *et al.* 1986). Sporocysts can survive for approximately 6-8 months in the environment (McKenna and Charleston 1994; Savini *et al.* 1996); they are resistant to freezing (Leek 1986; McKenna and Charleston 1992) and killed by desiccation (McKenna and Charleston 1994), although survival is also influenced by humidity (Savini *et al.* 1996). The impact of other environmental factors on sporocyst survival is not well known. Sheep are exposed and infected by consuming contaminated pasture, water or soil (Ford

1986). Parasites develop into macroscopic sarcocysts in the musculature which are detected and recorded during visual inspection of sheep carcasses at the slaughterhouse (Munday and Obendorf 1984b; Obendorf and Munday 1987). At slaughter, carcasses infected with sarcocystosis are trimmed, or in the case of highly infected carcasses, the whole carcass is condemned for human consumption (Australian and New Zealand Food Regulation Ministerial Council. Food Regulation Standing Committee 2007). Trimmed carcasses are then boned-out to facilitate sarcocyst removal, and consequently sold as a lower quality product. Macroscopic sarcocystosis results in economic losses for both sheep farmers and meat processors (Martínez-Navalón *et al.* 2012).

In 2007, Primary Industries and Regions South Australia initiated an ongoing slaughterhouse surveillance program to monitor sarcocystosis and other health conditions of sheep farmed across South Australia. Inspection data are recorded from any farm submitting sheep directly to two major slaughterhouses within the state. This provides continuous information, spanning a large and diverse geographical area, with substantial variation in environmental and climatic factors to investigate the ecology of cat-borne diseases.

Our objective was to analyse the slaughterhouse surveillance data to investigate: (1) the geographic distribution of sarcocystosis, and (2) identify potential location-dependent risk factors associated with the occurrence of the disease. We mapped the geographic distribution of sarcocystosis throughout South Australia and investigated potential associations with geographic and climatic factors. A better understanding of geographic and climatic factors associated with the presence of infection might support the development of targeted sarcocystosis control programs.

2.3 Materials and methods:

2.3.1 Study population: Slaughterhouse surveillance data

The sarcocystosis data used in our study were collated by Primary Industries and Region South Australia over the period January 2nd 2007 to December 31st 2017. This dataset includes all sheep that were directly consigned (i.e. there were no intermediate movements between farm and slaughterhouse) for slaughter at the Thomas Foods International slaughterhouses in Murray Bridge and Lobethal, South Australia.

The slaughterhouse surveillance dataset captures prevalence estimates by meat inspectors of visible sarcocysts in dressed carcasses at the flock-level (PIRSA 2018). In the remainder of the paper we use the term 'farm' to refer to a geographical area in which sheep are reared, and 'flock' as a consignment of sheep submitted for slaughter at a slaughterhouse. Using these definitions, multiple flocks can originate from a single farm throughout the eleven year study period. These data do not include information on visible sarcocysts in the offal of sheep and do not differentiate between carcasses with single or multiple sarcocysts.

We only analysed data collected for sheep that were greater than two years of age. The prevalence of macroscopic sarcocysts in lambs, defined as sheep less than two years of age, is very low due to the risk of infection being cumulative and sarcocysts taking time to grow to a visible size (Munday and Obendorf 1984a). We further restricted the study population to include only those sheep farms that were located to the south of the wild dog barrier fence in South Australia (Figure 2.1). The dog fence runs continuously for 2,132 km (5,614 km total fence length) across the middle of the state from east to west, and was built to protect sheep flocks from dingo (*Canis lupus dingo*) predation. Relatively few sheep farms are present to the north of the dog fence as a consequence of dingo predation and the effect of excluding these farms on the inferences drawn from our analyses was reasoned to be low. All sheep included in our study were assumed to have originated from extensive grazing systems as relatively few farms in South Australia operate other systems (feedlots or similar). We are not aware of any parasite treatments for sheep or cats that could influence the disease status of sheep or farms.

2.3.2 Data formatting and structure

The raw slaughterhouse surveillance data provided information at the flock level. Flock level data are linked back to their farm of origin by a unique farm identification code, which is given to all South Australian farms submitting sheep to the slaughterhouse. Farm identification codes are managed via a hierarchical system where they are grouped into zones, and zones grouped into regions. South Australia is divided into ten farm identification code regions; 1: Adelaide Hills/Fleurieu Peninsula, 2: Barossa Valley/Lower North, 3: Eyre Peninsula, 4: Kangaroo Island, 5: Lower South East, 6: Mid-South East, 7: Murray Mallee, 8: Northern Pastoral, 9: Upper-South East, and 10: Yorke Peninsula/Mid-North. In the remainder of this paper we use the term 'region' to refer to farm identification code region.

We used the slaughterhouse surveillance data on the total number of sheep within each submitted sheep flock and the estimated sarcocystosis prevalence for each flock, to back-calculate the number of sarcocystosis-positive sheep in each flock. A farm-level dataset was created by pooling sheep-level data to describe: (1) the number of sarcocystosis-positive sheep submitted by each farm over the eleven-year study period; (2) the number of sheep submitted by each farm (irrespective of disease status) over the eleven-year study period; (3) the farm-level period prevalence of sarcocystosis across the eleven-year study period; and (4) the latitude and longitude of the centroid of each farm. The farm-level data (farm locations and the information associated with each location) were used to create two marked point pattern datasets using the spatstat package (Baddeley *et al.* 2015a) in R version 3.5.1 (R Core Team 2018). In this context the term 'marked' refers to attribute information (e.g. the total number of sheep slaughtered by each farm over the study period) attached to each point location. Two marked point pattern datasets were created, one for the sarcocystosis-positive farms and one for the entire farm population at risk over the eleven year study period.

2.3.3 Data analyses

Case definition and spatial unit of interest

Our spatial unit of interest was the centroid of a given sheep farm. We considered a farm a case/sarcocystosis positive if one or more sheep submitted to the slaughterhouse by a particular farm over the entire study period had visible sarcocystosis.

Geographical distribution of sarcocystosis farms

The spatial distribution of a disease can be classified into two components: broad-scale trends (first-order effects) and spatial dependence/interaction (second-order effects) (Pfeiffer *et al.* 2008b). For example, broad-scale trends/first-order effects in the distribution of sarcocystosis would be predicted to occur due to temperature or rainfall influencing the survival time of *Sarcocystis* sporocysts in the environment, and spatial dependence/second-order effects, otherwise referred to as spatial autocorrelation, could occur if the disease status of one farm influenced the disease status of surrounding farms. To assess broad-scale trends and locate areas of increased period prevalence of sarcocystosis, we created a period

prevalence density surface. The numerator layer was the count of sarcocystosis-positive sheep per km^2. This layer was created by weighting each sarcocystosis-positive farm by the total number of sarcocystosis-positive sheep that the farm had submitted for slaughter over the eleven-year study period. The denominator comprised the total count of sheep per km^2. This layer was created by weighting each farm (irrespective of disease status) by the total number of sheep that the farm had submitted for slaughter over the eleven-year study period. In contrast to all other analyses, we created the sarcocystosis prevalence density map at the sheep-level to account for the large variation in the number of sheep submitted to the slaughterhouse by each farm, and the potential influence of this on within-farm estimated sarcocystosis period prevalence.

Density surfaces were created using the marked planar point pattern datasets and a kernel smoothing technique. Here we used a regular grid of 300 × 300 cells (2.9 km east-west x 3.5 km north-south) superimposed over the extent of our study area, with the standard deviation of the Gaussian kernel (that is, the bandwidth) fixed at 10 km for the positive-sheep density layer and 15 km for the population sheep density layer. Our reason for using a larger bandwidth for the sheep population at risk layer was to deal with the situation where, in areas where the density of the sheep population at risk was low, small changes in the number of sarcocystosis positive sheep led to unacceptable variation in the ratio of the two kernel estimates (Bailey and Gatrell 1995a; Bailey and Gatrell 1995b). The bandwidth for the sarcocystosis positive sheep density layer was determined using the cross-validation method (Wand and Jones 1994). Maps showing the number of sarcocystosis positive sheep per 100 sheep per square km based on the two sheep density layers (described above) were developed using the sparr package (Davies *et al.* 2018) in R. Using this same process, we then created annual period prevalence density maps for years 2007-2017 inclusive to assess the assumption of stationarity (Baddeley *et al.* 2015b).

The presence of spatial dependence or interaction at the farm-level was assessed by computing Ripley's empirical K-function (Ripley 1976; Ripley 1977) for sarcocystosis-positive and sarcocystosis-negative farms. The K-function identifies the distance over which dependence between points occurs (Pfeiffer *et al.* 2008a) and is defined as the expected number of points that are located within a distance *h* of an arbitrary selected point location, divided by the overall density of points (Ripley 1977). If dependence between points was

detected, sarcocystosis-positive farms would likely be surrounded by other sarcocystosis-positive farms and, for small values of distance *h*, K(*h*) would be relatively large. Conversely, if sarcocystosis-positive farms were regularly spaced, each sarcocystosis-positive farm would likely be surrounded by empty space and, for small values of distance, K(*h*) would be small. To facilitate inference, we created separate K-function plots for sarcocystosis-positive and sarcocystosis-negative farm locations. For each value of *h* we calculated the K-function difference as:

$$D(h) = K(h)_{positive} - K(h)_{negative} \quad \text{(Eq. 1)}$$

For a given *h*, if sarcocystosis-positive farm locations were spatially aggregated more than the sarcocystosis-negative farm locations then $D(h)$ will appear graphically as a peak, providing an indication of the nature of dependence and the distance over which it occurred within the data (Bailey and Gatrell 1995a; Pfeiffer *et al.* 2008a).

Risk factor analysis

We compared sarcocystosis-positive farm density (number positive farms per 100 farms per km^2) with raster maps of each of our hypothesised explanatory variables (Table 2.1). Explanatory variables were all expected to potentially impact the time to desiccation of *Sarcocystis* sporocysts in the environment, and consequently sporocyst survival, by increasing or decreasing sporocyst moisture loss, or rupturing or degrading the sporocyst wall in some way (Dubey *et al.* 2015). We included region (described above) to adjust for unaccounted for variation operating at the regional level. We did not include cat density (the definitive host of macroscopic ovine sarcocystosis) as no appropriate layer existed.

Table 2.1: Candidate explanatory variables hypothesised to influence the distribution of sarcocystosis.

Candidate explanatory variable	Resolution (m)	Date range (years)	Value range	Source
Average annual rainfall	5,000	1961 - 1990	134 - 1019 mm	(Australian Government Bureau of Meterology 2019)
Average count of days per annum with precipitation >1 mm	2,500	1961 - 1990	16 - 125 days	(Australian Government Bureau of Meterology 2019)
Average annual relative humidity measured at 9 am	10,000	1976 - 2005	46 - 78 %	(Australian Government Bureau of Meterology 2019)
Average count of frost days per annum (minimum daily temperature ≤ 0 ^{o}C)	5,000	1976 - 2005	0 - 32 days	(Australian Government Bureau of Meterology 2019)
Annual average of maximum daily temperature	2,500	1961 - 1990	17 - 29 ^{o}C	(Australian Government Bureau of Meterology 2019)
Annual average of daily sunshine duration	25,000	1990 - 2003	5 - 9 hrs	(Australian Government Bureau of Meterology 2019)
Clay content in the top 0-5 cm of soil	90		3 - 42 %	(Grundy *et al.* 2015)
Sand content in the top 0-5 cm of soil	90		37 - 95 %	(Grundy *et al.* 2015)
Soil pH	90		3.3 - 9	(Grundy *et al.* 2015)
Region			1 - 10	PIRSA[a]

[a] Primary Industries and Regions South Australia

We plotted sarcocystosis-positive farm density as a function of each of the hypothesised explanatory variables using the rhohat procedure (Baddeley *et al.* 2012) implemented in spatstat (Baddeley and Turner 2005). Explanatory variables were selected for multivariable modelling, based on the presence of a non-erratic (no irregular or unusual spikes), clearly defined and well-supported association with sarcocystosis-positive farm density in the rhohat plots. We tested for collinearity amongst risk factors using variance inflation factors (VIFs) implemented within the 'USDM' package (Naimi *et al.* 2014). Risk factors with VIFs exceeding a pre-selected threshold of three (Zuur *et al.* 2010) were excluded. The possibility of two-way interactions between non-collinear candidate explanatory variables were considered, and none were judged to be biologically plausible.

Poisson point process models fitted in spatstat are expressed in terms of the Papangelou conditional intensity function (Papangelou 1974; van Lieshout 2000) denoted by $\lambda(u, x)$. When referring to Poisson point process models and the Papangelou conditional intensity function, we use the terms intensity and density interchangeably, in an attempt to make our work more interpretable for non-statistically minded readers, although we recognise that

intensity is more readily used within the field of spatial statistics. We assumed that the density of sarcocystosis-positive farms was a loglinear function of parameters Φ and θ (Baddeley *et al.* 2015c):

$$log\lambda(u,x) = \Phi^T b(u) + \theta^T S(u,x) \quad \text{(Eq. 2)}$$

where $\Phi^T b(u)$ represents the broad scale (first order) trend component of the conditional intensity and $\theta^T S(u,x)$ represents the spatial dependence (second order) component. To capture non-linear associations between sarcocystosis-positive farm density and each of our hypothesised explanatory variables for multivariable modelling, continuous variables were categorised based on the rhohat plot trends (described above). To model broad-scale trends, we included an offset term representing the geographic distribution of all farms that submitted sheep for slaughter throughout the study period, in addition to each of the risk factors identified previously to be associated with sarcocystosis-positive farm density using the rhohat procedure. Our model offset term was log transformed so that it was on the appropriate scale for our loglinear model. To assess the need for a spatial dependence term in our model, we created a variogram of the standardised model residuals. In a well-fitting model, the residual variogram should be essentially flat, showing no evidence of spatial correlation (Isaacs and Srivistava 1989). Model selection involved manual backwards stepwise variable selection considering Akaike Information Criterion values (Akaike 1974).

Outputs from the point process model were estimated regression coefficients and their 95% confidence intervals (CI) for each of the parameterised explanatory variables. For those explanatory variables that varied on a continuous scale, the exponent of the regression coefficient is interpreted as the multiplicative effect of a one unit increase in the value of the explanatory variable on sarcocystosis-positive farm density. For categorical explanatory variables, the exponent of the regression coefficient is interpreted as the multiplicative effect increase or decrease in sarcocystosis-positive farm density for that level of the factor compared to the defined reference category. Residuals from our point process model were assessed using a series of diagnostic plots (lurking variable plots) to confirm goodness-of-fit and to identify outliers in the data; all plots were implemented in spatstat (Baddeley *et al.* 2008; Baddeley *et al.* 2005).

All figures were created in the spatstat (Baddeley *et al.* 2015a) and ggplot2 (Wickham 2009) packages in R V3.5.1 (R Core Team 2018).

2.4 Results:

Our restricted dataset represented a total of 3,865,608 sheep, two years or older, submitted for slaughter at the two study slaughterhouses originating from 17,341 flocks and from 4,204 farms across 385,468 km^2 of South Australia's land mass (Table 2.2, Figure 2.1) over the eleven-year study period. Period prevalence was low at the farm-, flock- and animal-level across the 11 year study period in all regions of the state, except Kangaroo Island (Table 2.2). On Kangaroo Island, the period prevalence of sarcocystosis was between 14 and 66 times greater than any other mainland region depending on which level of data hierarchy was compared (farm-level Vs. flock-level Vs. animal-level) (Table 2.2).

Table 2.2: Regional summary statistics at farm-, flock- and animal-level, showing number sampled, number positive to sarcocystosis and period prevalence over 2007-2017 period. Region numbers correspond to those shown in figure 2.2 and table 2.3.

Region	Total farms	Positive farms	Farm period prevalence % (95% CI)	Total flocks	Positive flocks	Flocks period prevalence % (95% CI)	Total sheep	Positive sheep	Sheep period prevalence % (95% CI)
1 - Adelaide Hills/Fleurieu Peninsula	162	10	6.2 (3.4, 11.0)	544	12	2.21 (1.14, 3.82)	90,474	420	0.46 (0.42, 0.51)
2 - Barossa Valley/Lower North	245	12	4.9 (2.8, 8.4)	815	13	1.60 (0.85, 2.71)	158,864	572	0.36 (0.33, 0.39)
3 - Eyre Peninsula	882	26	2.9 (2.0, 4.3)	3,987	27	0.68 (0.45, 0.98)	852,490	1,347	0.16 (0.15, 0.17)
4 - Kangaroo Island	310	266	85.8 (81.5, 89.3)	2,475	1,720	69.49 (67.64, 71.31)	502,559	165,244	32.9 (32.8, 33.0)
5 - Lower South East	214	12	5.6 (3.2, 9.5)	801	13	1.62 (0.87, 2.76)	209,835	394	0.19 (0.17, 0.21)
6 - Mid-South East	283	13	4.6 (2.7, 7.7)	1,111	16	1.44 (0.83, 2.33)	326,934	669	0.20 (0.19, 0.22)
7 - Murray Mallee	671	22	3.3 (2.2, 5.0)	2,480	26	1.05 (0.69, 1.53)	494,355	1,177	0.24 (0.22, 0.25)
8 - Northern Pastoral	327	7	2.1 (1.0 4.4)	1,496	10	0.67 (0.32, 1.23)	459,976	515	0.11 (0.10, 0.12)
9 - Upper-South East	347	15	4.3 (2.6, 7.0)	964	16	1.66 (0.95, 2.68)	264,417	764	0.29 (0.27, 0.31)
10 - Yorke Peninsula/Mid-North	763	30	3.9 (2.8, 5.6)	2,668	47	1.76 (1.30, 2.34)	505,704	1,578	0.31 (0.30, 0.33)
Total	**4,204**	**413**		**17,341**	**1,900**		**3,865,608**	**172,680**	

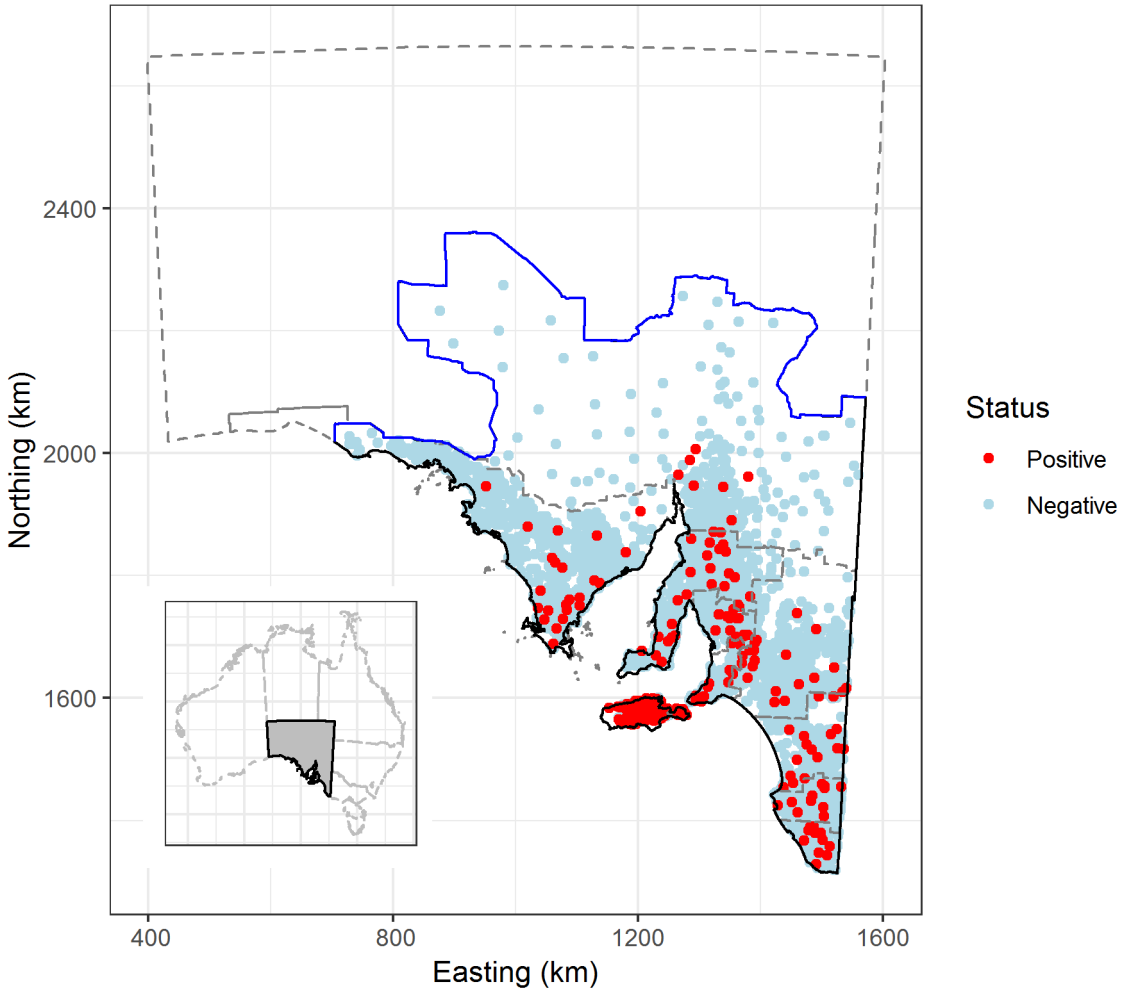

Figure 2.1: Sarcocystosis-positive and negative sheep farms, based on sheep submitted to the Thomas Foods International slaughterhouses in South Australia, 2007-2017. Solid black line defines the extent of the study region; the solid blue line represents the dog fence and outlines the northern and western boundary of the study region; the grey dashed line outlines farm identification code regions and the South Australian state border. Farm locations have been jittered by 5 km to obscure the identity of farm locations in remote areas. Insert - location of South Australia in relation to the remainder of Australia. Map projection EPSG: 3107 South Australian Lambert, GDA 94.

By visual inspection of annual sarcocystosis period prevalence density maps, there was no obvious or dramatic shift in the spatial distribution of sarcocystosis across years 2007-2017, suggesting a largely stationary point pattern. The average sarcocystosis period prevalence density map (years 2007-2017 grouped together) identified Kangaroo Island as having a substantially increased occurrence of sarcocystosis compared to the remainder of the state

(Figure 2). Using the empirical K-function, we identified spatial dependence in our dataset, consistent with clustering, up to a distance of 500 m from a given farm location.

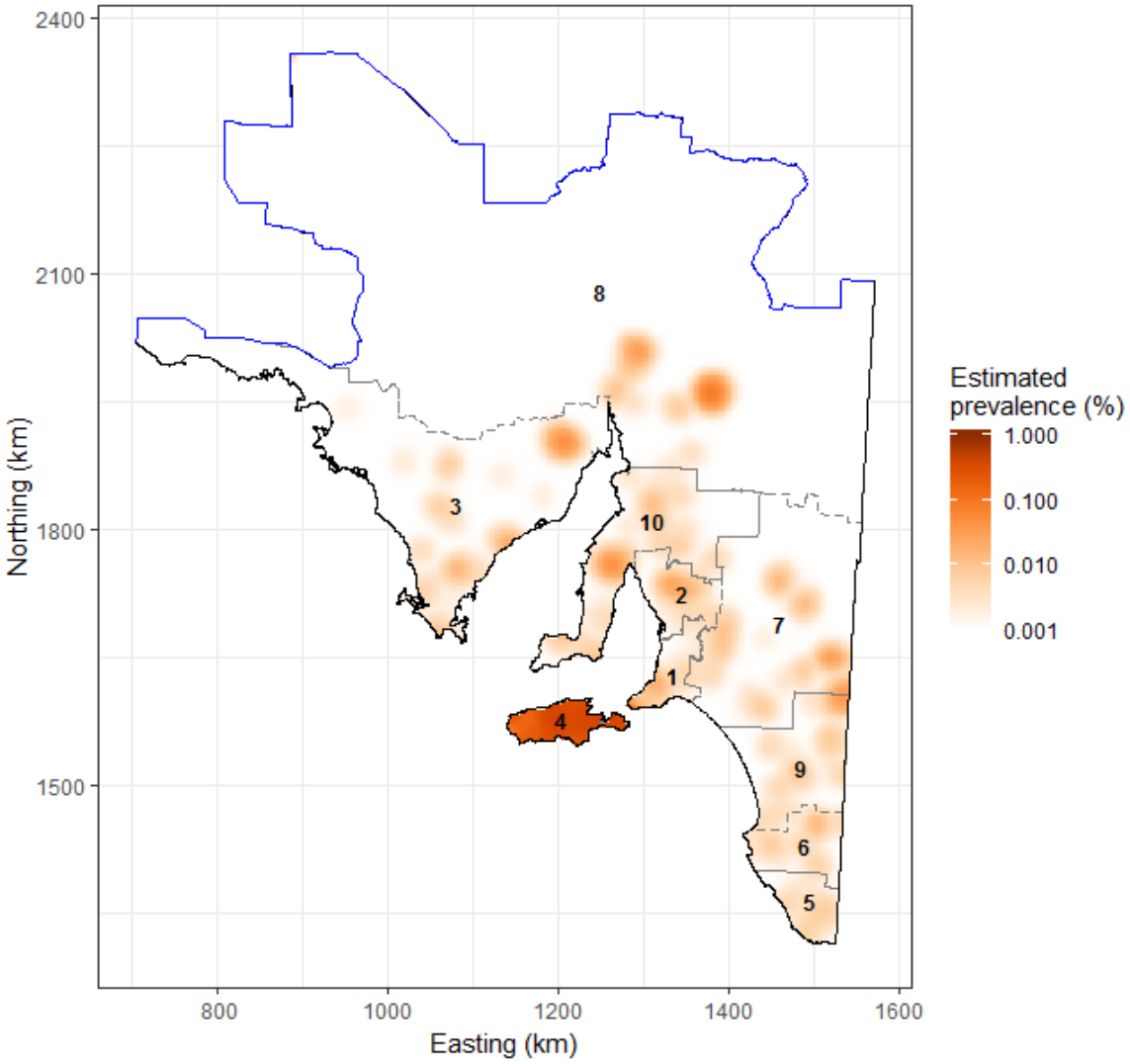

Figure 2.2: Raster image showing the estimated prevalence of sarcocystosis in sheep greater than two years of age submitted to the Thomas Foods International slaughterhouses in South Australia, 2007-2017. Data were log-transformed for plotting to facilitate detection of high- and low-risk areas, as the estimated prevalence of sarcocystosis on the mainland is relatively low. The solid blue line represents the dog fence and outlines the northern and western boundary of the study region; the grey dashed line delineates the farm identification code regions/numbers as listed in tables 2 and 3. Map projection is EPSG: 3107 South Australian Lambert, GDA 94.

Soil pH, clay content in the top 0-5cm of soil, and region were the only explanatory variables that significantly influenced (p-value < 0.05) sarcocystosis-positive farm density in the reduced Poisson point process model (Tables 2.3 and S2.1, Figure 2.3). One unit increases in soil pH decreased the density of sarcocystosis-positive forms by a factor of 0.86 (95% CI 0.78 to 0.95). In contrast, sarcocystosis-positive farm density increased by a factor of 1.45 (95% CI 1.10 to 1.92) where soil clay content was ≥ 16.5 % in the top 0-5 cm of soil relative to areas where clay content was < 14.5 % in the top 0-5 cm of soil. The density of sarcocystosis-positive farms on Kangaroo Island was 15.25 (95% CI 8.04 to 28.94) times greater than the density of sarcocystosis-positive farms located in the Adelaide Hills/ Fleurieu Peninsula. The empirical variogram lies within 95% posterior limits throughout the plotted region, demonstrating that the fitted model adequately accounted-for the second-order structure in the data (Figures 4). Whilst other explanatory variables appeared to be associated with sarcocystosis risk in the rhohat plots (Figure S2.1), they did not increase the explanatory power of our model. For example, soil sand content appeared to influence sarcocystosis-positive farm density (Figure S2.1), although spikes in the plot corresponded with soil sand content values found across the majority of Kangaroo Island.

Table 2.3: Estimated regression coefficients and their standard errors from the reduced Poisson point process model of variables associated with sarcocystosis-positive farm density. Data represent sheep more than two years old submitted to the Thomas Foods slaughterhouses in South Australia, 2007-2017. Bold - significant factors.

