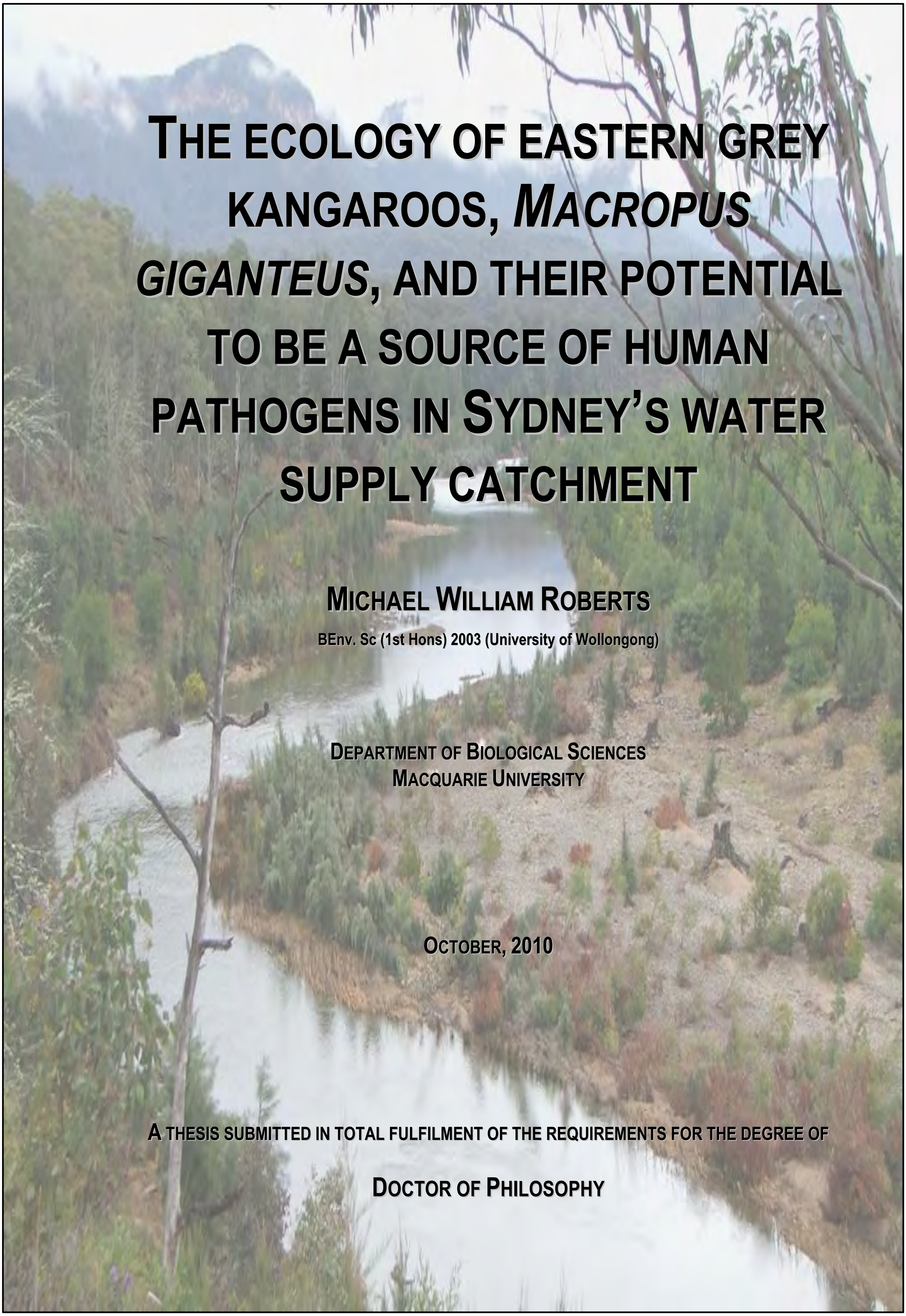

# THE ECOLOGY OF EASTERN GREY KANGAROOS, *MACROPUS GIGANTEUS*, AND THEIR POTENTIAL TO BE A SOURCE OF HUMAN PATHOGENS IN SYDNEY'S WATER SUPPLY CATCHMENT


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DOCTOR OF PHILOSOPHY

***"Nature does nothing uselessly."*** Aristotle
***"Disease is the retribution of outraged nature"*** Hosea Ballou

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# ABSTRACT

A clean water supply is of fundamental importance to human health. In July to September 1998, *Cryptosporidium* and *Giardia* were detected at levels of concern within Sydney's raw drinking water supply. *Cryptosporidium* and *Giardia* are parasitic protozoa that cause gastrointestinal illness and are commonly transmitted via faecal contamination. Illness is typically short-lived, but infection in immunocompromised hosts can become persistent and mortality can result. During the 1998 contaminated water supply incident, neither the source nor the genotypes of these pathogens were determined.

Following the commissioning of the Sydney Catchment Authority (SCA) and the prioritisation of research aimed at protecting the quality of Sydney's drinking water, the potential sources of *Cryptosporidium* and *Giardia* in the catchment were identified. The primary sources were bypasses from sewage treatment plants in high rainfall periods; livestock with unrestricted access to creeks and rivers, and feral and native animals residing adjacent to the water supply. Of these native animals, eastern grey kangaroos (*Macropus giganteus*) were found to harbour *Cryptosporidium* and *Giardia* at similar frequencies to the other potential sources. Populations within the inner catchment (adjacent to Warragamba Dam) were unmanaged, which highlighted the need to determine the extent to which this species was transmitting these pathogens to the water supply.

In this study, I aimed to quantify the ecology of eastern grey kangaroos to evaluate the risk of disease transmission from this species to Sydney's potable water supply. The specific aims of this research in relation to eastern grey kangaroos were to: 1) confirm the presence of zoonotic pathogens (e.g. *Cryptosporidum* and *Giardia*); 2) quantify the population density of the species and its influence on pathogen load calculation; 3) enumerate faecal deposition by the species in watershed habitats; 4) assess seasonal habitat use and movement patterns and 5) measure changes in the demography of the species. Inferences on the potential for the transmission of these diseases to humans via the potable water supply were drawn from the ecological data collected. The field surveys for this study took place between March 2004 and December 2007.

Eastern grey kangaroos harboured zoonotic strains of *Cryptosporidium* and *Giardia*. Preliminary analysis revealed the presence of both *C. parvum* and *C. hominis* (species known to be infective to humans) and faecal samples were positive for human-infective

*Giardia duodenalis*: assemblage A and B. In addition, the analysis of serum revealed that the population of eastern grey kangaroos were host of zoonotic strains of *Leptospira* and *Toxoplasma*. A simple risk assessment concluded that the presence of *Cryptosporidium* and *Giardia* in eastern grey kangaroos posed the largest risk to humans via their transmission to the potable water supply, whereas *Leptospira* and *Toxoplasma* were considered a lower risk due to different modes of transmission of these two diseases. Previous epidemiological studies conducted in 2002, had found that this population harboured only marsupial specific *Cryptosporidium* strains, making the results derived during the current study potentially very significant.

The population densities of eastern grey kangaroos in the Wollondilly River region were among the highest reported for this species (101 – 688 kangaroos per square kilometre). Previous risk assessments of waterborne disease transmission used lower density estimates of 200 kangaroos per square kilometre. Therefore, past assessments used density estimates less than half those typically present and kangaroo densities actually parallel the densities of other species that are considered important sources of pathogens, including livestock animals. Furthermore, because drought conditions were prevalent across the entire sampling period, population densities were presumably lower than in more favourable conditions.

The distribution and the extent of eastern grey kangaroo faecal excreta in relation to surface water was considered critical in the formulation of disease risk. The distribution of faecal material was related to pasture biomass, which was consistent with previous findings that eastern grey kangaroo defecate whilst actively foraging. Faecal deposition was highest in the grassland and woodland habitats and lowest in the riparian habitat. Although faecal deposition by eastern grey kangaroos was least in the riparian habitat, the rate of faecal excreta disappearance was slowest in this habitat suggesting that disease organisms may be preserved for longer periods.

Both the home range sizes and habitat preferences of eastern grey kangaroos are likely to substantially influence the density and proximity of faecal deposition to surface water and therefore disease risk. Eastern grey kangaroos were very localised, having small overlapping home ranges and limited dispersal from their capture locations. Female 95% Minimum Convex Polygon (MCP) home ranges (19.9 – 36.2 ha) were generally smaller than male 95% MCP home ranges (21.1 – 40.3 ha). The small home ranges in comparison to other studies within this species' temperate distribution were explained by the lack of current disturbance (e.g. farm dogs, hunting). The small home ranges exhibited by eastern grey kangaroos in this study reflect the high concentration of faecal material that they contribute to the floodplain and the potential for pathogens originating from this species to enter surface waters. Finite analysis of habitat selection showed that male kangaroos tended to utilise the riparian habitat more than females and visitation to this habitat was greatest at night during spring. These results were explained by the relatively high predation risk of the riparian habitat, sexual segregation and differences in the water balances and seasonal thermoregulation demands on this species at different times of the year. The analysis of habitat selection and movement patterns suggested that there were sex-based differences in the risk of disease transmission and that direct faecal deposition to surface water could occur, which would pose the highest and most direct zoonotic risk from this species.

The social structure of eastern grey kangaroos differed from previous studies due to the low levels of human interaction (e.g. hunting, farm dogs etc.) and the high densities of the species within the study area. A high proportion of groups observed contained more than six animals (mean 6.4 to 9.4 animals per group). The formation of large groups in close proximity to surface water could lead to higher transmission rates of disease. Births were recorded predominantly in January (47.6%), with none recorded in July or between September and November. There were high proportions of older animals in the population and the oldest animal determined from skull analysis was a female aged 21 years. The number of dependent juveniles (young-at-foot and pouch-young combined) ranged from 0.21 in February 2005 to 0.65 per adult female in October 2004. Autumn epidemics of cryptosporidiosis in the study population were previously linked to the presence of pouch-young in the population. Empirical data collected on the abundance of pouch-young concurrently with disease surveillance data suggested that a threshold in the number of pouch-young in the population may have to be attained before *Cryptosporidium* is detected.

The ecological information collected in this study allows water utility managers to identify natural processes that are either contributing to or reducing pathogen loads from wildlife sources (i.e. fluctuations in density, ranging patterns and utilisation of riparian zones). Such information can be used to help identify and prioritise management options that will be most effective. Ecological and epidemiological based risk assessments should be combined to formulate mathematical based models that are used to predict pathogen loads in water supply catchments to ensure that these are more accurate and reflect natural conditions.

# DECLARATION

I, Michael William Roberts, declare that this submission is entirely my own work and that, to the best of my knowledge it contains no written material written by another person, nor material which has been submitted for a higher degree to Macquarie University or any other institution, except where due acknowledgement has been made in the text.

Michael Roberts

10th October, 2010

# ACKNOWLEDGEMENTS

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This work was conducted under a NPWS scientific licence (11002) and Macquarie University Ethics Approval (2003/016, superseded by 2007/020).

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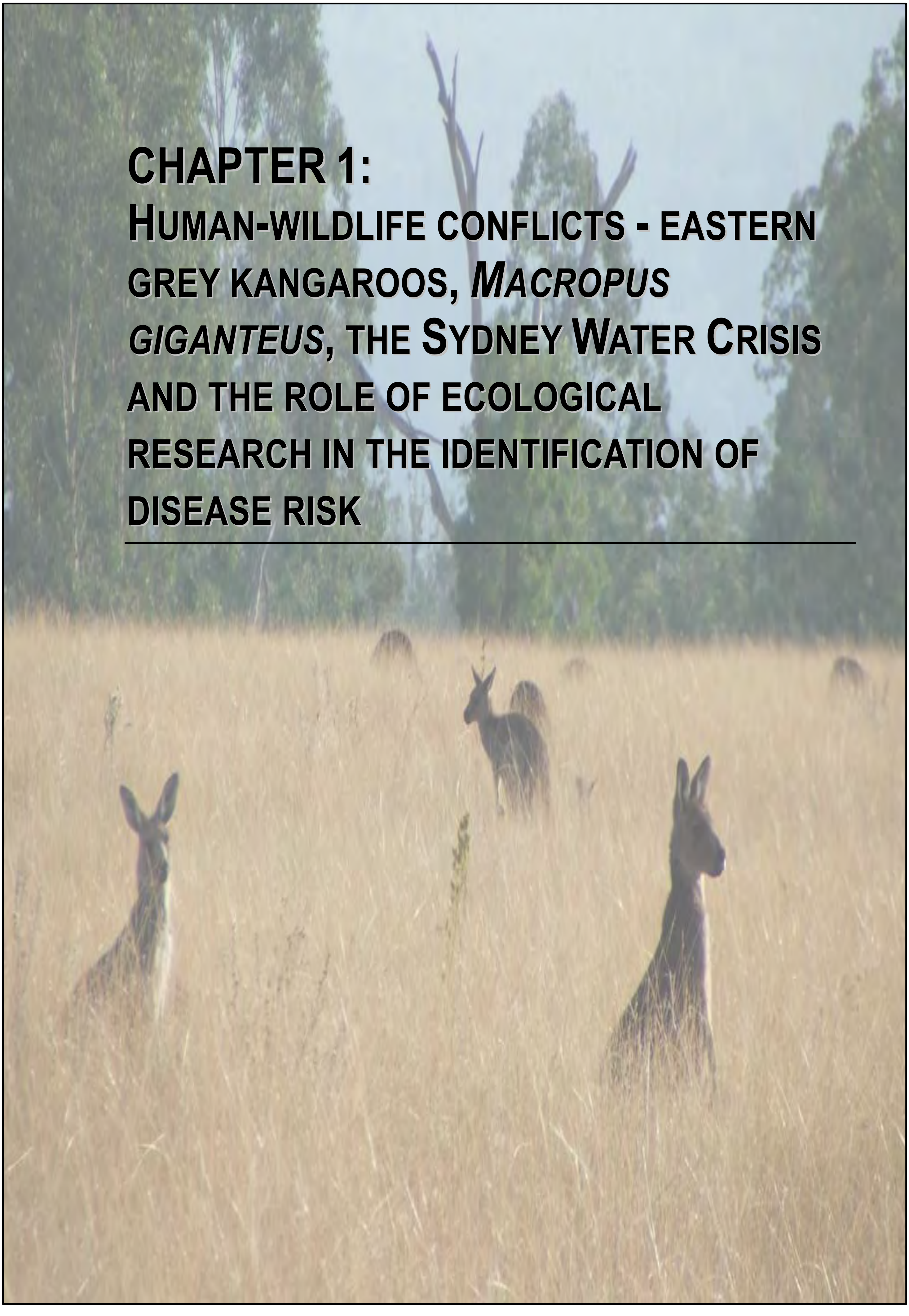
## HUMAN-WILDLIFE CONFLICTS - EASTERN GREY KANGAROOS, *MACROPUS GIGANTEUS*, THE SYDNEY WATER CRISIS AND THE ROLE OF ECOLOGICAL RESEARCH IN THE IDENTIFICATION OF DISEASE RISK



# CHAPTER 1: HUMAN-WILDLIFE CONFLICTS - EASTERN GREY KANGAROOS *MACROPUS GIGANTEUS*, THE SYDNEY WATER CRISIS AND THE ROLE OF ECOLOGICAL RESEARCH IN THE IDENTIFICATION OF DISEASE RISK

## *Preamble to Chapter 1*

This chapter provides the background, framework and rationale for the investigation of the potential for eastern grey kangaroos to be a source of the zoonotic pathogens *Cryptosporidium* and *Giardia* to water catchment areas. The impetus for this work arose from the Sydney Water Crisis of 1998 when these organisms were detected in the city's water supply. This chapter explains the need for ecological research of wildlife disease hosts or carriers in conjunction with pathogen epidemiology to completely evaluate the risk of zoonotic disease transmission via potable water supplies to the Sydney population. Included are details on the study area and the seasonal conditions during which the study was undertaken, as well as the history of pastoral activities on land surrounding the Warragamba Dam catchment.

## INTRODUCTION

Human-wildlife conflict can take on numerous dimensions. Conflicts result in impacts on humans, including losses to human enterprises and more direct impacts such as human deaths and injuries. Some recent global examples are presented in Table 1-1. A common theme driving human-wildlife conflict is that contact rates between humans and wildlife are increasing in response to the marginalisation and modification of wildlife habitats, increases in the human population and the consequent expansions of urban centres (Daszak *et al.* 2000), and changes in human attitudes and activities (Kellert *et al.* 1996). In addition, the increase in conflict can be attributed to the overabundance of some wildlife species in response to the creation of conservation reserves and a reduction in natural predation and hunting pressure (Moore *et al.* 2002, Augustine and deCalesta 2003). Social constraints on the lethal control of overabundant wildlife species have also played a significant role in the increase of these conflicts (Coulson 1998).

**Table 1-1 Summary of some recently reported examples of human-wildlife conflicts.**

| Human-wildlife conflict | Reported impacts | References |
|---|---|---|
| Elephants in Africa and Asia | • Crop raiding, trampling of crops and other impacts on subsistence agricultural practices and larger-scale agribusinesses such as palm oil and timber estates.<br>• Deaths and injuries to humans from trampling. | (Williams *et al.* 2001, Osborn and Parker 2003, Sitati and Walpole 2006) |
| Great Apes and Monkeys (Chimpanzees, Orangutan and Mountain Gorillas, Vervet Monkeys) in Africa and Asia | • Raiding of sugar, maize, banana plantations and oil palm agribusinesses.<br>• Attacks on people. | (Brennan *et al.* 2008, Baker *et al.* 2009, Hockings 2009) |
| Large felids (Tigers, Lions, Leopards, Jaguars and Panthers) in Asia, South America and Africa | • Predation of livestock.<br>• Attacks on people. | (Holmern *et al.* 2007, Gurung 2008, Sangay and Vernes 2008, Maclennan *et al.* 2009) |
| Bears in North America, South America and Europe | • Raiding of maize farms, beehives, orchards, rubbish bins and food store warehouses.<br>• Predation of livestock.<br>• Attacks on people. | (Beckmann and Berger 2003, Herrero *et al.* 2005, Wilson *et al.* 2006, Sangay and Vernes 2008) |
| Wolves and Coyotes in Europe, | • Predation of livestock. | (Meriggi *et al.* 1996, Tsewang *et al.* 2007, Harper *et al.* 2008, |

| Human-wildlife conflict | Reported impacts | References |
|---|---|---|
| Scandinavia and North America | • Attacks on people from wolves and farmers' dogs that are used to protect sheep. | Kaartinen *et al.* 2009) |
| Deer in North America and Europe | • Damage to crops and ornamental gardens<br>• Increases in vehicle-animal collisions leading to human injuries and mortalities | (Rouleau *et al.* 2002, Horsley *et al.* 2003, Sullivan and Messmer 2003, Stewart *et al.* 2007) |
| Macropodids and Possums in Australia and New Zealand | • Competition with domestic livestock.<br>• Damage to native and plantation forests<br>• Increases in vehicle-animal collisions.<br>• Attacks on people. | (Coulson 1982, Arnold *et al.* 1989, Dawson 1995, Cowan and Tyndale-Biscoe 1997, le Mar and McArthur 2005, Viggers and Hearn 2005, Coleman *et al.* 2006, ACT Parks Conservation and Lands 2010) |
| Dingo in Australia | • Predation on domestic livestock<br>• Attacks on people | (Allen and Sparks 2000, Fleming *et al.* 2001, Burns 2003) |
| Wombats in Australia* | • Damage to farm infrastructure.<br>• Injuries to livestock from falling in burrow networks. | (Borchard and Collins 2001) |

Notes: *injuries to livestock based on personal observation

The most commonly reported threat of wildlife species to human enterprise is through the destruction of agricultural crops and the depredation of livestock. For example, several studies on the habitat use and diet of white-tailed deer (*Odocoileus virginianus*) in North America have documented intense exploitation of agricultural landscapes, particularly where adjoining native forest has been removed (Rouleau *et al.* 2002, West and Parkhurst 2002, Stewart *et al.* 2007). In a 2002 survey of Virginian farmers, 58 percent reported damage to their corn and soybean crops that was attributable to white-tailed deer (West and Parkhurst 2002). Overabundant populations of Canada geese (*Branta candensis*) also impact negatively on agriculture in North America through their grazing and trampling of grain crops, pastures and spring seedlings (Reed *et al.* 1977). Recent expansions of wolf (*Canis lupus*) populations, following conservation efforts in Europe, have led to an increase in predation on sheep particularly in areas adjoining native forests (Kaartinen *et al.* 2009). In addition, the depredation of sheep, goats, horses and yak within agricultural areas in remote India by wild carnivores, such as snow leopard (*Uncia uncia*), Tibetan wolf (*Canis lupus chanku*), and Eurasian lynx (*Lynx isabellina*) has led to significant losses of valuable livestock for Trans-Himalayan pastoralists (Tsewang *et al.* 2007).

Human-wildlife conflict also results in significant economic losses to agribusinesses and timber production. Beaver (*Castor canadensis*) damage to tree plantations in south-eastern United States is estimated to exceed $22 million annually and deer (*Odocoileus* spp.) browsing causes an estimated $367 million loss per year for timber production in the northeast (Messmer 2000). Red and roe deer (*Cervus elaphus* and *Capreolus capreolus*) have been reported to impact on commercial timber harvests by over-browsing on seedlings, thus limiting regeneration of monetary species and by bark stripping of semi-mature trees. The total costs of their impacts on woodland plantations in the east of England have been estimated to be well over one million dollars per annum (Ward *et al.* 2004). Predatory wildlife in the United States annually kill more than 490,000 sheep and lambs, 83,000 goats, and 106,000 cattle, resulting in losses exceeding $73 million per year (Conover *et al.* 1995). Such economic losses lead to changes to policy, challenges for government and the direction of considerable amounts of research attention designed to minimise these conflicts and to maintain populations of wildlife in co-existence with people.

More direct impacts of human-wildlife conflicts include human fatalities and injuries resulting from animal-automobile collisions, bird-aircraft strikes and wildlife bites and attacks (Messmer 2000). There are several examples of such direct human-wildlife conflicts. Canada geese are the most common bird species involved in aircraft bird strikes in North America given their inclination to utilise urban areas and their large population sizes (York *et al.* 2000). Two recent examples of aircraft accidents involving Canada Geese include the US Airways Airbus 320 flight in 2009 that was retrieved from the Hudson River, and an E-3 Sentry Airborne Warning and Control System (AWACS) aircraft in Alaska, which resulted in 24 people being killed. In addition, white-tailed deer in the United States and kangaroo species in Australia are commonly involved in vehicle collisions resulting in serious injuries and deaths to humans (Nielsen *et al.* 2003, Lee *et al.* 2010).

The spread of disease from animal sources to humans is another direct form of human-wildlife conflict that can cause human mortality and illness. Prior to describing the aims of the current study, I provide some background on the worldwide importance of these diseases, with various examples, as well as the Sydney Water Crisis of 1998 with particular attention given to the role that eastern grey kangaroo (*Macropus giganteus*) may have played in this incident.

## ZOONOTIC DISEASES

The term ‗*zoonoses*' designates those infectious diseases that can be transmitted between humans and wild and domestic animals (Slingenbergh *et al.* 2004, Greger 2007). They represent some of the most significant infectious diseases as they comprise over 75% of emerging infectious diseases (EIDs) and are in part responsible for the resurgence of further diseases that were believed previously to be under control (Wilcox and Gubler 2005, Chomel *et al.* 2007, Greger 2007). A large proportion of these zoonotic diseases (72%) are of wildlife origin (hereafter ‗wildlife zoonoses') and this figure is expected to increase due to predisposing factors such as expanding global travel, trade, agricultural expansion, deforestation/habitat fragmentation, and urbanisation (Daszak *et al.* 2000). Consequently, wildlife zoonoses represent a significant burden on global economies and public health authorities worldwide, and surveillance is increasing internationally to prevent and mitigate the impacts of these diseases.

Some selected examples of recently emerging wildlife zoonoses are presented in Table 1-2. Wildlife species have long been recognised for the spread of important zoonoses to humans, for example, HIV AIDS, plague, rabies, and tularaemia. Alternatively, wildlife can also transmit these diseases to humans via livestock and domesticated animals. The emergence of diseases such as tuberculosis and brucellosis in humans, were likely to have –spilled over” from livestock to native wildlife and have recently –spilled back” to livestock (Figure 1-1). An increase in the interface and/or the rate of contact between humans, domestic animals, and wildlife populations thereby creates increased opportunities for spillover events to occur (Daszak et al. 2000, Chomel et al. 2007). Zoonoses may also be multi-directional, as is the case for the intra-specific transmission of brucellosis in bison and elk populations with occasional interspecies transmission also involving cattle, horses, and humans (Ryhan and Spraker 2010).

**Table 1-2 Examples of human-wildlife conflicts involving zoonotic diseases**

| Disease | Wildlife host | Main risks to humans | Reference |
|---|---|---|---|
| Highly Pathogenic (HP) avian influenza | Waterfowl, marine and other birds e.g. northern pintail (*Anas acuta*) | Movement of wild animals, infected animal products and the infection of domestic poultry | (Greger 2007, Koehler *et al.* 2008) |
| West Nile fever | Wild birds e.g. American Crow *Corvus brachyrhynchos* | Vector expansion, infection of domestic poultry, open air farming | (Ludwig *et al.* 2010) |
| Newcastle disease | Pigeons, waterfowl and other birds | Movement of wild animals and open air farming | (Douglas *et al.* 2007) |
| Rabies virus | Fox, raccoon, dog, wolf, bats and other mammals | Expansion or introduction of hosts, movements of wild animals | (Junior *et al.* 2008) |
| Salmonellosis | Wild vertebrates e.g. Wild brown rats Rattus | Wildlife overabundance and open air farming | (Webster and Macdonald 1995) |
| Tuberculosis | Wild boar, red and fallow deer, possums, badger and other wild mammals | Movement of wild animals, wildlife overabundance and open air farming | (Marcotty *et al.* 2009) |
| Brucellosis | Wild ruminants (e.g. North American Bison and deer) | Movement of wild and domestic animals | (Böhm *et al.* 2007) |
| Swine brucellosis | Wild boar and European brown hare | Open air farming | (Wyckoff *et al.* 2009) |
| Leishmaniasis | Wild canids | Vector expansion, movements of wild animals | (Quinnell and Courtenay 2009) |
| Echinococcosis/ hydatidosis | Wild canids | Expansion or introduction of hosts | (Miterpáková *et al.* 2009) |
| Hendra and Nipah virus | Fruit bats (e.g. Flying-foxes) | Open air farming, movement of wild animals | (Daszak *et al.* 2006) |
| Severe acute respiratory syndrome (SARS) | Microbats (*Rhinolophus* spp) and masked palm civets (*Paguma larvata*) | Bush meat trade, movements of wild animals, wet markets, open air farming | (Wang and Eaton 2007) |
| Toxoplasmosis | Wild mammals and birds (felids – definitive host) | Open air farming, consumption of undercooked meat, contamination of water supplies | (Aramini *et al.* 1995) |
| Ebola | Great apes | Bush meat and trade, movement of wildlife | (Georges-Courbot *et al.* 1997) |
| Cryptosporidiosis | Wild mammals | Contamination of water supplies | (Hunter and Thompson 2005, Power *et al.* 2005) |
| Giardiasis | Wild mammals | Contamination of water supplies | (Hunter and Thompson 2005) |

| **Disease** | **Wildlife host** | **Main risks to humans** | **Reference** |
|---|---|---|---|
| Leptospirosis | Wild mammals | Bush meat, movements of wildlife, open air farming | (Roberts *et al.* 2010) |

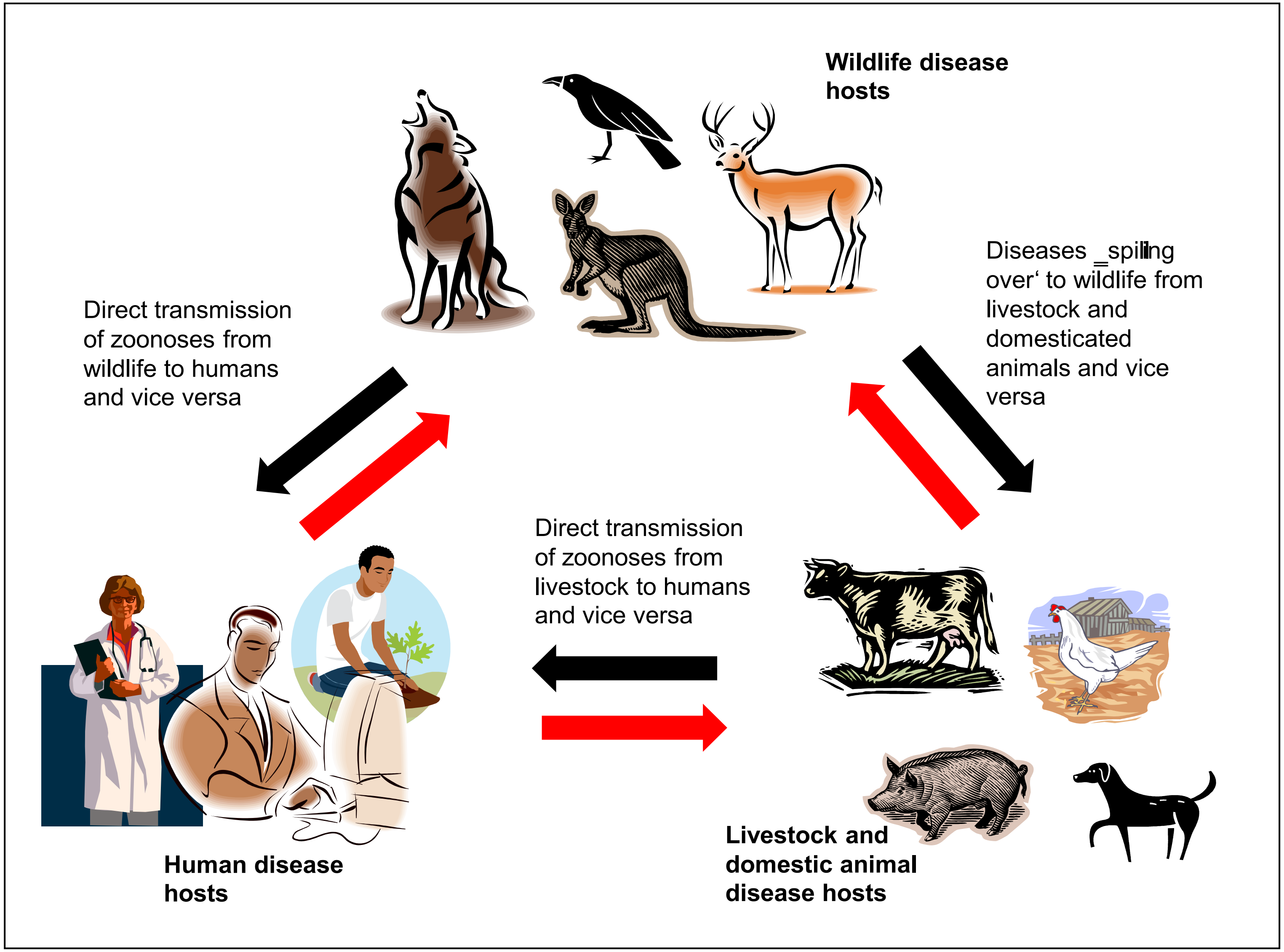

Wildlife disease hosts
Direct transmission of zoonoses from wildlife to humans and vice versa
Diseases ‗spilling over' to wildlife from livestock and domesticated animals and vice versa
Direct transmission of zoonoses from livestock to humans and vice versa
Human disease hosts
Livestock and domestic animal disease hosts

**Figure 1-1 Potential transmission pathways of zoonoses and the role of wildlife in this process**

## SYDNEY WATER CRISIS 1998

From July to September 1998, *Cryptosporidium* and *Giardia* were detected at concerning levels within Sydney‘s raw drinking water supply. *Cryptosporidium* and *Giardia* are parasitic protozoa that cause gastrointestinal illness, and are commonly transmitted via faecal contamination of water ways. Illness is typically short-lived, but infection in immunocompromised hosts can become persistent and mortality can result. During the contaminated water supply incident, neither the source nor the genotypes of these pathogens were determined. In addition, there was no consistent evidence of any increase in diarrhoeal disease yet large numbers of *Cryptosporidium* oocysts (the infective life stage of this pathogen) were apparently detected in the treated water supplied to consumers (Hrudey and Hrudey 2004). However, it was confirmed that poorer quality water arising from catchment runoff eventuated in the main storage reservoir and bypassed treatment facilities (Stein 2000).

The incident revealed significant levels of vulnerability to the Sydney water supply that had the capability to cause a large outbreak of disease (Hawkins *et al.* 2000). Three ‗boil water advisories‘ were issued and later withdrawn following three separate detections of these pathogens in Sydney‘s water supply. These were issued on the basis of other waterborne disease incidents involving *Cryptosporidium* and *Giardia*, such as that occurred in Milwaukee where several people died as a result (Mackenzie *et al.* 1994). The Sydney incident was estimated to have cost the New South Wales Government over $137 million and led to the resignation of both the Chairman and Managing Director of the Sydney Water Corporation and senior managers were also removed. Subsequently, the Sydney Water Corporation lost responsibility for major water supply assets and the Sydney Catchment Authority (SCA) was formed to manage the catchment and the bulk water supply to Sydney (Hrudey and Hrudey 2004).

The SCA manages a total of 21 storage dams (11 major dams), that hold more than 2.5 million mega-litres of water. Water stored in these dams is collected from five primary catchment areas covering an area of approximately 16,000 square kilometres. The Warragamba system, located 65 km southwest of Sydney in a narrow gorge of the Warragamba River, is the largest of the four catchments (Shoalhaven, Metropolitan, Woronora, Warragamba) supplying potable water to Sydney (Figure 1-2). This water currently supports a population of 4.5 million people. Water is collected from the catchment of the Wollondilly and Cox‘s Rivers, an area of 9,050 square kilometres, to form Lake Burragorang and Warragamba Dam. Water is drawn from this system and supplied to three Sydney Water operated filtration plants (Prospect, Orchard Hills and Warragamba), where it is treated and distributed to people residing in Sydney and the lower Blue Mountains.

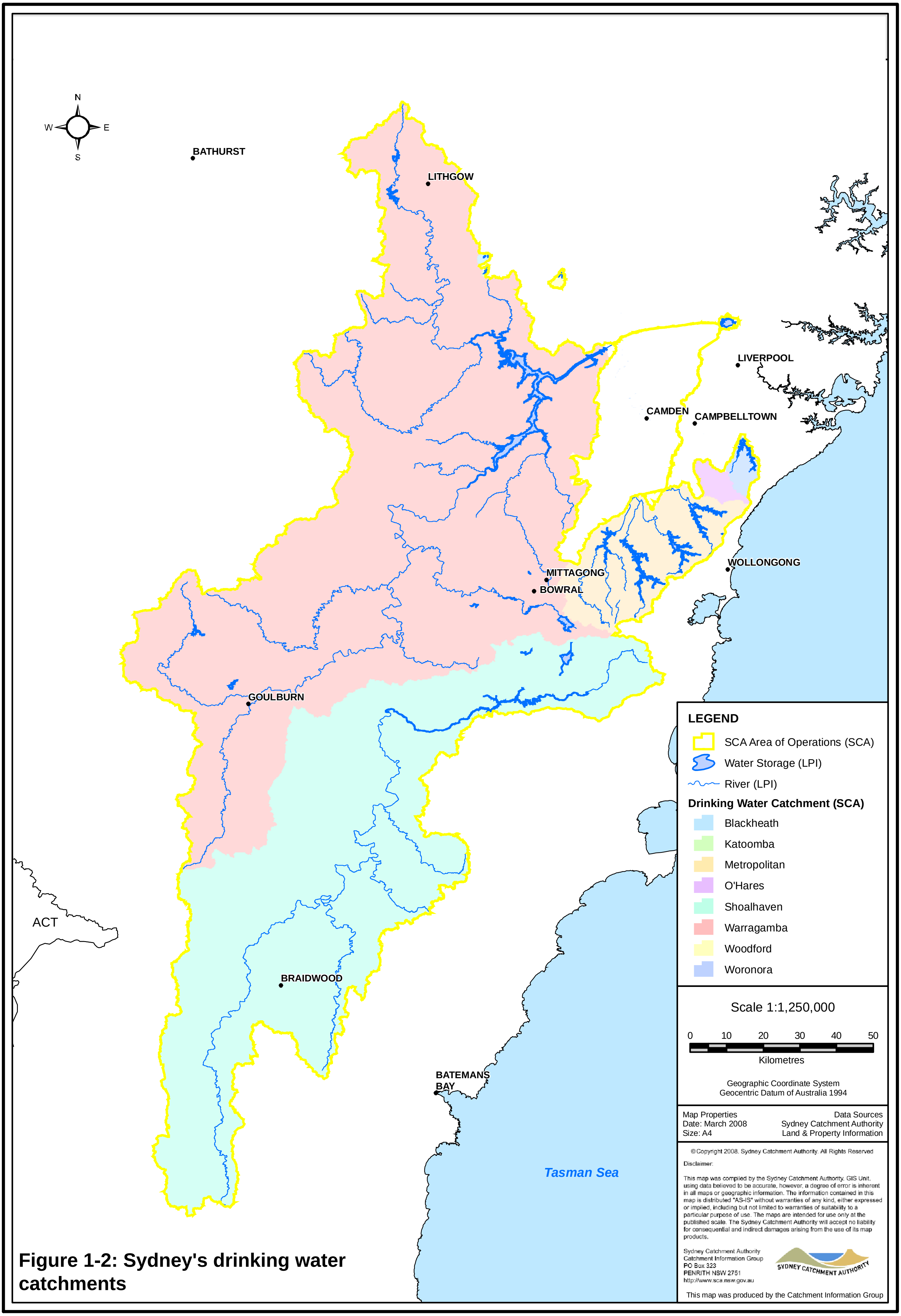



**Figure 1-2: Sydney's drinking water catchments**

### *McClellan Inquiry*

Following the first detection of *Cryptosporidium* and *Giardia*, the Premier of NSW ordered a public inquiry into the incident. This was chaired by Mr. Peter McClellan, QC. The ‗McClellan Inquiry' (McClellan 1998) concluded that there were several potentially significant sources of contamination of the water supply, including agricultural activities (sheep and cattle grazing with unrestricted access to streams, poultry farms, piggeries, dairies, saleyards, abattoirs and intensive horticulture), residential developments (sewage treatment plants, sewer overflows, on-site sewage management systems and stormwater runoff) and free ranging animals inhabiting the inner catchment lands (McClellan 1998). The inquiry also suggested that several potential wildlife hosts could be carriers of the organisms, including feral deer, pigs, goats, wild dogs, feral cats, foxes, horses and cattle as they were all known to *"infest*" parts of the catchment (McClellan 1998). In addition, McClellan specified in his inquiry that native animals, such as kangaroos, could also be a source of these pathogens (McClellan 1998, Stein 2000). Further, it was suggested that ―Heavy rains in the catchment which followed a period of significant drought carried the organisms into the stored waters ... During the recent heavy rainfall events, it is likely that run-off of animal manure and soil loads would have occurred from grazing lands denuded of pasture by 3-6 years of drought" (McClellan 1998).

The ‗McClellan Inquiry' mandated further research given the high level of scientific and medical uncertainty surrounding the sources of *Cryptosporidium* and *Giardia*. McClellan specified that future research would need to analyse the risk of each of the potential sources of contamination and address the following key pieces of information: the magnitude of contamination, frequency of the discharge (continuous versus run-off), type of contamination (human or animal), distance from the water off-take, and travel times during an event. Preliminary tests carried out during the inquiry indicated that contamination arose predominantly from herbivore faeces in Warragamba Dam, but also provided proof of infrequent human faecal contamination. It was concluded that the bulk of the *Cryptosporidium* and *Giardia* organisms detected had most likely originated from animals residing within the catchment and that zoonotic disease transmission could occur (McClellan 1998).

### *SCA's Multi-barrier Approach and the Pathogen Catchment Budget*

In response to the high level of scientific uncertainty surrounding the causes of the water supply incident, a multi-barrier approach was implemented by the SCA to protect the quality of raw (untreated) drinking water. This comprised addressing water quality risks at several different stages of water collection and storage to ensure that clean water was distributed from the catchments to the consumer, instead of relying solely on water treatment to circumvent potential water quality issues. A major stage in the multi-barrier approach was the designation of protected zones surrounding water storages, named Special Areas, that constitute areas of prohibited public access. In the Warragamba Special Area, this land composes a 3 km area bound by the top of the bank comprising Warragamba Dam (Figure 1-3). This area is jointly managed by the SCA for water quality purposes and by the NSW Department of Environment, Climate Change and Water (DECCW) as reserves for biodiversity conservation.

Another component of the multi-barrier approach was to identify and prioritise research to target the potential sources of pathogens within the hydrological catchments. In the early stages of this process and through the development of a conceptual model (Ferguson *et al.* 2003a), significant knowledge gaps, as alluded to in the ‗McClellan Inquiry' regarding the fate and origin of pathogens in drinking water catchments, were more clearly identified. Further, Ferguson *et al.* (2003b) identified the key processes governing pathogen fate and transport in surface water in the drinking water system. Collectively, this research identified the following finite knowledge gaps that precluded effective management: (i) characterisation of pathogen sources including native and feral animals and, septic seepage; (ii) quantifying the processes that determine pathogen fate and transport; (iii) development of tracing and tracking tools to identify the source of faecal contamination; and (iv) development of a model for the estimation of catchment pathogen and faecal indicator loadings from various sources over time and space (Ferguson *et al.* 2007). Process-based mathematical models (pathogen catchment budgets- PCB) were formulated to integrate all of the research information into a tool that could be used to assist catchment managers to prioritise pathogen control measures (Ferguson *et al.* 2007).

The high density and unmanaged status of the eastern grey kangaroo in the inner catchment (adjacent to Warragamba Dam) led to a need to determine the likelihood of this species being a transmission agent of *Cryptosporidium* and *Giardia* organisms to the water supply. This need was coupled with statements in the ‗McClellan Inquiry' that ‗animals have died around the Warragamba Dam catchment as a result of fires, starvation and drought..' thus posing a further zoonotic disease risk and pleading for answers regarding whether or not controlled culling of kangaroos should be undertaken to mitigate this risk (McClellan 1998). Furthermore, this species is probably capable of contributing significantly to watershed contamination, particularly because free ranging wildlife often has uncontrolled access to water supplies and riparian areas, making surface water protection difficult (Cox *et al.* 2005). Consequently, this species featured in the PCB and was compared against domestic livestock to assess the risk for the contribution of these organisms to the water supply.

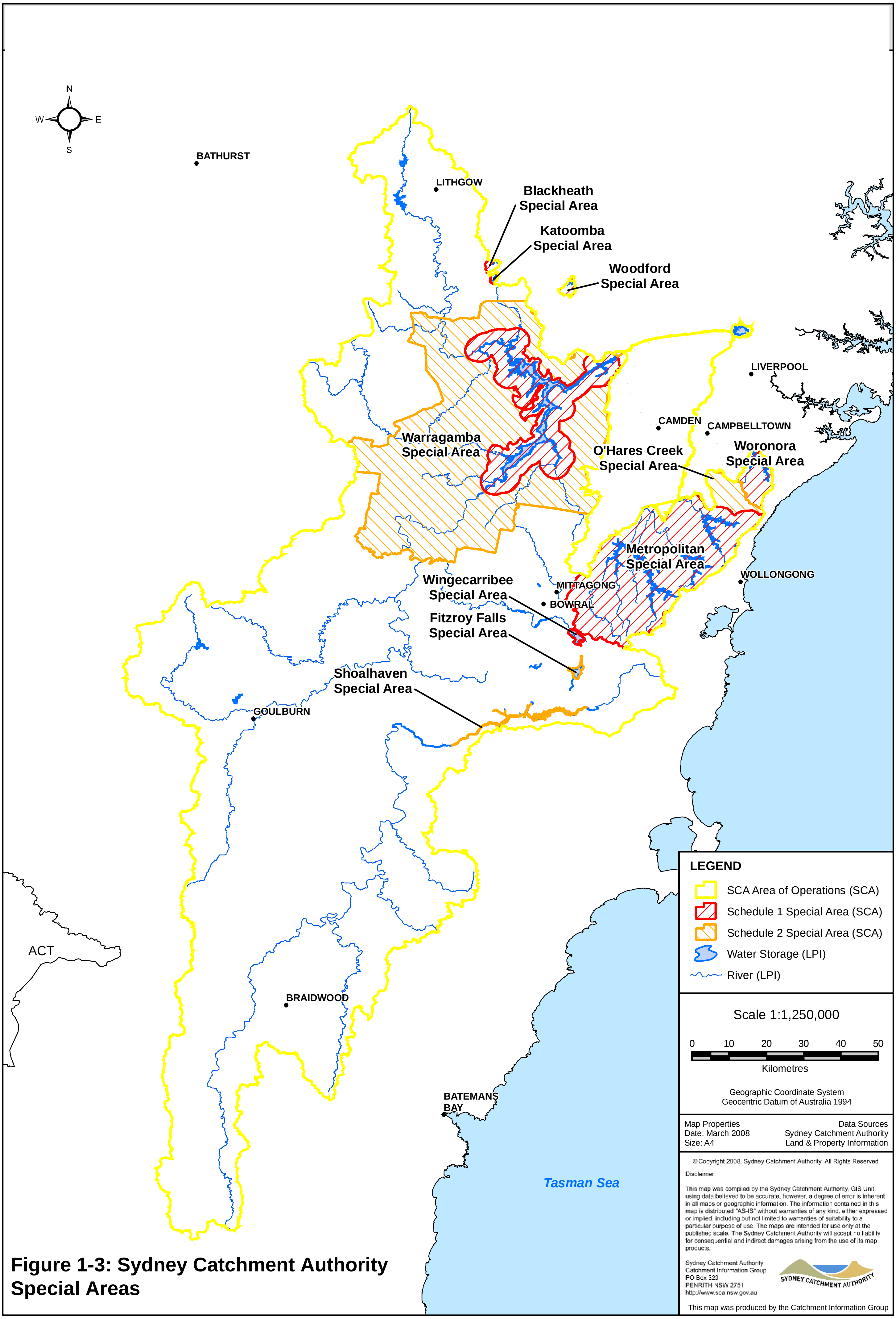



**Figure 1-3: Sydney Catchment Authority Special Areas**

## THE STUDY ANIMAL: EASTERN GREY KANGAROO (*MACROPUS GIGANTEUS*)

The eastern grey kangaroo (*Macropus giganteus*) is distributed throughout most of the eastern states and coastal regions, an area that supports over 85% of Australia‘s human population. It ranges from tropical areas in north Queensland, westward to the inland plains to southern Victoria and Tasmania (Dawson 1995). The range of this species is restricted by rainfall, with more than 250 mm of annual rainfall required to support the core distribution. However, in recent years the distribution of this species appears to be extending westward, due partly to the supply of watering points for cattle and sheep (Dawson *et al.* 2006). Eastern grey kangaroos occupy a range of habitats including woodland, shrubland, open forest and semi-arid mallee scrub (Poole 2002).

Eastern grey kangaroos are overabundant in many parts of their range and are commonly managed for ‗damage mitigation‘ against impacts that they may have on agriculture such as competition with livestock (ACT Parks Conservation and Lands 2010). Reasons for the overabundance of this species include land clearing for agriculture and pasture improvement, artificial provision of water, and dingo control (Pople and Grigg 1999). Eastern grey kangaroos are among four macropodid species that are commercially harvested for their skin and meat and some areas may be controlled strictly under licence (Department of Environment and Conservation 2006). In New South Wales, eastern grey kangaroos are managed across 16 ‗Kangaroo Management Zones‘ that cover more than 80% of the state. Recent population estimates of this species by aerial survey spanning these management zones totalled over 4 million animals, of which 5% were taken as commercial harvest (Department of Environment and Conservation 2006). Management of eastern grey kangaroos also occurs in other parts of the species‘ range to circumvent human-vehicle collisions and to reduce human-kangaroo conflict (ACT Parks Conservation and Lands 2010). Management of kangaroos is highly contentious; culling is prohibited within national parks and conservation reserves and can be undertaken only on private landholdings (Department of Environment and Conservation 2006). Thus, the management of eastern grey kangaroos in areas designated as conservation reserves, and in particular those that adjoin agricultural land, requires careful consideration to abate the impacts of this species and still meet conservation concerns (Coulson 1998, Viggers and Hearn 2005).

## *Eastern grey kangaroos in the Warragamba Special Area*

The eastern grey kangaroo occupies parts of the Warragamba Special Area, and in particular the Burragorang Valley, at very high densities (Figure 1-4) (DECC 2007). The high densities of kangaroos in this area are most likely a response to previous agricultural activities, including cattle and sheep grazing in the fertile and flat areas that occur adjacent to the Wollondilly River (Plate 1-1), that have increased the foraging habitat (water and forage) for this species. In addition, the cessation of culling by pastoralists and Aboriginal people, as well as a reduction in the abundance of predators, such as the dingo and dingo-dog hybrids (*Canis lupus dingo*, *C. l. dingo* x *C. l. familiaris*), has contributed to this increase (Plate 1-2). Agricultural activities in the Burragorang Valley ceased in most areas in the 1960s when the valley was flooded following the construction of Warragamba Dam. The Jooriland lease, located at the junction of the Wollondilly River and Lake Burragorang, was the last lease held in the area and was vacated in the early 1990s.

**Plate 1-1 Eastern grey kangaroo (*Macropus giganteus*) foraging in ex- pastoral areas adjacent to the Wollondilly River.**

**Figure 1-4: Predicted eastern grey kangaroo densities across the Greater Southern Sydney Region**

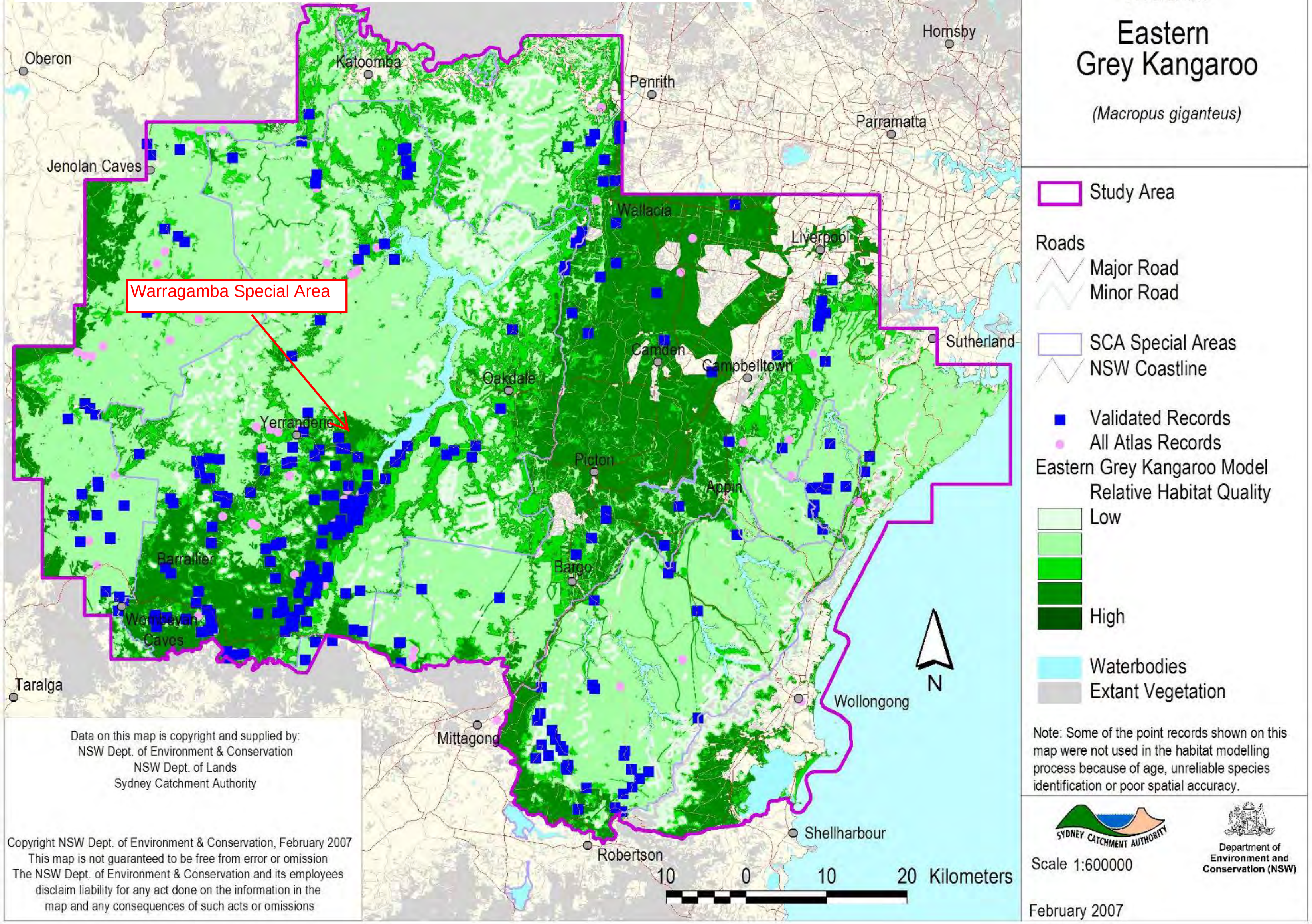


**Plate 1-2 Dingo and its hybrids (*Canis lupus dingo*, C. l. dingo x C. l. familiaris). This predator of the eastern grey kangaroo was controlled by pastoralists in the Warragamba Special Area and since the cessation of farming activities numbers of this species have been gradually increasing.**

In addition to any zoonotic disease risk, overgrazing by eastern grey kangaroos in the Burragorang Valley threatens the recovery and regeneration of Grassy Box Woodlands (DECC 2007). This habitat has been identified by the DECCW as a Priority Fauna Habitat as it supports many species of threatened woodland birds (e.g. speckled warbler and hooded robin) and contains components of the Threatened Ecological Community *White box - yellow box - Blakely's red gum grassy woodlands and derived native grasslands* (*White box yellow box Blakely's red gum woodland* in NSW) which is listed as Critically Endangered under the Commonwealth *Environment Protection and Biodiversity Conservation Act 1999* and Endangered under the NSW *Threatened Species Conservation Act 1995* (Plate 1-3). Given the importance of vegetative buffers for water quality, the overgrazing of this habitat by eastern grey kangaroos is also likely to increase run-off leading to increased sediment, nutrient and pathogen transport (DECC 2007).

**Plate 1-3** **Grassy Box Woodlands in the Warragamba Special Area. Eastern grey kangaroos may threaten the longevity of this community in the area due to their overgrazing of understorey plant species. Arrows indicate signs of overgrazing and dust bowls created by eastern grey kangaroos.**

Assessments of the zoonotic disease risk of eastern grey kangaroos to Sydney‘s water supply to date have had a microbiological and epidemiological focus. Macropodid species are generally susceptible to infections from marsupial-specific species and genotypes of *Cryptosporidum* (Power *et al.* 2005, McCarthy *et al.* 2008). The species are *Cryptosporidium fayeri* and *Cryptosporidium macropodium* (Power and Ryan 2008, Power 2010). Power *et al.* (2005) demonstrated a seasonal pattern of *Cryptosporidium* prevalence in eastern grey kangaroos residing in Sydney‘s watershed, with the highest oocyst counts in autumn. This peak may be related to the higher presence of pouch young at this time, because younger cohorts in other species (e.g. cattle) have been found to shed most oocysts (Atwill *et al.* 1999).

## THE ROLE OF THE ECOLOGY OF DISEASE HOSTS IN ASSESSING DISEASE RISK

Given the dynamic and diffuse nature of free-ranging wildlife pathogen sources compared to others in the hydrological catchment (e.g. sewage treatment plants and domestic livestock), different investigative techniques and skill sets are required to ascertain the pathogen risk. To accurately assess the potential for eastern grey kangaroos to transmit *Cryptosporidum* and *Giardia* to Sydney's water supply, detailed knowledge and an understanding of the species' ecology and interaction with surface waters is required. This information should ideally be combined with the seasonality expressed in epidemiology data and used in PCB models to precisely quantify the factors that cause pathogens to enter surface water and to develop risk and control strategies.

A conceptual model illustrating the role of several ecological parameters in the formulation of the zoonotic disease risk of eastern grey kangaroos adjacent to a water supply is presented in Figure 1-5. The following key pieces of ecological information are required:

- **Population density** – higher population densities of kangaroos result in larger amounts of faecal excreta deposited per unit area. As cryptosporidiosis and giardiasis are likely to act in a density dependent manner, the higher the density of kangaroos, the more likely animals are to be a stable reservoir of these diseases. In addition, animal density is a fundamental component of pathogen load calculations.
- **Distribution and volume of excreta** – concentrations of faecal excreta and their relative distribution with respect to surface water are critical in the assessment of the risk of pathogen entry to Sydney's water supply. For example, high concentrations of faecal excreta deposited in the riparian zone would represent a very high risk of pathogen dissemination.
- **Movement patterns and habitat preferences** - the movement patterns of a target species define the area it occupies in relation to water courses, which is critical for determining the concentration and distribution of faecal material across the watershed. In addition, determining the habitat preferences that are shown by kangaroos in relation to the riparian zone indicates the likely rate of direct faecal deposition into streams, and also provides a risk rating for other preferred habitats by assessing their distance from drinking water and the potential for them to trap pathogens.

- **Social structure** – previous research suggests that numbers of juveniles allow direct inferences to be made about the prevalence of *Cryptosporidium*. In addition, intrinsic social factors of a population such as group size and mortality patterns have broad implications for disease management such as transmission and symptomatic effects.

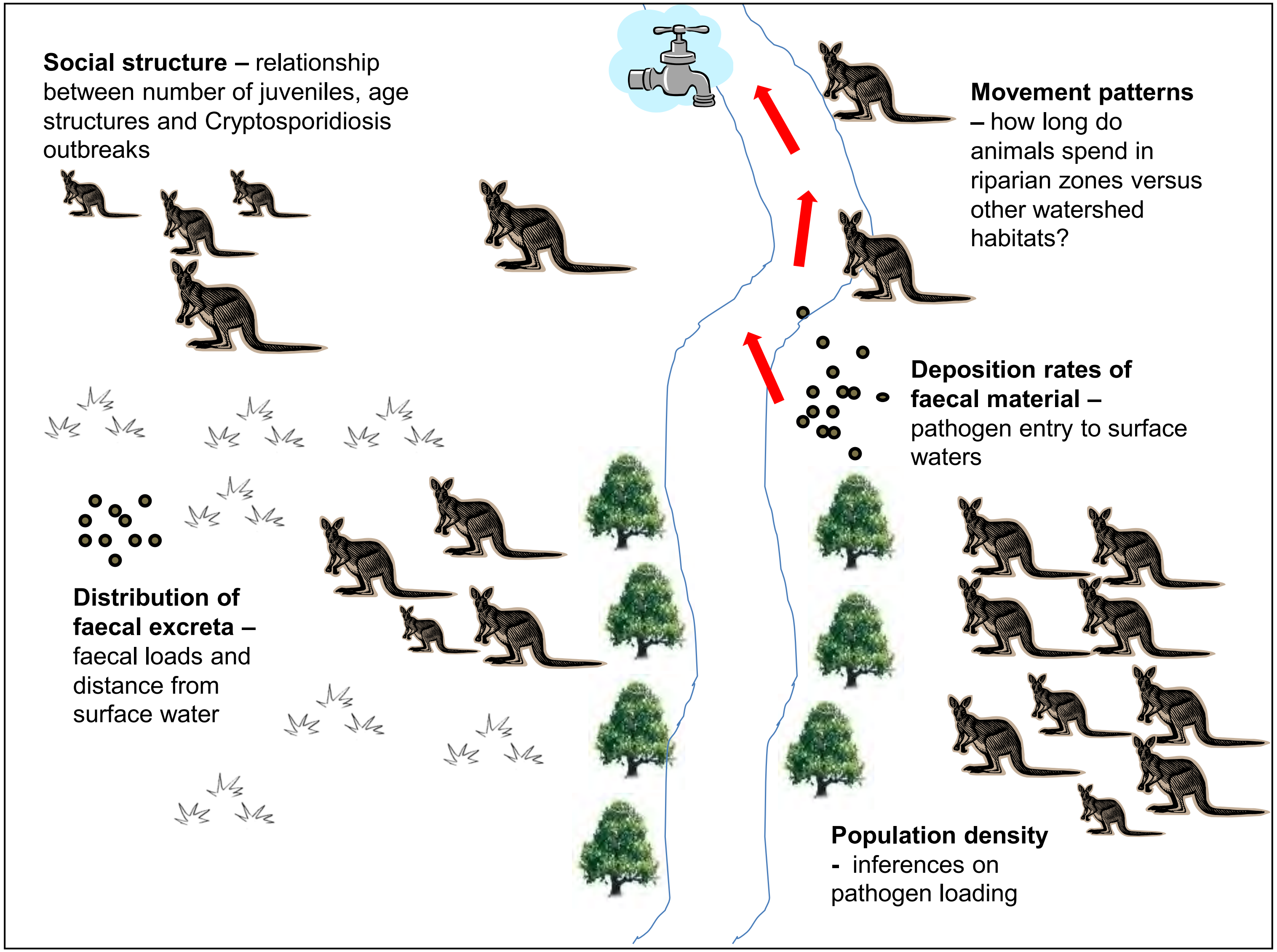

**Social structure** – relationship between number of juveniles, age structures and Cryptosporidiosis outbreaks
**Movement patterns** – how long do animals spend in riparian zones versus other watershed habitats?
**Deposition rates of faecal material** – pathogen entry to surface waters
**Distribution of faecal excreta** – faecal loads and distance from surface water
**Population density** - inferences on pathogen loading

Figure 1-5 Ecological factors associated with a host population of kangaroos that should be used to assess the risk of disease transmission to Sydney’s water supply catchment

## AIMS AND SCOPE OF THIS STUDY

The overall objective of this study was to examine the ecology of eastern grey kangaroos in Sydney's water supply catchment, in order to evaluate the potential for this species to disseminate zoonotic pathogens. This information is critical to the public health of the population of Sydney, after *Cryptosporidium* and *Giardia* were detected in the water supply in 1998 and subsequently, kangaroos were found to be source of these organisms.

The conceptual model presented in Figure 1-5 provides a framework of the investigation for this thesis. Thus, the risk of kangaroos transmitting these organisms to the water supply is gauged by the collection of ecological field data. Each of the parameters investigated (e.g. distribution of faecal material, population densities) will be synthesised with information from the literature pertaining to these attributes.

The thesis has been written as a series of extended papers intended for future stand-alone publication (Chapters 2 – 6) proceeded by this chapter that aims to be a general introduction (Chapter 1) and followed by a general conclusion (Chapter 7). In Chapter 2, I examine the prevalence of *Cryptosporidium* and *Giardia* to allow for the comparison of this information with the ecological data presented in subsequent chapters. I also assess the study population of kangaroos for other zoonotic diseases, such as *Toxoplasma* and *Leptospira*. I present a simple risk assessment for each of these diseases to be transmitted to the population of Sydney through the potable water supply, and given the wide ranging distribution of kangaroos, also the risk of these diseases being transmitted to the general community, recreational groups and people in agricultural settings. In Chapter 3, I assess the seasonal population densities of eastern grey kangaroos in the watershed of Sydney's catchment. I aimed to determine the population density thresholds that should be used for this species in future pathogen load modelling. I was particularly interested in the resilience of the population to the prevalent drought conditions. In Chapter 4, I compare the deposition of kangaroo faecal material between watershed habitats, with a particular emphasis on their proximity to surface waters of the catchment. I aimed to assess the factors influencing faecal deposition in these habitat types, which include pasture biomass and decay. In Chapter 5, I examine the movement patterns and habitat preferences of kangaroos and aim to infer the role of these parameters on faecal deposition by this species in the watershed. I also investigate the potential for the direct deposition of faeces by kangaroo into the water supply. Finally in Chapter 6, I investigate the social structure of kangaroos in Sydney's watershed. In this chapter, I consider the role of juvenile kangaroos in the dissemination of *Cryptosporidium* and *Giardia*, as it has been inferred in previous epidemiological research conducted on kangaroos, that the presence of pouch-young is responsible for autumn epidemics.

## STUDY AREA

I assessed the ecology of eastern grey kangaroo populations at three sites on the Wollondilly River; Murphy's Crossing, Jooriland and Douglas Scarp (34º11'1"S 150º18'22"E) 80 km south-west of Sydney. Jooriland and Murphy's Crossing are located on the western side of the river, Douglas Scarp to the east (Figure 1-6). Site selection was based on the locations of previous investigations of *Cryptosporidum* epidemiology. Power *et al.* (2004) analysed faecal excreta for *Cryptosporidium* between March 2000 and April 2002 at all of these sites and Radu and Slade (2007) carried out these investigations for two years between 2004 and 2005 at Murphy's Crossing only. The highest densities of this species in closest proximity to Warragamba Dam occur within these sites (DECC 2007). The field surveys for this study took place between March 2004 and December 2007.

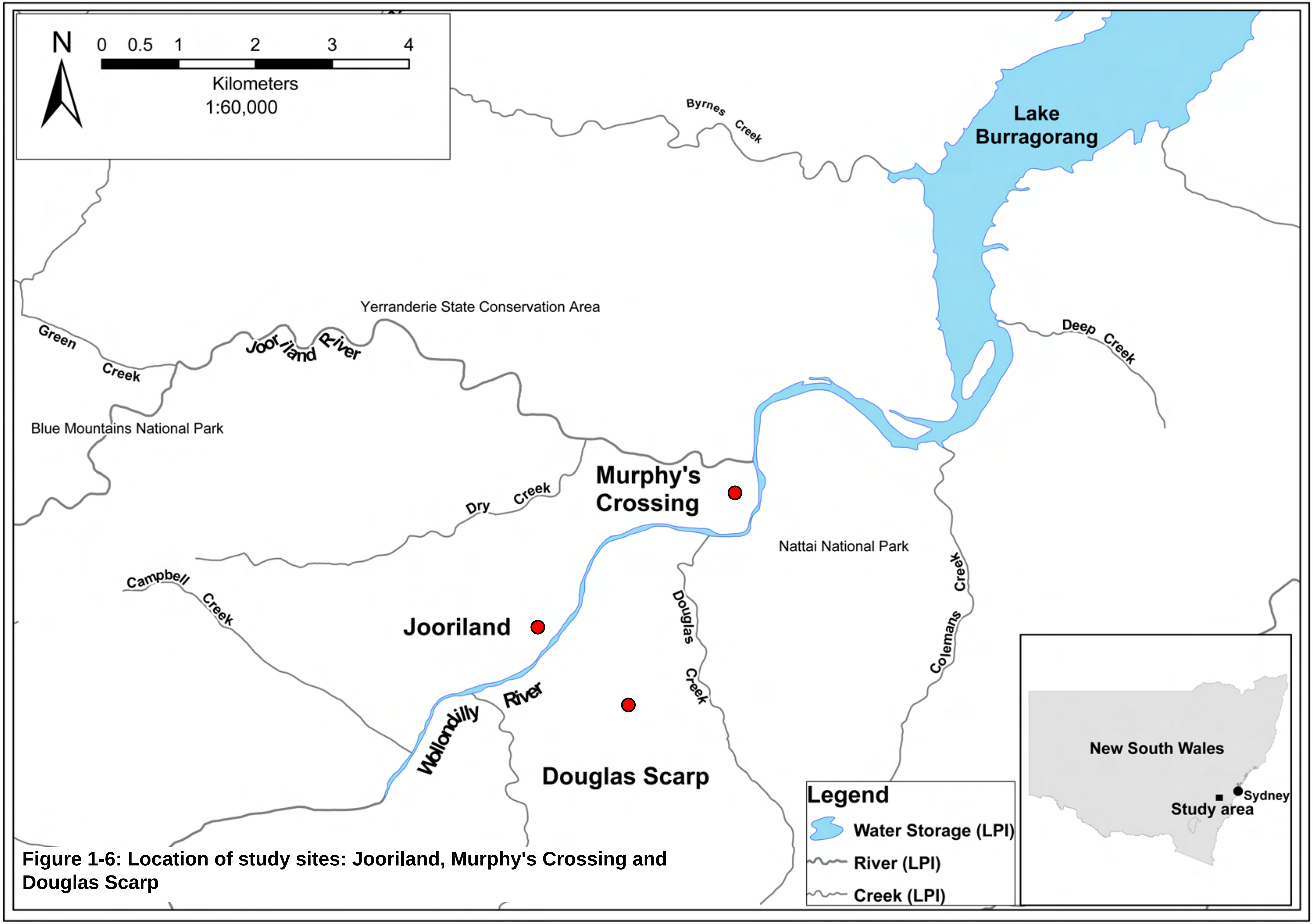



**Figure 1-6: Location of study sites: Jooriland, Murphy's Crossing and Douglas Scarp**

Murphy's Crossing, Jooriland and Douglas Scarp consist predominantly of previously cleared grassy areas (Plate 1-4), intermixed with scattered stands of open woodland (Grassy Box Woodland). The Wollondilly River and Jooriland River are fringed by riparian forest. The cleared areas are continuous in flood plain areas and are broken up by vegetated escarpments to the east (Wanganderry and Nattai Tablelands) and undulating hills to the west (Mt Egan and Jooriland Range). Jooriland and Murphy's Crossing are located in Kanangra-Boyd National Park and Douglas Scarp is located in Nattai National Park.

The climate of the study area is temperate; generally experiencing dry cold winters and warm and wet summers (Table 1-3). The long term average (± SD) annual rainfall for the study area is 602.8 (± 204.3) mm. The lowest rainfall in 19 years of data collection (1988 – 2007) was experienced in 2003 (one year before this study commenced) at 218 mm and the average (± SD) annual rainfall during the study period was 359.8 (± 40.3) mm, indicating that a severe drought was in operation (Figure 1-7). Frosts were relatively uncommon, and occurred most frequently in July and August 2006. The water levels in the Wollondilly River were regulated by transfers from the Shoalhaven River system, which peaked at 400 – 600 mega-litres per day during drought conditions.

**Table 1-3 Climate data for the Study Area collected from the Jooriland weather station. Frost days are days when temperature <0ºC was recorded**

| Year | Annual rainfall (mm) | Driest month | Wettest month | Mean min and max temp (ºC) (summer Dec - Feb) | Mean min and max temp (ºC) (winter June - Aug) | Frost days |
|---|---|---|---|---|---|---|
| **2004** | 350.7 | June (2.0 mm) | October (113.0 mm) | 16.8 – 31.0 | 6.5 - 18.5 | 1 |
| **2005** | 310.2 | January (2.0 mm) | February (135.5 mm) | 15.7 - 28.7 | 6.4 – 18.5 | 4 |
| **2006** | 406.5 | April, August (0 mm) | January (113.0 mm) | 16.4 - 30.1 | 6.2 - 17.3 | 13 |
| **2007** | 372.0 | August (0 mm) | June (181.0 mm) | 16.0 – 28.7 | 7.2 – 17.9 | 3 |
| *Average* | *359.8* | *August (30.5 mm)* | *February (75.6 mm)* | *16.2 – 29.6* | *6.3 - 17.8* | *NA* |

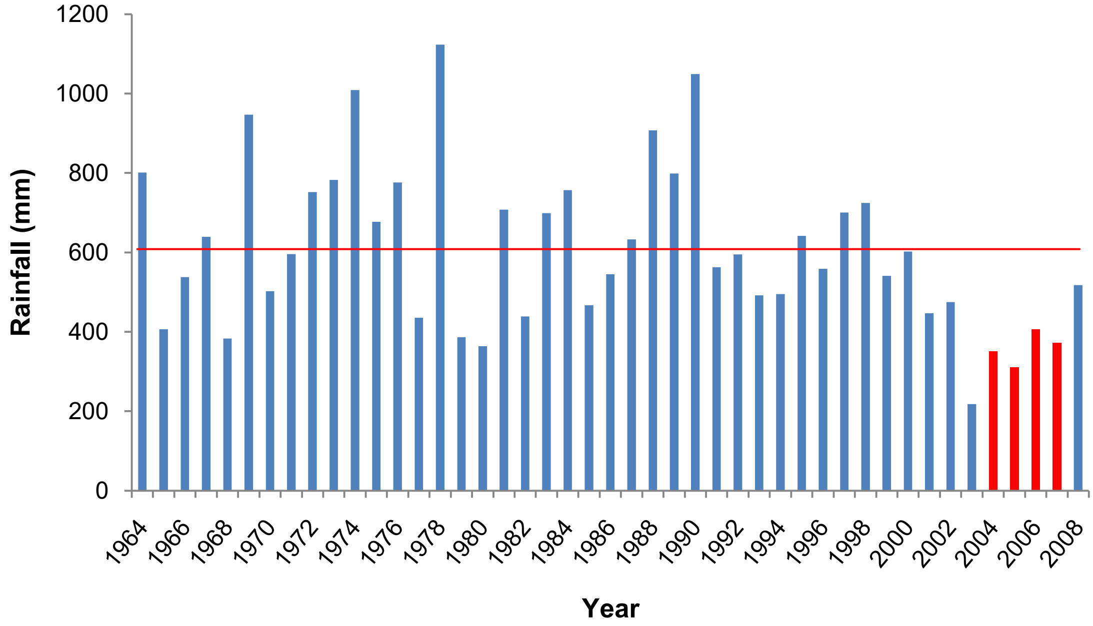



**Figure 1-7** **Annual rainfall in millimetres (mm) measured from Jooriland weather station. Red bars indicate study period (2004- 2007) and red horizontal line is annual average rainfall.**


**Plate 1-4** **A) Jooriland, B) Douglas Scarp and C) Murphy's Crossing**

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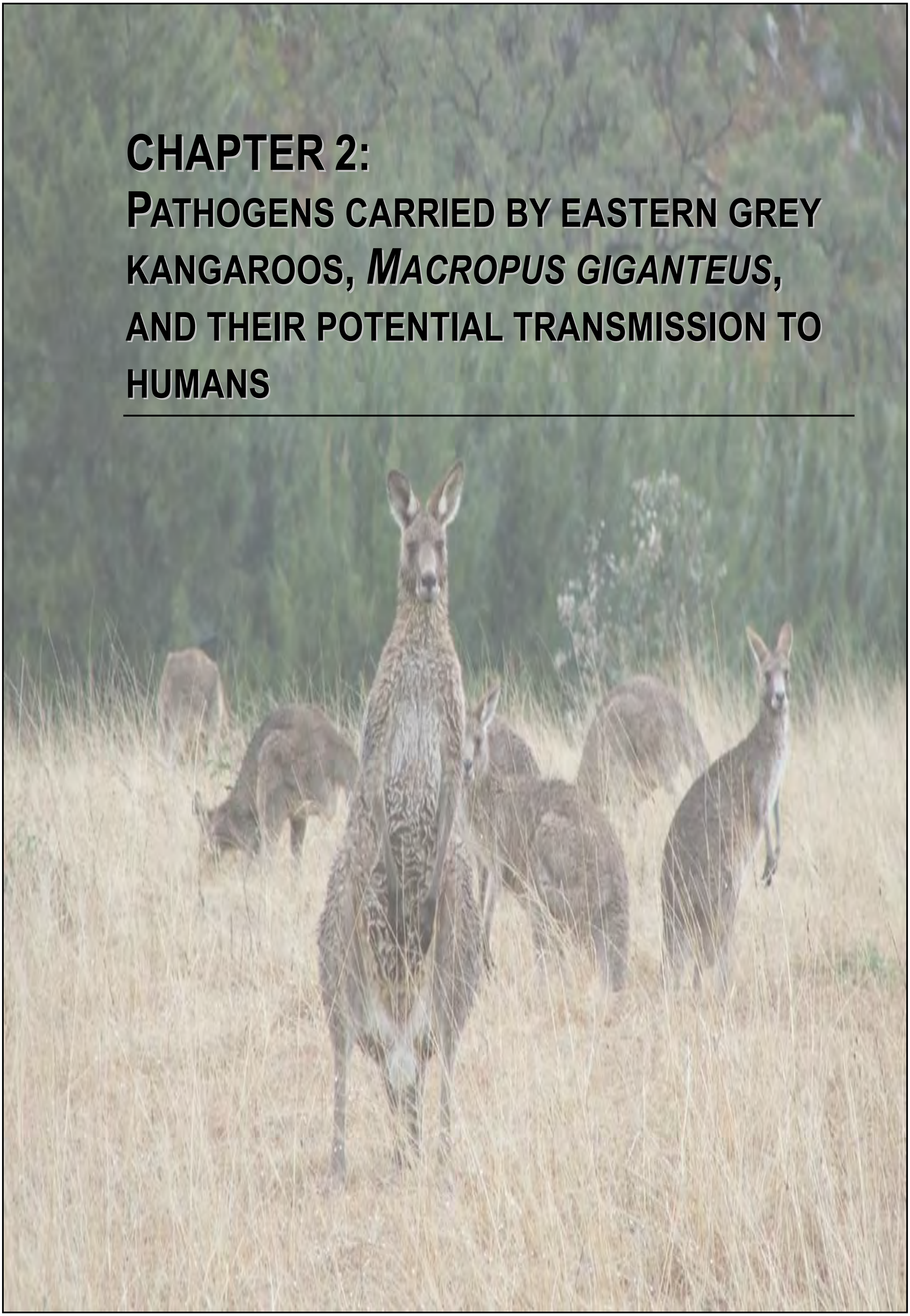
# CHAPTER 2:
# PATHOGENS CARRIED BY EASTERN GREY KANGAROOS, *MACROPUS GIGANTEUS*, AND THEIR POTENTIAL TRANSMISSION TO HUMANS



# CHAPTER 2: PATHOGENS CARRIED BY EASTERN GREY KANGAROOS, *MACROPUS GIGANTEUS*, AND THEIR POTENTIAL TRANSMISSION TO HUMANS

Components of this chapter have been submitted as:

Roberts, M.W, Smythe, L, Dohnt, M, Symonds, M and Slack, A. (2010) Serologic-based investigation of leptospirosis in a population of free-ranging eastern grey kangaroos (*Macropus giganteus*) indicating the presence of *Leptospira weilii* Serovar Topaz. *Journal of Wildlife Diseases*, **46**(2), 2010, pp. 564-569

M. Roberts (Macquarie University) collected serum from eastern grey kangaroos for Leptospira and *Toxoplasma* analysis and collected faecal material for *Cryptosporidium* and *Giardia* analysis. Lee Smythe, Michael Dohnt, Meegan Symonds and Andrew Slack (Queensland Health Scientific Services) performed microscopic agglutination tests (MAT) for the presence of *Leptospira* antibodies in the serum. Nevi Parameswaran (Murdoch University) performed the analysis of serum for *Toxoplasma*. Josephine Ng (Murdoch University) performed the analysis for *Cryptosporidium* and *Giardia* in faecal samples via ARC-linkage grant (LP0561862 - Using molecular tools to understand and control the transmission of *Cryptosporidium*). M. Roberts reported the assessment of the risk of these diseases in terms of environmental contamination, which forms the basis of this chapter.

## *Preamble to Chapter 2*

Eastern grey kangaroos, *Macropus giganteus*, were found to be source of *Cryptosporidium* and *Giardia* following the detection of these pathogens in Sydney‟s water supply in 1998. This chapter I aim to collect contemporary and concurrent epidemiological data on these pathogens from the same population that is the focus of the ecological-based investigations featured in the following data chapters. In addition, I examine the prevalence of other zoonotic diseases (e.g. *Toxoplasma* and *Leptospira*) that may be transmitted via the potable water supply of Sydney, and given the large and wide ranging distribution of eastern grey kangaroos, this chapter also assesses the disease risk to the general community, recreational groups and people in agricultural settings. Finally, I present a simple risk assessment that evaluates the likelihood and consequence of these diseases to be transmitted to humans.

## INTRODUCTION

The detection and surveillance of zoonotic diseases has received a considerable amount of attention recently, as it is considered crucial to improve public health worldwide and to mitigate the economic costs associated with treatment (Greger 2007, Jones *et al.* 2008). Human civilisations are becoming increasingly exposed to bacteria, protozoa, fungi, viruses, and parasites that cause zoonoses. The highest risk groups comprise people working with animals on a frequent basis including farmers, veterinarians, and abattoir workers (Stirling *et al.* 2008). However, members of the wider community are also at risk from zoonoses that can be transmitted via pets, by incidental contact with the disease-causing agent in the environment, and from consuming contaminated food and drinking water.

In a review of emerging infectious diseases, that is clusters of disease arising for the first time in a human population, approximately 72% of zoonoses were attributed to wildlife origin (Jones *et al.* 2008). Recent examples include the Napih virus in Malaysia, Severe Acute Respiratory Syndrome (SARS) in China, the West Nile virus in North America and the Hendra virus in Australia. The emergence of these diseases emphasises firstly, the significance of understanding the factors that increase contact between wildlife and humans and secondly, the need to obtain knowledge of the transport pathways of these diseases (Wilcox and Gubler 2005, Goss and Richards 2008, Thompson *et al.* 2009). Collectively, this information can be used to develop strategies for disease prevention and control (Jones *et al.* 2008).

An increase in the number of reported zoonoses originating from wildlife is potentially a response to the expansion of the human population and the consequent encroachment on wildlife habitat (Conrad *et al.* 2005). Changes in agricultural practices have also led to an increase in the emergence of some zoonoses, as a result of wildlife sharing pasture with livestock and from an increase in the local densities of wildlife surrounding farms (Chomel *et al.* 2007). Furthermore, a boom in recreational activities and ecotourism have also been implicated as the cause of a number of disease outbreaks, as humans are coming increasingly into contact with wildlife (Chomel *et al.* 2007, Greger 2007).

Waterborne zoonoses from drinking water represents an indirect pathway of disease transmission between wildlife and humans (Gajadhar and Allen 2004, Hrudey and Hrudey 2004). It represents high levels of concerns due to the very large and centralised water supply systems. Wildlife can contribute significantly to watershed contamination, particularly because animals often have uncontrolled access to water supplies and riparian areas, making surface water protection of a catchment difficult (Cox *et al.* 2005a).

The larger macropodid species have received a considerable amount of attention in response to their increasing interactions with humans in Australia (Coulson 1998). This has led to management problems associated with their overabundance in semi-urban areas as well as competition with domestic livestock in rural areas (Viggers and Hearn 2005). The eastern grey kangaroo (*Macropus giganteus*) is one of the most abundant large macropods co-inhabiting the landscape with humans in eastern Australia. As a consequence of this species‟ becoming occasionally overabundant, it is considered a pest species and requires management, particularly in reserves that are isolated or where dingoes and other population regulatory influences are not present. Management of this species is also required in agricultural areas where pastoral development has extended the area of suitable habitat (through clearing) and created permanent and more reliable water sources leading to very high local densities. Furthermore, it is also found in high densities in many drinking water catchments set aside for human use (Moore *et al.* 2002, Viggers and Hearn 2005, ACT Parks Conservation and Lands 2010) and thus represents a potentially significant source of zoonotic pathogens. Despite these attributes, there has been limited research on the zoonotic diseases that this species may be capable of transmitting to humans via drinking water storages, through associations with livestock or via indirect interactions with the wider community. Therefore, this chapter aims to:

1) Examine the prevalence of the parasites that are capable of being transmitted to humans by these means including *Cryptosporidium*, *Giardia*, *Toxoplasma* and *Leptospira* in a population of eastern grey kangaroos inhabiting a major component the watershed supplying Sydney‟s drinking water;
2) Determine the relative risk of the dissemination of these pathogens to the raw/untreated water supply of Sydney; and,
3) Given the high densities and wide distribution of eastern grey kangaroos, infer the risk of these pathogens being transmitted to the general community, recreational groups and people in agricultural settings.

Prior to describing the work carried out to meet these aims, I first provide a brief synopsis of the biology of each of the parasites to inform the directions taken in the field sampling and research design.

### *Cryptosporidium*

*Cryptosporidium* has recently become regarded as the most important waterborne human pathogen in developed countries. Over fifty waterborne outbreaks of *Cryptosporidium* associated with drinking water have been reported, with most of these occurring in North America, the United Kingdom and Japan (Fayer 2004). The largest of these outbreaks occurred during 1993 in Milwaukee, United States of America, where approximately 403,000 people were affected and 54 people were reported to have died from cryptosporidiosis (MacKenzie *et al.* 1994). *Cryptosporidium* also received some attention during the Sydney Water Crisis in 1998, when it was detected with *Giardia* at levels of concern in Sydney‟s water supply (Hrudey and Hrudey 2004). However, unlike the Milwaukee incident, community disease surveillance data from residents in Sydney, Wollongong and the lower Blue Mountains indicated that there was no increase in the reported level of illness during the period of the incident (Stein 2000).

*Cryptosporidium* is a genus of apicomplexan protozoan parasites with species that infect fish, amphibians, birds and mammals (Carey *et al.* 2004). The life cycle of species within the genus involves intracellular development in the gut wall of the infected host, with sexual and asexual reproduction. Transmission occurs via ingestion of thick-walled oocysts, which are shed in the faeces of the infected host (Figure 2-1). The oocyst is the environmentally stable stage and is able to survive and withstand many systems used to process and treat water and waste water. The resistance of the oocyst to commonly used disinfection methods, low infectious dose and modes of transmission all increase the risk of the transmission of this disease (Ryan *et al.* 2005). Cryptosporidiosis causes sub-clinical infection or an acute self-limiting diarrhoeal illness, but has the potential to cause prolonged and secretory diarrhoea in infants, the elderly and in the immunocompromised (e.g. AIDS patients). Similarly when the disease is contracted by animals, it can cause continued diarrhoea which in turn can lead to other problems such as prolapses, parasite overburden whilst the animal is under stress, dehydration and weight loss and if left untreated can lead to mortality (Carey *et al.* 2004).

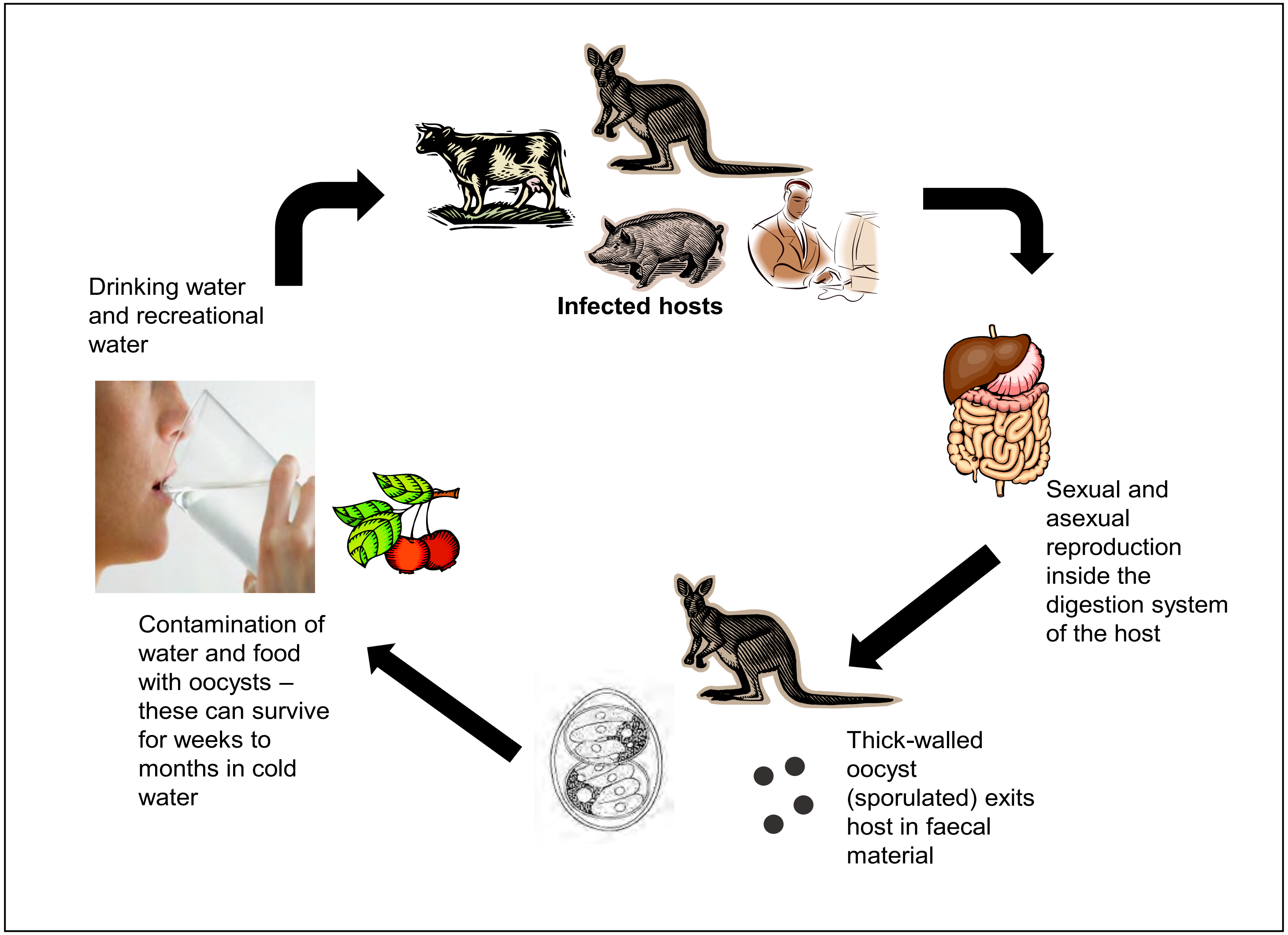

Infected hosts
Sexual and asexual reproduction inside the digestion system of the host
Thick-walled oocyst (sporulated) exits host in faecal material
Contamination of water and food with oocysts – these can survive for weeks to months in cold water
Drinking water and recreational water

**Figure 2-1 The life cycle of *Cryptosporidum*, involving development in the digestion system of the host and transmission of thick-walled oocysts via faecal excreta.**

The number of recognised *Cryptosporidium* species has undergone considerable revision since the organism was first discovered in 1912. This has followed the gathering of new molecular information that has enabled differentiation of genotypes into separable species and genotypes (Carey *et al.* 2004, Fayer 2004). Smith *et al.* (2007) documented sixteen species and the host specificity of these is notably variable (Table 2-1). For example, *C. parvum* is known to infect approximately 150 mammalian species, including humans. Other species such as, *C. baileyi*, *C. canis, C. felis*, *C. meleagridis*, and *C. muris*, that were previously thought be animal specific have also been found to infect humans and are therefore now considered zoonotic (Fayer 2004). However, the overwhelming majority of human infections are caused by either *C. parvum* or *C. hominis*, and a much smaller proportion is caused by *C. meleagridis, C. felis* and *C. canis.* Other zoonotic strains are generally found much less frequently as opportunistic infections of immunocompromised individuals. *C. hominis*, once thought to be human specific, has been reported to be zoonotic after it was found to infect dugongs, sheep and cattle (Morgan *et al.* 2000). The complicated epidemiology of *Cryptosporidium*, coupled with the fact that there are few distinguishing morphological features between individual species or genotypes makes the assessment of human infectivity risk difficult if *Cryptosporidium* is detected in raw water supplies.

**Table 2-1 *Cryptosporidium* species and the predominant type and minor hosts after Smith *et al.* (2007).**

| ***Cryptosporidium* species** | **Predominant type hosts** | **Minor hosts** |
|---|---|---|
| *Cryptosporidium andersoni* | Bovine (*Bos taurus*) | Sheep |
| *Cryptosporidium baileyi* | Chicken (*Gallus gallus*) | Ducks |
| *Cryptosporidium canis* | Dog (*Canis lupus familiaris*) | Humans |
| *Cryptosporidium felis* | Cat (*Felis catis*) | Humans, cattle |
| *Cryptosporidium suis* | Pigs (*Sus scrofa*) | Humans |
| *Cryptosporidium galli* | Birds | - |
| *Cryptosporidium hominis* | Human (*Homo sapiens*) | Dugongs, sheep, cattle |
| *Cryptosporidium meleagridis* | Turkey (*Meleagris gallopavo*) | Parrots |
| *Cryptosporidium molnari* | Fish | - |
| *Cryptosporidium muris* | Mouse (*Mus musculus*) | Humans and mountain goats |
| *Cryptosporidium nasorum* | Fish (*Naso literatus*) | - |
| *Cryptosporidium parvum* | 150 mammalian hosts (commonly | - |

| ***Cryptosporidium* species** | **Predominant type hosts** | **Minor hosts** |
|---|---|---|
| | found in cattle) | |
| *Cryptosporidium saurophilum* | Lizards | Snakes |
| *Cryptosporidium serpentis* | Snakes | Lizards |
| *Cryptosporidium varanii* | Emerald monitor lizard | - |
| *Cryptosporidium wrairi* | Guinea pig (*Cavia porcellus*) | - |

Following the Sydney Water Crisis in 1998, neither the source nor the genotypes of *Cryptosporidium* were identified (Hawkins *et al.* 2000). The „McClellan Inquiry" after the incident revealed that the most significant cause of the events was contamination from sources within the catchment (McClellan 1998, Stein 2000). These were alleged to be cattle, rural-residential development, native and feral animals. A number of studies following this incident have aimed to delineate the sources of *Cryptosporidium* and types of *Cryptosporidium* from each of these sources (Ryan *et al.* 2005). Investigations of native animals as a source of *Cryptosporidium* in the catchment have focussed on the prevalence of the organisms harboured by eastern grey kangaroos, after it was found that this species was potentially the largest contributor of *Cryptosporidium* in the inner catchment lands (Cox *et al.* 2005a).

Macropodid species are susceptible to infections from marsupial-specific genotypes of *Cryptosporidium* (Power *et al.* 2004, Power *et al.* 2005, McCarthy *et al.* 2008, Power 2010). These were *Cryptosporidium* "marsupial" genotype EGK 1, *Cryptosporidium* "marsupial" genotype EGK 2 and *Cryptosporidium* "marsupial" genotype II EGK 3 (Power *et al.* 2004; McCarthy *et al.* 2008). A subsequent revision of genotype I and genotype II has confirmed that these are genetically distinct from each other and from previously described species and strains. Consequently, the two marsupial genotypes were recently named as new *Cryptosporidium* species, *Cryptosporidium fayeri* (marsupial genotype I) (Ryan *et al.* 2008) and *Cryptosporidium macropodum* (Marsupial genotype II) (Power and Ryan 2008). Power *et al.* (2005) demonstrated a seasonal pattern of *Cryptosporidium* prevalence in eastern grey kangaroos residing in Sydney"s watershed, with the highest oocyst counts in autumn. Prevalence of *Cryptosporidium* in this species ranged from 0.32% up to 28.6% in the autumn months. Oocyst shedding from this species ranged from below 20/g faeces to as high as 2.0 $\times 10^6$/g faeces (Power *et al.* 2005). The autumn epidemics were related to the higher presence of pouch young at this time (Power *et al.*

2005), as previous studies have identified that younger cohorts of placental animal species (e.g. calves) generally shed most oocysts (Atwill *et al.* 1999). Therefore, the current study aims to determine whether eastern grey kangaroos have remained a non-zoonotic source of *Cryptosporidium* in Sydney‟s watershed and if the seasonal pattern previously identified by Power *et al.* (2005) has continued following an extended period of drought.

### *Giardia*

*Giardia* has gained a considerable amount of attention from water utilities and health authorities globally, as it causes an estimated 2 x $10^8$ disease cases per annum (Thompson 2004, McCarthy *et al.* 2008). It is also one of the most commonly encountered parasites of domesticated animals, especially livestock, dogs and cats, and numerous species of wild mammals and birds have been documented as hosts of *Giardia* (Thompson 2004). Analogous to cryptosporidiosis, giardiasis infection can result from the consumption of contaminated drinking water, or from unfiltered surface or groundwater systems impacted by surface runoff or sewage discharges (Thompson 2006). Furthermore, giardiasis can also commonly eventuate from the consumption of contaminated food or by direct contact with an infected host (Gagnon *et al.* 2006).

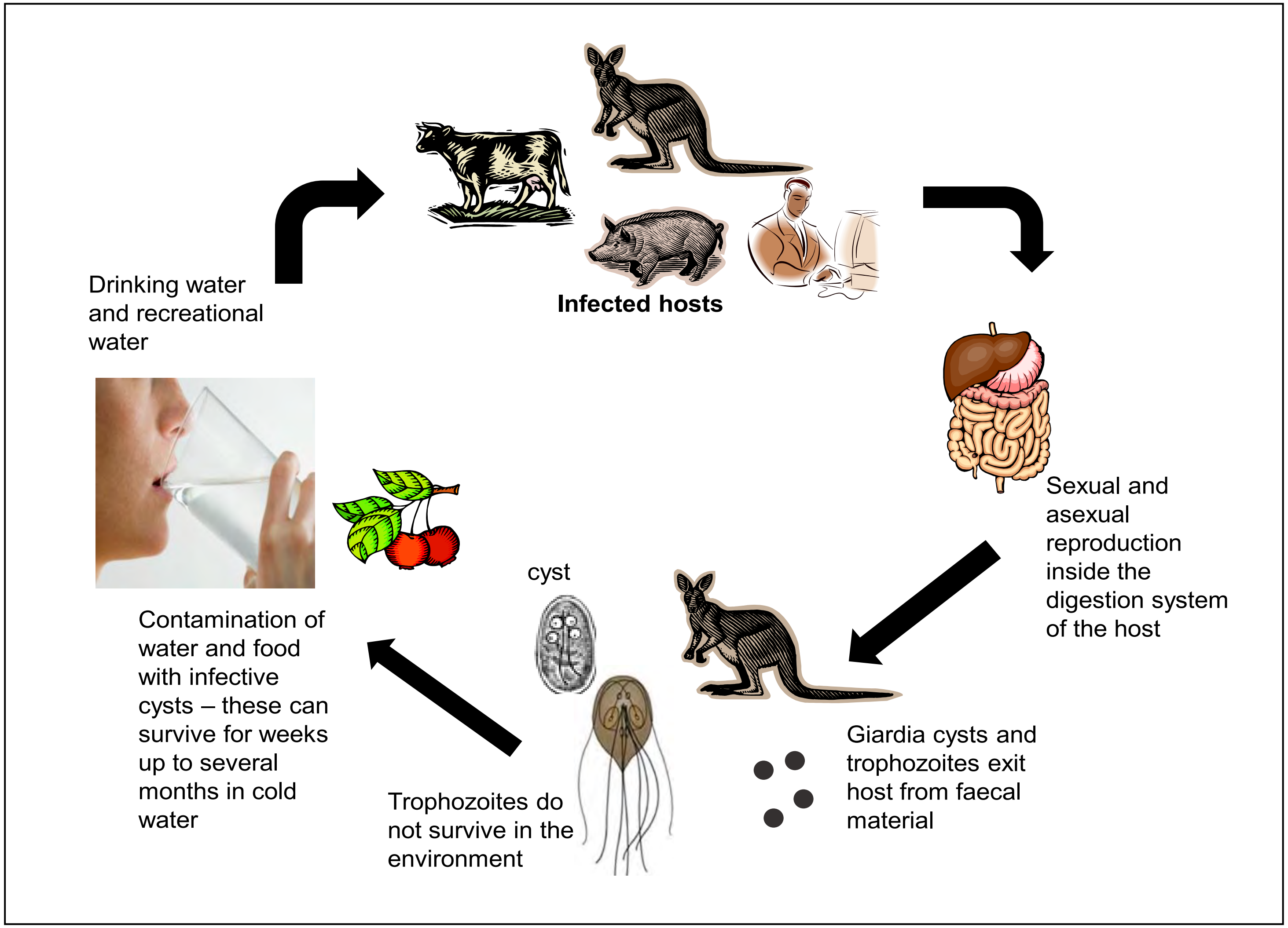
The life cycle of *Giardia* involves an active living form in the host‟s gut named the trophozoite and a dormant, robust and infective thick-walled cyst for transmission in the environment. The cyst is shed intermittently in large numbers in the faeces of the infected host (Figure 2-2). Ingestion of as few as ten cysts can cause infection, which in humans can comprise acute or chronic diarrhoea, weight loss, vomiting and fever. Left untreated, giardiasis may last for 10 days to three months or longer (Thompson 2004). The *Giardia* cyst can survive in fresh water for up to several months, but it is not as resistant as the *Cryptosporidium* oocyst, being vulnerable to many conventional water treatment processes, including filtration with chemical coagulation and chlorination (Hrudey and Hrudey 2004).

There are five known species of *Giardia* that have been differentiated based on trophozoite morphology. These are *Giardia duodenalis* (syn. *Giardia intestinalis*, *Giardia lamblia*) that infects a broad range of mammals, *G. muris* that infects mice and other rodents, *G. agilis* that infects amphibians, and *G. psittaci* and *G. ardeae* that infect birds (Thompson and Monis 2004). *G. duodenalis* has been split into seven assemblages on the basis of genetic information, with Assemblage A and B zoonotic (Table 2-2).

**Table 2-2 Assemblages and host range of *G. duodenalis*.**

| Assemblage/Genotype | Host range |
|---|---|
| A | Humans, livestock, cats, dogs, beavers, guinea pig, slow loris |
| B | Humans, slow loris, chinchillas, dogs, beavers, rats, siamang |
| C | Dog |
| D | Cattle, sheep, pigs |
| F | Cat |
| G | Domestic Rats |
| Muskrats/Voles | Wild rodents |

Relatively little is known about the influence of non-human hosts as a source of waterborne giardiasis (Thompson 2004). Several species of wildlife have been found to harbour *Giardia*, giving rise for their potential to be a source of waterborne giardiasis (Heitman *et al.* 2002, Trout *et al.* 2006, Bednarska *et al.* 2007, Paziewska *et al.* 2007). These have included zoonotic assemblages from wildlife species including deer, beavers and other rodent species in the northern hemisphere (van Keulen *et al.* 2002, Fayer *et al.* 2006, Bednarska *et al.* 2007).



Infected hosts
Sexual and asexual reproduction inside the digestion system of the host
Giardia cysts and trophozoites exit host from faecal material
cyst
Trophozoites do not survive in the environment
Contamination of water and food with infective cysts – these can survive for weeks up to several months in cold water
Drinking water and recreational water

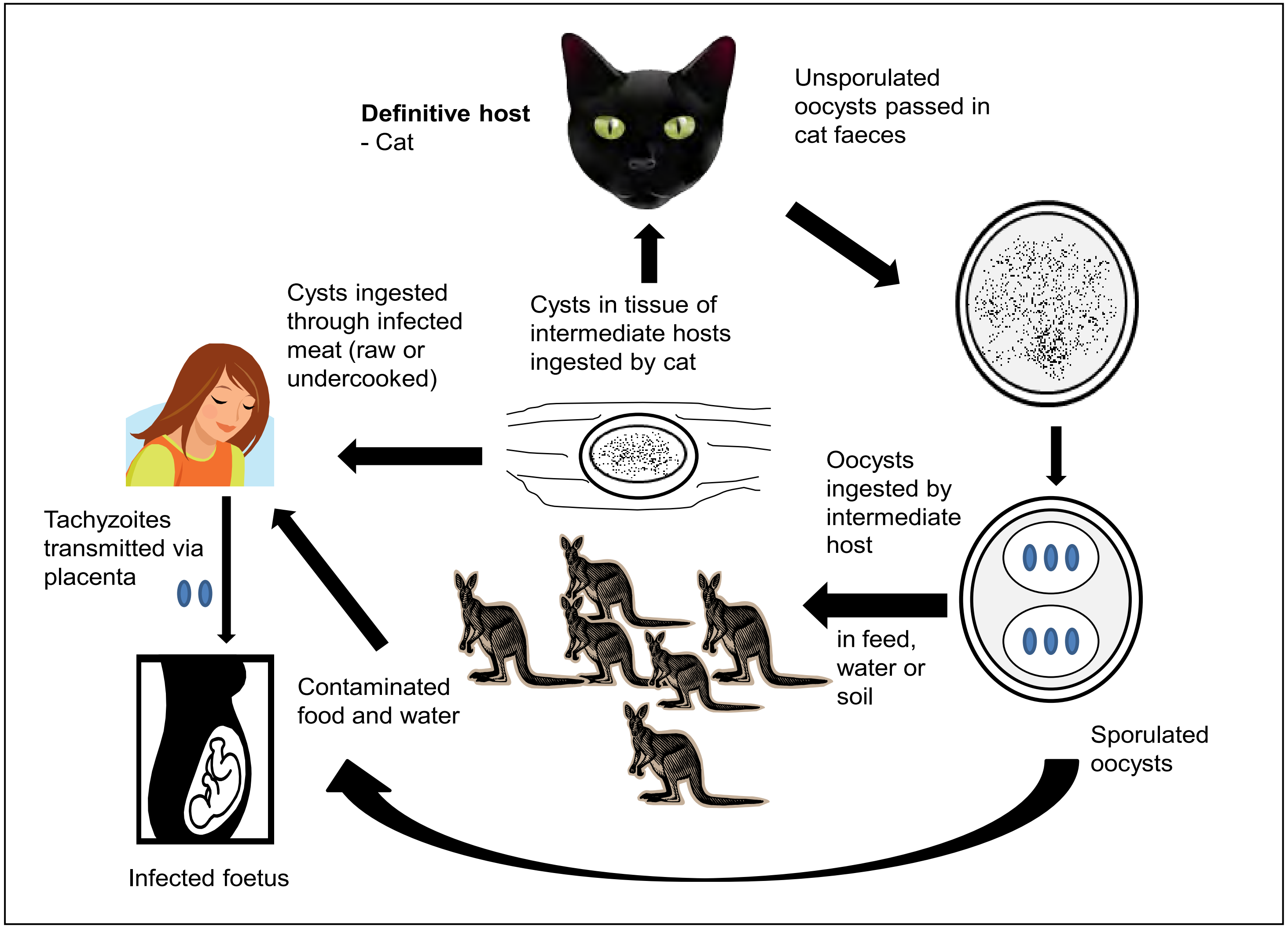
**Figure 2-2 The life cycle of *Giardia*, involving sexual and asexual reproduction in the host's digestion system and cyst shedding causing contamination of water and food.**

In Australia, McCarthy *et al.* (2008) found zoonotic *Giardia* assemblage A in western grey kangaroos, *Macropus fuliginosus*, inhabiting a watershed in Western Australia. This indicated that marsupials may potentially be a reservoir of zoonotic *Giardia* in Australia (McCarthy *et al.* 2008). However, despite high concentrations of cysts being detected in Sydney‟s raw water supply during the 1998 Sydney water crisis (McClellan 1998, Stein 2000), few studies have focused on the *Giardia* assemblages carried by marsupials in the eastern water catchments of Australia. Cox *et al.* (2005) screened a relatively small number of eastern grey kangaroo faecal samples collected from Sydney‟s watershed and did not detect *Giardia*. Therefore, given the paucity of information concerning the prevalence of *Giardia* in this species, a more intensive sampling program is required to ascertain if eastern grey kangaroos are a source of zoonotic *Giardia*.

### *Toxoplasmosis*

Toxoplasmosis has been found in an estimated one-third of the human population worldwide (Dubey 2004). Humans become infected with *Toxoplasma gondii* from the consumption of food or water contaminated by faeces from infected felids, consumption of underprepared meat of an infected host, or congenitally through the placenta during pregnancy (Aramini *et al.* 1999, Dubey 2010, Jones and Dubey 2010). *T. gondii* oocysts are extremely resistant to environmental conditions, including freezing, drying and heating up to 56$^{o}$C, along with chlorination.

The lifecycle of *Toxoplasma* is illustrated in Figure 2-3. Felidae, both domestic and wild, are the definitive hosts of *T. gondii*, shedding the environmentally resistant oocyst stage in their faeces (Dubey 1996, Aramani *et al.* 1998). Most other warm blooded animals, including humans, act as intermediate hosts of the disease, harbouring *T. gondii* as tissue cysts (Dubey 2004). These intermediate hosts ingest oocysts from contaminated food, water or soil. The oocysts multiply in the intestinal tract of the intermediate host, forming tachyzoites, which typically form cysts in the skeletal or heart muscles, the brain or the liver for the lifespan of the host (Dubey 1998). Infections in most healthy humans are asymptomatic or result in an influenza-like illness that may affect immunocompromised patients; then morbidity and mortality commonly occur (Conrad *et al.* 2005). Tachyzoites can be transmitted via the foetus during early pregnancy, potentially causing foetal death or symptoms such as mental retardation, hearing impairments or vision loss (Conrad *et al.* 2005). The life cycle is completed when cats consume the dormant cysts in the intermediate host and they are reactivated and eventually shed as oocysts again or sporulated oocysts are consumed from other cats (Dubey 1998). Feral cats are more susceptible to harbouring *T. gondii* than domestic cats because they more readily consume infected birds and mammals from the wild (Aramini *et al.* 1999).



Definitive host
- Cat
Unsporulated oocysts passed in cat faeces
Cysts in tissue of intermediate hosts ingested by cat
Cysts ingested through infected meat (raw or undercooked)
Oocysts ingested by intermediate host
in feed, water or soil
Sporulated oocysts
Tachyzoites transmitted via placenta
Contaminated food and water
Infected foetus

**Figure 2-3** **The life cycle of *Toxoplasma gondii*. Felids are the definitive host shedding oocysts in their faeces and intermediate hosts (e.g. kangaroos) store cysts in their tissue.**

There has been some recent work suggesting that toxoplasmosis has sub-clinical effects on a range of species, including humans. These effects make infected individuals more prone to taking risks. For example, infected rats have been studied to demonstrate a greater level of boldness towards cats (Berdoy *et al.* 2000), which may increase their susceptibility to predation and inadvertently complete the life cycle of *Toxoplasma*.

In recent times, there have been a few reported cases of human outbreaks of *T. gondii* associated with exposure to contaminated water sources (Aramani *et al.* 1998, Aramini *et al.* 1999, Sroka *et al.* 2006, Jones and Dubey 2010). One case in British Columbia showed that an estimated 2,894 to 7,718 individuals were affected with *T. gondii* in a waterborne outbreak associated with a municipal water supply on Vancouver Island. It was suspected that the faeces of domestic or feral cats, including cougars, entered the Vancouver reservoir (Hrudey and Hrudey 2004).

Australian marsupials are particularly vulnerable to the exposure of *T. gondii* having not evolved with felids before European settlement (Canfield *et al.* 1990, Moodie 1995, Dickman 1996). *T. gondii* causes acute and chronic infection in a number of Australian wild marsupials including possums, koalas, wombats and macropodid species (Dubey *et al.* 1988, Hartley *et al.* 1990, Hartley and English 2005, Eymann *et al.* 2006, Parameswaren *et al.* 2009). Therefore, the maintenance of *T. gondii* by high density kangaroo species residing near potable water sources is a potential concern for zoonotic disease dissemination. As an intermediate host of *T. gondii*, kangaroos are unable to transmit oocysts to water, but when infected with toxoplasmosis, the cyst may be consumed by feral cats when kangaroos are killed as prey or eaten as carrion (Molsher 2006, Parameswaren *et al.* 2009). Feral cats may then be capable of excreting potentially large numbers of oocysts into the watershed.

Another risk posed by the presence of *T. gondii* in kangaroo species is the human consumption of their meat, which represents an alternative food in Australia and in many countries worldwide (Parameswaren *et al.* 2009). This is further exacerbated because this meat is typically cooked underprepared or medium-rare, which reduces the likelihood of cyst degradation (Conrad *et al.* 2005). Although this risk is apparent, relatively few studies have focussed on the presence of *T. gondii* in commercially harvested kangaroo species.

### *Leptospirosis*

Leptospirosis is an emerging bacterial zoonotic disease of worldwide importance and is most prevalent in tropical countries (Lau *et al.* 2010). The disease is transmitted to humans either directly from the urine of a mammalian host or indirectly through contact with contaminated water and soil and infected body fluids or tissues of carrier animals (Slack *et al.* 2006). Free ranging wildlife species are most commonly involved in both the maintenance and the spread of leptospirosis to livestock and humans (Cox *et al.* 2005b).

The leptospires, enveloped in moisture, enter a host via portals such as damaged skin, mucous membranes, the lungs, or conjunctival membranes (Figure 2-4). Once inside the host, the host‟s metabolism permits the rapid growth of the bacterium. If the leptospires survive the innate immune response of the host, they will rapidly migrate to the blood stream and lymphatic system and the growth of the bacteria will continue to occur until an immune response occurs. Infections in humans can vary from being mild, where flu-like symptoms are exhibited, through to severe which may involve acute renal failure, jaundice and pulmonary haemorrhage; death can result in 10 to 50% of these cases (Dall'Antonia *et al.* 2008).

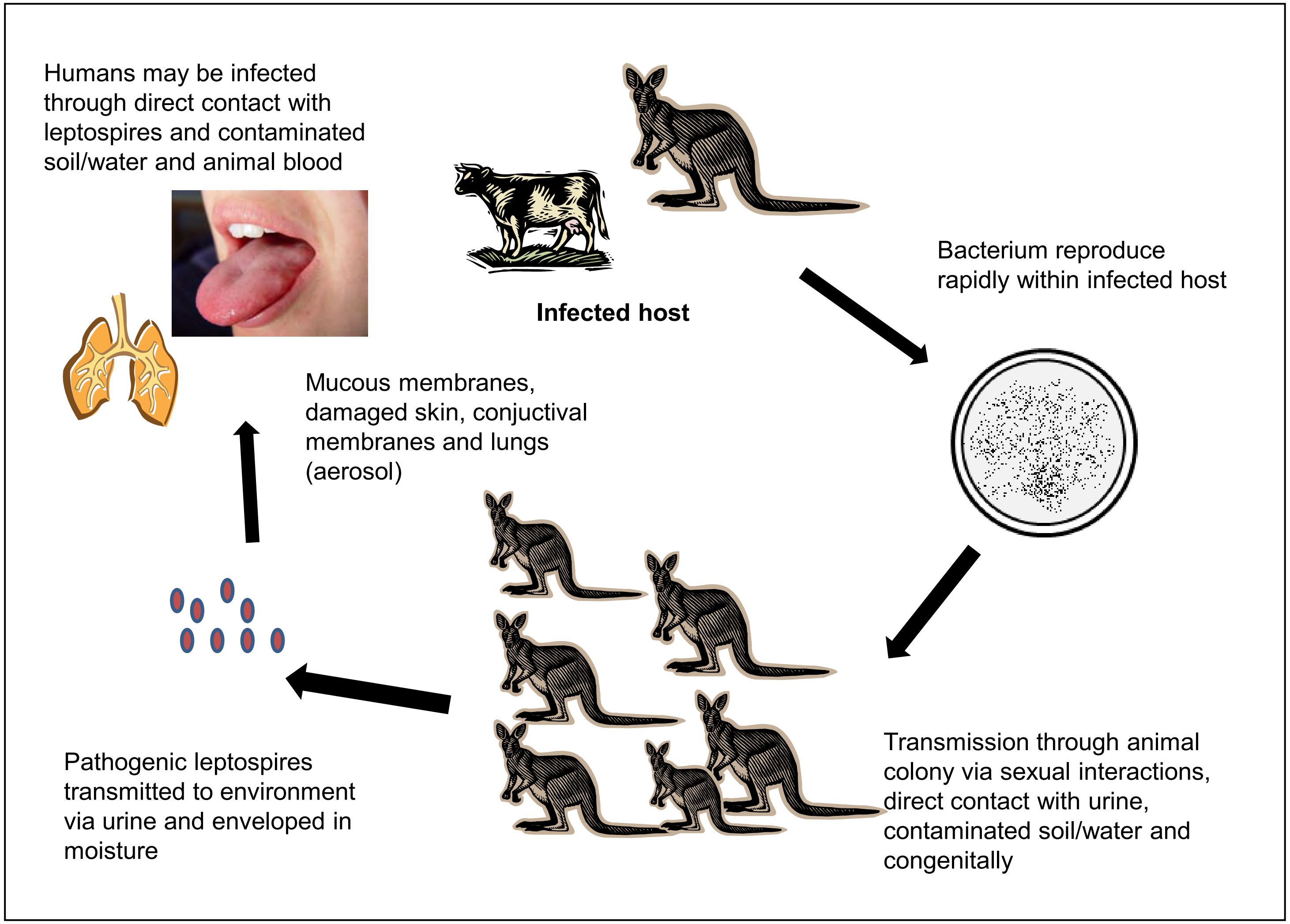

Humans may be infected through direct contact with leptospires and contaminated soil/water and animal blood
Infected host
Bacterium reproduce rapidly within infected host
Mucous membranes, damaged skin, conjuctival membranes and lungs (aerosol)
Transmission through animal colony via sexual interactions, direct contact with urine, contaminated soil/water and congenitally
Pathogenic leptospires transmitted to environment via urine and enveloped in moisture

Figure 2-4 The life cycle of Leptospira. Bacteria reproduce by binary fission inside the host and pathogenic leptospires are transmitted through urine into the environment.

Leptospirosis is as an occupational hazard for farmers, abattoir workers, veterinarians, cane farmers (acquired indirectly from rodents living in cane fields) and dairy workers through their elevated exposure to contaminated body fluids of animals being slaughtered or handled (Slack *et al.* 2006, Slack *et al.* 2007). In addition, recreational activities that involve contact with contaminated water or soil can also permit *Leptospira* to be transmitted; such groups include water-skiers, swimmers, campers, gardeners and bushwalkers (Monahan *et al.* 2009). There have been some reported cases of *Leptospira* infection from drinking water from rainwater catchment systems (Kats *et al.* 1991), smaller scale water systems (Cacciapuoti *et al.* 1987, Corwin *et al.* 1990, Ciceroni *et al.* 2000, Jena *et al.* 2004) and from water utility failures during floods (Ford 1999). However, these have typically occurred in developing countries or due to failure of water treatment processes, which have resulted in a reduced ability to eliminate bacteria.

Leptospirosis is a notifiable disease within Australia, where it was first reported in 1933 (Slack *et al.* 2006). Since 1991, there have been over 7,629 cases notified in Australia and twenty-three serovars have been isolated from human patients (Slack *et al.* 2007). These include *Leptospira interrogans* serovars (sv.) (Australis, Zanoni, Kremastos, Robonsoni, Broomi, Pomona, and Szwajizak), *L. kirschneri* (sv: Valbuzzi); *L. borgpetersenii* (sv: Arborea) and *L. weilii* (sv. Celledoni and Topaz). Some of these serovars have been detected in free ranging wildlife including brushtail possums, flying-foxes, feral pigs, rodents, bandicoots and platypus (Durfee and Presidente 1979, Mason *et al.* 1998, Cox *et al.* 2005b, Eymann *et al.* 2007, Loewenstein *et al.* 2008).

There is a limited understanding of the strains of *Leptospira* carried by macropodid species. Studies by Munday (1972) of three macropodid species found no antibodies and subsequent work by Durfee and Presidente (1979) against a limited panel of antigens showed similar results with only one wallaby demonstrating a low level titre to serovar Pomona. The work by Durfee and Presidente (1979) also involved a limited number of samples from the eastern grey kangaroos collected in Canberra, Australian Capital Territory and all yielded no evidence of leptospirosis. Therefore, it is essential that the prevalence of leptospirosis be investigated more thoroughly in this species to assess the possible effect on human health and domestic livestock.

## METHODS

### *Serum collection*

Blood serum was collected from captured eastern grey kangaroos from the same study area and by using the same capture methodology described in Chapter 1 and Appendix A. Briefly, between June 2004 and November 2006, blood serum was collected from eighty - seven eastern grey kangaroos residing in the Warragamba Catchment Area to examine the exposure of these animals to *Leptospira* and *Toxoplasma*. Each kangaroo was randomly captured using a Pneu-dart (Pneu-Dart® Inc., Williamsport USA) tranquilizer firearm from three sites on the Wollondilly River, Murphy‟s Crossing, Jooriland and Douglas Scarp (Figure 1-6). For each animal, a 1 ml Pneu-dart containing 5mgkg$^{-1}$ Zoletil® (1:1 zolazepam and tiletamine hydrochloride, Virbac, Sydney, Australia) was fired into the hindquarters from an optimum distance of 25-35 metres. Sedation was generally achieved within 10 minutes. Whilst sedated, each kangaroo was placed into a hessian bag and weighed, sexed, tagged, radio-collared and their body condition was noted. Body condition was indexed subjectively by assessing the fat circumference around the base of tail and scored from 1 (poor) to 5 (excellent). The age of the animal was generally determined by their size and classified into adult and sub-adult. Pouch young were not considered in this experiment but their presence was noted for observational analysis. Five millilitres of blood were collected from the lateral tail vein of each animal. Serum was separated and stored in temperatures less than $-10^{\circ}$C. After processing, animals were placed in the shade to recover, which generally occurred after two hours.

A total of eighty-seven serum samples were analysed for Leptospira antibodies and sixty-five were analysed for the presence of *Toxoplasma* as described in Appendix B. Briefly, the presence of *Leptospira* and *Toxoplasma* were detected using microscopic agglutination tests (MAT).

Contingency tables were used to examine differences in the prevalence of *Leptospira* and *Toxoplasma* antibodies in eastern grey kangaroos between sexes, body condition and in females, the presence or absence of pouch young (Snedecor and Cochran 1967). The relatively low number of sub-adult kangaroos captured in the study precluded a comparison in the prevalence of *Leptospira* or *Toxoplasma* antibodies between age groups.

### *Faecal sample collection*

Eastern grey kangaroo faecal samples were collected from the Warragamba Catchment Area between March and August 2006 to assess whether autumn *Cryptosporidium* epidemics occurred, as previously described by Power *et al.* (2005). Sampling was also conducted for *Giardia* at this time using the same samples.

Fresh faecal samples were collected from eastern grey kangaroos residing in close proximity to the Wollondilly River. A similar sampling strategy was adopted to that of Power *et al.* (2005) by setting the following criteria; (i) never more than one faecal sample was collected from the same „mob‟ of kangaroos, (ii) faecal collections were conducted only at grazing times (i.e. early morning and late afternoon) to ensure the freshness of samples and to negate any influence of their desiccation on *Cryptosporidium* and *Giardia* detection or presence, (iii) sample collection was as widespread as possible to avoid sampling the same individuals, and (iv) only discrete piles, likely to be from the same individual kangaroo, were collected. Faecal samples were placed in labelled specimen containers and were stored at less than -4$^{o}$C. Because faecal samples could not be assigned to individual kangaroos, it was not possible, as it was for *Leptospira* and *Toxoplasma*, to explore potential associations between the prevalence of *Giardia* and *Cryptosporidium* and sex or age classes of kangaroos.

Genotyping of faecal samples for *Cryptosporidium* and *Giardia* were performed as described in Appendix B.

### *Risk profile calculation*

The risk of the four pathogens being transmitted to humans was assessed primarily from the study population of eastern grey kangaroos and secondly, given that this species frequently co-inhabits the landscape with humans, it was also considered across the species‟ distribution. The following groups were included in the risk analysis:

- people drinking from Sydney‟s potable water supply;
- people undertaking outdoor recreational activities (e.g. camping, bushwalking);
- people associated with agricultural production; and,
- people with companion animals.

Assessment of the risk of the four pathogens being transmitted to each of the four groups above was based on four potential outcomes. Individual outcomes represented points along a gradient representing a low risk to a very high risk of pathogen transmission, whereby risk was determined from the product of the likelihood of the pathogens being transmitted and the consequence of the pathogens causing disease in each of four groups:

- Low risk: there is a low likelihood that the pathogen concerned will be transmitted to the respective group because of the low contact rate with the disease-causing agent present in kangaroos, and illness in humans will be negligible.
- Moderate risk: there is a moderate likelihood that the pathogen concerned will be transmitted to the respective group because of the moderate contact rate with the disease-causing agent present in kangaroos, and illness in the humans is possible.
- High risk: there is a high likelihood that the pathogen concerned will be transmitted to the respective group because of the high contact rate with the disease-causing agent present in the kangaroos, and illness in humans is highly likely.
- Very high risk: there is a very high likelihood that the pathogen concerned will be transmitted to the respective group because of the very high contact rate, and potentially direct contact, with the disease-causing agent and illness in humans is very likely.

The factors that were used to determine the likelihood of pathogen transmission were:

- the prevalence of the disease-causing agent in the kangaroos;
- published material on the associations of kangaroos (e.g. densities, movement patterns) with each of the four groups; and,
- persistence and longevity of the disease causing agent in the environment.

The risk categories that have been used represent the worst case scenario. For example, a breakdown of water treatment processes, high prevalence of the pathogen, inferior levels of water treatment or high public utilisation of camping grounds during holiday periods.

## RESULTS

### *Cryptosporidium*

From the 148 eastern grey kangaroo faecal samples collected, 16.3% (25) *Cryptosporidium* – positive samples were detected. All positive samples were collected during the autumn period (March – May). Genotyping at the 18S rRNA locus for the 25 positive samples showed that the isolates fell into three groups; *C. parvum* (16%), *C. hominis*-like (28%) and an unknown genotype closely related to *C. parvum* and *C. hominis* (56%) (Figure 2-5 and Figure 2-6). Unfortunately, these were generated only at the rRNA 18S locus. Attempts to amplify the samples at the HSP-70, Actin, Acetyl Co-A synthethase, COWP and GP-60 loci were unsuccessful and therefore the data should be interpreted cautiously and viewed as preliminary and as yet unsubstantiated (Una Ryan, Personal Communication, 2008).

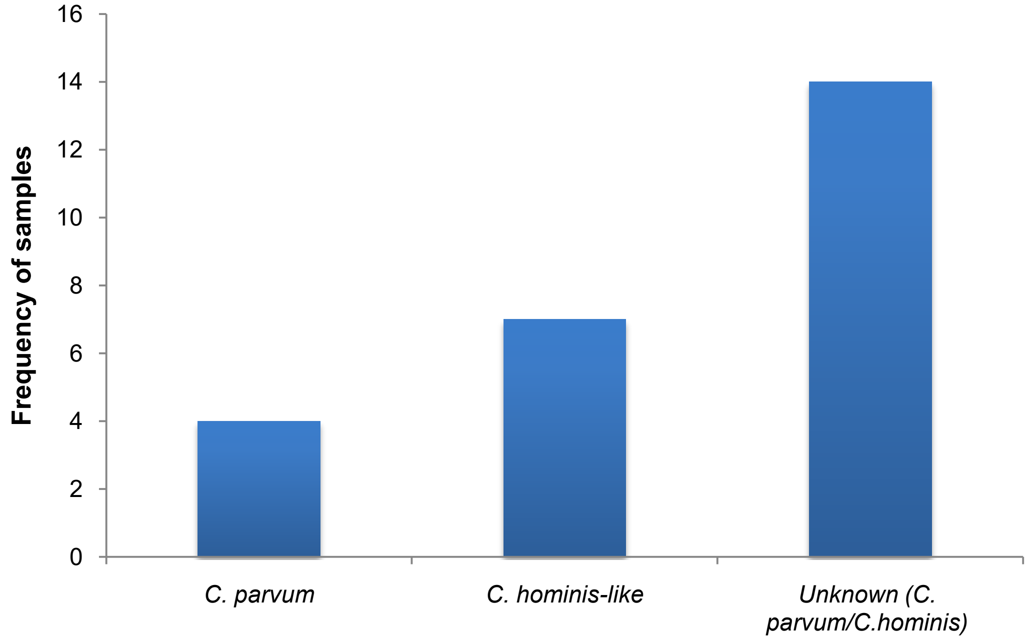
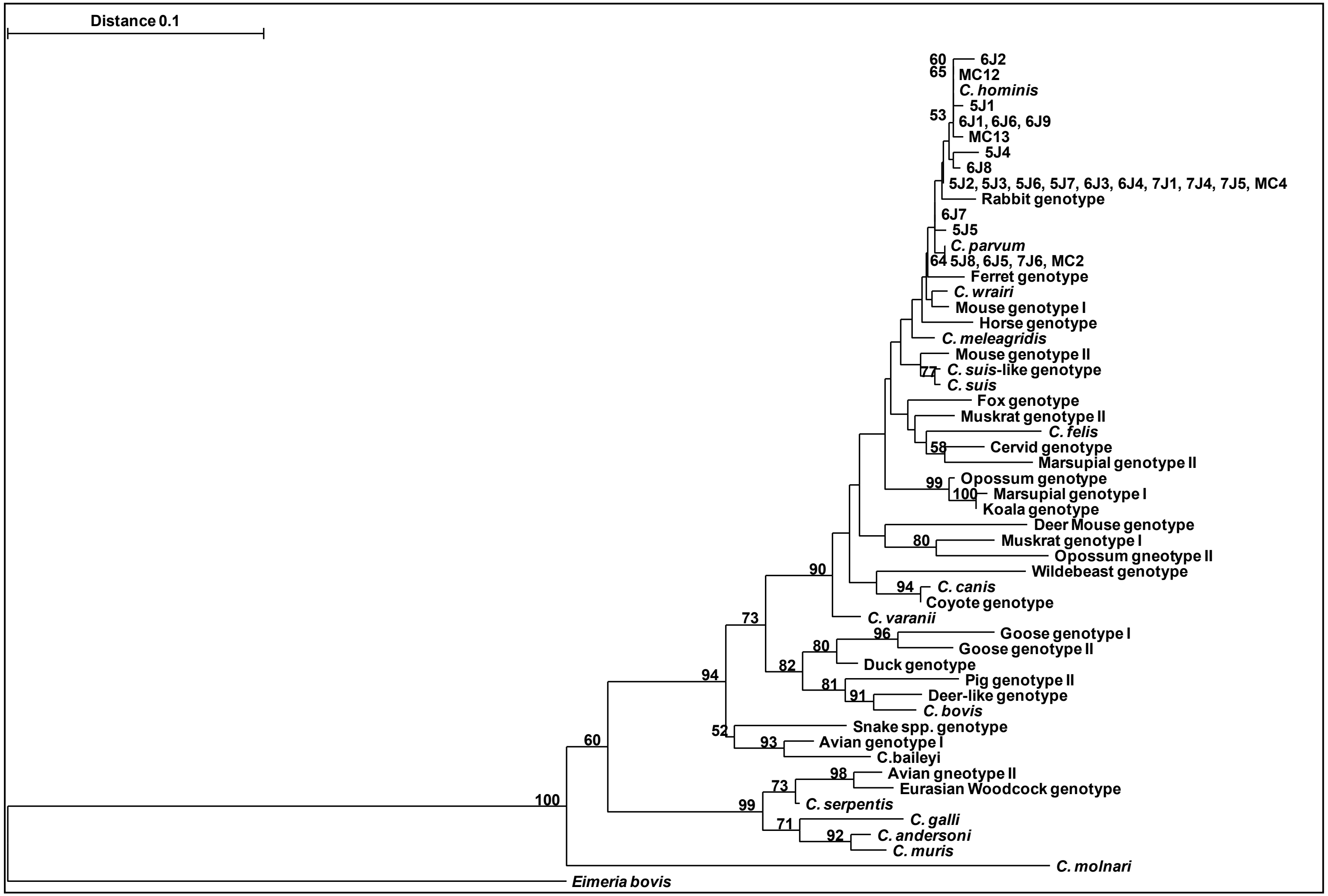



**Figure 2-5** **The breakdown of *Cryptosporidium* genotypes found in eastern grey kangaroo faecal samples.**



Distance 0.1
6J2
MC12
C. hominis
5J1
6J1, 6J6, 6J9
MC13
5J4
6J8
5J2, 5J3, 5J6, 5J7, 6J3, 6J4, 7J1, 7J4, 7J5, MC4
Rabbit genotype
6J7
5J5
C. parvum
5J8, 6J5, 7J6, MC2
Ferret genotype
C. wrairi
Mouse genotype I
Horse genotype
C. meleagridis
Mouse genotype II
C. suis-like genotype
C. suis
Fox genotype
Muskrat genotype II
C. felis
Cervid genotype
Marsupial genotype II
Opossum genotype
Marsupial genotype I
Koala genotype
Deer Mouse genotype
Muskrat genotype I
Opossum gneotype II
Wildebeast genotype
C. canis
Coyote genotype
C. varanii
Goose genotype I
Goose genotype II
Duck genotype
Pig genotype II
Deer-like genotype
C. bovis
Snake spp. genotype
Avian genotype I
C.baileyi
Avian gneotype II
Eurasian Woodcock genotype
C. serpentis
C. galli
C. andersoni
C. muris
C. molnari
Eimeria bovis

**Figure 2-6 Phylogenetic relationships of genotypes sequenced from *Cryptosporidium* – positive faecal samples (e.g. 5J2) inferred by the neighbour-joining method and previously recognised Cryptosporidium species (from various sources)**

### *Giardia*

A total of 12.2% (18/148) of the eastern grey kangaroo faecal samples collected were found to be *Giardia* positive. Amplification at the 18S rRNA gene revealed that the positive samples comprised four assemblages, all belonging to *G. duodenalis*: Assemblage A (44.4%), Assemblage B (11.1%), Assemblage C (22.2%) and Assemblage D (22.2%) (Figure 2-7).

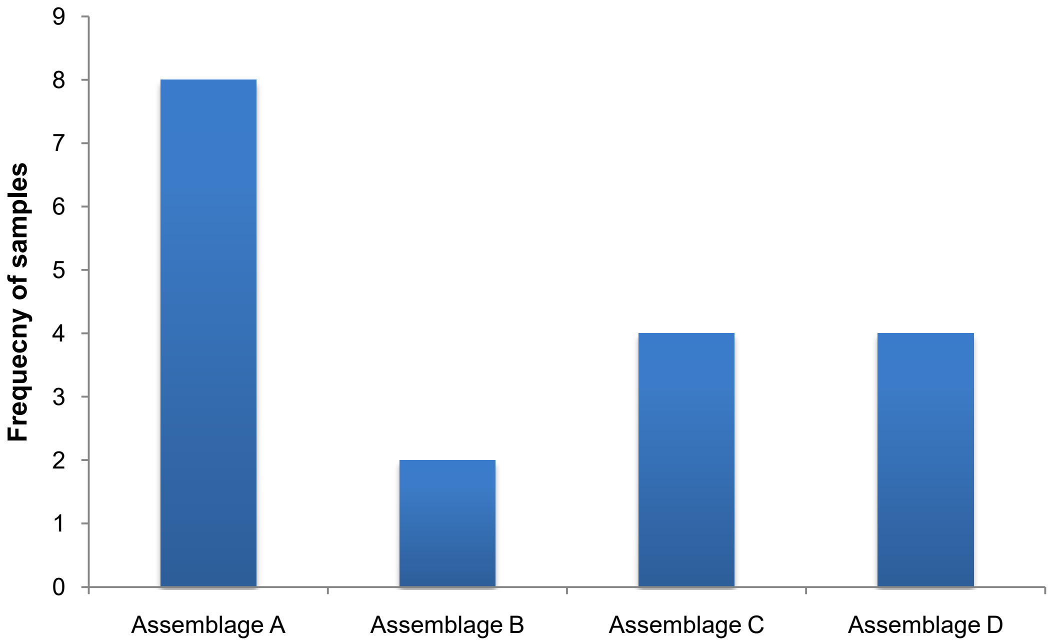



**Figure 2-7** ***Giardia duodenalis*** **assemblages found in eastern grey kangaroo faecal samples.**

### *Toxoplasma*

*Toxoplasma gondii* antibodies were detected in 3.1% (2/65) of the sera analysed. This represented two seropositive kangaroos, one adult female carrying pouch young (#38) and an adult male (#20). The number of seropositive animals was not large enough to perform statistical analysis for parameters such as age, sex and influence of the presence of pouch young.

## *Leptospira*

*Leptospira* antibodies were detected in 47.1% (41/87) of the sera analysed, with serovar Topaz identified in all seropositive kangaroos (41/41) (Table 2-3).The level of serovar Topaz antibody titres in individuals varied between 1:50 to 1:3,200. There were reactions observed against the serovars Tarassovi (14.9%), Shermani (3.4%), Australis (2.3%), Panama (1.1%) and Batavia (1.1%), but these were known cross reactions and not a result of multiple infections (Lee Smythe, personal communication 2008).

There was no significant difference in the prevalence of *Leptospira* antibodies between male and female eastern grey kangaroos ($x^2$ = 2; *d.f.* = 1; $P$ = 0.16), although 79.2% of males (19/24) compared with 34.9% of females (22/63) illustrated exposure. Body condition of eastern grey kangaroos had no effect on leptospirosis exposure ($x^2$ = 6.6; *d.f.* = 4; $P$ = 0.09) nor did the presence of pouch young in females ($x^2$ = 2.2; *d.f.* = 1; $P$ = 0.14). Females carrying pouch young had 42.9% exposure (15/35) compared with 25% of females not carrying (7/28).

**Table 2-3 Anti-bodies to *Leptospira* exhibited by 87 eastern grey kangaroos.**

| Leptospira serovar (titers) | Adult males (range of titres) | Adult females (range of titres) | Sub-adults (range of titres) |
|---|---|---|---|
| Topaz | 100-3200<br>n = 16 | 50-1600<br>n = 22 | 200<br>n = 3 |
| Shermani | 50<br>n = 1 | 50<br>n = 2 | - |
| Tarassovi | 50-800<br>n = 8 | 50-200<br>n = 4 | 50<br>n = 1 |
| Bataviae | - | 400<br>n = 1 | - |
| Panama | 100<br>n = 1 | - | - |

### ***Risk profile***

The risk of each of the four pathogens being transmitted to humans is presented Table 2-4.

**Table 2-4 Zoonotic disease risk profile of the four pathogens assessed in eastern grey kangaroo populations in Sydney's water supply catchment**

| Pathogen | Mode of transmission (Risk groups) | Risk | Justification |
|---|---|---|---|
| *Cryptosporidium* | Potable water supply | High | • High density of animals residing in close proximity to stored water<br>• Resistant to chlorination treatments<br>• Potentially human infective strains/genotypes found in the population<br>• Uncertain of oocyst counts, but previous studies indicate low levels<br>• High rates of oocyst degradation from source to off-take |
| | Recreational activities (e.g. camping, bushwalking etc.) | Medium | • Consumption of drinking water that may have been contaminated from runoff |
| | Farming | High | • Consumption of untreated or poorly treatment water<br>• Inter-species transmission to cattle and other livestock |
| | Companion animals | Low | • Little is known about interspecies transmission<br>• Species specific strains have generally been found in companion animals (e.g. dogs, cats) |
| *Giardia* | Potable water supply | Medium | • Organism is typically vulnerable to elaborate treatment processes in water supplies<br>• Zoonotic assemblages detected in study population |
| | Recreational activities (e.g. camping, bushwalking etc.) | Medium | • Potentially high loads entering water bodies that are not chlorinated<br>• Drinking un-chlorinated water |
| | Farming | Medium | • Drinking simply treated water<br>• Interspecies transmission of zoonotic strains to cattle may raise shedding intensity (esp. calves) |

| Pathogen | Mode of transmission (Risk groups) | Risk | Justification |
|---|---|---|---|
| | Companion animals | Medium | • Cats and dogs generally harbour their own genotypes<br>• Transmission of zoonotic strains to dogs is possible |
| *Leptospira* | Potable water supply | Very low | • Water sampling by government bodies<br>• High dilution factor and disinfection of bacterium likely |
| | Recreational activities (e.g. camping, bushwalking, hunting etc.) | High | • High exposure rates of zoonotic strain (*Leptospira weiili* sv.Topaz) present in high density populations of kangaroos<br>• Direct contact with habituated kangaroos in camping grounds<br>• Direct contact with dead or dying kangaroos (e.g. researchers, veterinarians)<br>• Indirect contact with contaminated water or soil |
| | Farming | Medium | • High utilisation of farming areas by kangaroos<br>• Associations with domestic livestock<br>• Direct contact with infected cattle and kangaroo carcasses<br>• *Leptospira weili* sv. Topaz has been found in domestic cattle<br>• Lack of hygiene |
| | Companion animals | Low | • Little is known about interspecies transmission |
| *Toxoplasma* | Potable water supply | Low | • High density of animals residing in close proximity to stored water<br>• Prevalence is low in population (1%), but fatal toxoplasmosis is possible following extended period of drought<br>• High density kangaroo populations will act as maintenance hosts of the disease, but oocysts can only be transmitted to stored water by definitive host (cat)<br>• Cat abundance relatively low |

| Pathogen | Mode of transmission (Risk groups) | Risk | Justification |
|---|---|---|---|
| | | | • Kangaroo is consumed as carrion by cats<br>• Oocysts entry to stored water from cat consumption of cysts<br>• Treatment processes in Sydney are superior to areas where water borne Toxoplasmosis has occurred (e.g.Vancouver Island case in Canada) |
| | Recreational activities (e.g. camping, bushwalking, hunting, water sports etc.) | Medium | • Kangaroo densities are quite high in many nature reserves and camping areas<br>• Kangaroos are illegally hunted<br>• Drinking water that may have been contaminated from runoff<br>• Ingestion of cysts from the consumption of underprepared kangaroo meat |
| | Farming | Medium | • High utilisation of farms by kangaroos<br>• Ingestion of cysts from eating uncooked or underprepared kangaroo meat<br>• Ingestion of oocysts by cattle sharing pasture with kangaroos after being consumed by cats<br>• Feral cat densities around farms are typically high<br>• Farm dogs eating kangaroo carcasses |
| | Companion animals | Medium | • High density populations encroaching on urban areas<br>• Ingestion of cysts by domestic cats from consumption of kangaroo carrion or kangaroo meat<br>• Ingestion of cysts by dogs eating kangaroo carcass/kangaroo pet meat |

## DISCUSSION

### *Cryptosporidium*

Although preliminary, this is the first study to identify *C. parvum* and a *C. hominis* – like genotype in eastern grey kangaroos. *C. parvum* has previously been found in approximately 150 mammalian hosts including humans, whereas *C. hominis* has only ever been detected in humans and dugongs (Morgan *et al.* 2000). Therefore, the results of this study suggest that eastern grey kangaroos may be involved in the zoonotic transmission of *Cryptosporidium*.