Explanatory variable	Coefficient (SE)	*P*-value	Density ratio[a] (95% CI)
Intercept	11.49 (0.42)		
Soil pH	**-0.15 (0.05)**	**0.003**	**0.86 (0.78, 0.95)** [b]
Soil clay content (%)			
< 14.5	Reference		1.0
≥ 14.5 < 16.5	0.24 (0.13)	0.07	1.27 (0.99, 1.63)
≥ 16.5	**0.37 (0.14)**	**0.008**	**1.45 (1.10, 1.92)**
Region (number – name): [c]			
1 - Adelaide Hills/Fleurieu Peninsula	Reference		1.0
2 - Barossa Valley/Lower North	0.09 (0.43)	0.83	1.09 (0.47, 2.52)
3 - Eyre Peninsula	-0.44 (0.39)	0.27	0.65 (0.30, 1.40)
4 - Kangaroo Island	**2.72 (0.33)**	**<0.001**	**15.25 (8.04, 28.94)**
5 - Lower South East	0.22 (0.44)	0.62	1.25 (0.52, 2.98)
6 - Mid-South East	-0.08 (0.43)	0.85	0.92 (0.40, 2.14)
7 - Murray Mallee	-0.19 (0.40)	0.65	0.84 (0.38, 1.82)
8 - Northern Pastoral	-0.85 (0.50)	0.09	0.43 (0.16, 1.14)
9 - Upper-South East	-0.07 (0.41)	0.86	0.93 (0.42, 2.08)
10 - Yorke Peninsula/Mid-North	-0.29 (0.38)	0.45	0.75 (0.35, 1.59)

SE: standard error; CI: confidence interval.

[a] Density ratio equals the exponent of the estimated regression coefficient for each explanatory variable.

[b] Interpretation: After controlling for the confounding effect of region in which a farm was located, one unit increases in soil pH decreased the density of sarcocystosis-positive farms by a factor of 0.86 (95% CI 0.78 to 0.95).

[c] Region refers to farm identification code regions used by Primary Industries and Regions South Australia, and corresponds to those shown in table 2.2 and figure 2.2.

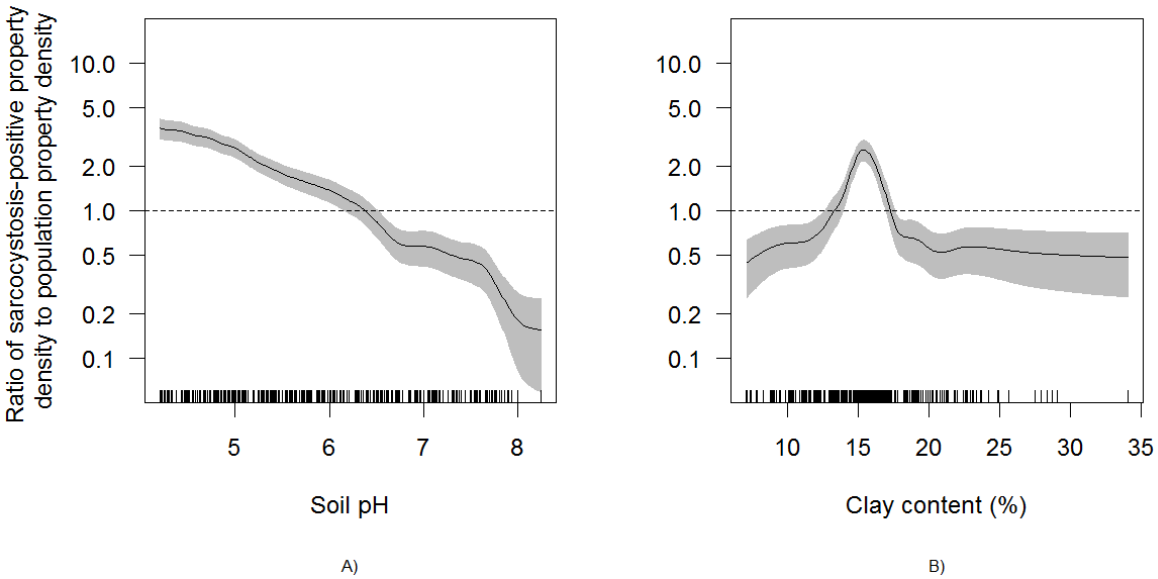

Figure 2.3: Ratio of sarcocystosis-positive farm density to population farm density as a function of soil pH (A) and clay content (%) in the top 0-5 cm of soil (B) estimated across South Australia using the rhohat procedure. The solid line shows function estimate; grey shading is pointwise 95% confidence band. The vertical dashes along the horizontal axis represent individual data points. The horizontal dashed line represents the null association (density of sarcocystosis-positive farms equals the density of all farms at risk). Interpretation: for those areas in the study area where soil pH was ~5 the intensity of sarcocystosis-positive farms was ~2 times that of all farms at risk. Data represent 4204 sheep farms that submitted sheep for slaughter during the period 2007-2017.

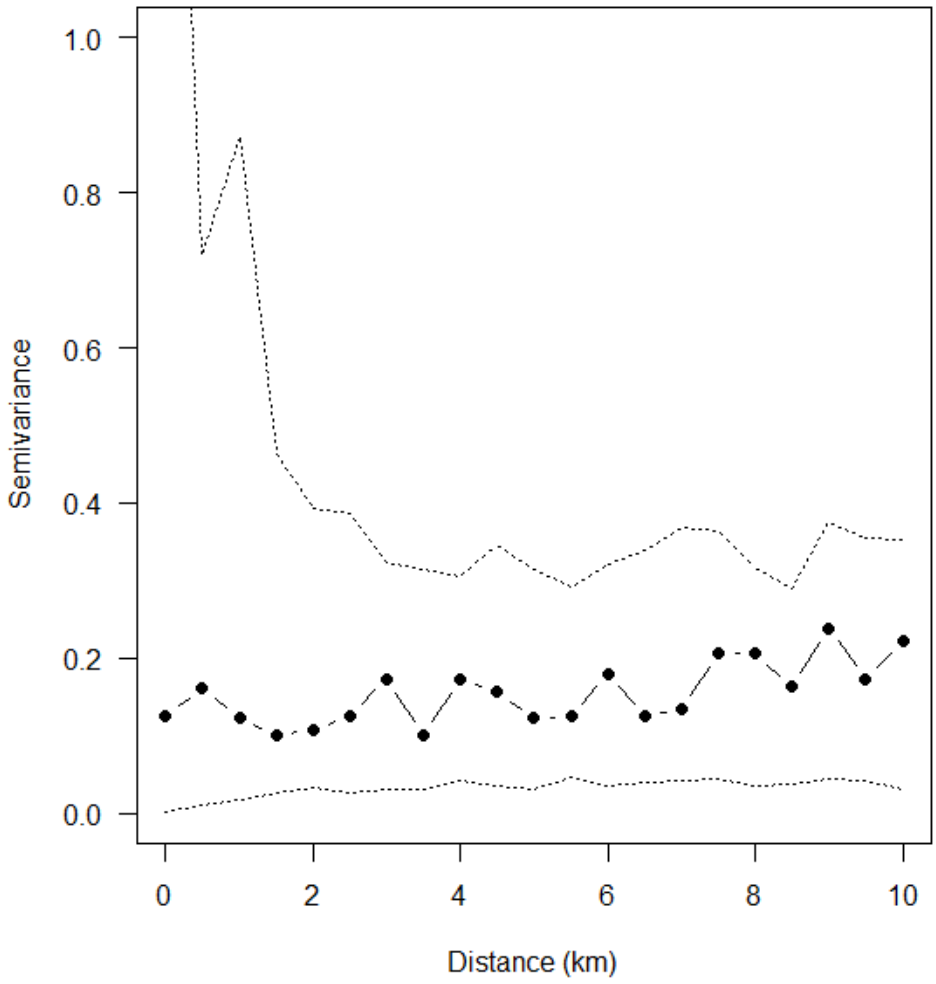

Figure 2.4: Empirical variogram fitted to the posterior mean of the standardised residuals from reduced Poisson point process model explaining sarcocystosis-positive farm density. Dashed lines show the pointwise 95% posterior intervals constructed from 999 simulated realisations of the fitted spatial model.

2.5 Discussion:

Our analyses show a marked heterogeneous distribution of macroscopic ovine sarcocystosis across South Australia with a clear hotspot on Kangaroo Island. Kangaroo Island had a modelled density of sarcocystosis-positive farms approximately 15 times higher than the Adelaide Hills/Fleurieu Peninsula region and 12 times higher than any other region. In addition to a regional difference, the occurrence of sarcocystosis was decreased by alkaline soils and increased by soil clay content. A one unit increase in soil pH corresponded to a 14 % reduction in the density of sarcocystosis-positive farms, and sarcocystosis-positive farm

density increased by approximately 45 % in soils with ≥ 16.5 % soil clay content in the top 0-5 cm of soil relative to soils with < 14.5 % soil clay content.

Kangaroo Island is situated approximately 14 km off the South Australian coast line, with the Adelaide Hills/Fleurieu Peninsula region being the closest mainland region. Both Kangaroo Island and the Adelaide Hills/Fleurieu Peninsula have similar Mediterranean climates (Jenkins 1985; Schwerdtfeger 2002), land uses (predominately agriculture) and vegetation communities (Jenkins 1985; Willoughby *et al.* 2001). One major difference between these two regions is the abundance of feral cats. We recently identified an approximately 11 fold greater relative abundance of feral cats on Kangaroo Island compared with the Fleurieu Peninsula (Taggart *et al.* In Press. [Chap. 7]). Cats are the definitive host of *S. gigantea* and *S. medusiformis* (Collins *et al.* 1979; Dubey *et al.* 1986), the parasites responsible for macroscopic sarcocystosis in sheep. We therefore suspect that region predominately acts as a proxy for cat density within the Kangaroo Island and Adelaide Hills/Fleurieu Peninsula regions, and likely across South Australia. We expect that active and consistent cat management on Kangaroo Island would produce long-term reductions in macroscopic sarcocystosis in the island's sheep, with cat eradication expected to result in the complete eradication of sarcocystosis by means of breaking the parasite's life cycle. Whilst other methods of reducing sarcocystosis burden on Kangaroo Island are potentially possible, such as collecting, burying or burning sheep carcasses and offal on farms, or treating all cats with anti-parasitic drugs, these methods are not practical or feasible, particularly at large geographic scales and where large populations of feral cats exist.

We believe our study is the first to describe the impact of soil pH and clay content on sarcocystosis in sheep, and first to provide evidence from the field suggesting that soil pH and clay content may influence the survival of *Sarcocystis spp*. sporocysts in the environment. Similar relationships have however been reported for parasites closely related to *Sarcocystis spp*. For example, there is evidence that increased soil pH decreases the probability of detecting oocysts of *Cryptosporidium spp*. in the soil (Barwick *et al.* 2003), although another study found no evidence for a relationship between soil pH and *Cryptosporidium spp.* oocyst viability (Kato *et al.* 2004). Similarly, soil clay content has previously been suggested, to influence the survival of *Toxoplasma gondii* oocyst in the environment (Van Knapen *et al.* 1995), and *Cryptosporidium parvum* oocysts are known to

associate with clay particles over time (McLaughlin *et al.* 2013) due to their higher cation exchange capacity (Balthazard-Accou *et al.* 2014), although experimental studies have suggested that *Cryptosporidium parvum* oocysts have reduced survival in silt clay loam, compared to silt loam and loamy sand (Jenkins *et al.* 2002). If the viability of *Sarcocystis spp.* sporocysts does decrease with increasing soil pH, as suggested by our study, the spreading of agricultural lime across pastures may be used to mitigate macroscopic sarcocystosis in sheep. Agricultural lime is commonly spread across pastures to increase soil pH and reverse soil acidification (Haynes and Naidu 1998) and could additionally be used to decrease the survival of *Sarcocystis spp*. sporocysts in the soil. The re-application of lime may however be necessary for long-term reductions in disease occurrence, deeming it un-feasible. Furthermore, the effectiveness of agricultural lime to reduce the survival of *Sarcocystis spp*. sporocysts at a large scale remains to be tested.

We found that the average count of frost days per annum (minimum daily temperature ≤ 0 ^{o}C) did not influence the density of sarcocystosis-positive farms, consistent with previous findings that *Sarcocystis spp.* sporocysts are resistant to freezing (Leek 1986; McKenna and Charleston 1992). *Sarcocystis spp.* sporocysts have previously been suggested to be killed by desiccation (McKenna and Charleston 1994; Savini *et al.* 1996) and candidate explanatory variables were selected based on predictions that they may influence the time to desiccation of sporocysts. However, the availability and resolution of candidate explanatory variable data may have negatively impacted on our ability to detect interacting factors and to more precisely tease out the influence of two competing variables in our analysis, for example the influence of rainfall or temperature Vs. cat density on sarcocystosis risk. We suspect that an adequate cat density data layer would be particularly beneficial in future analyses to help tease out possible variable interactions. Whilst soil sand content appeared to influence sarcocystosis risk in the rhohat plots, spikes in the plot corresponded with soil sand content values found across the majority of Kangaroo Island, explaining why this variable was not included in our reduced model.

We did not include a spatial dependence term in our model, due to the empirical variogram of the standardised residuals from the fitted model demonstrating no evidence of spatial clustering without the inclusion of this term, but we did identify spatial dependence in our data out to a distance of approximately 500 m using Ripley's empirical K-function. Spatial

dependence is most commonly observed for infectious diseases, and represents the influence of an infected property/animal on the disease status of surrounding properties/animals. Sarcocystosis is however a non-infectious disease, and hence spatial dependence in our data suggests that cats consume *Sarcocystis* sarcocysts in infected sheep on one property and subsequently shed infective sporocysts into the environment on adjacent properties out to a distance of approximately 500m. Whilst cat home ranges throughout our study region are known to have a greater radius than 500m (Bengsen *et al.* 2012), this distance may be broadly representative of the radius of the average cats' core home range area, particularly on Kangaroo Island where cat density is high (Taggart *et al.* In Press. [Chap 7]) and the majority of sarcocystosis-positive farms cluster.

Identifying the possible influence of soil pH and clay content on sarcocystosis is one example of where landscape scale studies can provide insights into little known aspects of the ecology of cat-borne diseases. One obvious limitation to conducting landscape scale studies is the availability of suitable landscape data of adequate scale and resolution. For example, we could not access an appropriate cat density or ultraviolet radiation layer that provided sufficient scale, resolution, quality, and variability across our study area to be of use in risk factor analysis; despite both variables being hypothesised to be important in explaining the distribution of sarcocystosis. In the absence of landscape scale data, proxies can provide appropriate insights if a strong association between the proxy and the outcome of interest has been demonstrated. The *Sarcocystis spp.* responsible for the development of macroscopic sarcocystosis in sheep are particularly closely related to *Toxoplasma gondii*, and both share similar biology and lifecycles. Consequently, we would predict that macroscopic sarcocystosis in sheep could potentially be utilised as a proxy of *T. gondii* infection in sheep, and that our findings are of relevance to other protozoal parasites other than *Sarcocystis spp*., although this association remains to be tested.

In our study, the influence of region on macroscopic sarcocystosis in sheep indicates unequal economic impacts throughout the sheep industry for this disease. We are aware of one regional slaughterhouse in South Australia that no longer processes Kangaroo Island sheep greater than two years of age due to its high occurrence of macroscopic sarcocystosis. Whilst we have highlighted two potential methods of reducing sarcocystosis burden in sheep, it is likely that cat management is currently the most feasible and sustainable.

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Conflict of interest statement:

We declare no conflict of interest.

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2.8 Chapter 2 supplementary material:

Table S2.1: Model selection process showing backwards step-wise variable selection considering AIC values. Bold model coefficients and p-values indicates statistical significance.

	Model 1		**Model 2**		**Model 3**		**Model 4**	
Variable	Coefficient (SE)	P-value	Coefficient (SE)	P-value	Coefficient (SE)	P-value	Coefficient (SE)	P-value
Intercept	11.52 (0.54)		11.66 (0.46)		11.63 (0.46)		11.49 (0.43)	
Soil pH	**-0.15 (0.05)**	**0.005**	**-0.16 (0.05)**	**0.004**	**-0.15 (0.05)**	**0.004**	**-0.15 (0.05)**	**0.003**
Average annual rainfall (mm)								
< 480	Reference							
≥ 480	0.12 (0.23)	0.61						
Average annual frost days								
< 1	Reference		Reference					
≥ 1	-0.11 (0.20)	0.59	-0.10 (0.20)	0.62				
Soil clay content (%)								
< 14.5	Reference		Reference		Reference		Reference	
≥ 14.5 < 16.5	0.20 (0.14)	0.14	0.20 (0.14)	0.14	0.20 (0.14)	0.14	0.24 (0.13)	0.07
≥ 16.5	0.29 (0.16)	0.07	0.29 (0.16)	0.06	0.30 (0.16)	0.06	**0.37 (0.14)**	**0.008**
Soil sand content (%)								
< 73	Reference		Reference		Reference			
≥ 73 < 77.2	-0.15 (0.16)	0.35	-0.15 (0.16)	0.32	-0.15 (0.16)	0.35		
≥ 77.2	-0.20 (0.17)	0.24	-0.20 (0.17)	0.22	-0.20 (0.17)	0.23		
Pic region								
Adelaide Hills/Fleurieu Peninsula	Reference		Reference		Reference		Reference	
Barossa Valley/Lower North	0.17 (0.45)	0.71	0.11 (0.43)	0.80	0.07 (0.43)	0.87	0.09 (0.43)	0.84
Eyre Peninsula	-0.30 (0.43)	0.48	-0.38 (0.40)	0.33	-0.39 (0.40)	0.32	-0.44 (0.39)	0.27
Kangaroo Island	**2.76 (0.33)**	**<0.001**	**2.77 (0.33)**	**<0.001**	**2.78 (0.33)**	**<0.001**	**2.72 (0.33)**	**<0.001**

Lower South East	0.26 (0.45)	0.55	0.27 (0.45)	0.55	0.24 (0.44)	0.59	0.22 (0.44)	0.62
Mid-South East	0.00 (0.45)	1.00	0.00 (0.45)	1.00	-0.06 (0.43)	0.90	-0.08 (0.43)	0.85
Murray Mallee	0.06 (0.47)	0.90	-0.05 (0.42)	0.91	-0.11 (0.40)	0.78	-0.18 (0.40)	0.65
Northern Pastoral	-0.70 (0.56)	0.21	-0.81 (0.52)	0.12	-0.89 (0.50)	0.77	-0.85 (0.50)	0.88
Upper-South East	0.10 (0.44)	0.83	0.06 (0.44)	0.90	-0.02 (0.41)	0.97	-0.07 (0.41)	0.86
Yorke Peninsula/Mid-North	-0.14 (0.43)	0.74	-0.23 (0.40)	0.56	-0.28 (0.38)	0.46	-0.29 (0.38)	0.45
AIC	4034.8		4033.1		4031.3		4028.8	

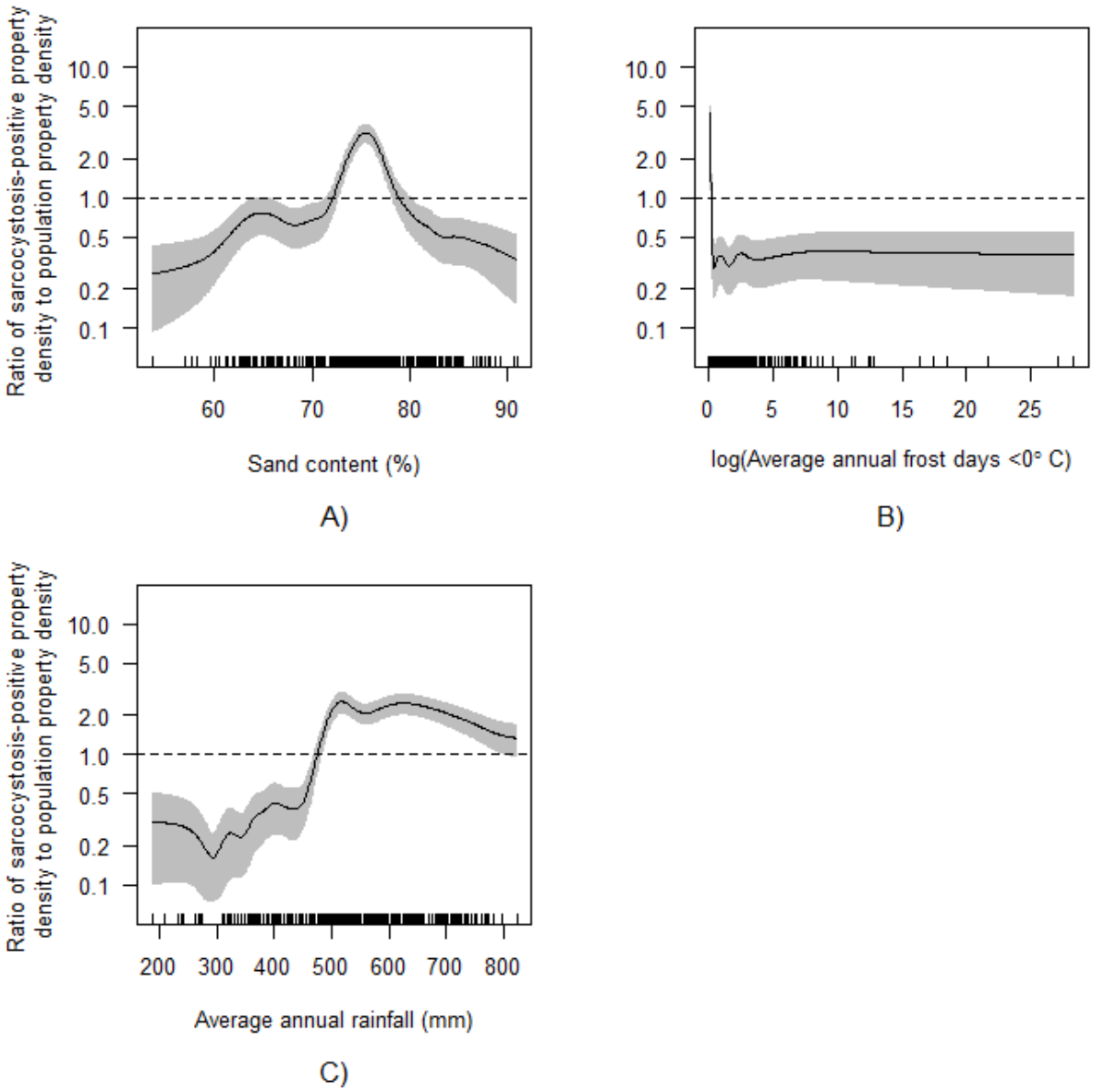

Figure S2.1: Rhohat plots for remaining candidate explanatory variables (excluding region) included in saturated model. Note that all other candidate explanatory variables described in methods were excluded prior to model construction due to coarseness of data or collinearity. Plots show ratio of sarcocystosis-positive farm density to population farm density as a function of soil sand content (A), average annual frost days <0 ^{o}C (B), and average annual rainfall (C), estimated across South Australia using the rhohat procedure. The solid line shows function estimate; grey shading is pointwise 95% confidence band. Vertical dashes along the x-axis represent individual data points, and provide an indication of where the function is based on raw data or purely predictive. Horizontal dashed line represents null association (intensity of sarcocystosis-positive farms equals the intensity of all farms at risk). Interpretation: for those areas in the study area where average annual rainfall was ~500mm the intensity of sarcocystosis-positive farms was ~2 times that of all farms at risk. Data represent 4,204 sheep farms during the period 2007-2017.

Chapter 3: Oesophageal sarcocystosis observed at slaughter provides a reliable and efficient proximate measure of *Toxoplasma gondii* seroprevalence in sheep

Statement of Authorship

Title of Paper	Oesophageal sarcocystosis observed at slaughter provides a reliable and efficient proximate measure of *Toxoplasma gondii* seroprevalence in sheep
Publication Status	☐ Published ☐ Accepted for Publication ☑ Submitted for Publication ☐ Unpublished and Unsubmitted w ork w ritten in manuscript style
Publication Details	Taggart, P. L., McAllister, M. M., Rutley, D. & Caraguel, C. G. B. Oesophageal sarcocystosis observed at slaughter provides a reliable and efficient proximate measure of *Toxoplasma gondii* seroprevalence in sheep. IN REVIEW – Small Ruminant Research.

Principal Author

Name of Principal Author (Candidate)	Patrick Taggart		
Contribution to the Paper	Formulated experimental study design. Sample collection, processing and testing. Formatted, edited, analysed and interpreted data. Wrote and revised manuscript.		
Overall percentage (%)	90		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	4th March 2019

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate in include the publication in the thesis; and

iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Milton McAllister		
Contribution to the Paper	Contributed to experimental study design. Some sample collection. Co-interpreted data. Revised manuscript. Co-supervised PhD candidate.		
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Name of Co-Author	David Rutley		
Contribution to the Paper	Facilitated access to slaughterhouse. Co-interpreted data. Revised manuscript.		
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Name of Co-Author	Charles Caraguel		
Contribution to the Paper	Formulated experimental study design. Some sample collection. Co-analysed and co-interpreted data. Revised manuscript. Supervised PhD candidate.		
Signature		Date	4th March 2019

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Oesophageal sarcocystosis observed at slaughter provides a reliable and efficient proximate measure of *Toxoplasma gondii* seroprevalence in sheep

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Keywords: *Sarcocystis*, *Toxoplasma*, *Ovis aries*, proxy, surveillance, risk indicator

3.1 Abstract:

Toxoplasmosis is a cat-borne parasitosis caused by the protozoal parasite *Toxoplasma gondii*, and is a major cause of reproduction failure in sheep. An essential part of successful disease management is effective surveillance. Slaughterhouse inspections provide opportunities to efficiently collect regular disease data from many animals across large geographic areas. However, *T. gondii* is not visually detectable and cannot be recorded at slaughterhouses. Macroscopic sarcocystosis is a disease of sheep that is visually detectable at slaughter, and is caused by two protozoan parasites that share similar lifecycles and ecology with *T. gondii*. We investigated if macroscopic sarcocystosis could act as a proximate measure for *T. gondii* infection in sheep to facilitate the efficient surveillance of *T. gondii* at large-scales. We sampled 560 sheep from 30 different farms and compared the presence of macroscopic sarcocystosis to *T. gondii* serostatus at the animal- and farm-level. At the animal-level, we found a weak association between *T. gondii* seropositivity and the presence of macroscopic sarcocysts in the oesophagus, OR = 1.76 (95 % CI: 1.17, 2.65; $_{\text{McFadden's}}$ R^2 = 0.01). At the farm-level, the seroprevalence of *T. gondii* was strongly correlated with oesophageal sarcocystosis prevalence (slope = 0.82, 95 % CI: 0.55, 1.09; R^2 = 0.56). No association was found between *T. gondii* serology and the presence of macroscopic

sarcocystosis in skeletal muscles at either the animal- or farm-level. For *T. gondii* surveillance in sheep at the farm-level, routine slaughter inspection and recording of macroscopic sarcocystosis in the oesophagi could be a reliable and efficient proximate measure.

3.2 Introduction:

Toxoplasmosis, the disease caused by the protozoal parasite *Toxoplasma gondii*, is recognised as one of the main infectious causes of sheep (*Ovis aries*) abortion (Dubey and Beattie 1988). Pregnancy and birthing problems due to toxoplasmosis predominately occur when a ewe is first infected during pregnancy. It is suggested that up to 23 % of aborted sheep foetuses result from *T. gondii* infection (Dubey 2009). *Toxoplasma gondii* infection is also associated with increased stillbirth and neonatal death (Dubey 2009). Consequently, toxoplasmosis is responsible for substantial economic losses to sheep producers. For example, the New Zealand sheep industry was estimated to have lost NZ$14 million due to *T. gondii* infection in 1992 (Charleston 1994).

To successfully manage disease, it is essential to implement effective surveillance (Thrusfield 2005). Effective disease surveillance requires an easy to conduct, high throughput and accurate method of data collection, which achieves extensive coverage of the population through both time and space. *Toxoplasma gondii* can be difficult to detect directly because of its patchy distribution within hosts (Parameswaran *et al.* 2009). The presence of *T. gondii* in a host is most frequently determined indirectly via the detection of immunoglobulin (Ig) G or IgM antibodies against the parasite in serum. Although serology is a well-established diagnostic procedure, it is not practical at large scales due to the cumulative labour and cost requirements; thus there is a need for alternative detection methods for monitoring *T. gondii* infection in sheep at these scales.