The highest human health risk of *C. parvum* and *C. hominis* in eastern grey kangaroos is their waterborne transmission, given that these have been implicated in a number of waterborne cryptosporidiosis outbreaks (Xiao *et al.* 2001, Chalmers *et al.* 2010). *Cryptosporidium* oocysts are much more robust than *Giardia* cysts, capable of surviving in the environment for extended periods, and able to withstand conventional water treatment by chlorination and granular based filtration processes (Carey *et al.* 2004). These factors coupled with the high density of eastern grey kangaroos in close proximity to one of the major inflows to Warragamba Dam, potentially exacerbate the risk of this species being a future source of *Cryptosporidium* contamination to Sydney"s water supply.

Power *et al.* (2005) reported repeated autumn epidemics of *Cryptosporidium* infection in eastern grey kangaroos at the same location as this study. The results of this earlier work were corroborated by the findings of the present study, in which *Cryptosporidium* was identified only during this period. Although sampling was not conducted during all seasons, this finding re-iterates that *Cryptosporidium* is potentially seasonal in eastern grey kangaroos. Power *et al.* (2005) hypothesised that these peaks arose in response to the high number of susceptible juvenile animals that are present at this time. Eastern grey kangaroos peak in breeding in spring and summer and juveniles are weaned 14-18 months after birth, coinciding with the autumn period. It is suggestive that the presence of *Cryptosporidium* in eastern grey kangaroos is rainfall-dependent, as rainfall will permit enough resources for the survival of the younger cohorts of this species. An unpublished Sydney Catchment Authority report assessing the *Cryptosporidium* peaks after Power *et al.* (2005) first identified them in 2000-2002, found the autumn peak again in 2004 but not in 2005 (Radu and Slade 2007). The lower level of rainfall experienced in 2003 and 2004 is the most plausible explanation for the absence of the autumn peak in 2005. Firstly, the lower rainfall during spring and early summer 2003/2004 potentially reduced the survival and reproductive output of kangaroos(Coulson 2008), resulting in a lower number of births. Secondly, the very dry winter of 2004 led to higher levels of juvenile mortality. Consequently, a reduction in the number of juveniles from a combination of these two factors resulted in the absence of the autumn peak during 2005. Higher rainfall during the two consecutive summers after this period potentially permitted an increase in juvenile survivorship and hence the *Cryptosporidium* detections in the current study. It appears, therefore, that the number of juveniles in the population and the factors affecting this are needed, so that accurate predictions of the risk of this species being important in *Cryptosporidium* transmission can be made.

The wild cattle co-inhabiting the study area with eastern grey kangaroos are the most probable reason for the presence of these previously unidentified *Cryptosporidium* isolates in this species. The kangaroos in this area experience little or no human intervention and it seems unlikely that they have been exposed to these isolates under natural conditions. Several studies have demonstrated that cattle can be infected with *C. hominis* and *C. parvum* (Peng *et al.* 1997, Darabus and Olariu 2003, Xiao and Ryan 2004) after being exposed to these isolates from human origins. Although wild, the cattle in the study area are more likely to have received recent exposure to humans, which is a likely explanation for their uptake of these isolates and their subsequent transmission to other animals. The potential maintenance and continued transmission of these isolates between cattle and kangaroos may raise the local shedding intensity of zoonotic *Cryptosporidium*.

The zoonotic potential of *C. parvum* has long been recognised (Ryan *et al.* 2005). A study conducted in rural New South Wales concluded that *C. parvum* was more prevalent than *C. hominis* in symptomatic human patients (Ng *et al.* 2008). Shared sub-genotypes of *C. parvum* between cattle and humans suggested that zoonotic transmission was occurring. Given the high resident densities of eastern grey kangaroos in rural areas (McCullough and McCullough 2000), further investigation into the sub-genotypes of *C. parvum* found in this study is required to determine if they are also shared with human cases. This is particularly imperative in rural settings, where potable water treatment is typically not as elaborate as in urban areas.

The „McClellan Inquiry" from the 1998 water incident concluded that in the Warragamba Catchment Area intensive and extensive animal husbandry, sewage treatment processes and unsewered urban areas were the highest risk activities in terms of their contribution of *Cryptosporidium* into the system (McClellan 1998). Although only preliminary, the results of this study allow for kangaroos to be also considered if *Cryptosporidium* is detected in Sydney‟s potable water in the future. Additionally, these findings illustrate the need for catchment management organisations and water testing utilities nationwide to monitor the genotypes of *Cryptosporidium* as a means of assessing the zoonotic potential of this diffuse pathogen source in all Australian drinking water catchments.

### *Giardia*

In Australia, the presence of *Giardia duodenilis* in wildlife species has been considered to constitute a low risk to public health because of the novel non-zoonotic genotypes they harbour (Adams and Thompson 2002, Thompson 2004). McCarthy *et al.* (2008) was the first study to refute this notion by identifying zoonotic *G. duodenilis* Assemblage A in western grey kangaroos, *Macropus fuliginosus*, from Western Australia. The current study corroborated this finding by identifying Assemblage A and B in eastern grey kangaroos. It also supports the hypothesis, proposed by McCarthy *et al.* (2008), that kangaroos may act as a reservoir for zoonotic *Giardia* in Australia. Furthermore, the risk of kangaroo species playing a role in the zoonotic transmission of *Giardia* is potentially elevated given the large cumulative range of *Macropus* spp., from the east to west of Australia, their high mobility and their high level interactions with humans on farm properties and as part of the kangaroo meat trade.

The occurrence of zoonotic assemblages of *G. duodenilis* in animal species has been perceived to be a result of their interaction with infected humans. For example, Bettiol *et al.* (1997) demonstrated that eastern barred bandicoots (*Perameles gunnii*) could be experimentally infected with *G. duodenilis* from a human source and in the case of domestic dogs, the zoonotic transmission of Assemblage A between pets and their owners has also been shown (Bugg *et al.* 1999). McCarthy *et al.* (2008) hypothesised that the occurrence of *Giardia* in western grey kangaroos was a response to their interaction with humans in an area that received high recreational use. This is not a plausible explanation for eastern grey kangaroos in this study because the studied population received very little or no intervention from humans. A more probable explanation for the presence of zoonotic *Giardia* in this species is that it arises from the interaction with wild cattle in the study area; cattle can harbour both Assemblage A and B, as well as their own „cattle genotype‟, Assemblage E (Trout *et al.* 2007, Geurden *et al.* 2008). These results could also be a residual effect of the former pastoral occupation of the study area, as kangaroos have potentially acquired these assemblages in the presence of humans and livestock, which are now currently endemic in the population. Although there are no formal data to substantiate any of these hypotheses, cattle and kangaroos were frequently observed co-inhabiting preferential grazing areas, where such transmission may currently take place.

The transmission of *G. duodenilis* Assemblage A and B from eastern grey kangaroos to cattle poses the highest health risk to humans. Cattle, and in particular calves, are vulnerable to infection with zoonotic genotypes of *Giardia* and it has been demonstrated that calves typically shed $10^5$ to $10^6$ cysts per gram of faeces (Xiao and Herd 1994). Therefore, if kangaroos were to transmit these genotypes to cattle, they may serve to amplify the numbers of the originally contaminating isolate and this could pose a more significant health risk to the handlers or indirectly as an important reservoir for waterborne giardiasis (Thompson 2004).

The ingestion of untreated, inadequately treated or simply chlorinated water is a significant contributor to the contraction of giardiasis (Gagnon *et al.* 2006). Waterborne giardiasis outbreaks in humans have most frequently occurred in unfiltered surface or groundwater systems impacted by surface runoff or sewage discharges (Thompson 2004). Therefore, given the specialised treatment of Sydney"s water from Warragamba Dam, the presence of *Giardia* in this supply poses very little risk to humans. However, the level of risk could sharply increase in the event of water treatment failure. Similarly, humans drinking simply treated water from camping grounds or from farm water supplies, where kangaroos occur, could be a potential and significant source of waterborne giardiasis.

Unexpectedly, *G. duodenilis* Assemblages C and D, which are typically dog-specific genotypes (Trout *et al.* 2006, Barutzki *et al.* 2007, Palmer *et al.* 2008) were detected in eastern grey kangaroos. These Assemblages have also been detected, surprisingly, in cats in two previous studies (McGlade *et al.* 2003, Palmer *et al.* 2008). Wild dogs were commonly sighted in the study area, which may suggest that interspecies transmission of *Giardia* genotypes may occur between these two species. However, without formal data it is difficult to ascertain whether these Assemblages are present in kangaroos as a result of patent infection or from mechanical transmission.

### *Toxoplasma*

This is the first study to report *T. gondii* in free ranging eastern grey kangaroos. The low prevalence in the study population is most probably related to the low feral cat abundance in the study area (DECC 2007), which has potentially led to kangaroos having a low encounter rate with this disease.

Clinical toxoplasmosis has been found in a variety of Australian marsupial species. Hartley and English (2005) reported a prevalence of 23.1% in common wombats (*Vombatus ursinus*) (6/23). A study on eastern barred bandicoots revealed that 6.7% of animals had antibodies to *T. gondii* (Obendorf *et al.* 1996), whereas a study of brushtail possums (*Trichosurus vulpecula*) living in an urban environment with relatively high interactions with domestic and feral cats, had similar seroprevalence of 6.3% (9/142) (Eymann *et al.* 2006). In captivity, most of the reported cases of toxoplasmosis in marsupials have been fatal (Dubey *et al.* 1988, Hartley *et al.* 1990, Basso *et al.* 2007, Portas 2010), which may provide a likely explanation for the low seroprevalence of *T. gondii* in this study because serum samples were collected only from live animals. Furthermore, *T. gondii* infection has been found to lead to high rates of infant mortality and abortions in a variety of animals including sheep, goats and western grey kangaroos (Dubey *et al.* 1988, Dubey 1996;2010). Therefore, the lack of juvenile kangaroos sampled in this study may have led to another bias in *T. gondii* seroprevalence in the population.

Fatal toxoplasmosis in animals is often exacerbated by nutritional and weather related stresses (Obendorf and Munday 1983). At the time of the study, eastern grey kangaroo mortality had just stabilised as the conditions started to improve following five years of drought. This may be another plausible explanation for the current low seroprevalence of *T. gondii*, if at the time of the drought seropositive individuals were weakened and died as a result of the poor ambient conditions. The two adult kangaroos that had *T. gondii* antibodies were both healthy and were not ill at the time of capture, further suggesting that *T. gondii* infection can be latent during better conditions and become clinically apparent and subsequently fatal upon the onset of drought. However, without data on the seroprevalence of animals before the onset of drought conditions, this hypothesis remains to be tested.

The consumption of underprepared kangaroo meat poses the highest human health risk for the contraction of toxoplasmosis. Eastern grey kangaroos are not permitted to be culled in this study area, but in other areas of this species" range population numbers are controlled in commercial harvests to reduce their impacts on farming. Game meat, including kangaroo meat, is traditionally undercooked to rare rather than well cooked, which means that *T. gondii* cysts are less likely to be neutralised. A case control study by Cook *et al.* (2000) in six European countries, demonstrated that the consumption of undercooked meat from livestock accounted for between 30 and 63% of *Toxoplasma* infections. This is particularly apparent for eastern grey kangaroos, where some 4 million individuals are harvested commercially each year in New South Wales for human and pet consumption within Australia and for overseas export (DEC 2006). It is clearly imperative that kangaroo meat is not consumed when undercooked, especially if it is sourced from areas where there are high densities of felids, and if the meat is consumed by pregnant women in whom congenital infection of the foetus may occur.

The presence of *T. gondii* in eastern grey kangaroos poses a low water quality risk to the potable supply of Sydney. As an intermediate host of *T. gondii*, kangaroos are unable to transmit oocysts (sporozoites) directly to the water supply. However, given the high density of this species and its close proximity to potable water, the eastern grey kangaroo poses a risk to water quality in terms of the potential maintenance of *T. gondii* at the cyst stage of its life cycle. This is particularly apparent because feral cats may consume kangaroo as carrion (Molsher 2006), whence the ingestion of cysts from infected kangaroos may occur. An individual cat is then capable of excreting millions of oocysts into the environment (Dubey 2004).

The waterborne outbreak of toxoplasmosis that occurred in British Columbia, Canada was preventable and is considered unlikely to occur in Sydney‟s water supply catchment. Firstly, a number of feral and domestic cats, later to be determined to have toxoplasmosis, were permitted to reside near the British Columbia reservoir and cougars, also heavily laden with *T. gondii* oocysts, utilised the watershed (Aramani *et al.* 1998, Hrudey and Hrudey 2004). Secondly, the absence of water filtration and the application of weak levels of disinfection, proved ineffective for *T. gondii* removal. In Sydney‟s water supply catchment, feral cats occupy the study area at low densities and are managed for biodiversity conservation and Sydney‟s water treatment is much more elaborate than the system used during the British Columbia incident, it consists of two stages capable of removing *T. gondii* - particulate removal by filtration and microorganism disinfection with chlorine (Hrudey and Hrudey 2004). The British Columbia case does raise concern that such an outbreak of *T. gondii* can occur where there are minimal barriers to ensure drinking water safety. This may be the case in farming communities, where water may not be treated nor filtered. For example, Sroka *et al.* (2006) concluded that 13.2% of farm water sources considered to be of high standard in Poland were infected with *T. gondii* oocysts. In addition, it does also raise concern about the limited understanding of feral cat ecology in relation to potable water supplies.

Overall, the prevalence of *Toxoplasma* in the studied population of eastern grey kangaroos poses a low risk in terms of human transmission. However, the presence of this disease in a highly mobile and high density species, such as eastern grey kangaroos, does raise concerns about the maintenance and spread of it across the Australian landscape. The following safeguards relevant to the spread of this disease from kangaroos to humans should be scrutinized at all times; continued feral cat control, cooking kangaroo meat to a minimum of 67°C before consumption, personal hygiene when handling potentially infected animals and effective filtration and disinfection of water supplies. In the case of pregnant women, extra precautions should be maintained to prevent infection of the foetus (Dubey 2004).

### ***Leptospira***

Leptospirosis is an emerging zoonotic disease with a worldwide distribution and *Leptospira weilii* serovar Topaz is a newly described serovar first isolated in the far north of Queensland, Australia. Given its novel nature, little is known about the epidemiology of this serovar. It was initially isolated in 1994 from both culture and serological methods from humans and two animal cases (bovine and long nosed bandicoot; *Perameles nasuta*) (Slack *et al.* 2007).The majority of *L. weilli* serovar Topaz infections have occurred in far north Queensland, with the remaining infections occurring in south-east Queensland and in Western Australia (Slack *et al.* 2007). The identification of *L. weilii* sv. Topaz in Western Australia and in New South Wales from this study, illustrates that this serovar may be more prevalent in the Australian environment than some of the more geographically isolated *Leptospira* serovars such as *L. interrogans* serovar Zanoni.

A significant proportion of the study population of eastern grey kangaroos (47%) and the high titres recorded indicate a current or recent infection with *L. weilii* sv. Topaz. Furthermore, the data suggest that *Leptospira* infection is endemic throughout the population of animals investigated. However, interpretation of single collection MAT titres for determination of carrier status in animals is difficult and needs the support of isolation or culture studies for confirmation of renal carriage.

Other *Leptospira* studies on Australian free ranging wildlife have reported much lower exposure rates. Mason *et al.* (1998), for example, reported 20% exposure of *L. interogans* sv. Pomona in feral pigs and Cox *et al.* (2005b) found that only 11% of flying foxes sampled had been exposed to *Leptospira* species in their study. Both of these animals exhibit large ranging patterns of up several square kilometres (Dexter 1999, Markus and Hall 2004). The population of kangaroos in this study were extremely localised with high resident densities occupying small home ranges sizes (refer Chapter 5). This has most probably led to a higher incidence of animal-animal interactions and therefore the higher prevalence of *Leptospira* in the population. Results from the study suggest that kangaroos may be a possible carrier if not, an incidental host for the disease agent. The impact of the disease on the health of the species is not understood.

Another plausible explanation for the high exposure of *Leptospira weilii* sv Topaz in eastern grey kangaroos is the species innate social behaviour. Eastern grey kangaroos are a gregarious species, forming large social groups of up 45 individuals in relatively small areas (Banks 2001). Kangaroos from the studied population were regularly observed feeding and drinking in these large groups, which may have resulted in much easier *Leptospira* transmission throughout the population. Furthermore, this rate of transmission potentially may have been facilitated by kangaroos sharing ephemeral puddles and disused farm dams, where they were frequently seen drinking. Also in this species, male kangaroos often travel alone from group to group to search for females in oestrous (Southwell 1984), a process which involves tasting the urine of receptive females (Kaufmann 1975, Woodward *et al.* 2006). This may have led to the higher exposure of *Leptospira* in males, as they are more likely to come into contact with the bacteria. Alternatively, *Leptospira* can also be transmitted by infected males from their semen (Kiktenko *et al.* 1976), and as males attempt to mate with several females, they may act as a mode of transmission from one population to another.

Although there are limited human interactions with eastern grey kangaroos in the study site, the high exposure of *L. weilii* sv Topaz in the population may pose some human health risks. Kangaroos within the studied population may transmit the disease to other kangaroos residing in areas where camping and other outdoor recreation activities are permitted (e.g. Yerranderie State Conservation Area). Humans may then be susceptible to *Leptospira* infection through direct contact with the urine of habituated kangaroos or by drinking from contaminated water supplies. Campers and outdoor recreation groups also may be vulnerable to infection from *Leptospira* shed by infected eastern grey kangaroos in water and soil. The highest risk of infection is probably through the interaction of eastern grey kangaroos with wild cattle, where they may be exposed to *Leptospira* at watering points, such as the former farm dams discussed earlier. The cattle may then rejoin farms, where they are capable of transmitting the infection to farmers. The lowest risk remains to the potable water supply of Sydney, due to the likely desiccation of *Leptospira* in water treatment processes and the high dilution factor (Lee Smythe, personal communication 2008).

*Leptospira* transmission to domestic livestock is possible throughout the entire distribution of the eastern grey kangaroo, given the high mobility and larger ranging patterns in the more arid regions of its range and its favoured utilisation of farms (Viggers and Hearn 2005). Conversely, where this species interacts heavily with humans in urban areas and tourist visitation sites, the potential for disease transmission is also apparent (Eymann *et al.* 2007, ACT Parks Conservation and Lands 2010). This risk of potential transmission to human populations is heightened in areas where high densities of this species reside in self limiting free ranging populations, as is the case for kangaroos in the current study. However, further research is warranted to determine if *L. weilii* sv. Topaz is endemic to the species across its distribution and not just exclusively to the study population. This will enable a better understanding of the role of eastern grey kangaroos in the transmission of *Leptospira*.

Eastern grey kangaroos may potentially act as a source of *Leptospira* infection, but its role, if any for interspecies transmission remains unclear. It could be proposed from the present study that because *L. weilii* sv. Topaz is present in a more mobile and evenly distributed animal host species, that eastern grey kangaroos are indeed responsible for the transmissions and infections from this strain. Furthermore, species such as long-nosed bandicoots are less likely to be responsible because of their patchy and much more restricted range. However, without culture data, it is difficult to draw conclusions about the role that eastern grey kangaroos play in the transmission of *Leptospira* to other species, including humans, which co-inhabit their range. The present findings remain significant because they have added to the knowledge of the distribution of a relatively newly discovered serovar.

## CONCLUSION

Analysis of each of the diseases surveyed in the studied population of eastern grey kangaroos has revealed that in most cases they pose a low to medium risk to humans through their transmission to the potable water supply of Sydney. However, eastern grey kangaroos may represent a potentially significant source of zoonotic pathogens across its large distribution given that this species co-inhabits many areas with humans, domestic livestock and other wildlife including pest species capable of harbouring many zoonoses. This is particularly apparent because of the marginalisation of this species" habitat, its protection in many nature reserves which allows population numbers to remain unchecked and its higher interactions with humans. The novel findings of this chapter will enable health authorities and water utilities to broaden their understanding of the sources of these diseases, particularly in the case of nationally notifiable diseases - cryptosporidiosis and leptospirosis. Further research is required into the factors governing the prevalence of these pathogens across the distribution of eastern grey kangaroos. This will enable the role, if any, that this species plays in zoonotic transmission of these diseases to be understood. Such studies should focus on areas within the distribution of eastern grey kangaroo that experience higher levels of human activity and in the case of *T. gondii*, its prevalence in commercially harvested areas.

The pathogens in the studied population need to be monitored closely given the proximity and high densities of eastern grey kangaroos near the Wollondilly River. Although there have been no reported outbreaks of these pathogens in Sydney to date, attributed to the drinking water supply, their maintenance in an unchecked and protected population may pose some future risks. The kangaroos essentially may be maintaining these pathogens until such time their transmission to humans occurs. Therefore, it is vital that water treatment processes and stringent water quality monitoring be maintained at a level to prevent contamination. Furthermore, it is essential that the hypothetical transmission routes of these diseases to humans from this population, for example from rogue or feral cattle, be managed immediately.

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# CHAPTER 3:
# POPULATION DENSITIES OF EASTERN GREY KANGAROOS, *MACROPUS GIGANTEUS*, IN WATERSHED AREAS OF SYDNEY'S WATER SUPPLY

# CHAPTER 3: POPULATION DENSITIES OF EASTERN GREY KANGAROOS, *MACROPUS GIGANTEUS*, IN WATERSHED AREAS OF SYDNEY'S WATER SUPPLY

## *Preamble to Chapter 3*

When the presence of an animal species is detrimental to humans and human livelihoods the scale of any impact is likely to be related to population density. In Chapter 2, I established that large numbers of eastern grey kangaroos reside adjacent to the Wollondilly River, a major source of Sydney"s potable water. I demonstrated that eastern grey kangaroos host four zoonotic diseases including *Cryptosporidium* and *Giardia* and that these pathogens were detected in the water supply in 1998. This chapter presents information specifically about the population density of this population of eastern grey kangaroos to help evaluate the disease risks.

In particular, I investigate the implications that variable population densities have on the Pathogen Catchment Budget model developed by Ferguson *et al.* (2007). This model assumes a fixed population size of eastern grey kangaroos when estimating the input of pathogens to watershed areas of Sydney"s drinking water catchment. I also discuss the relevance of population densities in relation to zoonotic disease management along with the implications of this information for biodiversity conservation and animal welfare.

## INTRODUCTION

The overabundance of animal populations has received a substantial amount of attention from wildlife managers and conservation biologists (Lunney *et al.* 2007). This interest has arisen from several studies that have found that the growth and expansion of certain animal populations can potentially have negative effects on endemic biota (Garrott *et al.* 1993) and human enterprise (Kitching 1986). Caughley (1981) defined four contexts in which the term „overabundance‟ can be understood when referring to an animal population: (1) when the animals threaten human life or well-being, (2) when the animals depress the densities of favoured species, (3) when the animals are too numerous for their own good, and (4) when their numbers cause ecosystem dysfunction. In terms of native biota, overabundant species may have negative impacts by reducing natural diversity via resource domination, introducing or spreading infectious diseases and parasites, changing species composition or the relative abundances of sympatric species, and even result in the total loss of species through local extinction.

Eastern grey kangaroos (*Macropus giganteus*) are the most widespread macropod species on the east coast of Australia (Dawson 1995); an area that supports approximately 80% of the country‟s human population. The species is occasionally considered to be overabundant in many parts of its range (Coulson 2001), including woodland and forest habitats adjoining agricultural areas (Hill 1981, Viggers and Hearn 2005), and in some isolated nature reserves (Coulson 2009). High densities of this species have been fuelled by the removal of predators such as the dingo, the absence of commercial harvests and hunting in many areas, and also by the artificial provision of food and water (Moore *et al.* 2002).

The population densities of eastern grey kangaroos in various parts of the species‟ range are presented in Table 3-1. The highest recorded densities occur in the Australian Capital Territory (ACT), which has recently introduced both lethal (e.g. shooting and euthanasia) and non-lethal (e.g. contraceptives) control measures (Fletcher 2007, ACT Parks Conservation and Lands 2010) to reduce population numbers and potential impacts. Densities in the ACT have been estimated to range between 167 and 500 animals per square kilometre (Perry and Braysher 1986, ACT Kangaroo Advisory Committee 1996, Banks *et al.* 2000, Fletcher 2007), with the most recent studies reporting densities at the upper end of this scale (Fletcher 2007). In other areas, particularly west of the Great Divide, eastern grey kangaroos are commercially harvested to reduce their potential impact on agriculture, and this results in lower population estimates in these areas (Cairns *et al.* 2008). In this case, state and federal government conservation agencies require kangaroo populations to be monitored (Pople *et al.* 2006).

**Table 3-1 The density of eastern grey kangaroos, *Macropus giganteus*, taken from various studies across their range.**

| Density (kangaroos km-$^2$) | Study area | Method* | Reference |
|---|---|---|---|
| 0.1 – 21.0 | Pastoral zone, QLD | Aerial surveys | Caughley and Grigg (1982) |
| 245 | Gippsland Lakes, VIC | Hayne (1949) and Morgan II (1979) line transect methods | Coulson and Raines (1985) |
| 167 | Tidbinbilla, ACT | Pellet counts | Perry and Braysher (1986) |
| 36.8 | Wallaby Creek, NSW | Absolute counts | Johnson and Jarman (1987) |
| 44 – 63<br>35 | Wallaby Creek, NSW<br>Coranderrk, NSW | Ground Distance surveys | Southwell (1994) |
| 1.1 – 12.6 | North-eastern NSW | Ground Distance surveys | Southwell *et al.* (1995) |
| 233 | Nature Reserves, ACT | Ground Distance surveys | ACT Kangaroo Advisory Committee (1996) |
| 5.3 | Eastern Highlands, NSW | Ground Distance surveys | Southwell (1997) |
| 36 | Reefs Hills, VIC | Morgan II line transect method | Morgan (1998); Meers and Adams (2003) |
| 0.4 – 11.0 | Western QLD | Aerial Distance surveys | Pople *et al.* (1998) |
| <1.0 – 28.9 | Idalia National Park, QLD | Aerial Distance surveys | McAlpine *et al.* (1999) |
| 52<br>93 | Yan Yean, VIC | Line transect<br>Mark re-sight | Coulson *et al.* (1999) |
| 180 - 480 | Namadgi, ACT | Observational | Banks *et al.* (2000) |
| 1.3 | Yathong, NSW | Ground Distance surveys | McCullough and McCullough (2000) |
| 0.4 – 16.9 | Epping State Forest, QLD | Ground Distance surveys | Woolnough and Johnson (2000) |
| 105 - 178 | Yan Yean, VIC | Line transect | Ramp and Coulson (2002) |
| 21 - 112 | Northern NSW and south-eastern QLD | Line transect | James (2003) |
| 9 – 16 | Cooma, Braidwood and Yass, NSW | Aerial Distance surveys | Pople *et al.* (2006) |

| Density (kangaroos km-$^2$) | Study area | Method* | Reference |
|---|---|---|---|
| 400 – 500 | Tidbinbilla and Googong, ACT | Ground Distance surveys | Fletcher (2007) |
| 5 - 10 | North-eastern NSW | Aerial Distance surveys | Cairns *et al.* (2008) |

*‟Distance‟ sampling refers to the methodology described by Buckland *et al.* (1993); Thomas *et al.* (2010)

High densities of eastern grey kangaroos have led to the instigation of a number of studies to assess the social, environmental and economic impacts of this species (Coulson 2001). These densities can have negative effects on the economic viability of rural properties through the competition with domestic livestock, create animal welfare issues of the kangaroos and other native animals, interfere with the maintenance of ecosystems that support their habitats, as well as other economic and social impacts (ACT Parks Conservation and Lands 2010). Table 3-2 summarises some of the effects and details how of each of Caughley‟s (1981) „overabundance‟ criteria apply to previously studied overabundant eastern grey kangaroo populations. An important economic impact of this species is from kangaroo-vehicle collisions; recent vehicle insurance claim statistics reveal that kangaroos are one of the major causes of animal related accidents in NSW (Table 3-3).

**Table 3-2 Caughley's (1981) overabundance criteria and how they apply to eastern grey kangaroos**

| Caughley (1981) criteria | Example | Reference |
|---|---|---|
| Human livelihood | Overgrazing, kangaroo-vehicle collisions | (ACT Parks Conservation and Lands 2010) |
| Depress densities of threatened species | Eastern barred-bandicoot (*Perameles gunnii*) | (Todd *et al.* 2002) |
| Animal welfare concerns | Exceeding carrying capacity leads to high winter mortality as a result of starvation | (Coulson *et al.* 1999) |
| Ecosystem dysfunction | Overgrazing of regrowth after a forest fire | (Meers and Adams 2003) |

**Table 3-3 NRMA insurance claims for collisions with animals in NSW during 2008**

| Animal | Number of insurance claims |
|---|---|
| **Kangaroos** | **6371** |
| Dogs | 669 |
| Wombats | 264 |
| Cattle | 235 |
| Cats | 169 |
| Sheep | 65 |
| Emu | 64 |
| Foxes | 62 |
| Horses | 60 |
| Deer | 52 |

Source: Illawarra Mercury - August 29, 2009

One further way that eastern grey kangaroos can be considered overabundant in accordance with Caughley‟s (1981) criteria is through the spread of diseases to humans (Thompson *et al.* 2010). Recent studies in a number of species have demonstrated that overabundance, through increased host aggregation, can facilitate disease transmission by reducing individual fitness and by increasing disease susceptibility, thus meeting more of Caughley‟s criteria (Gortázar *et al.* 2006). In addition, there are various examples of diseases where a clear relationship between density and disease prevalence has been demonstrated. Table 3-4 outlines some examples of how overabundance can benefit the spread and transmission of diseases.

**Table 3-4 Examples of infectious and parasitic wildlife diseases that benefit from overabundance of the host**

| Disease | Host species and transmission | Reference |
|---|---|---|
| Tick-borne encephalitis | Roe deer and other ungulates –prevalence increases with increasing hunting harvest | (Zenman and Benes 2004) |
| Classical swine fever | Wild boar – clear relationship between density and disease prevalence | (Rossi *et al.* 2005) |
| Johne‟s Disease | Red deer and other ungulates – abundant domestic livestock (sheep, cattle and goats) | (Deutz *et al.* 2005) |

| Disease | Host species and transmission | Reference |
|---|---|---|
| Gastrointestinal nematodes | Roe, red and fallow deer – prevalence increases with host density | (Santín-Durán *et al.* 2005) |
| Sarcoptic mange | Spanish Ibex – overabundance suspected as a risk factor | (González-Candela *et al.* 2004) |

In Chapter 2, I documented the prevalence of four zoonotic disease organisms in a population of eastern grey kangaroos, namely *Cryptosporidium*, *Giardia*, *Toxoplasma* and *Leptospira*. Of greatest concern to humans is the presence of *Cryptosporidium* and *Giardia*, as the study population resides in close proximity to Sydney‟s drinking water supply. These organisms produce zoonotic diseases that are transmitted by (oo)cyst-laden faecal material that contaminates water supplies. The study population of eastern grey kangaroos is potentially a significant source of these pathogens, due to its high resident numbers in close proximity to Sydney‟s drinking water; Lake Burragorang (Warragamba Dam). Furthermore, eastern grey kangaroos have unrestricted access to surface water of the catchment and have the ability to move to any parts of the watershed. This is in contrast with domestic animals that occur in the wider hydrological catchment and also being capable of transmitting these pathogens, such as domestic pigs, poultry and cattle, as these animal groups achieve high densities locally and thus only represent point sources of pathogens. Furthermore, the movement of domestic animals are more restricted than that of wild animals, which presumably limits the opportunity for disease transmission.

High densities of this species in the study site (Sydney‟s water supply catchment) have developed partly as a result of former farming practices, such as the modification of habitats to improve pasture and the provision of livestock watering points (dams). These activities dominated the Burragorang Valley up until between the 1970s and 1990s, after which farming ceased and these areas were compulsorily acquired by the Sydney Catchment Authority for the protection of the water supply. Currently, eastern grey kangaroos are unmanaged and they experience very minimal human interaction due to public exclusion for the protection of the water supply and the conservation value of this area as a National Park. The interplay of these factors has allowed high densities of this species to be maintained through the absence of hunting, predation by companion dogs and vertebrate pest management, which are factors that typically operate and influence densities of this species in other parts of their range (Pople *et al.* 2006).

### *Pathogen Catchment Budget*

In order to quantify, compare and predict the pathogen loads originating from point and diffuse sources within Sydney‟s drinking water catchment, a Pathogen Catchment Budget (PCB) model was developed (Ferguson *et al.* 2007). This model quantified the key processes affecting the generation and transport of microorganisms from humans and animals, namely *E. coli*, *Cryptosporidium* and *Giardia*, using land use and flow data, and also catchment specific information including the location of sewage treatment plants and density estimates of livestock and free ranging animals. The species considered to have the highest densities in the first application of the PCB model to the Sydney catchment are presented in Table 3-5

**Table 3-5 Animals with the highest densities represented in the Pathogen Catchment Budget**

| Animal species | Density ($km^{-2}$) |
|---|---|
| ***Domestic animals and livestock*** | |
| Cattle, grazing | 500 |
| Cattle, intensive | 2000 |
| Sheep | 500 |
| Pigs, domestic | 5000 |
| Dogs, domestic | 400 |
| Poultry | 5000 |
| Cats, domestic | 400 |
| ***Free-ranging wildlife*** | |
| **Kangaroos** | **200** |
| Pigs (feral) | 1 |
| Rabbits | 50 |
| Dogs (feral) | 0.25 |

Source: (Ferguson *et al.* 2007)

Animal density was used in the PCB to estimate the number of microorganisms entering surface waters of the catchment as follows:

$$I = APDdX$$

where $I$ is the input to surface water of the catchment of microorganism (e.g. *Cryptosporidium* oocysts), $A$ is the number of animals (density in number of animals per square kilometre), $P$ is the concentration (in microorganisms/kg) in the faecal material of an animal species, $D$ is the probability of a species defecating directly into a stream, $d$ is the amount of manure produced by an animal species and $X$ is the probability of a species accessing streams.

As a recognised potential source of pathogens within this model, a fixed population density (*A*) of eastern grey kangaroos was utilised within the PCB (Ferguson *et al.* 2007). However, unlike population numbers of livestock and other domestic animals in the water supply catchment that are typically regulated by stocking rates and through the artificial supply of food and water, densities of eastern grey kangaroos are known to fluctuate seasonally in response to a variety of limiting factors including rainfall (Pople 2003). This may greatly increase density and lead to pulses of microorganisms into the environment. Consequently, utilising fixed population estimates of this species within the PCB may potentially lead to inaccurate calculations of the pathogen loads originating from this potential zoonotic disease source.

Therefore the aims of this chapter were to:

1) Provide accurate estimates of population densities of eastern grey kangaroos at three sites adjacent to the Wollondilly River, a potential point source of *Cryptosporidium* and *Giardia*;
2) Determine the influence of season on population densities and evaluate to what degree these density estimates may affect the PCB calculations made previously for this species; and,
3) Ascertain the potential impacts of eastern grey kangaroo, both in terms of water quality and also conservation, at these sites.

## MATERIALS AND METHODS

Population sizes of eastern grey kangaroo were estimated using two different approaches, distance sampling and mark re-sighting. These two methods were adopted because they have previously proven effective at measuring densities of macropodids and large mammals (Minta and Mangel 1989, Southwell 1994, Thomas *et al.* 2010).

### *Distance sampling*

A total of eighteen permanently marked transect lines were established across the three study sites along the Wollondilly River; Murphy"s Crossing, Jooriland and Douglas Scarp (Chapter 1). Transect lines were placed systematically at regular intervals, with a random starting point, approximately 300 metres apart. The number and length of transect lines varied across the sites, based on the differences in their size and shape. Transects were approximately parallel and were kept as straight as possible by using a hand-held compass (Suunto, Finland) and a Global Positioning System (Garmin eTrex Venture, USA).

Transects were traversed in the period four hours after sunrise, for one week per month, between June 2004 and November 2005. On each day, a two person team consisting of an observer and a scribe was used to survey one transect per study site and the order the transects were traversed was determined systematically. The distance between the observer and scribe was kept to a minimum in order to reduce kangaroo movement before detection. The group size, radial distance and radial angle from the transect line to the location of the first sighting of an individual kangaroo, or the geometric centre for a group of kangaroos, was recorded. If a group was large and evenly spread, several distance and angle measurements were made and an average of these was used to calculate the perpendicular distance. Distances were measured by the observer using a laser range-finder (Bushnell Yardage Pro, USA) and angles were measured using a hand-held compass (Suunto, Finland). The definition of a kangaroo group was made after Southwell (1984) as an assemblage of animals, none being more than 50 metres from another, and all of which are able to maintain visual contact with each other. Single kangaroos, further than 50 metres from, and unable to maintain visual contact with other animals, were scored as groups of one. The scribe took note of the direction of kangaroo movements after they were counted to reduce the potential for double counting.

The perpendicular distances ($x$) of kangaroo groups to the transect lines were calculated using the radial angle (θ) and distance ($r$) and the equation $x = r\sin\theta$. For each month, the raw data, consisting of group sizes and perpendicular distances, were pooled across three months of each season in order to provide seasonal estimates of kangaroo density. Perpendicular distances and group sizes were analysed using DISTANCE 5.0 (Buckland *et al.* 1993, Thomas *et al.* 2010). This program assumes that all kangaroos present on the transect line are detected with certainty and calculates a probability of detection as the distance from the line increases. The detection function is difficult to model with extreme observations (outliers) present, consequently data truncation is necessary (Buckland *et al.* 1993). Perpendicular distances were truncated at the largest 5% to remove any outliers and to improve the accuracy of the fitted detection model. The program considered six models: uniform-cosine, uniform-simple polynomial, half normal hermite, half-normal – cosine, hazard rate-cosine, and hazard rate-simple polynomial. The model was chosen based on the lowest Akaike Information Criterion (AIC) value and chi-square goodness of fit criteria ($\alpha = 0.01$) using a Kolmogorov-Smirnov statistic (Buckland *et al.* 1993).

The effect of pooling monthly data into seasons was examined by assessing differences in the detection function ($f(\theta)$), the value of the probability of the density function at zero distance, between the three months comprising each season. The sum of the AIC values across survey months was larger than that from seasons (data pooled), indicating that the detection function did not differ between months, and that pooling of data into seasons was justified (Buckland *et al.* 2001). This process was also followed for pooling seasons and sites. Comparison of density values between seasons and sites was made by the $z$ – test (Buckland *et al.* 1993, Foccardi *et al.* 2002).

### *Mark re-sighting*

Mark resighting is a form of the Petersen or Lincoln index of mark re-capture, whereby after the initial capture animals are visually “recaptured” to compute a population estimate (Minta and Mangel 1989). This method assumes that 1) a known-sized sample of an animal population is marked and that the population in question is closed, that is there are no net losses (i.e. deaths or immigration) or gains (i.e. births or emigration); 2) sightings are obtained independently of the markings; and 3) the probability of resighting must be the same for all animals (Foccardi *et al.* 2002).

Between May and September 2005, kangaroos were captured and fitted with a unique colour combination of ear tags (Leader, Australia) (see Appendix A – Darting Protocol for capture methodology). From these, a total of 106 individually recognisable kangaroos were available for the mark re-sighting surveys. A summary of the total number of tagged animals in the population was compiled in the days preceding the re-sighting survey, and only these individuals were included in the analysis. In addition, the time duration between marking and resighting was kept to a minimum. Collectively, these precautions permitted the assumption that the kangaroo population was effectively closed between marking and re-sighting.

Two resighting surveys were conducted at Murphy‟s Crossing and Jooriland using the Distance sampling transects and service roads in August and October 2005. A total of 46 and 104 (composite) individually recognisable kangaroos were available for these two mark re-sighting surveys, respectively. The re-sighting occasions were considered independent from the marking process, as individual kangaroos were not captured from these transects or roads. Furthermore, the combination of utilising transects and roads permitted a broader and equal coverage of each study site, which increased the probability of detecting all animals. During each survey, observers recorded the total number of ear tagged kangaroos and the total number of kangaroos without tags.

The mark re-sight abundance of kangaroos at Murphy‟s Crossing and Jooriland was estimated using the Joint Hypergeometric Maximum Likelihood Estimator (JHE) in the program NOREMARK (Bartmann *et al.* 1987, White 1996). The immigration-emigration extension of this model was utilised, which relaxes the assumption that the population is geographically closed (but is still demographically closed), because some animals were noted outside the searchable area. Furthermore, this estimator assumes that on each sighting occasion each animal in the population has the same sighting probability as every other animal, but sighting probabilities can vary between occasions. This estimator also permits additional animals to be marked between sighting occasions, as occurred in this study.

Population estimates for the two surveys were compared using a two sample z-test (described above). NOREMARK provides 95% confidence intervals, but does not provide an estimate of variance. Therefore, after Shaughnessy *et al.* (2000) it was assumed that the 95% confidence interval was approximately four times the standard error of the abundance estimate. Estimates were converted to densities by dividing the abundance by the area of each site (Jooriland - 0.66 $km^{-2}$; Murphy‟s Crossing 0.86 $km^{-2}$).

### *Comparison of the two techniques*

The results originating from the two population size estimates were compared using the calculation of percent relative precision (PRP) (Seddon *et al.* 2003). The PRP is the difference between the estimated population size ($N$) and its 95% confidence intervals, where PRP = $50(CI_1-CI_2)/N$, and $CI_1$ and $CI_2$ are the upper and lower values of the confidence interval, respectively.

### *Rainfall*

The study area was experiencing significant drought conditions at the time of the study (refer Chapter 1). Monthly rainfall between September 2003 and November 2005 is presented in Figure 3-1. The driest conditions within this period were in autumn and winter (mean rainfall = 9.2 and 4.6 mm, respectively), and the wettest conditions were in spring and summer (mean rainfall = 45.6 mm and 40.0 mm, respectively).

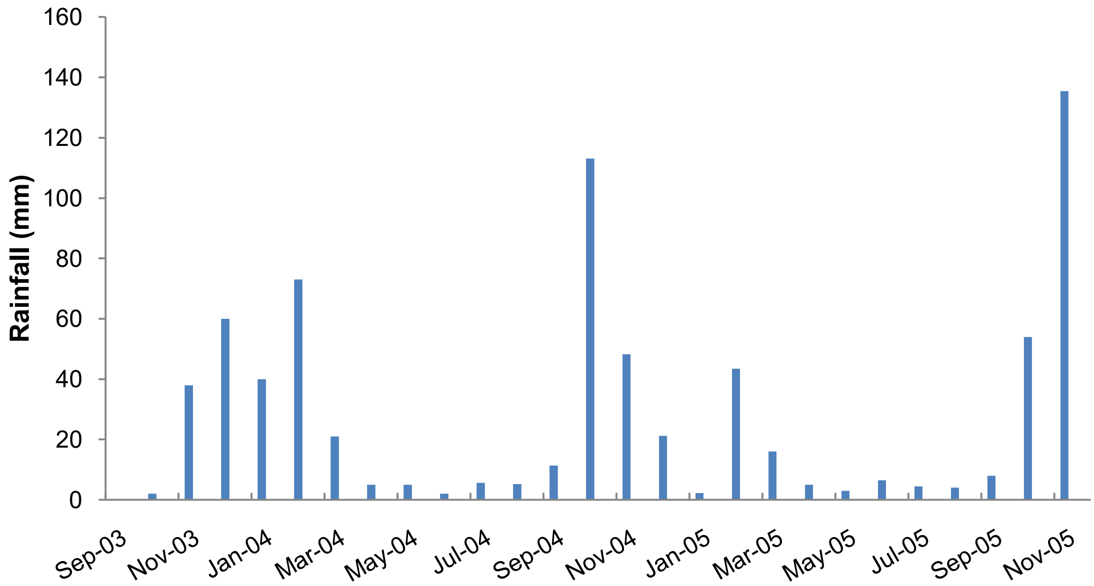


**Figure 3-1 Total monthly rainfall from September 2003 to November 2005 recorded from the Jooriland weather station.**

## RESULTS

### *Distance sampling*

The densities of eastern grey kangaroos between winter 2004 and spring 2005 at Murphy"s Crossing, Jooriland and Douglas Scarp are presented in Table 3-6. Densities (± s.e) at Murphy"s Crossing ranged from 101.6 (± 40.6) kangaroos km$^{-2}$ in autumn 2005 to 465.9 (199.6) kangaroos km$^{-2}$ in winter 2004. Density was lower in autumn 2005 than in winter 2004 ($z$ = 2.5, $P$<0.001), summer 2004/05 ($z$ = 2.3, $P$<0.001), winter 2005 ($z$ = -2.1, $P$<0.001) and spring 2005 ($z$ = -2.8, $P$<0.001). Jooriland densities ranged from 329.5 (± 91.7) kangaroos km$^{-2}$ in spring 2005 to 601.1 (± 88.0) kangaroos km$^{-2}$ in winter 2004. Densities were similar across all seasons ($P$>0.05) at Jooriland. Densities at Douglas Scarp ranged between 330.8 (± 64.6) in winter 2004 and 688.4 (± 179.0) in summer 2004/05. At this site, winter 2004 densities were lower than in summer 2004/05 ($z$ = -1.89, $P$<0.05) and winter 2005 ($z$ = -1.92, $P$<0.05).

The number of eastern grey kangaroos per square kilometre was similar between all sites during winter 2004 and spring 2005 ($P$>0.05). Densities were higher at Jooriland and Douglas Scarp than Murphy"s Crossing during spring 2004 ($z$ = -2.3, $z$ = -2.4, $P$<0.001, respectively) and autumn 2005 ($z$ = -3.3, $z$ = -3.2, $P$<0.001, respectively). Densities at Douglas Scarp were higher than at Jooriland in summer 2004/05 ($z$ = -1.7, $P$<0.05), and at Murphy"s Crossing in winter 2005 ($z$ = -1.9, $P$<0.05). Pooling estimates across all seasons revealed that densities at Jooriland and Douglas Scarp were significantly higher than at Murphy"s Crossing ($z$ = -2.8, $z$ = -2.3, $P$<0.05, respectively).

**Table 3-6 Seasonal population densities of eastern grey kangaroos by study site using Distance sampling**

| Season | Selected model | Animal density (heads km$^{-2}$) | %CV* | $n^{\#}$ | 95% Confidence interval | |
|---|---|---|---|---|---|---|
| ***Murphy's Crossing*** | | | | | | |
| Winter 2004 | Hazard-rate cosine | 465.9 | 42.9 | 50 | 202.5 | 1071.9 |
| Spring 2004 | Hazard-rate cosine | 236.3 | 40.3 | 37 | 108.4 | 514.8 |
| Summer 2004/05 | Uniform cosine | 356.6 | 28.8 | 51 | 202.1 | 629.2 |
| Autumn 2005 | Half-normal cosine | 101.6 | 40.0 | 40 | 47.1 | 219.0 |
| Winter 2005 | Half-normal cosine | 313.7 | 32.2 | 70 | 167.5 | 587.7 |
| Spring 2005 | Uniform cosine | 438.4 | 31.9 | 56 | 231.3 | 831.0 |
| Pooled | Half-normal cosine | 304.1 | 15.2 | 304 | 225.6 | 409.8 |
| ***Jooriland*** | | | | | | |
| Winter 2004 | Half-normal cosine | 397.6 | 27.3 | 56 | 232.3 | 680.4 |
| Spring 2004 | Half-normal cosine | 590.1 | 23.5 | 84 | 372.1 | 935.8 |
| Summer 2004/05 | Uniform cosine | 352.9 | 25.6 | 94 | 209.4 | 595.0 |
| Autumn 2005 | Half-normal cosine | 553.7 | 24.0 | 80 | 346.8 | 884.2 |
| Winter 2005 | Uniform cosine | 588.4 | 27.0 | 103 | 342.3 | 1011.5 |
| Spring 2005 | Uniform cosine | 329.5 | 27.8 | 65 | 190.2 | 570.6 |
| Pooled | Hazard-rate cosine | 531.9 | 12.3 | 481 | 417.8 | 677.1 |
| ***Douglas Scarp*** | | | | | | |
| Winter 2004 | Uniform cosine | 330.8 | 19.5 | 89 | 224.9 | 486.4 |
| Spring 2004 | Uniform polynomial | 508.7 | 18.2 | 111 | 354.7 | 729.5 |
| Summer 2004/05 | Uniform cosine | 688.4 | 26.0 | 90 | 413.3 | 1146.4 |
| Autumn 2005 | Uniform cosine | 439.4 | 22.7 | 79 | 280.7 | 689.2 |
| Winter 2005 | Uniform cosine | 685.6 | 25.2 | 74 | 416.1 | 1129.5 |
| Spring 2005 | Uniform cosine | 450.7 | 27.3 | 57 | 258.6 | 785.4 |
| Pooled | Half-normal hermit | 445.5 | 8.9 | 501 | 374.1 | 530.6 |

Notes: *%CV = percentage Coefficient of Variation, $^{\#}n$ = number of observations.

The pooled seasonal population densities of eastern grey kangaroos across the three study sites are presented in Figure 3-2. Overall, the spring 2005 population density was the highest at 547.2 kangaroos $km^{-2}$ and winter 2004 was lowest at 335 kangaroos $km^{-2}$. Population densities were greater in spring 2005 when compared with winter 2004 ($z$ = -2.2, $P$<0.05), autumn 2005 ($z$ = -1.7, $P$<0.05) and winter 2005 ($z$ = -1.7, $P$<0.05).

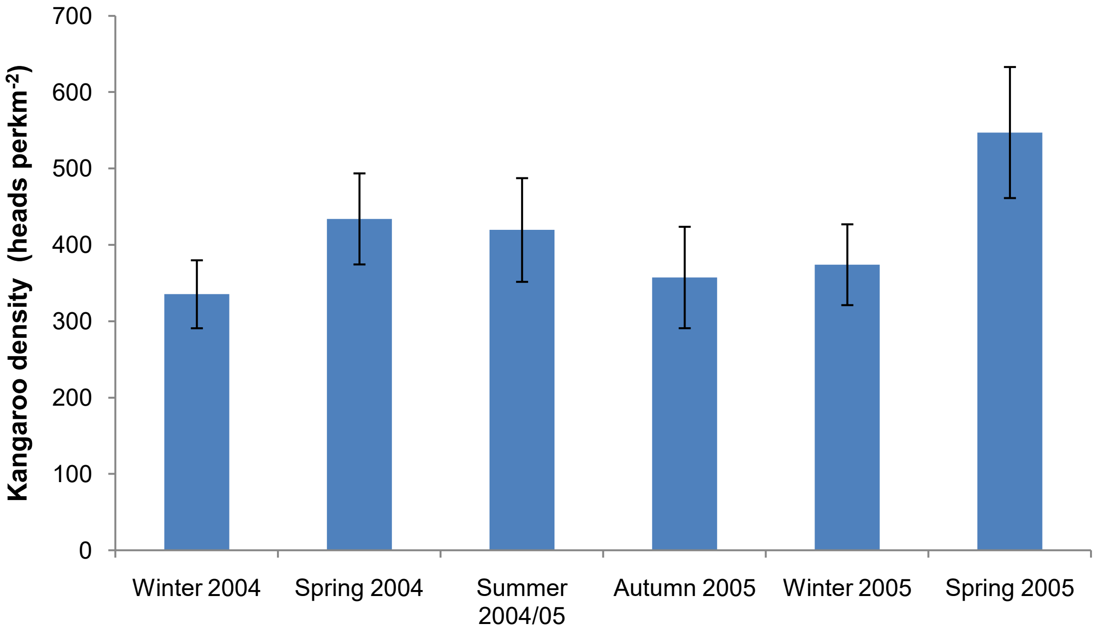


**Figure 3-2 Seasonal population densities of eastern grey kangaroos pooled across study sites using Distance sampling**

### *Mark re-sighting*

Population densities of eastern grey kangaroos from the two re-sighting surveys are presented in Table 3-7. In both surveys, densities were higher at Jooriland than at Murphy‟s Crossing (z = -4.7, z = -3.1, P<0.001, respectively). At Murphy‟s Crossing, densities were higher in October than in the August survey (z = -2.01, P<0.05), but were similar between the two surveys at Jooriland (z = 1.69, P>0.05).

**Table 3-7 Results from the mark-resight surveys**

| Site | Animal Density (heads km-2) | 95% Confidence interval | | Total marked animals | Marked animals sighted | Unmarked animals sighted |
|---|---|---|---|---|---|---|
| ***August*** | | | | | | |
| Murphy"s Crossing | 342.4 | 252.1 | 432.8 | 23 | 15 | 181 |
| Jooriland | 590.8 | 403.3 | 778.2 | 23 | 13 | 214 |
| ***October*** | | | | | | |
| Murphy"s Crossing | 410.2 | 310.7 | 509.9 | 49 | 25 | 158 |
| Jooriland | 505.8 | 433.3 | 578.2 | 53 | 39 | 208 |

### *Comparison of the two techniques*

Table 3-8 shows the *PRP* for the derived population densities for distance sampling and mark re-sighting. Overall, mark re-sighting provided more precise results than distance sampling at both sites and for the two surveys, with the most precise estimate obtained in spring 2005 (*PRP* = 14.3).

**Table 3-8 Percent relative precision (PRP) comparing the two population estimation techniques – Distance sampling and Mark-resight**

| **Murphy's Crossing** | | |
|---|---|---|
| **Survey** | **PRP_Distance sampling** | **PRP_Mark re-sight** |
| Winter 2005 | 67.0 | 26.4 |
| Spring 2005 | 68.4 | 24.3 |
| **Jooriland** | | |
| Winter 2005 | 56.9 | 31.7 |
| Spring 2005 | 57.7 | 14.3 |

## DISCUSSION

The population densities of eastern grey kangaroos in this study are one of the largest reported (refer to Table 3-1). In similar habitats, Fletcher (2007) found that the density of this species in the ACT (400 – 500 kangaroos per square kilometre) was within the lower range of that found in the current study. The major difference between the study conducted by Fletcher (2007) and this study was the level of human interaction, with the ACT site being close to human occupation, which may be a plausible explanation for the higher densities (up to 600 kangaroos per square kilometre) found in the current study. High densities have also been found in favourable environments such as defence sites in Canberra (up 560 per square kilometre at Belconnen Navel Base and 530 per square kilometre at Majura Training Area). Therefore, it is apparent that the study population has benefited from the interplay of the former farming practices that have artificially increased food and water availability and from lack of current human intervention, including hunting, commercial management and other human-animal conflicts common to semi-rural areas. This is supported by observations made by Water Board Officers, spanning across both farmed and non-farmed periods in the Burragorang Valley that suggests that the densities of this species are currently much higher than those previously observed (Dennis Ashton, personal communication 2009).

The highest densities of eastern grey kangaroos were recorded at Jooriland. This finding provides further support for the interpretation of the importance of former farming practices on population numbers of this species, as Jooriland was the last farming lease within the study area and thus contains a larger proportion of cleared areas and remnant farmland. Consequently, Jooriland is capable of supporting higher densities of resident kangaroos through its evidently increased carrying capacity. In addition, this site is also in closer proximity to water sources including the Wollondilly River and two farm dams, located to the north and south of where population estimates were carried out

Population densities of the larger macropodids are known to fluctuate in what has been described as „boom" and „bust" cycles (Letnic and Dickman 2006). The „boom" periods allow residents to achieve high densities in response to higher rainfall that supports better quality forage. As the study area was experiencing significant drought conditions at the time of the study (see Chapter 1), with the average rainfall 100-200 mm per year less than the longer term average, it would be reasonable to suggest that the estimated densities of eastern grey kangaroos are lower than what would be expected compared to those during more favourable conditions. The high levels of mortality that were observed during the study period provide support for this hypothesis (refer to Chapter 6). Thus, it is recommended that population densities of this species are calculated after more favourable conditions, to allow for a comparison with the „bust" cycle estimates provided here and to ascertain the maximum density that this population may reach.

The seasonal changes in the population densities of eastern grey kangaroo in the study area also reinforce the influence of the prevalent environmental conditions. Generally, the highest densities were found in spring and summer, when the highest average rainfall occurred. Conversely, when rainfall was least in autumn and winter, population densities were also lowest. During low rainfall periods, food resources were notably limited, which potentially caused the observed lower densities of this species and the higher mortality patterns at this time. However, although the population densities may vary slightly with season, they are by and large reasonably stable. This relative stability may reflect the localised nature of the study population, through the lack of any emigration or immigration between seasons, with any observed changes being attributable to mortality or fecundity. This hypothesis requires the support of home range analyses and an assessment of site fidelity, which is a focus of a subsequent chapter in this thesis (Chapter 5).

The high densities of eastern grey kangaroos recorded in this chapter may result in some previously unperceived biodiversity conservation issues in the study area. At other locations within the range of the eastern grey kangaroo it has been reported that high density populations have the potential to impact on other fauna species, including the eastern barred bandicoot (Todd *et al.* 2002) and the northern-hairy nosed wombat (Woolnough and Johnson 2000), as well as reduce the success of revegetation programs (Meers and Adams 2003). Although no data were collected formally on overgrazing by this kangaroos in my study, I did observe very limited regeneration of *Eucalyptus tereticornis* forest red gum, *Eucalyptus albens* white box, and within the understorey of woodland habitats. Overgrazing by eastern grey kangaroos in the Burragorang Valley may be affecting the understorey and ground layer of the grassy box woodlands that occupy these areas, including the numerous threatened species that rely on them (refer Chapter 1). This vegetation community is part of Box Gum Woodland (*White Box Yellow Box Blakely's Red Gum Woodland*), which has a critically endangered listing under the Commonwealth *Environment Protection and Biodiversity Conservation Act* 1999 and is endangered under the NSW *Threatened Species Conservation Act* 1995. In consequence, overgrazing by high density eastern grey kangaroo populations may continue to threaten the longevity of this community at a local scale.

High densities of eastern grey kangaroos play a potentially significant role in continuing the spread of weed species, which in part are relics of the former farming practices in the study area. These weed species include serrated tussock *Nassella trichotoma* and cobbler‟s pegs *Bidens pilosa*. Serrated tussock is typically known to be an unpalatable foraging species for herbivores, and its presence results in other grasses including the native kangaroo grass *Themeda australis*, weeping grass *Microlaena stipoides* and *Austrodanthonia* spp. being selectively grazed. Thus, the high grazing pressure on these preferred species may lead to the observed high prevalence of serrated tussock in some areas (Duncan Scott-Lawson, DECCW, personal communication 2006). The seed of cobbler‟s pegs typically attaches to the fur or skin of animals, and was commonly observed on captured eastern grey kangaroos during winter and early spring when this weed is known to seed. The attachment of the seed of cobbler‟s pegs onto kangaroos has potentially permitted high dispersal rates of this species throughout the study area. These changes in species distributions, reflected in continual shifts to introduced species and a lack of regeneration of the former farmland areas, may further exacerbate the impacts on threatened species that occupy these areas (NSW Department of Environment and Climate Change 2007). They apply also to several of Caughley‟s (1981) „overabundance‟ criteria.

### *Population densities and zoonotic disease management*

Population densities of a species are fundamental in gauging the risk of disease transmission and environmental contamination. Host density essentially provides the magnitude or scale at which the disease causing agent can be assessed, which ultimately informs management options designed to mitigate the risk. Table 3-9 summarises some of the key findings of this chapter together with the relevance of these to zoonotic disease management within Sydney‟s water supply catchment.

Radu and Slade (2006) conducted an epidemiological study of eastern grey kangaroos at Murphy‟s Crossing concurrently with the collection of data for this chapter and failed to identify the presence of *Cryptosporidium*. Radu and Slade (2006) related this negative finding to the prevailing drought conditions and the apparently low numbers of pouch young that they casually observed to be in the population. Given that many diseases are density dependent (Gortázar *et al.* 2006) including cryptosporidiosis (Wilson and Childs 1997), this finding may be further explained by the relatively low number of eastern grey kangaroos in the autumn period; the time that was previously identified by Power *et al.*

(2005) to be when this species expressed repeated epidemics of this disease. Although no population estimates were made during detection events in 2002 (Power *et al.* 2004) and again in 2006 (Chapter 2), higher rainfall preceding these periods (refer Chapter 1) would have supported higher population densities that facilitated the carriage of *Cryptosporidium* in the study population. The validity of this hypothesis remains untested and requires analysis of *Cryptosporidium* epidemiology in eastern grey kangaroos across both drought and higher rainfall periods to ascertain the relationship between host density and disease prevalence.

The high densities of eastern grey kangaroos within the study area may also increase their susceptibility to disease. Several studies have demonstrated the positive relationship between density and disease prevalence, such that with an increase in density, disease prevalence also increases (Gortázar *et al.* 2006). Overabundant wild boar (*Sus scrofa*), for example, have decreased health and fitness and are more susceptible to disease (Rossi *et al.* 2005). Eastern grey kangaroos within the study area experienced high mortality in severe drought conditions, which was exacerbated by high densities and reduced pasture biomass. It could be hypothesised that disease prevalence was highest during high mortality periods for two reasons; firstly, as a result of the increased uptake of parasites and pathogens as animals were grazing close to the ground (Altizer *et al.* 2006) and secondly the transmission of several diseases was high as animals were more susceptible to disease as a consequence of malnutrition and decreased immunity.

### *Population estimates of eastern grey kangaroos and the PCB*

The Pathogen Catchment Budget (PCB) developed by Ferguson *et al.* (2007) utilised a fixed population estimate for eastern grey kangaroos to estimate the output of *Cryptosporidium* oocysts from this species. The estimate used in the PCB model was 200 kangaroos per square kilometre, which was essentially the lowest population estimate found across my entire survey period. Hence, a more accurate reflection of the population density would be double this amount, which would have paralleled the densities of this species with other known sources of pathogens represented in the PCB including cattle grazing and sheep (500 animals per $km^{-2}$) (Table 3-5). However, as already discussed, the population estimates in this chapter are hypothesised to be lower than what would be expected under more ideal conditions; higher primary productivity following rainfall may in fact support much higher densities of this species. Consequently, the current position of eastern grey kangaroo in the PCB is an under-representation as it models only the minimum density of this species in drought conditions, rather than average density spanning across a range of climatic conditions. Based on these results, revision of the kangaroo densities used in the PCB model would be recommended for future analysis of pathogen loads in the Sydney drinking water catchment (Christobel Ferguson, personal communication 2010).

**Table 3-9 Relevance of the findings of this chapter to zoonotic disease management**

| Result | Implications for zoonotic disease management |
|---|---|
| **Seasonally stable high densities** | • Stable levels of potentially high pathogen loads present in the watershed<br>• Indicative of minimal net immigration/emigration of eastern grey kangaroos from study area (i.e. away from potable water supply)<br>• Lack of *Cryptosporidium* detected in periods concurrent to population estimates are indicative that a certain density threshold may have to be attained before this and other diseases are detected<br>• Repeated autumn epidemics – lack of *Cryptosporidium* detection during autumn may be linked to low population numbers of eastern grey kangaroos at Murphy"s Crossing at this time<br>• Host crowding has been linked to high prevalence of a number of diseases, including those with bacterial origins (e.g. *Leptospira*)<br>• High population densities were found in watershed areas (high risk areas for disease transmission from animal host to surface water), where favourable conditions also exist<br>• High densities may reduce fitness and make animals within the population more susceptible to disease, thus increasing the potential maintenance and spread of zoonoses |
| **Low densities during drought conditions and potential pathogen prevalence links to rainfall** | • Higher densities are probable after high rainfall (spring and summer) – this may lead to higher pathogen loads in comparison to those that were previously calculated from this species<br>• Lower densities used in the initial application of the PCB model – may be an under-representation for this species and hence pathogen loads may need to be recalculated<br>• *Cryptosporidium* may be density dependent – hence the absence of the disease in autumn when kangaroo density was low (Radu and Slade 2006)<br>• *Cryptosporidium* detections (Power *et al.* 2005 and Chapter 2) relate to higher rainfall periods when kangaroo density would be presumably higher.<br>• Drought may be a regulating factor for both eastern grey kangaroo density and hence *Cryptosporidium* dissemination from this species. |

## CONCLUSIONS

The results of this chapter help to explain the relationship between eastern grey kangaroo density and disease prevalence. Furthermore, this chapter indicates that there are a number of factors (e.g. rainfall) that influence the density of the free-ranging population studied, and that it cannot be considered fixed. Such complexity can be expected in natural systems. This is in contrast to domestic animals that are capable of transmitting *Cryptosporidium*, whereby densities are controlled artificially (e.g. stocking rates of cattle). Consequently, as density is an important factor in pathogen load calculations, the pathogen contribution of eastern grey kangaroos is likely to more variable than that from other potential sources.

This chapter provides evidence for the overabundance status of the study population of eastern grey kangaroos in accordance with Caughley"s criteria (Caughley 1981). However, in this study only the negative effects on human livelihood through the potential spread of disease was empirically tested. Therefore, further experimental studies are required to examine the suitability of other criteria listed in Caughley (1981) including ecological impacts (e.g. damage to box gum woodland and it"s fauna).

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# CHAPTER 4:

# FAECAL DEPOSITION BY EASTERN GREY KANGAROOS, *MACROPUS GIGANTEUS*, IN WATERSHED HABITATS ADJACENT TO SYDNEY'S WATER SUPPLY

# CHAPTER 4: FAECAL DEPOSITION BY EASTERN GREY KANGAROOS, *MACROPUS GIGANTEUS*, IN WATERSHED HABITATS ADJACENT TO SYDNEY'S WATER SUPPLY

## *Preamble to Chapter 4*

Knowledge of the quantity and distribution of faecal material originating from potential animal sources of faecal-oral route diseases is critically important for the management of water quality in drinking water catchments. In Chapter 2, I established that eastern grey kangaroos residing adjacent to the Wollondilly River, a major source of Sydney's potable water, are host to four zoonotic disease organisms including *Cryptosporidium*, *Giardia*, *Toxoplasma* and *Leptospira*. In Chapter 3, densities of this kangaroo population were shown to be very high and seasonally stable.

This chapter describes the quantity and distribution of faeces deposited by this species living in close proximity to the Wollondilly River. The role that several temporal and spatial factors may play in influencing faecal deposition, including faecal decay, pasture biomass and quality and animal density will also be discussed. The information collected in this chapter will provide the basis for accurate predictions of the pathogen loads originating from this species.

## INTRODUCTION

A recent worldwide increase in the number of zoonotic diseases has prioritised research into the transport pathways of these diseases to aid surveillance, prevention and cure (Chomel *et al.* 2007, Greger 2007, Field 2009). Faecal-oral route zoonotic diseases, such as those caused by *Cryptosporidium* and *Giardia*, are commonly transmitted to humans through the faecal contamination of water ways, as the critical life stages of these diseases (oocyst and cyst, respectively) are protected in the faeces of the host for many months before being dispersed by a combination of mechanical, biological and hydrological means (Davies *et al.* 2004, Davies *et al.* 2005). Direct animal faecal deposition in waterways and indirect deposition overland via surface runoff from land grazed by animals both contribute to event-related increases in the concentrations of in-stream waterborne pathogens in numerous watersheds (Ferguson *et al.* 2003, Davies *et al.* 2004, Castro-Hermida *et al.* 2009). Consequently, information regarding the faecal loads contributed by potential animal sources of these diseases, as well as the proximity of their faecal deposition to potable water, is critical to assess the zoonotic disease risk.

Eastern grey kangaroos (*Macropus giganteus*) are one of the most abundant macro fauna species occupying floodplain areas throughout their distribution (Coulson *et al.* 1999, Viggers and Hearn 2005, Fletcher 2007, ACT Parks Conservation and Lands 2010). Power *et al.* (2004) found that this species was a potential source of *Cryptosporidium* and Cox *et al.* (2005) determined that this species was a potential source of *Giardia* in Sydney‘s water supply catchment. This was corroborated by the findings presented in Chapters 2 and 3 of this thesis, which showed that as a potential host of these diseases, eastern grey kangaroos occupied flood plain habitats in Sydney‘s water supply catchment at very high densities.

The faecal loads deposited by eastern grey kangaroos in a floodplain environment can vary both temporally and spatially and cannot be considered fixed. The factors that potentially influence this variability include population density (Ramp and Coulson 2002), diet through resource supply and quality as this species preferentially selects forage with the highest nitrogen content (Taylor 1984), microbial activity, faecal decay rates (Johnson and Jarman 1987), climate including the history of floodplain inundation (Wilson *et al.* 2010) and the costs of foraging or dispersal such as competition and predation (Ramp and Coulson 2002). The importance of each factor may differ in accordance with the proximity of certain habitats from surface water, which can alter the faecal loads contributed to the watershed. Similarly, the potential impacts of disease micro-organisms on water quality may also vary, as inactivation may occur after faecal material is exposed to high levels of solar radiation, and under the attack of soil microbes (Robertson *et al.* 1992, Li *et al.* 2010).