Within the sheep industry, visual inspection of carcasses at slaughterhouses provides an opportunity to efficiently collect disease information at large scales (Thrusfield 2005). However, *T. gondii* causes microscopic tissue cysts that are not detectable visually during slaughter inspection. Macroscopic sarcocystosis is a similar parasitosis that causes visually detectable lesions in sheep. This condition is caused by *Sarcocystis gigantea* or *S. medusiformis*, both of which are closely related to *T. gondii*. All three parasites (*T. gondii*, *S. gigantea*, *S. medusiformis*) have two-host predator-prey lifecycles. They share the same

definitive host, the domestic cat (*Felis catus*), that when infected sheds an egg type stage of the parasite in their faeces (Collins *et al.* 1979; Dubey *et al.* 1986). Sheep act as an intermediate host for all three parasites, and are infected by consuming contaminated food, water or soil (Aramini *et al.* 1999; Ford 1986; Hill and Dubey 2002). Within sheep, *T. gondii* forms microscopic cysts in muscle and neurological tissues, containing bradyzoites. *Sarcocystis gigantea* and *S. medusiformis* infection in sheep results in the development of large macroscopic cysts (sarcocysts) in the musculature, containing bradyzoites (Munday and Obendorf 1984; Obendorf and Munday 1987). Sarcocysts are trimmed during post-slaughter processing for aesthetic reasons (Australian and New Zealand Food Regulation Ministerial Council. Food Regulation Standing Committee 2007). Bradyzoites of each species remain dormant in the sheep's tissues for the life of the animal, and the parasites' lifecycle is completed when a cat consumes contaminated tissues of an infected sheep. These similarities in the biology of *T. gondii* and macroscopic sarcocystosis, suggests that they may co-occur, this is to say that sheep exposed to cat faeces may be more likely to contract either or both parasitoses. If this is the case, then visually detectable macroscopic sarcocysts could act as a proximate measure for *T. gondii* surveillance in sheep populations.

To explore the association between *T. gondii* and macroscopic sarcocystosis infection in sheep, we recorded the presence of sarcocysts in slaughtered sheep and simultaneously collected blood for *T. gondii* serology. If the suspected association is confirmed, sarcocystosis surveillance data from sheep slaughterhouses may provide an efficient means to indirectly monitor the occurrence of *T. gondii* in sheep consignments with extensive time and space coverage. Information on macroscopic sarcocystosis prevalence is currently available at large-scales in some countries (Primary Industries and Regions South Australia 2019), although not available universally (Martínez-Navalón *et al.* 2012). This existing data may prove advantageous for the management of *T. gondii* in sheep or when assessing the impact of feral cat control programs on the cat-borne disease burden in sheep.

3.3 Materials and methods:

3.3.1 Sampling and data collection

We conducted a cross-sectional survey at the Thomas Foods International slaughterhouses in Murray Bridge and Lobethal, South Australia, during the December 2016-January 2017 and February-March 2018 periods respectively. Only sheep originating from Kangaroo Island,

South Australia, were sampled, as both *T. gondii* infection and macroscopic sarcocystosis are known to be prevalent on the island (O'Donoghue *et al.* 1987; Taggart *et al.* 2019. [Chap 2]) due to its high cat density (Taggart *et al.* In Press. [Chap 7]). The inclusion of sheep from Kangaroo Island only, minimised the probability of sampling largely nil or low prevalence consignments and improved the probability of covering the full prevalence spectrum, from 0-100 %, for both parasitoses. Sheep greater than two years of age (sheep with more than two adult teeth) were targeted because the visual detection of macroscopic sarcocystosis is easier in older animals where cysts have had the time to develop to a larger size (Munday and Obendorf 1984).

At the study slaughterhouses, sheep carcasses were visually inspected by certified meat inspectors. When macroscopic sarcocystosis was detected in skeletal muscles, carcasses were stamped and redirected for trimming. Within sampled consignments, every tenth animal was selected along the processing line according to a systematic random sampling protocol. Investigators were able to record the presence of sarcocystosis stamps on the selected carcasses, collect heart blood, and observe the presence of sarcocystosis in the corresponding oesophagus. Sheep consignments were identified by a unique consignment number and property identification code. The property identification code allows consignments to be traced back to the farm of origin.

Blood samples were allowed to clot at ambient temperature for approximately five hours prior to being centrifuged at 4,000 g for five minutes. Sera were stored at -20 ^{o}C for up to 12 months prior to serological testing. Serum samples experienced 1 freeze/thaw cycle prior to testing.

3.3.2 Toxoplasma gondii antibody test

Sera were tested for *T. gondii* IgG antibodies using a commercially available modified agglutination test (MAT) following the manufacturers protocol (Toxo-Screen DA, BioMerieux, Marcy-l'Etoile, France). The MAT is based on the direct agglutination of fixed *T. gondii* tachyzoites of the RH strain, with sera pre-treated with 2-mercaptoethanol to neutralise IgM antibodies (Desmonts and Remington 1980; Dubey and Desmonts 1987). Immunoglobulin G antibodies typically take greater than one week to develop, but once developed persist for the remainder of the animal's life, consequently this test may give false negative results

during early stages of acute infection (Dubey and Crutchley 2008). Sera were screened at 1:40 and 1:4,000 dilutions, and classified as positive if agglutination occurred at either dilution. The diagnostic sensitivity and specificity of the MAT in sheep is 92.6 % (95 % CI: 85.2 %, 96.9 %) and 95.5 % (95% CI: 89.9 %, 98.7 %) respectively (Mainar-Jaime and Barberan 2007). With each assay we included positive and negative control sera from known infected (challenged with approximately 7,000 oocysts) and uninfected sheep, and a second negative phosphate buffered saline control sample. A sheep was classified as positive if the observed agglutination of *Toxoplasma* formed a mat covering about half or more of the well base. We are not aware of any serologic cross-reactivity problems with the MAT (Dubey 2016b; Gondim *et al.* 2017), and there is no vaccine for *T. gondii* within Australia which could potentially confound the presence of antibodies in sheep.

3.3.3 Data analysis

All analyses were implemented within the lme4 (Bates *et al.* 2015) package within the statistical program R version 3.5.1 (R Core Team 2018). To compare parasite prevalences, we used McNemar's chi-squared test to account for paired data.

Animal-level association

We use 'animal-level' to refer to the comparison of infection status within individual animals. Animal-level analyses were conducted using mixed effect logistic regression to assess the strength of association (using odds ratios) between *T. gondii* serostatus and the presence of macroscopic sarcocystosis in skeletal muscles, oesophagus, or oesophagus and/or skeletal muscles. We use 'oesophagus and/or skeletal muscles' to represent macroscopic sarcocystosis presence in an animal irrespective of site. Each model included *T. gondii* serostatus as the dependent variable and macroscopic sarcocystosis as a fixed effect. To account for expected clustering of *T. gondii* serostatus within-farms, farm was included as a random effect. Consignment was not included as a random effect as it was highly collinear with farm (mostly one consignment per farm). To explore the pathogenesis of *S. gigantea* and *S. medusiformis*, we additionally estimated the association between macroscopic sarcocystosis presence in skeletal muscles and in the oesophagus using the same mixed model structure. For all mixed effect logistic regression models, we converted subject-specific odds ratios to population-averaged odds ratios to allow interpretation of the influence of fixed effects across all farms using the formula (Dohoo *et al.* 2009b):

$B^{PA} = B^{SS} / \sqrt{(1 + 0.346 * \sigma^2)}$ (Eq.1)

Where B^{PA} is the population-averaged coefficient, B^{SS} is the subject-specific coefficient, and σ^2 is the farm-level variance. McFadden's pseudo R^2 was calculated for each model to assess goodness of fit (McFadden 1973).

Farm-level association

We averaged individual animal infection status data within each farm to obtain within-farm prevalence. We use 'farm-level' to refer to the comparison of the two parasitoses within an individual farm. We corrected for MAT misclassification by calculating the true within-farm *T. gondii* seroprevalence using the formulae (Dohoo *et al.* 2009a):

$seroprevalence_{true} = (seroprevalence_{apparent} + DSp - 1) / (DSe + DSp - 1)$

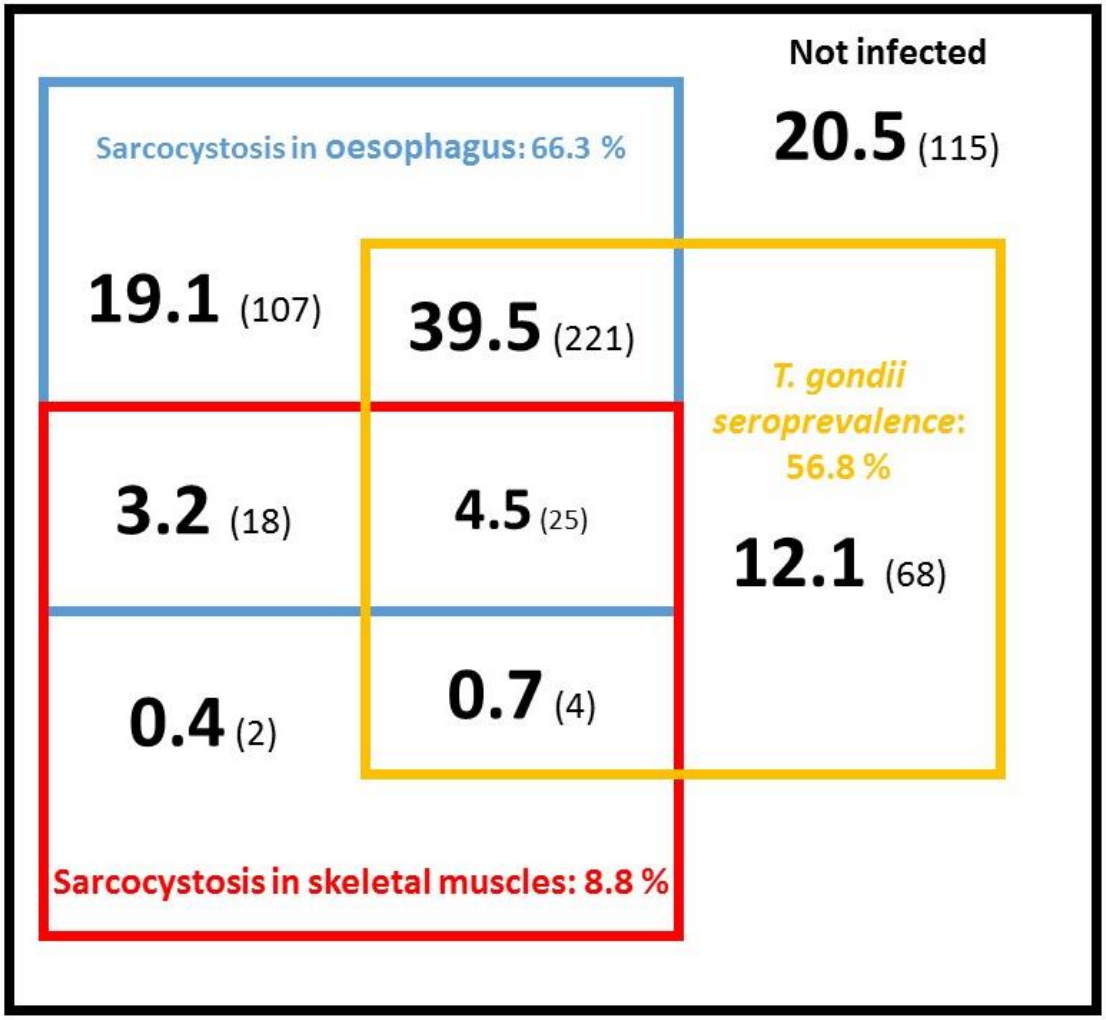
(Eq.2)

where *DSe* is diagnostic sensitivity and *DSp* is diagnostic specificity of the MAT.

Farm-level analyses were conducted using linear regression. We included *T. gondii* within-farm seroprevalence as the dependent variable and macroscopic sarcocystosis within-farm prevalence in skeletal muscles, oesophagus, or oesophagus and/or skeletal muscles as the explanatory variable. Individual farms were allocated weights to account for variation in the number of sheep sampled from each farm.

3.4 Results:

We randomly sampled approximately 10% of one or more consignments from approximately 10% of Kangaroo Island farms, totalling 560 sheep from 30 different farms (median number of sheep sampled per farm: 15, range of the number of sheep sampled per farm: 2-50). For 22 farms we sampled only a single consignment, and for 8 farms we sampled two consignments. The apparent prevalence of macroscopic sarcocystosis in the oesophagus of sheep (66.3%) was higher than the seroprevalence of *T. gondii* (56.8%) ($_{McNemar's}$ $p < 0.001$), but *T. gondii* seroprevalence was higher than the apparent prevalence of macroscopic sarcocystosis in skeletal muscles (8.8%) ($_{McNemar's}$ $p = < 0.001$) (Figure 3.1). Forty five percent of sampled sheep had coinfection with both *T. gondii* and *Sarcocystis spp* (Figure 3.1). Macroscopic sarcocystosis was much more prevalent in the oesophagus (66.3%) than in skeletal muscles (8.8%) ($_{McNemar's}$ $p <0.001$) (Figure 3.1).



Note: *Toxoplasma gondii* seroprevalence represents the number of infected and uninfected animals prior to adjusting for MAT diagnostic sensitivity and specificity.

Figure 3.1: Venn diagram showing percentage of sheep infected with sarcocystosis and *Toxoplasma gondii*. Numbers in brackets represent the total number of sheep sampled.

3.4.1 Animal-level association

We found significant associations at the animal-level between *T. gondii* serostatus and the presence of macroscopic sarcocystosis in the oesophagus, or oesophagus and/or skeletal muscles (Table 3.1, models B and C), but no association with sarcocystosis in skeletal muscles (Table 3.1, model A). For approximately 65% of sheep, their macroscopic sarcocystosis status matched their *T. gondii* sero-status (65.2% match when considering sarcocystosis in the oesophagus and/or skeletal muscles, 64.5% match when considering sarcocystosis in oesophagus) (Figure 3.1). When the association was significant, macroscopic sarcocystosis did not explain a large amount of the variability in *T. gondii* based on McFadden's pseudo R^2 values (Table 3.1, model B and C), which is said to be > 0.2 when

logistic model fit is good (Hensher and Stopher 1979). We additionally found a significant association between the presence of macroscopic sarcocystosis in the oesophagus and in skeletal muscles at the animal level (Table 3.1, model D). This model explained a larger amount of the variability in our data based on McFadden's pseudo R^2.

Table 3.1: Mixed effect logistic regression models exploring the association between *Toxoplasma gondii* seropositivity and macroscopic sarcocystosis presence at the animal-level. The association between the locations of detection of macroscopic sarcocystosis was also explored.

Model	Response disease	Explanatory disease	Odds Ratio$_{SS}$ (95% CI)	Variance of 'consignment' random effect	Odds Ratio$_{PA}$ (95% CI)	*P*-value	R^2
A	*T. gondii* serostatus	Macro. sarcocystosis in skeletal muscles	0.87 (0.44, 1.73)	1.11	0.89 (0.50, 1.60)	0.70	0.0002
B	*T. gondii* serostatus	Macro. sarcocystosis in oesophagus	1.86 (1.19, 2.92)	0.90	1.76 (1.17, 2.65)	0.01	0.01
C	*T. gondii* serostatus	Macro. sarcocystosis oesophagus &/or skeletal muscles	1.97 (1.25, 3.11)	0.90	1.86 (1.23, 2.80)	0.00	0.01
D	Macro. sarcocystosis in skeletal muscles	Macro. sarcocystosis in oesophagus	3.70 (1.47, 9.32)	0.57	3.19 (1.41, 7.25)	0.01	0.53

Note: analyses described in table are based on 560 sheep greater than two years of age from 30 different farms on Kangaroo Island, South Australia.
$_{SS}$: subject specific estimate
$_{PA}$: population averaged estimate
R^2: McFadden's pseudo R^2

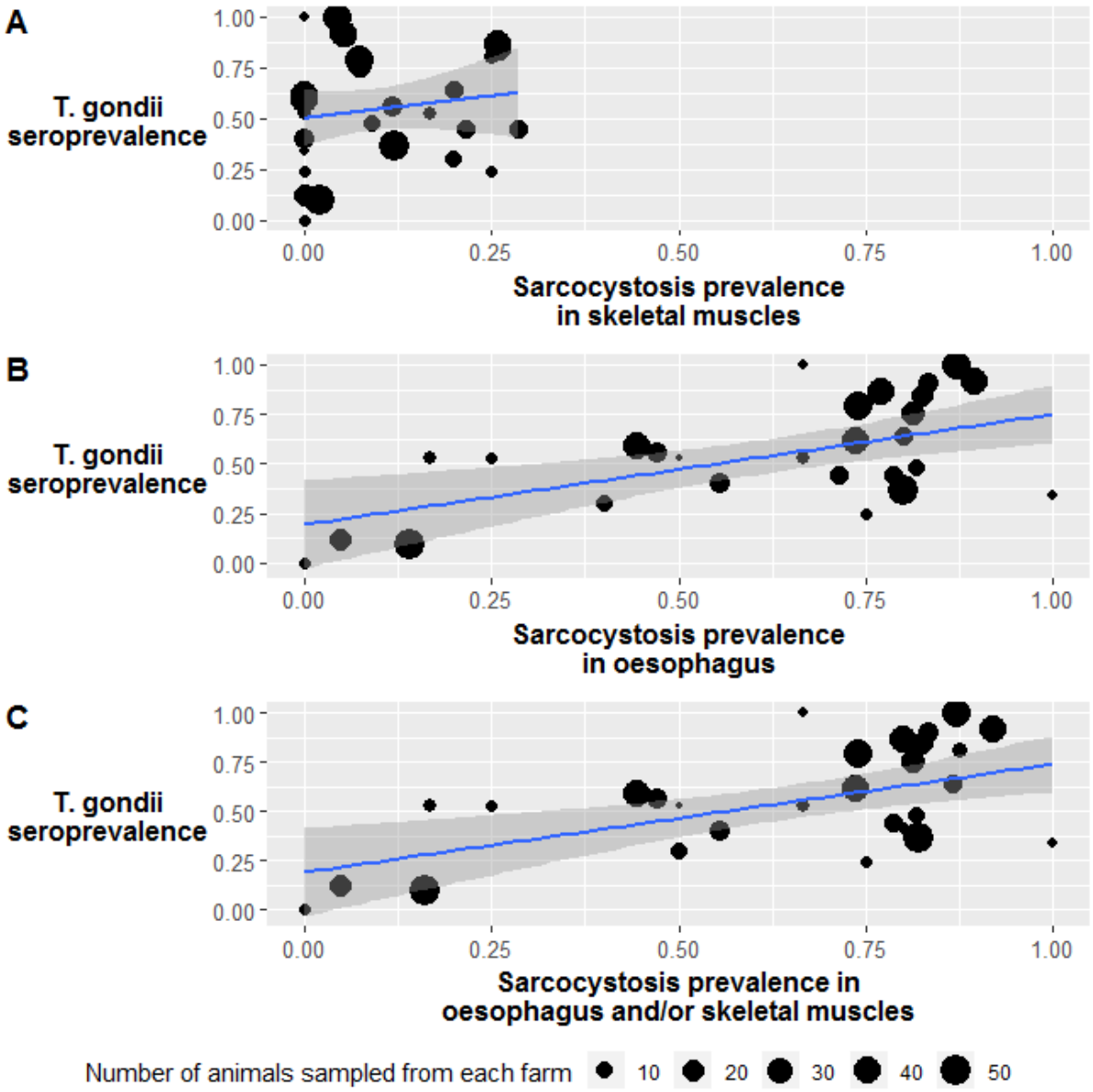
3.4.2 Farm-level association

We found a significant correlation between *T. gondii* seroprevalence and macroscopic sarcocystosis prevalence in the oesophagus, or oesophagus and/or skeletal muscles (Table 3.2, model F and G; Figure 3.2B and 3.2C), but not when considering macroscopic sarcocystosis in skeletal muscles (Table 3.2, model E; Figure 3.2A). The two models including macroscopic sarcocystosis in the oesophagus, or oesophagus and/or skeletal muscles in the sheep (Table 3.2, models F and G), explained a reasonable amount of the variability in our data based on R^2 values obtained from linear regression.

Table 3.2: Weighted linear regression models exploring the association between *Toxoplasma gondii* seroprevalence and macroscopic sarcocystosis prevalence at the farm-level.

Model	Outcome variable	Explanatory variable	Slope (95% CI)	Intercept (95% CI)	*P*-value	R^2
E	*T. gondii* serostatus	Macro. sarcocystosis in skeletal muscles	0.62 (-0.50, 1.74)	0.54 (0.40, 0.68)	0.29	0.04
F	*T. gondii* serostatus	Macro. sarcocystosis in oesophagus	0.82 (0.55, 1.09)	0.07 (-0.12, 0.25)	<0.001	0.56
G	*T. gondii* serostatus	Macro. sarcocystosis in oesophagus &/or skeletal muscles	0.80 (0.52, 1.07)	0.07 (-0.13, 0.26)	<0.001	0.54

Note: analyses described in table are based on 560 sheep greater than two years of age from 30 different farms on Kangaroo Island, South Australia.



Note: Data obtained from 560 sheep greater than two years of age from 30 different farms on Kangaroo Island, South Australia.

Figure 3.2: Linear regression (blue line) and 95% CI band (grey shading) showing relationship between *Toxoplasma gondii* corrected seroprevalence and macroscopic sarcocystosis prevalence at the farm-level.

3.5 Discussion:

When health information are unavailable or difficult to obtain, proximate measures can be useful alternatives to monitor disease progress over time and space. However, to be of value, proximate measures must demonstrate a strong and systematic association with the true measure of interest. Through recording macroscopic sarcocystosis presence in sheep and simultaneously collecting blood for *T. gondii* serology, we show an association between

these two parasitoses. At the animal-level, relatively low odds ratios and R^2 values, moderate agreement (65%) between sarcocystosis and *T. gondii* status, and significant McNemar's tests suggest these associations were not strong. However, this association was strong at the farm-level and when considering oesophagi sarcocysts (slope: 0.82, R^2: 0.56). Therefore, recording oesophageal macroscopic sarcocystosis in sheep at slaughterhouses functioned as a reliable and efficient proximate measure of *T. gondii* seroprevalence. However, these records should be averaged, interpreted and monitored at the farm-level.

Both macroscopic sarcocystosis in the oesophagus and *T. gondii* infection co-occur with cats, and are associated via this common link. The relationship between these two parasitoses via a common exposure as opposed to an interactive relationship would explain why the relationship increased in strength at a higher level (farm-level Vs. animal-level). At the animal-level a strong association between both parasitoses would occur if a large majority of sheep had matching infection statuses. This could occur due to the co-occurrence of both parasitoses or an interactive relationship where one infection increases the probability of the other. However, if one infection influenced the probability of the other, our farm-level association would be driven largely by processes occurring at the animal-level, and hence we would expect a similar strength of association at both the animal- and farm-levels. Instead, our results suggest that the relationship between these two parasitoses is primarily driven by co-occurrence at the farm-level.

When macroscopic sarcocystosis was observed in skeletal muscles, there was no association with *T. gondii* infection. Few sheep (8.8%) had visible sarcocysts in skeletal muscles, whist over half (56.8%) were seropositive to *T. gondii* (Figure 3.1). This may be explained by other findings and further understanding the parasite lifecycles. We found an association between macroscopic sarcocystosis in skeletal muscles and oesophagus, suggesting that that they do not occur independently. *Sarcocystis gigantea* cysts are first visible in the oesophagus, larynx and tongue, and later in the infection, are detectable in skeletal muscles (Dubey *et al.* 2015b; Munday and Obendorf 1984), either due to the slower growth of cysts in skeletal muscles or the subsequent spread of the parasite to skeletal muscles. In contrast, *S. medusiformis* cysts are first visible in the laryngeal muscles, abdominal muscles and diaphragm; their subsequent detection in other tissues is not well documented (Dubey *et al.* 2015b; Obendorf and Munday 1987). As only 12 % of sheep (6 of 49) showed cysts in skeletal muscles only

without cysts in the oesophagus (Figure 3.1), we suspect that the majority of macroscopic sarcocystosis in skeletal muscles of sheep (43 of 49, 88%) is due to the progression of an initial *S. gigantea* infection in the oesophagus, rather than *S. medusiformis* directly infecting skeletal muscles. The occurrence of sarcocystosis in the skeletal muscles, in the majority of cases, cannot therefore be associated with exposure to cat faeces/sporocysts, but is rather due to the stage or severity of an infection with *S. gigantea*. Hence, the lack of association between sarcocystosis in skeletal muscles and *T. gondii* seroconversion observed in our study.

The seroprevalence of *T. gondii* infection, and prevalence of macroscopic sarcocystosis in the oesophagus and skeletal muscles additionally highlight interesting aspects of parasite ecology and areas for future research. The prevalence of macroscopic sarcocystosis in the oesophagus of sheep was higher than its prevalence in skeletal muscles and *T. gondii* seroprevalence. This higher prevalence of macroscopic sarcocystosis in the oesophagus relative to in skeletal muscles of sheep suggests that *S. gigantea* is more transmissible than *S. medusiformis*. The relative transmissibility of these two parasites may be explained by oesophageal sarcocysts being more easily accessed and consumed by cats compared to those on or in skeletal muscles. Alternatively, the higher prevalence of macroscopic sarcocystosis in the oesophagus of sheep compared to *T. gondii* infection may be explained by the frequency with which cats shed these parasites. *Sarcocystis gigantea* sporocysts are shed by cats following each exposure to the parasite (Dubey *et al.* 2015a), although *T. gondii* oocysts are predominately shed only once in a cats lifetime following their initial infection (Dubey 2016b). This would result in more consistent shedding of *S. gigantea* sporocysts by cats, and more intermittent shedding of *T. gondii* oocysts, resulting in the environmental burden of macroscopic sarcocystosis being higher than that for *T. gondii*.

The high seroprevalence of *T. gondii* documented in this study is, to our knowledge, equivalent to the highest seroprevalence recorded in Australia. Munday (1975) recorded a *T. gondii* seroprevalence of 61.7 % in 144 sheep from Tasmania. Kangaroo Island and Tasmania are two of Australia's three largest Islands; both are known to have a high seroprevalence of *T. gondii* in cats (Fancourt and Jackson 2014). Kangaroo Island is additionally known to have a high seroprevalence of *T. gondii* in macropods (Taggart *et al.* In Press. [Chap 4]), and a high abundance of cats (Taggart *et al.* In Press. [Chap 7]), and Tasmania is known to have a high

seroprevalence of *T. gondii* in quolls (Fancourt *et al.* 2014). As *T. gondii* can infect and cause health problems in most warm-blooded animals (Dubey 2016a), the high seroprevalence of *T. gondii* in sheep, cats, macropods and quolls, and the high abundance of cats on these islands, suggests a high risk of infection and health problems associated with *T. gondii* for other livestock, wildlife and humans. On Tasmania, evidence exists in macropods demonstrating that *T. gondii* impacts the health of other species (Obendorf and Munday 1983), but on Kangaroo Island little is known of the prevalence or impacts of *T. gondii* in other species. It is thus important to determine if other species on Kangaroo Island, including humans, experience high rates of infection with, or adverse health impacts from *T. gondii* infection.

In South Australia, meat inspectors estimate and record the within-consignment prevalence of macroscopic sarcocystosis in the skeletal muscles of sheep only, not in the oesophagi. Our findings suggest that the prevalence of macroscopic sarcocystosis in sheep oesophagi at the farm-level provides a more reliable proximate measure of *T. gondii* seroprevalence. Accordingly, we suggest to record macroscopic sarcocystosis in the oesophagi of sheep at slaughterhouses and validate oesophageal sarcocystosis as a proximate measure for *T. gondii* seroprevalence at the farm-level elsewhere. This will enable the monitoring of *T. gondii* in sheep populations at minimal cost to the industry and authorities, and prove advantageous when assessing the impact of *T. gondii* control programs in sheep.

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Conflict of interest statement:

We declare no conflict of interest.

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Chapter 4: Variation in *Toxoplasma gondii* seroprevalence: effects of site, sex, species and behaviour between insular and mainland macropods

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Name of Principal Author (Candidate)	Patrick Taggart		
Contribution to the Paper	Formulated experimental study design. Sample collection, processing and testing. Formatted, edited, analysed and interpreted data. Wrote and revised manuscript.		
Overall percentage (%)	90		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
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Co-Author Contributions

By signing the Statement of Authorship, each author certifies that

i. the candidate's stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate to include the publication in the thesis; and

iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

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Variation in *Toxoplasma gondii* seroprevalence: effects of site, sex, species and behaviour between insular and mainland macropods

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4.1 Abstract:

Context: Feral cats threaten wildlife conservation through a range of direct and indirect effects. However, most studies that evaluate the impacts of feral cats on species of conservation significance focus on direct impacts such as predation; few studies consider the indirect impacts of cat-borne disease. *Toxoplasma gondii*, a cat-borne parasite, causes both acute and latent disease in a range of wildlife species, and macropods are particularly susceptible. Kangaroo Island is Australia's third largest island and supports a high density of feral cats and high seroprevalence of *T. gondii* in multiple species relative to the mainland. This suggests Kangaroo Island has high environmental contamination with the parasite and a high risk of infection for other species.

Aims: We aimed to describe *T. gondii* seroprevalence in culled and road-killed macropods to assess the effects of island vs mainland location, sex, species, and behaviour.

Methods: Macropod sera were tested for *T. gondii* IgG antibodies using a commercially available modified agglutination test.

Key results: The seroprevalence of *T. gondii* in culled western grey kangaroos (*Macropus fuliginosus*) was significantly higher on the island (20%, 11/54 positive) than on the mainland (0%, 0/61 positive). There was no difference in *T. gondii* seroprevalence between culled and road-killed (21%, 21/102 positive) kangaroos from the island. The seroprevalence of *T. gondii* was significantly higher in female (32%, 12/38 positive) than in male (13%, 8/60 positive) kangaroos, but we observed no sex effect in tammar wallabies (*Macropus eugenii*), and no effect of species.
Conclusions: The higher *T. gondii* seroprevalence in insular macropods support previous reports of higher *T. gondii* exposure in other Kangaroo Island fauna. The lack of difference in *T. gondii* seroprevalence between culled and road-killed kangaroos suggests *T. gondii* positive animals are not more vulnerable to road mortality, in contrast to that suggested previously.
Implications: Our findings suggest greater potential adverse conservation impacts due to toxoplasmosis on the island relative to the mainland. In light of a recent study demonstrating higher cat abundance on the island compared to the mainland, the higher observed *T. gondii* seroprevalence in insular macropods is likely a consequence of higher cat density.

4.2 Introduction:

Feral cats (*Felis catus*) threaten wildlife conservation globally through predation, resource competition, disruption of migration and seed dispersal pathways, induced behavioural changes, hybridisation and disease (Medina *et al.* 2014). For example, predation by feral cats has been implicated as the primary cause of failure of the reintroduction of multiple species of macropod (Hardman *et al.* 2016), and competition for food by feral cats is reported to reduce the reproductive success of predatory seabirds (Courchamp *et al.* 2003). When evaluating the mechanisms by which feral cats threaten species of conservation significance, however, the majority of studies focus on the direct and seemingly most apparent impacts of cats, such as predation, and to a lesser extent competition (Medina *et al.* 2014). By comparison, few studies consider the impacts of cat-borne disease when evaluating threats to wildlife.

Toxoplasmosis, the disease caused by the parasite *Toxoplasma gondii*, causes significant health problems in wildlife worldwide. For example, *T. gondii* infection can influence rodent

behaviour (Vyas *et al.* 2007), cause mortality in lagomorphs (Sedlák *et al.* 2000) and new world monkeys (Dietz *et al.* 1997), and cause respiratory, neurological and gastrointestinal problems, and mortality in Australian marsupials (Canfield *et al.* 1990). Macropods are particularly susceptible to *T. gondii* infection and consequently toxoplasmosis, with disease documented in both captive and free ranging populations. For example, wild Tasmanian pademelons (*Thylogale billardierii*) and Bennett's wallabies (*Macropus rufogriseus rufogriseus*) develop severe blindness and incoordination (Obendorf and Munday 1983), and tammar wallabies (*M. eugenii*) experimentally infected with *T. gondii* experience acute mortality (Lynch *et al.* 1993; Reddacliff *et al.* 1993). However, in some other species, the majority of *T. gondii* infections are asymptomatic (Dubey 2016b), therefore it is possible that many infections in macropods may produce no clinical signs.

Felids are the only known definitive host of *T. gondii*. After consuming infected prey, cats shed the oocyst stage in faeces (Hutchison *et al.* 1971). Oocysts are environmentally resistant and remain viable under favourable conditions for 18 months or longer (Frenkel *et al.* 1975; Yilmaz and Hopkins 1972). Any warm blooded animal can act as an intermediate host and becomes infected by consuming food, water or soil contaminated with oocysts (Aramini *et al.* 1999; Hill and Dubey 2002). Within the intermediate host's digestive tract, oocysts transform into tachyzoites, which rapidly divide within the host cells, causing them to rupture and releasing tachyzoites to infect other host cells (Dubey 2016a). Tachyzoites disseminate via blood and lymph, and their rapid multiplication and spread during the early stages of infection may cause tissue necrosis, organ failure, and death in severe cases (acute toxoplasmosis). In less severe cases, the extensive multiplication of tachyzoites is suppressed by the host's immune system, causing the parasite to transform into microscopic cysts in muscle and neurological tissues that contain bradyzoites. Bradyzoites remain dormant in the tissues for life (latent toxoplasmosis); the lifecycle of *T. gondii* is then restarted when a naïve felid consumes bradyzoites in the tissues of an infected intermediate host.

Latent toxoplasmosis may cause behavioural abnormalities in some intermediate host species. For example, in rodents the innate fear of feline pheromones becomes an attraction in latently infected animals (Vyas *et al.* 2007). Behavioural abnormalities due to latent toxoplasmosis have also been speculated to explain differences in the seroprevalence of *T.*

gondii between road-killed and culled macropods by reducing their reaction time (Hollings *et al.* 2013).

Kangaroo Island, in southern Australia, supports a number of threatened and endemic wildlife species, but it also has a high relative abundance of feral cats (14.6 cats site^{-1}) compared to the adjacent mainland (1.39 cats site^{-1}) (Taggart *et al.* In Press. [Chap. 7]). The seroprevalence of *T. gondii* in feral cats (*Felis catus*) and sheep (*Ovis aries*) on the island is substantially higher than on the Australian mainland (Fancourt and Jackson 2014; O'Donoghue *et al.* 1987; Taggart *et al.* In Review. [Chap 3]). This suggests a comparatively high level of environmental contamination with *T. gondii* on the island and high risk of infection to other species. However, in some ecosystems a high seroprevalence of *T. gondii* has been recorded in some species, but not others sampled from the same location (Dubey *et al.* 2006; Murata *et al.* 2018). This highlights the need to investigate *T. gondii* seroprevalence separately for different wildlife species on Kangaroo Island and the adjacent mainland, particularly for species which could be expected to suffer from toxoplasmosis..

We conducted a *T. gondii* serosurvey in macropods on Kangaroo Island and a climatically similar location on the adjacent mainland. Western grey kangaroo (*M. fuliginosus*) from both locations were tested for *T. gondii* exposure in conjunction with culling for population control. Road-killed kangaroos and tammar wallabies (animals killed by collision with motor vehicles along roads) were also tested opportunistically to compare *T. gondii* seroprevalence to animals killed by culling, to investigate the potential for behavioural differences in macropods with latent toxoplasmosis. Based on studies in cats and sheep (Fancourt and Jackson 2014; O'Donoghue *et al.* 1987), we anticipated *T. gondii* seroprevalence to be 2-3 times higher in kangaroos on the island than on the adjacent mainland.

4.3 Materials and methods:

We followed the CONSISE guidelines for the reporting of seroepidemiologic studies outlined in Horby *et al.* (2017).

4.3.1 Study regions

Kangaroo Island (-35.8015 °; 137.9752 °) is located 13.5 km south west of the Fleurieu Peninsula (-35.5886 °; 138.1986 °), the closest region of mainland South Australia. The study areas will be referred to here as 'island' and 'mainland', respectively. Both the island and

mainland sites experience similar Mediterranean climates (Jenkins 1985; Schwerdtfeger 2002) and are comprised of similar land uses and vegetation types, predominately cropping and pasture land interspersed with patches of native vegetation (largely low *Eucalyptus* spp. woodlands). However, relative cat abundance on the island (14.6 cats site^{-1}) is known to be substantially higher than on the mainland (1.39 cats site^{-1}) (Taggart *et al.* In Press. [Chap. 7]), which could be expected to influence *T. gondii* seroprevalence in intermediate hosts (de Wit *et al.* 2019).

4.3.2 Culled kangaroo sampling on island and mainland

Kangaroos were sampled in conjunction with cull programs on two separate landholdings (2.4 km^2 each) on the Dudley Peninsula, Kangaroo Island, and a single landholding (44.5 km^2) at the tip of the Fleurieu Peninsula on mainland South Australia. Approximately ten kangaroos were sampled on the mainland, and then immediately on the island (1-2 days later), at approximate two-month intervals for a period of 12 months. We estimated the age of kangaroos as adult, young-at-foot or pouch young. Although information was obtained on kangaroo sex, this information could not be linked back to individual serum samples (sex information was collected one day post serum collection).

4.3.3 Road-killed macropod sampling on island

Road-killed kangaroos and wallabies were predominately sampled (83%) along the Hog Bay Road where vehicle traffic is greatest. As the collectors regularly drove the same stretch of road, the majority of blood samples were collected and frozen whole within 24 hrs of the animal's death. We recorded the sex and species of sampled carcasses, prior to dragging them away from the road to ensure they were not sampled twice. All road-killed macropods were considered adults as we could not differentiate between adults and young-at-foot post-death.

4.3.4 Blood collection and processing

We collected blood from culled macropods within minutes after death. Blood was collected via cardiac puncture where possible. For road-killed animals, where the thoracic cavity was damaged or blood was coagulated, we opened the thoracic cavity and collected blood or haemolysed fluids from within the cavity or via direct cardiac puncture. Blood samples from road-killed macropods were usually very haemolysed or diluted by other bodily fluids.

Blood samples from culled kangaroos were allowed to clot at ambient temperature, centrifuged at 4,000 g for 5 mins and extracted sera stored at -20 ^{o}C until serological testing. Whole blood from road-killed macropods were first stored at -20 ^{o}C for up to 3 months after collection, prior to being defrosted, centrifuged, and sera collected and stored until serological testing as described above. All serum samples were frozen for periods of 1-6 months and underwent two freeze/thaw cycles prior to serological testing. Long-term storage of frozen sera or whole blood does not significantly modify the interpretation of *T. gondii* serologies (Dard *et al.* 2017; Fancourt *et al.* 2014).

4.3.5 Toxoplasma gondii antibody test

Sera were tested for *T. gondii* IgG antibodies using a commercially available modified agglutination test (MAT) (Toxo-Screen DA, bioMe´rieux, Marcy-l ´ Etoile, France) following the manufacturer's guidelines. The MAT is based on the direct agglutination of fixed *T. gondii* tachyzoites of the RH strain, with sera pre-treated with 2-mercaptoethanol to neutralize IgM antibodies (Desmonts and Remington 1980; Dubey and Desmonts 1987). Immunoglobulin G antibodies typically take greater than one week to develop, but once developed persist for the remainder of the animal's life, consequently this test may give false negative results during the first week of infection (Dubey and Crutchley 2008). It is not known how quickly IgG antibodies degrade in carcasses exposed to ambient environmental conditions. Sera were screened at 1:40 and 1:4,000 dilutions, and classified as positive if agglutination occurred at either dilution. A dilution was classified as positive when agglutination of *Toxoplasma* formed a mat covering about half of the well base. In each assay, we included a positive kangaroo control, the positive and negative goat controls provided by the manufacturer, and a negative phosphate buffered saline (PBS, pH 7.2) control. The kangaroo positive control was sourced from a captive western grey kangaroo showing neurological signs (head tilt) consistent with toxoplasmosis and that tested positive to *T. gondii* using the MAT in a different laboratory at a dilution of 1: 64,000.

The diagnostic sensitivity and specificity of the MAT in western grey kangaroos and tammar wallabies is unknown, but is expected to be high based on the test performance in other species (Mainar-Jaime and Barberan 2007). Diagnostic test misclassification in our study was expected to be non-differential, potentially reducing our power to detect between-group differences in *T. gondii* seroprevalence (Dohoo *et al.* 2009). We are not aware of any

serologic cross-reactivity with the MAT or the direct agglutination test (Dubey 2016a; Gondim *et al.* 2017).

4.3.6 Data analysis

Seroprevalence between groups of macropods were compared using Fisher's exact tests or Chi-squared tests using the (n-1) adjustment as recommended (Campbell 2007). We tested for seroprevalence differences associated with sex, location (island or mainland), species, and behaviour (culled vs roadkill). To test if kangaroo sex confounded the effect of behaviour, we compared the sex ratio between the road-kill and cull groups using the (n-1) chi squared test. Confidence intervals for seroprevalence estimates are reported as binomial exact. All statistical analyses were performed in R version 3.5.1 (R Core Team 2018).

4.4 Results:

We examined 65 culled kangaroos from the mainland, 67 culled and 102 road-killed kangaroos from the island, and 76 road-killed tammar wallabies from the island (Table 1).

4.4.1 Sex effect

We found a significant difference in *T. gondii* seroprevalence between road-killed male and female kangaroos, but not between road-killed male and female wallabies (Table 1).

4.4.2 Island effect

Toxoplasma gondii seroprevalence in culled adult kangaroos from the island was significantly greater than in culled adult kangaroos from the mainland (Table 1).

4.4.3 Species effect

We found no difference in *T. gondii* seroprevalence between road-killed kangaroos and road-killed wallabies (Table 1).

4.4.4 Behaviour effect

We found no difference in *T. gondii* seroprevalence between adult culled and road-killed kangaroos from the island (Table 1), and the sex ratio of these two groups did not differ (P = 0.44), indicating that our behavioural analysis was not confounded by kangaroo sex.

Table 4.1: Seroprevalence of *Toxoplasma gondii* in culled and road-killed western grey kangaroos (*Macropus fuliginosus*) and tammar wallabies (*M. eugenii*) from Kangaroo Island and the adjacent Australian mainland by age and sex.

				Mainland			Kangaroo Island		
Sample type	**Species**	**Age**	**Sex**	***N***	**Seroprevalence %**	**95% CI (lower, upper)**	***N***	**Seroprevalence %**	**95% CI (lower, upper)**
Culled animal	Kangaroo	Adult		61	0[C]	0, 6	54	20[C, E]	12, 33
		Young at foot		0	0	0, 0	5	0	0, 43
		Pouch young		4	0	0, 49	8	25	7, 59
Road-killed animal	Kangaroo	Adult	All				102	21[D, E]	149, 29
		Adult	Male				60	13[A]	7, 24
		Adult	Female				38	32[A]	19, 48
		Adult	Unknown				4	25	1, 7
	Wallaby	Adult	All				76	15[D]	8, 24
		Adult	Male				39	15[B]	7, 30
		Adult	Female				31	10[B]	3, 25
		Adult	Unknown				6	33	10, 70

[A]Test for sex effect in kangaroos, P = 0.03
[B]Test for sex effect in wallabies, P = 0.48
[C]Test for island effect in kangaroos, P = <0.001
[D]Test for species effect, P = 0.29
[E]Test for behaviour effect, P = 0.97

4.5 Discussion:

We found a significantly higher seroprevalence of *T. gondii* in kangaroos from Kangaroo Island compared to those from the directly adjacent Australian mainland, as predicted. This finding is consistent with previous studies in cats and sheep which identified higher seroprevalence on the island than the mainland (Fancourt and Jackson 2014; O'Donoghue *et al.* 1987; Taggart *et al.* In Review. [Chap 3]), but the magnitude of difference in *T. gondii* seroprevalence between the island and mainland was larger than expected. Our finding of no infection on the mainland was unexpected and warrants further investigation. As felids are definitive hosts of *T. gondii* and the only hosts known to shed the oocyst stage of the parasite in their faeces following infection, we suggest that the high seroprevalence of *T. gondii* in macropods on the island is likely driven by the island's high cat abundance (14.6 cats site^{-1}) compared to the mainland (1.39 cats site^{-1}) (Taggart *et al.* In Press. [Chap. 7]).

The observed *T. gondii* seroprevalence in western grey kangaroos from the island is consistent with a previous seroprevalence survey in this species in Western Australia, however our mainland seroprevalence was much lower than that reported in the Western Australian survey. Parameswaran *et al.* (2009) reported a *T. gondii* seroprevalence of 15.5% in 219 free-ranging adult western grey kangaroos from the metropolitan region of Perth in Western Australia. This is comparable to the level of infection observed on Kangaroo Island in our study (20%), but much greater to that observed on the mainland (0%) (Table 1). This suggests that environmental contamination with *T. gondii* shows large variation on the Australian mainland, and whilst the seroprevalence of *T. gondii* on Kangaroo Island appears to be much greater than the South Australian mainland, it may be comparable to other regions of mainland Australia. Reasons for the comparable *T. gondii* seroprevalence between Kangaroo Island and the metropolitan region of Perth can only be speculated, but could include similarities and or differences in a combination of factors such as cat density and feeding ecology, climate, or soil characteristics. Whilst other studies have assessed *T. gondii* infection in free-ranging western grey kangaroos, only few animals were sampled (Pan *et al.* 2012), precluding any robust comparison.

Toxoplasma gondii infection did not seem to be driving higher rates of road-kill in kangaroos as a consequence of behavioural impacts due to latent toxoplasmosis. We found no difference in the seroprevalence of *T. gondii* between culled and road-killed kangaroos, in contrast to our predictions that seroprevalence would be higher in road-killed animals.

These predictions were founded from Hollings *et al.* (2013) who speculated that reduced reaction time in Tasmanian pademelons (*Thylogale billardierii*) due to latent toxoplasmosis may have explained the higher seroprevalence of *T. gondii* they found in road-killed compared to culled animals. Whilst we are not aware of any evidence in macropods that would support their hypothesis, other studies may provide alternative explanations for their findings. A higher seroprevalence of *T. gondii* may have been observed in road-killed relative to culled macropods due to acute disease causing them to become uncoordinated or impacting their vision (Obendorf and Munday 1983). Cat activity may also concentrate along roadsides following the decline of Tasmanian devils (*Sarcophilus harrisii*) that regularly use roads to traverse the landscape (Fancourt *et al.* 2015), subsequently resulting in higher *T. gondii* oocyst contamination and Hollings *et al.* (2013) observed higher prevalence of *T. gondii* in macropods along roadsides relative to those culled away from roadsides. However, regardless of the mechanism, we found no evidence to support any hypothesis which might explain a difference in *T. gondii* seroprevalence between road-killed and culled macropods. As we collected substantially more road-kill samples than Hollings *et al.* (2013), we suggest that their results may have arisen due to a low number of road-killed samples.

Our finding of no difference in *T. gondii* seroprevalence between kangaroos and wallabies contradicts what would be expected based on the ecology and physiology of these species. Western grey kangaroos and tammar wallabies are both solely herbivorous and have many similarities in their diet and feeding ecology. However, smaller macropod species such as wallabies, are generally considered to consume a greater proportion of browse and forbs in comparison to their larger counterparts, the kangaroos, which consume a greater proportion of grass (Telfer and Bowman 2006; Wann and Bell 1997). Accordingly, kangaroos would be expected to spend a greater portion of their time grazing closer to the ground than do wallabies, increasing their relative risk of exposure to *T. gondii* oocysts. Western grey kangaroos are also known to live approximately 25% longer than tammar wallabies (Ahlert 2002; Inns 1982; Norbury *et al.* 1988), and as the risk of *T. gondii* infection increases with age (Jones *et al.* 2001; Van der Puije *et al.* 2000), kangaroos would be expected to have a greater average cumulative risk of infection. Some evidence also exists suggesting that wallabies are more susceptible to acute toxoplasmosis than kangaroos (Johnson *et al.* 1989; Lynch *et al.* 1993; Reddacliff *et al.* 1993); this would be expected to result in greater mortality of acutely infected wallabies, thereby removing them from the population and leaving a higher

proportion of negative wallabies to be sampled. Furthermore, wallabies are known to be within the size range of cat prey items (Fancourt 2015), so if *T. gondii* infection influences their behaviour, then this could make them more susceptible to cat predation as has been suggested for rodents and other species (Poirotte *et al.* 2016; Vyas *et al.* 2007). If this occurs, this predation bias would not impact kangaroos due to their larger size excluding them as a possible cat prey item. Whilst the true seroprevalence of *T. gondii* in kangaroos and wallabies may differ, the lack of difference observed in our study despite reasonable sample sizes of both species suggests that the described factors combined do not contribute to a large difference in *T. gondii* seroprevalence. Additional sampling of both species is likely required to uncover relatively small but true difference in *T. gondii* seroprevalence between kangaroos and wallabies in our study.

We found a higher seroprevalence of *T. gondii* in female compared to male kangaroos, but no effect of sex in wallabies. This difference in *T. gondii* seroprevalence between sexes, and the order of the difference (higher seroprevalence in females compared to males), is consistent with studies in sheep and goats (Teshale *et al.* 2007; Van der Puije *et al.* 2000), and has even been previously reported in western grey kangaroos (Parameswaran *et al.* 2009). Parameswaran *et al.* (2009) suggested this difference in *T. gondii* seroprevalence between male and female kangaroos likely resulted from differences in their feeding ecology, where females graze shorter grass than do males (Newsome 1980), increasing their risk of infection. However, Teshale *et al.* (2007) suggested that the difference in *T. gondii* seroprevalence between male and female goats was likely due to differences in their average age, and Van der Puije *et al.* (2000) suggested the same difference in seroprevalence in sheep and goats was likely due to females being more susceptible to infection than males (Ahmad *et al.* 2015; Alexander and Stimson 1988; Ntafis *et al.* 2007) and an enhanced immune responses in males (Kittas *et al.* 1984). Any of these alternative explanations for the observed difference in *T. gondii* seroprevalence between male and female kangaroos in our study may be plausible, particularly if macropod culling disproportionately removes larger and older male kangaroos from the population.

Like all field and serological studies, our study has inherent limitations and biases. Two limitations in particular may have influenced the results of our serological assays. Both culled and road-killed macropod samples were collected based on convenience, and may

misrepresent the seroprevalence of *T. gondii* in the population relative to randomly collected samples. In an attempt to overcome this, the sampling of culled kangaroos was conducted over as large an area as practical based on active population control programs at the time of sampling. Whilst road-kill sampling was restricted to busy roads and routes regularly travelled by sample collectors, *T. gondii* seroprevalence estimates from these samples were equivalent to those from culled samples, suggesting minimal bias due to non-random sampling. Road-kill sampling and sampling from animals carcasses may additionally underestimate true seroprevalence due to the reduced sample quality impacting on serological results (Tryland *et al.* 2006). However, the majority of road-killed kangaroo blood samples were collected within 24 hours of the animal's death, reducing the probability of antibody denaturation. Furthermore, serological results from bloody fluids collected shortly after the animals' death, like those collected from road-killed carcasses in this study or meat juice in other studies, are known to closely match serological results completed on serum from the same animals (Glor *et al.* 2013). Similarly, serological results from frozen whole blood and serum collected shortly after an animal's death are known to produce equivalent serological results (Fancourt *et al.* 2014). The dilution of blood with other bodily fluids and the gradual degradation of antibodies in carcasses, for example due to heat, autolysis, or bacterial putrefaction, will at some point impact on the accuracy of the serological test, although we do not believe that this has had a large influence on our serological results in road-killed macropods.

High *T. gondii* seroprevalence is known to, or would be expected to be, associated with a high prevalence of the disease toxoplasmosis (Dubey *et al.* 2012). Accordingly, our study suggests greater potential adverse conservation impacts due to toxoplasmosis on the island relative to the mainland. Furthermore, our study is consistent with previous studies suggesting that Kangaroo Island provides a favourable environment for the proliferation of *T. gondii* relative to the Australian mainland (Fancourt and Jackson 2014; O'Donoghue *et al.* 1987; Taggart *et al.* In Press. [Chap. 7]; Taggart *et al.* In Review. [Chap 3]). This is of particular concern for other wildlife species on Kangaroo Island due to the known health impacts of *T. gondii* infection (Hillman *et al.* 2015). We suggest that *T. gondii* seroprevalence surveys be performed to assess the infection rate in a greater range of island fauna to help assess disease risk and inform management actions. The high level of *T. gondii* infection in kangaroos on the island however does not appear to be contributing towards the high rate

of road-killed kangaroos on the island. Current evidence suggests that the increased cat abundance on Kangaroo Island compared to the adjacent mainland is likely the primary driver of the increased prevalence of *T. gondii* in island fauna.

Conflict of interest statement:

We declare no conflict of interest.

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Chapter 5: Unexpectedly low *Toxoplasma gondii* seroprevalence in rodents on a large island with high endemicity in larger mammals

Statement of Authorship

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Contribution to the Paper	Formulated experimental study design. Sample collection, processing and testing. Formatted, edited, analysed and interpreted data. Wrote and revised manuscript.		
Overall percentage (%)	90		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
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Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate in include the publication in the thesis; and

iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

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Unexpectedly low *Toxoplasma gondii* seroprevalence in rodents on a large island with high endemicity in larger mammals

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5.1 Abstract:

Toxoplasmosis is a disease caused by the cat-borne parasite *Toxoplasma gondii* that is known to negatively impact many species of wildlife globally. We conducted a seroepidemiologic survey for *Toxoplasma* to investigate the potential risk of cat-borne disease to small- and medium-sized mammals on an island and the adjacent mainland, and to compare seroprevalence to the high rates of infection known in large mammals on the island. We tested for *Toxoplasma* exposure in 100 animals across three species on the island and 211 animals across seven species on the mainland using two serological tests (MAT and IFAT). In contrast to previous findings of high seroprevalence in kangaroos, cats and sheep on the island, *Toxoplasma* seroprevalence in small- and medium-sized mammals on the island was low and did not differ to that on the mainland. We found no seropositive animals in any species tested in either region, with the exception of a single bush rat (*Rattus*

fuscipes) on the island. The low observed *Toxoplasma* seroprevalence on the island was also in contrast to our predictions based on the island's high abundance of cats, but the observed low seroprevalence on the mainland was consistent with low cat abundance in this region. We suggest the hosts' short lifespan, low exposure to *Toxoplasma* oocysts, and/or rapid direct and indirect impacts of disease contribute to a low incidence and duration of infection in the species tested on the island, resulting in the low observed *Toxoplasma* seroprevalence.

5.2 Introduction:

Predation is often considered the main pathway through which feral cats (*Felis catus*) impact on faunal communities (Woinarski *et al.* 2014). For small- and medium-sized terrestrial mammals, declines in abundance or geographic range thought to be associated with cats are generally presumed to be the result of predation (Risbey *et al.* 2000; Woinarski *et al.* 2010). However cats are known to have diverse impacts on wildlife communities through a range of mechanisms. A global review of the impacts of feral cats on endangered vertebrates on islands concluded that cats impacted wildlife communities through eight major pathways; 1) predation; 2) competition for food; 3) competition for habitat; 4) disruption of migration; 5) disruption of seed dispersal; 6) induced behavioural changes; 7) hybridisation; and 8) disease (Medina *et al.* 2014). Some studies have hypothesised the importance of cat-borne disease in the widespread decline of mammals (Abbott 2006; Thompson *et al.* 2010), for example due to toxoplasmosis (Lynch *et al.* 1993; Obendorf and Munday 1983). Therefore, it is important to also assess the potential risk of cat-borne disease to mammals of conservation significance.

Toxoplasmosis is a disease caused by the cat-borne parasite *Toxoplasma gondii*. Felids are the definitive host of the parasite and shed the oocyst stage of the parasite in faeces when first infected. Intermediate hosts of *T. gondii* can be any warm-blooded animal and are infected by consuming contaminated food, water or soil (Aramini *et al.* 1999; Hill and Dubey 2002). Within the gut of the intermediate host, oocysts excyst to release sporozoites which invade host cells, transform into rapidly multiplying tachyzoites, and cause cell lysis to release tachyzoites to infect other cells (Uzal *et al.* 2016). This process results in the death of the host cell and is responsible for the development of acute disease, which can lead to a range of debilitating symptoms and can even cause mortality. However, in the majority of

infections, the tachyzoite stage of the parasite is suppressed by the intermediate host's immune system, causing the parasite to develop into microscopic intracellular cysts that contain bradyzoites in muscles and neural tissues (Dubey 2016). Bradyzoites remain dormant in the intermediate host tissues for the life of the animal, but are associated with latent disease (Weiss and Kim 2000). The parasite's lifecycle is restarted when bradyzoites are consumed by a cat predating or scavenging an infected intermediate host (Zulpo *et al.* 2018).

Both acute and latent toxoplasmosis are known to negatively impact on the health and welfare of Australian mammals and closely related species. For example, experimental infection of *T. gondii* is lethal in bandicoots (Bettiol *et al.* 2000) and tammar wallabies (Reddacliff *et al.* 1993), is known to influence rodent behaviour in such a way that is speculated to increase their likelihood of being predated by cats (Berdoy *et al.* 2000; Vyas 2013; Vyas *et al.* 2007), and may also influence the timing of bandicoot activity (Bettiol *et al.* 2000).