There is currently very little known about the amount or rate of faecal deposition of eastern grey kangaroos in Sydney‘s water supply catchment. The calculation of faecal deposition from high density populations of this species is a fundamental component for determining pathogen loads (Ferguson *et al.* 2004) through Pathogen Catchment Budgets (PCB) (Ferguson *et al.* 2007a) and other mathematical models (refer Chapter 3). Therefore, the aims of this chapter were to:

1) Calculate the faecal loads deposited by eastern grey kangaroos in three broadly defined watershed habitats including; riparian, woodland and grassland;
2) Determine the influence of several environmental factors on the faecal loads deposited by eastern grey kangaroos in each of these habitats including decay rates, pasture quantity and quality;
3) Assess the use of alternative water sources as a management tool to reduce eastern grey kangaroo faecal loads near potable water; and,
4) Examine the habitat use of eastern grey kangaroos at the foraging scale using the distribution of their faecal material.

In this chapter, eastern grey kangaroo faecal loads are defined as the dry mass of excreta deposited per unit area and scaled by time interval.

## MATERIALS AND METHODS

### *Faecal pellet loads*

Eastern grey kangaroo faecal loads were calculated at Murphy's Crossing and Jooriland between November 2004 and March 2006. These sites were chosen as they comprised representative sections of the three major habitats in the area; riparian, woodland and grassland in close proximity to the Wollondilly River and thus areas that represented places in the watershed where the transport of disease was highly likely.

A pilot study in October 2004 was used to determine the number of plots and their size in each habitat by ―over-sampling" the amount of survey effort. This comprised selecting 20 plots in riparian and 14 plots in woodland and grassland using three different radius circles (1, 2, and 3 metres). Plots were spaced systematically along transects every 20 metres in the grassland and riparian habitats and a random number generator was used to determine the placement of plots by using a grid system in the woodland habitat.

A technique developed by Bros and Cowell (1987) using the standard error of the mean to resolve the statistical power was used to determine the number of plots required in each habitat. This method uses a Monte-Carlo simulation to generate a number of samples versus their coefficient of variation (or power). The sample size at which the coefficient of variation does not decrease substantially (e.g. <5%) with an increase in sample size is taken as the minimum sample size required. This analysis was conducted in SAS (SAS Institute Inc. USA).

On the basis of the lowest achievable coefficient of variation, six plots were chosen for the grassland habitat, 14 plots were chosen for riparian habitat and 10 plots were chosen for the woodland habitat. All plots were 2 metres in radius (Figure 4-1, Figure 4-2 and Figure 4-3).

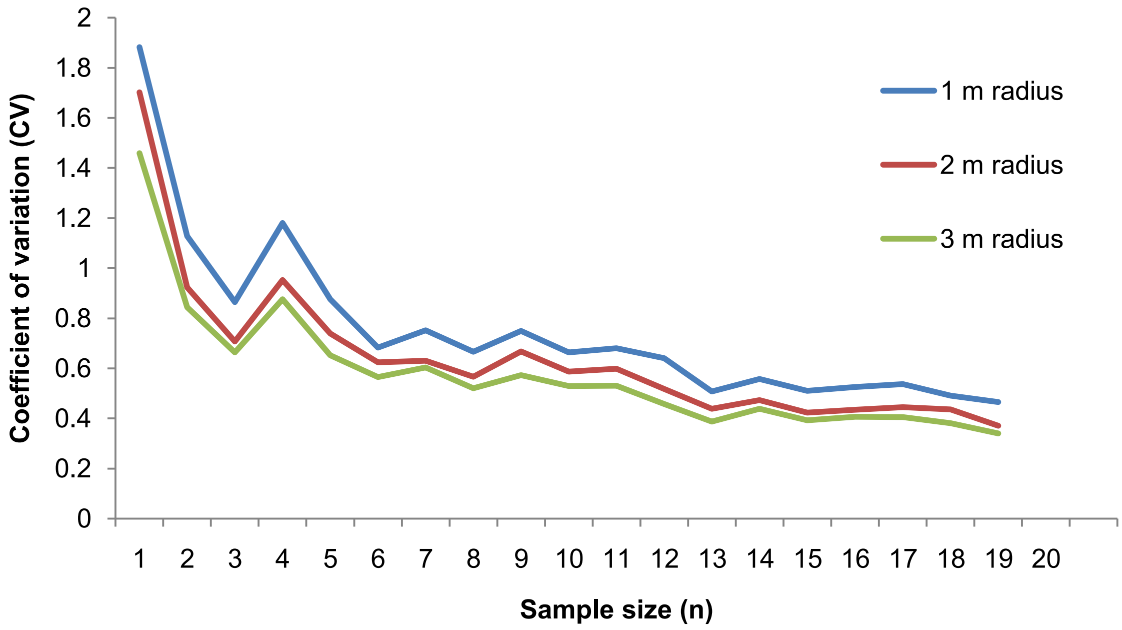



**Figure 4-1** **Relationship between Coefficient of Variation and sample size for the faecal accumulation plots in the riparian habitat.**

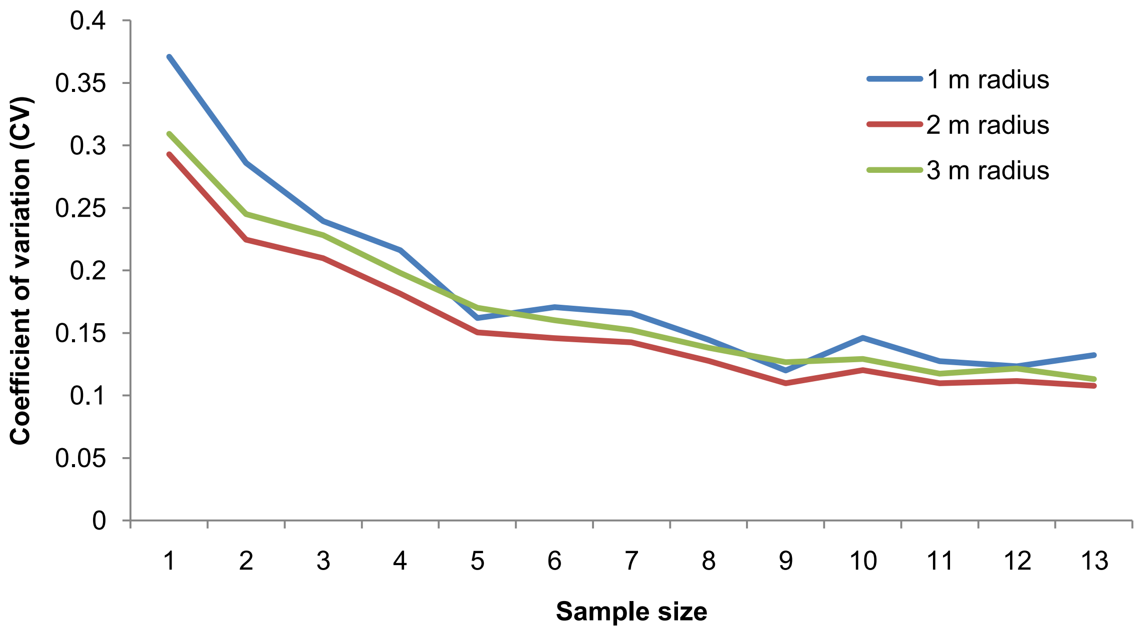



**Figure 4-2** **Relationship between Coefficient of Variation and sample size for faecal accumulation plots in the grassland habitat.**

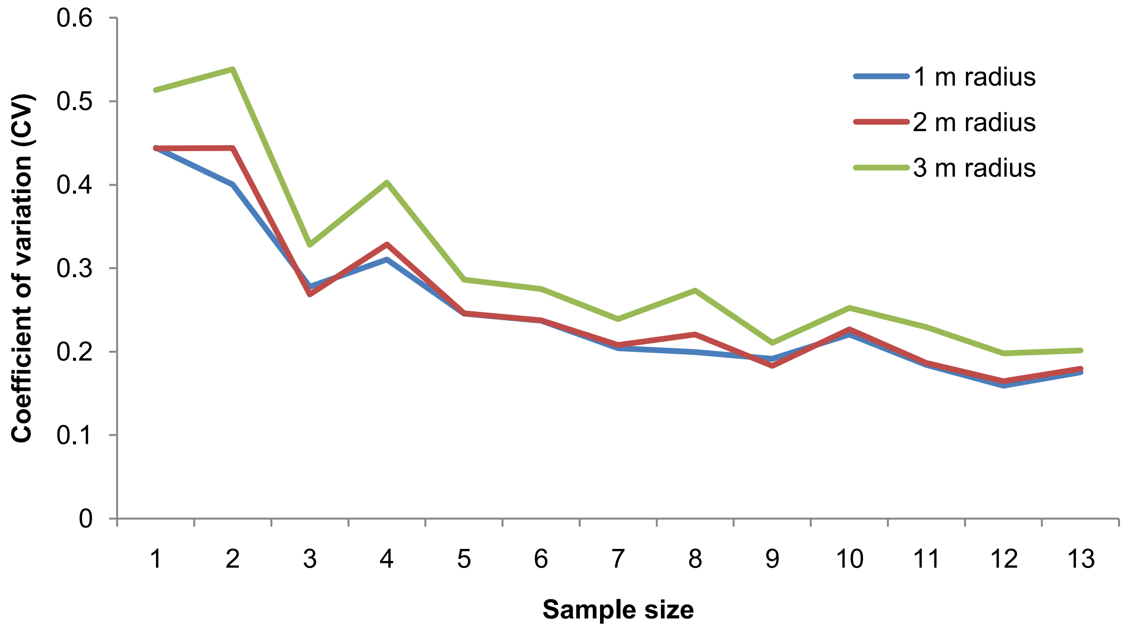



**Figure 4-3** **Relationship between Coefficient of Variation and sample size for faecal accumulation plots in the woodland habitat.**

To determine the relative usage of alternative water sources present in the study area by eastern grey kangaroos, six faecal accumulation plots were also established around Jooriland dam. This number of plots was chosen as the area surrounding the dam predominantly consisted of grassland habitat. The placement of plots surrounding the dam was determined by a random number generator, and the proportion of a set distance of 200 metres from the centre of the dam along directional bearings.

Faecal loads were estimated in all habitats and surrounding Jooriland dam by counting individual intact pellets and by dry weight. Monthly faecal loads was assessed by dividing the dry weights by the total number of days between sampling (gramsm$^{-2}$day$^{-1}$) after they were initially cleared in September 2004. Intact pellets were only considered as they are more likely to accommodate pathogens having not dried or been exposed to ultraviolet radiation. The dry weight of pellets was determined using an analytical balance (0.0001g accuracy) after they were dried at 80ºC for eight hours. To assess the relative utilisation of these habitats by other fauna, these measurements were also made for the common wombat (*Vombatus ursinus*), red-necked wallaby (*Macropus rufogriseus*); swamp wallaby (*Wallabia bicolor*), common wallaroo (*Macropus robustus*) and the feral pig (*Sus scrofa*). Reliable identification of pellets and scats was aided by using keys provided in Triggs (2004).

Differences in faecal accumulation between the surveyed months were assessed using repeated measures MANOVA. Differences between the surveyed months in the faecal loads deposited in the individual habitats were assessed using ANOVA of square root transformed data. Post-hoc Tukey HSD tests were used to examine which of the surveyed months was different from each other.

### *Faecal decay*

Decay rates of eastern grey kangaroo faecal material was assumed to equate to the breakdown of micro-organisms (oocysts and cysts). This assumption was made due to the oocysts and cysts being protected and encased within intact faecal pellets and when these pellets decay their desiccation would presumably lead to a loss in the viability of these micro-organisms (i.e. they are exposed to the elements such as ultraviolet radiation and drying). Secondly, the decay rate of faeces was used as a surrogate for the breakdown of oocysts and cysts due to the apparent difficulties associated with measuring the persistence of these micro-organisms in remote field situations. To measure decay, freshly deposited pellets were collected each month for 12 consecutive months and placed in known locations within each habitat. Approximately 150 pellets comprising three lots of 50 were established in each habitat. Separating pellets into three groups was done to ensure that decay measurements would resemble near natural conditions. Pellets were colour coded for each month using a finite amount of nail varnish to distinguish the pellets from those freshly deposited within the plots and from those established in previous months. The number of intact pellets (i.e. showing no signs of breakage) remaining at the end of each month was recorded (more frequent trips were made if decay was fast). The study was carried out from November 2004 to October 2005 inclusive and counts of remaining pellets continued each month until October 2006.

Survivorship analysis was used to estimate the persistence rate of the pellets in each habitat following the methods described by Caughley (1980) and Hone and Martin (1998). Fresh pellets recorded each month were considered a cohort and estimates of survival ($lx$) and mortality rate ($qx$) were calculated. Survival (persistence rate) was estimated, as $lx+1/lx$, for the following periods: less than five days, 30 days, 60 days, 90 days and 120 days. Estimates of persistence rate could not always be made for the latter two time periods due to fast decay. These standard times were chosen as they represent critical time periods for the assessment of zoonotic disease persistence, and thus were able to provide a baseline for risk analysis of zoonotic disease entry into surface water from the three habitats. Rates of decay were compared between habitats and months using contingency tables of raw frequencies.

The influence of environmental variables, such as rainfall, temperature and humidity (collected from a weather station at Jooriland) on faecal decay in each of the three habitats was assessed by using partial correlation analysis. The partial correlation is a measure of linear dependency between two variables, where the influence of a third variable is ‗partialled out”. In this way, each of the environmental variables was ‗partialled out” to determine their full influence on faecal decay (Hone and Martin 1998).

***Pasture quality and quantity***

Pasture quality and quantity was measured in each of the three habitats seasonally through the collection and analysis of digital photographs. Following a similar methodology to that described by Ramp and Coulson (2002), a five point graduated ‗greenness‘ scale and the percentage cover of twenty, haphazardly placed, square metre quadrats was used to visually assess forage quality between habitats and over time. Plant quality (protein content) has been previously linked to moisture content and plant greenness (Ramp and Coulson 2002). Pasture quality was inferred using a scale from 0 ‗poor quality‘ through to 1 ‗good quality‘ and four categories were used: 0, 0.25, 0.50, 0.75 and 1 (Table 4-1). Pasture quantity was measured using the percentage cover of grasses (to the nearest 10%) from the same one square metre quadrats. One way analysis of variance of arcsine transformed data was used to differentiate pasture quality and quantity between habitats. Post-hoc Tukey HSD tests were used to explore any differences found.

**Table 4-1 Pasture quality ‘five point’ greenness scale reference photos (a) 0%, (b) 25%, (c) 50%, (d) 75% and (e) 100%**

(a)

(b)

(c)

(d)

(e)

## Results

### *Faecal loads*

Faecal loads deposited by eastern grey kangaroos varied between the time of the year and habitat at Jooriland and Murphy's Crossing, indicating that the kangaroos preferentially shift habitats at the microhabitat scale in time and space. Across the two sites average faecal deposition was highest in the winter months within the grassland habitat and highest in the summer months in the woodland habitat, with faecal deposition consistently lowest in the riparian habitat (Figure 4-4 and Figure 4-5). Faecal deposition surrounding the Jooriland dam remained high throughout the course of the study (Figure 4-6).

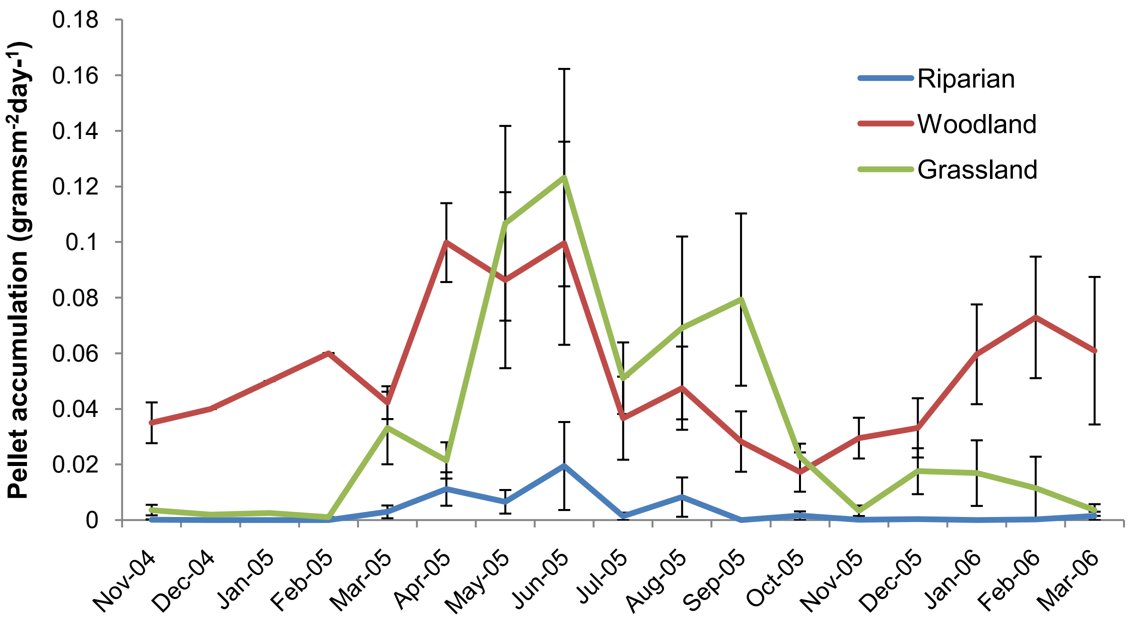



**Figure 4-4** **Mean faecal deposition rate and loads (gramsm$^{-2}$day$^{-1}$) of eastern grey kangaroos at Jooriland in each habitat over time. Error bars represent 95% confidence intervals**

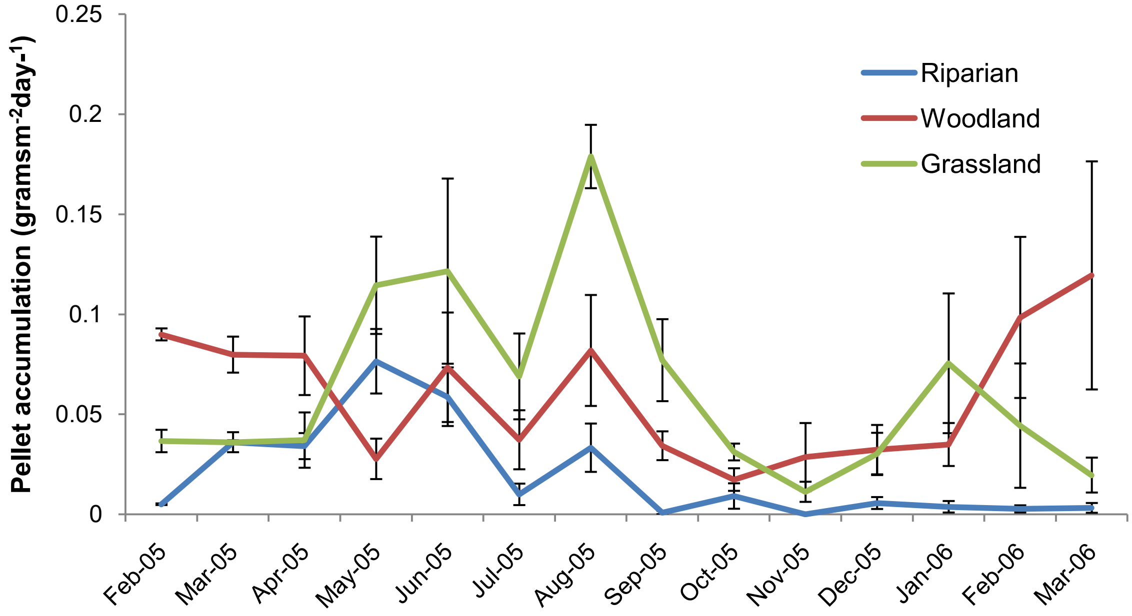



**Figure 4-5** **Mean faecal deposition rate and load (gramsm$^{-2}$day$^{-1}$) of eastern grey kangaroos at Murphy's Crossing in each habitat over time. Error bars represent 95% confidence intervals.**

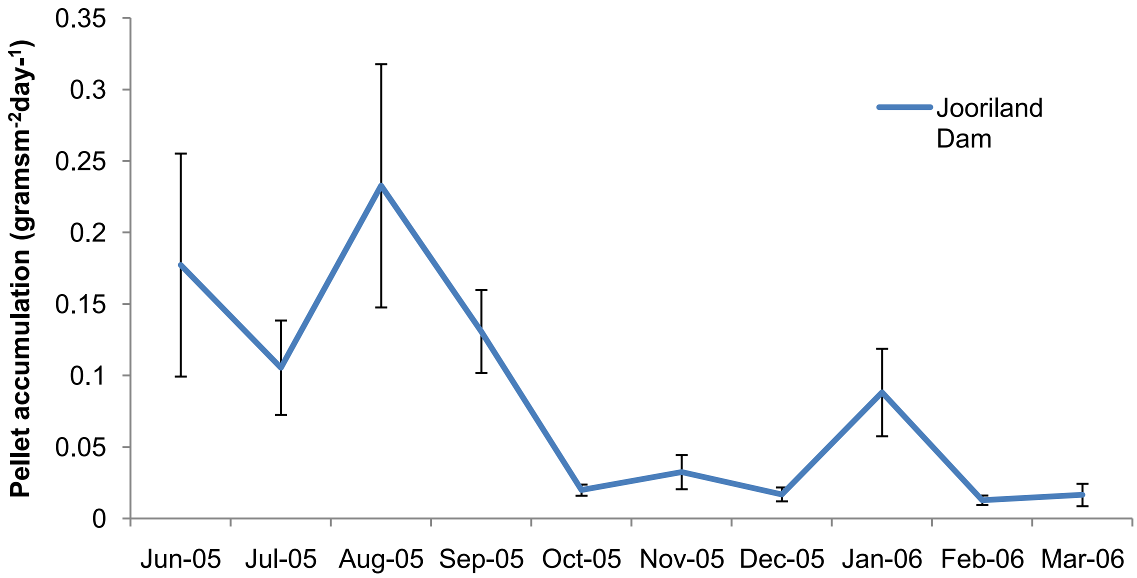



**Figure 4-6** **Mean faecal deposition rate and load (gramsm$^{-2}$day$^{-1}$) of eastern grey kangaroo surrounding Jooriland Dam over time. Error bars represent 95% confidence intervals.**

The total volume of faecal material deposited by eastern grey kangaroos pooled across the three major habitats in the watershed areas ranged from 32.9 kgkm$^{-2}$day$^{-1}$ to 242.2 kgkm$^{-2}$day$^{-1}$ at Jooriland and 39.8 kgkm$^{-2}$day$^{-1}$ to 294 kgkm$^{-2}$day$^{-1}$ at Murphy's Crossing (Figure 4-7).

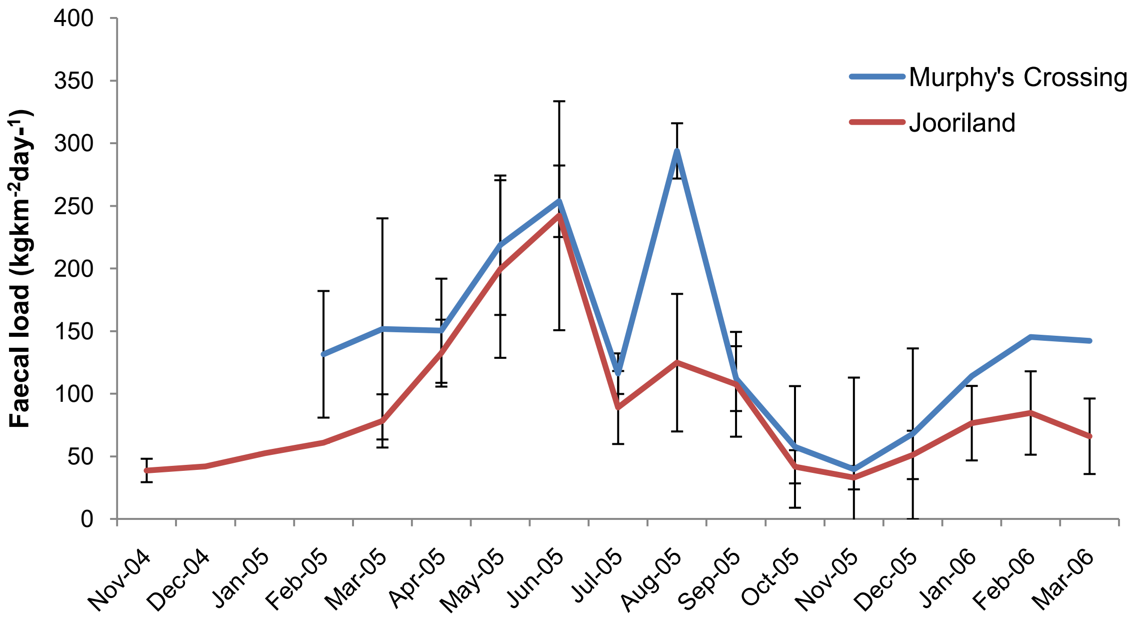



**Figure 4-7 Mean total faecal loads (kgm$^{-2}$day$^{-1}$) of eastern grey kangaroos at Murphy's Crossing and Jooriland. Error bars represent 95% confidence intervals.**

At Jooriland, MANOVA revealed a significant difference in the faecal loads between riparian and woodland habitats across the surveyed months and a significant time and habitat interaction (Table 4-2). Similarly, faecal loads differed significantly between grassland and riparian habitats across surveyed months and over time. There was no difference observed between the faecal loads deposited in the woodland and grassland habitats. At Murphy's Crossing, MANOVA showed a significant time and habitat interaction between the faecal loads deposited in grassland and woodland habitats and riparian and woodland habitats. At both sites there were significantly higher faecal loads deposited by eastern grey kangaroos surrounding the Jooriland farm dam when compared with the riparian habitats.

**Table 4-2 Eastern grey kangaroo faecal deposition repeated-measures MANOVA results.**

| Habitat comparison | Effect | *F* | *d.f.* | *P* |
|---|---|---|---|---|
| **Jooriland** | | | | |
| Riparian/Woodland | Time | 209.7 | 16,3 | **0.0057** |
| | Time*Habitat | 174.5 | 16,3 | **0.0075** |
| Riparian/Grassland | Time | $2.9 \times 10^5$ | 16,1 | **0.005** |
| | Time*Habitat | $2.9 \times 10^5$ | 16,1 | **0.005** |
| Grassland/Woodland | Time | 62.5 | 16,1 | 0.356 |
| | Time*Habitat | 31.6 | 16,1 | 0.484 |
| Riparian/Dam | Time | 1322.4 | 9,5 | **<0.0001** |
| | Time*Habitat | 1253.8 | 9,5 | **<0.0001** |
| **Murphy's Crossing** | | | | |
| Riparian/Woodland | Time | 5.6 | 13,6 | 0.13 |
| | Time*Habitat | 9.4 | 13,6 | **0.04** |
| Riparian/Grassland | Time | 112.0 | 13,2 | 0.06 |
| | Time*Habitat | 79.8 | 13,2 | 0.08 |
| Grassland/Woodland | Time | 15.1 | 13,2 | 0.34 |
| | Time*Habitat | 217.6 | 13,2 | **0.03** |
| Riparian/Dam | Time | 207.7 | 9,5 | **<0.0001** |
| | Time*Habitat | 147.4 | 9,5 | **<0.0001** |

Within the individual habitats at Jooriland, faecal loads differed between surveyed months in the grassland habitat ($F_{16,85}$ = 3.1; $P$<0.001) with the highest faecal loads recorded in May and June 2005 and the lowest recorded in the summer months of 2004/05. Post-hoc Tukey HSD tests revealed that faecal loads in May and June 2005 were significantly different from those in November 2004 and 2005 and March 2006. Faecal loads were similar between surveyed months for the woodland ($F_{16,153}$ = 1.41; $P$>0.05) and the riparian habitats ($F_{16,153}$ = 1.32; $P$>0.05). Faecal loads surrounding the Jooriland farm dam were different between the surveyed months ($F_{9,40}$ = 3.87; $P$<0.0001) with the highest deposition occurring in August 2005 and the lowest in February 2006.

Within the individual habitats at Murphy's Crossing, faecal loads differed between the surveyed months in the riparian habitat ($F_{13,126}$ = 10.53; $P$<0.0001) with the highest loads recorded in May and June 2005 and the lowest recorded between November 2005 and March 2006. Post-hoc Tukey HSD tests revealed that the faecal loads recorded in May and June 2005 were significantly different from a majority of the other surveyed months. Similarly, there was a significant difference observed in the faecal loads deposited within the grassland habitat between the surveyed months ($F_{13,70}$ = 3.27; $P$<0.001) with the highest levels recorded in June and August 2005 and the lowest in November 2005 and March 2006. Post-hoc Tukey HSD tests revealed that the faecal loads recorded in November 2005 were significantly different from those in May and August of the same year. Faecal loads deposited in the woodland habitat did not vary between the surveyed months ($F_{13,126}$ = 1.39; $P$>0.05).

The common wombat was the only species found to deposit comparable quantities of faecal material as eastern grey kangaroos in riparian habitats. At both sites, there was a general trend for there to be higher faecal loads detected in winter than in the summer months (Figure 4-8). Significant differences in the faecal loads deposited by common wombats in the riparian habitat were detected between the sites and survey months (Table 4-3). Similarly, there were significant differences between the faecal loads deposited by eastern grey kangaroos and common wombats, but no interaction between time, species and site. Feral pig faecal material was detected only in the riparian habitat during the winter months.

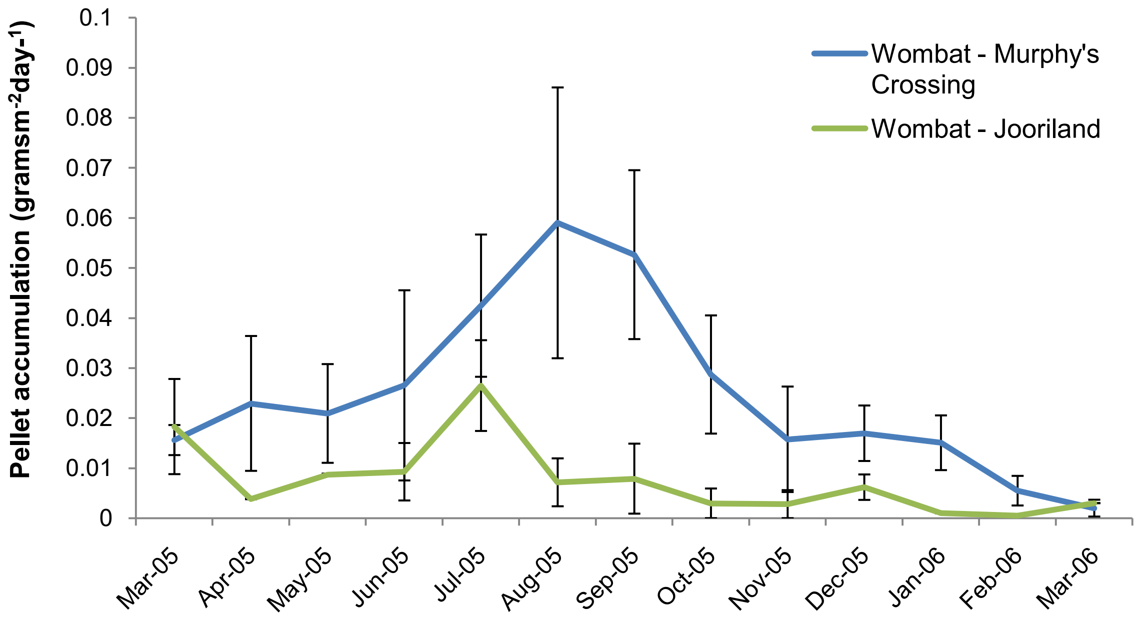



**Figure 4-8** **Mean faecal deposition rate (gramsm$^{-2}$day$^{-1}$) of common wombats in the riparian habitats of Jooriland and Murphy's Crossing over time. Error bars represent 95% confidence intervals**

**Table 4-3** **Repeated-measures MANOVA results comparing common wombat faecal deposition between sites and with eastern grey kangaroo**

| **Effect** | ***F*** | ***d.f.*** | ***P*** |
|---|---|---|---|
| **Common wombat** | | | |
| Time | 34.5 | 12,7 | **<0.001** |
| Time*Site | 9.5 | 12,7 | **0.02** |
| **Eastern grey kangaroo vs. common wombat** | | | |
| Time | 4.2 | 12,24 | **<0.001** |
| Time*Site | 1.8 | 12,24 | **0.004** |
| Time*Species*Site | 0.9 | 12,24 | 0.111 |
| Time*Species | 2.5 | 12,24 | **<0.001** |

### *Faecal decay*

The decay of eastern grey kangaroo faecal material was generally faster in the summer months and slower in winter. The maximum duration a pellet remained intact was in excess of 15 months and the minimum was less than one day. In relation to habitats, faecal material set aside in the grassland habitat decayed faster than in woodland, with the faecal material in the riparian habitat decaying the slowest overall (Table 4-4, Table 4-5 and Table 4-6).

**Table 4-4 The time, in days, of specified proportions of eastern grey kangaroo faecal pellets remaining intact in the grassland habitat.**

| | Proportion remaining intact | | | | |
|---|---|---|---|---|---|
| **Month when fresh** | **0.90** | **0.75** | **0.50** | **0.25** | **0.10** |
| November | <2 | 4 | 30-36 | 70-80 | 100-120 |
| December | <1 | <1 | 1 | 3-5 | 40-70 |
| January | <1 | <1 | 1 | 2-3 | 7-9 |
| February | <1 | <1 | 1-2 | 3 | 5-10 |
| March | <1 | 1-2 | 2 | 5 | 30-45 |
| April | <1 | <1 | <1 | 1-2 | 3 |
| May | 15-20 | 35-40 | 65-70 | 130-140 | 150-160 |
| June | 10-20 | 20-30 | 40-45 | 120 | 200-230 |
| July | 50-55 | 70-80 | 90-110 | 175-190 | 340-360 |
| August | 25-30 | 35-40 | 45-50 | 60-70 | 105-115 |
| September | 7-15 | 15-25 | 40-50 | 130-140 | >394 |
| October | <1 | 1-2 | 2-3 | 5-15 | 20-30 |

**Table 4-5 The time, in days, of specified proportions of eastern grey kangaroo faecal pellets remaining intact in the riparian habitat.**

| | Proportion remaining intact | | | | |
|---|---|---|---|---|---|
| **Month when fresh** | **0.90** | **0.75** | **0.50** | **0.25** | **0.10** |
| November | 1-30 | 1-30 | 30-35 | 80-100 | 105-110 |
| December | 1-25 | 1-25 | 25-30 | 60-70 | 70-90 |
| January | 1-15 | 1-15 | 15-20 | 30-35 | >300 |
| February | <1 | 2-3 | 3-4 | 10-13 | 40-45 |
| March | 1-2 | 2-3 | 10-14 | 20-25 | 75-90 |
| April | <1 | 1-2 | 1-2 | 2-3 | 17-20 |
| May | 40-50 | 150 | 210 | >215 | >215 |
| June | 35-40 | 100-110 | >182 | >182 | >182 |
| July | 60-70 | 75-80 | >143 | >143 | >143 |
| August | 40-45 | 45-50 | 113 | >113 | >113 |
| September | 10-20 | 20-28 | >90 | >90 | >90 |
| October | 1-2 | 4-5 | 30-35 | >64 | >64 |

**Table 4-6 The time, in days, of specified proportions of eastern grey kangaroo faecal pellets remaining intact in the woodland habitat.**

| | Proportion remaining intact | | | | |
|---|---|---|---|---|---|
| **Month when fresh** | **0.90** | **0.75** | **0.50** | **0.25** | **0.10** |
| November | 2 | 4 | 40-60 | 75-85 | 96-100 |
| December | 1-2 | 4 | 20-30 | 40-47 | 50-60 |
| January | 1-2 | 2-8 | 8-11 | 15-23 | 50-56 |
| February | 1-2 | 5-10 | 5-10 | 13 | 65-73 |
| March | <1 | <1 | 1-2 | 10-14 | 25-30 |
| April | <1 | <1 | <1 | <1 | 1-2 |
| May | 5-10 | 30-35 | 145-160 | 350-400 | >500 |
| June | 3-5 | 38-42 | 145-150 | 320-325 | >470 |
| July | 60-65 | 150-160 | 205-240 | >431 | >431 |

| | Proportion remaining intact | | | | |
|---|---|---|---|---|---|
| **Month when fresh** | **0.90** | **0.75** | **0.50** | **0.25** | **0.10** |
| August | 34-36 | 40-50 | 113-130 | >401 | >401 |
| September | 3-10 | 10-28 | 30-60 | >378 | >378 |
| October | 4 | 5-20 | 5-20 | 5-20 | 20-30 |

The shape of decay curves varied throughout the course of the study (Figure 4-9). Faecal material that was fresh in January and February showed sharp declines to zero (Figure 4-9 c-d). Faecal material that was fresh in June, July and August showed a much slower decay rate and decay curves had clear broad shoulders (Figure 4-9 h-i).

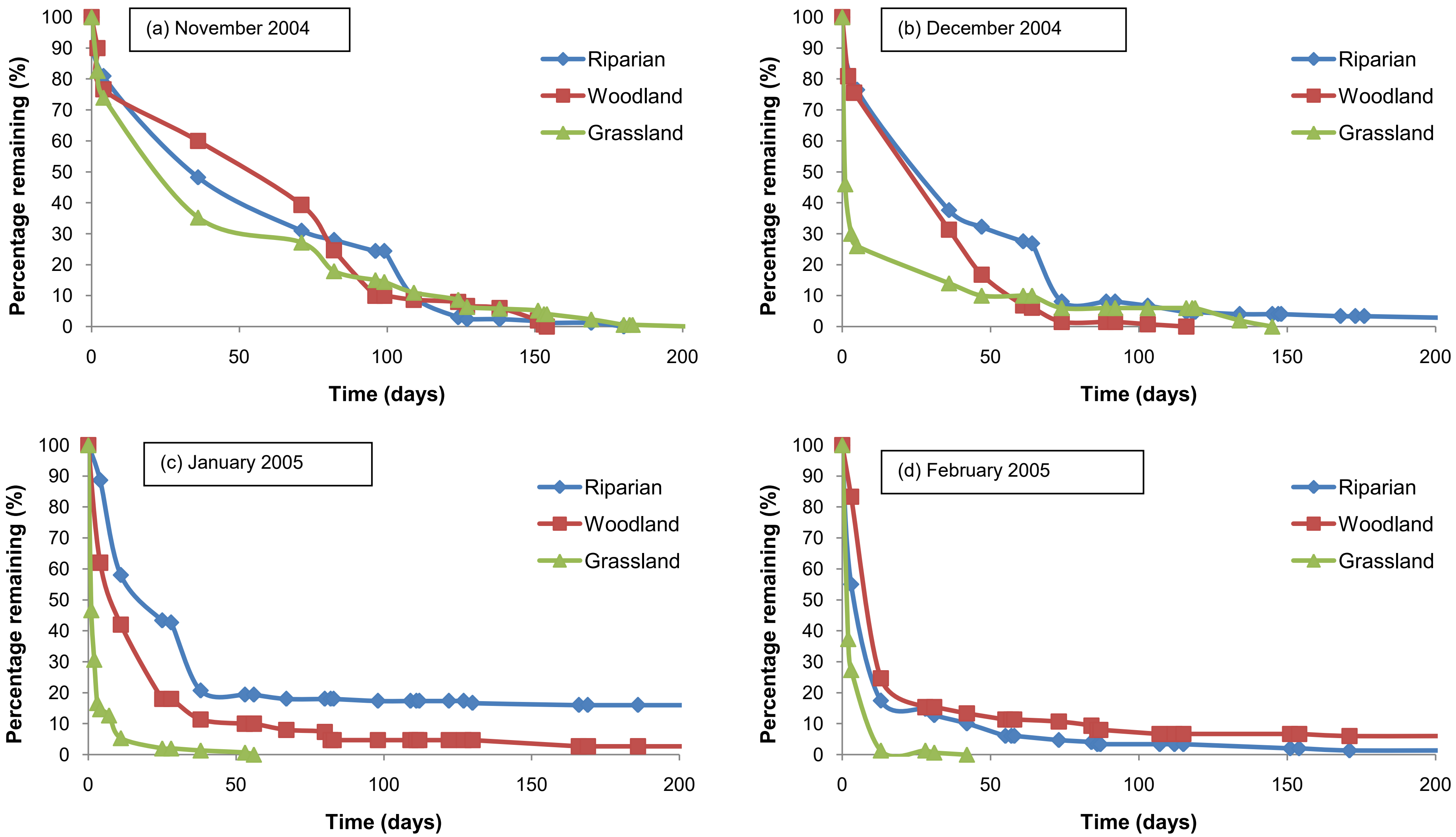

(a) November 2004
(b) December 2004
(c) January 2005
(d) February 2005
Riparian
Woodland
Grassland
Percentage remaining (%)
Time (days)

Figure 4-9 Faecal decay curves (percentage remaining over time in days) of fresh eastern grey kangaroo faecal pellets for 11 consecutive months commencing (a) November 2004 and concluding in (l) October 2005

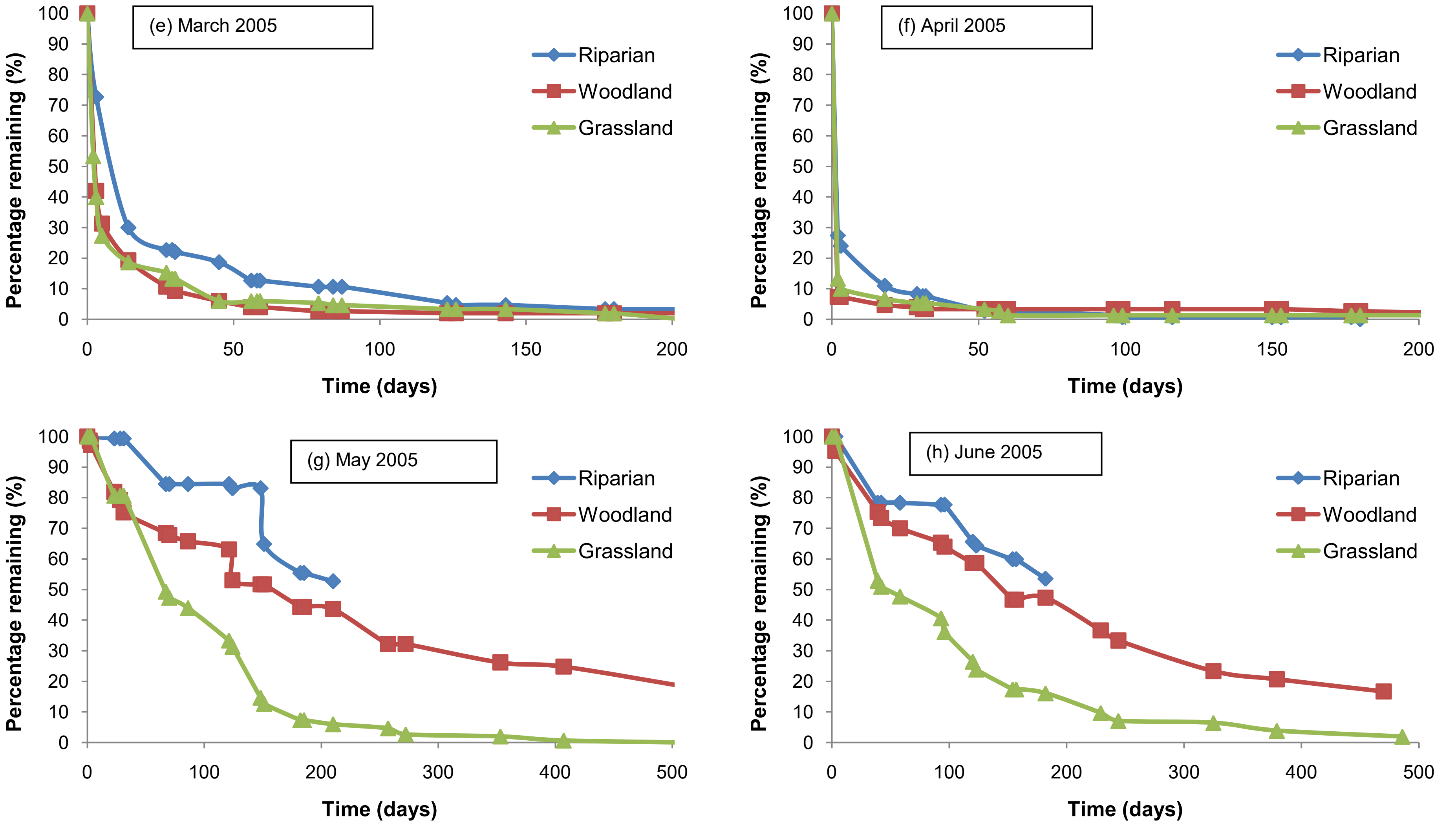
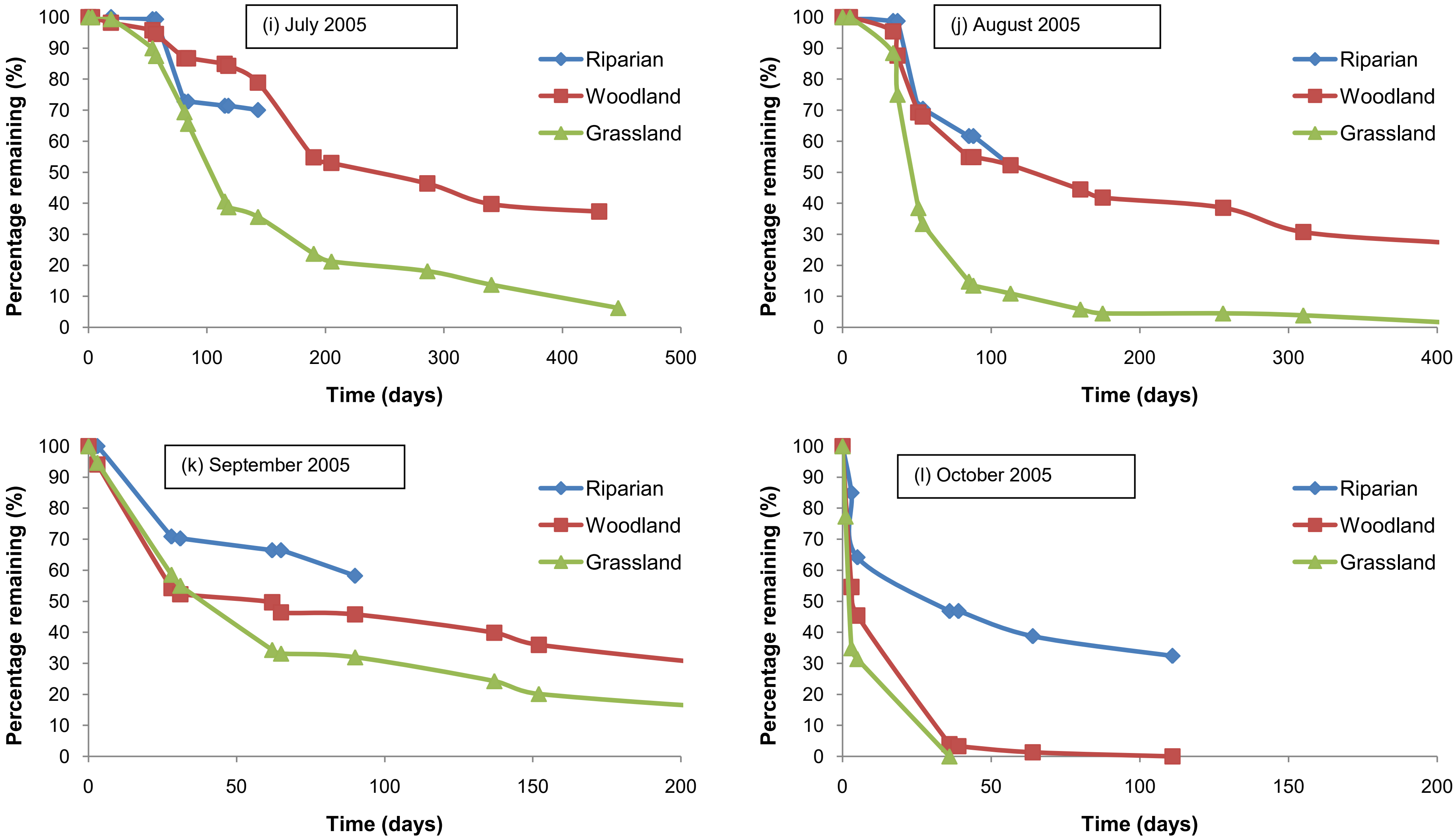

(e) March 2005
Riparian
Woodland
Grassland
Percentage remaining (%)
Time (days)
(f) April 2005
Riparian
Woodland
Grassland
Percentage remaining (%)
Time (days)
(g) May 2005
Riparian
Woodland
Grassland
Percentage remaining (%)
Time (days)
(h) June 2005
Riparian
Woodland
Grassland
Percentage remaining (%)
Time (days)

Figure 4-9 (continued) Faecal decay curves (percentage remaining over time in days) of fresh eastern grey kangaroo faecal pellets for 11 consecutive months commencing (a) November 2004 and concluding in (l) October 2005



(i) July 2005
(j) August 2005
(k) September 2005
(l) October 2005
Riparian
Woodland
Grassland
Percentage remaining (%)
Time (days)
0
10
20
30
40
50
60
70
80
90
100
0
100
200
300
400
500
50
150

Figure 4-9 (continued) Faecal decay curves (percentage remaining over time in days) of fresh eastern grey kangaroo faecal pellets for 11 consecutive months commencing (a) November 2004 and concluding in (l) October 2005

Faecal decay was faster in the grassland habitat and slower in the riparian habitat. The proportion of eastern grey kangaroo faecal material that persisted in the first five days differed between grassland and woodland ($x^2$ = 59.00, *d.f.* = 11, *P* <0.0001), between grassland and riparian ($x^2$ = 217.17, *d.f.* = 11, *P*<0.0001) and woodland and riparian ($x^2$ = 49.61, *d.f.* = 11, *P*<0.0001). Similarly, after one month the proportion of faecal material remaining also varied between grassland and woodland ($x^2$ = 83.04, *d.f.* = 11, *P*<0.0001), grassland and riparian ($x^2$ = 169.69, *d.f.* = 11, *P*<0.0001) and woodland and riparian ($x^2$ = 93.49, *d.f.* = 11, *P*<0.0001). In relation to the environmental variables, the survival rate of faecal material during the first five days and in the first month was significantly negatively correlated with mean minimum and maximum temperatures in all habitats and with mean maximum humidity in the woodland habitat only (Table 4-7). When the effect of rainfall on the decay of faecal material was controlled (partial correlation), the relationships with mean minimum and maximum temperatures were also significant, with the exception of faecal material in the first five days in the woodland habitat. Some partial correlations of rainfall with humidity were also significant in the woodland and riparian habitats.

**Table 4-7 Correlation coefficients of the relationship between survival rate of faecal pellets in the first five days (< 5 days) and first month since being fresh, and environmental variables.**

For correlation analysis, *d.f.* = 11, for partial correlation analysis, *d.f.* = 10; * *P*<0.05. Variable controlled in partial correlation analysis is shown in parentheses.

| Environmental variable | Survival rate in < 5 days | Survival rate in first month |
|---|---|---|
| **Grassland** | | |
| Mean min. temp. (C) | -0.78* | -0.90* |
| Mean max. temp. (C) | -0.77* | -0.88* |
| Mean min. humidity (%) | -0.23 | -0.32 |
| Mean max. humidity (%) | -0.40 | -0.24 |
| Rainfall (mm) | -0.32 | -0.30 |
| Partial correlations | | |
| Mean min. temp. (rain) | -0.75* | -0.91* |
| Mean max. temp. (rain) | -0.74* | -0.87* |
| Mean minimum humidity (rain) | -0.15 | -0.26 |
| Mean maximum humidity (rain) | -0.46 | -0.29 |
| Rainfall (min. temp.) | 0.11 | 0.39 |
| Rainfall (max. temp.) | 0.02 | 0.18 |

| Environmental variable | Survival rate in < 5 days | Survival rate in first month |
|---|---|---|
| Rainfall (min. humidity) | -0.28 | -0.23 |
| Rainfall (max. humidity) | -0.40 | -0.34 |
| **Woodland** | | |
| Mean minimum temperature (C) | -0.51* | -0.79* |
| Mean maximum temperature (C) | -0.50* | -0.77* |
| Mean minimum humidity (%) | -0.13 | -0.22 |
| Mean maximum humidity (%) | -0.50* | -0.39 |
| Rainfall (mm) | -0.29 | -0.25 |
| Partial correlations | | |
| Mean minimum temperature (rain) | -0.44 | -0.79* |
| Mean maximum temperature (rain) | -0.43 | -0.75* |
| Mean minimum humidity (rain) | -0.04 | -0.16 |
| Mean maximum humidity (rain) | -0.55* | -0.42 |
| Rainfall (min. temp) | -0.05 | 0.26 |
| Rainfall (max. temp) | -0.10 | 0.14 |
| Rainfall (min. humidity) | -0.27 | -0.20 |
| Rainfall (max. humidity) | -0.40 | -0.31 |
| **Riparian** | | |
| Mean minimum temperature (C) | -0.57* | -0.84* |
| Mean maximum temperature (C) | -0.58* | -0.82* |
| Mean minimum humidity (%) | -0.10 | -0.38 |
| Mean maximum humidity (%) | -0.40 | -0.43 |
| Rainfall (mm) | -0.13 | -0.36 |
| Partial correlations | | |
| Mean minimum temperature (rain) | -0.58* | -0.82* |
| Mean maximum temperature (rain) | -0.58* | -0.80* |
| Mean minimum humidity (rain) | -0.07 | -0.31 |
| Mean maximum humidity (rain) | -0.42 | -0.50* |
| Rainfall (min. temp) | 0.21 | 0.14 |

| Environmental variable | Survival rate in < 5 days | Survival rate in first month |
|---|---|---|
| Rainfall (max. temp) | 0.16 | 0.00 |
| Rainfall (min. humidity) | -0.11 | -0.28 |
| Rainfall (max. humidity) | -0.19 | -0.44 |

### *Pasture quality and quantity*

Pasture quality was significantly different among habitats throughout different sampling periods (Figure 4-10) and was generally highest during warmer periods of the year (February 2005, October 2005 and January 2006) compared with cooler months. In the autumn and winter months of 2005 (March through to August), pasture quality was higher in the riparian habitat than in the woodland and grassland habitats (Table 4-8). Conversely, in spring and summer (September through to February) the mean pasture quality in all habitats increased and the differences between the habitats were less apparent, with the exception of October 2005 and January 2006, when riparian and woodland had significantly higher pasture quality, respectively.

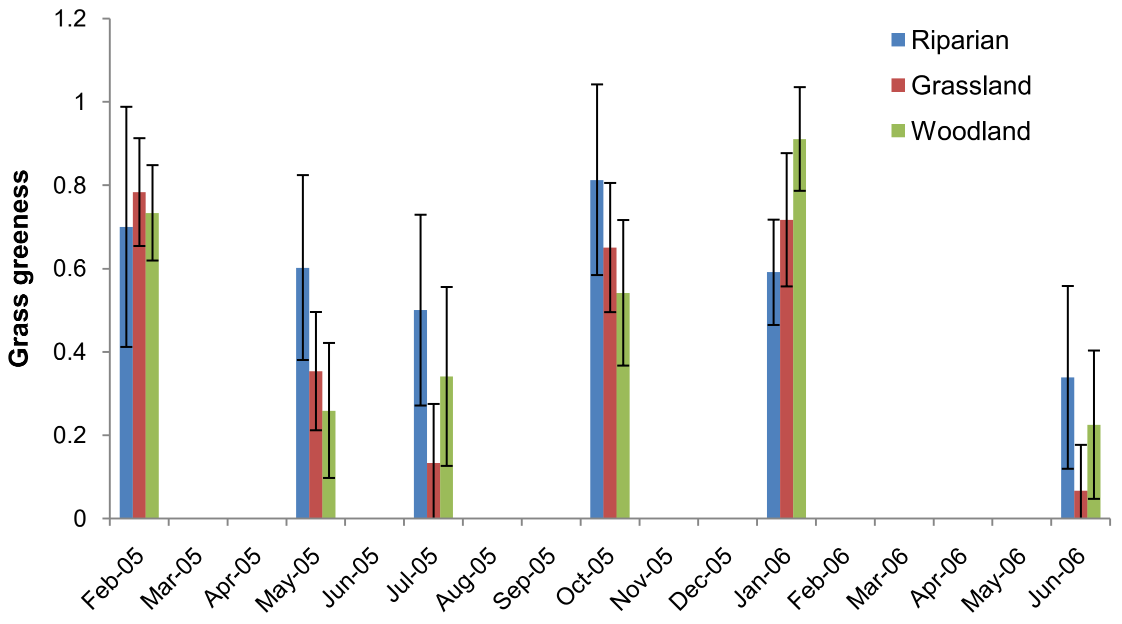



**Figure 4-10 Mean pasture quality in all habitats between February 2005 and June 2006**

Pasture quantity did not differ significantly among the habitats throughout the different sampling periods (Figure 4-11). Grassland had more pasture than woodland and riparian habitats throughout the sampling period. The riparian habitat contained similar amounts of pasture quantity as the woodland habitat in all sampling periods, with the exception of February 2005 and January 2006, when the woodland habitat had greater pasture quantity.

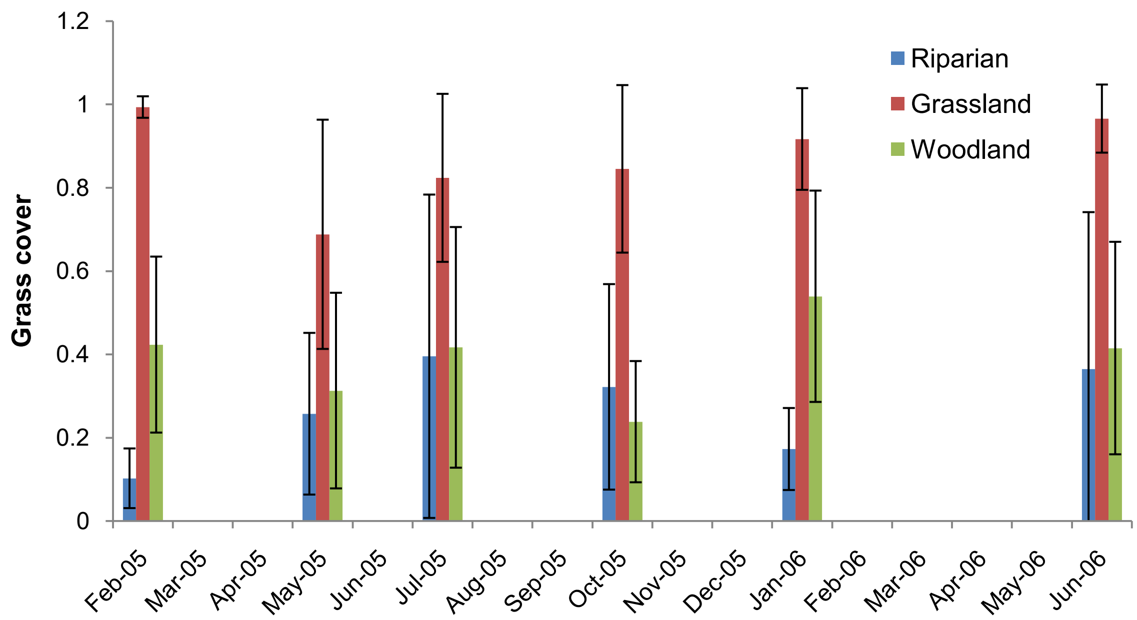



**Figure 4-11 Mean grass cover in all habitats between February 2005 and June 2006**

**Table 4-8 One-way ANOVA of arc-sine transformed grass greenness scores and percentage grass cover data among habitats for each sampling occasion.**

Significant results (P<0.05) are shown in bold. Post-hoc analysis ranks the habitats from lowest to highest, those not significantly different are shown as =, while those significantly different are shown as <

| **Parameter** | **Sampling Month** | ***F*** | ***P*** | **Tukey HSD** |
|---|---|---|---|---|
| **Pasture quality** | | | | |
| | February 2005 | 0.39 | 0.6795 | G=R=W |
| | May 2005 | 24.69 | **<0.0001** | **G=W<R** |
| | July 2005 | 22.99 | **<0.0001** | **G<W<R** |
| | October 2005 | 22.24 | **<0.0001** | **G=W<R** |
| | January 2006 | 16.33 | **<0.0001** | **R=G<W** |

| Parameter | Sampling Month | *F* | *P* | Tukey HSD |
|---|---|---|---|---|
| | June 2006 | 19.30 | **<0.0001** | **G<W=R** |
| **Pasture quantity** | | | | |
| | February 2005 | 381.45 | **<0.0001** | **R<W<G** |
| | May 2005 | 26.97 | **<0.0001** | **R=W<G** |
| | July 2005 | 18.89 | <0.0001 | **R=W<G** |
| | October 2005 | 80.97 | <0.0001 | **R=W<G** |
| | January 2006 | 53.89 | <0.0001 | **R<W<G** |
| | June 2006 | 77.39 | <0.0001 | **R=W<G** |

## DISCUSSION

### *Foraging scale habitat selection*

Eastern grey kangaroos clearly demonstrated preferential selection of habitats at the foraging scale within the study area. As kangaroo species have been previously reported to defecate primarily whilst actively feeding (Caughley 1964), the results of this study provide an indication of the total time spent foraging by eastern grey kangaroos in each of the habitats. Consequently, this study suggests that eastern grey kangaroos spend considerably more time foraging in the woodland and grassland habitats compared with the riparian habitat. In addition, as previous studies have successfully utilised faecal load calculations as a relative index of abundance for eastern grey kangaroos (Perry and Braysher 1986, Southwell 1989), the results are indicative of the numbers of kangaroos utilising each of the surveyed habitats as low and high in the riparian and grassland habitats, respectively.

The quantity of faecal material deposited by eastern grey kangaroos in the grassland and woodland habitats was primarily reflective of the higher pasture biomass in these habitats rather than pasture quality. The woodland and grassland habitats appeared to be more fertile compared with the riparian habitat, as they contained improved areas of pasture on basalt clays and supported higher yields of pasture species than the alluviums (gravels and sand) found in the riparian zones (NSW Department of Mines 1966). In addition, the blanketing nature of River She Oak *Casuarina cunninghamiana* subsp. *cunninghamiana*, the dominant tree species in the riparian areas, inhibited the growth of understorey vegetation, rendering most of the area where this species dominates grass free.

There was an interchange between the peaks of faecal material deposited by eastern grey kangaroos in the grassland and woodland habitats throughout the sampling period. The seasonal shifts in the time spent foraging by kangaroos between these habitats is potentially a result of their increased requirement for shelter in the woodland habitat during spring and summer, versus increased time spent foraging in the more open grassland habitats during winter. In this study eastern grey kangaroos were less likely to seek shelter from the riparian habitat during spring and summer, an outcome that reflects the balance sought between time spent foraging and the requirement for thermoregulation (Dawson *et al.* 2000). Eastern grey kangaroos have been reported to spend more time foraging in grassy habitats when forage deteriorates (Hill 1982), which is a likely explanation for the higher faecal loads deposited in the grassland habitat during the winter period. However, other studies have reported that eastern grey kangaroos drop fewer faecal pellets during drought in response to poorer quality diet (Perry and Braysher 1986). The high faecal loads deposited in the grassland habitat during winter in this study may be in response to the higher concentrations and subsequent densities of animals foraging in these areas.

High levels of eastern grey kangaroo foraging in the woodland habitat may pose some biodiversity concerns, as this habitat is part of the nationally listed Critically Endangered Box Gum Woodland community. Eastern grey kangaroos and other macropodids may reduce the success of revegetation programs due to their apparent overgrazing (Pople and McLeod 2000, Meers and Adams 2003). The high densities of eastern grey kangaroos in the Burragorang Valley may be affecting the understorey and ground layer of this community, which could result in a cumulative threatening process that compromises the conservation goals of the area (NSW Department of Environment and Climate Change 2007).

The low faecal deposition by eastern grey kangaroos in the riparian habitat may also be associated with the costs of foraging in this habitat (Maguire *et al.* 2006). This habitat potentially represents a predation risk for eastern grey kangaroos and would not be considered a ‗safe refuge‘ from predators such as dingoes, given the steep sandy banks, limited visibility and restricted escape routes. Alternatively, the former farm dams present in the study area are more likely to be utilised for drinking and foraging by resident eastern grey kangaroos, due to the high pasture biomass surrounding them, as well as lower predation risk associated with increased visibility. The higher faecal loads deposited in close vicinity to the Jooriland farm dam compared with the riparian habitat corroborates this view, whereby the predation risks associated with foraging and drinking can be counterbalanced by forming large social groups (Banks *et al.* 2000, Banks 2001) that were observed in these areas.

The apparent link between moisture content of forage and pasture quality has potential implications on the requirement of eastern grey kangaroos to drink and thus frequent the riparian habitat, such that water loss can be offset by the moisture content of the available pasture in surrounding habitats (Grice and Beck 1994, Dawson *et al.* 2000). The water requirements of eastern grey kangaroos would have been lowest in the spring and summer months of the study, when pasture quality and moisture content was highest in the grassland and woodland habitats. Jarman and Evans (2010) considered leaf water content of common pasture species as a source of free water for the eastern grey kangaroo and concluded that animals would be less likely to drink water if the leaf water content remained above 70%. The lower eastern grey kangaroo faecal load in the riparian habitat during spring and summer when pasture quality was highest provides support for this view. In addition, higher densities of eastern grey kangaroos were observed in the riparian habitat during winter, as this habitat sustained higher pasture quality and the requirement for animals to drink from the river would have increased during this time (i.e. when the kangaroos would be especially water-limited).

Several researchers have suggested that eastern grey kangaroos conform to the Ideal Free Distribution (IFD) (Maguire *et al.* 2006). This theory implies that animals should distribute themselves among the available habitat patches according to the density of conspecifics and the depletion of resources, such that better quality patches of forage are preferentially chosen at a landscape scale. Other researchers have found some departures from this theory in relation to eastern grey kangaroos, such that competitive interferences occur in high quality patches and conspecifics redistribute themselves to poorer patches where competition is less intense. Although no study was conducted to test the conformity of this population of eastern grey kangaroos to the IFD theory, the faecal loads contributed to the different habitats on a temporal and spatial scale suggest that the IFD theory applies. In addition, the mild difference in eastern grey kangaroo faecal deposition between Jooriland and Murphy's Crossing may be explained by the IFD, such that differences in densities between the sites may influence the way in which fine scale habitat utilisation occurs. Generally, however, the high density study population of eastern grey kangaroos has preferentially utilised the highest quality grassland habitat and at certain times of the year (most apparent in winter) there has been a spill over to poorer quality patches when concentrations of animals in this habitat have reached a certain threshold and competitive interactions presumably have increased.

Faecal decay has had a previously unperceived influence on the spatial and temporal variation in the eastern grey kangaroo faecal loads in each habitat. Several studies have used faecal deposition rates to measure and make generalisations on habitat utilisation of this species at the foraging scale (Ramp and Coulson 2002;2004), but have not considered faecal decay as a correction factor for the amount of faeces found on the foraging ground. The results of this study suggest that faecal decay is fastest in the grassland habitats and slowest in the riparian habitats, with decay occurring more readily in the warmer months of the year compared with the colder months. Faecal decay was correlated significantly with temperature in all habitats, and this would appear to be a function of the presence and increased activity of dung beetles (family Scarabaeidae) in the warmer periods of the year that breakdown the faecal material at a faster rate. Faecal decay was slowest in the riparian habitat as it provided the most shelter from sunlight and comprised sandy soils that did not appear good dung beetle habitat.

The exponential rate of faecal decay experienced during the summer months needs to be considered in future experiments examining the foraging scale utilisation by this species. For instance, one cannot accurately infer the foraging habitat utilisation of the species in the grassland habitat during summer at the sampling intensity used, as faecal material is present on the ground for only a short period of time. Consequently, in the current study, utilisation of the grassland habitat during this time may have been much greater than what the results have revealed. However, the reasons for the apparent differences in faecal loads between habitats at this time would remain, in that animals would still be seeking shelter in the woodland habitats for thermoregulation, but may forage for longer and unmeasured periods of time at night in the grassland habitat to avoid the heat of the day. Estimating this relative time using faecal loads may be confounded by diurnal differences in the defecation rates exhibited by this species and a reduction in the faecal material deposited during the night (Johnson *et al.* 1987).

***Faecal deposition and zoonotic disease management***

The low quantity of eastern grey kangaroo faecal material in the riparian habitat, closest to receiving waters of the catchment water supply, has strong implications for the management of *Cryptosporidium* and *Giardia* sources in Sydney's water supply catchment. Given its close proximity, the eastern grey kangaroo faecal material (encasing potential zoonotic micro-organisms) present in this habitat would likely enter the surface water after a large rainfall event in overland surface runoff or following an increase in the flow and height of the Wollondilly River (Davies *et al.* 2004). Table 4-9 summarises the implications of the eastern grey kangaroo faecal deposition results on zoonotic disease management.