Kangaroo Island in southern Australia is Australia's third largest island (4,405 km^2) and is of high conservation value; although much of its high conservation value fauna is under threat from the high abundance of feral cats on the island (Taggart *et al.* In Press. [Chap 7]). About half of the island retains remnant native vegetation (Willoughby *et al.* 2001) and supports multiple species of threatened terrestrial mammal, including the southern brown bandicoot (*Isoodon obesulus obesulus*), Kangaroo Island echidna (*Tachyglossus aculeatus multiaculeatus*), and Kangaroo Island dunnart (*Sminthopsis griseoventer aitkeni*). Cat predation is known to be a major threat to small- and medium-sized terrestrial mammals on the island, as these species comprise a large proportion of cat diets (Hodgens *et al.* 2019), and cat predation is known to have negative impacts on population recruitment in some threatened island fauna (Rismiller 1999; Rismiller and McKelvey 2000). While other possible cat-associated threats to the conservation of small- and medium-sized terrestrial mammals on the island are currently unknown, it is speculated that disease, specifically *T. gondii* infection, may pose a major threat to their conservation as its seroprevalence is known to be high in cats, sheep and macropods on the island compared to the adjacent Australian mainland (Fancourt and Jackson 2014; O'Donoghue *et al.* 1987; Taggart *et al.* In Review. [Chap 4]). This is of particular concern as some threatened island fauna would be expected

to suffer acute mortality from *T. gondii* infection based on the impacts of infection shown for related species (Bettiol *et al.* 2000).

We conducted a seroepidemiologic survey for *T. gondii* in small- and medium-sized mammals on Kangaroo Island and the adjacent mainland to: 1) investigate the potential risk of cat-borne disease to the conservation of small- and medium-sized terrestrial mammals on Kangaroo Island relative to the adjacent mainland; and 2) compare seroprevalence in small- and medium-sized mammals to the high rates of infection known in large mammals on the island. We predicted *T. gondii* seroprevalence to be 2-3 times higher in mammals on the island compared to the mainland, as suggested by previous studies in other species (Fancourt and Jackson 2014; O'Donoghue *et al.* 1987; Taggart *et al.* In Review. [Chap 4]). We also predicted *T. gondii* seroprevalence in small and medium sized mammals on Kangaroo Island to be relatively high, based on the relatively high seroprevalence of *T. gondii* shown in cats, sheep and macropods on the island (approximately 20-60% positive) (O'Callaghan *et al.* 2005; Taggart *et al.* In Review. [Chap 4]; Taggart *et al.* In Review. [Chap 3]).

5.3 Materials and methods:

We followed the CONSISE guidelines for the reporting of seroepidemiologic studies outlined in Horby *et al.* (2017).

5.3.1 Study sites

The Dudley Peninsula on Kangaroo Island (-35.8015^{o} N; 137.9752^{o} E) and the tip of the adjacent Fleurieu Peninsula (-35.5886 ^{o} N; 138.1986 ^{o} E) on mainland South Australia were selected as study regions as described in Taggart *et al.* (In Review. [Chap 4]). The close proximity of these regions assisted in controlling for potentially confounding climatic conditions which may contribute to the differential survival of *T. gondii* oocysts in the environment, and consequently differences in *T. gondii* seroprevalence in small- and medium-sized mammals. The Dudley Peninsula is located 13.5 km south west of the Fleurieu Peninsula, the closest region of mainland South Australia. The study regions are hereafter referred to as 'island' and 'mainland', respectively. Both locations experience similar Mediterranean climates (Jenkins 1985; Schwerdtfeger 2002).

We sampled small- and medium-sized mammals at four sites on the island and four sites on the mainland. Study sites were chosen to be representative of the remnant vegetation types on the island and mainland and all comprised patches of this vegetation surrounded by agricultural land. All study sites within each region were considered spatially independent for all species sampled and feral cats based on previously reported home range data (Table S1).

5.3.2 Animal capture

Animals were trapped in cage, Elliot or pit-fall traps baited with peanut butter and rolled oats. Traps were deployed along linear transects within native vegetation patches at intervals of 20 m. The number, layout and type of traps used was not standardised across sampling periods for logistical reasons.

5.3.3 Blood sampling and processing

We collected blood from all trapped mammals, except house mice (*Mus musculus*), via jugular venepuncture whilst animals were anaesthetised. Blood was collected from restrained house mice directly into a plain sample tube following incision of the sub mandibular vein (Francisco *et al.* 2015; Golde *et al.* 2005). All captured animals were marked to facilitate the identification of recaptures and to avoid animals being sampled multiple times.

Blood samples were processed, sera collected and stored as described by Taggart *et al.* (In Review. [Chap 4]). Serum samples experienced two freeze/thaw cycles prior to testing. Long-term sera storage does not significantly modify the interpretation of *T. gondii* serology (Dard *et al.* 2017).

5.3.4 Toxoplasma gondii antibody test

Modified agglutination test (MAT)

Sera were tested for *T. gondii* immunoglobulin G (IgG) antibodies using a commercially available MAT (Toxo-Screen DA, bioMe´rieux, Marcy-l ´Étoile, France) as described in detail by Taggart *et al.* (In Review. [Chap 4]). The MAT is based on the direct agglutination of fixed *T. gondii* tachyzoites of the RH strain, with sera pre-treated with 2-mercaptoethanol to neutralize IgM antibodies (Desmonts and Remington 1980; Dubey and Desmonts 1987).

Immunoglobulin G antibodies typically take greater than one week to develop, but once developed persist for the remainder of the animal's life. Consequently, this test may give false negative results within the first week of infection (Dubey and Crutchley 2008). Sera were screened at 1:40 and 1:4,000 dilutions, and classified as positive if agglutination occurred at either dilution. With each assay we included positive and negative control sera from known infected (intra-peritoneal inoculation with *T. gondii* tachyzoites) and uninfected mice, a positive control from a western grey Kangaroo (*Macropus fuliginosus*) described by Taggart *et al.* (In Review. [Chap 4]), the positive and negative control goat sera provided by the manufacturer, and a negative phosphate buffered saline (PBS, pH 7.2) control. A serum sample was classified as positive when agglutination of *Toxoplasma* formed a mat covering about half or more of the well base.

The MAT has not been evaluated in the species surveyed, but we assumed its diagnostic sensitivity and specificity to be fair based on high sensitivity and specificity results shown in other species (Mainar-Jaime and Barberan 2007). To our knowledge there are no meaningful cross-reactivity problems with the MAT (Dubey 2016; Gondim *et al.* 2017).

Indirect immunofluorescent antibody test (IFAT)

To detect potential false negative results, all mice (*Mus musculus*) sera that tested negative to the MAT were re-tested using a modified IFAT. The testing procedure is described in detail by Dabritz *et al.* (2008) and Fredebaugh *et al.* (2011). The lack of appropriate control sera and secondary antibodies prevented us from performing the IFAT on sera from other surveyed species.

Slides with twelve 5 mm wells (Thermos Fisher Scientific, Scoresby, Victoria, Australia) were prepared in the laboratory with the use of a modified technique from Miller *et al.* (2001). Tachyzoites were spotted onto antigen wells, dried, fixed in methanol, and dried again.

Serum samples were diluted with phosphate buffered saline to a titre of 1:20, and 20 µl placed into each slide test well. Each slide contained a *T. gondii* positive and negative mouse (*Mus musculus*) control. Slides were incubated for 30 min at 37 ^{o}C and 5 % CO_2. After each

incubation, slides were rinsed in IFA rinse buffer (pH 9), and then soaked in the buffer for a further ten minutes.

Wells were then treated with 20 µl of a 1:100 dilution of anti-mouse antibody (anti-mouse IgG Dylight 488, Thermo Fisher Scientific, Scoresby, Victoria, Australia) for 30 minutes at 37 ^{o}C, and rinsed and soaked in buffer as before. Slides were coverslipped with a mixture of 50% glycerol and 50% rinse buffer, and examined under a fluorescence microscope. A sample was classified as positive if all edges around the tachyzoites glowed bright green, or negative if the entire edge of the tachyzoite did not fluoresce (Paré *et al.* 1995).

5.3.5 Data analysis

We tested for differences in the proportion of *T. gondii* positive and negative mice and bush rats (*Rattus fuscipes*) from the island and the mainland regions using Fisher's exact test. We were unable to perform the same comparison for species captured and sampled in only one region. For all species surveyed, we present *T. gondii* seroprevalence and binomial 95 % confidence intervals.

5.4 Results:

We examined 100 small- and medium-sized mammals from three species on the island and 211 small- and medium-sized mammals from seven species on the mainland (Table 5.1). Only a single bush rat (*Rattus fuscipes*) from the island was seropositive to *T. gondii* using the MAT; all other animals from both the island and the mainland were seronegative. All mice (*Mus musculus, n = 55*) were also seronegative with the IFAT. *Toxoplasma gondii* seroprevalence could only be compared between the island and mainland for house mice and bush rats (the only species captured at both locations). We found no difference in *T. gondii* seroprevalence between the island and mainland for either mice or bush rats ($_{\text{Fisher's exact}}$ p = 1.00 and 0.22 respectively).

Table 5.1: *Toxoplasma gondii* seroprevalence in surveyed small- and medium-sized mammals on the Dudley Peninsula, Kangaroo Island, and the adjacent Fleurieu Peninsula, mainland South Australia.

	Mainland		Island		
Species	*N*	Seroprevalence % (95 % CI)	*N*	Seroprevalence % (95 % CI)	**P**
House mouse (*Mus musculus*)	19	0 (0, 16.8)	36	0 (0, 9.6)	1.00
Black Rat (*Rattus rattus*)	10	0 (0, 27.8)			
Bush rat (*Rattus fuscipes*)	169	0 (0, 2.2)	46	2 (0.1, 11.3)	0.22
Swamp rat (*Rattus lutreolus*)	2	0 (0, 65.8)			
Common brushtail possum (*Trichosurus vulpecula*)			18	0 (0, 17.6)	
Common ringtail possum (*Pseudocheirus peregrinus*)	1	0 (0, 94.9)			
Southern brown bandicoot (*Isoodon obesulus obesulus*)	6	0 (0, 39.0)			
Yellow-footed antechinus (*Antechinus flavipes*)	4	0 (0, 49.0)			

5.5 Discussion:

All mammal species surveyed on the mainland were seronegative for *T. gondii* as predicted. This finding is consistent with the mainland's relatively low abundance of cats, the parasite's definitive host (Taggart *et al.* In Press. [Chap 7]). However, the observed low seroprevalence of *T. gondii* in all small- and medium-sized mammal species tested on the island was in contrast to our predictions, and is inconsistent with the island's relatively high cat abundance (Taggart *et al.* In Press. [Chap 7]). Additionally, the observed low seroprevalence of *T. gondii* in small- and medium-sized mammal species on the island is inconsistent with previously reported seroprevalence in macropods (Taggart *et al.* In Review. [Chap 4]), cats (O'Callaghan *et al.* 2005) and sheep (O'Donoghue *et al.* 1987; Taggart *et al.* In Review. [Chap 3]).

A similar disparity between small mammals and larger sympatric species was also observed on the island of Grenada in the West Indies, where *T. gondii* seroprevalence in rodents was reported to be 0.8% (Dubey *et al.* 2006) and 0.6% (Murata *et al.* 2018), despite a high seroprevalence (>23%) in humans (Asthana *et al.* 2006), dogs (Dubey *et al.* 2008), cats (Dubey *et al.* 2009), pigs (Chikweto *et al.* 2011), chickens (Chikweto *et al.* 2017; Dubey *et al.* 2005) and mongoose (Choudhary *et al.* 2013). These studies from Grenada are of particular relevance to ours as both Grenada (349 km^2) and Kangaroo Island are insular environments

and small relative to the size of a country or continent. The relatively small area of both islands reduces the probability of large climatic or environmental variation across the islands that may influence *T. gondii* oocyst survival. Therefore, all the animals sampled on these islands likely existed in similar environments, and were all likely exposed to a similar level of *T. gondii* contamination, despite the large disparity in *T. gondii* seroprevalence between small animals, such as rodents, and larger sympatric species. Dubey *et al.* (2006) and Murata *et al.* (2018) both suggested that the low seroprevalence of *T. gondii* in rodents from Grenada could be explained by rodents not being important in the epidemiology of *T. gondii* on the island. However, we suggest the reasons for the comparatively lower *T. gondii* seroprevalence in rodents from Kangaroo Island and Grenada are more complex, and the importance of rodents in the epidemiology of *T. gondii* depends on the ecology of the parasite's hosts and their interactions.

Based on a high seroprevalence of *T. gondii* (>20%) previously reported for multiple species on Kangaroo Island, we speculate that the true *T. gondii* prevalence in all species sampled on the island is greater than 0%. The prevalence of a parasite in a population is a combination of the incidence and duration of infection in the host population (Dohoo *et al.* 2009). Accordingly, an observed low seroprevalence of *T. gondii* in a species could result from one of four scenarios; 1) small sample size or non-representative sampling; 2) a low incidence of *T. gondii* infection in the host; 3) a short duration of *T. gondii* infection in the host; or 4) a combination of low incidence and short duration of *T. gondii* infection in the host. For some species tested on the island (house mice and brushtail possums) our sample size appears to have been too small to detect the low but positive rate of infection that is likely present, based on the high seroprevalence reported in other species (Fancourt and Jackson 2014; O'Donoghue *et al.* 1987; Taggart *et al.* In Review. [Chap 4]). This is reflected by seroprevalence confidence intervals for these species ranging from 0% to >9%.

A low incidence of *T. gondii* infection in rodents could result from the comparatively short lifespan of rodents. As the incidence of *T. gondii* infection is cumulative, its prevalence in animals increases with age (Jones *et al.* 2001; Van der Puije *et al.* 2000). Accordingly, it is expected that for animals with short life expectancy, such as rats and mice (average lifespan in wild approximately 1 year (Ballenger 1999; Gillespie 2004)), *T. gondii* seroprevalence will

be low compared to animals with longer life expectancies, such as sheep (6 years) and macropods (up to 10 years) (Miller 2002)).

Alternatively, a low incidence of *T. gondii* infection in rodents could result from low transmission of *T. gondii* to rodents. In most cases, rodents rarely consume tissues of warm blooded animals (Daniel 1973; Traveset *et al.* 2009; Whitaker Jr 1966), therefore the major route of *T. gondii* transmission to rodents would be through the consumption of oocysts shed in cat's faeces. Accordingly, a low incidence of *T. gondii* infection in rodents suggests rare consumption of oocysts. This could arise if rodent feeding ecology on Kangaroo Island or Grenada is not conducive to the consumption of oocysts. For example, if rodents on these islands predominately consume food sources above ground level as opposed to foraging for invertebrates and seeds at or below ground level, their exposure to *T. gondii* oocysts would be low. Whilst we have no information on rodent feeding ecology from either Kangaroo Island or Grenada, studies from Marion Island, south of South Africa, suggest that rodents spend a large proportion of their time foraging at or below ground level (Gleeson and Van Rensburg 1982) and would be expected to be regularly exposed to *T. gondii* oocysts in the environment if they were present. Therefore, we would expect rodents on both of Kangaroo Island and Grenada to be regularly exposed to *T. gondii* oocysts whilst foraging. This suggests rodent feeding ecology is unlikely to be a major contributing factor to their observed low *T. gondii* seroprevalence.

A low incidence of *T. gondii* infection in rodents could also arise if cats defecate non-randomly across the landscape creating areas of low oocyst burden and areas of high oocyst burden. In our study, we captured and sampled all animals in remnant patches of native vegetation, but not in open areas. In contrast, previous studies found a high seroprevalence of *T. gondii* in sheep (O'Donoghue *et al.* 1987; Taggart *et al.* In Review. [Chap 3]) and macropods (Taggart *et al.* In Review. [Chap 4]) on Kangaroo Island, both of which spend the majority of their time grazing in open areas. If cats defecate less frequently in sylvatic environments (native vegetation patches) relative to domestic environments (paddocks and fields), this could result in reduced shedding of *T. gondii* oocysts into sylvatic environments and a separate and distinct sylvatic and domestic *T. gondii* transmission cycle. Whilst we are unsure if or why cats may defecate less frequently in sylvatic environments, if this is the case, our sampling would have inadvertently targeted rodents in areas of low *T. gondii*

contamination. This could have contributed to the low observed *T. gondii* seroprevalence in rodents, and the underestimation of true population prevalence.

As infection with *T. gondii* is known to last for the duration of the host's life (Dubey 2016), a short duration of infection could result from the comparatively short lifespan of a host species, or the swift and efficient removal of seropositive animals from the population. For example, mice (*Mus musculus*) and rats (*Rattus spp.*) are known to succumb to acute toxoplasmosis (mice more than rats) to some extent following the consumption of *T. gondii* oocysts (Dubey 1996; Dubey 2006). Thus it would be expected that some of the infected rodents are removed from the population due to death. This would reduce the duration of *T. gondii* infection for positive animals, leaving a greater proportion of negative animals to be sampled. Latent toxoplasmosis in rodents is also known to decrease their innate fear, and even cause them to become attracted to cat odour (Vyas *et al.* 2007). It has been suggested that this attraction potentially increases the probability that infected rodents will be depredated by cats (Vyas 2013), thereby reducing the duration of *T. gondii* infection in rodents. As we are not aware of any evidence to suggest cats defecate preferentially in open relative to vegetated areas, we suggest that the low seroprevalence of *T. gondii* in rodents on Kangaroo Island, and likely Grenada, is due to the short lifespan of rodents and the rapid direct and indirect impacts of disease on rodent mortality. Consequently, we believe that *T. gondii* infection is an important cat-associated threat to the survival of small terrestrial mammals.

Assessing the importance of an intermediate host species in the epidemiology of *T. gondii* in a particular ecosystem requires consideration of the ecology of both the intermediate host species and cats, and their interactions in that ecosystem. For example, Kangaroo Island is known to have a high density of cats (Bengsen *et al.* 2011; Taggart *et al.* In Press. [Chap 7]) and unpublished data suggests that rodents and small mammals are the most frequently observed prey item in cat gastrointestinal tracts on the Island (Hodgens *et al.* 2019). This is consistent with feral cat diets on islands worldwide, with mice, rats and rabbits comprising the major dietary items (Bonnaud *et al.* 2011). Therefore, in contrast to conclusions made by Murata *et al.* (2018) and Dubey *et al.* (2006) regarding rodents not being important in the epidemiology of *T. gondii* on Grenada, we suggest that rodents must be important in the

epidemiology of *T. gondii* on Kangaroo Island due to the high number of cats on the island and frequency with which they consume rodents relative to other food items.

The potentially fatal attraction of rodents to cat odour (Berdoy *et al.* 2000), and the possibility that dead infected rodents are consumed by cats further suggest that rodents are likely important in the epidemiology of *T. gondii* on Kangaroo Island and Grenada, despite the low observed seroprevalence. High cat densities on Kangaroo Island increases the probability that *T. gondii* positive rodents attracted to cat odour will be depredated, and that this mechanism of parasite-induced behavioural change, to increase its own transmission via carnivorism, is highly efficient. Cats are also known to occasionally scavenge (Forsyth *et al.* 2014; Paltridge *et al.* 1997) and cats on Kangaroo Island are no exception (Hodgens *et al.* 2019). Accordingly, it is reasonable to assume that rodents that have recently died of acute toxoplasmosis might be opportunistically scavenged by cats, as suggested elsewhere (Strang 2018). On islands such as Kangaroo Island and Grenada, where cats are the largest (Kangaroo Island) or one of the largest (Grenada) mammalian carnivores, the frequency with which they scavenge would be expected to increase due to carrion being readily available and/or accessible in the absence of competition from larger predators (Cunningham *et al.* 2018). These two mechanisms may additionally help explain how rodents can remain important in the epidemiology of *T. gondii*, despite its seroprevalence in rodents often being low (Dabritz *et al.* 2008).

We additionally found *T. gondii* seroprevalence in common brushtail possums on Kangaroo Island to be 0%, consistent with previous studies in this species on the island (O'Callaghan and Moore 1986). Brushtail possums are omnivorous, but vegetation comprises the majority of their diet (Cochrane *et al.* 2003; Evans 1992; How and Hillcox 2000). On Kangaroo Island, possums frequently forage on the ground (P. L. Taggart personal observation) so could be expected to be frequently exposed to *T. gondii* oocysts. However, despite foraging on the ground, studies elsewhere show that brushtail possums predominately consume vegetation above ground level (Cochrane *et al.* 2003; Evans 1992; How and Hillcox 2000), as opposed to grazing vegetation close to the soil surface where *T. gondii* oocysts are deposited in cat faeces. Additionally, some evidence suggests that *T. gondii* infection in brushtail possums may result in acute toxoplasmosis and their subsequent death (Eymann *et al.* 2006). This suggests a short duration of infection in brushtail possums due to the death of positive

animals, consequently contributing to the observed low seroprevalence in our study. It is unknown to what degree other factors described above (biased predation of *T. gondii* infected brushtail possums by cats or sylvatic transmission cycle) may contribute to the low *T. gondii* seroprevalence observed in brushtail possums in our study. It is unlikely that a short lifespan is a major contributing factor as the average lifespan of possums in the wild is thought to be approximately seven years (Meyer 2000).

A high *T. gondii* seroprevalence does not necessarily mean that the species will be negatively impacted by infection or that the species is important in the epidemiology of the parasite. For example, whilst high *T. gondii* seroprevalence has been shown in eastern quolls (*Dasyurus viverrinus*) in Tasmania, infection does not impact quoll survival (Fancourt *et al.* 2014). Conversely, low seroprevalence of *T. gondii*, as we have observed here, does not necessarily mean that the species is not negatively impacted by infection, that it is not being infected, or that the species is unimportant in the epidemiology of the parasite. We found a low seroprevalence of *T. gondii* in all species tested on both the island and mainland. However, when we take into consideration; 1) the ecology of the species sampled; 2) the ecology of cats, the definitive host of *T. gondii*; 3) interactions between the species sampled and cats; and 4) previous evidence of negative health impacts due to *T. gondii* infection in the species sampled and related species, we conclude that *T. gondii* infection is an important cat-associated threat to the survival of small and medium-sized terrestrial mammals on the island. Additionally, we conclude that small mammals are important in the epidemiology of *T. gondii* on Kangaroo Island despite the low observed *T. gondii* seroprevalence in these species. When assessing the impact of a parasite on a host species, or the importance of a host in the epidemiology of a parasite, it is important not to be misled by the prevalence of the parasite in the intermediate host species (McCallum and Dobson 1995), but carefully consider the prevalence of the parasite in the intermediate host species in conjunction with its known impact on the host species and related species, and both the definitive and intermediate host ecology.

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Conflict of interest statement:

We declare no conflict of interest.

5.7 References:

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5.8 Chapter 5 supplementary material:

Table S5.1: Home range data for the species sampled, similar species and cats on the Fleurieu Peninsula, mainland South Australia, and the Dudley Peninsula, Kangaroo Island, South Australia.

Species	Study region	Home range size (km^2)	Publication
Common brushtail possum (*Trichosurus vulpecula*)	South-west Western Australia, Australia	0.01 - 0.02	How and Hillcox (2000)
Common brushtail possum (*Trichosurus vulpecula*)	Melbourne, Victoria, Australia	0.01	Harper (2006)
Ringtail possum (*Hemibelideus lemuroides*)	Atherton Tablelands, Queensland, Australia	0.002 - 0.01	Wilson *et al.* (2007)
Southern brown bandicoot (*Isoodon obesulus*)	Perth, Western Australia, Australia	0.003 - 0.08	Broughton and Dickman (1991)
Eastern-barred bandicoot (*Perameles gunnii*)	South-eastern Tasmania, Australia	0.02 - 0.04	Mallick *et al.* (2000)
Dusky antechinus (*Antechinus swainsonii*)	Snowy Mountains, Victoria, Australia	0.002 - 0.01	Sanecki *et al.* (2006)
Bush rat (*Rattus fuscipes*)	Snowy Mountains, Victoria, Australia	0.005 - 0.02	Sanecki *et al.* (2006)
House mouse (Mus musculus)	Wellington, New Zealand	0.006 – 0.0262	Fitzgerald *et al.* (1981)
Domestic/feral cat (*Catus felis*)	Kangaroo Island, South Australia, Australia	4	Bengsen *et al.* (2012)

Chapter 6: No evidence of *Toxoplasma gondii* exposure in South Australian Koalas (*Phascolartos cinereus*)

Statement of Authorship

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Contribution to the Paper	*Formulated experimental study design. Some sample collection and processing. Sample testing. Formatted, edited, analysed and interpreted data. Wrote and revised manuscript.*
Overall percentage (%)	*80*
Certification:	*This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.*
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By signing the Statement of Authorship, each author certifies that:

i. *the candidate's stated contribution to the publication is accurate (as detailed above);*
ii. *permission is granted for the candidate in include the publication in the thesis; and*
iii. *the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.*

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No evidence of Toxoplasma gondii exposure in South Australian koalas (*Phascolarctos cinereus*)

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6.1 Abstract:

Infection with the cat-borne parasite *Toxoplasma gondii* has been detected in numerous Australian marsupials and can lead to severe disease (toxoplasmosis) in some cases. The seroprevalence of *Toxoplasma* on Kangaroo Island, South Australia, has been reported to be higher than the South Australian mainland in macropods, cats and sheep, suggesting an increased risk of infection on this island. However, *Toxoplasma* seroprevalence in small- and medium-sized terrestrial mammals was almost zero on the island and did not differ to that on the mainland. We surveyed *Toxoplasma* seroprevalence in koala (*Phascolarctos cinereus*) populations on the island and on the mainland and assessed their risk of infection and their role in the life cycle of *Toxoplasma*. All screened koalas from the island (n = 94) and mainland (n = 63) were seronegative. This represents the largest *Toxoplasma* seroprevalence survey in this species and provided sufficient evidence to confidently demonstrate freedom from parasite exposure in both the island and mainland populations at the time of the survey. Due to koalas being extensively arboreal and predominately consuming tree foliage

they appear to be at negligible risk of *Toxoplasma* infection. Furthermore as koalas are rarely consumed by cats, we suggest they have a minor role in the parasites lifecycle.

6.2 Introduction:

The disease toxoplasmosis is caused by the parasite *Toxoplasma gondii* and can have adverse effects on wildlife health (Obendorf and Munday 1983). Felids are the definitive host of *T. gondii* and any warm-blooded animal is said to be an intermediate host (Dubey 2016). Intermediate hosts are primarily infected via the consumption of oocysts (shed in cat feces) in contaminated food or water, or by the consumption of bradyzoites in infected tissues (Dubey 2016). *Toxoplasma gondii* is particularly virulent in Australian marsupials. In wild marsupial populations, toxoplasmosis has been reported to cause severe blindness and incoordination, and fatalities in Tasmanian pademelons (*Thylogale billardierii*) and Bennett's wallabies (*Macropus rufogriseus rufogriseus*) (Obendorf and Munday 1983). Similarly in captive marsupial populations, toxoplasmosis has been reported to cause respiratory, neurological and gastrointestinal problems, and even fatalities in macropods, wombats (*Vombatus ursinus*), possums, dasyurids, numbats (*Myrmecobius fasciatus*), bandicoots (*Perameles gunnii*), bilbies (*Macrolis lagolis*) and koalas (*Phascolarctos cinereus*) (Bettiol *et al.* 2000; Canfield *et al.* 1990).

The seroprevalence of *T. gondii* in macropods, cats (*Felis catus*) and sheep (*Ovis aries*) is substantially higher on Kangaroo Island than the Australian mainland (Fancourt and Jackson 2014; O'Donoghue *et al.* 1987; Taggart *et al.* In Press. [Chap 4]), likely due to the island's higher abundance of cats (Taggart *et al.* In Press. [Chap. 7]). This suggests a higher level of environmental contamination with *T. gondii* and a higher risk of infection to sympatric wildlife. However, *T. gondii* seroprevalence in small- and medium-sized terrestrial mammals was estimated to be negligible (Taggart unpublished data). No studies have investigated *T. gondii* seroprevalence in wild populations of koalas. We aimed to estimate the seroprevalence of *T. gondii* in South Australian koalas and assess whether it is higher on Kangaroo Island relative to the mainland. In addition, we aimed to assess the risk of *T. gondii* infection in koalas and their role in the life cycle of *T. gondii.* Due to their ecology and life history, we expected a relatively low *T. gondii* seroprevalence in koalas, but a higher seroprevalence on the island compared to the mainland.

6.3 Materials and methods:

6.3.1 Study sites

Kangaroo Island (-35.788172, 137.213681) is located off the coast of South Australia, and approximately 120 km south west of the Mount Lofty Ranges (-34.96671, 138.695233), which supports the closest mainland koala population. Large populations of koalas are found on Kangaroo Island and the adjacent mainland. The study regions are hereafter referred to as 'island' and 'mainland' respectively. Both the island and mainland regions experience similar Mediterranean climates (Jenkins 1985; Schwerdtfeger 2002), suggesting climatic differences are unlikely to confound *T. gondii* seroprevalence in our study.

6.3.2 Animal capture and sampling

Koalas were captured and sera obtained for other purposes as described by Fabijan *et al.* (2019) in April 2016 (mainland koala sampling) and February 2017 (island koala sampling). Koalas from both regions were captured using the flag capture method described by Duka and Masters (2005). Only female koalas were captured on the island as part of the state governments Koala Sterilization Program (Duka and Masters 2005). Both male and female koalas were captured and sampled on the mainland. All captured koalas were sexed, and aged by tooth wear according to Martin and Handasyde (1999). Blood was collected by cephalic or femoral venepuncture whilst animals were anaesthetised. Blood samples were allowed to clot, centrifuged at 4,000 *g* for 5 min and extracted sera stored at -80 C until serological examination.

6.3.3 *Toxoplasma gondii* modified agglutination test (MAT)

Serological testing was conducted as described in Taggart *et al.* (In Press. [Chap 4]). Sera were tested for *T. gondii* immunoglobulin G (IgG) antibodies using a commercially available MAT (Toxo-Screen DA, bioMérieux, Marcy-l'Étoile, France) following the manufacturers guidelines. Sera were screened at 1:40 and 1:4,000 dilutions, and classified as positive if agglutination occurred at either dilution. A dilution was classified as positive when agglutination of *Toxoplasma* formed a mat covering about half or more of the well base. A positive kangaroo control described in Taggart *et al.* (In Press. [Chap 4]), the positive and negative goat control provided by the manufacturer, and a negative phosphate buffered saline (PBS, pH 7.2) control were included with each essay. The diagnostic sensitivity and specificity of the MAT in koalas is unknown, but is expected to be fair based on test performance in other species (Mainar-Jaime and Barberan 2007).

6.3.4 Data analysis

We estimated T. gondii seroprevalence and binomial 95% confidence intervals stratified by age, sex, and sampling location.

We also estimated the probability of freedom from *T. gondii* seropositivity in koalas from the island and mainland using the approach outlined by Cameron *et al.* (2014). The probability of freedom from *T. gondii* seropositivity was unknown prior to sampling, therefore, an initial value of 50% was used in our model. We assumed that the MAT had perfect detection as its diagnostic sensitivity and specificity has not been evaluated in koalas. Similarly, no published information is available on the risk of *T. gondii* exposure across koala sexes or age classes, accordingly, we assumed risk to be constant. We estimated the probability of freedom for a range of design seroprevalence values (minimum seroprevalence of *T. gondii* expected in the koala population if it is exposed) to explore the robustness of the survey data. Freedom from *T. gondii* exposure was deemed demonstrated if the modelled probability of freedom was ≥95%.

6.4 Results:

We screened 94 koala sera from the island and 63 from the mainland for *T. gondii* IgG antibodies (Table 1). All koalas were negative to *T. gondii* and all control samples tested as expected.

Table 6.1: Seroprevalence of *Toxoplasma gondii* in South Australian koalas (*Phascolarctos cinereus*) stratified by sex, age category and sampling location.

		Age category*							
Population	**Sex**	I	II	III	IV	V	VI	***N***	**Seroprevalence % (95% CI)**
Kangaroo Island	All	4	11	18	37	21	3	94	0 (0, 3.9)
	Male	0	0	0	0	0	0	0	-
	Female	4	11	18	37	21	3	94	0 (0, 3.9)
Mount Lofty Ranges	All	7	8	11	18	11	8	63	0 (0, 5.7)
	Male	3	6	5	11	6	4	35	0 (0, 9.9)
	Female	4	2	6	7	5	4	28	0 (0, 12.1)

*An increase in age category corresponds to an increase in koala age. Age categories are described by Martin and Handasyde (1999)Martin and Handasyde (1999)Martin and Handasyde (1999).

Estimates of the probability of freedom from *T. gondii* exposure for the island and mainland, and the combined populations, are reported for a range of design seroprevalence values in Table 2. We collected enough seronegative evidence to demonstrate freedom (probability of freedom ≥95%) from *T. gondii* exposure if the design seroprevalence was ≥4% on the island, ≥5% on the mainland, and ≥2% in the combined populations.

Table 6.2: Estimates of the probability of freedom from *Toxoplasma gondii* exposure in 157 koalas (*Phascolarctos cinereus*) (all seronegative) from Kangaroo Island and the Mount Lofty Ranges (mainland) in South Australia. Bold – probability of freedom ≥95%.

Design prevalence	Kangaroo Island	Mount Lofty Ranges	Combined populations
1%	72.0%	65.3%	82.9%
2%	87.0%	78.1%	**96.0%**
3%	94.6%	87.2%	**99.2%**
4%	**97.9%**	92.9%	**99.8%**
5%	**99.2%**	**96.2%**	**100.0%**
10%	**100.0%**	**99.9%**	**100.0%**

6.5 Discussion:

Our screening of 157 koalas for *T. gondii* exposure represents the largest serological survey undertaken in this species. No koalas from either population were seropositive to *T. gondii*. We are 96% confident that the combined island and mainland koala population was free from *T. gondii* infection at the time of the survey given that the design prevalence is ≥2% and assuming that the MAT was perfectly accurate. As we only sampled females on the island, we cannot exclude the possibility of an effect of sex on *T. gondii* seroprevalence in island koalas. However, we consider it unlikely given all mainland koalas were seronegative irrespective of sex.

Before interpreting the risk of *T. gondii* infection in koalas or the role they play in its life cycle, it is important to consider; 1) the ecology of koalas; 2) the ecology of cats; 3) interactions between koalas and cats; and 4) any known impacts of *T. gondii* on koalas or related species. As koalas have an extensively arboreal lifestyle, predominately consume the foliage of *Eucalyptus* spp. trees (Moore and Foley 2000), and typically only come to the ground to move between trees or when ill, they would be expected to have very limited

exposure to *T. gondii* oocysts which are deposited on the ground in cat feces. Previous studies have demonstrated that captive koalas have died from toxoplasmosis (Canfield *et al.* 1990; Dubey *et al.* 1991; Hartley *et al.* 1990). Therefore, *T. gondii* infection in wild koalas would likely cause mortality and swiftly remove infected animals from the population. This could result in a survival bias where only negative animals remained for sampling. In addition to the nil apparent *T. gondii* seroprevalence, koalas are unlikely to be depredated as a food source by cats or other carnivore species that are subsequently consumed by cats, reducing the probability that an infected koala would contribute to sustained transmission of *T. gondii*. Therefore, we conclude that the risk of *T. gondii* infection in wild koalas is negligible and that koalas have a minor role in the life cycle of *T. gondii* in South Australia.

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Conflict of interest statement:

We declare no conflict of interest.

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Chapter 7: Evidence of significantly higher island feral cat abundance compared to the adjacent mainland

Statement of Authorship

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Name of Principal Author (Candidate)	Patrick Taggart		
Contribution to the Paper	Formulated experimental study design. Data collection and processing. Formatted, edited, analysed and interpreted data. Wrote and revised manuscript.		
Overall percentage (%)	85		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	4th March 2019

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate in include the publication in the thesis; and

iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

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Contribution to the Paper	Contributed to experimental study design. Co-interpreted data. Revised manuscript. Co-supervised PhD candidate.		
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Evidence of significantly higher island feral cat abundance compared to the adjacent mainland

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7.1 Abstract:

Context: Feral cats (*Felis catus*) impact the health and welfare of wildlife, livestock and humans worldwide. They are particularly damaging where they have been introduced into countries such as Australia and islands like New Zealand, where native prey species evolved without feline predators. Kangaroo Island, in South Australia, is Australia's third largest island and supports a number of threatened and endemic species. Cat densities on Kangaroo Island are thought to be greater than densities on the adjacent South Australian mainland, based on one cat density estimate on the island that is higher than most estimates from the

mainland. The prevalence of cat-borne disease in cats and sheep is also higher on Kangaroo Island than the mainland, suggesting higher cat densities. A recent continental-scale spatial model of cat density predicted that cat density on Kangaroo Island should be about double that of the adjacent mainland. However, although cats are believed to have severe impacts on some native species on the island, other species that are generally considered vulnerable to cat predation have relatively secure populations on the island compared to the mainland.
Aims: Our study aimed to compare feral cat abundance between Kangaroo Island and the adjacent South Australian mainland using simultaneous standardised methods. Based on previous findings, we predicted that the relative abundance of feral cats on Kangaroo Island would be approximately double that on the South Australian mainland.
Methods: We used standardised camera trap surveys to simultaneously estimate the relative abundance of feral cats on Kangaroo Island, and the adjacent South Australian mainland. Survey data were analysed using the Royle-Nichols abundance-induced heterogeneity model to estimate feral cat relative abundance at each site.
Key results: Cat abundance on the island was estimated to be over ten times greater than that on the adjacent mainland.
Key conclusions: Consistent with predictions, cat abundance on the island was greater than on the adjacent mainland. However, the magnitude of this difference was much greater than expected.
Implications: Our findings show that the actual densities of cats at local sites can vary substantially from predictions generated by continental-scale models. Our study also demonstrates the value of estimating abundance or density simultaneously across sites using standardised methods.

7.2 Introduction:

Feral cats (*Felis catus*) threaten the conservation of many vertebrates worldwide (Hardman *et al.* 2016; Medina *et al.* 2011). The impacts of cats on wildlife can be both direct, through predation (Hardman *et al.* 2016), and indirect, through competition (Courchamp *et al.* 2003), disease transmission (Canfield *et al.* 1990), and induced behavioural changes (Bonnington *et al.* 2013). The effects of cats are demonstrated by wildlife responses in cat-free refuges (Moseby *et al.* 2009; Short *et al.* 1999), and the numerous reports worldwide of the devastating conservation impacts of introduced cats on islands that were once cat-free (Burbidge and Manly 2002; Medina *et al.* 2014). The island continent of Australia is one such

example where cats, since their introduction in the 18th century, are thought to have contributed to the extinction of 22 species of native mammal and have been implicated in declines in the distribution and/or abundance of a further 46 threatened species and 29 near threatened species (Woinarski *et al.* 2015).

Island fauna are particularly susceptible to the impacts of feral cats (Medina *et al.* 2011). The increased vulnerability of island fauna to the impacts of feral cats has been attributed to several potentially compounding factors, including high levels of endemicity (Myers *et al.* 2000), prey naivety (Salo *et al.* 2007), and the inability for populations to be replenished by immigration (Laurance 2008). Islands are also considered to provide favourable conditions for increased cat densities due to a combination of lower competition and predation from larger predators, fewer diseases, and greater food availability (Legge *et al.* 2017). These conditions may allow cat populations to reach higher densities on islands than on nearby mainland areas.

Kangaroo Island, in South Australia, is Australia's third largest island (4405 km^2). Like many islands, Kangaroo Island supports a number of threatened and endemic species, including the southern brown bandicoot (*Isoodon obesulus obesulus*), hooded plover (*Charadrius cucullatus*), Rosenberg's goanna (*Varanus rosenbergi*), Kangaroo Island short-beaked echidna (*Tachyglossus aculeatus multiaculeatus*) and Kangaroo Island dunnart (*Sminthopsis griseoventer aitkeni*). Cat densities on the island are often claimed to be greater than densities on the adjacent South Australian mainland. This claim is supported by: 1) cat density estimates on Kangaroo Island of 0.7 cats km^{-2} (Bengsen *et al.* 2011a), greater than many cat density estimates from the Australian mainland (Legge *et al.* 2017); 2) a higher seroprevalence of *Toxoplasma gondii* in cats (range 87% - 89%) and sheep (range 17% - 33%) on Kangaroo Island compared to the mainland across multiple different tests (cats: range 0% - 75%; sheep: range 4% - 30%) (Fancourt and Jackson 2014; O'Donoghue *et al.* 1987); and 3) the significant conservation impacts cats have on some Kangaroo Island species, such as the endemic short-beaked echidna (Rismiller 1999; Rismiller and McKelvey 2000). In contrast to this, some species that would be expected to be vulnerable to cat predation such as the southern brown bandicoot and bush stone curlew (*Burhinus grallarius*) have been suggested to have relatively secure populations on Kangaroo Island when compared to populations on mainland South Australia (Gates and Paton 2005; Paull 1995), suggesting that cat impacts

(and their density) on the island are similar to or less than those on the mainland. However, no comparisons of cat populations between Kangaroo Island and the mainland using standardised methods or conducted simultaneously have been reported. Given the lack of evidence regarding cat density on Kangaroo Island, a study that compares relative cat abundance on the island and adjacent mainland at the same time would be valuable, and inform conservation action.

To compare the relative abundance of cats on Kangaroo Island and the adjacent mainland, we used standardised camera trap surveys to simultaneously estimate the relative abundance of cats. Based on previously reported strong correlations between relative abundance and density (Clare *et al.* 2015; Linden *et al.* 2017), and cat density estimated for Kangaroo Island (Bengsen *et al.* 2011a) and modelled for Kangaroo Island and the mainland (Legge *et al.* 2017), we predicted that the relative abundance of cats on Kangaroo Island would be approximately double that of the mainland.

7.3 Materials and methods:

7.3.1 Study areas

The Dudley Peninsula on Kangaroo Island (-35.8015^{o} N; 137.9752^{o} E), and the tip of the adjacent Fleurieu Peninsula (-35.5886 ^{o} N; 138.1986 ^{o} E) on mainland South Australia (Figure 6.1), were selected because they were the closest points of Kangaroo Island and the mainland, with the most similar climatic conditions. The Dudley Peninsula is located 13.5 km south west of the Fleurieu Peninsula (Figure 6.1). The study areas are hereafter referred to as 'island' and 'mainland', respectively. The island and mainland experience similar Mediterranean climates (Jenkins 1985; Schwerdtfeger 2002), with the mainland having a higher 2010-2015 average annual rainfall (mainland = 699mm per year and island = 528mm per year) (Australian Government Bureau of Meterology (BOM) 2017). Both regions are comprised of similar land uses and vegetation types, predominately cropping and pasture land interspersed with patches of native vegetation (largely low *Eucalyptus* spp. woodlands).

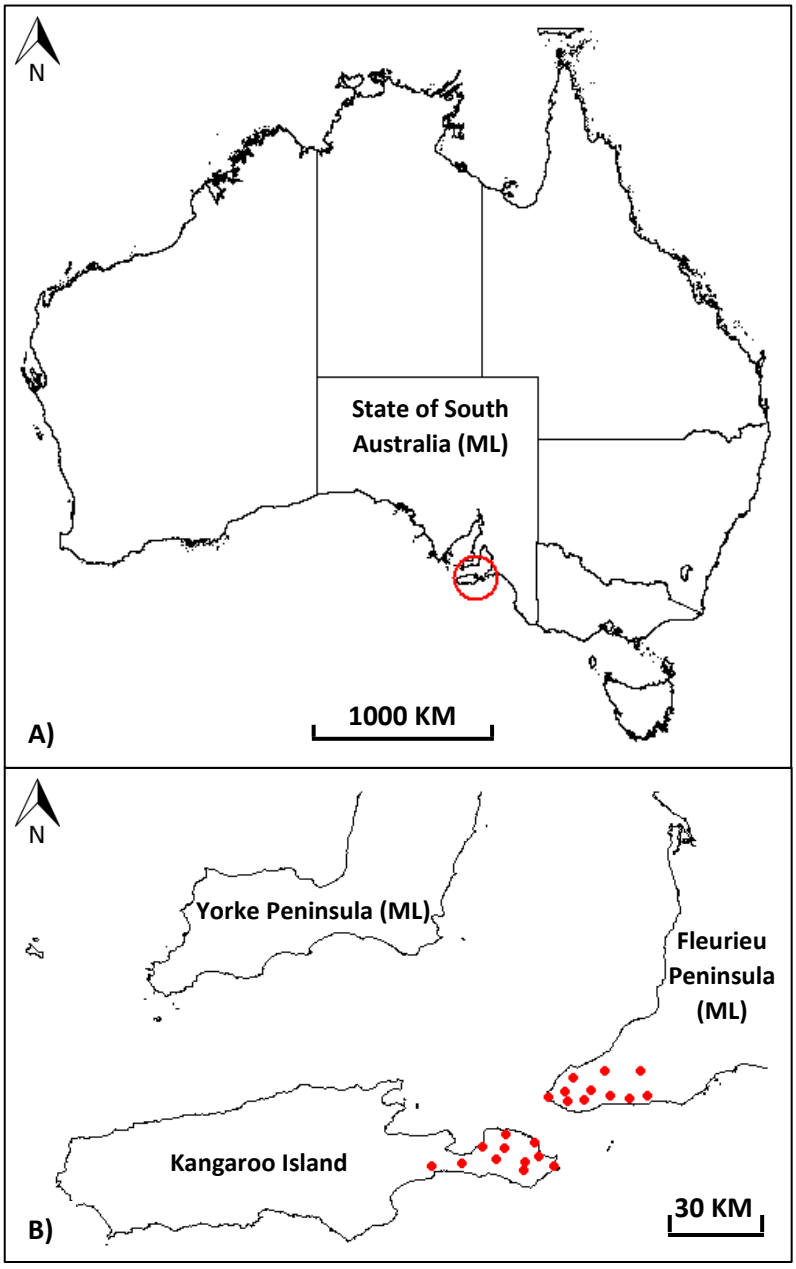

Figure 7.1: A) Location of study areas (red circle) in relation to the Australian continent. B) Location of the 11 camera sites (red dots) on the Dudley Peninsula (Kangaroo Island) and of the 11 camera sites on the adjacent Fleurieu Peninsula (mainland South Australia). ML: mainland South Australia. For an interactive map of the camera sites access https://rpubs.com/a1701399/341574.

Camera traps were set at 11 sites on the island and 11 sites on the mainland (Figure 6.1). Camera sites were spaced at least 4 km apart to increase the likelihood of spatial independence based on cat home ranges on Kangaroo Island which average 5.2 km^2 (Bengsen *et al.* 2012). Camera sites were selected to include a structural interface between native vegetation and adjacent cleared land (predominately grazing pastures) to optimise cat

detection which is often greater in open areas such as roads and trails adjacent to suitable habitats (Meek *et al.* 2012; Read *et al.* 2015) (Figure 6.2).

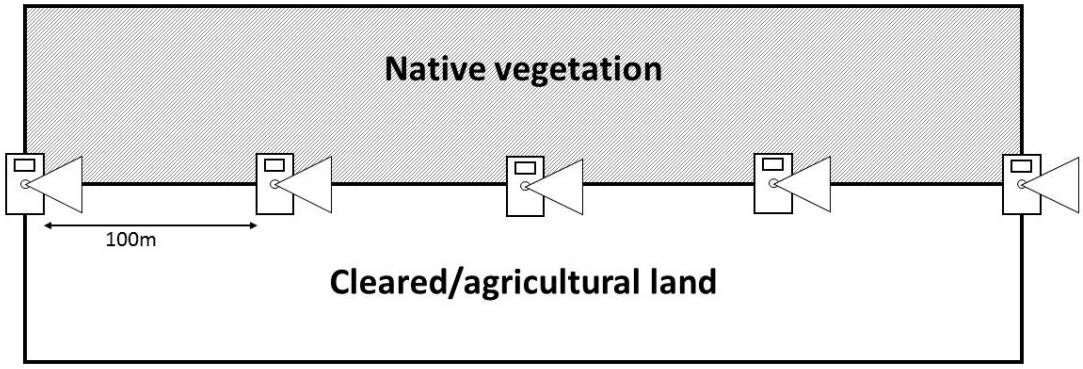

Figure 7.2: Study site layout, showing five camera traps deployed along a structural interface between native vegetation and cleared/agricultural land. All cameras face the same direction along the structural interface and are spaced 100m apart.

7.3.2 Camera trap surveys

At each of the 22 camera sites, five cameras were deployed simultaneously approximately 100 m apart on the open ground directly adjacent to the native vegetation for 24 days (September 17th to October 10th 2016). We deemed this to be an appropriate length of time to maintain population closure. All cameras at each site faced the same direction, parallel to the structural interface (Figure 6.2). During the study period the mean daily rainfall and temperature on the island was 2.2 mm and 16.2 ^{o}C respectively compared to 5.7 mm and 15.1 ^{o}C on the mainland. On both the island and mainland, we used ScoutGuard DTC560K infrared cameras at three sites, ScoutGuard 565F white-flash cameras at three sites, Moultrie M-999i infrared cameras at three sites and Reconyx HC550 white-flash cameras at two sites. Camera placement at each site was chosen to maximise cat detections, with cameras attached to wooden stakes at a height of 400 mm. No habitat modification was required to optimise camera performance.

We used both a visual and olfactory lure. Lures were placed 3m in front of the cameras and consisted of coloured turkey feathers (n = 3) tied together and suspended from a second wooden stake 500-800 mm above the ground (Bengsen *et al.* 2011a), and 50 mL of tuna oil poured on the ground at the base of the wooden stake (Elizondo and Loss 2016). The visual

lure was used to serve as an attractant if/when the tuna oil scent deteriorated. Combining two different lures increased the likelihood of detecting both visually stimulated and food-motivated individuals. All cameras and lures were deployed by the same operator (PLT).

Cameras recorded three pictures in rapid succession following each trigger, with further sets of three pictures taken until movement stopped. Camera trigger delay (time between subsequent images of same individual) and speed (time difference between when animal is detected and image is captured) varied between camera models, ranging between 0.5-1 sec and 0.2-1.2 sec respectively. All images were stamped with the date and time.

7.3.3 Image processing and data analysis

All images were sorted and mammal species were identified by a single person (PLT) using the image browser ExifPro 2.1 (Kowalski 2011). If a camera's orientation changed during the course of deployment such that the lure was no longer clearly in view, we stopped recording and data were coded as missing. Any images where species could not be determined confidently were excluded from the analyses. All analyses were performed in the statistical program R version 3.4.4 (R Core Team 2018).

Cat detection event

We calculated the rate of cat detections per camera night. We first plotted a histogram of the time difference between consecutive cat detections at a particular camera location (individual camera) on a particular day (Figure S6.1). For instance, the time difference between the first and the second cat detection at that camera location on that day, or between the third and the fourth cat detection at that camera location on that day. To distinguish if multiple cat detections within a short period (< 60 mins) were part of the same cat detection event or a different cat detection event, we compared the markings of each cat captured to determine if it was the same or a different individual. If multiple cat detections within a short period were deemed to be part of the same cat detection event, we excluded the subsequent detection of the same individual.

To describe and compare the rate of cat detections camera^{-1} night^{-1} on the island and the mainland, we used a general index (GI) model (Bengsen *et al.* 2011b). This method calculates an index and variance components on the basis of the number of detection events observed

at each camera location on each day by using linear mixed-effects models with random effects for camera location and day (Engeman 2005). We modelled the counts of cat detections at a camera location using a GI model with location (island or mainland) as a fixed effect, and Poisson error distributions and log-link functions. Indices were calculated as the back-transformed expected counts for the period of camera deployment, which described the expected number of cat observations camera^{-1} day^{-1}. Parameters were estimated by the restricted maximum likelihood, using the R package 'lme4' version 0.999375-18 (Bates *et al.* 2015).

Relative abundance of cats

We divided the camera surveys into 24 one-day (midnight-midnight) sampling occasions. Daily sampling occasion data was used to create site-specific detection histories by recording cat presence (1) or absence (0) each day. Cat detections were pooled across all cameras at each site (1 = one or more cats detected by any of the five cameras at the site on that day, 0 = no cats were detected on any camera on that day), providing the majority of cameras (3-5 cameras) remained undamaged/unmoved on that day. Once three or more cameras at a site had been damaged/moved cat presence/absence data was recorded as missing from that site for the remainder of the deployment period.

To calculate site-specific relative abundance estimates of cats for each study area, we used the Royle-Nichols (RN) abundance-induced heterogeneity model (Royle and Nichols 2003), implemented using the 'unmarked' package version 0.11-0 (Fiske and Chandler 2011). The RN model is an extension of the MacKenzie *et al.* (2002) occupancy modelling approach which does not require the individual identification of animals. Instead, the RN model assumes a positive association between a species' abundance and its detection probability and exploits variation in detection probability attributable to variation in abundance to estimate the relative abundance of the species at a site (Royle and Nichols 2003). This method of analysis accounts for situations when a species may be present but not detected (MacKenzie *et al.* 2002), and assumes that all inter-site variation in the detection probability for and abundance of a given species can be explained by site or observation level covariates.

We constructed nine nested candidate models using three covariates. Camera model (ScoutGuard DTC560K, ScoutGuard 565F, Moultrie M-999i, and Reconyx HC550) was included in candidate models as a covariate on detection due to known differences in detection probability between camera makes and models (Fancourt *et al.* 2018; Meek *et al.* 2015). To account for the possibilities that olfactory lure effectiveness declined with age post deployment or cat behaviour differed between the island and mainland, we additionally included lure age and location (Island Vs. mainland) as covariates on detection. To test for differences in cat abundance between the island and mainland, location was included as a covariate on abundance in all models excluding the null model. As there was no reason to expect interaction between covariates, we did not include interaction terms in our models. Model parsimony was compared using Akaike Information Criterion values, corrected for small sample size (AICc).

To test the robustness of our RN abundance model, we ran a model sensitivity analysis. The strength of the relationship between the probability to detect ≥ 1 individual of the target species (p) and true abundance (N) is strongly influenced by the observer's ability to detect a given individual of the target species if they are present (r) and N (Noon *et al.* 2012). When r is high for a given N, the relationship between p and N breaks down. For example, the relationship between r and N may break down if a site is by chance located close to the centre of a cat's home range, where it is likely to spend a disproportionate amount of time, in which case heterogeneity in r may more strongly reflect cat activity as opposed to true abundance, N. By re-running our analysis with 12 two-day, 8 three-day and 6 four-day sampling occasions (data not presented) we were able to determine how increasing the value of r would influence the observed relationship between p and N. We found that sampling occasions of two and three days produced near identical abundance estimates and confidence intervals compared to sampling occasions of one day, suggesting that our model was below saturation and within the realms of functional dependence. In contrast, sampling occasions of four days produced substantially different abundance estimates with much wider confidence intervals, indicating the model was saturated and a breakdown in the relationship between p and N had occurred. This gave us confidence that the use of one-day sampling occasions produced a functionally dependent relationship between our detection estimates and true site abundance.

7.4 Results:

For 130 of the 176 (73.9%) daily surveys (one-day period at one site) the sites were fully operational (5 of 5 cameras at a site remained undamaged/unmoved) for the 24 day camera deployment. For 38 of the 176 (21.6%) daily surveys a site had up to two cameras damaged or moved, but was still considered operational (3 or 4 of 5 cameras at a site remained undamaged/unmoved) on that day. In eight of the 176 (4.5%) daily surveys the site was considered not operational (1 or 2 of 5 cameras at a site remained undamaged/unmoved) and cat presence/absence data were recorded as missing on these days.

7.4.1 Cat detections

We recorded a total of four cat detections on the mainland and 84 cat detections on the island over 1320 camera-nights in both study areas. The expected number of cat detections camera^{-1} day^{-1} on the mainland (0.002, 95% CI: <0.001, 0.006) was approximately 95 % lower than the expected number of detections camera^{-1} day^{-1} on the island (0.037, 95% CI: 0.024, 0.059) (z = -5.39, p = <0.001). We excluded one cat detection on the island from analyses as it was classified as being part of another cat detection event based on being only seven minutes after a previous detection (Figure S6.1) and the cats both having very similar and distinctive markings. All other cat detections were separated by at least three hours (Figure S6.1). Only a single cat was captured in any detection event.

7.4.2 Relative abundance of cats

Based on AICc values, the model including camera model as a covariate on detection and location as a covariate on abundance best fitted our data (Model 2, Table 6.1). Using this model, we estimated the relative abundance of cats site^{-1} on the island at 14.6 (95% CI: 7.51, 28.3). This estimate was over ten times higher than the estimated relative abundance of cats site^{-1} on the mainland, 1.39 (95% CI: 0.46, 4.19) (Figure 6.3). The large difference in estimated relative abundance between the island and mainland was consistent with the large difference in expected number of cat observations per camera per day. Camera model explained some of the inter-site variation in cat detection probability (Figure 6.4).

Table 7.1: Royle-Nichols abundance-induced heterogeneity models for cat abundance, and their associated model fit.

LL = log likelihood;

K = total number of parameters in model.