**Table 4-9 Summary of faecal deposition results of eastern grey kangaroos and their implications for zoonotic disease management in the Sydney water supply catchment**

| *Result* | **Implications for zoonotic disease management** |
|---|---|
| **Faecal deposition rates – low in riparian zones but generally high in areas supporting grassy habitats** | • Low quantity of eastern grey kangaroo faecal material entering surface water from vegetated riparian corridors<br>• Bare soils down slope of deposition areas i.e. most riparian zones in the catchment - highest risk (Davies *et al.* 2004, Davies *et al.* 2005, Ferguson *et al.* 2007b)<br>• Faecal-oral route disease transmission (inter and intra-species) would be highest in grassland habitat (Hutchings *et al.* 2002, Judge *et al.* 2005)<br>• Trade-off between reducing grassy habitat to reduce eastern grey kangaroo faecal loads and increasing the ability to trap pathogens on vegetated slopes |
| **Faecal decay – high disappearance rate in grassland, low in riparian zones; fast in warmer months and slow in cooler months** | • Faecal attrition rates are clearly linked to the inactivation of *Cryptosporidium* oocysts and *Giardia* cycts (Ferguson *et al.* 2007b)<br>• Presence and abundance of dung beetles would be potentially correlated with a decrease in *Cryptosporidium* and *Giardia* dissemination from animal sources (Nichols *et al.* 2008), however there is some evidence that dung beetles increase the dispersion of these micro-organisms as they survive dung beetle digestion and excrement (Christobel Ferguson, personal communication 2010).<br>• Highest oocyst/cyst preservation in cooler months of the year and also in riparian zones when faecal material is preserved for longer periods |
| **Faecal loads surrounding alternative water sources – higher loads around Jooriland Dam when compared to riparian zones** | • Closing of artificial watering points is a commonly utilised population control technique<br>• Higher faecal loads surrounding artificial watering points versus riparian zones<br>• Closing artificial watering points may inflate eastern grey kangaroo faecal loads in riparian zones |
| **Other species of zoonotic disease risk – e.g. common wombats** | • Common wombat had significantly higher faecal loads in riparian zones than eastern grey kangaroos<br>• Related to the number of burrows in this habitat<br>• Feral pigs, the source of several potential human pathogens (Choquenot *et al.* 1996), had very low faecal loads in the riparian habitat – reflective of current control campaigns despite their requirements for daily access to water (Dexter 1999) |

The deposition and decay results for eastern grey kangaroo faecal material identify a series of interrelated factors that need to be considered when assessing the zoonotic disease risk of this species in Sydney‘s water supply catchment. Firstly, the low faecal deposition rates in the riparian habitat are suggestive of a lower risk of this species contributing zoonotic pathogens to receiving waters. Secondly, the slower decomposition rates of faecal material in the riparian habitat may counteract the low risk, as any potential oocysts/cysts may be preserved in this area for longer periods of time. Thirdly, any grassy areas in close proximity to receiving waters may still contain high volumes of eastern grey kangaroo faecal material, as the volume of faecal material found in a habitat is a function of pasture biomass and the time spent actively foraging by this species.

The faecal deposition and decay rate data collected in this chapter are integral to the accurate calculation of pathogen loads originating from eastern grey kangaroos. In the first application of the Pathogen Catchment Budget (PCB) in Sydney‘s water supply catchment (Ferguson *et al.* 2007a), this model utilised fixed values for the volume of manure produced by kangaroos and assumed that faecal decay occurred after one day of being deposited. In converse, this study showed that faecal deposition and decay varied according to the time of year and the distance from surface waters of the catchment on the basis of the presence of suitable foraging areas. Based on the results of this study, revision of the faecal loads and decay rates used in the PCB model would be recommended for future analysis of pathogen loads in the Sydney drinking water catchment (Christobel Ferguson, personal communication 2010). This would allow for the seasonality in these parameters to be expressed to attain an understanding of the pulses of micro-organisms originating from this species.

Faecal decay in this study was assumed to equate to breakdown of micro-organisms (oocysts and cysts). A recent oocyst inactivation study assessing *Cryptosporidium* infectivity under different climatic regimes (Li *et al.* 2010) has provided support for this assumption. This study showed that *Cryptosporidium* oocysts are completely non-infectious after 14 24-h cycles when exposed to a 30°C and after 70 24-h cycles of 20°C. In contrast, however, oocysts remained infectious after 90 days when exposed to 10°C. These results roughly parallel fast decay periods (e.g. summer and grassland areas) and slow decay periods (e.g. winter and riparian areas) as low and high survival of oocysts, respectively. Thus given the accuracy of this assumption, it is recommended that it be used in the future application of the PCB to permit more accurate predictions of pathogen loads.

Power *et al.* (2005) found that *Cryptosporidium* oocyst shedding from eastern grey kangaroos ranged from below 20/g faeces to as high as 2.0 $x10^6$/g faeces (Power *et al.* 2005). Taking into account the worst case scenario, the total volume of eastern grey kangaroo faecal material that may enter surface waters of the catchment was estimated as a maximum of 250 $kgkm^{-2}day^{-1}$. Using data on the number of oocysts shed from kangaroos, this quantity of faecal material may contain between 5 and $1 \times 10^{13}$ *Cryptosporidium* oocysts. If this scenario ever eventuated, it would be more likely to occur in the cooler months of the year when faecal decay was determined to be slowest, and following high rainfall facilitating the transport of oocysts.

Previous research has explored the transport and fate of *Cryptosporidium* oocysts in watershed environments (Davies *et al.* 2004, Davies *et al.* 2005, Ferguson *et al.* 2007b). Under simulated rainfall events, these studies demonstrated that oocysts would move further distances if deposited on bare soils and slopes compared with grassy and flatter areas. Ferguson *et al.* (2007) also showed that *Cryptosporidium* oocysts were transported from one-week old cow faecal pats, albeit at a lower intensity than if not locked up in faeces. In the context of the current study, transport of *Cryptosporidium* oocysts from eastern grey kangaroo faecal material may be potentially high in riparian habitats for three reasons. Firstly, most soils in this habitat are bare due to the blanketing nature of Swamp Oak Forest. Secondly, the coarse sandy soils present in the riparian habitat would be less conducive to oocyst infiltration and thus would promote prolonged inactivation at the surface (Davies *et al.* 2005). Thirdly, riparian habitats are naturally down slope of the grassland habitats which contained the highest faecal loads. The latter effect would be exacerbated in winter, when the highest density of faecal material was deposited in the grassland habitat and pasture biomass was lowest, thus reducing the vegetative buffer between this habitat and receiving waters. Slack-water deposits containing eastern grey kangaroo faecal material were commonly observed after high rainfall events (in excess of 20 mm in a 24 hour period) during this period, thus corroborating this view.

Faecal-oral route diseases, such as those caused by *Cryptosporidium* and *Giardia*, are commonly transmitted to conspecifics from the host through the ingestion of faecal material during foraging. Herbivores are forced to graze faeces-contaminated grass swards in both agricultural and natural conditions (Hutchings *et al.* 2002, Judge *et al.* 2005). As a result, there exists the potential for inter-species transmission between wildlife species and wildlife and domestic species. However, a recent study has suggested that eastern grey kangaroos demonstrate aversion of faecal material by avoiding areas contaminated with faeces from conspecifics and choosing forage of lower quality and reducing nutrient uptake (Garnick *et al.* 2010). Garnick *et al.* (2010) also found that eastern grey kangaroos were more likely to move outside areas contaminated with faecal material to forage. The subsequent increase in foraging path would potentially increase the spread of disease/pathogens at a landscape scale, which in turn would be reflected in an increase in home range size (see Chapter 5). This pattern would be more pronounced during winter in the current study, as there was a higher concentration of eastern grey kangaroos foraging in the grassland habitats at this time and animals would have to range further to avoid areas contaminated with faecal material.

The closure of artificial watering points has been used as a population control technique for macropodid species, but with mixed success (Fukuda *et al.* 2009). Artificial watering points that are present in the study area are a relic of former farming practices, and have no doubt increased the carrying capacity of the area for wild herbivores such as eastern grey kangaroos. Faecal accumulation surrounding the Jooriland farm dam was much higher compared with the riparian habitat. Consequently, in terms of zoonotic disease management in the watershed, the presence of artificial watering points may potentially reduce the faecal loads present in close proximity to surface waters, and lower the zoonotic disease risk originating from this species and other species of zoonotic disease risk such as feral pigs (*Sus scrofa*). Mitigation of kangaroo numbers adjacent to stored surface water may be necessary in the future but careful consideration is required before passive population control measures of eastern grey kangaroos, such as closure of artificial watering points, are implemented.

Common wombats are known to harbour *Cryptosporidium* and *Giardia* (Cox *et al.* 2005, Power 2010), thus the high quantities of wombat faecal material in riparian habitats poses a potential zoonotic disease risk. Common wombats deposited more faecal material in the riparian habitat than eastern grey kangaroos, which is probably a direct consequence of the high number of wombats in this habitat rather than their requirement to drink (Jarman and Evans 2010). Roger *et al.* (2007), through the use of landscape models, predicted that most common wombat burrows occurred adjacent to watercourses and stream bank gullies, which is in accord with this study. Jarman and Evans (2010) concluded that common wombats could obtain most of their free water requirements from common pasture species instead of drinking. In addition, the riparian habitat has a much larger foraging niche for wombats in comparison to eastern grey kangaroos, as wombats consume roots of trees and shrubs, in addition to native grasses, sedges and rushes

(Rishworth *et al.* 1995). Further research into the animal densities, pathogen excretion, faecal deposition rates and decay are required to formulate pathogen models for this species. In addition, simulated trials of pathogen decay in the habitats used in this study would be another factor worthy of more research.

## CONCLUSION

The faecal deposition results provided in this chapter are pivotal to assessing the zoonotic disease risk of eastern grey kangaroos in Sydney's water supply catchment. The quantity and distribution of eastern grey kangaroo faecal material in close proximity to the Wollondilly River has been described. The results suggest that eastern grey kangaroos contribute stable quantities of faecal material in the study area and that deposition and accumulation rates are driven by a suite of temporal and spatial factors, such as pasture biomass and quality, faecal decay rates and animal density. The lower eastern grey kangaroo faecal loads in riparian habitat compared with the grassland and woodland habitats is an important finding with respect to management of water quality since it indicates that the likelihood of direct deposition of faecal material from this species is low.

The number of faecal pellets deposited in each habitat was related directly to forage biomass and grazing preferences by eastern grey kangaroos, with previous studies showing that this species primarily defecates whilst actively feeding. Consequently, the low eastern grey kangaroo faecal loads present in the riparian habitat appear to be a direct result of the low pasture biomass. Conversely, the interchange between the peaks exhibited in faecal loads present in the woodland and grassland habitats are due to the presence of available forage and balancing the requirement for shelter and nutrient uptake.

The relationship between the presence of eastern grey kangaroo faecal material and pasture biomass provides some insight into potential management options that may be utilised to mitigate the waterborne zoonotic disease risk and other impacts that this species may have on biodiversity. These include a reduction in the amount of the grassland habitat through revegetation of Box Gum Woodland and Alluvial Floodplain Forest (riparian habitats), an increase in the vegetative buffer along riparian corridors to encompass both the area adjacent to surface water and the top of the bank, the continuation of alternative water sources that are present in the study area, and the creation of more artificial water points at some distance away from surface waters of the catchment. This will ensure both a sustainable reduction in the density of eastern grey kangaroos and deposition of faecal loads from this species in close proximity to the drinking water supply.

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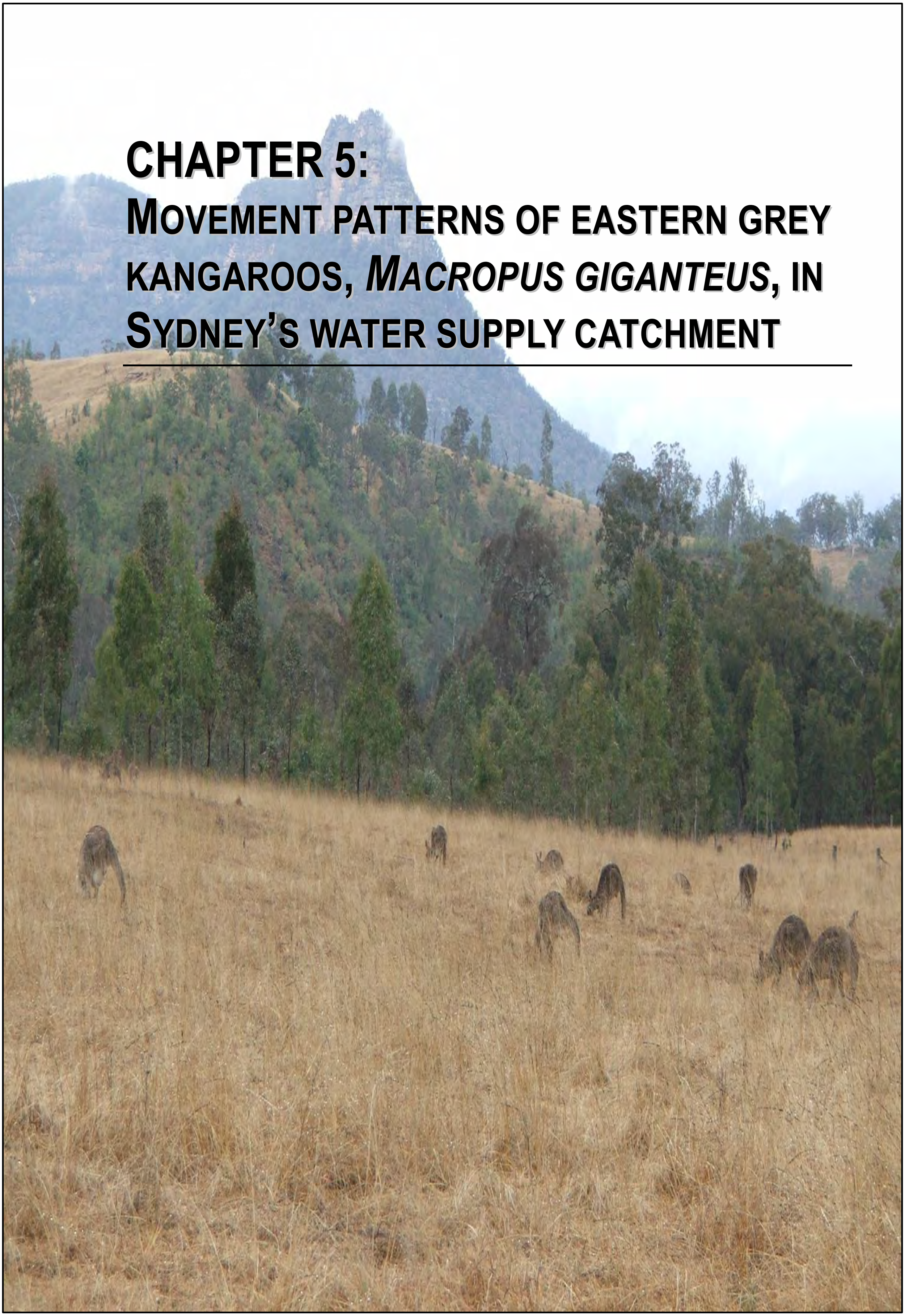
# CHAPTER 5:
# MOVEMENT PATTERNS OF EASTERN GREY KANGAROOS, *MACROPUS GIGANTEUS*, IN SYDNEY'S WATER SUPPLY CATCHMENT



# CHAPTER 5: MOVEMENT PATTERNS OF EASTERN GREY KANGAROOS, *MACROPUS GIGANTEUS*, IN SYDNEY'S WATER SUPPLY CATCHMENT

## *Preamble to Chapter 5*

The spatial distribution and landscape utilisation of wildlife disease hosts is vital information required for assessing zoonotic disease risks. In Chapter 2, I established that eastern grey kangaroos residing adjacent to the Wollondilly River, a major source of Sydney‟s potable water, were host to four zoonotic diseases including organisms responsible for two faecal-route diseases: *Cryptosporidum* and *Giardia*. In Chapter 3, densities of the kangaroo population were found to be very high and seasonally stable. In Chapter 4, the deposition and distribution of faecal material originating from this species with respect to surface water was determined.

This chapter first aims to describe movement patterns and habitat selection of eastern grey kangaroos in Sydney‟s water supply catchment. I then use this information to infer the influence of movements on the distribution of kangaroo excreta material. I also discuss the lack of scientific certainty about parameters used to predict pathogen loads from this species, such as animal‟s access to streams and the potential for direct faecal deposition to surface water. The information on movement patterns and habitat utilisation collected in this chapter will allow water utility managers to identify catchment processes that either contribute to or reduce pathogen loads. These data will inform landuse managers where management options will be most effective in reducing pathogen contamination of the potable water supply.

## INTRODUCTION

Emerging infectious diseases are a key threat to public health, with many caused by zoonotic pathogens of wildlife origin (Daszak et al. 2000, Jones et al. 2008). Ebola, SARS, West Nile virus, Hantavirus, Avian Influenza and Lyme disease are some of the recently emerged zoonotic diseases (Dizney 2008). Other examples in Australia include the transmission of Hendra virus and Lyssavirus from Grey-headed Flying Foxes to horses and humans (Field 2009). These examples highlight that the spatial distribution and landscape utilisation of wildlife disease hosts, coupled with the associated environmental distribution of their excretory products in relation to humans and livestock, are critical factors in the assessment of zoonotic disease risk (Boehm *et al.* 2008). Any variations expressed in the movement patterns of a wildlife disease host can have broad implications for disease spread, transmission and the implementation of safeguards to prevent re-infection.

*Cryptosporidium* and *Giardia* are zoonotic pathogens that are capable of causing gastrointestinal illness in human and other mammalian populations. The illness is relatively short-lived, but it is debilitating in healthy subjects when clinical symptoms are apparent and can cause mortality in immunocompromised hosts (Woodall 2009). The transmissive form of these organisms (oocyst and cyst, respectively) travels in surface waters, either directly entering streams through sewage discharge or indirectly from the overland transport of contaminated faecal material during or after high rainfall events from land grazed by livestock or wildlife (Davies et al. 2004, Ferguson et al. 2004, Ferguson et al. 2007b). *Cryptosporidium* is considered to be more of a concern to water management authorities than *Giardia* due to its higher environmental resilience and resistance to conventional water treatment processes such as chlorination (Reynolds *et al.* 2008).

In 1998, high concentrations of *Cryptosporidium* and *Giardia* were detected in Sydney"s drinking water distribution system, following an extended period of higher than average rainfall (Hrudey and Hrudey 2004). A subsequent inquiry ("McClellan Inquiry") concluded that there were several potential sources of contamination including, cattle, residential developments and free ranging animals inhabiting the inner catchment lands (McClellan 1998). The inquiry suggested that several potential wildlife hosts could be carriers of these parasites, namely feral deer, pigs, goats, wild dogs, feral cats, foxes, horses and cattle, as they were all known to *"infest*" parts of the catchment (McClellan 1998). In addition, McClellan specified in his inquiry that native animals, such as kangaroos could also be a source of these pathogens (McClellan 1998, Stein 2000).

Ferguson et al. (2007a) developed a pathogen catchment budget (PCB) model to describe the processes affecting the generation, fate and transport of pathogens originating from these sources within Sydney‟s hydrological catchment. Twenty-seven variables were used to describe the processes that influence pathogen loads including the number of people in urban centres connected to sewage treatment plants (STPs), the stocking rates of domestic animals, densities of free ranging animals, the volumes of their excreta, and the probability of their access to streams (Ferguson *et al.* 2007a). Fixed default values were chosen for these factors for each animal type and hence the variation in pathogen loads generated by these dynamic processes was potentially underestimated (Fraser et al. 1998, Ferguson et al. 2007a). The uncertainty associated with the "access to streams" factor in the PCB model is particularly problematic for free ranging wildlife hosts since natural processes and age and sex differences, amongst others, govern variations in their movement patterns.

The behavioural ecology of free ranging animals in drinking water catchments plays a significant role in determining levels of the pathogen loads that enter receiving waters (Ferguson 2005). Several aspects are particularly important. Firstly, the movement patterns and home range of the target species define the area it occupies in relation to water courses in their normal activities of feeding, mating and caring for young. Secondly, range size and dispersal rates influence the concentration and distribution of faecal material across the watershed. Thirdly, habitat preferences of the host in relation to the riparian zone indicates the rate of direct faecal deposition into streams (Collins *et al.* 2007), and also provide a risk rating for other preferred habitats by assessing their distance from raw drinking water and the potential for them to trap pathogens (Davies et al. 2003). Finally, this information can be used collectively to develop control campaigns for species infected with the pathogen (Woodroffe *et al.* 2009) or mitigation measures to reduce pathogen loads delivered to surface waters.

The eastern grey kangaroo (*Macropus giganteus*) is the most abundant free-ranging large mammal in Sydney‟s water supply catchment. This species is capable of excreting relatively high numbers of *Cryptosporidium* oocysts and *Giardia* cysts (Cox et al. 2005, Power et al. 2005), especially where animals inhabit former pastoral areas in close proximity to source drinking water. Previous studies of this species have found that home range varies as a function of productivity (Table 5-1). The larger range sizes are associated with drier locations in the west of the geographical range (McCullough and McCullough 2000) and, conversely, the smaller ranges are often found in the mesic and more productive locations in the east (Moore et al. 2002, Viggers and Hearn 2005). Some populations of this species are relatively sedentary (Jaremovic and Croft 1987), whereas others are nomadic (Jarman and Taylor 1983). Adult females often exhibit high degrees of site fidelity, whereas males have much larger home ranges and dispersal patterns (Jarman and Taylor 1983). The eastern grey kangaroo is principally a grazing animal, exhibiting preferences for areas that have an adequate supply of grasses and forbs (Taylor 1984, Moore et al. 2002, Poole 2002), with adjoining woodland and forest with sufficient lateral cover for protection from predators and for resting during the middle of the day (Coulson 2009).

**Table 5-1 Home range sizes of eastern grey kangaroos from published literature**

| Location | Landscape | Mean range size (ha) | Method[B] | Reference |
|---|---|---|---|---|
| Canberra, ACT | Agricultural/open woodland | 43.2[A] | 95% MCP | Viggers and Hearn (2005) |
| Melbourne, VIC | Agricultural/open woodland | 42.9[A] | 95% MCP | Moore *et al*. (2002) |
| Cool temperate, NSW | Open forest | 30.1 F, 23.2 M | 95% Ellipse | Jaremovic and Croft (1987) |
| New England Tablelands, NSW | Agricultural | 430 F, 470M | 90% MCP | Jarman and Taylor (1983) |
| Semi-arid, NSW | Agricultural | 528 F, 1117 M | 95% Kernel | McCullough and McCullough (2000) |

Notes: A – Home ranges were measured from females only; B – method of home range estimation MCP – minimum convex polygon; F – female, M – male

The movement patterns of eastern grey kangaroos residing in Sydney‟s water supply catchment need to be understood to ensure that pathogen load calculations for this species are accurate. The faecal deposition and distribution of this species (Chapter 4), coupled with animals‟ access to streams cannot be considered fixed, as they are determined by movement and dispersal patterns as well as landscape utilisation. As such, this information needs to be incorporated into PCB models to gain a clearer understanding and interpretation of faecal loads in areas where disease transmission to the watershed is highly likely and thus to predict the pathogen loads originating from this species. Therefore, the aims of this chapter were to:

1) Investigate the seasonality and gender differences in home range of eastern grey kangaroos inhabiting the watershed,
2) Examine the extent of site fidelity and dispersal to estimate the time spent by eastern grey kangaroos in watershed areas versus other areas where the risk of disease transmission to the water supply would be significantly reduced; and,
3) Assess the preferences exhibited by eastern grey kangaroos for available habitats near the potable water supply and in particular, any preferences for high disease transmission risk areas such as riparian zones, to infer the potential for direct faecal deposition into surface waters.

It was also of some interest to compare home ranges and movements of eastern grey kangaroos in the study area with these reported in studies elsewhere.

## MATERIALS AND METHODS

### *Study area and capture methodology*

The study area and capture methodology are described in Chapter 1 and Appendix A, respectively. Briefly, for this component of the study, thirty-four adult eastern grey kangaroos were captured using a tranquiliser firearm at three sites on the Wollondilly River within Sydney‟s hydrological catchment. Twenty-two of these were radio-collared from Jooriland and Murphy‟s Crossing and 12 were GPS-collared from these sites and Douglas Scarp. These sites were chosen for this component of the study, as they comprised representative sections of the major habitats present in Sydney‟s water supply catchment in close proximity to the Wollondilly River.

### *Radio telemetry and GPS data-loggers*

Between June 2005 and November 2006, 22 adult eastern grey kangaroos, comprising 15 females and 7 males, were fitted with VHF radio-collars (Plate 5-1 a) (Sirtrack, Havelock North, New Zealand) and tracked in the Murphy‟s Crossing and Jooriland areas. The collars operated between the frequencies 150 – 151 MHz, emitting an individual signal at 40 pulses $min^{-1}$ with a battery life of approximately 24 months. The number of animals radio-tracked at any one time varied from nineteen to twenty-one due to animal dispersal or mortality.

Eastern grey kangaroos were located using a hand-held collapsible three element Yagi antenna and a portable TR2 receiver (Telonics, Mesa, Arizona, USA). Directional signals were recorded using a hand-held compass (Suunto, Finland) from pre-determined radio-tracking stations and, when resources permitted, two observers were used to record bearings simultaneously (Plate 5-1 b). These stations were generally 200 m apart and at high elevations in the landscape to maximise reception and reduce signal bounce. Locations of individual animals were determined by triangulating between 3-5 bearings, and if necessary additional bearings were recorded if they were found to not intersect at approximately right angles (White and Garrott 1990). Stations were at least 100 m from radio-collared kangaroos to ensure that they were not disturbed during the tracking period. Bearings were taken as quickly and as accurately as possible (White and Garrott 1990), so that for each eastern grey kangaroo the collection of all bearings took approximately 10-15 minutes. Radio fixes were taken at four different times of the day (06.00 h, Midday, 18.00 h and Midnight) for 6 days each month for approximately 12 months. LOAS (Location Of A Signal; Ecological Software Solutions, 1998-2005, USA) was used to determine locations of individual animals by calculating the centre point of 95% error ellipses. Points were rejected if there was a significant departure from the 95% ellipse error ellipse. Using the Lenth estimator available in LOAS, the average size of the 95% error ellipse was less than 0.01 $km^2$. Signals were validated by placing two transmitters at known locations; one in open grassy areas and one in the riparian zone of the Wollondilly River. Repeated bearings (n =10 fixes) were taken from various stations surrounding the study area and the average error for each transmitter was determined to be 50.9 and 95.6 m for the open grassy and riparian areas, respectively.

GPS data-logger collars were used to supplement radio-tracking, as they can collect data continuously day and night for long periods, making them potentially much more accurate and less resource-dependent than VHF radio-collars. Six animals, 3 males and 3 females, were fitted with automated GPS collars (Sirtrack, Havelock North, New Zealand) on two occasions (totalling 12 animals) from June to September (winter) and September to November (spring), 2006. These collars were pre-programmed to record the animal"s location every 45 minutes, with a battery life of approximately 60 days. The collars also comprised a VHF assembly, operating in the frequency range of 150-151 MHz and emitting an individual signal of 40 pulses $min^{-1}$. A mortality sensor was added to each transmitter, whereby a rate of 80 pulses $min^{-1}$ was emitted if there was no movement after 24 hours. After approximately 60 days, each animal was radio-tracked; its location determined and recaptured to remove the GPS collar.

The GPS receiver had an accuracy of ± 6 metres R50 CEP. The R50 CEP infers that 50% of the locations were within a circle of ± 6 metres in diameter. Erroneous locations were identified by the dilution of precision (DOP) value provided with each fix. High DOP values (greater than 12) indicated poor satellite geometry and these location fixes were removed from the analysis. The high precision and the ability to identify incorrect location estimates indicated that differential correction of GPS locations was unnecessary.

(a)

(b)

**Plate 5-1** **(a) Captured eastern grey kangaroo fitted with VHF radio-transmitter and (b) Triangulation procedure from vantage points in the study area**

### *Home range delineation*

Location estimates of radio and GPS-collared animals were modelled using the ADEHABITAT function of the program R(R Development, Vienna, Austria, 2007) to calculate bivariate normal kernel (using the least squares cross validation LSCV smoothing factor) and minimum convex polygon (MCP) home range sizes. The kernel estimator mathematically converts the location co-ordinates into contour lines or areas with varying probabilities of utilisation (Worton 1989). The MCP is formulated by connecting the outermost points to form a convex polygon. As the MCP is the most commonly used home range estimator, it also served as a comparison with other kangaroo home range studies. Isopleths of 95% and 50% were calculated for both models to represent home and core ranges respectively.

Home ranges were calculated for all seasons for radio-collared kangaroos and in winter and spring for GPS-collared kangaroos. These two groups were analysed separately to avoid any potential bias that might arise from pooling such different methods. The minimum number of locations required to achieve stability in the home range estimates was assessed by plotting home range area against the number of locations to examine the asymptote (i.e. area observation-curve, after Harris *et al*. (1990). All efforts were made to obtain at least 30 location estimates per animal to achieve stable kernel home ranges (Seaman *et al.* 1999). However, this process was used as a guide because the home range size for some individual eastern grey kangaroo did not completely stabilise even when the number of estimated locations exceeded 1000.

Data were log transformed after inspection of residual plots revealed skewed distributions for both the home and core ranges. A repeated measures analysis of variance (ANOVA) was used to detect any seasonal effects on home range and core range size. Similarly, ANOVA was employed to test for differences in male and female range sizes. Post-hoc Tukey HSD tests were utilised to identify the source of differences in comparisons yielding significant results, with significance taken as $\alpha \leq 0.05$.

### *Site fidelity*

The percentage overlap of seasonal home range (winter: June to August; spring: September to November; summer: December to February and autumn: March to May) for each individual was used to measure the site fidelity exhibited by radio-collared eastern grey kangaroos (Kernohan *et al.* 2001). Seasonal home ranges were paired and their overlap was calculated as follows: *[(the percentage of the home range of season$_1$ that overlaps the home range of season$_2$) + (the percentage of the home range of the season$_2$ that overlaps the home range of season$_1$)]/2*. Percentage data were transformed using arcsine square-root to test for differences between seasons and between sexes using ANOVA. Post-hoc Tukey HSD tests were applied to distinguish any significant results. Overlap of seasonal home ranges was determined using the Patch Analyst Tool in ArcView 3.2 (ESRI, Redlands CA, USA).

### *Habitat selection*

To assess habitat selection, it was first necessary to define the limits of the study area used by the study population of kangaroos. This was described by a composite 95% kernel home range of all collared kangaroos, following the recommendations of Sawyer *et al* (2006). The percent coverage of habitats within this composite home range was determined from vegetation maps (NPWS 2003) and by using the Patch Analyst Tool in ArcView 3.2. The errors of fixed radio-collars were incorporated into this analysis and were applied as buffer distances around the linear and patchy habitats. As for the home range delineation, habitat selection was analysed separately for radio-collared and GPS-collared individuals. The composite home ranges of collared kangaroos initially comprised a total of six habitat types; for analysis these were then collapsed into the following four habitats (with corresponding NPWS (2003) vegetation community) based on percentage cover:

1) **Grassland** (Cleared/Modified Lands) – This habitat comprised large areas of grass devoid of canopy tree species, and former farmland (Plate 5-2 a). There were occasional Forest Red Gum (*Eucalyptus tereticornis*) and Grey Box (*E. moluccana*) trees, as well as juveniles of the latter species planted in small rows for regeneration. The native groundcover species consisted of Kangaroo grass (*Themeda australis*), Weeping grass (*Microlaena stipoides*), Sprawling Bluebell (*Wahlenbergia gracilis*), Open summer grass (*Digitaria diffusa*) and *Danthonia* spp. The exotic weeds included Serrated Tussock (*Nassella trichotoma*), Chilean Needle Grass (*Nassella neesiana*), Common couch (*Cynodon dactylon*), Tall Fleabane (*Conyza* spp), Cobblers Pegs *(Bidens pilosa)* Stinking Roger (*Tagetes minuta*). This habitat had the highest percentage cover in the study area, occupying 65.1% of the ranges of radio-collared kangaroos and 58.5% of the ranges of GPS-collared animals.

2) **Open Woodland** (Devonian Red Gum- Ironbark Woodland, Devonian Red-Gum – Grey Box Woodland) – This vegetation complex contains the White Box-Yellow Box-Blakely"s Red Gum Woodland (Box-gum Woodland) Endangered Ecological Community which is listed under the NSW *Threatened Species Conservation Act* 1995 (TSC Act) and is Critically Endangered under the Commonwealth *Environment Protection and Biodiversity Conservation Act* 1999 (EPBC Act) (Plate 5-2 b). This habitat was highly fragmented within the study area and comprised a mixture of low and tall open woodland dominated by Forest Red Gum (*E. tereticornis*), Grey Box (*E. moluccana*), Yellow Box (*E. melliodora*) and White Box (*E. albens*). There was a low tree layer consisting of Port Jackson Fig (*Ficus rubiginosa*), Hickory Wattle (*Acacia implexa)* and Kurrajong (*Brachychiton populneus* subsp. *populneus*). The understorey encompassed a shrubby layer of Wallaby Weed (*Olearia viscidula*) and Blackthorn (*Bursaria spinosa*) and a dense and diverse ground layer of Kidney Weed (*Dichondra repens*), Rough saw-sedge (*Gahnia aspera*) and Spiny-headed Mat-rush (*Lomandra longifolia*), Barbed-wire Grass (*Cymbopogon refractus*), Purple Wire Grass (*Aristida ramosa*), *Microlaena stipoides,* Threeawn Speargrass (*Aristida vagans*), *Oxalis perennans,* Hairy Panic (*Panicum effusum*), Small St John"s Wort (*Hypericum gramineum*), *Digitaria diffusa,* Paddock Lovegrass (*Eragrostis leptostachya*) and Forest nightshade (*Solanum prinophyllum*). Minor differences were noted in this habitat with changes in elevation. This habitat was predominantly located on the floodplain and formed mosaic patches surrounding the grassland habitat. This habitat covered 21.2% of the study area occupied by radio-collared kangaroos and 19.3% of the area used by GPS-collared kangaroos.

3) **Riparian Forest** (Tablelands River Oak Forest) – This habitat was dominated by a narrow ribbon of River She Oak (*Casuarina cunninghamiana* subsp. *cunninghamiana*), minor associations of various *Eucalyptus* species (*Eucalyptus tereticornis,* Thin-leaved Stringybark *E. eugenioides,* Narrow-leaved Ironbark *E. crebra,* Grey Gum *E. punctata,* and White-topped Box *E. quadrangulata*) and small trees including Sandpaper Fig (*Ficus coronata*) and Water Gum (*Tristaniopsis laurina*) (Plate 5-2 c). The understorey consisted of herbs and grasses such as *Microlaena stipoides, Dichondra repens* and *Lomandra longifolia*. This habitat was found bordering the Wollondilly River, its tributaries and major drainage lines. It covered 10.1% of the study area occupied by radio-collared individuals and 13.7% of the area used by GPS-collared individuals.

4) **Scarp Woodland** (Douglas Scarp Woodland) – This open grassy woodland has a canopy layer of Narrow-leaved Ironbark (*Eucalyptus crebra*) and Black Cypress Pine (*Callitris endlicheri*) on rocky elevated scarps (Plate 5-2 d). Coast Myall (*Acacia binervia*) makes up most of the shrub layer. The lowest stratum is scattered with herbs and grasses such as Mulga Fern (*Cheilanthes sieberi* subsp. *sieberi*), *Pomax umbellata,* and *Lomandra filiformis* subsp. *coriacea*. This habitat was least used by kangaroos, covering 2.7% of the ranges of radio-collared animals and 8.5% of the ranges of those with GPS collars.

The distribution of these communities in the study area is shown in Figure 5-1.

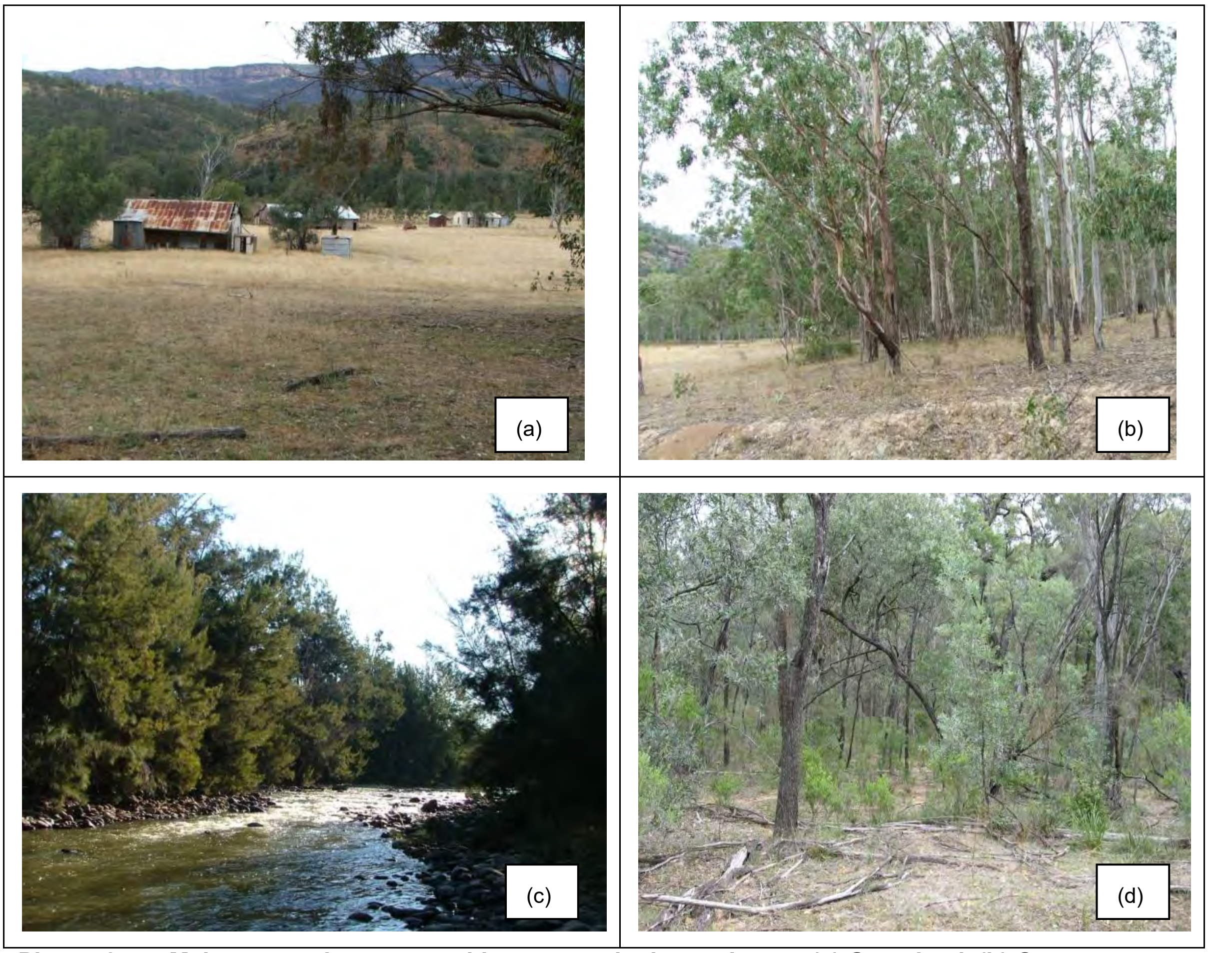



**Plate 5-2 Major vegetation communities present in the study area (a) Grassland, (b) Open woodland, (c) Riparian forest, (d) Scarp woodland**

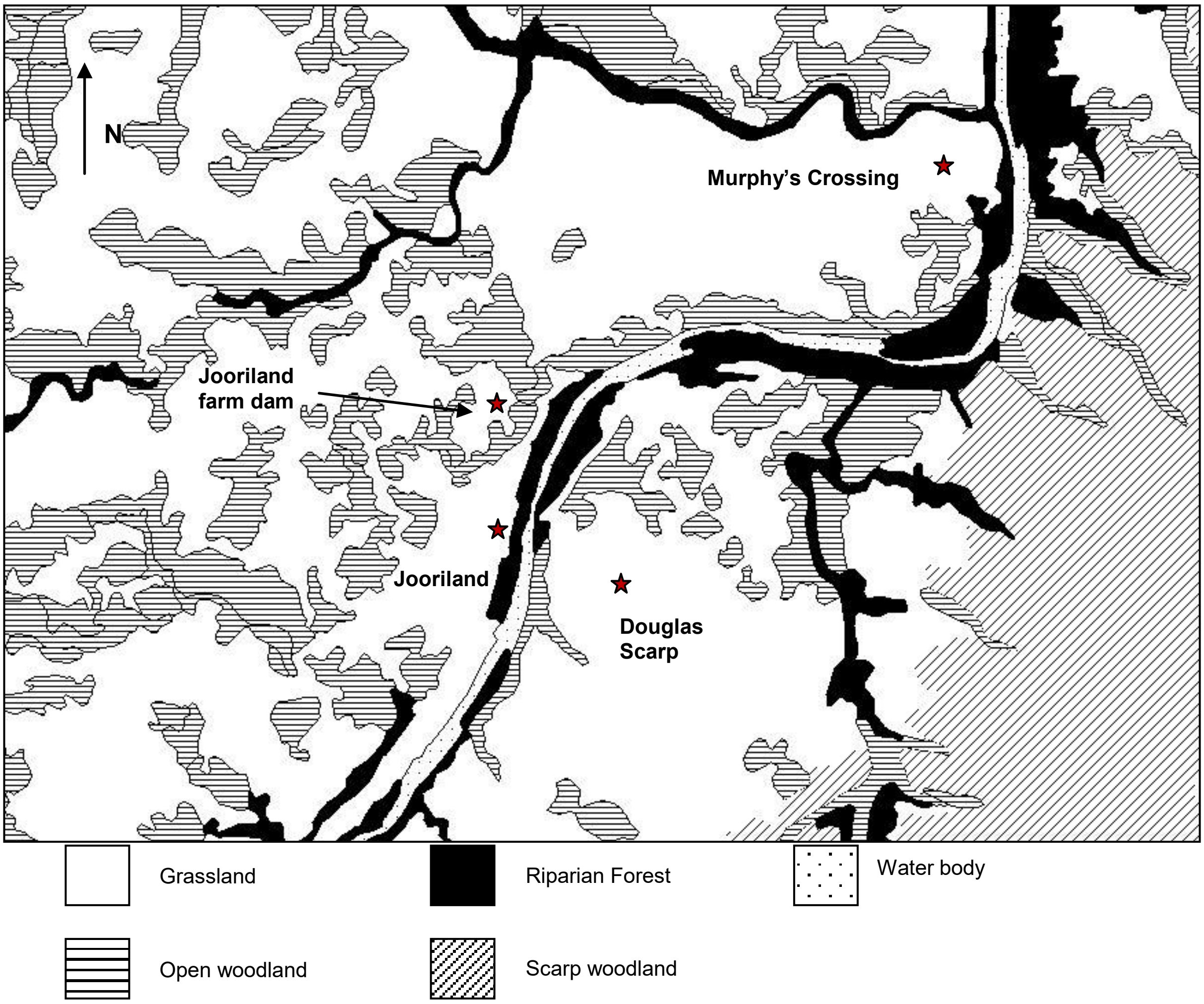



**Figure 5-1 Distribution of habitats in the study area**

Habitat preferences of the collared kangaroos were assessed at two spatial scales: (1) within the study area as a whole (the second order selection of Johnson 1980), and (2) within each individual"s home range (the third order selection of Johnson (1980)). At the second order of selection (Johnson 1980), the habitat composition within the 95% kernel for each kangaroo was compared with the habitat composition of the study area. At the third order of selection, the location for each kangaroo in each habitat type was compared with the proportion of each habitat type in the 95% kernel home range.

At these two scales of selection, compositional MANOVA analysis was used to determine whether habitat preferences differed from random ($P$<0.05) among seasons using a Wilks" Lambda test. When habitat use deviated significantly from random ($P$<0.05), compositional analysis was used to develop a ranking of habitat preference from the habitats least exploited to those most exploited (Aebischer *et al.* 1993). The preference ranking of habitats was constructed using a *4 X 4* preference ranking matrix, ranking habitat types in order of their use and testing whether the habitat type in each row was used significantly more or less than the habitat type in the column. To assess differences between ranks, paired *t*-tests were calculated to compare mean utilisation between all pairs of habitats.

The ranking of habitat preference was done by comparing the proportional use of each habitat type to a reference class k by the log transformed ratio of habitat proportions for each animal (Aitchison 1982, Dickson and Beier 2002):

$$yij = \ln(xij \;/\; xik) \;\; (i = 1, \dots., n; j = 1, \dots, D; j \neq k,$$

where $xij$ describes proportional use by individual $i$ of the *j*th of $D$ habitat types, and $n =$ number of individual animals. Using the proportional use of habitats by animals as the sample unit, rather than individual locations, it is possible to overcome issues associated with the lack of independence of sequentially recorded locations, low samples of locations and, in some situations, problems where not all of the habitats are used by every individual. When an individual"s utilisation of a habitat was 0, this value in the matrix was replaced with a number less than 0.1 times the smallest observed value for that habitat (Aebischer *et al.* 1993).These analyses were performed in *COMPANA* (R Development, Vienna, Austria, 2007). Males and females were pooled because sample sizes were too small to permit comparisons (Aebischer *et al.* 1993).

Third order habitat selection was assessed further by describing diurnal and nocturnal utilisation of habitats separately. For radio-collared animals, location estimates were divided into day (06.00 – 18.00 h) and night (18.00-06.00 h), whereas the richer data set of the GPS-collared kangaroos permitted further stratification into early morning (0.00 – 06.00 h), morning (06.00 – 12.00 h), midday (12.00 – 18.00 h) and night (18.00 – 24.00 h). Compositional analysis was performed as described previously.

To assess the utilisation of alternative water sources in the study area by eastern grey kangaroos, the home ranges of GPS-collared animals captured from the Jooriland area were examined. The proportion of locations within a 10 ha buffer of the Jooriland farm dam (Figure 5-1) was compared with the proportion of locations in riparian forest within each animal"s home range. ANOVA of arcsine-transformed data was used to assess significant differences between the utilisation of these areas.

## RESULTS

### *Home range*

A total of 94 individual home ranges were calculated from 23 radio-collared kangaroos (Figure 5-2). The seasonal stabilised mean kernel home range size for males varied from 52.9 ha in autumn to 155.3 ha in spring. Mean home range size for females ranged from 65.7 ha in summer to 116.4 ha in winter (Table 5-2). Male home range size was larger than that for females in summer ($F_{1,19}$ = 6.3; $P$ = 0.02) and autumn ($F_{1,19}$ = 8.31; $P$ = 0.01), but the home range sizes of males and females were similar in winter ($F_{1,17}$ = 0.0003; $P$ = 0.986) and spring ($F_{1,17}$ = 3.56; $P$ = 0.076). Female home range size was different between seasons ($F_{3,60}$ = 6.76; $P$ = 0.0004), with winter and autumn home ranges significantly larger than those in the other seasons. Correspondingly, male home range size also differed between seasons ($F_{3,32}$= 3.89; $P$ = 0.0006). Post-hoc Tukey HSD analysis showed that the autumn home range size was larger than that in spring ($P$ < 0.05), and the summer and winter home ranges were similar ($P$ > 0.05). Home range results using the MCP method revealed similar trends, but tended to give considerably smaller values.

Core kernel range sizes of radio-collared male kangaroos varied from 14.1 ha in spring to 30.3 ha in autumn. The mean female core range was smallest in summer (15.0 ha) and largest in winter (29.2 ha) (Table 5-2). The core range size varied seasonally ($F_{3,76}$ = 3.68; $P$ = 0.015) when pooled for all kangaroos. Male core ranges were larger than female core ranges in summer ($F_{1,19}$ = 6.71; $P$ = 0.02) and autumn ($F_{1,19}$ = 7.96; $P$ = 0.01), but not in spring ($F_{1,17}$ = 3.07; $P$ = 0.098) or winter ($F_{1,17}$ = 0.87; $P$ = 0.872). The core ranges of females differed between seasons ($F_{3,60}$ = 4.29; $P$ < 0.0001), and post-hoc Tukey HSD tests showed that the winter core range was larger than in summer and autumn, while the spring core range was similar ($P$ > 0.05). Male core range size did not differ between seasons ($F_{3,32}$ = 2.76; $P$ = 0.07), although the comparison approached statistical significance. As for the home range results, MCP core ranges yielded similar trends to the kernel procedure, but tended to give considerably smaller values.

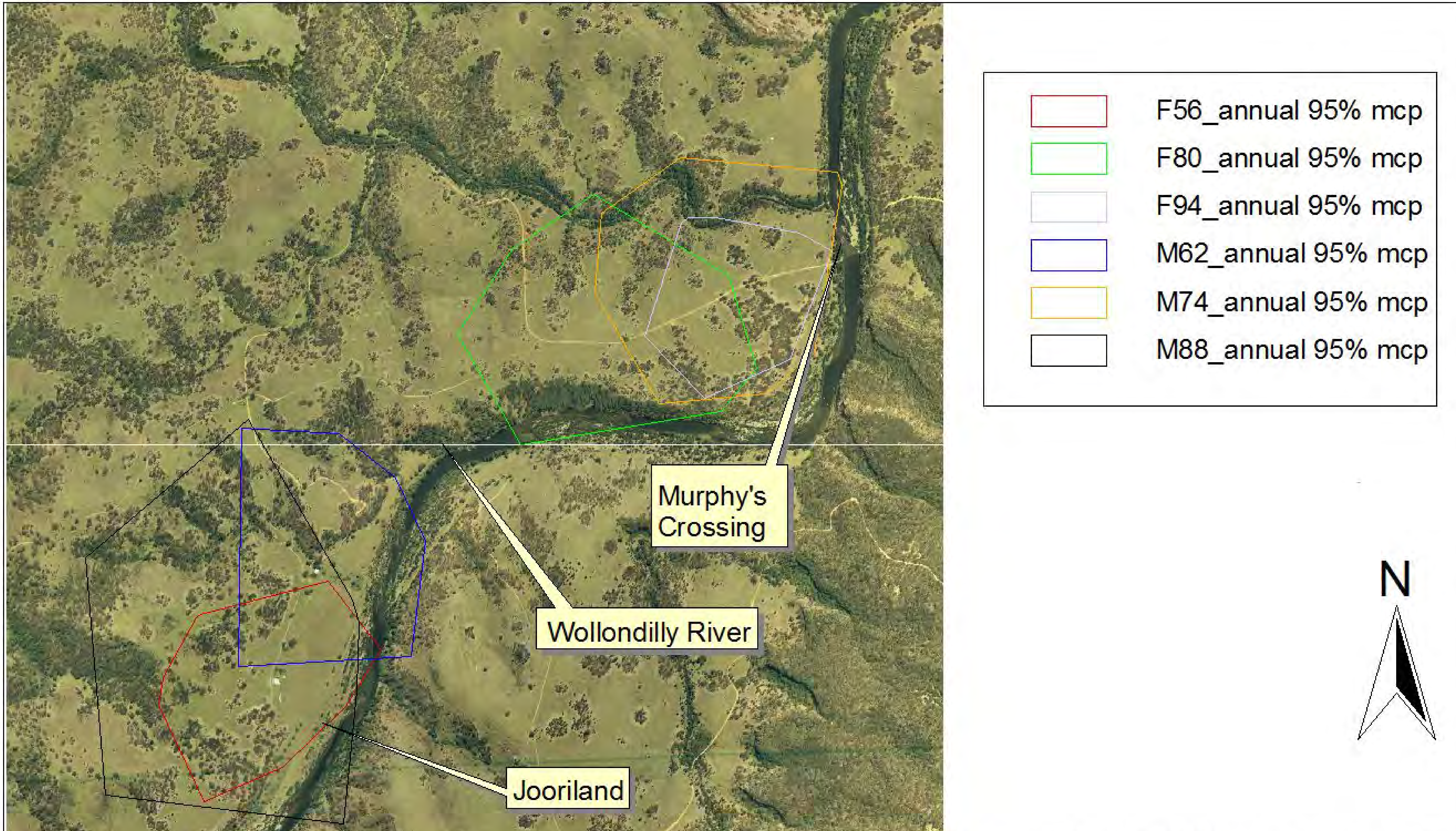



Figure 5-2 - 95% MCP home ranges of six radio collared eastern grey kangaroos

**Table 5-2 Mean (se) stabilised annual and seasonal home range sizes and core range sizes (ha) of radio-collared eastern grey kangaroos**

M - Male, F – Female; home ranges are 95% kernel (KE) and 95% minimum convex polygon (MCP); core ranges are 50% KE and 50% MCP. Radio-tracking periods were Annual (December 2005 – November 2006; n = 17), summer (December – February; n = 21), autumn (March – May; KE, n = 18; MCP, n = 17), winter (June – August; n = 19) and spring (September – November; KE n = 19, MCP n = 18).

| Season | KE home range (ha) | KE core range (ha) | MCP home range (ha) | MCP core range (ha) |
|---|---|---|---|---|
| Annual – M | 127.8 (17.8) | 23.8 (1.4) | 66.5 (12.6) | 13.6 (0.7) |
| Annual – F | 88.5 (5.4) | 16.9 (0.9) | 45.0 (2.8) | 9.4 (0.6) |
| Summer – M | 115.9 (25.8) | 24.6 (3.7) | 40.3 (8.7) | 8.4 (2.0) |
| Summer – F | 65.7 (6.1) | 15.0 (1.6) | 21.4 (2.2) | 5.8 (0.9) |
| Autumn – M | 155.3 (31.6) | 30.3 (5.3) | 31.7 (4.7) | 6.5 (0.8) |
| Autumn – F | 92.3 (7.8) | 19.5 (1.6) | 23.7 (2.2) | 4.7 (0.6) |
| Winter – M | 127.4 (40.6) | 24.7 (5.3) | 37.5 (13.2) | 9.2 (2.0) |
| Winter – F | 116.4 (16.0) | 23.2 (2.4) | 36.2 (5.0) | 7.9 (1.2) |
| Spring – M | 52.9 (6.7) | 14.1 (2.4) | 21.1 (4.5) | 6.7 (2.1) |
| Spring – F | 69.8 (5.3) | 17.9 (1.4) | 19.9 (1.7) | 5.5 (0.7) |

A total of 12 individual home ranges were calculated from GPS-collared eastern grey kangaroos (Figure 5-3, Figure 5-4 and Table 5-3). The mean kernel home range sizes of GPS- collared male kangaroos ranged from 50.5 ha in spring to 78.4 ha in winter. Mean kernel home range sizes for GPS-collared females varied from 53.5 ha in spring to 89.8 ha in winter (Table 5-3).

Home ranges did not differ between spring and winter ($F_{1,10}$ = 0.25; $P$ = 0.625). No gender difference was detected in home range size in winter ($F_{1,4}$ = 0.37; $P$ = 0.57) or spring ($F_{1,4}$ = 0.11; $P$ = 0.76). Neither male ($F_{1,4}$ = 0.90; $P$ = 0.40) nor female ($F_{1,4}$ = 0.004; $P$ = 0.98) home ranges differed between seasons. MCP results were of similar magnitude as the kernel home range estimates.

The mean kernel male core ranges varied from 9.5 ha in spring to 18.6 ha in winter and the mean female core ranges from 12.0 ha in spring to 20.5 ha in winter. Male and female core ranges were similar in both winter ($F_{1,10}$ = 0.42; $P$ = 0.55) and spring ($F_{1,10}$ = 0.60; $P$ = 0.43). Neither male ($F_{1,4}$ = 0.92; $P$ = 0.39) nor female ($F_{1,4}$ = 0.01; $P$ = 0.93) core ranges differed between seasons. MCP core range results were of similar magnitude as the kernel core range estimates (Table 5-3).

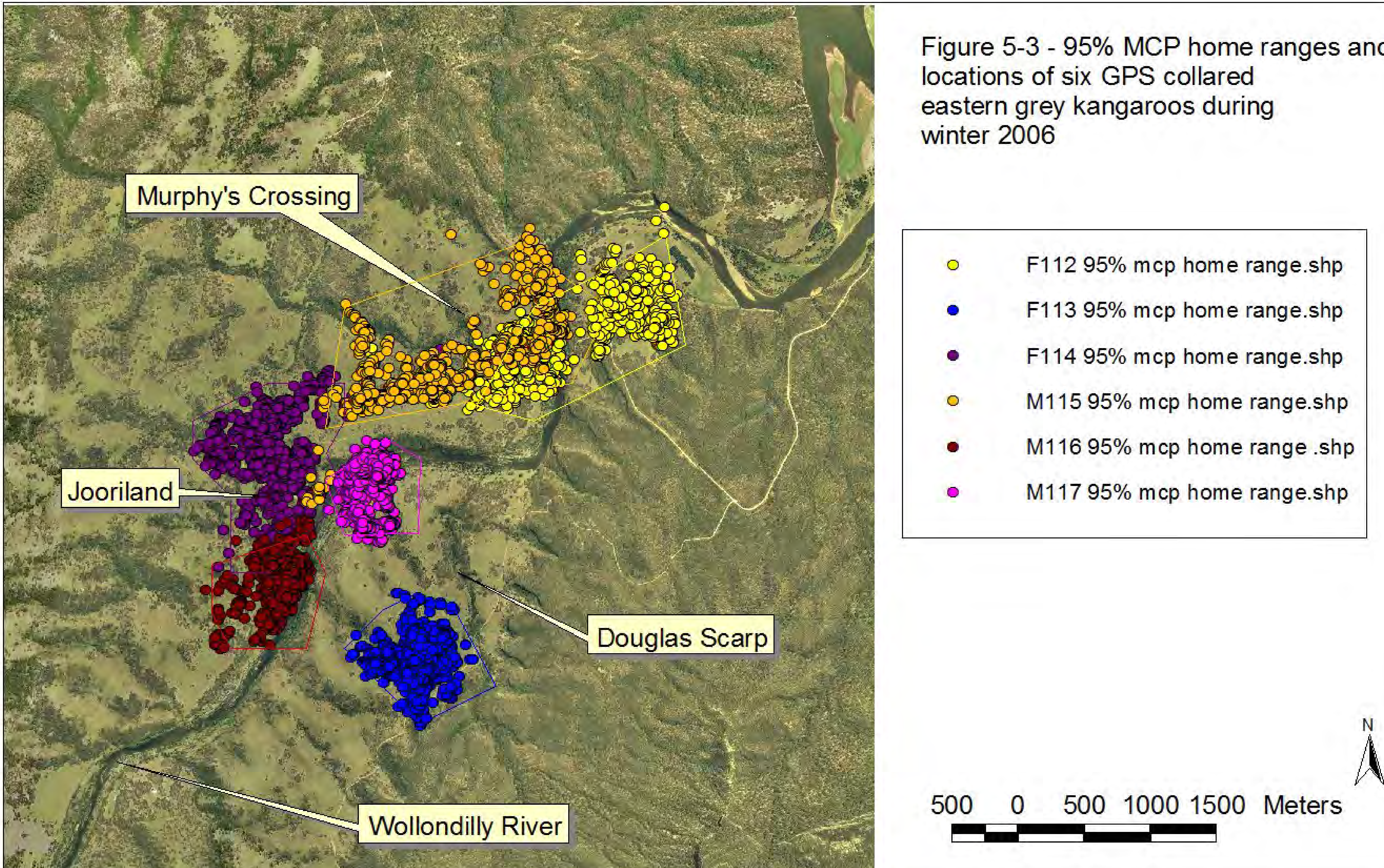



Figure 5-3 - 95% MCP home ranges and locations of six GPS collared eastern grey kangaroos during winter 2006

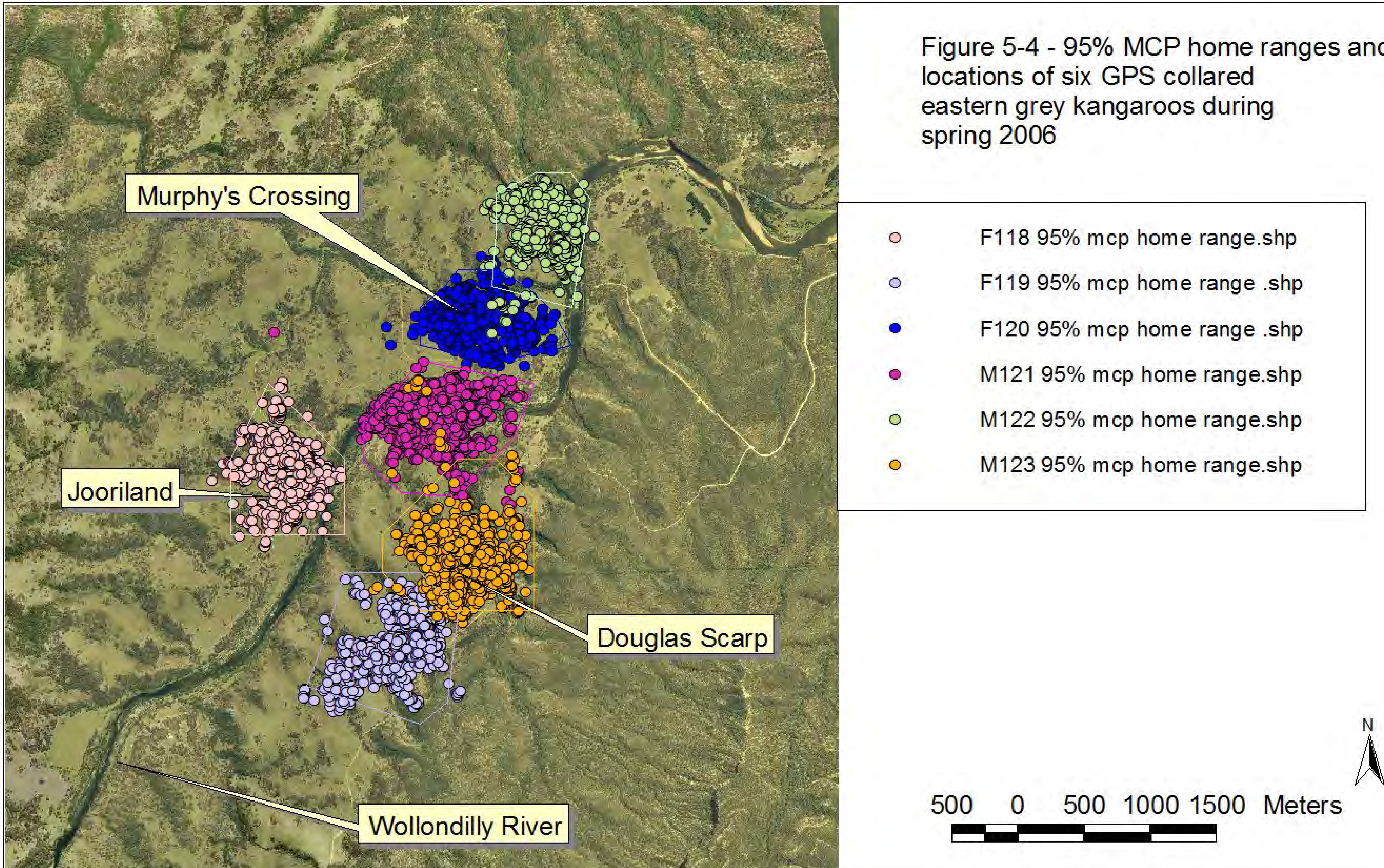



Figure 5-4 - 95% MCP home ranges and locations of six GPS collared eastern grey kangaroos during spring 2006

**Table 5-3 Mean (se) stabilised seasonal home range and core range sizes of GPS-collared eastern grey kangaroos**

M - Male, F – Female; home ranges are 95% kernel (KE) and 95% minimum convex polygon (MCP); core ranges are 50% KE and 50% MCP. GPS collars were deployed on two (2) occasions in winter (*n* = 6) and spring (*n* = 6).

| Season | KE home range (ha) | KE core range (ha) | MCP home range (ha) | MCP core range (ha) |
|---|---|---|---|---|
| Winter – M | 78.4 (53.6) | 18.6 (13.8) | 69.9 (44.9) | 29.4 (16.4) |
| Winter – F | 89.8 (30.5) | 20.5 (7.7) | 80.4 (23.6) | 29.4 (16.4) |
| Spring – M | 50.5 (7.6) | 9.5 (1.1) | 45.5 (5.4) | 10.3 (2.0) |
| Spring – F | 53.5 (6.4) | 12.0 (2.4) | 52.5 (8.4) | 14.0 (3.1) |

The kernel core and home ranges of GPS-collared eastern grey kangaroos were smaller than those of radio-collared animals for the winter and spring period. These discrepancies may relate to the smaller sample sizes (number of animals) of the GPS-collared sample compared with the radio-collared sample, the higher number of more accurate location fixes that were collected using the GPS collar technology and/or the suitability of the models used to calculate GPS-collared home ranges leading to much tighter kernel contours and restricted minimum convex polygons than the home range results derived using radio-collars (Table 5-4).

**Table 5-4 Number of location estimates used to compute home range for GPS-collared and radio-collared eastern grey kangaroo**

| Season | Mean (±SD) number of fixes per radio-collared animal | Mean (±SD) number of fixes per GPS-collared animal |
|---|---|---|
| Winter – M | 29.8 (± 8.7) | 1822.0 (± 166.3) |
| Winter – F | 30.1 (± 3.8) | 1696.7 (± 162.7) |
| Spring – M | 28.0 (± 5.0) | 1631.6 (± 548.1) |
| Spring – F | 29.0 (± 4.1) | 1963.0 (± 100.6) |

### *Site fidelity, dispersal patterns and forays*

Radio-collared eastern grey kangaroos exhibited high fidelity to their home ranges, with individual seasonal home ranges overlapping on average by 58% (Table 5-5). There was no difference between the overlap of seasonal home ranges ($F_{5,114}$ = 0.74; P = 0.59). Seasonal home range overlap for individual males was highest between winter and spring

(64.6%) and lowest between autumn and winter (48.4%). Seasonal home range overlaps for individual females were consistently high across all seasons, with the highest between autumn and spring (65.7%) and the lowest between summer and spring (59.7%).

**Table 5-5 Mean percentage overlap of home ranges of eastern grey kangaroos of both sexes combined and of males and females separately, in each of the season pairings.**

| | Percentage overlap (%) | | |
|---|---|---|---|
| **Paired season** | **Sexes combined** | **Male** | **Female** |
| Summer/Autumn | 59.8 | 48.4 | 64.2 |
| Summer/Winter | 60.8 | 50.8 | 64.9 |
| Summer/Spring | 60.6 | 63.0 | 59.7 |
| Autumn/winter | 61.0 | 48.5 | 61.5 |
| Autumn/spring | 68.1 | 60.3 | 65.7 |
| Winter/spring | 66.0 | 64.6 | 64.1 |

Dispersal by eastern grey kangaroos out of the study area was minimal. Only one male (M66) left the site and established a home range 2 km upstream of his original position. Several radio-collared animals exhibited forays away from their home ranges, but these were often short lived, with most lasting until the next tracking period. These forays were also commonly recorded from GPS-collared eastern grey kangaroos, as well as home range extensions and exploratory patterns that would not have otherwise been measured using the traditional radio tracking techniques and sampling intensity. The details of these forays are summarised in Table 5-6 and selected examples are presented in Figure 5-5 – 5-7.