Model	Model covariates - detection covariate (det.), abundance covariate (abun.)	LL	K	AICc	ΔAICc	AICc Wt
2	Camera (det.), location (abun.)	-156.98	6	331.96	0	0.64
3	Location (det.), location (abun.)	-162.62	4	335.75	3.79	0.10
5	Camera (det.), location (det.), location (abun.)	-156.58	7	335.77	3.82	0.09
6	Camera (det.), lure age (det.), location (abun.)	-156.92	7	336.46	4.51	0.07
1	Location (abun.)	-164.55	3	336.51	4.56	0.07
7	Lure age (det.), location (det.), location (abun.)	-162.60	5	339.19	7.23	0.02
4	Lure age (det.), location (abun.)	-164.51	4	339.51	7.56	0.01
8	Camera (det.), lure age (det.), location (det.), location (abun.)	-156.53	8	341.07	9.11	0.01
null		-174.97	2	354.60	22.64	0.00

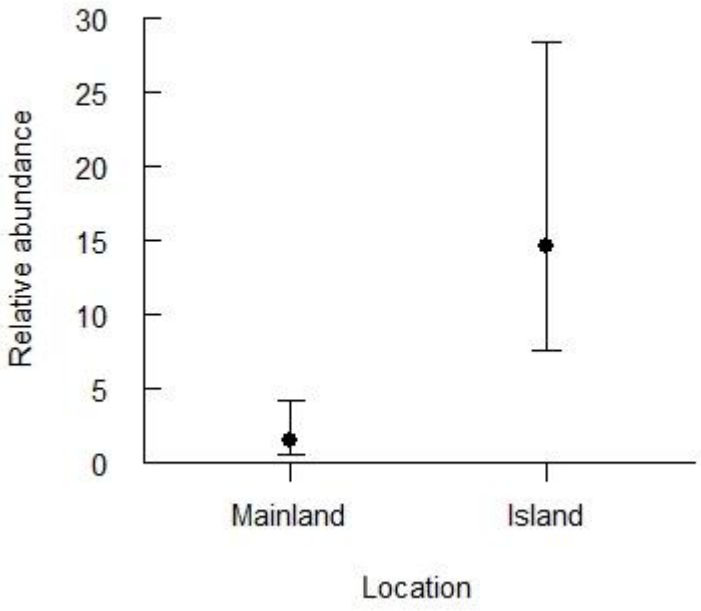

Figure 7.3: Relative cat abundance per site (± 95% confidence interval) on the island (Dudley Peninsula, Kangaroo Island, South Australia), and the mainland (Fleurieu Peninsula, mainland South Australia), estimated across 24 repeated one-day sub-surveys.

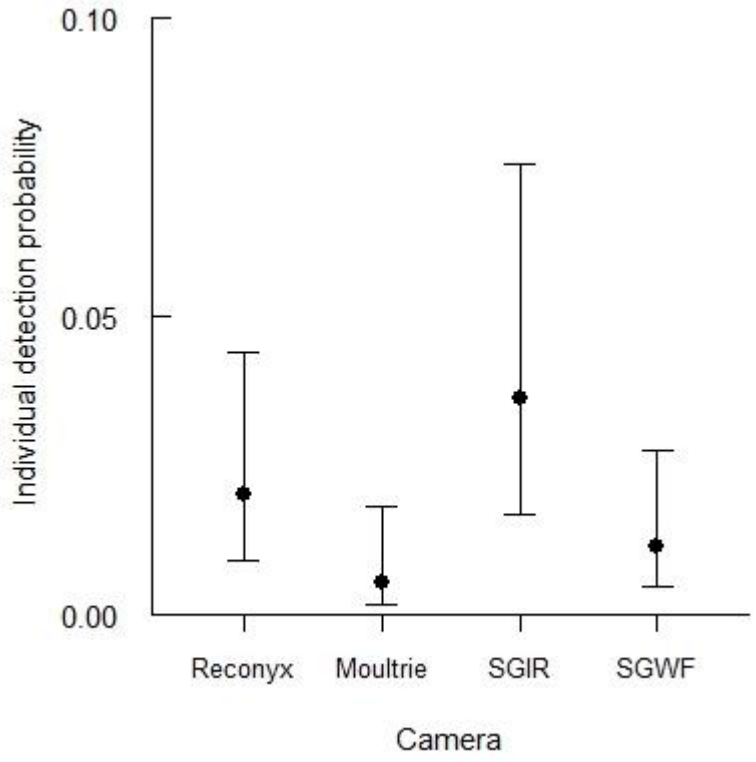

Figure 7.4: Individual cat detection probability (probability that a given individual is detected in a single one-day sub-survey, r) (± 95% confidence interval) by camera model (SGIR = ScoutGuard infrared, SGWF = ScoutGuard white-flash).

7.5 Discussion:

Comparisons of cat density estimates from a number of island and mainland sites in Australia, collected at different times and using different methods, suggest that cat densities on islands are higher than on the mainland. But, this study is the first to collect information on relative cat abundance from an island and the adjacent mainland at the same time and using the same methods. We estimated the mean relative abundance of cats per camera site to be over ten times greater on the island (14.6 cats site^{-1}) compared to the mainland (1.39 cats site^{-1}) (Figure 6.2). This finding supports previous evidence/suggestions of higher cat abundance on Kangaroo Island (Bengsen *et al.* 2011a) compared to the mainland, but is substantially greater in magnitude than predictions from a continental-scale model of cat density on the mainland and islands (Legge *et al.* 2017).

We could not identify any reason to suggest the observed difference in cat abundance between the island and mainland was an artefact of our study design. In our analysis, models allowing cat detection to vary between the island and mainland were not supported. We appreciate that the low number of cat detections on the mainland may have given us low

power to detect differences in cat detection between the island and mainland. However, our camera locations were chosen to optimise cat detections (Meek *et al.* 2012; Read *et al.* 2015), suggesting that the absence of cats at some mainland sites was not due to differences in detection probability, but rather that cat abundance was approaching zero. This evidence supports a true difference in cat abundance between the island and mainland study areas, and that cat density on the island compared to the mainland is much greater than previously suggested (Bengsen *et al.* 2011a).

Potential reasons for the observed difference in relative cat abundance may include direct and/or indirect factors. Cats may be less abundant on the mainland because of higher predation and competition compared to the island. Neither the red fox (*Vulpes vulpes*) nor dingo (*Canis familiaris*) have ever been reported on Kangaroo Island. The red fox is abundant and the largest mammalian predator in the absence of dingoes at our mainland study area. It is known that foxes can attack and kill cats on the mainland (Molsher 1999), but the frequency and population level impact of these occurrences is unknown. In other studies, cat activity indices have been shown to be negatively correlated with fox activity indices, again suggesting that foxes may influence cat abundance and/or activity (Read and Bowen 2001; Risbey *et al.* 2000). Foxes may also influence cat populations via competition for food when it is a limited resource, as suggested by substantial overlap in their dietary niches (Catling 1988; Glen *et al.* 2011). No other native predators are known to be present at our mainland study sites at densities sufficient to have a population level impact on cats. At best, we can only hypothesise that the presence of foxes on the mainland may influence the behaviour and abundance of cats, as speculated previously (Risbey *et al.* 2000), but it seems unlikely that foxes could be the sole cause of the very large difference in relative cat abundance on the island and mainland reported here.

Abundant food resources and/or greater hunting success on Kangaroo Island may also support a higher cat abundance compared to the mainland. An additional food source that is more readily available on Kangaroo Island compared to the adjacent mainland is medium sized mammals. Kangaroo Island has high densities of some medium-sized mammals (i.e. macropods (*Macropus fuliginosus fuliginosus* and *Macropus eugenii*) and common brush tail possums (*Trichosurus vulpecula*)), contributing to a high incidence of roadkill on the island (Leeuwenburg 2004) and a high rate of lethal population control of these species. These

animals, and their carcasses provide an abundant and easily accessible food source for cats. A higher seroprevalence of *T. gondii* in wildlife on Kangaroo Island, like that shown in cats and sheep (O'Donoghue *et al.* 1987; O'Callaghan *et al.* 2005), could further increase prey species' susceptibility to cat predation (Berdoy *et al.* 2000). Islands themselves additionally often support higher levels of productivity than mainlands due to their increased ratio of coastal, compared to non-coastal, habitats. Biomass from marine food webs enter terrestrial webs through shore drift of algal wrack, carrion, and colonies of seabirds (Polis and Hurd 1996). Whilst the island and mainland regions in our study likely do not differ greatly in their received volume of algal wrack or carrion, Kangaroo Island does support substantial waterfowl breeding sites, which could provide similar food resources and are absent within the mainland study region. Small cat home ranges and high densities are associated with increased and more predictable landscape productivity/food resources (Bengsen *et al.* 2016). Our results, showing high relative cat abundance on the island compared to the mainland suggest that Kangaroo Island presents a more productive and predictable environment than the adjacent mainland.

A more favourable climate and more suitable habitat, less lethal control or lower incidence of infectious diseases on Kangaroo Island may potentially be other contributing factors to the observed difference in cat abundance. However, due to the close proximity of our two study areas (13.5 km at closest distance), and the fact that cat density estimates on the mainland show large variation even between areas with similar climates (Legge *et al.* 2017), we consider it unlikely that climatic differences would be a key factor explaining the difference in cat abundance between the two regions. On Kangaroo Island and the mainland, cat populations are sometimes managed via live trapping and opportunistic shooting at a local scale. However, from anecdotal reports, the level of lethal cat management on Kangaroo Island is considerably higher than the nearby Fleurieu Peninsula. Although this may be a consequence of Kangaroo Island's higher cat abundance, the lethal control of cats is nonetheless a conflicting explanation for the observed difference between cat abundance. Occupancy rates of cats are known to be lower in topographically complex habitats (scree, cliffs and rock outcrops where elevation varies greatly over relatively short distances) (Hohnen *et al.* 2016) because of their lower hunting efficacy in these habitats (McGregor *et al.* 2015). But, the complexity of the topography does not differ much in our two study areas.

We are not aware of any evidence or studies that have reported a differential burden in feline infectious diseases between the two areas.

As well as having implications for predation rates, high cat abundance is likely to increase the impacts of cat-borne diseases. Cats are the definitive host of the parasite *Toxoplasma gondii,* which causes the disease toxoplasmosis. The relative high cat abundance on Kangaroo Island that we report here is consistent with the high *T. gondii* prevalence in intermediate hosts on Kangaroo Island (O'Donoghue *et al.* 1987; O'Callaghan *et al.* 2005), because cat density correlates with *T. gondii* prevalence across sites (Dubey *et al.* 1997; Mateus-Pinilla *et al.* 1999). This parasite is known to have substantial health, welfare and economic impacts on wildlife, livestock and humans (Dubey 2016).

The probability of detecting a cat at a site, given they were present at the time of sampling (detection probability), varied according to camera model. Similar results demonstrating differences between camera models in their ability to detect the target species when it is present have been reported previously (Fancourt *et al.* 2018; Heiniger and Gillespie 2018). These results highlight the importance of standardised camera trap surveys that use the same model camera, or that account for differences in camera model via analytical methods or careful study design. Besides camera model, detection probability depends on a number of key factors that must also be considered in the study design phase, including camera detection system, camera placement and orientation, camera triggering and recovery, camera trap settings, temperature differentials, accurate animal identification, behavioural responses of the animals to the cameras (Meek *et al.* 2015) and sympatric species such as predators and competitors (Fancourt 2016).

Our study was limited to a single season (spring) and our results and extrapolations are therefore limited to this season. Differences in relative cat abundance between the island and mainland may be more or less pronounced at different times of the year due to seasonal fluctuations in food resources, breeding, competition or predation pressure. Future studies comparing cat population abundance or density between Kangaroo Island and the mainland may overcome some of the potential limitations of our study by using other analytical techniques such as spatial capture-recapture.

The results of this study indicate that the relative abundance of feral cats is more than ten-fold higher on Kangaroo Island than the adjacent mainland. This suggests potentially large differences in the conservation impacts of cats between these two areas. Reductions in cat abundance and density on Kangaroo Island will benefit wildlife through reductions in predation, competition, and disease transmission (Hardman *et al.* 2016; Risbey *et al.* 2000), with additional benefits for livestock and human health (Dubey *et al.* 1997; Fredebaugh *et al.* 2011; Mateus-Pinilla *et al.* 1999; Mateus-Pinilla *et al.* 2002; Wallace *et al.* 1972). Our study highlights the importance of simultaneous standardised population comparisons to ensure that conservation management actions are informed and appropriate at local scales.

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Conflict of interest statement:

We declare no conflict of interest.

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7.8 Chapter 7 supplementary material:

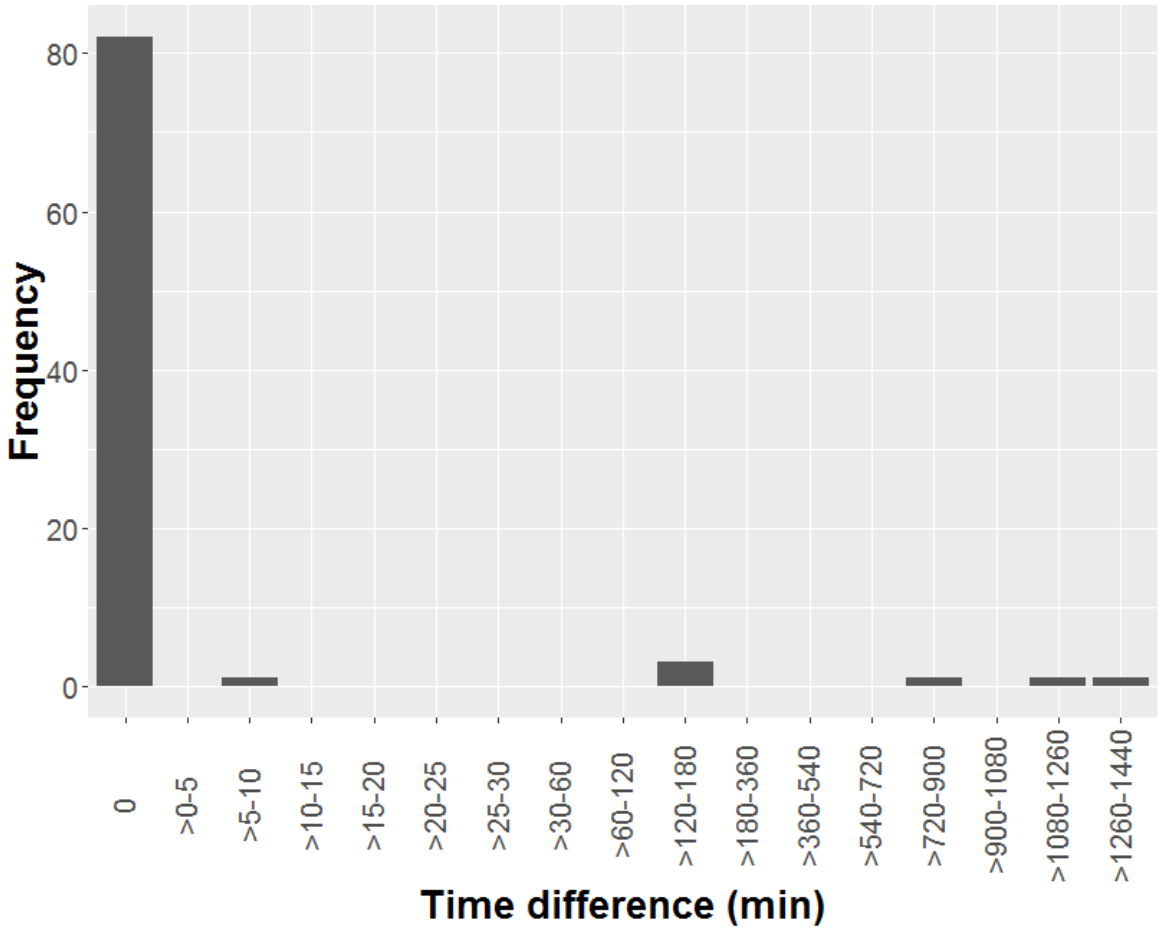

Figure S7.1. Time difference between consecutive cat detections at a particular camera location. Time difference ranges from 0-24 hours and is in increments of three hours from 180 mins onwards.

Chapter 8: Discussion

The purpose of my study was to develop a better understanding of the ecology of *T. gondii*, *S. gigantea* and *S. medusiformis* to enable better control of these cat-borne parasitoses. To achieve this I first identified an appropriate region within which to study these cat-borne parasitoses and applied a multidisciplinary approach to investigating my three thesis aims outlined in Chapter 1. In this final chapter, I align my findings with my thesis aims and then describe my recommendations for the management of these cat-borne parasitoses and the benefits their management will have for humans, livestock and wildlife.

8.1 Aim 1: Confirm that the prevalence of *T. gondii* and macroscopic sarcocystosis is higher in sheep on Kangaroo Island compared to the adjacent South Australian mainland

Sheep are the only known intermediate host of *Sarcocystis gigantea* and *S. medusiformis*. Using government surveillance data collected for skeletal muscles of sheep at slaughterhouses over the 2007-2017 period, we identified Kangaroo Island as a strong cluster of macroscopic ovine sarcocystosis compared to the South Australian mainland (Taggart *et al.* 2019. [Chap 2]). Ovine sarcocystosis was 14 times more frequent on the island than in any other South Australian agricultural region (Taggart *et al.* 2019. [Chap 2]).

At the farm-level, we show a strong association between macroscopic ovine sarcocystosis in the oesophagus and *T. gondii* infection, suggesting that *T. gondii* infection may also cluster on Kangaroo Island relative to the adjacent Australian mainland (Taggart *et al.* In Review. [Chap 3]). This is consistent with previous evidence showing a higher *T. gondii* seroprevalence in sheep on Kangaroo Island relative to the South Australian mainland (O'Donoghue *et al.* 1987). However, I was unable to provide strong evidence for a cluster of *T. gondii* infection in sheep on Kangaroo Island as I could not show an animal- or farm-level association between *T. gondii* infection and macroscopic ovine sarcocystosis in skeletal muscles (Taggart *et al.* In Review. [Chap 3]), which were the data used in my spatial analysis of sarcocystosis (Taggart *et al.* 2019. [Chap 2]).

8.2 Aim 2: Investigate if the seroprevalence of *T. gondii* is higher in wildlife on the island

Through sampling macropods, small- and medium-sized mammals, and koalas on Kangaroo Island and the adjacent South Australian mainland, we show that *T. gondii* seroprevalence is higher relative to the mainland in species other than sheep. The seroprevalence of *T. gondii* in culled western grey kangaroos on Kangaroo Island was 20.4%, compared to 0% on the mainland (Taggart *et al.* In Press. [Chap 4]). These findings are consistent with the high seroprevalence of *T. gondii* found in cats (*Felis catus*) on Kangaroo Island relative to the mainland (Fancourt and Jackson 2014). We were unable to demonstrate a higher seroprevalence of *T. gondii* on Kangaroo Island compared to the mainland in small- and medium-sized mammals (Taggart *et al.* Chap. 5), or koalas (Taggart *et al.* In Press. [Chap 6]).

8.3 Aim 3: Investigate what factors influence the ability of *T. gondii*, *S. gigantea* and *S. medusiformis* to thrive within an ecosystem?

8.3.1 Key ecological factors

Cat density

I suggest that cat density is the foremost factor that influences the ability of cat-borne parasitoses to thrive within an ecosystem, and is likely the leading explanation for the observed higher prevalence of cat-borne parasitoses on Kangaroo Island. My spatial analysis of sarcocystosis in sheep across South Australia showed a large effect size for Kangaroo Island and suggested that the island supports an unmeasured confounder, which was not included in our spatial analysis and is much more prominent on the island relative to the mainland (Taggart *et al.* 2019. [Chap 2]). Based on our cat abundance comparison revealing over ten times higher cat abundance on the Dudley Peninsula, on Kangaroo Island, relative to the Fleurieu Peninsula, on mainland South Australia (Taggart *et al.* In Press. [Chap. 7]), I argue that this unmeasured confounder is likely cat density. This high cat density on Kangaroo Island may be the consequence of a number of factors, including low depredation of or competition with cats, high food resources, favourable climate or habitat, low lethal control of or disease burden for cats, all of which are discussed in detail in Taggart *et al.* (In Press. [Chap. 7]).

Few studies exists demonstrating that the prevalence of cat-borne parasitoses in intermediate hosts scales with cat density. However, one recent study by de Wit *et al.* (2019) compared cat density and *T. gondii* seroprevalence in humans on seven Mexican islands.

Although they found that cat presence was a necessary factor, *T. gondii* seroprevalence in humans did not scale with cat density (de Wit *et al.* 2019). The lack of a continuous relationship between cat density and *T. gondii* seroprevalence reported by de Wit *et al.* (2019) questions the importance of cat density compared to presence only – can few cats shed sufficient oocysts to saturate the environment spatially and temporally? The results reported by de Wit *et al.* (2019) may be explained by the high mobility and long lifespan of humans, and the lifelong persistence of *T. gondii* IgG antibodies. As a consequence of these three factors human seroprevalence values reported by de Wit *et al.* (2019) are strongly influenced by the duration of infection and process that may have occurred off of the islands, for example infections may have occurred off the islands and imported contaminated food products are subjected to off-island environments. I suggest a better understanding of if and how *T. gondii* seroprevalence scales with cat density may be gained by studying seroprevalence in rodents or other animals confined to the seven islands and with shorter lifespans to better reflect the incidence of infection instead of the duration of the infection (or seropositivity).

Intermediate host characteristics

We tested *T. gondii* seroprevalence in macropods, small- and medium-sized mammals, and koalas on Kangaroo Island and the adjacent mainland to provide insights into how the biological attributes of the intermediate host may influence *T. gondii* seroprevalence. Whilst we were unable to control potential confounding factors and infer with confidence, the screening of multiple species from the same locations but with different life history characteristics provided insights into the influence of host factors on parasite transmission.

Western grey kangaroos have similar life histories to sheep in that they are large mammals, with a similar lifespan (10 years vs. 6 years respectively (Miller 2002)) and similar feeding behaviour (grazers). Accordingly, we found *T. gondii* seroprevalence in both kangaroos (20.4%) (Taggart *et al.* In Press. [Chap 4]) and sheep (56.8%) (Taggart *et al.* In Review. [Chap 3]) on the island to be high. In contrast, all brushtail possums were seronegative to *T. gondii*. Brushtail possums are a species that has a similar lifespan (approximately 7 years (Meyer 2000)) to kangaroos and sheep, spends a large amount of their time on the ground on the island, but forage vegetation above ground level (Cochrane *et al.* 2003; Evans 1992; How and

Hillcox 2000), reducing its ingestion of soil contaminated with *T. gondii* oocysts. The contrast in *T. gondii* seroprevalence between intermediate host species suggests that feeding ecology is likely a determinant factor that influences parasite transmission and ecology. Similarly, we sampled koalas on Kangaroo Island (Taggart *et al.* In Press. [Chap 6]), a species that has a longer lifespan (approximately 13 years (Dubuc and Eckroad 1999)), but is arboreal, occupying a vertically separated and distinct niche to that of kangaroos and sheep. All koalas were also seronegative to *T. gondii*, suggesting that the niche or distribution occupied by the intermediate host is another determinant factor contributing to *T. gondii* transmission and ecology.

Lastly, we sampled rodents (Taggart *et al.* Chap. 5), which are much smaller in size and have a much shorter lifespan (approximately 1 year (Ballenger 1999; Gillespie 2004)), but also forage directly on the ground and amongst the soil. Unexpectedly, we found that *T. gondii* seroprevalence in house mice (0%) and bush rats (2%) was negligible, suggesting that the lifespan of the intermediate host is a determinant factor favouring *T. gondii* transmission. As described in Taggart *et al.* Chap. 5), the suggestion that intermediate host lifespan is a key factor is supported by several studies showing that seroprevalence increases with age (Jones *et al.* 2001; Van der Puije *et al.* 2000).

8.3.2 Other contributing factors

Climatic factors

We were unable to show that climatic variables such as rainfall, humidity and temperature influence the occurrence of macroscopic ovine sarcocystosis (Taggart *et al.* 2019. [Chap 2]), but our sarcocystosis prevalence density map showed very few positive farms in the north of South Australia where the climate is hot and dry and cats are known to be present (Legge *et al.* 2017). It is possible that our analysis was unable to detect the importance of climatic factors due to the strong influence of the disproportionately high number of sarcocystosis-positive farms within the Kangaroo Island region. However, studies showing that sporocysts and oocysts are killed by severe and extended desiccation (Dubey *et al.* 2015; Dubey 2016a; Savini *et al.* 1996) suggest that climatic factors would influence the prevalence of cat-borne parasitoses, but do not provide insights into the importance of climatic relative to other factors. Whilst our spatial analysis did not identify climatic factors, similar studies assessing

the spatial distribution of macroscopic sarcocystosis or *T. gondii* infection may demonstrate otherwise if data show less disparity in infection prevalence across regions. Alternatively, by excluding the Kangaroo Island region and re-running our spatial analysis of sarcocystosis, we may provide further insight into the influence of climatic factors on the occurrence of this condition.

Environmental factors

In our spatial analysis of macroscopic sarcocystosis, we show that environmental variables, such as soil pH and clay content may influence the prevalence of macroscopic ovine sarcocystosis and *T. gondii* infection (Taggart *et al.* 2019. [Chap 2]). Our spatial analysis of sarcocystosis in sheep revealed that the frequency of infection increased with decreasing soil pH and increasing soil clay content (Taggart *et al.* 2019. [Chap 2]). We proposed that lower soil pH and higher clay content likely impact the occurrence of macroscopic ovine sarcocystosis by favouring the survival of *Sarcocystis spp.* sporocysts in the environment (Taggart *et al.* 2019. [Chap 2]). As *T. gondii* is closely related to and shares similar lifecycle and ecology with *S. gigantea* and *S. medusiformis*, I would expect that these soil characteristics also impact on the survival of *T. gondii* oocysts in the environment. However, due to the additional protective shell in *T. gondii* oocysts relative to *Sarcocystis spp.* sporocysts (Dubey *et al.* 2015; Dubey 2016a), the impacts of soil pH and clay content on the desiccation and survival of *T. gondii* oocysts may be less pronounced than their impacts on the survival of *Sarcocystis spp.* sporocysts.

8.3.3 Untested factors

Cat feeding ecology

Regional differences in the feeding ecology of cats could result in profound differences in the prevalence of cat-borne parasitoses, although such differences are rarely considered. For example, sub-ordinate scavengers consume carrion more frequently in regions where the abundance of dominant scavengers has been suppressed relative to regions where dominant scavenger populations are intact (Olson *et al.* 2012). Kangaroo Island lacks larger mammalian scavengers, such as the red fox (*Vulpes vulpes*), and is therefore an example of a region where sub-ordinate scavengers, such as cats, may exhibit increased scavenging (Figure 7.1). If the prevalence of cat-borne parasitoses in this carrion is higher than their prevalence in

other typical dietary items of cats, for example live small mammals, it would be expected that cats in regions where dominant scavenger populations are suppressed or absent would more frequently shed these parasites into the environment. Therefore, I would expect an increase in the relative prevalence of cat-borne parasitoses in the region lacking dominant scavengers. Cats have been shown to scavenge on Kangaroo Island (Hodgens *et al.* Unpublished Data) and the prevalence of *T. gondii* in macropod carrion on the island is known to be higher than in typical cat dietary items, such as small and medium sized mammals (Taggart *et al.* Chap. 5; Taggart *et al.* In Press. [Chap 4]). Consequently, the scavenging of macropod carrion by cats on Kangaroo Island would contribute to increased transmission of *T. gondii*.

Figure 8.1: Feral cat (*Felis catus*) feeding on macropod carcass on Kangaroo Island. Photo: Patrick Taggart, University of Adelaide.

Cat defecation behaviour

Regional differences in cat defecation behaviour could contribute to differences in the prevalence of cat-borne parasitoses. Cats are known to bury a large proportion of their faeces (Panaman 1981). Whilst the reason for this behaviour is not well-known, it is hypothesised to be associated with scent marking, hygiene or social rank (Ishida and Shimizu 1998). If this behaviour is associated with scent marking, it may be influenced by the

presence or absence of predators, as suggested for other species (Swihart 1991). The South Australian mainland and Kangaroo Island are examples of this, where cats may bury their faeces more frequently on the mainland to avoid being detected by larger predators, and more frequently deposit their faeces on the ground surface on the island due to being the largest mammalian predator and having little fear of being detected by larger predators. This could influence the transmission of cat-borne parasitoses, such as *T. gondii* and macroscopic sarcocystosis. All of *T. gondii*, *S. gigantea* and *S. medusiformis* rely heavily on faecal-oral transmission. Consequently, the burying of faeces would be expected to limit the dispersal of oocysts/sporocysts during adverse weather compared to unburied faeces deposited on the surface of the ground (Figure 7.2). If oocysts/sporocysts dispersed further from unburied faeces this would influence the level of environmental contamination with *T. gondii*, *S. gigantea* and *S. medusiformis*.

Figure 8.2: Feral cat (*Felis catus*) faeces deposited on the surface of the ground. Photo: Pat Hodgens, Terrain Ecology.

Factors influencing cat abundance

It could also be argued that factors influencing cat abundance have a direct influence on the prevalence of cat-borne parasitoses. In Taggart *et al.* (In Press. [Chap. 7]), we suggest a number of factors which may influence cat abundance, including differences in red fox abundance, cat food resources, climate, frequency of lethal cat control, and diseases lethal to cats. The relative importance of each of these factors in influencing cat abundance would require strictly structured experiments to adequately test. However, I suggest that differences in the abundance of larger mammalian predators and cat food resources are

likely important to explaining differences in cat abundance (Taggart *et al.* In Press. [Chap. 7]). These two factors, implicitly linked, could thus be important indirect drivers of the high prevalence of cat-borne parasitoses.

8.3.4 Testing causality

In this thesis I suggest the most likely causal factor and hypothesise several others that may favour the proliferation of cat-borne parasitoses (Taggart *et al.* In Press. [Chap. 7]; Taggart *et al.* 2019. [Chap 2]). As discussed above and shown in chapter 6, the presence/absence of cats is known to be strongly associated with the prevalence of cat-borne parasitoses (Taggart *et al.* In Press. [Chap. 7]). However, the relationship between soil characteristics and the prevalence of macroscopic sarcocystosis and *T. gondii* are speculative.

Identifying a causal relationship between soil characteristics and cat-borne parasitoses prevalence ideally requires multiple steps, including controlled experiments. A specified number of oocysts/sporocysts should be superficially buried in soil of varying but defined pH and clay content, similar to the work by McKenna and Charleston (1994). At specified time points oocyst/sporocyst survival would be measured by their ability to excyst in vitro. Demonstrating that differences in oocyst/sporocyst survival in the environment translate to meaningful differences in the prevalence of cat-borne parasitoses in natural populations would be difficult to achieve. Such an experiment would require multiple large areas, initially of equivalent oocyst/sporocyst burden and of equivalent cat density, but varying soil pH or clay content. Due to the difficulties involved in demonstrating that reduced oocyst/sporocyst survival translates to reduced prevalence of cat-borne parasitoses, correlative evidence from modelling (Taggart *et al.* 2019. [Chap 2]) is likely the closest we will come to demonstrating causality. As discussed, soil pH can be raised via the application of agricultural lime (Taggart *et al.* 2019. [Chap 2]). Accordingly, the application of lime to soils could be expected to reduce the prevalence of cat-borne parasitoses. However, this also requires demonstration. Similar to that described above, a reduction in oocyst/sporocyst survival due to the application of agricultural lime could be demonstrated within the laboratory, but demonstrating that this translates to meaningful reductions in the prevalence of cat-borne parasitoses would be difficult.

Differences in cat feeding ecology and defecation behaviour between the island and mainland could be investigated, but whether this translates to differences in the prevalence of cat-borne parasitoses would also be difficult to test. As described above, scavenging behaviour would be expected to be the main aspect of cat feeding ecology that may differ between the island and mainland. Camera traps are often used to assess scavenging behaviour (Forsyth *et al.* 2014) and could be placed on carcasses on both the island and mainland simultaneously to observe the frequency with which they are utilised by cats (Figure 7.3). For best results, I recommend using cameras with sufficient image resolution to facilitate the individual identification of cats and deploying cameras in video mode to ensure cats are truly feeding on carcasses as opposed to hovering around them without feeding. To assess if increased scavenging translates to increased prevalence of cat-borne parasitoses, it is also important to know whether the seroprevalence of *T. gondii* in the carrion on which cats are feeding is higher than in other typical cat dietary items.

Figure 8.3: Camera trap placed on macropod carcass to investigate how they are utilised by scavengers. Photo: Patrick Taggart, University of Adelaide.

Differences in cat defecation behaviour between the island and mainland could be assessed by searching for and collecting faecal deposits from multiple cats across multiple habitats in both regions. The important part of testing this hypothesis would be to determine whether the dispersal of oocysts/sporocysts is limited by the burial of faeces compared to faeces deposited on the ground surface. The dispersal of oocysts/sporocysts from buried and unburied faeces could be assessed in the laboratory similar to described above. Cat faeces free of *T. gondii* and *Sarcocystis spp*. should be homogenised with a defined number of oocysts/sporocysts. For the buried treatment, replicates of the homogenised faecal matter should then be superficially buried and for the unburied treatment, replicates of the homogenised faecal matter should be deposited in the same environment on the ground surface. Both buried and unburied faeces should be subjected to artificial rain and wind, followed by collecting soil samples at increasing distances from faecal deposits and testing soil samples for parasite DNA.

8.4 Recommendations for the management of cat-borne parasitoses

8.4.1 Cat control

At a large scale and for domestic and wild animals (including wildlife), I suggest that the control of cats is currently the most efficient and acceptable method of managing *T. gondii* infection in Australia and in most countries without native felids due to the control of intermediate hosts or manipulation of the environment being less ethical and achievable. However, for the management of *T. gondii* infection in humans, current evidence suggest that the complete eradication of cats, as opposed to reductions in their density, is necessary (de Wit *et al.* 2019). In countries without native felids, the importance of cat control to the reduction of *T. gondii* infection and impacts cannot be overstated. Based on current knowledge of *T. gondii* biology, the eradication of cats from environments with no native felids would be expected to result in the eventual complete elimination of *T. gondii* and associated health impacts in all species. However, as *T. gondii* can be transmitted vertically (Parameswaran *et al.* 2009), horizontally (Boyer *et al.* 2011; Hill *et al.* 2011), and sexually (Dass *et al.* 2011), the complete elimination of *T. gondii* and associated impacts in all species would not occur immediately. Similarly, cat control would be expected to reduce the prevalence of macroscopic sarcocystosis in sheep, and the complete eradication of cats would be expected to result in the elimination of this condition. As sheep are the only known intermediate host of *S. gigantea* and *S. medusiformis*, and these parasites are not

transmitted horizontally and would be expected to be rarely transmitted vertically (Moré *et al.* 2009), the complete elimination of macroscopic sarcocystosis in sheep following cat eradication would occur more rapidly. I predict that the complete elimination of macroscopic sarcocystosis in sheep following cat eradication would occur within approximately two generations (of sheep).

The control of cats (*Felis catus*) is conducted across varying scales using numerous tools and techniques, depending on the aims and goals of the control program (Campbell *et al.* 2011; Denny and Dickman 2010; Robertson 2008). At small scales, such as individual households, cats and their associated parasitoses can be managed via: changing litter boxes daily to ensure *T. gondii* oocysts cannot sporulate and become infective prior to their disposal; feeding cats only dry, canned or cooked food; and eliminating all opportunities cats have to hunt (Dubey 2016a). This can be achieved by keeping cats indoors at all times, confined to cat runs, or installing cat-proof fences that confine cats to a particular household (Oscillot 2019). At medium scales, where lethal cat control is less accepted or palatable, for example urban stray cat populations, cats are regularly controlled via trap-neuter-release programs (Tan *et al.* 2017). However, the effectiveness of these programs is debated (Kilgour *et al.* 2017), and de-sexing does not stop cats from shedding oocysts or sporocysts into the environment. For this reason, I do not recommend trap-neuter-release for the management of cat-borne parasitoses. At large scales (e.g. geographic regions, ecosystems or larger), cats are most frequently managed via lethal control. Lethal control of cats is achieved by many tools and techniques, including poison baiting, hunting/shooting, disease, detector dogs followed by euthanasia, kill traps and trapping followed by euthanasia (Campbell *et al.* 2011). Whilst other techniques, such as non-surgical contraception (Robertson 2008), gene drive (Hammond *et al.* 2016) or grooming traps (Read 2019), may be possible in the future, these techniques are not currently available.

8.4.2 Hygiene

To reduce *T. gondii* infection in humans it is possible to manipulate aspects of parasite exposure that are difficult to manipulate for most other species, for example a high standard of personal and food hygiene. Washing ones hands and vegetables prior to eating to avoid oocyst contamination would be expected to reduce the incidence of *T. gondii* infection in humans (Dubey 2016a). Similarly, avoiding raw or undercooked meat which may harbor live

T. gondii bradyzoites (Bobić *et al.* 2007), and freezing meat prior to eating to kill bradyzoites would be expected to contribute to the management of this parasitosis in humans (Dubey 1988). In livestock, similar precautions can be taken to reduce the incidence of *T. gondii* infection to some extent. For example, keeping cats out of barns and away from livestock grain stores (Dubey 2016c), and providing adequate livestock watering points to minimise the use of surface water for drinking (Vesco *et al.* 2007) would all be expected to reduce the incidence of *T. gondii* and macroscopic ovine sarcocystosis in livestock.

8.4.3 Vaccines

For the management of *T. gondii* and its associated impacts, vaccines produced from live attenuated and/or genetically modified *T. gondii* organisms are available to reduce congenital transmission (O'Connell *et al.* 1988; Wilkins *et al.* 1988). Experimental vaccines have also been produced to reduce the formation of *T. gondii* tissue cysts in livestock (Dubey *et al.* 1991) and to stop the shedding of oocysts by cats (Ramakrishnan *et al.* 2019). In New Zealand, France, Ireland and the United Kingdom, vaccines produced from live attenuated *T. gondii* organisms are used regularly for disease management purposes in sheep (Garcia *et al.* 2014). The use of the vaccine has resulted in a 4% mean increase in lambing percentage, and much greater increases (>25%) where high levels of challenge of susceptible sheep occur (Charleston 1994). Vaccines designed to reduce the formation of tissue cysts are aimed at minimising the transmission of *T. gondii* to humans via the consumption of bradyzoites in meat. Despite these vaccines producing positive results and reducing *T. gondii* tissue cyst formation (Dubey *et al.* 1991), to my knowledge they have never been commercially available. Vaccines to stop the shedding of oocysts by cats can work at local scales (Mateus-Pinilla *et al.* 1999; Mateus-Pinilla *et al.* 2002). However, in their current form these vaccines are experimental only and not commercially available. Irrespective of their availability, I do not consider these vaccines practical disease management options due to their storage and transport requirements, or where the vaccine would need to be delivered to large populations of feral, stray or wild felines for disease management to be effective. To my knowledge no *T. gondii* vaccines are currently available for any species in Australia due to the country's strict biosecurity policies forbidding the importation of a live vaccine.

We are not aware of any vaccines designed for the management of macroscopic sarcocystosis in sheep. Whilst economic estimates have suggested that this condition could

be costing the Spanish sheep industry € 20 million annually (Martínez-Navalón *et al.* 2012), research into the impacts and cost of macroscopic sarcocystosis to the sheep industry are scarce. It is thus difficult to ascertain if vaccine production would be economically viable.

8.4.4 Manipulation of soil characteristics

For broad-land sheep grazing, the application of agricultural lime to pastures may facilitate the management of *T. gondii* and macroscopic sarcocystosis infection (Taggart *et al.* 2019. [Chap 2]). Different soil types require varying amounts of lime to produce a 1 unit increase in soil pH, with typical application rates ranging from 1-5 tonnes per acre (Anderson *et al.* 2013). The current cost of agricultural lime for supply and application to pasture is approximately AUD $80 per tonne. Therefore, the application of agricultural lime to pastures, specifically for the management of macroscopic sarcocystosis, could be costly, and feasibility remains to be tested. However, for landholders currently applying lime to pastures, a reduction in the impacts of cat-borne parasitoses in sheep flocks would appear to be an added and previously unrecognised benefit. For landholders considering applying lime to pastures, these potential benefits are encouraging.

8.4.5 Improved farm practices

For macroscopic sarcocystosis, sheep are the only known intermediate host. Therefore, it is theoretically possible to break the lifecycle of *S. gigantea* and *S. medusiformis*, and reduce the prevalence of this condition, by picking up and disposing of all sheep offal and carcasses on farm via burying or burning to prevent scavenging by cats. Under the right circumstances, where farmers work collaboratively across a large scale, the efficient removal of all sheep offal from farms could result in the complete elimination of this condition. Whilst the removal of sheep offal from farms is recommended by industry and government agencies for the management of macroscopic sarcocystosis in sheep (Primary Industries and Regions South Australia 2018), the large scale of many farms makes this impractical, and/or cultural shifts in farmer attitudes would be required for this to be effective.

8.5 Expected benefits from the management of cat-borne parasitoses

In this thesis I have measured and investigated the prevalence of *T. gondii* infection and macroscopic ovine sarcocystosis (Taggart *et al.* In Press. [Chap. 7]; Taggart *et al.* In Press. [Chap 6]; Taggart *et al.* In Press. [Chap 4]; Taggart *et al.* In Review. [Chap 3]; Taggart *et al.*

2019. [Chap 2]). I have not measured disease prevalence or frequency, although it is presumed that they increase with an increasing prevalence of infection, as suggested elsewhere (Dubey *et al.* 2012). I describe the many health, welfare, economic, social and or conservation impacts these two cat-borne parasitoses can have on wildlife, livestock and humans. The management of these two cat-borne parasitoses would subsequently be expected to reduce the impacts described, and have positive health, welfare, economic and or conservation outcomes for impacted species. However, detecting reductions in disease prevalence or frequency could be challenging. In sheep, humans and wildlife, the impacts of both acute and latent toxoplasmosis are often undiagnosed for a wide variety of reasons (Dubey 2016b). On Kangaroo Island specifically, detecting reductions in disease prevalence or frequency in humans would be difficult due to the relatively small population (approximately 4,700 people) reducing statistical power to detect real differences. Slaughterhouse surveillance data on macroscopic sarcocystosis in sheep provides the best available method of observing and demonstrating the benefits of disease management efforts (Taggart *et al.* 2019. [Chap 2]). This data provides a large quantity of information on the baseline disease prevalence through time (Taggart *et al.* 2019. [Chap 2]) and its collection will continue into the future, documenting reductions in disease prevalence associated with management efforts. On Kangaroo Island, the management and or elimination of cat-borne disease would result in the largest refuge from toxoplasmosis and macroscopic ovine sarcocystosis in the world, excluding Antarctica. This could only contribute to a thriving environment for wildlife, livestock and people on the island.

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Appendix 1: Camera trap flash type does not influence feral cat behaviour

Statement of Authorship

Title of Paper	Camera trap flash type does not influence feral cat behaviour
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Principal Author

Name of Principal Author (Candidate)	Patrick Taggart		
Contribution to the Paper	Formulated experimental study design. Data collection and processing. Formatted, edited, analysed and interpreted data. Wrote and revised manuscript.		
Overall percentage (%)	90		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	4th March 2019

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate in include the publication in the thesis; and

iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Bronwyn Fancourt		
Contribution to the Paper	Contributed to experimental study design. Co-interpreted data. Revised manuscript. Co-supervised PhD candidate.		
Signature		Date	4th March 2019

Name of Co-Author	David Peacock		
Contribution to the Paper	Contributed to experimental study design. Co-interpreted data. Revised manuscript. Co-supervised PhD candidate.		
Signature		Date	4th March 2019

Camera trap flash type does not influence feral cat (*Felis catus*) behaviour

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Keywords: feline; avoidance, monitor, detect, invasive species, pest

A1.1 Abstract:

Camera trapping is now the most commonly used technique for indexing feral cat (*Felis catus*) and predator populations. Camera flash type has previously been suggested to influence animal behaviour and their redetection by similar cameras, with white-flash cameras being shown to reduce the probability of redetecting some species. We investigated the influence of camera flash-type on the behaviour of feral cats by categorising their behavioural response to white-flash and infrared-flash cameras and assessing the frequency with which individual cats were redetected by the same white-flash camera or a different white-flash camera at the same site following their initial detection. We found no evidence that flash type had any influence on the cats' observed behavioural responses towards cameras, or that cats captured by white-flash cameras avoided redetection. Our findings suggest that white-flash cameras are suitable for the detection and redetection of cats, and provide better quality images from which to identify individual cats.

A1.2 Introduction:

Camera traps are one of the most commonly used tools to monitor wildlife populations. They are advantageous due to their ability to be deployed for extended periods of time, increasing the probability of detecting rare or cryptic species (McCallum 2013; Meek *et al.* 2015b). Predators, especially feral cats (*Felis catus*), are inherently difficult to study due to their secretive, nocturnal and far-ranging behaviour, low population densities, and association with hard to access locations (Gese 2001). Accordingly, camera trapping is the most commonly used technique for indexing cat and predator populations (Burton *et al.* 2015; Legge *et al.* 2017; Rovero *et al.* 2013).

When relying on data from camera trap surveys, it is important to have a thorough understanding of how cameras themselves can influence the behaviour of the target species, their detectability and redetection (Meek *et al.* 2015a). White-flash cameras are reported to provide better quality images than infrared-flash cameras, facilitating the identification of individual animals where desired (Bengsen *et al.* 2011; Glen *et al.* 2013; McGregor *et al.* 2015). However, white-flash cameras have also been shown to reduce the redetection of some species (Schipper 2007), with some authors suggesting this might also be the case for felids (Cove and Jackson 2011; Glen *et al.* 2013; Wegge *et al.* 2004). Few studies, however, have quantified or demonstrated negative behavioural responses of feral cats towards white-flash cameras. In this study, we investigated the influence of camera flash-type on the behaviour of feral cats by categorising their response to white- and infrared-flash cameras. In addition, we aimed to assess the frequency with which individual cats were redetected by either the same camera or another camera of the same flash-type at the same site, following their initial detection.

A1.3 Methods:

A1.3.1 Camera trap deployment

Five camera traps and corresponding lures were set at each of 11 spatially independent sites on the Dudley Peninsula on Kangaroo Island, South Australia (-35.8015^{o} N; 137.9752^{o} E), for 24 days as described in Taggart *et al.* (In Press. [Chap. 7]). All cameras and lures were deployed at all sites simultaneously using standardised methods, with cameras set on open ground along the interface between native vegetation and cleared land, and lures (suspended turkey feathers and tuna oil) placed 3 m in front of all cameras. All cameras at

each site faced the same direction and were spaced at 100 m intervals, parallel to the structural interface.

The Dudley Peninsula has a high abundance of feral cats compared to the adjacent Australian mainland (Taggart *et al.* In Press. [Chap. 7]), increasing the probability of detecting sufficiently high numbers of cats on cameras. We used ScoutGuard 565F white-flash cameras at three sites, Reconyx HC550 white-flash cameras at two sites, ScoutGuard DTC560K infrared-flash cameras at three sites and Moultrie M-999i infrared-flash cameras at three sites. Only a single camera model was deployed at any one site. Overall there were 25 white-flash cameras compared to 30 infrared-flash cameras. Cameras were programmed to record three pictures in rapid succession following each trigger, with further sets of three pictures taken until movement stopped. All images were stamped with the date and time. Identical, fully charged batteries were used for all cameras to minimise and standardise any noise produced by cameras when triggered (Meek *et al.* 2014).

A1.3.2 Data analysis

All cats detected were classified into detection events. Multiple cat detections within a short period (< 60 min) were treated as a single cat detection event unless individual cats were distinguishable by their unique pelage markings. We then classified the behaviour of each cat in each detection event as 1) fled: if the cat appeared to flee from the camera following the initial trigger/image; 2) wary: if the cat appeared aware of the camera's presence (e.g turned its head and looked directly at the camera) but its behaviour otherwise remained unchanged; 3) unaware: if the cat did not appear to notice the camera or alter its behaviour; or 4) approach: if the cat was aware of the camera and approached it following the first trigger/image. We considered the classifications flee (#1) and wary (#2) to be negative cat behavioural responses towards the camera that could have implications for camera flash selection in future studies and the classifications unaware (#3) and approach (#4) to be non-negative cat behavioural responses towards the camera that were less likely to have implications for camera flash selection. If incomplete or insufficient images for a detection event precluded us from classifying a cat's behaviour as described, these detection events were excluded from further analysis.

To determine if white-flash cameras have a larger negative influence on cat behavioural responses towards cameras relative to infrared-flash cameras, we compared the proportion

of negative and non-negative behaviours on white- and infrared-flash cameras in a 2x2 contingency table using a Chi-square test. To increase resolution in our analysis, we additionally compared the proportion of all four classified cat behavioural responses to white- and infrared-flash cameras in a 2x4 contingency table using a Fisher's exact test. This second test allowed us to account for the possibility that cat behaviour was influenced by camera flash type in a manner not predicted (for example, flee behaviours were higher on white-flash cameras than on infrared-flash cameras, but wary behaviours were higher on infrared-flash cameras than on white-flash cameras). As there is low variation in noise produced between different camera trap models and as field conditions are expected to mask camera noise further (Meek *et al.* 2014), we considered it highly unlikely that camera trap noise would confound the relationship between flash type and cat behaviour.

To investigate the possibility that either flash-type caused lasting or cumulative impacts on cat behaviour, we compared individual cat redetection rates within each site for both flash types.

A1.4 Results and discussion:

We captured a total of 85 cat detections, 84 of which were classified as separate detection events. One cat detection was excluded as it occurred only seven minutes after a previous detection and both cats had similar markings. White-flash cameras produced 17 events (i.e. 0.03 (95% CI: 0.02-0.04) per camera-night), whereas infrared-flash cameras produced 67 events (i.e. 0.09 (95% CI: 0.07-0.10) per camera-night). This difference between flash-types could be due to a number of factors. First, the camera models used to test responses to white-flash in our study (Reconyx and ScoutGuard) are possibly less sensitive than the camera models used to test responses to infrared-flash (Moultrie and ScoutGuard), irrespective of flash type (Taggart *et al.* In Press. [Chap. 7]). For example, Reconyx infrared-flash cameras have been shown to be far less sensitive and record far fewer species detections than other model infrared-flash cameras (Fancourt *et al.* 2018). Second, the sites selected for the white-flash cameras may have inadvertently supported lower cat densities than the sites selected for infrared-flash cameras. This has the potential to confound the relationship between the redetection of cats on white- and infrared-flash cameras, but is unlikely to influence our comparison of cat behavioural responses to white- and infrared-flash cameras as it relies on proportional differences.

We were able to classify the cat's behavioural response towards the camera in 73 of the 84 cat detection events (Table 1). On several occasions, cats turned their head towards the camera (both white- and infrared-flash cameras on three occasions each), or approached the camera following the first photo (infrared-flash cameras only on two occasions), indicating that they had seen and/or heard the camera trigger. No cat was observed fleeing from a camera.

Table A1.1: Cat behavioural responses towards white- and infrared-flash camera traps

Camera trap flash type	**Classified behavioural response of cat towards camera flash**							
	Flee		Wary		Unaware		Approach	
	Total	%	Total	%	Total	%	Total	%
White	0	0	3	18	14	82	0	0
Infrared	0	0	3	5	51	91	2	4

Table A1.2: Negative (flee and wary classification) and non-negative (unaware and approach classification) cat behavioural responses towards white- and infrared-flash camera traps

Camera trap flash type	**Classified behavioural response of cat towards camera flash**			
	Negative		Non-negative	
	Total	%	Total	%
White	3	18	14	82
Infrared	3	5	53	95

We found no evidence that white-flash cameras had a larger negative influence on cat behavioural responses towards cameras relative to infrared-flash cameras ($X^2 = 2.61$, df = 1, p = 0.11, Table 2), or that the proportion of any of the four classified cat behaviours differed between white- and infrared-flash cameras ($_{\text{Fisher's exact}}$ p = 0.18, Table 1). However, our results should be interpreted with caution due to the small number of cat detections recorded on white-flash cameras. Cats may be able to see both flash types due to their highly sensitive vision (Gekeler *et al.* 2006; Meek *et al.* 2014). Despite some infrared-flash cameras being marketed as 'semi-covert', 'covert', or 'no glow' by the manufacturer, the fact that some animals' vision extends into the infrared spectrum potentially renders attempts to filter or reduce infrared detection by animals as ineffective for these species (Meek *et al.* 2014). This likely explains the lack of a difference in cat behavioural responses towards white- and infrared-flash cameras observed in our study. We recommend that future studies run longer camera surveys with more cameras to increase the likelihood of cat detection and redetection.

We found no evidence that white-flash cameras reduced the likelihood of cats being redetected though our sample size was small. Based on individual cat identification, we estimated a minimum of nine individual cats were detected. Seven of the nine cats were redetected, one on the same camera, four on a different white-flash camera at the same site and two on the same and a different white-flash camera at the same site. The poor quality of many infrared-flash images precluded identification of individual cats and any analysis of redetection. Whilst we acknowledge that the data cannot be analysed, we are not aware of evidence to suggest that the redetection rates of cats on infrared-flash cameras is significantly higher than the observed redetection rate of cats for white-flash cameras in our study. Future studies investigating the influence of camera flash type on animal behaviour should use a paired camera design (e.g. paired white- and infrared-flash camera both at the same location and pointing at the same field of view) to ensure equal opportunity of species detection and minimise the potential confounding factors discussed earlier.

Our findings have implications for monitoring and managing feral cats. White-flash cameras are often avoided by wildlife managers surveying cats due to a perceived negative impact on the cat's behaviour. However, we found no evidence that white-flash cameras are any more likely to negatively influence the behaviour of cats than infrared-flash cameras. Given the improved image quality provided by white-flash cameras (and the improved ability to identify individual cats where required), we suggest that white-flash cameras could provide an improvement over infrared-flash cameras for sampling cat populations.

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Conflict of interest statement:

We declare no conflict of interest.

A1.6 References:

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Appendix 2: Oral conference and workshop presentations derived from this thesis

Presenting author is highlighted in bold font.

1. **Taggart, P. L.**, McAllister, M. M., Rutley, D. & Caraguel, C. G. B. Investigating a novel method of monitoring *Toxoplasma* infection in Australian sheep flocks, Sheep Connect South Australia, Adelaide, South Australia, March 2019
2. **Taggart, P. L.**, Fancourt, B. A., Peacock, D. E., McAllister, M. M. & Caraguel, C. G. B. Look what the cat dragged in! Explaining the increased prevalence of cat-borne diseases on Kangaroo Island, South Australian Wildlife Health Network, Adelaide, South Australia, October 2018
3. **Taggart, P. L.**, Fancourt, B. A., Peacock, D. E., Stevenson, M. R., Bengsen, A. J., Hodgens, P., Read, J. L., Firestone, S. M., McAllister, M. M. & Caraguel, C. G. B. Look what the cat dragged in: correlates of an increased prevalence of cat-borne diseases on an island compared to the adjacent mainland, Ecological Society of America, New Orleans, America, August 2018
4. **Taggart, P. L.**, Stevenson, M. R., Firestone, S. M., McAllister, M. M. & Caraguel, C. G. B. Risk factors associated with sarcocystosis in South Australia: Describing the spatial distribution of a cat-borne disease, Advanced Spatial Epidemiology Workshop, Massey University, Palmerston North, New Zealand, February 2018
5. **Taggart, P. L.**, Fancourt, B. A., Peacock, D. E., McAllister, M. M. & Caraguel, C. G. B. Toxoplasmosis and sarcocystosis in sheep on Kangaroo Island, South Australia, South Australian Sheep Industry Blueprint Meeting, Adelaide, South Australia, November 2017
6. **Taggart, P. L.**, Fancourt, B. A., Peacock, D. E., Bengsen, A. J., Hodgens, P., Read, J. L., McAllister, M. M. & Caraguel, C. G. B. Investigating the increased occurrence of cat-borne disease on Kangaroo Island, South Australia, Wildlife Disease Association Australasian Section Conference, Falls Creek, Victoria, September 2017
7. **Taggart, P. L.**, Stevenson, M. R., Firestone, S. M., McAllister, M. M. & Caraguel, C. G. B. Risk factors associated with sarcocystosis in South Australia: Describing and explaining the spatial distribution of a cat-borne disease, Wildlife Disease Association Australasian Section Conference, Falls Creek, Victoria, September 2017

8. **Taggart, P. L.**, Fancourt, B. A., Peacock, D. E., McAllister, M. M. & Caraguel, C. G. B. Investigating correlates of *Toxoplasma gondii* infection to explain its higher prevalence on Kangaroo Island, Australian Mammal Society Conference, Alice Springs, Northern Territory, September 2016
9. **Taggart, P. L.**, Fancourt, B. A., Peacock, D. E., McAllister, M. M. & Caraguel, C. G. B. Investigating correlates of *Toxoplasma gondii* infection to explain its higher prevalence on Kangaroo Island, Natural Resource Management Science Conference, Adelaide, South Australia, April 2016
10. **Taggart, P. L.**, Fancourt, B. A., Peacock, D. E., McAllister, M. M. & Caraguel, C. G. B. Biology, ecology and impacts of macroscopic *Sarcocystis* in sheep, Kangaroo Island One Health Workshop, Adelaide, South Australia, June 2015
11. **Taggart, P. L.**, Fancourt, B. A., Peacock, D. E., McAllister, M. M. & Caraguel, C. G. B. *Toxoplasma gondii* and macroscopic *Sarcocystis* on Kangaroo Island, Kangaroo Island One Health Workshop, Adelaide, South Australia, June 2015