**Table 5-6 Forays exhibited by GPS-collared eastern grey kangaroos**

| Individual | Maximum distance of foray (km) | Length of foray (days) | Comments |
|---|---|---|---|
| F112 | 1.3 | 2 | Home range spanned both sides of the Wollondilly River for most of the sampling period. |
| F114 | 1.2 | 0.2 | One larger foray and several smaller forays outside core home range |
| M115 | 1.1 | 0.5 | One foray to Jooriland for 12 hours between 07:50 and 19:52 |
| M122 | 0.5 | 0.5 | One foray across Jooriland river to Murphy"s Crossing between 21:50 and 0750 |

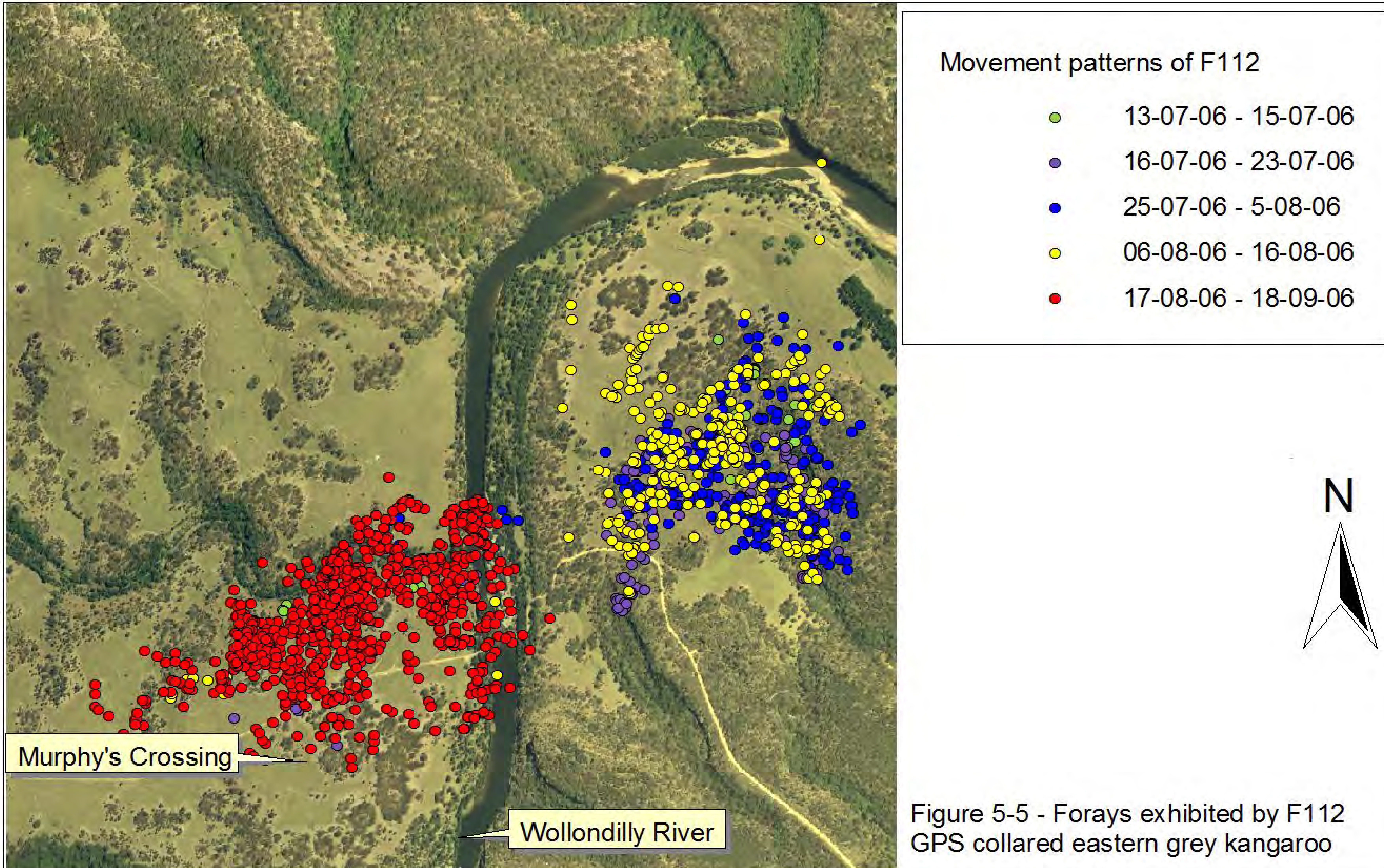



Figure 5-5 - Forays exhibited by F112 GPS collared eastern grey kangaroo

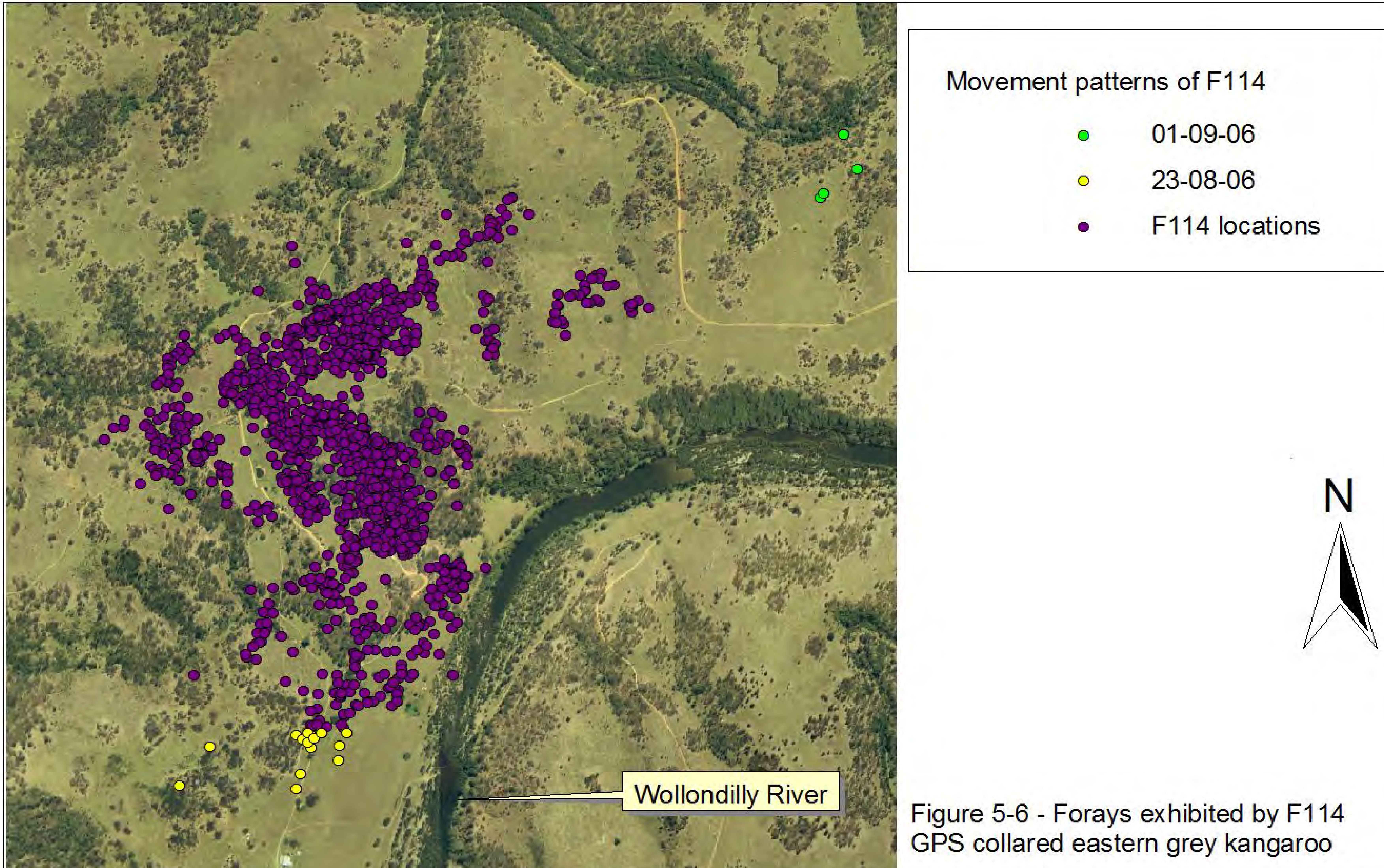


Figure 5-6 - Forays exhibited by F114 GPS collared eastern grey kangaroo

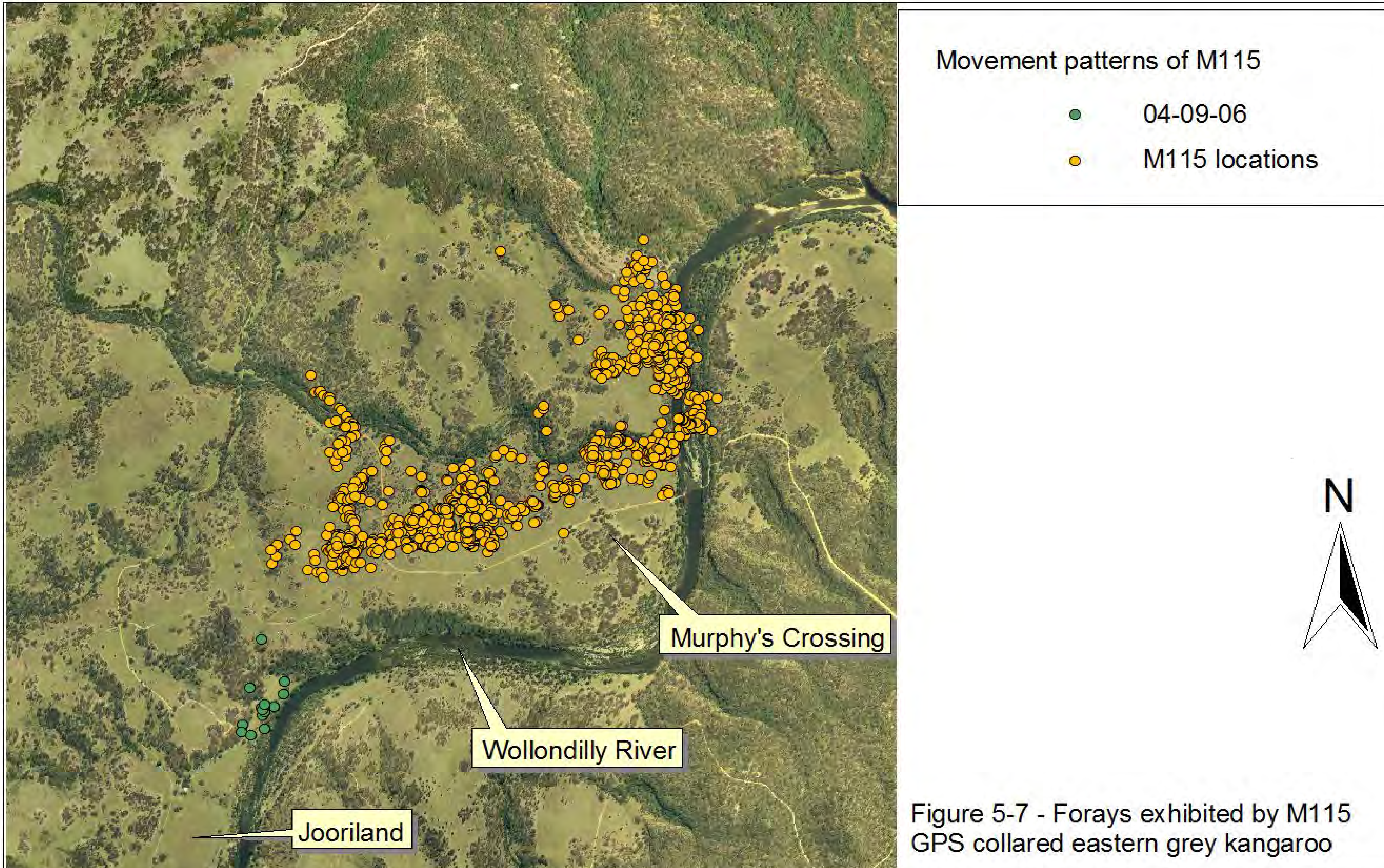



Figure 5-7 - Forays exhibited by M115 GPS collared eastern grey kangaroo

### *Habitat utilisation*

At the second order of habitat selection, radio-collared eastern grey kangaroos demonstrated significant non-random use of habitats in all seasons (summer: $\lambda = 1.62 \times 10^{-4}$; $P < 0.0001$, autumn: $\lambda = 0.036$; $P < 0.0001$, winter: $\lambda = 5.55 \times 10^{-5}$; $P < 0.0001$, spring: $\lambda = 3.84 \times 10^{-4}$; $P < 0.0001$). Compositional analysis revealed that in all seasons the grassland habitat constituted the most preferred habitat, followed by open woodland, riparian forest and then scarp woodland (Table 5-7 and Figure 5-8). The proportion of the grassland habitat occurring within individual home ranges in relation to its availability in the study area was larger than that of open woodland in spring and winter ($P < 0.05$), but not in summer and autumn ($P > 0.05$). Open woodland was preferred over riparian forest and scarp woodland ($P < 0.05$) in all seasons. Riparian forest occupied a larger area of the home range than scarp woodland in all seasons ($P < 0.05$), except in summer when the proportional difference in coverage of these two habitats in individual home ranges was negligible ($P > 0.05$). Riparian forest encompassed a small proportion of home ranges throughout the study period, but was used more frequently during autumn and winter ($F_{3, 76} = 3.29$; $P < 0.05$). Post-hoc Tukey HSD tests revealed that riparian forest was preferred more in autumn and winter when compared against summer ($P < 0.05$).

**Table 5-7 Simplified ranking matrix for radio-collared eastern grey kangaroos at the second-order of selection scale (comparing habitat composition in individual home ranges against habitat availability in the study area)**

[a] Cells in the matrix consist of mean differences in the log ratios of used and available habitats for all eastern grey kangaroos divided by the SE (i.e., t-values). At the intersection of the row i and of the column j, there is a "+" when the habitat i is used more than the habitat in the column, and "-" otherwise. When the difference is significant, the sign is tripled at a α =0.05.

[b] Rank is equal to the sum of the positive values in each row. Higher ranks indicate a more preferred habitat.

| | Habitat type | | | | |
|---|---|---|---|---|---|
| **Habitat type[a]** | **Grassland** | **Open woodland** | **Riparian forest** | **Scarp woodland** | **Rank[b]** |
| Summer | | | | | |
| Grassland | 0 | + | +++ | +++ | 3 |
| Open woodland | - | 0 | +++ | +++ | 2 |
| Riparian forest | --- | --- | 0 | + | 1 |
| Scarp woodland | --- | --- | - | 0 | 0 |
| Autumn | | | | | |
| Grassland | 0 | + | +++ | +++ | 3 |

| | Habitat type | | | | |
|---|---|---|---|---|---|
| **Habitat type[a]** | **Grassland** | **Open woodland** | **Riparian forest** | **Scarp woodland** | **Rank[b]** |
| Open woodland | - | 0 | +++ | +++ | 2 |
| Riparian forest | --- | --- | 0 | +++ | 1 |
| Scarp woodland | --- | --- | --- | 0 | 0 |
| Winter | | | | | |
| Grassland | 0 | +++ | +++ | +++ | 3 |
| Open woodland | --- | 0 | +++ | +++ | 2 |
| Riparian forest | --- | --- | 0 | +++ | 1 |
| Scarp woodland | --- | --- | --- | 0 | 0 |
| Spring | | | | | |
| Grassland | 0 | +++ | +++ | +++ | 3 |
| Open woodland | --- | 0 | +++ | +++ | 2 |
| Riparian forest | --- | --- | 0 | +++ | 1 |
| Scarp woodland | --- | --- | --- | 0 | 0 |

Although there were not enough data to analyse male and female kangaroo habitat preferences separately, there were trends to suggest that sex differences in second order selection existed (Figure 5-8). For example, males tended to utilise the riparian habitat more than females in autumn and winter and open woodland in spring and summer. In addition, there was a trend to suggest that females utilised the grassland habitat more than males.

GPS -collared eastern grey kangaroos also illustrated significant non-random utilisation of habitats at the second order scale of selection (winter: $\lambda$ = 0.04; P < 0.05, spring: $\lambda$ = 0.08; $P$ < 0.05). During winter, the grassland habitat was the most preferred habitat (range 52-82% of individual home ranges), followed by open woodland (range 9 – 30%), riparian forest (range 0-28%). The least preferred was scarp woodland (range 0-9%) (Table 5-8 and Figure 5-9). During winter, the grassland habitat was more preferred than scarp woodland ($P$< 0.05) and in spring both the grassland habitat and open woodland occupied larger areas in home ranges than scarp woodland ($P$ < 0.05). The utilisation of the riparian habitat by GPS-collared eastern grey kangaroos was similar between winter and spring ($F_{1,11}$ = 0.24, $P$ = 0.62). GPS-collared male eastern grey kangaroos tended to utilise riparian and woodland habitats more than females, and females also had a tendency to exploit the grassland habitat more than males (Figure 5-9).

**Table 5-8 Simplified ranking matrix for GPS-collared eastern grey kangaroos at the second-order of selection scale (comparing habitat composition in individual home ranges against habitat availability in the study area)**

[a] Cells in the matrix consist of mean differences in the log ratios of used and available habitats for all eastern grey kangaroo divided by the SE (i.e., t-values). At the intersection of the row i and of the column j, there is a "+" when the habitat i is used more than the habitat in the column, and "-" otherwise. When the difference is significant, the sign is tripled at a α =0.05.

[b] Rank is equal to the sum of the positive values in each row. Higher ranks indicate a more preferred habitat.

| | Habitat type | | | | |
|---|---|---|---|---|---|
| **Habitat type**[a] | **Grassland** | **Open woodland** | **Riparian forest** | **Scarp woodland** | **Rank**[b] |
| Winter | | | | | |
| Grassland | 0 | + | + | +++ | 3 |
| Open woodland | - | 0 | + | + | 2 |
| Riparian forest | - | - | 0 | + | 1 |
| Scarp woodland | --- | - | - | 0 | 0 |
| Spring | | | | | |
| Grassland | 0 | + | + | +++ | 3 |
| Open woodland | - | 0 | + | +++ | 2 |
| Riparian forest | - | - | 0 | + | 1 |
| Scarp woodland | --- | - | - | 0 | 0 |

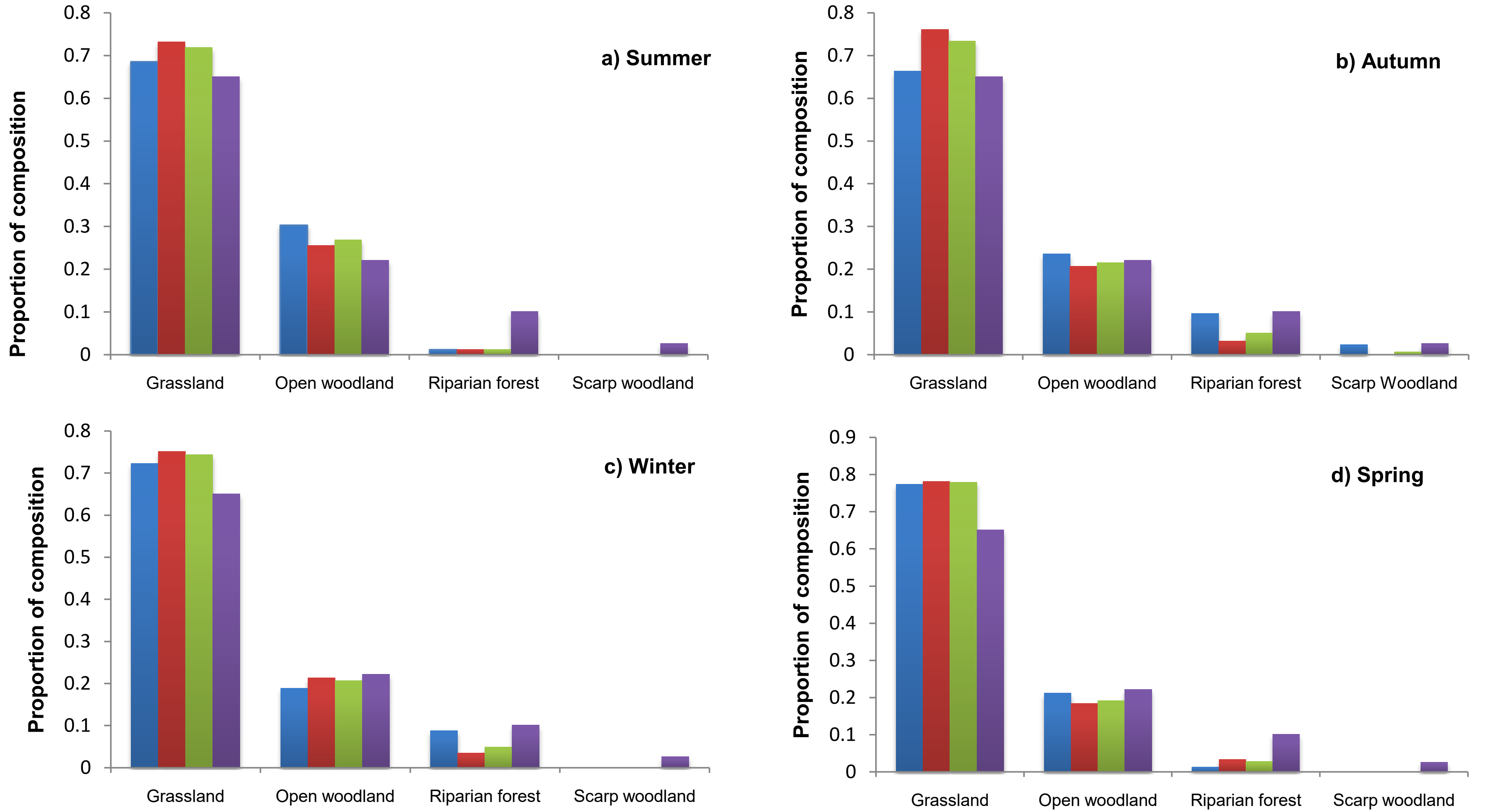



**Figure 5-8** **Second order habitat selection (comparing habitat composition in individual home ranges against habitat availability in the study area) exhibited by individual radio-collared eastern grey kangaroos for a) summer, b) autumn, c) winter and d) spring. Blue and red bars indicate mean proportional habitat composition for males and females respectively, green bar is males and females combined mean proportional habitat composition and the purple bar indicates the availability of the habitats in the study area.**

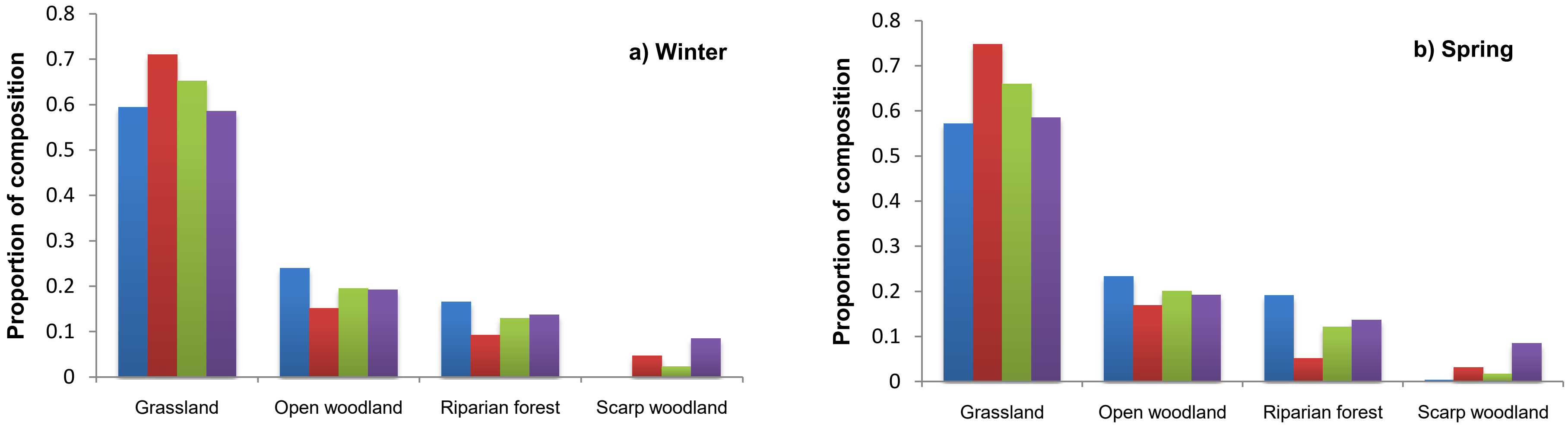



**Figure 5-9** **Second order habitat selection (comparing habitat composition in individual home ranges against habitat availability in the study area) exhibited by individual GPS-collared eastern grey kangaroos for a) winter and b) spring. Blue and red bars indicate mean proportional habitat composition for males and females respectively, green bar is males and females combined average and the purple bar indicates the availability of the habitats in the study area.**

Compositional analysis of radio locations within the home ranges (i.e. third order selection) of radio-collared eastern grey kangaroos did not reveal a significant departure from random use in all seasons (summer: $\lambda = 0.491$; $P = 0.059$, autumn: $\lambda = 0.809$; $P = 0.199$, winter: $\lambda = 0.821$; $P = 0.198$, spring: $\lambda = 0.904$; $P = 0.567$) (Figure 5-10). However, there were trends to suggest that in autumn and winter male and female radio-collared eastern grey kangaroos showed preferences for the riparian habitat and females demonstrated preferences for open woodland in summer and spring.

Similarly, GPS-collared eastern grey kangaroos did not demonstrate selection at the third order scale during winter ($\lambda = 0.269$; $P = 0.179$) or in spring ($\lambda = 1.59 \times 10^{13}$; $P = 0.939$) (Figure 5-11). However, female GPS-collared kangaroos tended to demonstrate preferences for open woodland and males and females tended to show preferences for riparian forest during both sampling periods.

The home ranges of five GPS-collared eastern grey kangaroo encompassed the Jooriland farm dam. The proportion of locations collected from these animals within a 10 ha vicinity of the Jooriland farm dam relative to the total within their home ranges ranged from 0 to 20% (Table 5-9). The difference in the utilisation between Jooriland farm dam and riparian forest was negligible ($F_{1,8} = 2.05$, $P = 0.19$). The utilisation of the Jooriland farm dam environment by F114 is presented in Figure 5-12 and by F118 in Figure 5-13.

**Table 5-9 Utilisation of alternative water sources by GPS-collared eastern grey kangaroo in comparison to riparian forest**

[a] F = female, M = male, [b] proportion of locations collected from within a 10 ha radius of the Jooriland Dam

| Individual[a] | Proportion of locations – Jooriland Dam (%)[B] | Proportion of locations – riparian forest (%) |
|---|---|---|
| F118 | 2.0 | 9.0 |
| F114 | 20.6 | 5.4 |
| M121 | 0 | 18.0 |
| M115 | 0 | 11.3 |
| M116 | 0 | 12.2 |

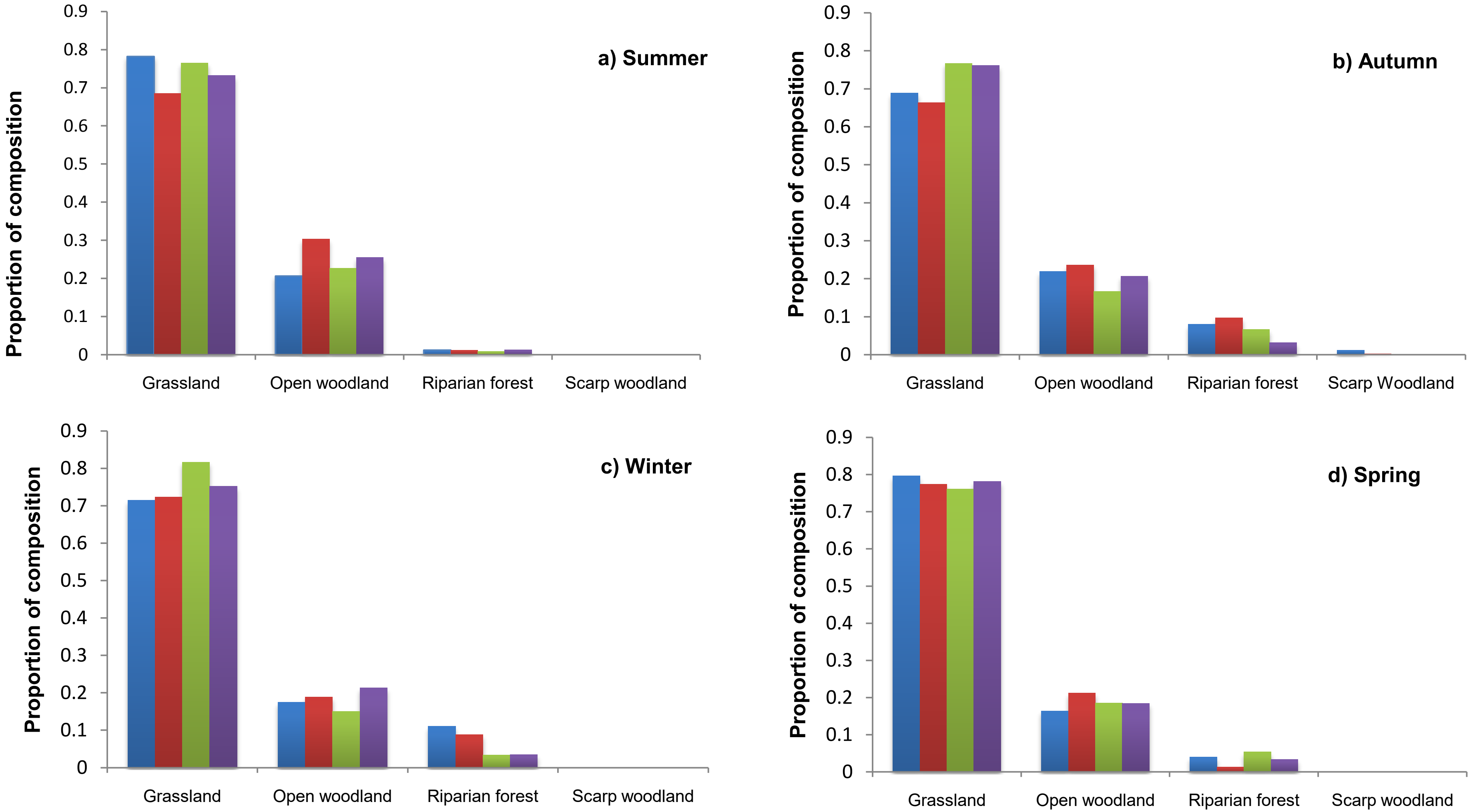



**Figure 5-10 Third order habitat selection (comparing habitat composition of individual radio-locations with the habitat composition of individual home ranges) exhibited by radio-collared eastern grey kangaroos for a) summer, b) autumn, c) winter and d) spring. Blue and red bars indicate the mean male and female composition of radio locations respectively and the green and purple bars indicate the mean habitat composition in male and female home ranges, respectively.**

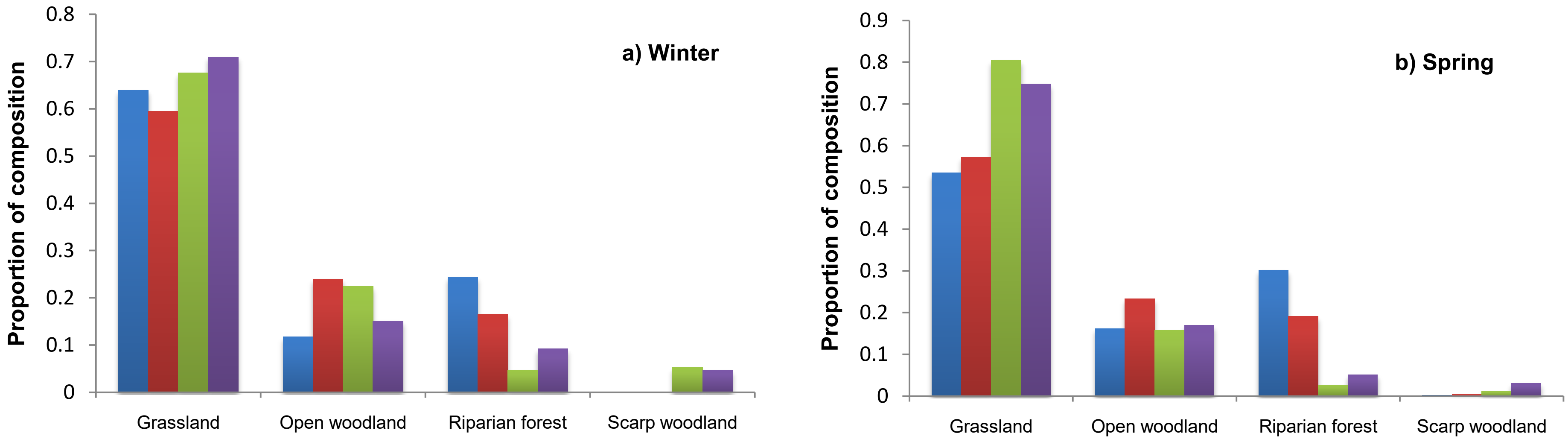



**Figure 5-11** **Third order habitat selection (comparing habitat composition of individual GPS locations with the habitat composition of individual home ranges) exhibited by GPS-collared eastern grey kangaroos for a) winter and b) spring. Blue and red bars indicate the mean male and female composition of locations respectively and the green and purple bars indicate the mean habitat composition in the home ranges of males and females respectively.**

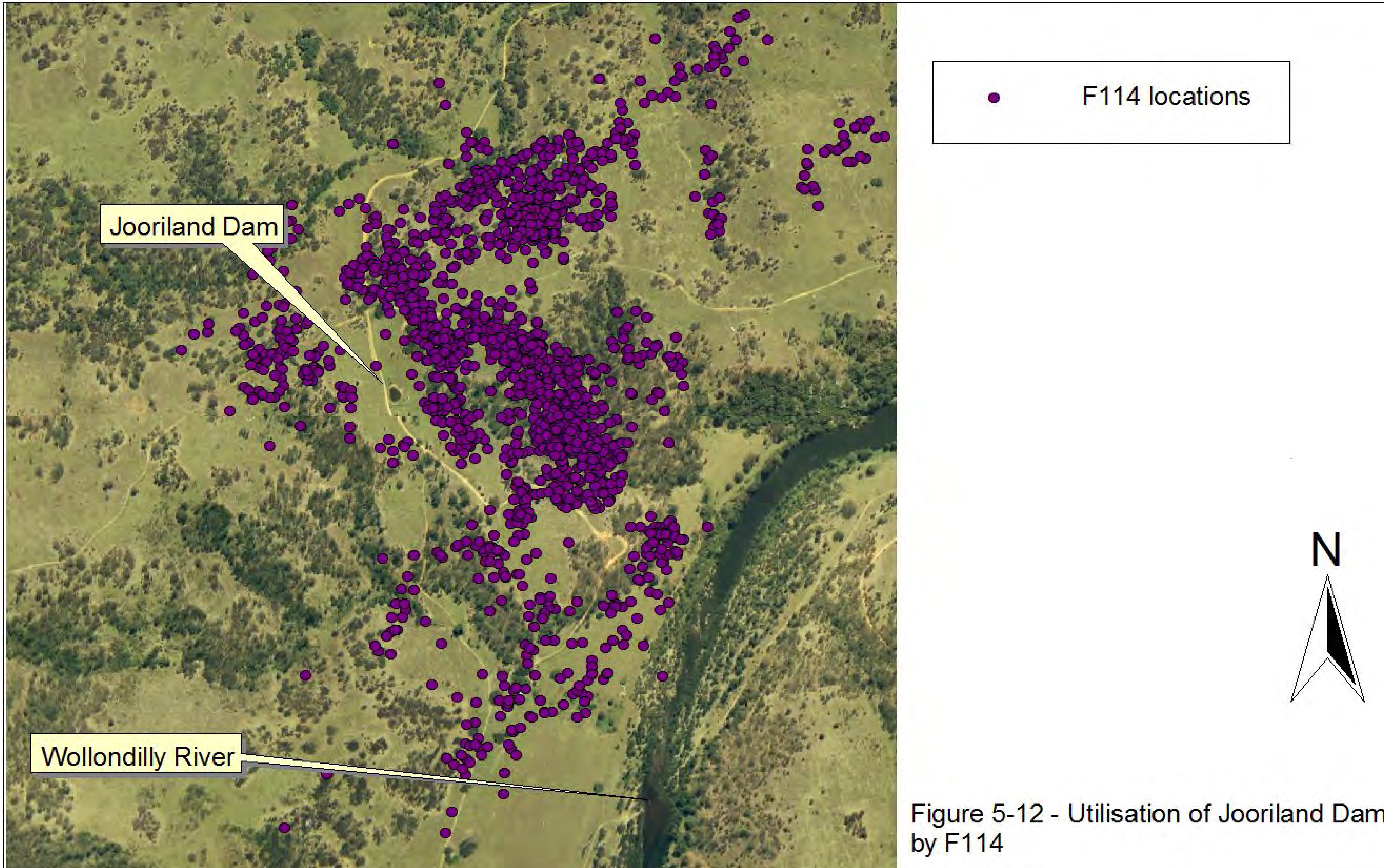



Figure 5-12 - Utilisation of Jooriland Dam by F114


Figure 5-13 - Utilisation of Jooriland Dam by F118

Figure 5-14 illustrates the diurnal patterns of habitat use at the third order for radio-collared and GPS-collared kangaroos, respectively. For radio-collared kangaroos, third order selection was only exhibited during the day in summer ($\lambda$ = 0.361; $P < 0.05$) and at night during spring ($\lambda$ = 0.391; $P < 0.01$). During the day in summer, eastern grey kangaroos preferred open woodland over the grassland and riparian forest, where grassland was significantly more preferred than riparian forest ($P < 0.05$). At night in spring, eastern grey kangaroos preferred grassland over open woodland ($P < 0.05$) and the riparian habitat was more preferred than open woodland (Table 5-10). The lack of radio-locations in the scarp woodland habitat precluded any third order analysis within this habitat.

**Table 5-10 Simplified ranking matrix for radio-collared eastern grey kangaroos at the third-order of selection scale (comparing habitat composition of individual radio-location with the habitat composition of individual home ranges) for during the day in summer and at night in spring**

[a] Cells in the matrix consist of mean differences in the log ratios of used and available habitats for all eastern grey kangaroos divided by the SE (i.e., t-values). At the intersection of the row i and of the column j, there is a "+" when the habitat i is used more than the habitat in the column, and "-" otherwise. When the difference is significant, the sign is tripled at a α =0.05.

[b] Rank is equal to the sum of the positive values in each row. Higher ranks indicate a more preferred habitat.

| | Habitat type | | | |
|---|---|---|---|---|
| **Habitat type[a]** | **Grassland** | **Open woodland** | **Riparian forest** | **Rank[b]** |
| Summer-day | | | | |
| Grassland | 0 | - | +++ | 1 |
| Open woodland | + | 0 | +++ | 2 |
| Riparian forest | --- | --- | 0 | 0 |
| Spring - night | | | | |
| Grassland | 0 | +++ | + | 2 |
| Open woodland | --- | 0 | - | 0 |
| Riparian forest | - | + | 0 | 1 |

GPS-collared eastern grey kangaroos did not demonstrate third order selection at any of the four periods of the day ($P > 0.05$). However, they tended to utilise the grassland habitat more in the late evening and night and utilised riparian forest more during the middle of the day in winter and spring and also at night during spring (Figure 5-15).

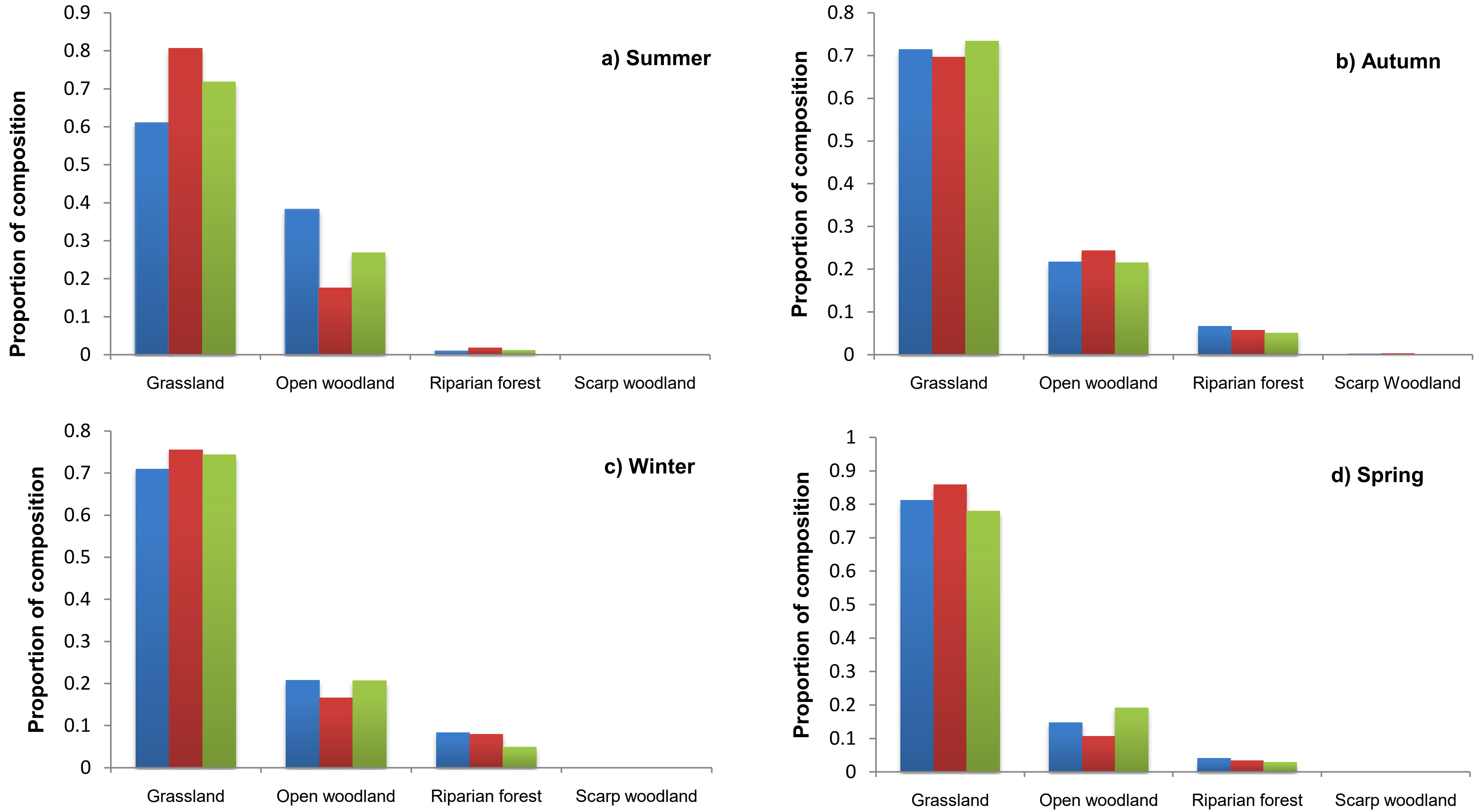



**Figure 5-14** **Third order habitat selection of radio-collared eastern grey kangaroos by day and night a) summer, b) autumn, c) winter and d) spring. Blue and red bars indicate the mean day and night composition of radio locations respectively and the green bar indicates the mean habitat composition of individual home ranges.**

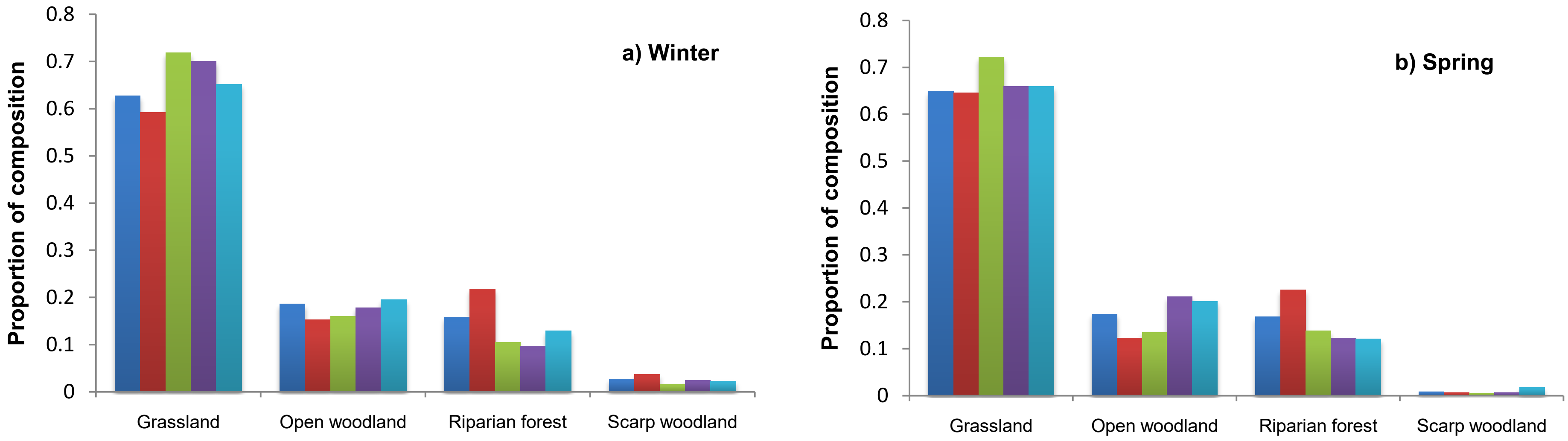



**Figure 5-15** **Third order habitat selection of GPS-collared eastern grey kangaroos at four periods of the day (0600 – 1200, 1200 – 1800, 1800 – 2400 and 2400 – 0600) in the Warragamba Special Area for a) winter and b) spring. Blue, red, green and purple bars represent 0600 – 1200, 1200 – 1800, 1800 – 2400 and 2400 – 0600 periods, respectively; aqua bar indicates the mean habitat composition of individual home ranges.**

## DISCUSSION

### *Home range and movement patterns*

Making direct comparisons between home range studies of eastern grey kangaroos is confounded by the different time periods, locations and methodologies that have been used. However, it appeared that eastern grey kangaroos in this study had small home range sizes compared with other populations from temperate parts of their range. In a similar environment to that studied here, Moore *et al*. (2002) found that the mean 95% MCP for female kangaroos was 42.9 hectares. These researchers concluded that female eastern grey kangaroos used adjacent farmland and that the resultant disturbance by farmers, dogs and domestic stock probably contributed to the slightly larger ranges (Moore *et al.* 2002). Disturbance was also noted as a potential factor increasing the range sizes of kangaroos inhabiting a farm property in the New England Tablelands (Jarman and Taylor 1983). Kangaroos residing in Sydney‟s water supply catchment had very minimal interaction with humans and there were no farms near the study area, which potentially contributed to their smaller ranging behaviour. Jaremovic and Croft (1987) reported 95% ellipse home ranges of 20 - 30 hectares for males and females. These smaller ranges were most probably a response to the higher rainfall (and hence primary productivity) at their study site, which was double that of the annual average rainfall in Sydney‟s water supply catchment (Chapter 1). Furthermore, the smaller home ranges found by Jaremovic and Croft (1987) potentially arose, in part, from their technique of marking the positions of visually sighted tagged animals in intensively sampled areas. This technique leads to biases in the resighting of animals close to their capture locations and consequently the under sampling of animals that make larger movements (McCullough and McCullough 2000).

The seasonal home ranges of eastern grey kangaroos followed a predictable pattern, although this regularity has not been well documented for this species in other studies. Generally, much larger home ranges occurred in winter and autumn than in summer and spring. This finding was corroborated by Viggers and Hearn (2005) in their study of eastern grey kangaroos in Canberra, Australian Capital Territory, that showed a reduction in home range size during the warmer seasons. This occurs in other taxa also (Tufto et al. 1996, Dexter 1999) and has been related to an increase in pasture biomass in spring and summer. For example, roe deer *Capreolus capreolus* and feral pig *Sus scrofa* increase their home range size when resources become sparse (Tufto et al. 1996, Dexter 1999). In the present study, eastern grey kangaroos foraged widely during the drier autumn and winter periods when food availability was low (Chapter 4), and conversely concentrated their movements in spring and summer when resources and their reliance to be near adequate cover were high. This result is perhaps supported by optimal foraging theory (McNair 1982). This theory suggests that more time should be spent foraging in good patches than in poor ones; thus the abundance of high quality food in spring and summer in this study likely influenced the higher usage of grassy floodplain areas and concentrated eastern grey kangaroo movements (Taylor 1984).

Males of the larger macropodid species typically have larger home ranges and movements than their female counterparts (Jarman and Taylor 1983, Jarman 1991, Coulson 2009). This reflects their larger body size, thus their greater food requirements and the need to forage over larger areas to find adequate food supplies (McNab 1963). Males may also exhibit larger ranges often with multiple activity centres to maximise encounters with receptive females, and hence fitness (Dexter 1999). Eastern grey kangaroos exhibit peak breeding during summer, which may explain the larger summer and autumn home range size of males in this study if they were searching actively for females in oestrus (Poole 2002). Antagonistic interactions between rival males and courtship behaviour were frequently observed in the study area during this period, which supports this claim. In other seasons, the ratio of male to female home range size was comparatively smaller than in other studies (McCullough and McCullough 2000). This may be reflected by the higher year round productivity of the site in contrast to that of harsher environments, which nullify the need for males to range further to meet their higher metabolic requirements and, as females are concentrated in the few areas, the need to range further to increase the number of offspring sired. Alternatively, another hypothesis is that sex differences in home range size are larger in high rainfall areas (Fisher and Owens 2000). This may explain the similarity between male and females range size in this study as extreme dry conditions were common and good conditions were infrequent during the sampling period. The summer and spring home ranges of females were smaller than those in other seasons because most females (60%) were carrying large pouch young or rearing newly emergent young at foot. At such a time, females generally separate from larger groups and may reduce their range size (Jaremovic and Croft 1987, Martin et al. 2007). Several solitary females with pouch young and young-at-foot were observed in the study area at these times, lending support to this view.

The high site fidelity and low dispersal rates indicated that eastern grey kangaroos were relatively localised in the watershed. This high degree of site fidelity has also been reported in the Allied rock-wallaby (*Petrogale assimilis*) (Horsup 1994). Horsup (1994) hypothesised that permanent occupation of an area allows an accumulation of knowledge about the location of good foraging areas, shelter positions and escape routes from predators. The principles behind this hypothesis are applicable to eastern grey kangaroos residing in Sydney"s water supply catchment, particularly because all of these requirements are met in this small area and due to the limited disturbance that these animals experience in relation to other areas in their range (Viggers and Hearn 2005, Martin et al. 2007). The high rate of fidelity was also indicated by the lack of animals dispersing from the site, with only one male (M66) leaving and setting up a home range two kilometres upstream from the study area. Several kangaroos exhibited forays outside their established home ranges, but these were often very short lived with some lasting only until the next tracking period. These forays consisted of movements across the Wollondilly River and north and south of their capture locations, but kangaroos showed a reluctance to move outside the valley floor. Eastern grey kangaroos are reported to generally avoid steep slopes (Taylor 1984), which helps to explain this finding. Furthermore, the presence of steep slopes to the east and the west of the study area may also limit the size of the home ranges, and thus may explain the small linear home ranges demonstrated by eastern grey kangaroos in this study. Animals exhibiting these short-term forays may have been disturbed by resident wild dogs in the study area, moving away from their capture location temporarily until after the threat of predation had passed.

Home range and movement pattern results were observed to be different when using the two different techniques; GPS and VHF radio telemetry. Firstly, the differences in the number of data points that could be obtained using each technique was highlighted in the similarity of the 95% MCP home range of VHF radio-collared eastern grey kangaroos and the 50% core range of GPS-collared eastern grey kangaroos (average n = 25 fixes for VHF and n = 1000 for GPS). This reflects the suitability of the different models that are used to estimate home range (MCP and kernel), such that with the increase in the sampling intensity larger exploratory patterns demonstrated by GPS-collared eastern grey kangaroos were captured in the home range calculation, which influences the larger home range size derived by using the MCP method. Conversely, forays exhibited by radio-collared eastern grey kangaroos are excluded from the analysis as extreme points or outliers in the MCP calculation and varying probabilities of utilisation in the kernel calculation were less clustered. This is an important finding, as the GPS technology demonstrated a more accurate and detailed map of home range and movement patterns because of the larger number of data points available then could be obtained from radio telemetry. The GPS method was also shown to be less labour intensive and therefore less intrusive to the animal population being studied. Similar comparative findings between GPS technology and radio telemetry for the study of dingoes have been reported (Purcell *et al.* 2006).

The advantages of continuous collection of location data from GPS collars were apparent in the analysis of forays and home range extensions of collared eastern grey kangaroos. One of the many criticisms made of the home range study of eastern grey kangaroos conducted by Viggers and Hearn (2005) was the low sampling intensity of that study, as data were collected over just six days in a two week period to formulate a seasonal home range estimate (Martin *et al.* 2007). Martin *et al*. (2007) supported this claim by comparing two weeks of location data with data collected a full season from one female eastern grey kangaroo from data in Moore *et al*. (2002). The results of this comparison showed a four-fold increase in the core and home range sizes between the two periods (Martin *et al*. 2007). This was corroborated by the examination of the home range of F112, a GPS-collared eastern grey kangaroo in this study that showed that her home range for most of the survey period spanned both sides of the Wollondilly River, whereas during the last month only one side of the river was used. Consequently, if this animal was radio-tracked only for the last month, the home range would have been one quarter the full size measured over a period of approximately 60 days. These results highlight the need for the random collection of locations over a longer period when radio-tracking is used to fully quantify the extent of eastern grey kangaroo home range size.

Martin *et al*. (2007) showed that the LSCV smoothing on kernel-based home range estimates substantially underestimates range size for small sample sizes, but produced more accurate estimates as sample sizes of locations increased. The results of this study have taken this observation one step further by the apparent differences shown in the kernel estimates derived between radio-tracking and GPS-data loggers. The kernel estimates of GPS-collared eastern grey kangaroos were consistently smaller than those of the radio-collared animals, despite the much larger sample sizes used to formulate the home range of these animals. In addition, inspections of the area-observation curves when in excess of 1000 locations were collected showed a lack of asymptote, which suggests continual shifts in home range in response to the ability of an increased sampling frequency to detect fine scale exploratory movements. The tendency for the area-observation core range curves of GPS-collared eastern grey kangaroos to reach asymptote quicker than the home range curves provides support for this view. These results warrant further investigation and perhaps call for a re-definition of the „home range" of eastern grey kangaroos, since it is often the case that researchers must choose between a few very expensive GPS collars that can provide many locations and several inexpensive radio collars that will provide few numbers of locations and high error rates without the use of substantial resources in the field (Girrard *et al.* 2006).

***Habitat preferences***

The study area offers ideal habitat for eastern grey kangaroos as described by Caughley (1964), in that it contains mosaic patches of lateral cover interspersed with large open, grassy areas providing forage (Coulson 2009). The preference for the grassland habitat by eastern grey kangaroos at the second order of selection was expected given the good foraging opportunities that this habitat supplies. The open woodland habitat appeared to be used merely as a complementary habitat by eastern grey kangaroos, providing shelter for thermoregulation in the warmer seasons. This was reflected by the strong preference that eastern grey kangaroos showed for the grassland habitat in the cooler seasons. Eastern grey kangaroos are known to spend more time in grassland areas when forage deteriorates (Hill 1981;1982). in this study in winter, when the quality of forage was poor, instead of moving into more sheltered habitats to rest, kangaroos continued to graze at a lower intensity throughout the day (Clarke *et al.* 1989).

Eastern grey kangaroos in this study showed no preference for scarp woodland for two potential reasons; firstly, because of their reluctance to utilise steep slopes (Taylor 1984) or secondly, because this habitat was mostly on the eastern side of the study area and collared kangaroos predominantly had home ranges on the western side. The latter possibility is least likely as GPS-collared animals captured adjacent to this habitat also generally avoided it. Moreover, direct observations suggested that eastern grey kangaroos used this habitat primarily as a „stop over" on their way to habitats offering better foraging opportunities.

An interesting finding from this study was the low preference for riparian forest by eastern grey kangaroos. On the whole, this habitat was probably not perceived as „safe refuge" by eastern kangaroos, given the steep sandy banks, limited visibility and restricted escape routes from predators (e.g. dingoes) that it offered. The greater preference for open woodland over riparian forest at most times of the year may reflect the closer proximity to foraging opportunities and lower predation risk of the woodland habitat (Banks 2001). In addition, the open woodland habitat provides a higher level of lateral cover and better visibility, a requirement of eastern grey kangaroos to reduce predation risk (Coulson 2009). Banks (2001) established that eastern grey kangaroos foraged further from cover in areas where foxes were removed, an observation that was also made in this study, whereby the high number of resident dingoes in the area influenced the close proximity of kangaroos to woodland habitats whilst foraging.

The predation risk associated with the utilisation of the riparian forest may also be explained by the higher usage of this habitat by male eastern grey kangaroos relative to that shown by females. The threat from predators, such as the dingo, has been suggested to be least for large uninjured male kangaroos (Jarman and Wright 1993, Wright 1993). In addition, male kangaroos exhibit numerous defences to combat predation risk, including the utilisation of water as a refuge and aggressive self-defence (Jarman and Wright 1993, Wright 1993). Consequently, the reluctance of female collared animals to utilise riparian areas supports this view. Furthermore, on several occasions male eastern grey kangaroos were observed retreating to the Wollondilly River within the study area to escape dingo predation, thus the close proximity of the riparian forest may provide an easy route to this potential refuge.

The higher preference for riparian habitat by males during winter and autumn was supported by observations made of „bachelor groups" in this area (Chapter 6). Chapter 6 describes the formation of these „bachelor" in the non-mating season (autumn and winter) as males dissociated away from female dominated groups at the time that most females were likely to be in anoestrous (Dawson 1995, Coulson 2009) . This behaviour has been described as habitat segregation, a form of sexual segregation in which males and females exhibit the use of different habitats outside the breeding season (Conradt 1998, MacFarlane 2006). The occurrence of habitat segregation is further supported by habitat selection results from this chapter that have revealed that females tended to utilise open woodland and grassland more than males at these times of year.

Seasonal differences in habitat selection were exhibited by eastern grey kangaroos in this study. At the second order of habitat selection, in winter and spring radio-collared eastern grey kangaroos showed similar preferences for the available habitats, ranking grassland before woodland, with riparian forest the least preferred habitat. This result is suggestive of the time spent foraging by eastern grey kangaroos: firstly, in winter animals would spend more time in grassy habitats searching for adequate supplies of nourishment (Hill 1981) and also when the thermoregulation requirement (i.e. water loss) for shelter from open woodland is decreased; and secondly, in spring and in accordance with optimal foraging theory, animals would spend more time foraging in good patches of fresh green grass (McNair 1982). This in turn would influence the larger amounts of time and greater preference for the grassland habitat exhibited by female eastern grey kangaroos, most carried pouch-young and would presumably have higher energy requirements in response to lactation (Hume 1999, Krockenberger 2003, Munn and Dawson 2006). The faecal deposition, pasture quality and biomass results (Chapter 4), as well as direct observations of these patterns corroborate this hypothesis.

Diel differences in habitat selection were demonstrated by GPS-collared eastern grey kangaroos by day and night, during summer and spring respectively. The preference shown for open woodland during the day in summer is reflective of the thermoregulation requirements of this species seeking shade to reduce water loss and heat loads (Dawson 1995, Dawson et al. 2000, Dawson et al. 2006). The higher preference for riparian forest demonstrated by eastern grey kangaroos in spring at night may be reflective of their drinking requirements. However, as this species can extract water from higher quality feed (Dawson et al. 2006, Jarman and Evans 2010), this result was considered unusual given the high quality forage that was present at this time. Three alternative explanations may provide support for these results. Firstly, the higher abundance of juveniles (present in the population as newly emergent young-at-foot and mature pouch young) and the elevated water requirements (up to 2.5 fold) of this age cohort may necessitate eastern grey kangaroos drinking more frequently at this time to address their higher evaporative water requirements (Munn and Dawson 2004). Munn and Dawson (2004) reported the increased water requirements of juvenile red kangaroos (*Macropus rufus*) in comparison with adults, and given that there are some similarities in the metabolism between these species (Dawson *et al.* 2006), this pattern is expected in eastern grey kangaroos also. Secondly, as foraging bouts and other movements are generally restricted to the late evening and night at this time of year to reduce water loss (Dawson *et al.* 2006), given the relative proximity of water sources from preferential shade in the open woodland habitat, visitation to the Wollondilly River may also occur at this time. Dawson *et al*. (2006) in a comparative study of eastern grey and red kangaroos showed that eastern grey kangaroos partitioned foraging and non-foraging (locomotion and drinking) to different times of the day, in which drinking mostly occurred during late evening (i.e. 18.00 and 23.00 h) which supports this view. Thirdly, drinking at night may also be an anti-predator behaviour exhibited by eastern grey kangaroos to reduce the predation risk (Halle 1993) associated with utilisation of the riparian zone. An observation during the spring nocturnal radio-tracking sessions of large eastern grey kangaroo mixed sex mobs resting in the

Wollondilly River instead of individual animals provides support for this view. Such mobs would increase vigilance to offset predation risk (Banks 2001) and thus satisfy thermoregulatory requirements and water balances.

***Movement patterns, habitat utilisation and zoonotic disease management***

Overall, the movement patterns of eastern grey kangaroos, and in particular their high degree of site fidelity, in Sydney"s water supply catchment indicates that they probably contribute a fairly stable rate of supply of faecal material to the watershed. These results extend the faecal deposition results presented in Chapter 4 through the collection of specific information regarding movement patterns, habitat selection and landscape utilisation by this species. Table 5-11 summarises the results of this chapter on their relevance to zoonotic disease management.

The high concentration of faecal material that eastern grey kangaroos contribute to the floodplain and the high potential for pathogens originating from this species that could enter surface waters reflects the small home ranges found in this study (see also Chapter 4). A very high percentage of the home range areas of collared eastern grey kangaroos encompassed the floodplain of the Wollondilly River, which collectively forms the high risk area for faecal entry into surface waters. Consequently, the largest concentration of faecal material in the floodplain would be in spring and summer when home ranges of eastern grey kangaroos are smallest and much more dispersed in winter and autumn, when home ranges are larger. Furthermore, the more sedentary nature and smaller home ranges of female eastern grey kangaroos at most times of the year may mean that females deposit a higher concentration of faecal material in their home ranges within the watershed compared with males.

The habitat preferences exhibited by eastern grey kangaroos are directly comparable to the faecal deposition results in Chapter 4. Thus, the time spent in habitats translates to the amount of faecal material deposited, being highest in the grassland habitat, then open woodland, and lowest in riparian forest and scarp woodland. The seasonal differences in habitat utilisation also support the faecal deposition results, as eastern grey kangaroos were found to spend more time in the grassland habitat during winter and spring and more time in the open woodland habitat during summer.

**Table 5-11 Summary of eastern grey kangaroo movement patterns and habitat selection results and their implications for zoonotic disease management in the Sydney water supply catchment**

| Result | Implications for zoonotic disease management |
|---|---|
| **Small home range size of eastern grey kangaroos** | • High concentration of faecal loads contributed to the watershed<br>• Faecal-oral route disease transmission (inter and intra-species) would be highest in floodplain areas due to the high concentration of this species occupying small home ranges in these areas |
| **High site fidelity for floodplain areas, males more likely to disperse than females** | • High level of home range overlap between individuals – high intra-species transmission of disease<br>• Faecal loads originating from females are likely to be more concentrated than males due to restricted movement patterns and higher faecal output rates (Johnson *et al.* 1987)<br>• Males may transmit diseases more broadly across the landscape given their larger exploratory patterns<br>• Site fidelity and lack of dispersal out of site – population is easy to manage if required but, large amounts of time spent near the water supply leads to higher potential for pathogen entry to surface waters. |
| **Habitat preferences - grassland > open woodland > riparian forest > scarp woodland** | • Preference exhibited for habitats is comparable to faecal deposition rates and distribution (Chapter 4)<br>• Highest utilisation of grassland areas occurred in winter and spring, whereas the open woodland habitat was used more frequently in summer<br>• Highest faecal loads are likely to occur in floodplain areas during winter and early spring in response to thermoregulation and metabolic energy requirements of this species |
| **Low preference for riparian forest – males more likely to occupy this habitat and some preferences were shown during spring at night.** | • Males most probably represent a lower zoonotic disease risk than females given their lower faecal output rates (Johnson *et al.* 1987) – trade-off between their higher utilisation of riparian forests versus their lower faecal output than females<br>• Females in riparian forest habitat at night represents a high zoonotic disease risk due to their higher faecal output rates at this time<br>• Juveniles are most abundant in spring, have higher water requirements at this time and they are more susceptible to Cryptosporidiosis and have higher shedding intensities of this disease<br>• Higher water requirements of juveniles would restrict their foraging distances from water sources<br>• Direct deposition of faecal material into surface waters is possible despite the low rate of faecal accumulation found the riparian habitat – 20% of GPS-collared animal locations were found in the riparian habitat and animals were found to swim across the Wollondilly River |

| Result | Implications for zoonotic disease management |
|---|---|
| **Utilisation of riparian forest and the area around the Jooriland farm dam was similar** | • Closing of artificial watering points is a commonly utilised population control technique<br>• Riparian habitat was utilised at similar level to the Jooriland farm dam<br>• Closing artificial watering points may inflate eastern grey kangaroo faecal loads in riparian zones |
| **Requirements for animals to drink** | • Driven by time of year, age and sex (males more likely to drink than non-lactating females and juvenile have higher water requirements than adults)<br>• Females less likely to drink directly after rainfall – limiting *Cryptosporidium* and *Giardia* dissemination to the water supply |

The output rate of faecal pellets by eastern grey kangaroos has been reported to vary between sexes (females 6.6 pellets $h^{-1}$ and males 3.1 pellets $h^{-1}$) and time of day, being lowest during the middle of the day when kangaroos are least active and highest in mid-afternoon when kangaroos commence foraging (Johnson *et al.* 1987). Thus, the higher preference for riparian habitat by males may represent a lower zoonotic disease risk than if females showed similar preferences. Conversely, at the third order of habitat selection, in spring eastern grey kangaroos showed a higher preference for riparian forest at night. This may reflect a higher zoonotic disease risk through higher faecal deposition in these areas at this time. Furthermore, as this result may reflect the higher water requirements of juveniles at a time when they are most abundant (Munn and Dawson 2004), the risk may be higher given the increased susceptibility of this age cohort to Cryptosporidiosis and their higher rates of oocyst dissemination compared with adults (Power *et al.* 2005). Additionally, this risk may be confounded by the requirement of juveniles to drink more water than adults for thermoregulation, which would also restrict their foraging distances from water.

The results of the habitat selection study suggest that faecal deposition by eastern grey kangaroos may occur directly into surface waters, despite the low to negligible faecal accumulation levels that were found to occur in the riparian habitat (Chapter 4). The riparian habitat comprised approximately 20% of the GPS-collared eastern grey kangaroo home ranges and several animals were found to traverse across the Wollondilly River on numerous occasions. The low levels of faecal material in the riparian forest habitat reflected the low pasture biomass (Chapter 4), as eastern grey kangaroos primarily defecate whilst foraging (Johnson *et al.* 1987). However, in some parts of the study area the close proximity of good foraging opportunities with respect to the riparian forest may lead to a higher probability of direct faecal deposition if animals drink from the river directly after food consumption. Furthermore, as it has demonstrated for cattle crossing agricultural streams (Davies-Colley et al. 2002), eastern grey kangaroos may contribute higher levels of pathogens when traversing across the Wollondilly River. The concentration of location estimates of GPS-collared eastern grey kangaroos provided support for this hypothesis, however, direct faecal deposition into surface water would be difficult to quantify.

The use of artificial water sources by macropodid species has been well documented and, as such, closure of such sources is a common method to reduce the density of the subject species (Fukuda *et al.* 2009). The location information of GPS-collared eastern grey kangaroos suggested that there was no difference between the utilisation of riparian forest and Jooriland farm dam. Male eastern grey kangaroos were more likely to utilise riparian forest than females, as previously discussed, and females were more likely to not frequent riparian zones with only half of GPS-collared females recording locations in or near this area. Although these results could be sampling artefacts in that animals captured near the Jooriland farm dam were found to utilise it, they are highly suggestive of the importance of artificial water sources in water-borne zoonotic disease management. Furthermore, if these alternative water sources were to be closed, there is the potential that higher densities of eastern grey kangaroos may rely on the Wollondilly River for water.

The requirement of collared eastern grey kangaroos to drink water and thus visit the riparian zones or alternative water sources revealed interesting results. The two female GPS-collared eastern grey kangaroos captured from Douglas Scarp were found to not visit any water sources throughout the course of the study. This contradicts results from Dawson *et al*. (2006) that eastern grey kangaroos drank every two days. I suggest three plausible reasons for my results. Firstly, Douglas Scarp does not contain any alterative water sources and is geographically further away from the Wollondilly River. In contrast, collared males from Douglas Scarp did frequent the Wollondilly River due to their larger ranging patterns, segregation from females, aggressive anti-predator defence mechanisms, and higher water requirements. Secondly, eastern grey kangaroos can satisfy their water requirements through the extraction of free water from forage (Jarman and Evans 2010), thus negating the need to visit water sources. This is an unlikely explanation for the winter ranging patterns of F113 as the pasture quality at this time was poor and, as suggested by Jarman and Evans (2010), eastern grey kangaroos need to drink when the moisture content of their forage drops below 70%. Thirdly, rainfall may create temporary water sources for resident eastern grey kangaroos in the form of puddles and moist pasture can adequately satisfy their water requirements. This is a likely explanation as there was some rainfall during the survey and observations of eastern grey kangaroos drinking from ephemeral water holes were commonly made throughout the course of the study. This is an important finding as it suggests that eastern grey kangaroos are unlikely to need or be near catchment supply water after rainfall events, thus limiting the dissemination of *Cryptosporidium* and *Giardia* to the water supply (Ferguson *et al.* 2007a). Collectively, this information highlights that there are several factors influencing the requirement for eastern grey kangaroos to drink from the Wollondilly River and these need to be considered in the assessment of the zoonotic disease risk that they pose to Sydney‟s water supply and PCB model formulation.

## CONCLUSION

The movement pattern results allow supporting evidence for interpretation of the faecal deposition and distribution results provided in Chapter 4. The stable contribution of faecal loads by eastern grey kangaroos in areas adjacent to the Wollondilly River are a direct consequence of the small home ranges and restricted dispersal patterns demonstrated by individuals within this population. However, the actual rates can still be expected to vary somewhat, and to be influenced by several factors: 1) the prevailing climatic conditions and pasture quality, with larger ranges expected in drier seasons. This would result in a less concentrated distribution of faecal material as it is spread over a larger area, 2) gender differences in movement patterns, with males typically exhibiting larger ranges than females, and 3) landscape characteristics of the watershed, with steeper areas being generally avoided by kangaroos and flatter areas preferred, which results in floodplain areas being heavily utilised. Furthermore, the exploratory patterns and home range extensions demonstrated by some animals would also affect the distribution of faecal material as these may relate to a myriad of factors such as predator avoidance, seeking mates or resource availability. Consequently, the faecal loads that are deposited by eastern grey kangaroos should not be considered fixed, as is the case in the current PCB models.

The habitat preferences exhibited by eastern grey kangaroos correlate broadly with the faecal deposition results (Chapter 4). Individual eastern grey kangaroos showed strong preferences for grassland habitat due to the high level of foraging opportunities it provides, and open woodland habitat for sheltering and predator avoidance resources. The faecal deposition results reflect the time spent actively grazing by this species, which was much higher in the grassland habitat and lower in the woodland habitat. Eastern grey kangaroos exhibited a low preference for the riparian habitat at most times of the year and this was corroborated by the low quantity of faecal material deposited in this habitat. However, males utilised this habitat more than females, and in summer it was utilised more than the open woodland. In addition, during spring there were some preferences shown for riparian forest at night. Consequently, the low levels of faecal deposition by this species that were found in the riparian zones (Chapter 4) suggest that: 1) the higher utilisation of this habitat by male eastern grey kangaroos and their lower faecal output rates; and 2) the potential for direct faecal deposition by this species into surface water given that 20% of GPS-collared animal locations occurred in this habitat and animals frequently swam across the river.

The “access to streams” factor in the PCB model is governed by the habitat preferences of the target animal species, and in particular the preferences exhibited for riparian zones. The frequency of eastern grey kangaroo visitation to riparian zones is dictated by several factors. Firstly, the water requirement of this species, as for example, juvenile animals have higher water requirements than adults, which may have influenced the preference shown for riparian areas during spring when this age cohort was most abundant. Secondly, the predation risk of the riparian habitat and thirdly, quality and quantity of forage in the riparian habitat (Chapter 4).

The movement patterns and habitat selection of eastern grey kangaroos in Sydney"s water supply catchment provide some insight into the potential management options that may be utilised to mitigate the zoonotic disease risk, as well as other impacts that this species may have on biodiversity. The regeneration and subsequent expansion of the open woodland habitat (Box gum woodland) in floodplain areas will have two primary benefits. First, eastern grey kangaroos more readily utilise this habitat for shelter than riparian forest, thus attracting kangaroos away from surface water. Second, it will reduce the carrying capacity of the area and reduce herbivore densities through the reduction of grassy areas. Furthermore, a trial of the effects of the location and placement of artificial water sources at varying distances from the Wollondilly River is required to ascertain the effect on macropodid movement and density, and distribution of faecal loads in floodplain areas.

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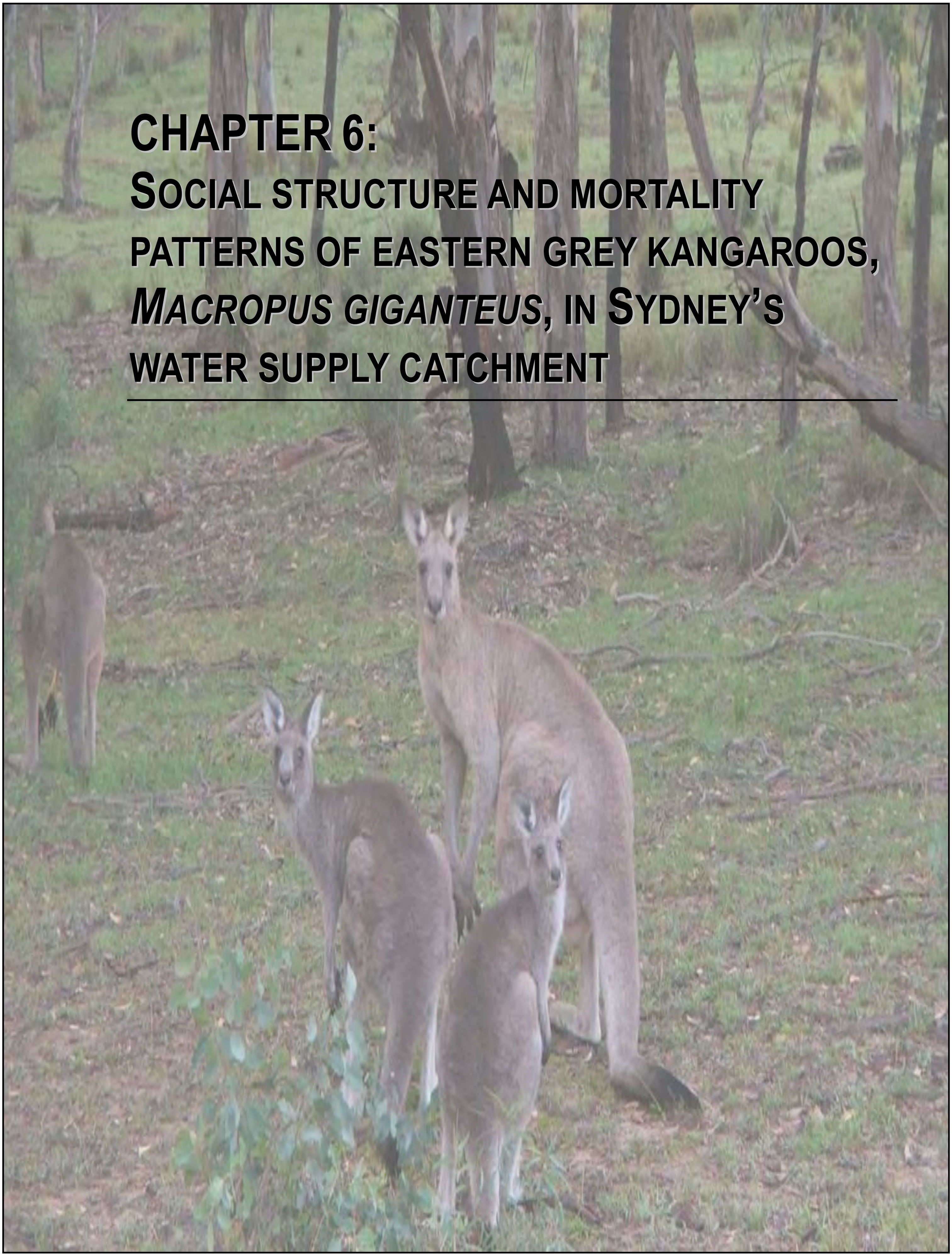
# CHAPTER 6: SOCIAL STRUCTURE AND MORTALITY PATTERNS OF EASTERN GREY KANGAROOS, *MACROPUS GIGANTEUS*, IN SYDNEY'S WATER SUPPLY CATCHMENT



# CHAPTER 6: SOCIAL STRUCTURE AND MORTALITY PATTERNS OF EASTERN GREY KANGAROOS, *MACROPUS GIGANTEUS,* IN SYDNEY'S WATER SUPPLY CATCHMENT

## *Preamble to Chapter 6*

In Chapter 2, I established that eastern grey kangaroos residing adjacent to the Wollondilly River were hosts to four zoonotic disease organisms. The subsequent chapters demonstrated that kangaroos are at a high enough density to be important sources of these pathogens to Sydney"s water supply (Chapter 3, 4 & 5),. This chapter presents information on the social structure and mortality patterns of this species in the catchment area,

In this chapter I investigate group size, group composition, timing of reproduction and mortality patterns of eastern grey kangaroos, and their relevance to zoonotic disease management. In particular, I investigate previous suggestions that the presence of pouch young is responsible for the reported autumn epidemics of cryptosporidiosis that have been found in this population. Finally, I explored my *apriori* expectation that the social system of the eastern grey kangaroo in the catchment area would differ to elsewhere because of the absence of humans and overt human disturbance in the catchment area.

## INTRODUCTION

The social systems exhibited by humans and other animal species have been associated with the rate of disease transmission and disease prevalence in a myriad of studies (Table 6-1) In particular, host proximity is recognised as having significant effects on the transmission of contagious or contact-transmitted pathogens and parasites (Ezenwa 2004, Gudelj and White 2004). In many animal species the social factors that drive pathogen loads appear to be seasonal, as neighbour - to – neighbour contacts vary predictably with shifts in reproductive, dispersive, aggressive, amicable or other behaviours. For instance, the aggregation of gorillas (*Gorilla beringei*) around seasonal food resources has been associated with the high prevalence of the Ebola virus (Pinzon *et al.* 2004), while increased prevalence of helminth parasites in *Antechinus* spp. in winter appears to be driven by the increased ranging behaviour of males at that time and the consequent increase in encounter rates with other individuals (Lee and Cockburn 1985). Furthermore, bacterial eye infections in house finches (*Carpodacus mexicanus*) have been related to seasonal flocking and the recruitment of immunologically naïve juveniles in autumn (Hosseini *et al.* 2004).

The relationship between the group size of a species and disease prevalence has been well studied, as grouping promotes close contact between individuals (Ezenwa 2004, Altizer *et al.* 2006). Social grouping varies according to the seasonal availability of resources for a wide range of species. For instance, in dry periods animals may congregate around spatially-limited foraging sites or water supplies, which support a greater potential for disease transmission. Epizootics of rabies in skunks (*Mephitis mephitis*) and jackals (*Canis mesomelas*), for example, have been correlated with seasonal host crowding (Loveridge and Macdonald 2001). Also, larger group sizes tend to increase the level of interactions between individuals that are infected with others that are susceptible to a particular disease, which in turn may elevate the pathogen load in the population (Loehle 1995). Ezenwa (2004) demonstrated that grouping behaviour had a strong influence on nematodes and coccidias in bovid species in Africa. This researcher concluded that nematode strongyles and coccidia oocysts occur at higher intensities in gregarious rather than in solitary bovid species. Furthermore, the transmission of faecal-oral route diseases may be exacerbated in gregarious herbivore species, as it has been reported previously that foraging leads to a higher uptake of infectious diseases (Hutchings *et al.* 2006). These observations suggest that knowledge of the social organisation and grouping behaviour of animal species should help to predict and understand patterns of associated disease prevalence.

**Table 6-1 Pathogens/parasites in humans and other vertebrates that have social drivers for their transmission and prevalence**

| Host | Pathogen/disease | Timing of outbreak | Social parameter | Reference |
|---|---|---|---|---|
| Humans | Measles | Spring and autumn | Aggregation of children at the commencement of school calendar | (Fine and Clarkson 1982) |
| Humans | Rotavirus | Winter | Aggregation of children | (Cook *et al.* 1990) |
| Red fox (*Vulpes vulpes*) | Rabies | Winter | Breeding season and dispersal, which increases contact between individuals | (Macdonald and Voigt 1985) |
| Jackals (*Canis mesomelas*) | Rabies | Winter | Breeding season and dispersal, which increases contact between individuals<br>Young males were more vulnerable to diseases due to larger exploratory patterns | (Loveridge and Macdonald 2001) |
| Skunks (*Mephitis mephitis*) | Rabies | Early spring and autumn | Seasonal host crowding<br>Early spring epidemics related to juvenile dispersal and increased potential contact with raccoons<br>Autumn peaks in prevalence related to breeding season | (Guerra *et al.* 2003) |
| Gorillas (*Gorilla beringei*) | Ebola hemorrhagic fever (EHV virus) | During dry conditions following the wet season | Aggregation of animals around seasonal food resources (e.g. fruiting trees) | (Pinzon *et al.* 2004) |
| Harp seals (*Phoca groenlandica*) | Phocine distemper virus | Location dependent | Linked to the period when this species hauls out and aggregates on beaches | (Swinton *et al.* 1998) |
| House finches (*Carpodacus mexicanus*) | Bacterial eye infection (*Mycoplasma gallisepticum*) | Autumn | Recruitment of young animals that are immunologically naïve | (Hosseini *et al.* 2004) |

| Host | Pathogen/disease | Timing of outbreak | Social parameter | Reference |
|---|---|---|---|---|
| | | | Seasonal flocking | |
| Antelope | Nematode infections | Unknown | Territorial individuals had higher prevalence than non-territorial animals<br>Suggestive of sedentary nature of these animals in defended territories and their exposure to infective stages accumulating in the environment | (Altizer *et al.* 2003) |
| Cat (*Felis catus*) | Feline immunodeficiency virus (FIV) and feline leukemia virus (FeLV) | Unknown | Exclusively found in male cats as a result of fighting activities to maintain dominant status | (Fromont *et al.* 1994) |
| Rabbits (*Oryctolagus cuniculus*) | *Mycobacterium avium* subsp. *paratuberculosis* | Unknown | Young rabbits consuming contaminated milk and ingestion of faecal material during weaning process | (Judge *et al.* 2006) |
| Canadian Geese (*Branta canadensis maxima*) | Highly pathogenic Avian Influenza | Unknown | Young ducks are more likely to carry and transmit the disease | (Greger 2007) |

Besides group size, there are several other social factors that may foster an increased level of contact between a pathogen and susceptible animals within a population. These may include mating systems and the sex and age structures in a population. However, few studies have attempted to correlate these factors with disease. Ezenwa (2004) demonstrated a relationship between exposure to disease, territoriality and social class in bovid species. In this study, territorial males had a higher prevalence of coccidia than bachelor males and this was hypothesised to be a result of their sedentary nature within their defended territories and also their suppressed immune systems as a consequence of mate guarding. In another study, Loveridge and Macdonald (2001) showed that jackals had a higher prevalence of rabies during the breeding season. This was related, firstly, to increases in home range size and subsequent overlaps with neighbouring individuals and secondly, to the usage of peripheral areas by juvenile males that increased their contact with other population members. Collectively, these studies highlight the importance of combining epidemiological studies with population ecology to obtain an understanding of disease prevalence and transmission. In addition, these examples emphasise that it may be difficult or misleading to assess the dynamics of disease prevalence without consideration of the heterogeneity in transmission rate that is likely to be imposed by the social structure of a species.

In Chapter 2, I described four zoonotic diseases that were found in my study population of eastern grey kangaroos (*Macropus giganteus*). The transmission and prevalence of these diseases have been found to be linked to elements of host social structure in a wide range of animal species (Table 6-2).

**Table 6-2 Social structure linkages with zoonoses identified in eastern grey kangaroos**

| Disease and transmission mode | Social driver | Reference |
|---|---|---|
| *Cryptosporidium* – faecal-oral route disease, ingestion of oocysts in contaminated food and water | Presence of juveniles in a range of species<br>e.g. Dairy cattle<br>*Cryptosporidium* was detected only in calves and heifers. | (Coklin *et al.* 2007) |
| *Giardia* – faecal-oral route disease, ingestion of cysts from contaminated food and water | Presence of juveniles<br>e.g. Dairy cattle<br>Higher prevalence in young cattle (< 6 months of age) that were not fed colostrum or not separated from the | (Wade *et al.* 2000) |

| Disease and transmission mode | Social driver | Reference |
|---|---|---|
| | herd at birth<br>Disease risk decreased with increase in age<br>Higher prevalence during summer housing of bred heifers on pasture within 2 months of calving | |
| *Toxoplasma* – faecal-oral route disease in cats (definitive host) and through the ingestion of cysts from the muscle and nervous tissue fibre in the intermediate host (e.g. kangaroos) | Mortality of the intermediate host<br>e.g. cat, mink and red fox consumption of infected squirrels, rabbits, muskrats and „Alala (*Corvus hawaiiensis*). Such ingestion increases the availability of this disease as carrion is consumed by carnivores (in particular by feral cats that can excrete the oocyst stage of this disease) | (Smith and Frenkel 1995, Work *et al.* 2000) |
| *Leptospira* – bacteria encased in urine droplets, ingestion, broken skin | Breeding season and tight social groups<br>e.g. Brushtail possums (Trichosurus vulpecula)<br>Agonistic contacts and general close social contacts. This disease can also be sexually transmitted. | (Caley and Ramsey 2001) |

As has been demonstrated in other species, the transmission and prevalence of these diseases also would be very likely influenced directly by the social systems of the eastern grey kangaroo. The only exception is *Toxoplasma*, as the social system of the cat (the definitive host) would influence the transmission and prevalence of this disease organism most directly. However, because cats have been reported to scavenge on kangaroo carrion (Triggs et al. 1984, Catling 1988) and prey on juvenile macropodid species (Fisher et al. 2001), as an intermediate host of this disease the mortality patterns and the presence of juvenile eastern grey kangaroos would be likely to indirectly influence the availability of *Toxoplasma* cysts in the environment (Chapter 2).

The social systems of the eastern grey kangaroo have been thoroughly investigated. A large suite of studies has concluded, collectively, that this species has highly seasonal and predictable social traits (see Coulson 2009). Therefore, the patterns in the timing of these traits and the factors that influence them will be important to elucidate the potential links that social structure has with the prevalence and transmission of zoonotic disease in this species. This will in turn enable the risk of environmental contamination for each of the zoonotic diseases to be adjudicated.

### *The social structure of the eastern grey kangaroo*

Eastern grey kangaroos are gregarious. They form open membership groups, and the groups consist of non-random associations between individuals that may constantly join and leave (Jarman and Coulson 1989, Coulson 2009). The average numbers of animals in eastern grey kangaroo groups range from two to ten (Table 6-3). The typical relatedness between group members is unclear, but recent studies suggest that pairings of individual females and young may be kin (Dawson 1995). Larger groups may form to permit the early detection of potential predators (Banks *et al.* 2000, Banks 2001), in open habitats (Heathcote 1987), and also to concentrate on limited resources (e.g. feed, water and shelter) (Kaufmann 1975, Dawson 1995). Group sizes may therefore fluctuate seasonally according to the interplay of these factors. As such, given the evidence that larger group sizes promote disease transmission through close contact (Guerra *et al.* 2003), determining when larger aggregations are most likely may indicate when transmission of the identified diseases potentially will be highest.

**Table 6-3 Mean group sizes of eastern grey kangaroos from published literature**

| Mean group size | Study area | Reference |
|---|---|---|
| 3.7 | Bonalbo, NSW | (Kaufmann 1974) |
| 2.5 – 5 | New England Tablelands, NSW | (Taylor 1982) |
| 2.8 – 10.5 | New England Tablelands and Nocoleche, NSW | (Southwell 1984b) |
| 4.0# | Entire distribution of species | (Dawson 1995) |
| 3.3 - 9.1 | Wallaby Creek, NSW | (Clarke *et al.* 1995) |
| 3.2 | Grampians, Victoria | (Coulson 1999) |
| 2.8 | Yathong Nature Reserve, NSW | (McCullough and McCullough 2000) |
| 3.5 - 8 | Namadgi, ACT | (Banks 2001) |

Notes - # represents an average of group sizes abstracted from several studies that are not specified in this reference

Eastern grey kangaroos can breed at any time of the year, but they are typically seasonal breeders with peaks in spring and summer (Dawson 1995). During favourable conditions females may mate when the pouch young are less than six months old, with the resulting embryo held in diapause in response to lactational inhibition. This embryo is released following vacation of the incumbent pouch young from the pouch or following the premature loss of that young due to drought-related mortality or predation. Females do not normally enter oestrus again immediately after birth, but may return to oestrus 11 days after the loss of pouch young (Poole 2002). The first exit from the pouch occurs at approximately 9 months and during what is referred to as the „in-out" period, the pouch young grows rapidly and from the pouch begins eating grass. Permanent exit from the pouch occurs at approximately 10.6 months, when the young-at-foot continue to suckle from the mother for another seven to eight months longer, but the level of suckling decreases as the young-at-foot increases the uptake of herbage (Dawson 1995). Fletcher (2007) determined that in south-eastern Australia young were born predominantly in summer and large pouch young emerged in the following spring. Data on the reproductive biology of this species from the ACT shows very high levels of fecundity at high population densities and low per capita food availability. This trend is probably typical of populations of this species in temperate climates.

The presence and number of juvenile eastern grey kangaroos in a population probably has important implications for the risk of *Cryptosporidium* transmission. A large body of research has found that both *Cryptosporidium* and *Giardia* are more prevalent in younger cohorts in a range of animal species (Shiibashi et al. 2006, Coklin et al. 2007), with resultant higher shedding intensities being found in these generations. In earlier work, Power *et al*. (2005) also noted that the autumn epidemics of *Cryptosporidium* in eastern grey kangaroos are probably linked to the usually high abundance of pouch young at this time. Despite the likely correlation, however, the work of Power *et al*. (2005) was purely epidemiological and did not quantify the seasonal abundance of pouch young at the time of their study to verify their suggestion. To confirm the nature of the disease-young host association, it is therefore considered essential to collect empirical data on the abundance of pouch young concurrently with epidemiological studies. This is a key objective of the present chapter.

Mortality of eastern grey kangaroos peaks during drought periods, with more males succumbing than females due to their larger metabolic requirements and greater ranging movements (Dawson 1995). Several studies also have found asymmetric patterns in the mortality of this species with age, with death rates of animals less than two years old being greater than those of older animals (Quin 1989, Fletcher 2007). Death of sub-adult animals has been hypothesised to result from starvation during dry winters, as animals in this age cohort have relatively high intake requirements for digestible energy compared with older animals (Fletcher 2007). This trend has been observed in many other herbivore species and is a defining demographic characteristic in regulating population size (Fletcher 2007). Therefore, there is threefold importance in assessing mortality patterns for disease interpretation. Firstly, due to many diseases being density-dependent (Hone and Donnelly 2008), mortality may indirectly influence the prevalence of an expressed disease by reducing population size. Secondly, when mortality is high an increase in the number of kangaroo carcasses may lead to the direct entry of pathogens into the environment (Work et al. 2000), and thirdly, mortality may be a surrogate measure for disease prevalence, as disease is a common cause of mortality in macropodids, especially in drought conditions (Dawson 1995).

Given the importance of social structure in the expression and interpretation of disease, this chapter aims to explore the disease - social structure association in eastern grey kangaroos in the Sydney water catchment area. Specific aims are to:

1) Examine the social structure of eastern grey kangaroos, in particular patterns of group size, age, sex ratio and the abundance of juveniles;
2) Examine age and sex specific mortality patterns;
3) Infer the relevance of patterns of social structure for the management zoonotic disease in Sydney‟s water supply catchment; and,
4) Provide new insights into the social structure of the target species in a population that receives very little disturbance or human intervention.

## MATERIALS AND METHODS

### *Group composition*

A four-wheel drive vehicle was used for observations of group size and composition at Douglas Scarp, Murphy"s Crossing and Jooriland. For consistency, the vehicle was driven at a constant low speed (10 -15 km/h) along a route that covered all of the roads at these sites. Monthly surveys were conducted between July 2004 and June 2005 (with the exception of August 2004 and May 2005) in the period two hours before dusk. Previous research has demonstrated this to be the peak foraging time of kangaroos, when they are often found in open habitats (Coulson 2009). Restricting observations to this period permitted consistency between observations and allowed a standardised measure of attainable group sizes in high risk areas for disease transmission to surface water. When a group of kangaroos was detected, the location, group size, sex and approximate age (adult, young-at-foot and pouch young) of the group members were noted. The definition of a group was maintained as described in Chapter 3, i.e. as animals within 50 m of each other. Young animals that had not permanently left the pouch of their mother were not included in the calculation of group size. These observations were aided by the use of a spotting scope (Nikon 80A series 15-45x/20-60x) and binoculars (Australian Geographic 4 - 60x) at a minimum distance of approximately 50 – 60 m. This setback distance minimised disturbance and reduced the chance of kangaroos taking flight.

### *Timing of reproduction*

A total of eighty-nine female eastern grey kangaroos were captured between June and September 2005 and 2006 using a tranquiliser firearm and the sedative Zoletil 100® as described by the protocol in Appendix A. The pouch of females was inspected for pouch young (PY) and the condition of the pouch was noted (clean, dirty, teats elongated/everted/not everted). Pes, leg and head measurements were taken on all PY using vernier callipers. The approximate age of the PY was determined using growth curves derived for the head, leg and foot measurements (Poole *et al.* 1982).

### *Mortality*

A total of 256 eastern grey kangaroo skulls were collected from Murphy‟s Crossing, Jooriland and Douglas Scarp between 2004 and 2007. Skulls were collected on a monthly basis during routine field work activities and typically whenever carcasses of kangaroos, which had died from natural causes, were found within these sites. The skulls were cleaned, if necessary, by boiling them for about four hours in a slow cooker with enzymatic washing powder. Skulls were then dried at 80°C for ten to fifteen minutes in an oven.

The age of each kangaroo was determined using molar progression. This is the process by which, as teeth wear down they move forward relative to the zygomatic process and are shed from the jaw. The rate at which this process occurs slows with an increase in age and eventually all but the rear molars are lost (Kirkpatrick 1964, Dawson 1995).

Cleaned skulls were photographed using a digital SLR camera (Canon 300DF) perpendicular to the line of vision. A reference line, tangential to the anterior rims, was drawn and the number of molars in front of the line was determined. The proportion of any molars that were bisected by the reference line was determined by using a grid template divided into ten equal portions in Microsoft PowerPoint®. The template was re-sized to the same size as the molar that was bisected by the reference line and the proportion in front of it was estimated to the nearest 0.1. The number of molars in front of the reference line, averaged for both sides of the jaw, was used to determine the overall molar index for each kangaroo. This process involved differentiation of molars from pre-molars to accurately assess age (Figure 6-1). The age of the skull was then determined using reference data prepared by Kirkpatrick (1964) (Figure 6-2). 95% confidence limits of the age in years were ± 0.2.

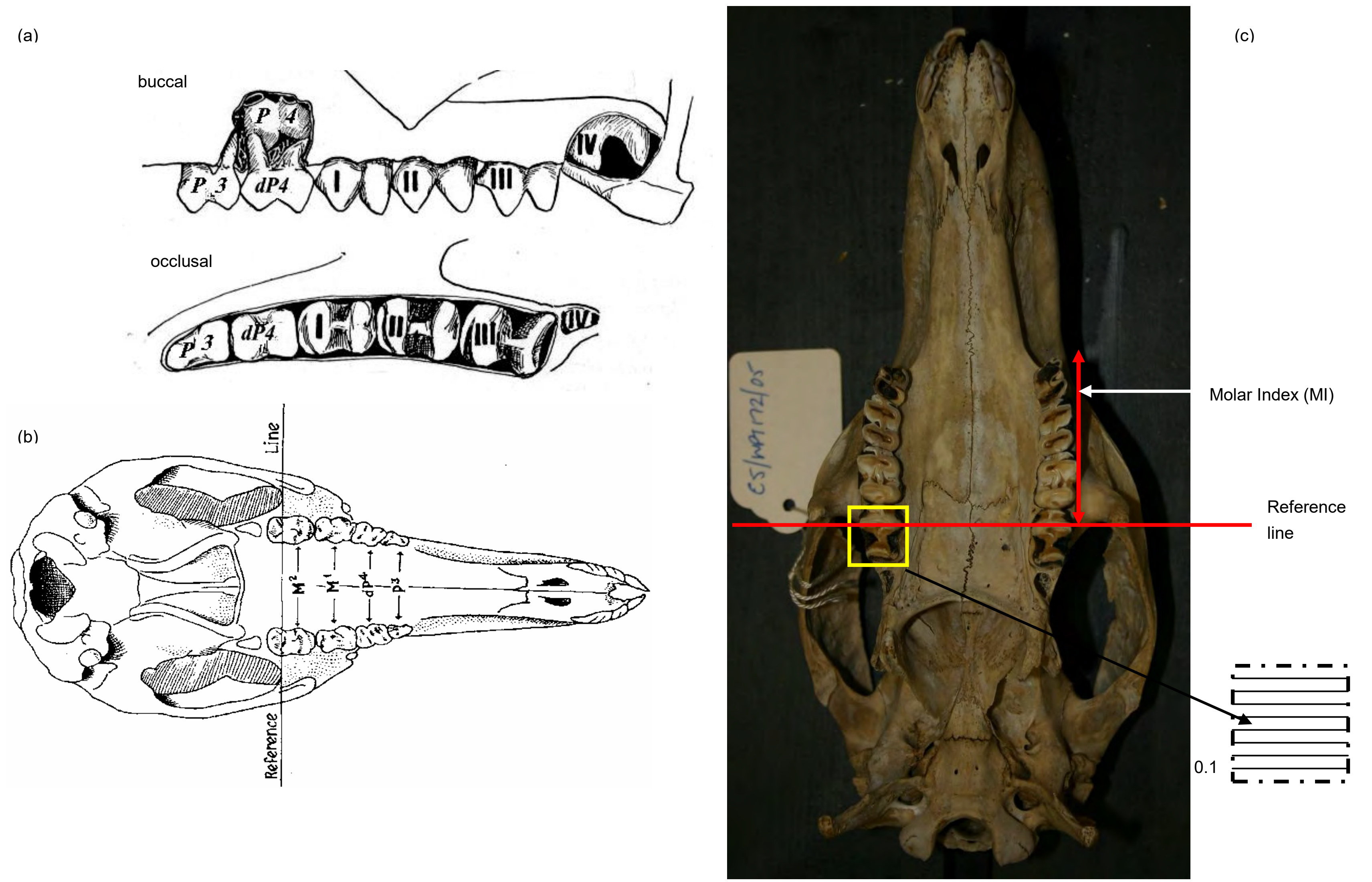



**Figure 6-1** **(a) Dentition of eastern grey kangaroos showing the differentiation between dP4 and P4 (b) Process by which molars are counted forward of the reference line (c) Photograph of skull illustrating measurements made in Microsoft Powerpoint®**

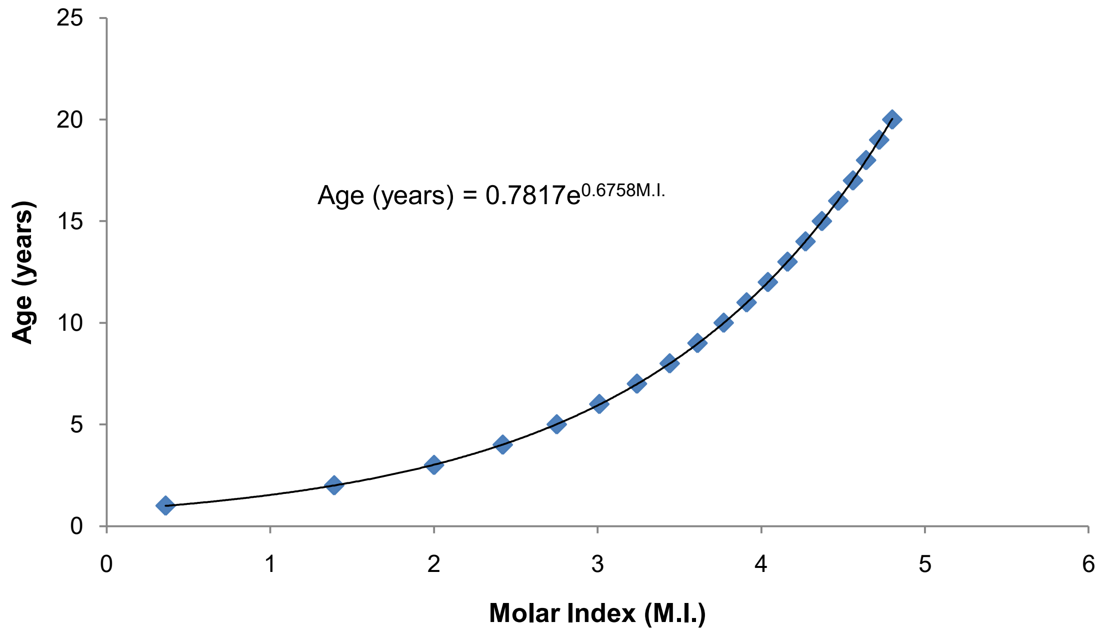



**Figure 6-2** **The relationship between molar index (MI) and age in years of eastern grey kangaroo skulls (Kirkpatrick 1964)**

In circumstances where the sex of the kangaroo carcasses could not be determined in the field, the basal length (i.e. from the anterior tips of the premaxillae to the anterior rim of the foramen magnum) and the molar index (M.I.) were used to establish the sex of the carcass by reference data, as defined by Kirkpatrick (1964, 1967). This relationship is based on variations associated with the size of skulls at particular ages as a result of sexual dimorphism in this species. If the basal length of a skull was less than 14.6 centimetres, or if the molar index was less than 1.25, the sex of the carcass could not be differentiated (Figure 6-3). Therefore, animals falling in this age category were pooled together and classified as juveniles.

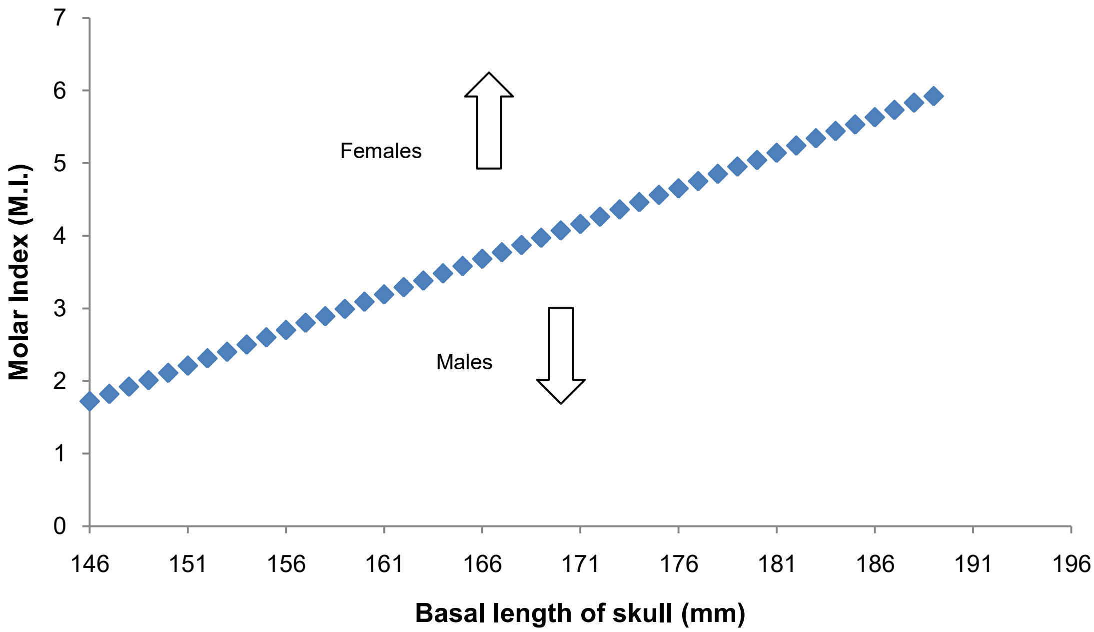



**Figure 6-3** **The relationship between basal length (mm) and molar index (MI) in the determination of the sex of eastern grey kangaroo skulls (Kirkpatrick 1967)**

## *Data analysis*

Individual groups were considered as replicates for each monthly survey for mean group size and age-sex category because they were not derived from repeated measures during a single observation session. Groups containing individuals of unknown sex were excluded from further analysis. The effect of the observation time on group size was determined by ANOVA, and Tukey HSD *post-hoc* testing was used to compare any significant results. Group size data were log-transformed to ensure compliance with the assumptions of ANOVA.

To test for differences in the sex and age composition of eastern grey kangaroos between the survey months, a two-way chi-squared test of the raw frequencies was used. To determine how adults and juveniles comprised groups of different sizes, analysis was carried out in the same way as Arnold *et al*. (1991) by categorising all the possible adult/juvenile combinations (e.g. group size = 3: two males and female) in group sizes ranging from 1 to greater than 5. Differences in these grouping formations between survey months were assessed using a chi-squared test on raw frequencies.

A chi-squared test on the raw frequencies was used also to test for differences in mortality between sexes, age and year of collection using the number of skulls that were identified to fall within each age category.

In all chi-squared tests, significant cells were identified by analysis of the cell chi-square and the expected cell count. Partial chi-square values and cell chi-square values were used to assess which groups contributed the most to any differences that were found.

## RESULTS

### *Group size*

Group sizes ranged from 1 to 84 across the months surveyed, with most of the groups observed comprising more than six animals (Table 6-4). Mean (± SD) group size ranged from 6.3 (± 0.7) in July 2005 to 9.4 (± 3.3) in September 2004 (Figure 6-4). Group sizes were not significantly different between the observation months ($F_{9,903}$ = 1.31; $P$ = 0.23).

**Table 6-4 Group sizes of eastern grey kangaroos between July 2004 and June 2005 expressed as proportions of observations per month.**

| Group Size | Observation month | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | July | Sept | Oct | Nov | Dec | Jan | Feb | March | April | June |
| 1 | 18.0 | 15.4 | 21.9 | 21.1 | 12.7 | 13.3 | 9.1 | 14.4 | 23.0 | 21.6 |
| 2 | 12.4 | 38.5 | 9.4 | 15.4 | 10.1 | 18.3 | 16.7 | 16.9 | 18.1 | 14.4 |
| 3 | 9.0 | 3.8 | 6.3 | 8.0 | 5.1 | 9.2 | 13.6 | 11.1 | 9.6 | 8.8 |
| 4 | 10.1 | 7.7 | 6.3 | 6.3 | 3.8 | 5.8 | 7.6 | 5.1 | 3.6 | 10.4 |
| 5 | 10.1 | 3.8 | 3.1 | 8.6 | 13.9 | 8.3 | 4.5 | 5.1 | 4.8 | 6.4 |
| 6 | 9.0 | 3.8 | 15.6 | 3.4 | 15.2 | 7.5 | 4.5 | 9.3 | 7.2 | 7.2 |
| >6 | 31.5 | 26.9 | 37.5 | 37.1 | 39.2 | 37.5 | 43.9 | 38.1 | 33.7 | 31.2 |
| Maximum group size | 57 | 84 | 23 | 74 | 77 | 49 | 47 | 68 | 62 | 57 |

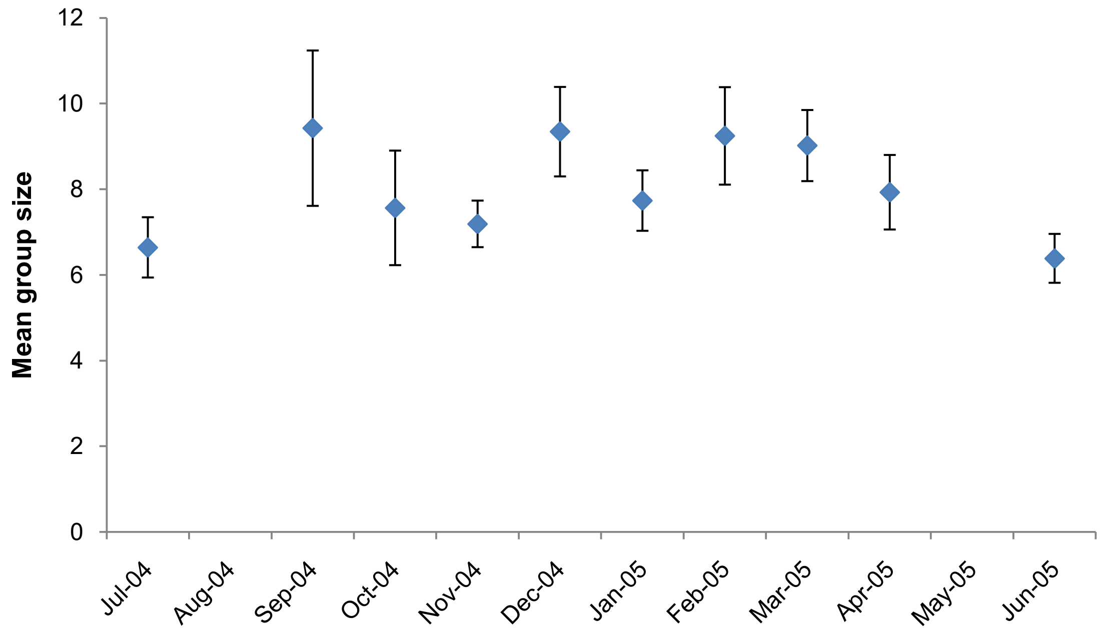



**Figure 6-4** **Mean (± SD) group size of eastern grey kangaroos between July 2004 and June 2005.**

### *Group composition*

The sex ratios of eastern grey kangaroos ranged from 2 to 3 females to every male, with the exception of November 2004 when the ratio almost reached parity (Figure 6-5). Overall, the female biased sex ratio was significant ($x^2$ = 89.6, *d.f* = 9, *P*<0.001), with fewer adult males than expected by chance and a high proportion of females observed. The high weighting of females compared to males was most apparent June 2005 (cell $x^2$ = 7.2). Conversely, there were more males within groups in November 2004 (cell $x^2$ = 30.3) and in March 2005 (cell $x^2$ = 3.9).

The number of dependent juveniles (YAF and PY combined) ranged from 0.21 in February 2005 to 0.65 per adult female in October 2004 (Figure 6-5). There was no significant differences in the number of juveniles present between the survey months ($x^2$ = 9, *d.f.* = 9, *P*>0.05). When analysing PY separately, the number of PY per female ranged from 0.03 in January 2005 to 0.22 in October 2004. This was also found to be non- significant between the sampling months ($x^2$ = 9, *d.f.* = 9, *P*>0.05).

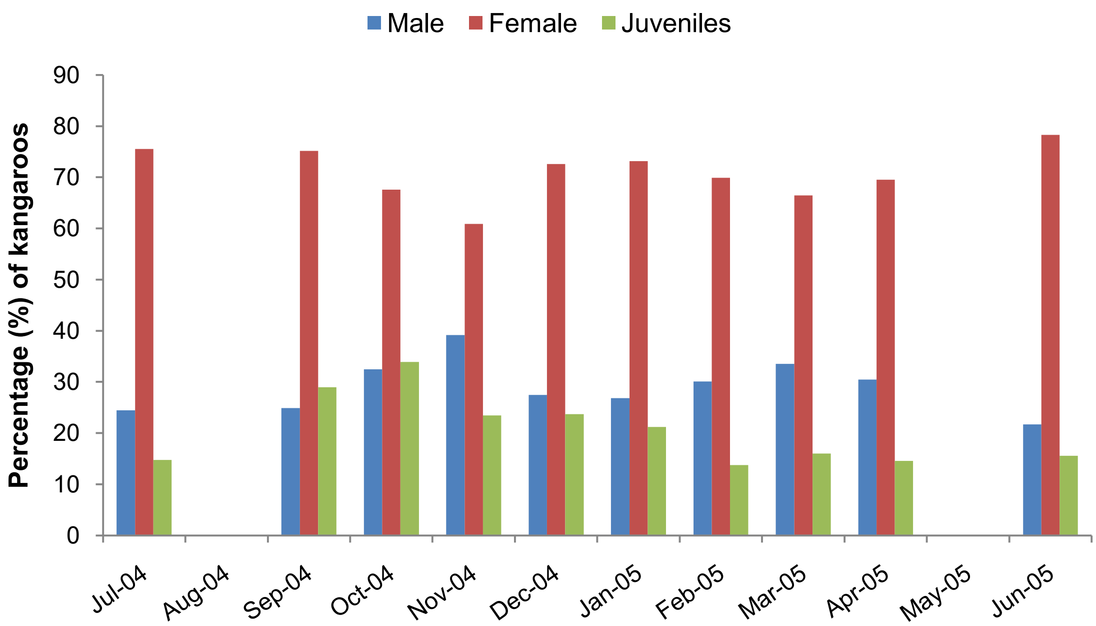



**Figure 6-5** **Percentage of males, females and juvenile eastern grey kangaroos in groups recorded from July 2004 to June 2005**

An analysis of how adults and juveniles comprised groups of different sizes is shown in Table 6-5. As a summary, the grouping patterns of adult males, adult females and juveniles are discussed separately:

***Adult males****:* These were found to be solitary more than expected in October, November and March. In groups of 2, adult males associated more with females than expected in November and June and less than expected with juveniles in all months. In groups of 3, adult males were found in mixed groups (females and juveniles) more than expected in summer. In groups of 4 and more, males occurred in mixed groups and in groups with the opposite sex in most seasons. Males were commonly found to associate with peers more than expected in October, November, March and April when in groups of 2, in July, March and April when in groups of 3 and in July and March when in groups of greater than 4.

***Adult females****:* These were observed to be solitary more than expected in July and in the summer months. When in groups of 2, females were associated with juveniles more than expected between October through to February and less than expected with adult males with the exception of November. In groups of 3, females associated with two males more than expected in October, November and March, with other females and males in November and January through to March and with females and juveniles in July, September, December and January. In groups greater than four, females were found in mixed groups or in groups with the opposite sex in most seasons. Females associated with peers more than expected during July and February to June when in groups of 2, in July, February and June when in groups of 3 and in July when in groups greater than four.

***Juveniles***: Juveniles associated with females as expected in most of the survey months. They were found to be solitary more than expected from July to October and in December and January. Juveniles associated with peers more than expected in June and July when in groups of 2, but as the group size increased juveniles were typically found in mixed groups. However, this occurred less than expected in the autumn and winter months when groups comprising opposite sexes were observed more than expected.

### *Reproduction*

The estimated month of birth of 42 pouch young between 2005 and 2006 is illustrated in Figure 6-6. Births were recorded predominantly in January (47.6%), with none recorded in July or between September and November. The sex ratios of pouch young were female biased with 62% female and 38% male.

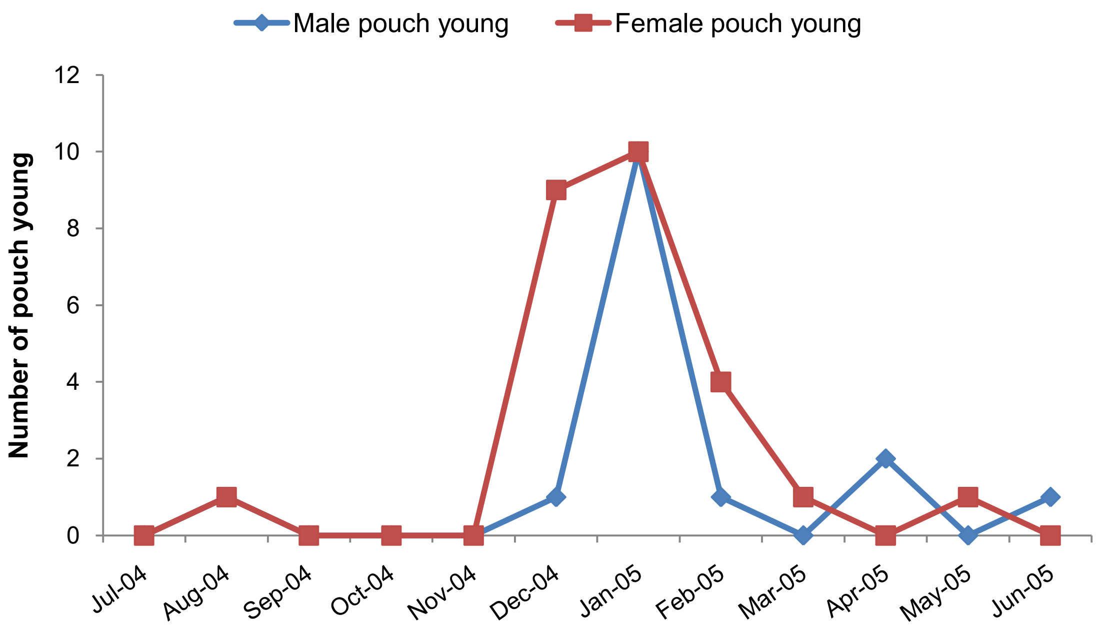



**Figure 6-6** **The number of estimated births and pouch young sex from 89 captured eastern grey kangaroos between July 2004 and June 2005.**

**Table 6-5 The composition of eastern grey kangaroo groups from July 2004 – June 2005**

Notes: Numbers refer to percentage (%) contribution within each grouping; „+"and „-" refers to whether there was more (+) or less (-) than expected within each grouping

| | July | Sept | Oct | Nov | Dec | Jan | Feb | Mar | Apr | June |
|---|---|---|---|---|---|---|---|---|---|---|
| Group size = 1 | | | | | | | | | | |
| Adult females | $56.3^{+}$ | $25.0^{-}$ | $0^{-}$ | $21.6^{-}$ | $60.0^{+}$ | $62.5^{+}$ | $66.7^{+}$ | $29.4^{-}$ | $57.9^{+}$ | $59.3^{+}$ |
| Adult males | $37.5^{-}$ | $50.0^{-}$ | $85.7^{+}$ | $78.4^{+}$ | $10.0^{-}$ | $31.3^{-}$ | $33.3^{-}$ | $70.6^{+}$ | $42.1^{-}$ | $37.0^{-}$ |
| Juveniles | $6.3^{+}$ | $25.0^{+}$ | $14.3^{+}$ | $0^{-}$ | $10.0^{+}$ | $6.2^{+}$ | $0^{-}$ | $0^{-}$ | $0^{-}$ | $3.7^{-}$ |
| Group size = 2 | | | | | | | | | | |
| Two adult females | $72.3^{+}$ | $20.0^{-}$ | $0^{-}$ | $14.8^{-}$ | $12.5^{-}$ | $18.2^{-}$ | $45.5^{+}$ | $45.0^{+}$ | $40.0^{+}$ | $55.5^{+}$ |
| Adult female and juvenile | $0^{-}$ | $30.0^{-}$ | $66.6^{+}$ | $33.4^{+}$ | $75.0^{+}$ | $59.1^{+}$ | $36.3^{+}$ | $25.0^{-}$ | $20.0^{-}$ | $11.1^{-}$ |
| Adult female and adult male | $18.2^{-}$ | $20.0^{-}$ | $0^{-}$ | $37.0^{+}$ | $12.5^{-}$ | $18.2^{-}$ | $18.2^{-}$ | $15.0^{-}$ | $20.0^{-}$ | $22.2^{+}$ |
| Two adult males | $0^{-}$ | $20.0^{-}$ | $33.3^{+}$ | $14.8^{+}$ | $0^{-}$ | $4.5^{-}$ | $0^{-}$ | $15.0^{+}$ | $20.0^{+}$ | $5.6^{-}$ |
| Adult male and juvenile | $0^{-}$ | $10.0^{+}$ | $0^{-}$ | $0^{-}$ | $0^{-}$ | $0^{-}$ | $0^{-}$ | $0^{-}$ | $0^{-}$ | $0^{-}$ |
| Two juveniles | $9.1^{+}$ | 0 | $0^{-}$ | $0^{-}$ | $0^{-}$ | $0^{-}$ | $0^{-}$ | $0^{-}$ | $0^{-}$ | $5.6^{+}$ |
| Group size = 3 | | | | | | | | | | |
| Three adult females | $37.5^{+}$ | $0^{-}$ | $0^{-}$ | $0^{-}$ | $0^{-}$ | $18.2^{-}$ | $33.3^{+}$ | $7.7^{-}$ | $12.5^{-}$ | $45.4^{+}$ |
| Adult female and two males | $0^{-}$ | $0^{-}$ | $100.0^{+}$ | $21.4^{+}$ | $0^{-}$ | $0^{-}$ | 11.1 | $46.1^{+}$ | $25.0^{-}$ | $9.1^{-}$ |
| Adult male and two females | $12.5^{-}$ | $0^{-}$ | $0^{-}$ | $35.8^{+}$ | $0^{-}$ | $27.3^{+}$ | $44.4^{+}$ | $15.4^{+}$ | 0 | $9.1^{-}$ |
| Three adult males | $12.5^{+}$ | $0^{-}$ | $0^{-}$ | $0^{-}$ | $0^{-}$ | $0^{-}$ | $0^{-}$ | $7.7^{+}$ | $25.0^{+}$ | $0^{-}$ |
| Adult male, adult female and | $12.5^{-}$ | $0^{-}$ | $0^{-}$ | $21.4^{+}$ | $25.0^{+}$ | $18.2^{+}$ | $11.1^{-}$ | $15.4^{-}$ | $12.5^{-}$ | $18.2^{+}$ |

| | July | Sept | Oct | Nov | Dec | Jan | Feb | Mar | Apr | June |
|---|---|---|---|---|---|---|---|---|---|---|
| juvenile | | | | | | | | | | |
| One adult female and two juveniles | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Three juveniles | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Two adult females and juvenile | $25.0^{+}$ | $100.0^{+}$ | $0^{-}$ | $21.4^{-}$ | $75.0^{+}$ | $36.3^{+}$ | $0^{-}$ | $7.7^{-}$ | $25.0^{+}$ | $18.2^{-}$ |
| Two adult males and juvenile | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Group size = 4 | | | | | | | | | | |
| Adult males | $22.3^{+}$ | $0^{-}$ | $0^{-}$ | $0^{-}$ | $0^{-}$ | $0^{-}$ | $0^{-}$ | $16.7^{+}$ | $0^{-}$ | $0^{-}$ |
| Adult females | $44.4^{+}$ | $0^{-}$ | $50.0^{+}$ | $0^{-}$ | $0^{-}$ | $0^{-}$ | $0^{-}$ | $0^{-}$ | $33.3^{+}$ | $30.7^{+}$ |
| Opposite sex only | $0^{-}$ | $0^{-}$ | $0^{-}$ | $9.1^{-}$ | $33.3^{+}$ | $28.6^{+}$ | $40.0^{+}$ | $50.0^{+}$ | $33.3^{+}$ | $23.1^{+}$ |
| Mixed (adults and juveniles) | $33.3^{-}$ | $100.0^{+}$ | $50.0^{-}$ | $90.1^{+}$ | $66.6^{+}$ | $81.4^{+}$ | $60.0^{+}$ | $33.3^{-}$ | $33.3^{-}$ | $46.2^{-}$ |
| Group size =5 | | | | | | | | | | |
| Adult males | $33.3^{+}$ | $0^{-}$ | $0^{-}$ | $0^{-}$ | $0^{-}$ | $0^{-}$ | $0^{-}$ | $0^{-}$ | $0^{-}$ | $0^{-}$ |
| Adult females | $11.2^{+}$ | $0^{-}$ | $0^{-}$ | $0^{-}$ | $0^{-}$ | $0^{-}$ | $0^{-}$ | $0^{-}$ | $0^{-}$ | $25.0^{+}$ |
| Opposite sex only | $33.3^{-}$ | $0^{-}$ | $0^{-}$ | $20.0^{-}$ | $27.3^{-}$ | $30.0^{-}$ | $0^{-}$ | $50.0^{+}$ | $100^{+}$ | $37.5^{+}$ |
| Mixed (adults and juveniles) | $22.2^{-}$ | $100.0^{+}$ | $100.0^{+}$ | $80.0^{+}$ | $72.7^{+}$ | $70.0^{+}$ | $100.0^{+}$ | $50.0^{-}$ | $0^{-}$ | $37.5^{-}$ |
| Group size >5 | | | | | | | | | | |
| Adult males | $5.4^{+}$ | $0^{-}$ | $0^{-}$ | $0^{-}$ | $0^{-}$ | $0^{-}$ | $0^{-}$ | $1.8^{+}$ | $0^{-}$ | $0^{-}$ |
| Adult females | $5.4^{+}$ | $0^{-}$ | $5.9^{+}$ | $0^{-}$ | $2.3^{+}$ | $0^{-}$ | $0^{-}$ | $3.6^{+}$ | $0^{-}$ | $4.2^{+}$ |

| | July | Sept | Oct | Nov | Dec | Jan | Feb | Mar | Apr | June |
|---|---|---|---|---|---|---|---|---|---|---|
| Opposite sex only | $36.6^{+}$ | $12.5^{-}$ | $11.8^{-}$ | $22.5^{-}$ | $20.9^{-}$ | $12.9^{-}$ | $31.3^{+}$ | $17.8^{-}$ | $38.2^{+}$ | $41.7^{+}$ |
| Mixed (adults and juveniles) | $52.6^{-}$ | $87.5^{+}$ | $82.3^{+}$ | $77.5^{+}$ | $76.7^{+}$ | $87.1^{+}$ | $68.7^{-}$ | $76.8^{+}$ | $61.8^{-}$ | $54.1^{-}$ |

### *Age and sex specific mortality*

The longest-living eastern grey kangaroo determined from skull analysis was a female aged 21 years, whereas the longest-living male was estimated to be 19.7 years old. Male mortality was found to be highest in the 10 – 11 year age category (21.2%) and for females mortality peaked between the 10 – 11 and the 12 – 13 year age categories (15.3%). Sixty-eight percent of males and 60.4% of females died between the ages of nine and 15 years (Figure 6-7). Overall, mortality between male and female kangaroos for each age category was not significantly different ($x^2$ = 0, *d.f.* = 18, *P*>0.05).

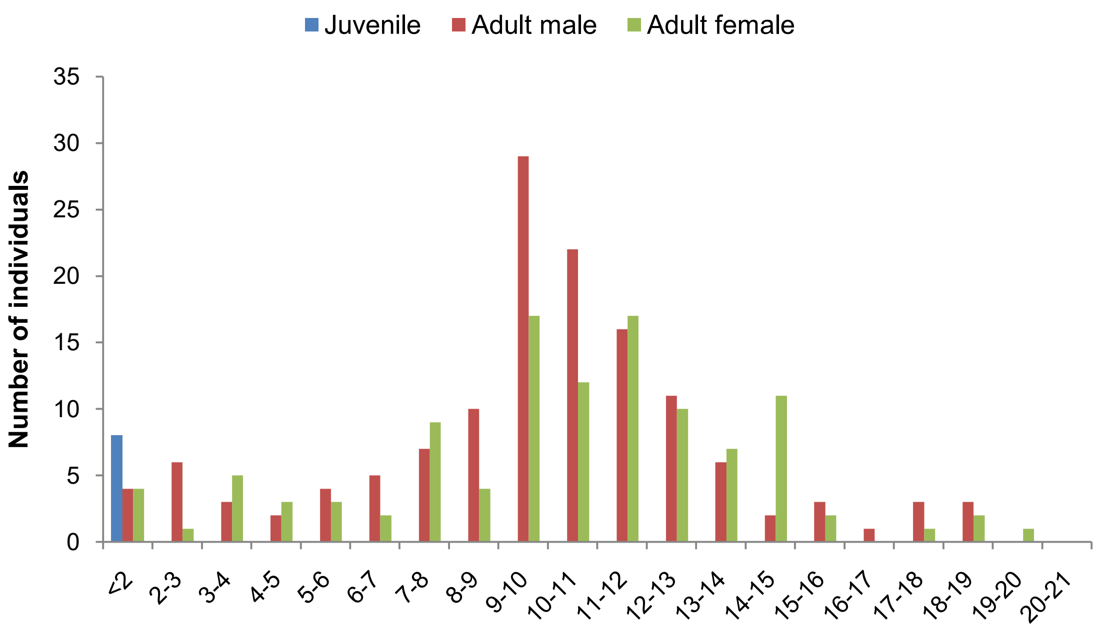



**Figure 6-7 Sex and age mortality distribution of eastern grey kangaroo determined from skulls collected between 2004 and 2007**

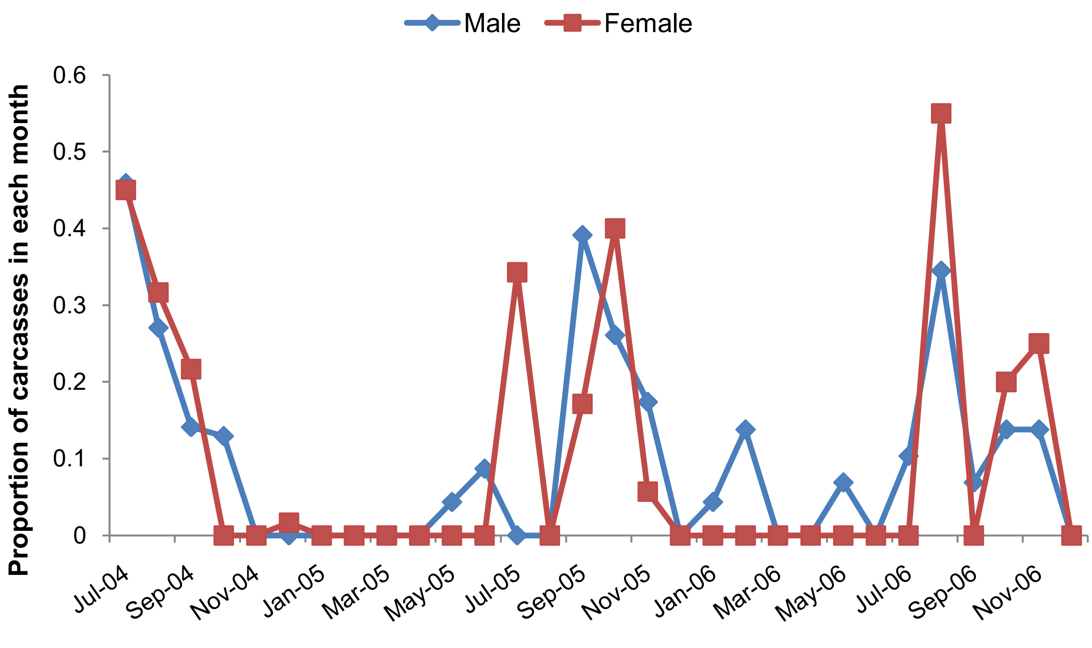
The average age at death and the proportions of male and female eastern grey kangaroo skulls found in the study area between 2004 and 2007 are presented in Table 6-6. More male skulls were found than female skulls in 2004 and 2006 – 2007, but not in 2005. Overall, this result was significant ($x^2$ = 6.6, *d.f.* = 2, *P* = 0.03) with more male skulls found in 2004 (cell $x^2$ = 0.48) and 2006 – 2007 (cell $x^2$ = 0.21) than expected by chance and more female skulls found in 2005 (cell $x^2$ = 2.75) than expected by chance. The average age of male and female kangaroos at death were very similar between the survey years, with females living slightly longer than males overall.

**Table 6-6 Adult eastern grey kangaroo mortality between 2004 and 2007 based on the analysis of skulls**

Notes: „+"refers to significant result

| | Males | | Females | |
|---|---|---|---|---|
| Year | Average age of males (± SD) | % | Average age of females (± SD) | % |
| 2004 | 10.0 (± 3.0) | $58.6^{+}$<br>n = 85 | 10.9 (± 3.0) | 41.4<br>n = 60 |
| 2005 | 12.7 (± 4.2) | 39.6<br>n = 23 | 11.8 (± 5.8) | $60.4^{+}$<br>n = 35 |
| 2006-2007 | 11.2 (± 4.4) | $59.2^{+}$<br>n = 29 | 11.6 (± 3.8) | 40.8<br>n = 20 |
| Overall | 10.7 (± 3.6) | 54.5 | 11.2 (± 3.7) | 45.6 |

Eastern grey kangaroo carcasses were found predominantly in winter in each of the three years of collection (Figure 6-8). An exception of this pattern was in 2005, when most deaths occurred in spring. The patterns in the timing of male and female mortality were similar in most years, with the exception of winter 2005 when female mortality was higher and in autumn 2006 when male mortality was more evident.




Notes: Figures quoted represent fresh carcasses only

**Figure 6-8 Proportion of eastern grey kangaroo carcasses found per month**

## DISCUSSION

### *Factors influencing the large group sizes*

The group sizes exhibited by eastern grey kangaroos in this study are within the upper range of those previously reported for this species (Table 6-3). Given the high densities of this species in the study area (see Chapter 3) these results provide further support for the positive correlation that has been found between density and group size in previous work (Taylor 1982, Southwell 1984b, Heathcote 1987, Clarke et al. 1995). The results may suggest also that predator avoidance and site productivity have an influence on the formation of large group sizes of eastern grey kangaroos in Sydney‟s water supply catchment (Taylor 1982, Southwell 1984b, Heathcote 1987), as discussed further below.

It has been recognised previously that eastern grey kangaroos are sensitive to the pressures exerted by predators and form larger groups to share the costs of vigilance (Jarman and Coulson 1989). Banks (2001) demonstrated experimentally the impact of predators on group formation by finding that eastern grey kangaroos exhibited larger group sizes in fox-removal sites as compared to control sites where foxes were not removed. Known predators of eastern grey kangaroos, wedge-tailed eagles (*Aquila audax*), red foxes (*Vulpes vulpes*), feral pigs (*Sus scrofa*), dingoes and their hybrids (*Canis lupus dingo*, *C. l. dingo* x *C. l. familiaris*) were observed in the study area. Dingoes were the most frequently observed predator in the study area (at least two per week per month), and several dingo attacks on eastern grey kangaroos were witnessed. These attacks were made on solitary animals or on kangaroos in small groups (<3 animals), lending some support to the idea that larger groups may be more vigilant or at least less prone to being attacked. Furthermore, the large group sizes of kangaroos found in this study and the absence of human activities in the study area, such as from hunting and the presence of companion animals, suggest that dingoes may be exerting a higher level of predator pressure on the study population than reported elsewhere.

Although I did not make any formal observations on the anti-predator behavioural strategies of eastern grey kangaroos, casual observations made during the course of fieldwork help to corroborate the suggestion that dingoes exerted a high degree of pressure on the study population. Eastern grey kangaroos in the study area were frequently observed scanning their surroundings when foraging and drinking. This behaviour was particularly apparent when kangaroos were drinking in the riparian zone of the Wollondilly River, where only one kangaroo would drink at any one time and the other group members would carry out surveillance. Expression of this particular behaviour may reflect the high predation risk of this activity, due to the steep sandy banks and the lack of predator escape routes in the riparian area. Clarke *et al*. (1995) showed similarly that individual kangaroos within a group would often switch between foraging and surveillance as an anti-predator strategy, and in another study by Jarman (1987), individual eastern grey kangaroos were found to spend less time being vigilant and more time feeding while in large groups.

Environmental conditions may also favour the formation of large groups in eastern grey kangaroos. Kaufmann (1974) and Kaufmann (1975) found that group sizes of this species were largest in areas of better pasture or when kangaroos congregated around limited resources. As the study area was experiencing significant drought conditions at the time of this study (see Chapter 1), with food resources notably very limited, this may be a plausible explanation for the large groups that I observed. In support of this, observations of the group dynamics of eastern grey kangaroos took place when they were foraging in open habitats, which are the most fertile sites in the study area. On one particular occasion after drought-breaking rain, one kangaroo group was observed to comprise over 200 animals on the fertile floodplains of the study area. This observation supports the environmental condition hypothesis, as this large group most probably consisted of several smaller groups that had congregated in the area to maximise nutrition from the available fresh green grass.

### *Sex ratios and mortality*

The adult female-biased sex ratios found in this study accord with numerous studies conducted on other large herbivore species (e.g. Clutton-Brock and Lonergan 1994). Furthermore, a significant female bias has been reported in every study conducted on the sex ratios of eastern grey kangaroos (Jarman and Coulson 1989, Quin 1989). These sex biases are particularly pronounced, even for a sexually dimorphic species; male eastern grey kangaroos are larger than females and thus their faster growth rates and in turn larger metabolic requirements make them more vulnerable to mortality (Clutton-Brock and Lonergan 1994, Coulson 1997). They also appear to be acutely pronounced where shortages of food, high population densities and higher male dispersal generate male-bias in mortality rates (Clutton-Brock and Lonergan 1994, Coulson 1997). Chapter 5 demonstrated that home ranges of males and females were similar, making male dispersal a less likely explanation for the female sex ratio bias. A more likely explanation, however, is the influence of drought on male mortality. The impact of drought was indicated by the sex analysis of skulls, which showed that significantly more males died than females in 2004, which was the driest year when compared to the long-term average rainfall for the study area. Alternatively, the female bias observed in pouch young (62%) could also influence adult sex ratios.

Sex ratios of eastern grey kangaroos neared parity in November 2004. This was most probably in response to males joining female-dominated groups to seek mating opportunities at a time that coincided with the peak in the breeding season (Poole 2002). There was also an increase in the number of males in the study area during the breeding season that was evident from observations of tagged and collared males (Chapter 5). Outside this period, the sex composition of groups shifted back to approximately three females to every male; males also associated frequently with their peers when the group size was greater than three. Taken together, these results suggest that (i) segregation occurs between males and females when adult females are likely to be in anoestrous, and males spent more time with their peers as a result; and (ii) during the peak breeding season transient males enter the study area and compete with resident males for potential mating opportunities. The latter possibility is supported by the home range results in Chapter 5, whereby of the male- collared kangaroos only one was transient and left the study area. Furthermore, collared and tagged males that were considered to be residents (i.e., those with overlapping home ranges and which were commonly sighted) were involved in fighting encounters with males not previously observed in the study area.

The age estimates of eastern grey kangaroo skulls revealed that animals in the study area were relatively longer-lived in comparison to those in other free ranging populations. Wilson (1975) found that eastern grey kangaroos, shot by professional shooters during commercial harvests across western New South Wales, were dominated (78%) by individuals less than 5 years old. Quin (1989) compared the age structures of kangaroos in the Yan Yean area, Victoria that were found dead from natural causes (dead samples) with those that were shot (mortality sample) during drought conditions. When analysing the age structures of the mortality sample, Quin found that very high proportions (97%, 94% and 71% for 1961, 1962 and 1963, respectively) of the study population were less than 2 years old. Furthermore, age distributions of the dead sample mirrored these results as up to 80% of skulls collected belonged to animals that were less than three years old; just 2% belonged to animals that were older than 10 years. Jaremovic and Croft (1991) similarly found high mortality of newly weaned animals (18 months). The higher mortality of this age group was hypothesised to be caused by dry winters, a vulnerable time for kangaroos, particularly if the biomass of forage is low. The results of the present study differ from those of previous investigations because, despite the severe drought conditions that were operating at the time of this study, more old animals died relative to young animals in the population.

The mortality patterns of several herbivore species have also been linked to population density (Owen-Smith 2006). Fletcher (2007) noted a large proportion of eastern grey kangaroos dying in the 1-2 year cohort (58% of the total mortality was attributed to the 1-year old age cohort compared to an average of 1.8% in each year between two to 15). This population, in the Australian Capital Territory, had a very similar density of resident animals to the study population, about five animals per hectare.

The presence of older animals in the mortality sample in this study may reflect the absence of human activities in the study area, such as hunting. These activities have been reported to skew age ratios in macropodid species that are commercially harvested (Olsen and Low 2006), and also in trophy species elsewhere (Parker et al. 2002). Alternatively, predation of younger animals may have occurred more frequently than was indicated by the skull samples. If the skulls of younger animals are cached or destroyed by predators more readily than those of older animals (M. Roberts personal observation), the age estimates of skulls presented here may have been biased towards older animals. In support of this possibility, Fletcher (2007) claimed that the lack of younger animals in his mortality samples may have been due to the rapid disappearance and subsequent difficulty of detection of sub-adult carcasses in comparison to those of older and larger animals.

Summarising all these observations, the age and sex specific mortality patterns expressed in this species in the study area could be dictated by three factors. Firstly, the operative influence of hunting on a population may skew age ratios, leading to an over-representation of younger animals in the population as older animals are constantly removed. Since the cessation of farming activities, hunting and culling have not been in operation in the study area for 15 to 20 years (Dennis Ashton, personal communication 2009), and the lack of human interaction with the study population has continued as a result of prohibited public access to the area to protect the quality of the stored water. Secondly, high densities have been linked to winter starvation and high juvenile mortality. The density of the study population is very similar to that reported by Fletcher (2007), and thus is unlikely to have influenced age and sex specific mortality. In addition, the collection of skulls from the study area spanned a period of severe drought, and similar patterns to those recorded in other studies should have been observed. Finally, the absence of juvenile animals in the mortality sample may reflect their carcasses being undetected due to their caching by predators or rapid disintegration. I consider this last possibility unlikely, because the study area was searched thoroughly for carcasses each month and, being relatively flat and open, a representative sample of skulls of all ages most likely would have been detected. Thus, the absence of human activities in the study area, such as hunting, most probably led to the higher proportion of older animals in the mortality sample. I suggest, furthermore, that based on supporting observations, this is also representative of age structures in the population.

The longevity of individual eastern grey kangaroos in this study area was also documented in 1986 in a report to the Water Board (now Sydney Catchment Authority). This report presented in Figure 6-9 detailed observations of large-scale mortality of older animals during winter drought conditions. Analysis of skulls using molar progression at this time indicated that most deceased individuals were 8 -10 years old, and that some animals were aged over ten years. This finding further supports the theory that the absence of human activities has led to older age structures, as farming practices in the Burragorang Valley ceased in most areas some two decades before this mortality event was reported (Dennis Ashton, personal communication 2009). Moreover, the presence of older eastern grey kangaroos provides evidence that the favourable conditions of the study area may permit animals to live longer in comparison to other populations of this species, but also that mortality in winter droughts is common.

Winter mortality of eastern grey kangaroos during drought conditions has commonly been reported across the range of this species (Banks et al. 2000). These peaks in mortality, typically, are responses to shortages in food supplies, which lead to starvation combined with the symptomatic effects of increased parasite loads induced by food stress (Dawson 1995). I also observed higher mortality in the study population during early spring, which may have been in response to the over-consumption of fresh green forage after the dry winters. Although bloating and stress-related mortality have been known to occur in many domesticated animals following the introduction of lush green grass, hay and legume-rich feed (Hancock 1953), this possibility is unsubstantiated in kangaroo species.

Geoff Smith.
15 O'Shannassy Street,
MOUNT PRITCHARD. N.S.W. 2170.

Phone. 602 0748.

Officer in Charge.
Water Board Catchment Area.

Dear Sir,

In relation to recent inspection of Kangaroos in the East and West Wollondilly Area Burragorang Valley.

Your concern for the welfare of these animals is appreciated. My findings indicate that the cause of the deaths of a number of animals recently in this area was the result of a number of factors.

Firstly some 15 skulls were collected, and molar eruption indicates that all these Kangaroos were aged 8 to 10 years, some well over ten years.

This area has a heavy burden of Intestinal Parasites as indicated by samples of droppings collected.

The recent sudden cold snap at the end of winter, and the combination of age and the level of Intestinal Parasites was the major contributing factor causing these deaths. This is consistent to the lifestyle of free ranging Kangaroos.

The balance of the animals within the Boards Catchment Area are relatively young, ranging from 2 to 6 years, and they are in prime condition, food is abundant within their range.

Another problem that could arise at the end of a long dry period without any green food could be a form of Muscular Dystrophy caused by a Selinium Deficiency.

With your permission I would like to monitor the condition of these animals in six months, and again toward the end of next winter.

Free ranging Kangaroos tend to be self regulating in relation to numbers within their range, over a period of say 5 years you could expect to see very volatile increases and reduction of numbers depending on the prevailing conditions usually toward the end of each season.

Kindest Regards.

Geoff. Smith

**Figure 6-9 Letter to the Water Board documenting large-scale mortality in the study population in 1986**

### *Group composition and reproduction*

The composition of eastern grey kangaroo groups of different sizes was highly variable in this study. The association of females with juveniles between October and February was expected, as this coincides with the time that young emerge from the pouch; they are then dependent on mothers until they are fully weaned at approximately 18 months (Banks 2001). Also expected was the timing of the associations of male and female kangaroos in spring and summer, which as already discussed is the peak in the breeding season of this species (Poole 2002). Females also enter oestrus shortly after their young cease suckling and at this time males were often observed checking the reproductive status of females and battling over potential reproduction opportunities. Males associated with females more than expected on some occasions during the winter months, most probably in response to the mortality of pouch young during drought conditions followed by females entering oestrus at this time.

The tendency of both sexes of eastern grey kangaroos to be observed alone in this study was noticed also, and investigated, by Southwell (1984c). He suggested two alternative hypotheses for this phenomenon; the first is that solitary animals are avoiding close proximity and communication with other animals, and the second that solitary animals are in transit to another group. This first hypothesis is a highly probable explanation for female kangaroos in this study, as they were typically found alone in the summer months when they may have been attempting to avoid harassment from male kangaroos seeking mating opportunities (Croft 1989). Adult females were also found to be solitary when they had young-at-foot; this behavioural trait has been reported commonly in several other species of macropodids. This behaviour apparently reduces the chance of the mother losing contact with young (Croft 1981), which I observed on one occasion when a mother – young pairing was broken when the pair were in a group comprising more than 20 kangaroos.

The second hypothesis proposed by Southwell (1984c) received more support in male eastern grey kangaroos. Males were commonly observed alone in November, when they were in the process of moving from group to group to check the reproductive status of females. Such movements were observed also in radio-collared male kangaroos during this time (Chapter 5).

The solitary nature of female eastern grey kangaroos as a strategy to avoid male harassment requires further investigation after a disturbing observation of forced copulation in this study. In January 2005, I observed a female in oestrus being harassed by seven sub-adult males. The female kangaroo struggled to escape this harassment and made loud vocalisations in an attempt to warn off the males. Despite the apparent struggling from the female kangaroo, the males continued to physically restrain her and persisted to alternate with each other to carry out forced copulation. The female kangaroo eventually escaped and the males continued to follow her until she fell to the ground. This aggressive behaviour has never been previously reported in macropodids, but has been documented in non-human primate species, as sub-adult males carry out forced copulation (referred to as “sneak rape”) in response to their hormonal suppression (Bercovitch et al. 1987, Clutton-Brock and Parker 1995, Maggioncalda and Sapolsky 2002, Harrison and Chivers 2007).

In the larger macropodid species, hierarchies usually are thought to be formed between adult males to establish dominance, with the dominant male then siring most offspring produced in the population (Poole 2002). The behaviour exhibited by the sub-adult male eastern grey kangaroos does not fit this pattern. Firstly, there was no established dominance rank between the sub-adult males, and nor was there a large male present to establish dominance. Secondly, the potential death of this female kangaroo as a result of hyperthermia, dehydration and/or myopathy would overcome any selective advantage that might otherwise accrue to males in a male dominance system that resulted in the siring of offspring. One suggestion for this possibly aberrant behaviour, however, is that it may be related to the high density of the study population and the unbalanced adult sex ratio, such that when females enter oestrus rather synchronously, sub-adult males sneak rare mating opportunities in the absence of dominant males.

The formation of same-sex groups outside the breeding season has been previously reported in macropodids (Coulson 2009) and other sexually dimorphic species (Bowyer 2004). In this study, the formation of “bachelor” groups, as already mentioned, occurred only in the non-mating season as males dissociated from female-dominated groups at the time that most females were likely to be in anoestrous (Dawson 1995). Sexual segregation at this time has been previously described in western grey kangaroos, *Macropus fuliginosus* (McCullough and McCullough 2000, MacFarlane and Coulson 2005). MacFarlane and Coulson (2005) described this behaviour also in red kangaroos, *Macropus rufus*, although it is less marked in this species. The behaviour exhibited by male eastern grey kangaroos was in accordance with these previous studies (Coulson 2009), as bachelor males joined mixed groups and began associating with individuals of the opposite sex at the onset of the mating season.

The “bachelor” groups in this study typically formed in marginal habitats of the study site and at some distance from other kangaroo groups. These marginal habitats were located on the edge of the riparian area and supported poorer quality forage (Chapter 4). In addition, animals of both sexes demonstrated very little preference for these areas at other times of the year (Chapter 5). This behaviour has been described as habitat segregation, a form of sexual segregation in which males and females use different habitats outside the breeding season (Conradt 1998, MacFarlane 2006).

Four hypotheses have been proposed to explain why sexual segregation occurs in sexually dimorphic macropodids and other species such as social ungulates (see MacFarlane 2006). The first is the reproductive – strategy hypothesis, which suggests that the different ecological requirements of males and females bring about sexual segregation: males select areas where nutritious resources are high to maximise growth, and females select areas where predation risk is low for their dependent offspring. The second is the sexual – dimorphism body size hypothesis. This implies that differences in body size, metabolic rates and digestion are responsible for sexual segregation: males select habitats that have more abundant, lower quality food supplies, whereas females select habitats that provide high quality food that is more easily digestible. The third is the social factors hypothesis. This recognises that there are differences in the social attributes of males and females, and predicts that males spend more time together to learn fighting skills and to establish rank in the hierarchy, whereas females devote time solely towards maternal investment. The fourth and final hypothesis, tested by MacFarlane (2006) in western grey kangaroos, relates to differences in activity budget between the sexes: it predicts that males spend less time being active than females, by foraging less and laying down more, which leads to a lack of unity within mixed groups.

Although data were not gathered formally to identify the hypothesis that best explains sexual segregation by eastern grey kangaroos in this study, all hypotheses appear relevant. Firstly, the reproductive – strategy hypothesis may provide a partial explanation. Although males selected areas with poor quality forage, females with pouch young occupied habitats that posed the lowest predation risk when compared with males, as the riparian areas lacked lateral cover and comprised steep sand banks with a low degree of predator refuge and limited prey escape routes. Secondly, the sexual – dimorphism hypothesis may be slightly more relevant as males were found in areas of poorer quality forage than females. However, food supplies were not abundant due to drought conditions, which probably led to the observed poorer condition of males in the bachelor groups. Thirdly, the social factors hypothesis is also relevant as fighting was observed occasionally between males in the bachelor groups and appeared unrelated to potential mating opportunities as there were no females in close proximity (Plate 6-1). Finally, the activity budget hypothesis is probably most relevant for my results, as males in bachelor groups occupied small home ranges and showed limited activity in comparison with females (Chapter 5). MacFarlane (2006) rejected this hypothesis for sexual segregation in western grey kangaroos because activity levels were similar between males and females and there was no evidence that activity asynchrony led to differences in cohesion in mixed sex (control) and male groups. However, a major difference between MacFarlane (2006) and this study, besides the different species that were studied, is that western grey kangaroos were culled to maintain densities below four animals per square kilometre. Culling may have biased the activity levels of males and females in MacFarlane"s work; as such, the application of the activity budget hypothesis in this study warrants further investigation.

Like males, the adult females in this study were recorded grouping together at various times of the year. This behaviour has been reported previously in this species and may reflect kin groupings (Jaremovic and Croft 1991, Jarman 1994). These published studies used tagged animals to identify grouping behaviour and did not have genetic information to confirm that females were related. Unfortunately, there was no genetic analysis of tagged animals in this study either. However, repeated observations of tagged female kangaroos are consistent with the notion that they may have been kin; certain individuals were often seen together and used overlapping home ranges (Chapter 5) that suggest long-term associations and potential relatedness.

**Plate 6-1** **Males from the same bachelor group fighting with no females in close proximity**

The reproductive patterns of eastern grey kangaroos at the study site were highly seasonal, in accordance with other studies of this species in south-eastern Australia (Quin 1989, Fletcher 2007). Although usually peaking in summer, breeding is affected also by the availability of food resources, as food influences the survival of pouch young. During drought conditions, higher than average mortality of pouch young would result in their replacement after about 30 days (Poole 2002). This was evident from the analysis of rainfall data from 2005 (Chapter 1), which showed that there was only 2.2 mm of rain during January, when most births were estimated to have occurred, and less than 10 mm of rain over the following winter. Consequently, these dry conditions most likely led to high mortality of pouch young shortly after birth in summer, thus resulting in another pulse in autumn. However, as dry conditions prevailed, continual replacement of pouch young probably occurred throughout the year, a result corroborated by a relatively constant ratio of juveniles to adult females across the survey months and a paucity of dependent young. A similar pattern was also observed in this species by Fletcher (2007) during drought conditions.

When pouch young were most abundant, they were most frequently found in groups comprising larger numbers of animals. Southwell (1984c) also noted that females with pouch young were rarely unaccompanied. This may be related to predator-avoidance, as discussed earlier, as an increase in group size elevates vigilance for the vulnerable adult female – pouch young pairings (Banks 2001). To date, vigilance behaviour of adult females with pouch young has not been adequately described. However, Pays and Jarman (2008) suggested that the time spent being vigilant by females with pouch young may be profitable given the constraints of carrying a heavier load and the difficulty of shepherding young back into the pouch during a surprise predator attack. Therefore, females with pouch young may benefit from being in larger groups by using collective vigilance as a predator awareness and detection strategy.

### *Social structure and zoonotic disease management*

The influence of social organisation in arbitrating the interaction between eastern grey kangaroos and the identified zoonotic diseases they carry has important implications for wildlife management and public health (Loehle 1995). Table 6-7 summarises the social attributes of eastern grey kangaroos and their apparent linkages with zoonotic disease management.

The results of most relevance for zoonotic disease management were centred on the abundance of pouch young and young-at-foot in the population. This is because accumulating evidence suggests that younger cohorts have higher shedding intensities of *Cryptosporidium* and *Giardia* (Coklin et al. 2007); the diseases that pose the highest risk to Sydney‟s water supply (Chapter 2). Power *et al*. (2005) first isolated *Cryptosporidium* from free ranging eastern grey kangaroos in this study area and linked repeated autumn epidemics to the presence of pouch young (Power *et al.* 2005). However, a subsequent unpublished epidemiological study (Radu and Slade 2006), conducted concurrently to the collection of data for this chapter, failed to identify the presence of *Cryptosporidium*. These researchers linked this to the prevailing drought conditions and the apparently low numbers of pouch young that they observed casually to be in the population. Although pouch young were not estimated by Power *et al*. (2005) when *Cryptosporidium* was detected, the results of this chapter support the conclusions drawn by Radu and Slade (2006) that the absence of *Cryptosporidium* during this later period was due to the drought-induced paucity of pouch young. Furthermore, if indeed the presence of pouch young is directly attributable to the detection of *Cryptosporidium*, then the results also suggest that a threshold in the number of pouch young in the population may have to be attained before *Cryptosporidium* is detected.

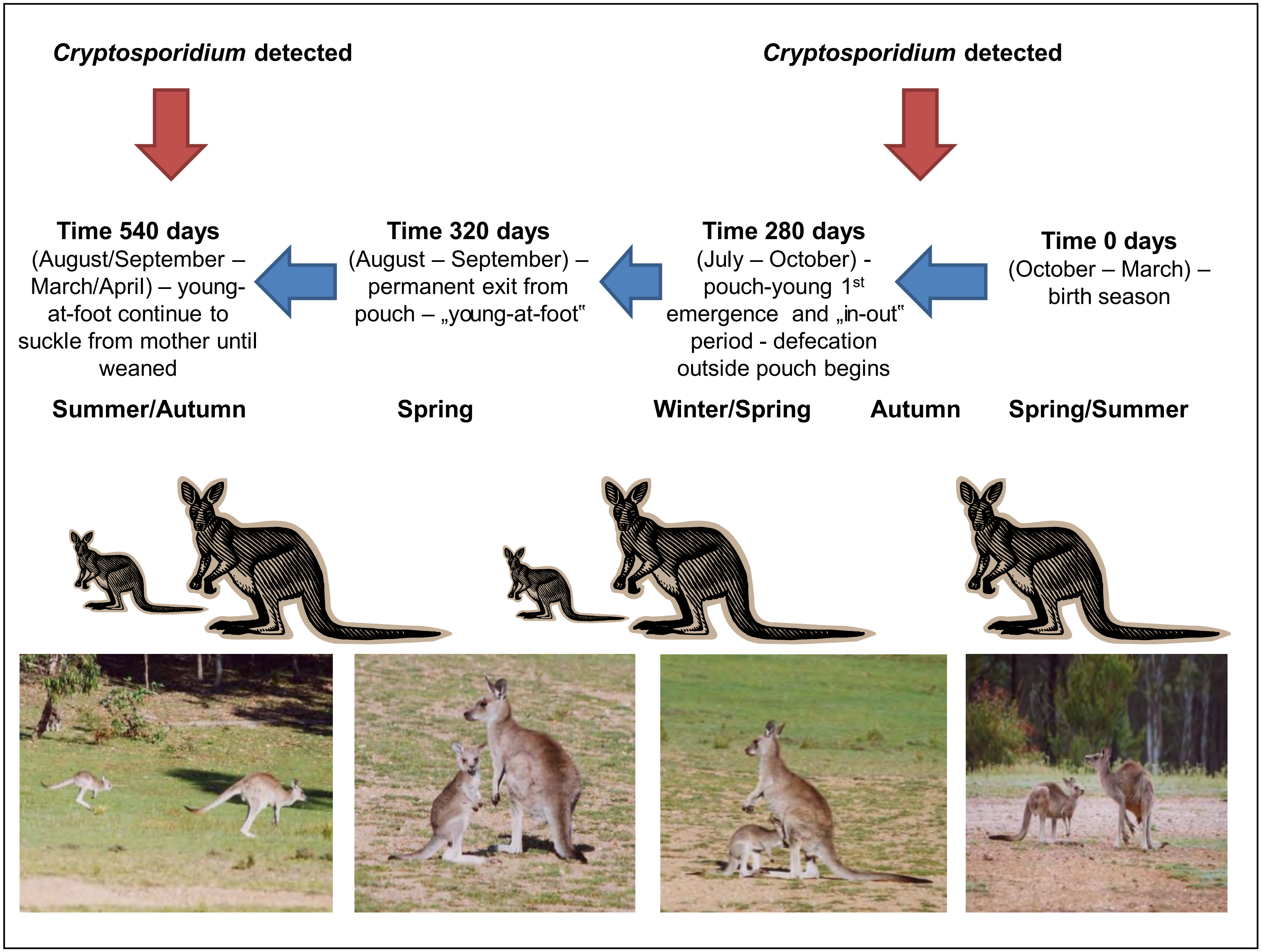
**Table 6-7 Summary of the relevance of the findings of this Chapter for zoonotic disease management**

| Result | Implications for zoonotic disease management |
|---|---|
| Large open membership groups | • Increased level of direct contact between individuals and the transmission of disease<br>• Defecation occurs when foraging (Ramp and Coulson 2002) – increased uptake of oocysts and cysts when foraging occurs in large groups<br>• Elevated prevalence of the disease-causing agent and increased risk of environmental contamination<br>• Large groups concentrate on limited resources in close proximity to surface water during drought– this increases potential runoff of pathogens due to the absence of grass<br>• The presence of pouch-young is related to larger group sizes – increased disease transmission to conspecifics due to the increased susceptibility and higher shedding intensity of younger animals to Cryptosporidium to Giardia (Coklin et al. 2007)<br>• Host crowding has been linked to high prevalence of a number of diseases including those with bacterial origins (e.g. *Leptospira*)<br>• Disease transmission increased due to open membership through the continual shifts in group members |
| High proportion of older animals in the population | • Older animals may be more susceptible to disease due to a decreased immune response (Barquero et al. 2007)<br>• High reproductive capacity of population – relatively higher *Cryptosporidium* and *Giardia* risk in conditions that permit the presence of pouch young |
| Seasonality in pouch young (PY) | • Repeated autumn peaks of *Cryptosporidium* have been linked to the presence of pouch young in previous studies (Power et al. 2005)<br>• Concurrent epidemiological studies in 2004 and 2005 did not detect *Cryptosporidium* (Radu and Slade 2006). This was most likely in response to drought conditions that:<br>• led to an overall reduction in the number of juveniles in the population<br>• limited the survival of *Cryptosporidium* oocysts in the environment<br>• *Cryptosporidium* and *Giardia* were detected in 2006 (Chapter 2), upon the return of wetter and more favourable conditions, which would have supported higher juvenile survivorship<br>• Targeted control of disease management by reducing pouch young abundance<br>• Cat predation of juvenile kangaroos may increase the maintenance of Toxoplasma if this age class has a higher prevalence of this disease (Fisher et al. 2001, Miller et al. 2003). |

| Result | Implications for zoonotic disease management |
| --- | --- |
| Female biased sex ratios | • Increased reproductive potential and higher abundance of pouch young during optimal conditions<br>• Population management – disease control may be targeted at reducing the number of females or by using non-lethal techniques (e.g. deslorelin implants) to slow production of pouch young (Herbert et al. 2004) |
| Mortality patterns | • Peaks in winter – may increase the maintenance of Toxoplasma, as cats consume kangaroo carrion that may be contaminated with cysts (Work et al. 2000)<br>• Toxoplasmosis has been reported to be sometimes fatal in this species and other Australian fauna (Miller et al. 2003) – increased probability of infected carrion |

The finding of *Cryptosporidium* in the population during 2006 (Chapter 2) provides further support for the theory linking pouch young with the prevalence of this disease. Although there were no formal estimates of pouch young made during 2006, casual observations suggested that there were many pouch young in the population at this time (approximately 1:1 adult female to juvenile ratio) in comparison to the previous year"s survey. Pouch young survival would have been supported by above average rainfall in summer 2005/06, that led to the return of *Cryptosporidium* in large enough numbers to be detected (i.e. beyond the suggested hypothetical threshold).

The age at which pouch young acquire cryptosporidiosis requires further investigation. The detection of *Cryptosporidium* in the population during autumn suggests that young of two different ages may be responsible for shedding this pathogen (Figure 6-10); (i) pouch young 5-8 months of age born in the preceding summer and/or (ii) weaned young aged about 14-18 months. Firstly, adult females clean the cloaca of their young immediately after attachment (Jarman 1994), thus the sampled prevalence of *Cryptosporidium* in the study population may relate to the degree of immunity individual adult females („mother"), if pouch young aged 5-8 months are infected with this pathogen. Secondly, as pouch young typically commence grazing during weaning up until they are aged 18 months, the uptake of pathogens at this time is likely, but excretion of this pathogen would depend on immunity of individuals at this age. Therefore, further studies are required to determine if young to mother transmission occurs and at what age individuals develop immunity to cryptosporidiosis, as these factors may potentially play a significant role in the dissemination of this disease into the environment.



*Cryptosporidium* detected
*Cryptosporidium* detected
Time 540 days
(August/September – March/April) – young-at-foot continue to suckle from mother until weaned
Time 320 days
(August – September) – permanent exit from pouch – „young-at-foot"
Time 280 days
(July – October) - pouch-young 1st emergence and „in-out" period - defecation outside pouch begins
Time 0 days
(October – March) – birth season
Summer/Autumn
Spring
Winter/Spring
Autumn
Spring/Summer

**Figure 6-10 The potential link between autumn epidemics of Cryptosporidiosis and the presence of juvenile eastern grey kangaroos**

The number of eastern grey kangaroo carcasses present at any one time has important implications for the lifecycle of *Toxoplasma gondii*. As an intermediate host of this disease organism, kangaroos store the cyst in their muscle and nervous tissue and the life cycle is completed by cats consuming infected kangaroo meat (Prestrud et al. 2007). The results of this chapter have shown that there is an all year round supply of kangaroo carrion, with peaks observed during winter periods. Thus, the maintenance of toxoplasmosis in eastern grey kangaroo is likely to be highest in winter, when there is increased availability of potentially infected carrion for cats and other carnivores to consume (Prestrud et al. 2007). Furthermore, the likelihood of carcasses being infected with *Toxoplasma* cysts could also be higher, given that toxoplasmosis has been reported to be sometimes fatal in this species (Miller et al. 2003) and other Australian marsupials (Obendorf and Munday 1990).

The prevalence of a number of bacterial diseases has been related to host crowding (Barlow 1991). Therefore, the large group sizes that were exhibited by the study population may foster *Leptospira* transmission, as animals were regularly observed feeding and drinking from ephemeral dams and puddles in these large groups. In addition, the movement of male kangaroos from group to group to search for females in oestrus (Southwell 1984a), a process that involves tasting the urine of receptive females (Kaufmann 1975, Woodward et al. 2006), may also perpetuate the spread of this disease. Thus, the presence of male kangaroos in groups during the breeding season may be an influential factor in the determination of the prevalence of this disease in the population. Furthermore, transmission of *Leptospira* during the breeding season may be heightened as it is also known to be transmitted by sexual contact (Kiktenko 1976).

Given the close proximity of the sampled floodplain environments to surface water, the formation of large open membership groups by eastern grey kangaroos at these locations has significant implications for disease risk. Larger group sizes promotes disease transmission through close contact (Guerra *et al.* 2003) and in particular increases the transmission of faecal-oral route diseases, such as *Cryptosporidium* and *Giardia*, between individual animals and may raise the dissemination of such diseases to the water supply. This risk would be presumably more pronounced in groups containing pouch young.

## CONCLUSION

The results of this chapter have contributed to an overall understanding of the social factors influencing the potential origin, disease persistence and transmission of zoonotic diseases in eastern grey kangaroos. This information is relevant to health authorities, water managing authorities and conservation agencies, as it allows prediction of when these diseases are likely to be prevalent in the environment. When used in conjunction with epidemiological studies, this work also allows assessment of the risk associated with disease transmission originating from eastern grey kangaroos.

Although there is paucity of information concerning the social drivers of the diseases identified in the study population of eastern grey kangaroos, the social systems in a variety of species have been linked to disease transmission and prevalence. Thus, given this accumulating body of evidence, it appears that social systems, and in particular host proximity, are strong contributors to the prevalence and transmission of these four diseases. Furthermore, as it has been indicated for *Cryptosporidium* in this chapter, the recruitment of immunologically naïve juveniles into the population is also important in the analysis of the risk of occurrence of this disease.

This chapter has highlighted the importance of studying the social behaviour of the host in order to gain an appreciation of the origin and prevalence of the disease-causing agent. In most epidemiological studies, concurrent empirical studies on the social structure of the host have been lacking; this has often led to inconclusive interpretations concerning disease dynamics and limits our ability to predict the future reoccurrence of the particular pathogen identified.

Management actions to contain diseases are typically directed at the disease-causing agent, the host population, or a component of the environment with the key objective to reduce transmission and exposure (Cross et al. 2009). Control methods that are directed at the host population are limited in their approach, without due consideration of how the host"s behavioural and social traits may influence the diseases dynamics. Therefore, using information derived from this chapter, future control efforts to manage the diseases identified in eastern grey kangaroos can potentially be directed at certain age groups, such as by non-lethal limitation of the numbers of pouch young (e.g. Herbert et al. (2004)), targeting certain locations (e.g. removing carcasses from the watershed) or times of the year that are potentially most important for the propagation and spread of the disease in question.

The absence of human activities and the high density of the study population of eastern grey kangaroos potentially influenced the novel social traits that were found. Previous ecological studies of this species have traditionally been conducted on lower density populations coupled with the presence of human activities that probably alter kangaroo behaviour (e.g. Moore *et al*. (2002)). These factors, together with a stable population of dingoes in the study area, provide a perfect opportunity to study the predator avoidance strategies that have been identified in this species. Furthermore, the impact of high population densities on the social systems exhibited by this species warrants further investigation, particularly in view of the age structures and unusual reproductive behaviours that were observed throughout the course of the study.

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# CHAPTER 7:
# GENERAL CONCLUSIONS

# CHAPTER 7: GENERAL CONCLUSIONS

## *Preamble to Chapter 7*

A clean water supply is of fundamental importance to human health. Waterborne pathogens such as *Cryptosporidium* and *Giardia* can compromise the ability of water supply systems to meet these needs. In this chapter, I provide a brief summary of key findings from the preceding data-based chapters, and synthesise the results to evaluate the overall risk that eastern grey kangaroos pose in terms of disseminating zoonotic disease to consumers of the Sydney water resource. I also provide some suggestions for future management to safeguard the water supply in future.

## EASTERN GREY KANGAROOS: ECOLOGY AND POTENTIAL FOR SPREADING ZOONOTIC DISEASE

Eastern grey kangaroos, *Macropus giganteus*, residing in the Warragamba Special Area were a potential source of pathogens detected in Sydney's water supply in 1998. Preliminary risk assessments focussed on the microbiology of these diseases in populations of eastern grey kangaroos living near major rivers that flowed into Warragamba Dam (the source of 80% of Sydney's water supply). Very little was known about the ecology of this species in the catchment area including the population density, movement patterns, habitat preferences and social structure; all of which are key facets of information required to gain understanding of how these animals interact with surface water and subsequently for formulating risk assessments. In this study, both the epidemiology of potential zoonotic diseases carried by this species and their ecology were investigated in former pastoral areas on the Wollondilly River. The results provide an ecologically-based risk assessment approach to determine if eastern grey kangaroos are a zoonotic disease threat to the 4 million consumers of Sydney's water supply. The collection of such ecological information revealed that the zoonotic disease profile of this species was more complex than first thought.

Densities of eastern grey kangaroos were both very high and localised within Sydney's water supply in the study area. The densities of this species calculated in Chapter 3 (mean seasonal densities ranged between 335 and 547 kangaroos $km^{-2}$) were among the highest reported for this species. The densities of eastern grey kangaroos recorded in Canberra by Fletcher (2007) (400 – 500 animals $km^{-2}$ ) were comparable to those found in this study, while the next highest densities reported were at Gippsland Lakes (Coulson and Raines (1985) (245 kangaroos $km^{-2}$), followed by the Yan Yean Reservoir in Melbourne (Ramp and Coulson (2002) (178 kangaroos $km^{-2}$). Densities are potentially higher in Sydney's water supply catchment because eastern grey kangaroos residing here experience very little human interaction in comparison to other studied populations. In addition, it appeared that despite high levels of mortality during this study (Chapter 6), eastern grey kangaroos remained relatively unchecked by extreme drought conditions. The high resilience demonstrated by this species provides support for their adaptation to such conditions, which has been assisted by the presence of large areas of former pasture and plentiful supplies of artificial water sources that occur in the Warragamba Special Area.

What density would eastern grey kangaroos reach in the study sites following higher rainfall periods? It could be assumed that, on the basis of the previously reported positive responses of other kangaroo populations to rainfall, the calculated population densities of eastern grey kangaroos in the study sites were not the highest that could be attained. The average annual rainfall during the study period was approximately 250 mm less than the long-term average, which had an effect on the kangaroos in the study sites by significantly reducing pasture quality and quantity (Chapter 4). Thus, it would be expected that an increase in rainfall would lead to an increase in pasture biomass and in turn a short-term increase in the carrying capacity of the study sites. Subsequently, this would lead to an increase in the potential pathogen load carried by this species and an increase in the prevalence of potential zoonoses within Sydney's water supply catchment. However, without data collected on the densities and pathogen prevalence of this species following higher rainfall periods, this suggestion remains inconclusive. It is possible, for example, that higher densities of well-nourished and healthy animals could shed smaller per capita parasite loads than lower densities of poorly-nourished and stressed animals with compromised immune systems (Lee and McDonald 1985).

The McClellan Inquiry identified a high level of scientific uncertainty associated with the sources of *Cryptosporidium* and *Giardia* eventuating from the Sydney Water Crisis in 1998. In relation to eastern grey kangaroos being a source of these pathogens, a combination of several years of epidemiological research on *Cryptosporidium* and *Giardia* (Power *et al.* 2004, Power *et al.* 2005, Radu and Slade 2007) and ecological investigations of this species (this study) have now provided some certainty: eastern grey kangaroos carry both *Cryptosporidium* and *Giardia* and have the potential to disseminate these organisms to the water supply. This species cannot therefore be ruled out as a cause of human illness from the drinking water supply in Sydney's water supply catchment. Despite this, however, the culmination of all this information does not comprise a 'pass or fail' test. On the contrary, the ecological information collected in this study allows water utility managers to identify natural processes that are either contributing to or reducing pathogen loads from such wildlife pathogen sources (i.e. fluctuations in animal density, ranging patterns and utilisation of riparian zones) and thus permitting where the allocation of management options will be most effective. For example, mitigation of kangaroo numbers and faecal loads adjacent to stored surface water by closing artificial watering points requires careful consideration on the basis of data collected in this study, as the dependence on the Wollondilly River by kangaroos may increase in their absence.

The analysis of serum collected from eastern grey kangaroos revealed the presence of other zoonotic diseases such as *Leptospira weilli* serovar Topaz and *Toxoplasma gondii*

(Chapter 2). The discovery and occurrence of these diseases for the first time in this species currently represents a poorly recognised zoonotic disease risk given the close proximity of the study population to the largest city in Australia and the wide ranging distribution and overabundant status of the eastern grey kangaroo along the eastern coastline of the country. The high prevalence of *L. weilii* serovar Topaz in the study population of eastern grey kangaroos (approx 47%) and the previously known distribution of this serovar highlight the complexity of this unperceived risk. It was initially isolated in 1994 from both culture and serological methods from humans and two animal cases (bovine and long nosed bandicoot; *Perameles nasuta*) and the majority of *L. weilli* serovar Topaz infections have occurred in far north Queensland, with the remaining infections occurring in south-eastern Queensland and in Western Australia. The Topaz serovar had never been previously reported in New South Wales. Consequently, it is possible that as *L. weilii* sv. Topaz is present in a more mobile and evenly distributed animal host species that eastern grey kangaroos are responsible for the transmission and infection resulting from this disease. Furthermore, it is considered imperative that rogue cattle present within the Burragorang Valley are controlled to prevent the spread of this disease to humans via the human-livestock interface.

The finding of *Toxoplasma gondii* in the eastern grey kangaroo has practical implications for the management of this species across its distribution as it is harvested for its meat (Department of Environment and Conservation 2006). The consumption of under-prepared kangaroo meat poses the highest human health risk for toxoplasmosis. However, further research in consultation with food authorities is required to determine the distribution of *T. gondii* across commercial harvest management zones to fully ascertain this risk.

The data collected during this study suggest overall that eastern grey kangaroos pose a relatively low risk of zoonotic disease transmission to Sydney's water supply. That is, they contribute fairly low quantities of faecal excreta (Chapter 4 – up to 0.12 $gm^{-2}day^{-1}$) to the watershed and even lower volumes in the riparian zones. However, movement pattern data (Chapter 5) suggest that direct faecal deposition to surface water is possible and, given sufficient rainfall, up to 240 $kgkm^{-2}day^{-1}$ of kangaroo faecal excreta could enter surface water and potentially contribute to waterborne disease contamination.

## THE ROLES OF OTHER SPECIES IN SPREADING OR REDUCING RISKS OF ZOONOTIC DISEASE

The Warragamba Special Area is jointly managed by the Sydney Catchment Authority (SCA) for water quality protection and by the Department of Environment, Climate Change and Water (DECCW) for biodiversity conservation. The results of this study fit this duality for the following reasons. Firstly, the control of feral animals including rogue cattle that are present in the Warragamba Special Area to mitigate their impacts on native biota would also limit the cross-transmission of potentially human-infective species of *Cryptosporidium* and *Giardia* from these animals to eastern grey kangaroos, and thus benefit water quality. The potential maintenance and continued transmission of these human-infective strains between cattle (low density and high prevalence of disease) and kangaroos (very high density) is of large concern as it is likely to elevate the local shedding intensity of zoonotic *Cryptosporidium* and *Giardia* in close proximity to surface water. Secondly, overgrazing by eastern grey kangaroos in the Burragorang Valley has been identified by the DECCW as a potential threat to the recovery of the state and federally listed threatened ecological community; Box Gum Woodland. This vegetation community constitutes a relatively large area within the study sites and plays a vital role in reducing surface flow leading to sedimentation and pathogen transport to surface water. Consequently, the rehabilitation and revegetation of this community within the Burragorang Valley will allow it to provide these functions, as well as to permit the long-term reduction in kangaroo numbers in response to a decrease in the quantity of grassy areas and the overall carrying capacity of the land.

Predators such as red foxes and the dingo are considered to maintain lower population numbers of macropodids across their distributions (Coulson 2009). This is particularly the case for the dingo, in which the study sites contained a moderately-sized population of this species and its hybrids with domestic dogs. Dingos were commonly observed attempting to prey upon older males and younger animals, although only the latter would have any potential regulatory effect on the population density of this species. Observations of the social structure of eastern grey kangaroos made during the course of the study, including the formation of large mobs for shared predator vigilance, as well as the high life expectancy of these animals (oldest recorded animal was a female aged 21 years), suggest that eastern grey kangaroos have successfully developed strategies to combat the predation risk posed by dingos. However, it is expected that if population numbers of dingoes and other wild dogs increase, they will likely have a much larger influence on the maintenance of kangaroo numbers, particularly during drought, through the predation of younger and weaker individuals. Consequently, if dingo control is ever contemplated within the study area, the DECCW will need to consider the likely increase of kangaroo numbers in the formulation of management strategies (DECC 2007).

## RECOMMENDATIONS AND FINAL CONCLUSIONS

As a component of the SCA's multi-barrier approach to protect water quality, routine water quality monitoring for *Cryptosporidium* and *Giardia* is conducted at the Warragamba Dam wall on a daily basis. Given the diffuse nature of wildlife pathogen sources, such as eastern grey kangaroos, it is highly recommended that water utility managers consider annual monitoring of these sources using a combination of ecological and epidemiological techniques. The contemporary collection of this information should involve analytical techniques within laboratories accompanied by the use of the most recent ecological tools (e.g. GPS collars). This study provides a robust ecological approach that can be used to assess the risk of any wildlife species considered to be a source of human pathogens (e.g. feral pigs and deer) in Sydney's water supply catchment.

The development of models designed to quantify pathogen loads within drinking water catchments are currently evolving on the basis of the supply of new information (Ferguson *et al.* 2007). In the first application of the Pathogen Catchment Budget (PCB) in Sydney's drinking water catchments, Ferguson *et al*. (2007) utilised fixed values for animal densities, faecal volumes, and to determine the frequency of animals accessing streams to quantify pathogen loads originating from wildlife species. Using the eastern grey kangaroo as an example, the results of this study suggest that these parameters cannot be considered fixed as natural conditions (e.g. rainfall, presence of predators) dictate their magnitude, and ecological-based studies spanning a range of conditions are required to accurately quantify pathogen loads. Based on these results, refinement of the PCB model would be recommended for future analysis of pathogen loads in the Sydney drinking water catchment. Furthermore, this study provides the baseline for the accurate pathogen load calculations of various other wildlife species that are of zoonotic disease concern in the catchment area.

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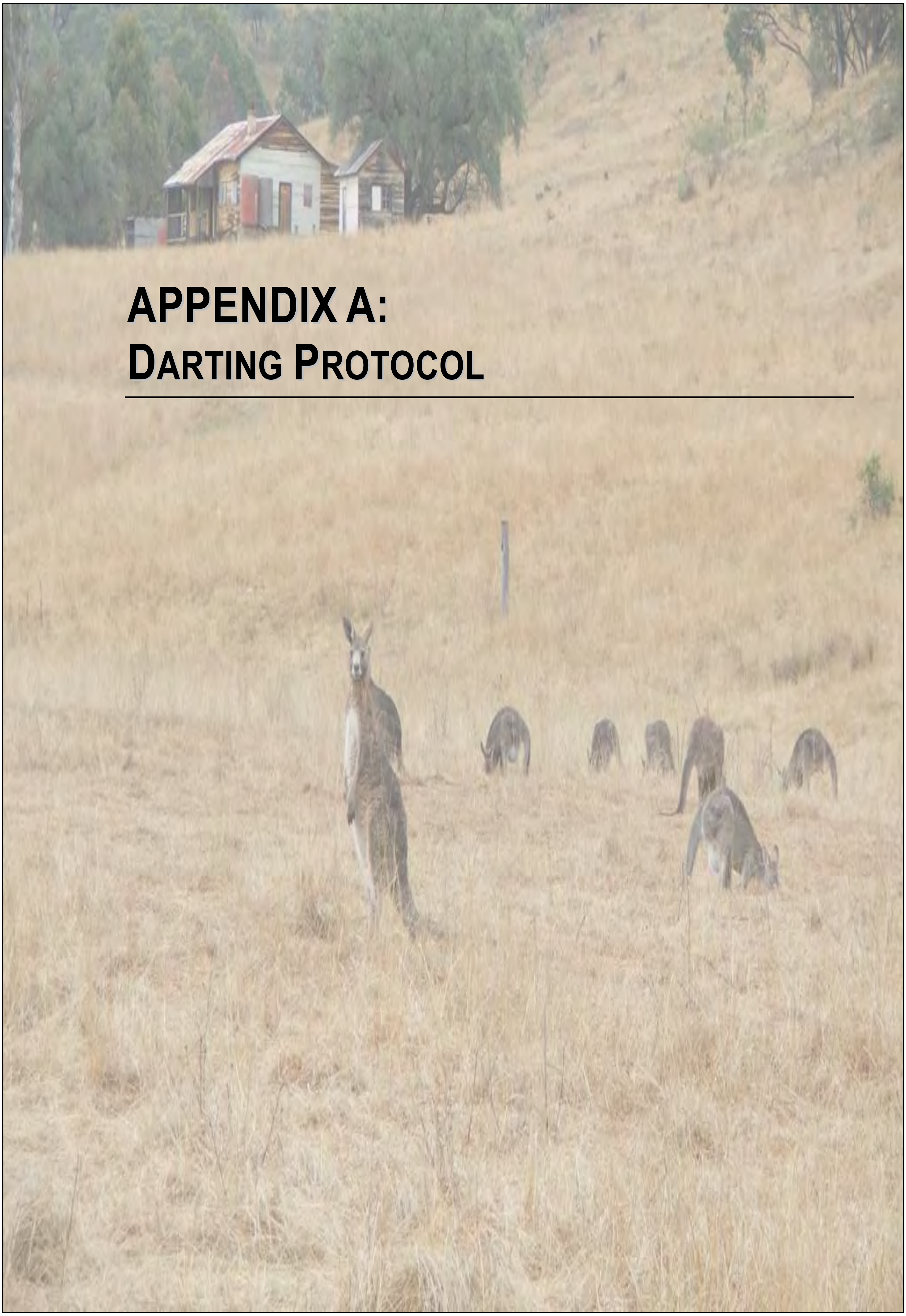
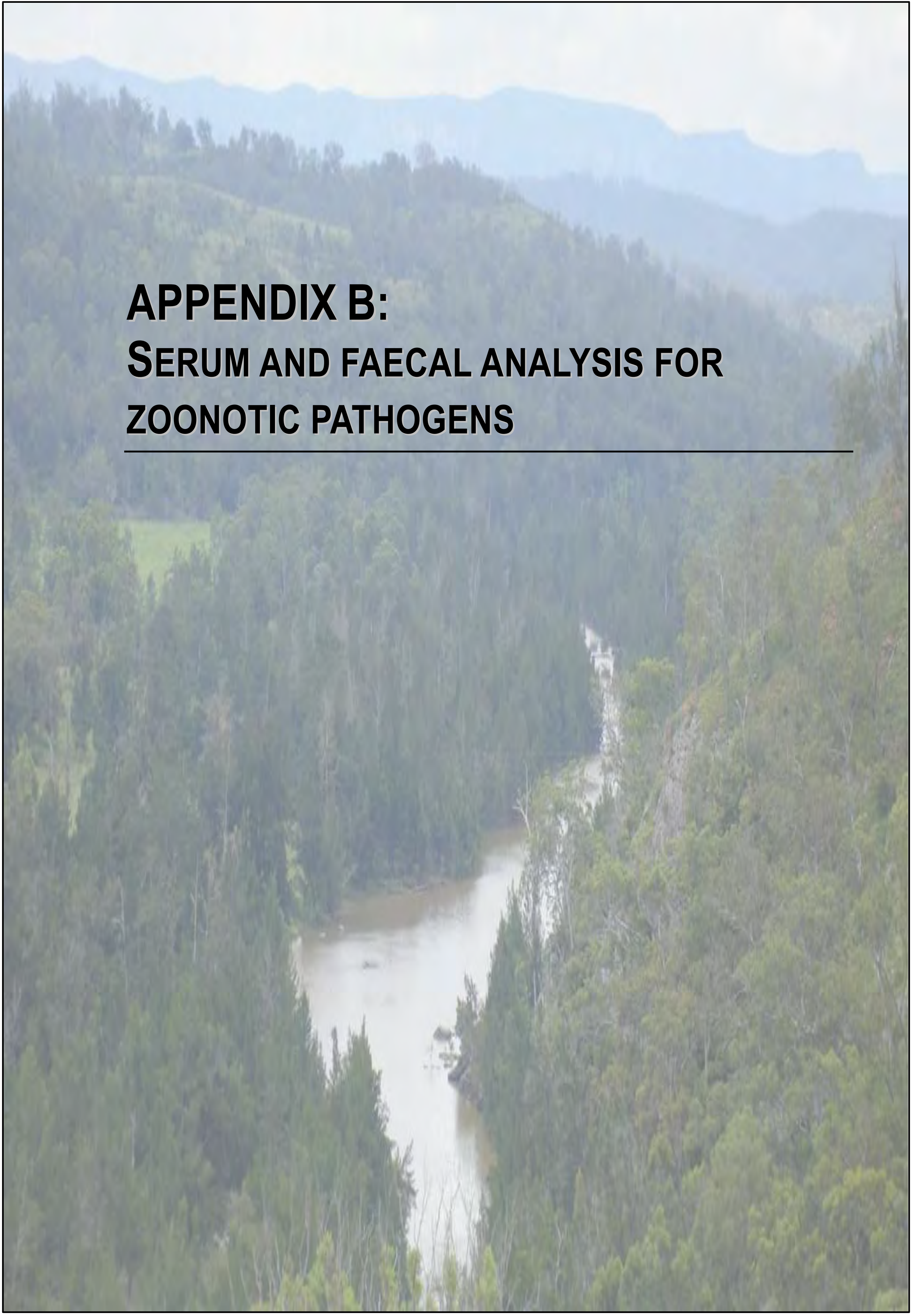
# APPENDIX A:
# DARTING PROTOCOL



**Appendix A: Darting Protocol has been removed as it contains published material under copyright. Removed contents published as:**

Roberts, M. W., Neaves, L. E., Claassens, R., Herbert, C. A. (2010) Darting eastern grey kangaroos: a protocol for free-ranging populations. In Coulson, G. & Eldridge, M. (Eds.), *Macropods: The Biology of Kangaroos, Wallabies and Rat-kangaroos,* pp. 325-339. CSIRO Publishing.

# APPENDIX B:
# SERUM AND FAECAL ANALYSIS FOR ZOONOTIC PATHOGENS



## *Leptospira*

The sera were screened against a reference panel of twenty-two *Leptospira* serovars using the microscopic agglutination test (MAT). The starting dilution for each sample was 1:25 and positive samples were diluted down to an end point. Titers of 1:50 or higher were regarded as evidence of past or current infection.

The MAT panel of antigens represented those serovars (sv.) that had previously been isolated in mainland Australia of the *Leptospira interrogans* (sv. Australis, Ballico; Pomona, Pomona; Hardjo, Hardjoprajitno; Zanoni, Zanoni; Kremastos, Kremastos; Robinsoni, Robinson; Canicola, Hond Utrecht IV; Copenhageni, M 20; Medanensis, Hond HC; Grippotyphosa, Moskva V and Szwajizak, Szwajizak), *L. borgpeterseni* (sv. Tarassovi, Perepelitsin), *L. weilii* (sv.Topaz, 94/7990/3 and Celledoni, Celledoni,), and *L. kirschneri* (sv. Bulgarica, Nicolaevo), and the exotic serovars, *L. interrogans* (sv. Bataviae, Swart; Djasiman, Djasiman), *L. santarosai* (sv. Shermani, 1342 K), *L. borgpeterseni* (sv.Ballum, Mus 127 and Javanica, Veldrat Batavia 46), *L. kirschneri* (sv. Cynopteri, 3522 C), and *L. noguchi* (sv. Panama, CZ 214).

## *Toxoplasma*

Sera were tested using the commercially available MAT (Toxo-Screen DA, bioMerieux, France). Sera were tested at two different sera dilutions; 1:40 and 1:4000, according to the manufacturer's instructions. The positive and negative control sera included in the kit were used in each round of samples tested, in addition to an antigen control comprised of PBS. A serum sample was determined to be *T. gondii* positive when an agglutination reaction was observed at a serum dilution of at least 1:40, based on the manufacturer's directions.

## *Cryptosporidium and Giardia*

### *DNA Extraction*

DNA was extracted directly from faeces using the Mobio Powersoil DNA kit with minor modifications to the manufacturer's protocol. Briefly, the samples were freeze (liquid nitrogen)-thawed (100°C) 5 times followed by 10 minutes of boiling. This is to ensure lysis of the thick-walled *Cryptosporidium* oocyst and release of DNA. The final elution volume was adjusted to 50 μl Buffer 6 from the kit manufacturer’s recommended volume of 100 μl Buffer 6 in order to increase DNA concentration.

***PCR amplification and sequence analysis at the 18S rRNA gene locus***

All faecal samples were screened for the presence of *Cryptosporidium* and *Giardia* by amplifying the 18S rRNA gene of the parasite using a two-step nested PCR.

For *Cryptosporidium*, the primary PCR amplified a ~763 bp product using the forward primer 18SiCF2 (5'- GAC ATA TCA TTC AAG TTT CTG ACC-3') and the reverse primer 18SiCR2 (5'-CTG AAG GAG TAA GGA ACA ACC-3') for the primary reaction and the nested forward 18SiCF1 (5'-CCT ATC AGC TTT AGA CGG TAG G-3') and nested reverse 18SiCR1 (5'-TCT AAG AAT TTC ACC TCT GAC TG-3') primers for the secondary reaction . The PCR mixture consisted of 0.01-1.0ng of DNA, 200 µM (each) deoxynucleotide triphosphates (dNTP) (Fisher Biotech, Perth, Australia), 1 x DNA polymerase reaction buffer (Fisher Biotech), 1.5 mM $MgCl_2$ (Fisher Biotech), 0.5 U of *Tth plus* DNA polymerase (Fisher Biotech) and 12.5 µM of forward and reverse primers in a total of 25µL reaction. Fifty-three PCR cycles (94 ºC for 30 sec, 58 ºC for 30 sec, 72 ºC for 45 sec) were carried out in an Applied Biosystems Gene Amp PCR System 2700 thermocycler with a preliminary cycle (95 ºC for 2 min, 58 ºC for 1 min, 72 ºC for 2 min) and a final extension (72 ºC for 5 min). For the secondary PCR, a fragment of ~587bp was amplified using 1 µl of primary PCR product. The conditions for the secondary PCR were identical to the primary. Positive secondary PCR products were sequenced directly in the reverse direction. For samples that were PCR negative, a second internal nested PCR, amplifying a fragment of ~300bp, was carried out using the forward primer 18SiF (5'- AGT GAC AAG AAA TAA CAA TAC AGG -3') and the reverse primer 18SiR (5'- CCT GCT TTA AGC ACT CTA ATT TTC -3') (Morgan *et al* 1997). Briefly, the PCR mixture consisted of 1 µl of primary PCR product, 200 µM (each) deoxynucleotide triphosphates (dNTP) (Fisher Biotech, Perth, Australia), 1x DNA polymerase reaction buffer (Fisher Biotech), 1.5 mM $MgCl_2$ (Fisher Biotech), 0.5 U of *Tth plus* DNA polymerase (Fisher Biotech) and 12.5 µM of forward and reverse primers in a total of 25µL reaction. The thermal cycling conditions were identical to that of the primary PCR.

For *Giardia*, a primary PCR product of ~292 bp was amplified using the forward primer RH11 (5'-CAT CCG GTC GAT CCT GCC-3') and reverse primer RH4LM (5'-GTC GAA CCC TGA TTC TCC G-3'). The PCR mixture consisted of 0.01-1.0ng of DNA, 200 µM (each) deoxynucleotide triphosphates (dNTP) (Fisher Biotech, Perth, Australia), 1 x DNA polymerase reaction buffer (Fisher Biotech), 2.0 mM $MgCl_2$ (Fisher Biotech), 0.5 U of *Tth plus* DNA polymerase (Fisher Biotech), 1.25 µl Dimethyl Sulfoxide (DMSO) and 12.5 µM of forward and reverse primers. Fourty-five PCR cycles (94 ºC for 30 sec, 58 ºC for 20 sec, 72 ºC for 45 sec) were carried out in an Applied Biosystems Gene Amp PCR System

2700 thermocycler with a preliminary cycle (94 ºC for 2 min, 58 ºC for 1 min, 72 ºC for 2 min) and a final extension (72 ºC for 7 min). For the secondary PCR, a fragment of ~130 bp was amplified using 1 µl of primary PCR product and nested forward primer Giar18SER (5'-GAC GCT CTC CCC AAG GAC-3') and reverse primer Giar18SIR (5'-CTG CGT CAC GCT GCT CG-3'). The secondary reaction was the same as for the primary reaction with omission of DMSO. Thermal cycling conditions were as for the primary PCR with two modifications; annealing temperature was raised to 60 ºC, with the temperature time increased to 30 seconds. Positive secondary PCR products were sequenced directly in the reverse direction.

***Sequence and Phylogenetic Analysis***

Amplified DNA fragments were separated by gel electrophoresis and purified using the MoBIO Ultraclean15 Kit. Briefly, the excised gel fragments were placed in a 1.5ml Eppendorf tube and DNA purification was carried out according to manufacturer's protocol. The purified PCR products were then sequenced using an ABI Prism$^{TM}$ Dye Terminator Cycle Sequencing kit (Applied Biosystems, Foster City, California) according to the manufacturer's instructions with the exception that the annealing temperature was raised to 58ºC for *Cryptosporidium* and 60ºC for *Giardia*. Sequences were analyzed using Chromas 2.3 (Technelysium Pty Ltd). Additional *Cryptosporidium* and *Giardia* sequences were obtained from GenBank and aligned using ClustalW (http://clustalw.genome.jp).

# APPENDIX C:
## SCIENTIFIC PUBLICATIONS

*Journal of Wildlife Diseases*, 46(2), 2010, pp. 564–569


# Serologic-based Investigation of Leptospirosis in a Population of Free-ranging Eastern Grey Kangaroos (*Macropus giganteus*) Indicating the Presence of *Leptospira weilii* Serovar Topaz

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ABSTRACT: Eastern grey kangaroos (*Macropus giganteus*) are one of the most abundant large macropodids sharing the landscape with humans. Despite this, little is known about the prevalence of *Leptospira* carriage within this species and the role that they may partake in the transmission of this disease in Australia. The sera of 87 free-ranging eastern grey kangaroos, captured in the Warragamba Catchment Area, Sydney, Australia, from June 2004 to November 2006, were screened against a reference panel of 22 *Leptospira* serovars using the microscopic agglutination test (MAT). Leptospiral antibodies were detected in 47% (41 of 87) of serum samples collected. *Leptospira weilii* Topaz, a newly emergent serovar in Australia, was detected in all seropositive kangaroos (41 of 41; 100%). The sex and tail-fat body condition index of kangaroos appeared to have no significant effect on the exposure to the disease. This serologic-based study is the first reported for *L. weilii* serovar Topaz in New South Wales, to our knowledge, having previously been isolated only in humans and two other animal species (bovine and long-nosed bandicoot [*Perameles nasuta*]) in Western Australia and Queensland. The potential role of eastern grey kangaroos in the maintenance and zoonotic spread of the disease to livestock and humans is discussed.

*Key words:* Eastern grey kangaroo, *Leptospira weilii*, leptospirosis, *Macropus giganteus*, Topaz, zoonotic.

Leptospirosis is an emerging, bacterial, zoonotic disease of worldwide importance (Cox et al., 2005). Transmission to humans has been reported to occur from direct contact with the urine of a mammalian host or indirectly through contact with contaminated water, soil, or infected body fluids or tissues of carrier animals (Slack et al., 2006). Free-ranging wildlife species are most commonly involved in both the maintenance and the spread of leptospirosis to livestock and humans (Cox et al., 2005).

Leptospirosis is a notifiable disease within Australia, where it was first reported in 1933 (Slack et al., 2006). Since 1991, there have been more than 7,629 cases notified in Australia, and 23 different serovars have been isolated from human patients (Slack et al., 2007). These include *Leptospira interrogans* (serovars Australis, Zanoni, Kremastos, Robonsoni, Broomi, Pomona, and Szwajizak), *Leptospira kirschneri* (serovar Valbuzzi); *Leptospira borgpetersenii* (serovar Arborea), and *Leptospira weilii* (serovar Celledoni and Topaz). Some of these *Leptospira* species have been detected in free-ranging wildlife that are often found to interact with humans, including the common brushtail possum (*Trichosurus vulpecula*), feral pigs (*Sus scrofra*), flying foxes (*Pteropus* spp.), rodents (*Rattus* spp.), and the long-nosed bandicoot (Munday, 1972; Durfee and Presidente, 1979; Mason et al., 1998; Banks, 2001; Cox et al., 2005; Slack et al., 2006; Eymann et al., 2007).

The larger macropodid species have received considerable attention in response to their increasing interactions with humans within Australia (Coulson, 2001). This has led to management problems associated with their overabundance in semiurban areas and with their competition with domestic livestock in rural areas (Viggers and Hearn, 2005). However, given the propensity of this species to cohabit areas with humans and to share pasture with domestic livestock, there have been limited studies conducted on the presence of anti-*Leptospira* antibodies in these species. Studies by Munday (1972) of three macropodid species found no antibodies, and subsequent work by Durfee and Presidente (1979) against a limited panel of antigens showed similar results with only one Bennetts wallaby (*Macropus rufogriseus*) demonstrating a low level titer to serovar Pomona. This work by Durfee and Presidente (1979) also involved samples from the eastern grey kangaroos (*Macropus giganteus*) collected in Canberra, Australia, and all yielded no evidence of leptospirosis.

Eastern grey kangaroos are one of the most abundant, larger macropodid species in Australia. This species has a broad and almost continuous distribution along the east coast, extending west to the inland plains, where annual average rainfall exceeds 250 mm (Dawson, 1995). Eastern grey kangaroos exhibit preferences for open grasslands that adjoin woodland providing lateral cover. These requirements are most frequently met in forested nature reserves that adjoin agricultural areas (Moore et al., 2002). In this article, we investigate the antibody prevalence to *Leptospira* species in eastern grey kangaroos, and we analyze the role, if any, that this species has in the maintenance of this bacteria and the likelihood of it posing a source of environmental contamination.

Between June 2004 and November 2006, blood serum was collected from 87 eastern grey kangaroos residing adjacent to the Wollondilly River in the Warragamba Catchment Area (34°11′1″S, 150°18′22″E), approximately 95 km southwest of Sydney, Australia. This area is a major part of Sydney's hydrologic catchment, Lake Burragorang, which supplies approximately 80% of the city's potable water. Public access to the study area is restricted for the protection of water quality. Camping is permitted in designated areas within Yerranderie State Conservation Area, which lies outside the public exclusion zone surrounding Lake Burragorang.

Eastern grey kangaroos reside within the study area with relatively low levels of competition and predation. These factors, together with large areas of improved pasture from former farming practices, have fuelled high densities of this species. These areas also support a large diversity of habitats and other fauna species including those which have been introduced to Australia such as, wild dogs (*Canis lupus familiaris*), farm-escaped cattle (*Bovis taurus*), feral pigs (*Sus scrofa*), cats (*Felis catus*), and foxes (*Vulpes vulpes*).

Eastern grey kangaroos were captured using a Pneu-dart (Pneu-Dart®, Williamsport, Pennsylvania, USA) tranquilizer firearm and the sedative Zoletil 100® (5 mg/kg, 1:1 zolazepam and tiletamine hydrochloride, Virbac, Sydney, Australia). Sedation was generally achieved within 10 min. During sedation, each kangaroo was placed into a hessian bag and weighed, sexed, tagged, radiocollared, and their body condition was noted. Body condition was indexed subjectively by assessing the tail-fat of each kangaroo and scored from 1 (poor) to 5 (excellent). The age of the animal was classified into adult and sub-adult on the basis of their body mass relative to size. Pouch young were not considered in this experiment. Blood (6 ml) was collected from the lateral tail vein of each animal using a 10-ml syringe and winged infusion kit. Serum was separated and stored in temperatures less than −10 C. After processing, animals were placed in the shade to recover, which generally occurred after approximately 2 hr.

The sera were screened against a reference panel of 22 *Leptospira* serovars using the microscopic agglutination test (MAT; Stallman, 1982). The starting dilution for each sample was 1:25, and positive samples were diluted down to an endpoint. Titers of 50 or higher were regarded as evidence of past or current infection.

The MAT panel of antigens represented *Leptospira* serovars that had previously

TABLE 1. Antibodies to *Leptospira* exhibited by 87 eastern grey kangaroos residing in the Warragamba Catchment Area, Australia.

| Leptospirae serovar (titers) | Adult males (*n*/range of titers) | Adult females (*n*/range of titers) | Subadults (*n*/range of titers) |
|---|---|---|---|
| Topaz | 16/100–3,200 | 22/50–1,600 | 3/200 |
| Shermani | 1/50 | 2/50 | 0 |
| Tarassovi | 8/50–800 | 4/50–200 | 1/50 |
| Bataviae | 0 | 1/400 | 0 |
| Panama | 1/100 | 0 | 0 |

been isolated in mainland Australia of the *L. interrogans* (serovar Australis-Ballico; Pomona-Pomona; Hardjo-Hardjoprajitno; Zanoni-Zanoni; Kremastos-Kremastos; Robinsoni-Robinson; Canicola–Hond Utrecht IV; Copenhageni–M 20; Medanensis–Hond HC; Grippotyphosa–Moskva V; and Szwajizak-Szwajizak), *L. borgpeterseni* (serovar Tarassovi-Perepelitsin), *L. weilii* (serovar Topaz–94/7990/3 and Celledoni-Celledoni,), and *L. kirschneri* (serovar Bulgarica-Nicolaevo), and the exotic serovars of *L. interrogans* (serovar Bataviae-Swart; Djasiman-Djasiman), *L. santarosai* (serovar Shermani–1342 K), *L. borgpeterseni* (serovar Ballum–Mus 127 and Javanica–Veldrat Batavia 46), *L. kirschneri* (serovar Cynopteri–3522 C), and *L. noguchi* (serovar Panama–CZ 214).

Contingency tables were used to examine the differences in the prevalence of anti-*Leptospira* antibodies in eastern grey kangaroos among sexes and body condition, and in females, the presence or absence of pouch young (Snedecor and Cochran, 1967). The relative number of juvenile kangaroos captured in the study precluded a comparison in the prevalence of anti-*Leptospira* antibodies among age groups.

*Leptospira* antibodies were detected in 47% (41 of 87) of the sera analyzed with serovar Topaz identified in all seropositive kangaroos (41 of 41; Table 1).The level of serovar Topaz antibody titers in individuals varied from 50 to 3,200. There were reactions observed against the serovars Tarassovi (15%), Shermani (3%), Australis (2%), Panama (1%), and Bataviae (1%), but these were known cross-reactions and not a result of multiple infections. These previously identified cross-reactions are common, and the highest titer detected is not necessarily the infective serovar (Levett, 2001).

There was no significant difference in the prevalence of anti-*Leptospira* antibodies between male and female eastern grey kangaroos ($x^2$=2, $P$=0.16, df=1), although 79% of the samples collected from males (19 of 24), compared with 35% of those collected from females (22 of 63), indicated *Leptospira* exposure. Body condition of the kangaroos had no effect on leptospirosis exposure ($x^2$=6.6, $P$=0.09, df=4) nor did the presence of pouch young in females ($x^2$=2.2, $P$=0.14, df=1. Female kangaroos carrying pouch young had 43% exposure (15 of 35) to *Leptospira* compared with 25% of females not carrying pouch young (7 of 28).

Leptospirosis is an emerging zoonotic disease with a worldwide distribution, and *L. weilii* serovar Topaz is a newly described serovar first isolated in the far north of Queensland, Australia. Given its novel nature, little is known about the epidemiology of this serovar (Slack et al., 2007). It was initially detected in 1994 from both culture and serologic methods in humans and two animal cases (bovine and long nosed bandicoot; Slack et al., 2007). Most of the *L. weilii* serovar Topaz infections have occurred in far north Queensland, with the remaining infections occurring in southeast Queensland and in Western Australia (Slack et al., 2007). The identification of *L. weilii* serovar Topaz in Western Australia and in New South Wales, Australia, from this study, illustrates that this serovar may be more prevalent in the Australian environment than some of the more geographically isolated *Leptospira* serovars, such as *L. interrogans* serovar Zanoni.

The large proportion of the study population of eastern grey kangaroos with leptospiral antibodies (47%) and the high titers recorded suggest a current or recent infection with *L. weilii* serovar Topaz. The data suggest that *Leptospira* infection is endemic throughout the population of kangaroos investigated. However, the risk of disease transmission by the species is dependent on the ability of the animals to act as a carrier. This was not been determined in this study.

Other *Leptospira* studies on Australian free-ranging wildlife have reported lower exposure rates. Mason et al. (1998), for example, reported 20% exposure of *L. interrogans* serovar Pomona in feral pigs, and Cox et al. (2005) found that only 11% of flying foxes sampled had been exposed to *Leptospira* species in their study. Both of these animals can exhibit large ranging patterns of up several tens of square kilometers in area (Dexter, 1999; Markus and Hall, 2004). The study population of eastern grey kangaroos was extremely localized, with high resident densities occupying very small home range sizes. This may have led to a higher level of animal-animal interactions and, therefore, a higher prevalence of *Leptospira* in the population that were sampled.

Another plausible explanation for the high exposure of *L. weilii* serovar Topaz in eastern grey kangaroos is the species innate social behavior. Eastern grey kangaroos are a gregarious species, forming large social groups of up 45 individuals in relatively small areas (Banks, 2001). Kangaroos from the studied population were regularly observed feeding and also drinking from ephemeral dams and puddles in these large groups, which may have acted as a mode of *Leptospira* transmission throughout the population. Also in this species, male kangaroos often travel from group to group to search for females in estrous (Southwell, 1984), a process which involves tasting the urine of receptive females (Kaufmann, 1975; Woodward et al., 2006). The latter may have not only facilitated the transmission of *Leptospira* within the study population, but to other areas because male kangaroos are known to disperse several tens of kilometers to search for receptive females (Dawson, 1995).

If kangaroos are proven to be reservoirs or carriers of the organism, humans and other animals could be at risk of infection through direct contact with the urine of infected kangaroos or from handling carcasses of this species where it is commercially harvested (Pople et al., 2003). Although there are limited human interactions with eastern grey kangaroos in the study area (public access is prohibited to the study area), the incidence of *L. weilii* serovar Topaz in this population may pose disease transmission risks because of the presence of cattle that escape from farms and graze within the study area. These cattle were frequently observed to be associating with eastern grey kangaroos and may be exposed to *Leptospira* at watering points, such as ephemeral puddles and former farm dams, which they share with this species.

Further research is required to determine if *L. weilii* serovar Topaz is endemic to the species across their distribution and not just exclusively to the study population. The risk of eastern grey kangaroos facilitating transmission of the disease across their distribution may be higher where population densities attain high levels and the resultant interaction of this species with humans increases (A.C.T Kangaroo Advisory Committee, 1997; Eymann et al., 2007).

It may be proposed from the present study that because *L. weilii* serovar Topaz is present in a more mobile, abundant, and evenly distributed animal host species, eastern grey kangaroos could play a previously undetermined role in the broader distribution of this particular serovar. Furthermore, the isolation of the serovar Topaz in another native animal (long-nosed bandicoot) provides further support for the suggestion made by Slack et al. (2007) that it may be endemic to Australia.

The findings of this study are significant because they have added to our knowledge of the distribution and identification of a potential new carrier for the recently discovered *Leptospira* serovar Topaz. However, in a future study, there is a need to confirm whether eastern grey kangaroos are carriers or intermittent hosts because this will influence the actual infection risk to other animals and humans exposed to this species. This can be achieved through the analysis of isolation data, which will confirm renal carriage in this species. Furthermore, given the potential for *Leptospira* transmission from eastern grey kangaroos to cattle and the previous finding of bovine infections with the Topaz serovar, sample collection should focus on areas supporting such interactions.

This work would not have been possible without the assistance of numerous volunteers (including D. Brennan, J. Cook, and L. Neaves) and the staff from the Sydney Catchment Authority (SCA; M. Gilmour, J. Ray, M. Krogh, and I. Wright). We thank C. Banffy from the Parks and Wildlife Division of New South Wales Department of Environment, Climate Change and Water (PWD-DECCW) and D. Ashton and A. Simson from Sydney Catchment Authority (SCA) for assisting with the approval process for tranquilizer use in the study area. We are very appreciative of the staff at the SCA Warragamba Catchment Office (B. Waldron, L. Gallen, G. Capararo, U. Manna, J. MacCormick, and T. Kondek) for permitting and facilitating access to the study area and thank D. Scott-Lawson and S. Mills from PWD for their assistance in the field. We thank C. Herbert for her assistance with capturing kangaroos. This work was conducted under a PWD scientific license (11002) and Macquarie University AEC ethics approval (2003/016). This project was supported by SCA funding to Macquarie University (D.W.C.).

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**The following article has been removed as it contains published material under copyright. Removed contents published as:**

Roberts Michael W., Dexter Nick, Meek Paul D., Hudson Matt, Buttemer William A. (2006) Does baiting influence the relative composition of the diet of foxes?. Wildlife Research 33, 481-488.

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# ANIMAL RESEARCH AUTHORITY

**AEC Reference No**.: **2007/020**
**Approval Duration**: 18 August 2007 to 17 August 2008

To: **Mr Michael Roberts (PI)**
7 Penterong Way
Haywards Bay
Phone: 0414 50 70 80
Email: **michael.roberts@sca.nsw.gov.au**

Is authorised by:

MACQUARIE UNIVERSITY to conduct the following research:

**Title of the project: COLLECTION OF RADIO COLLARS FROM EASTERN GREY KANGAROOS FROM EXPIRED PROTOCOL (2003/016)**

**Type of animal research and description of project**:
Research (Wildlife) – The aim of the project is to safely remove radio collars previously fitted to Eastern grey kangaroos under approval 2003/016. (1) Animals with VHF collars will be identified in the field under suitable conditions. (2) Animals will be approached within a distance of 20 to 40 m and darted with zolazepam HCl approx. 5mg/kg i.m. (3) Animal will be observed from a distance until sufficiently sedated to approach. (4) The collar will be removed, blood sample collected, reproductive status assessed, weighed, pes measured and faeces collected opportunistically. (5) Animals will be placed in a shaded area with eyes covered, away from other animals and observed every 15 minutes until recovered and moved away.

**Species of animal**: Eastern grey kangaroo *(Macropus giganteus)*

**Number**: 15 (to be recaptured)

**Location**: Warragamba Special Area, Sydney's Hydrological Catchment

**Amendments considered by the AEC during last period:** N/A

As approved by and in accordance with the establishment's Animal Ethics Committee.

MACQUARIE UNIVERSITY AEC

**Approval was granted subject to compliance with the following conditions:**

*Approval is granted for a period of one year to encourage safe removal of collars and to allow for unforeseen delays and unfavourable weather conditions.*

**(This authority has been issued as the above conditions have been addressed to the satisfaction of the AEC)**

Being animal research carried out in accordance with the Code of Practice for a recognised research purpose and in connection with animals (other than exempt animals) that have been obtained from the holder of an animal suppliers licence.

This authority remains in force from **16 August 2007** to **15 August 2008**, unless suspended, cancelled or surrendered, **and will only be renewed upon submission of a new application to the AEC at the end of this period.**

_______________________________________ **Date:**________________
**Assoc. Prof. R Harcourt**
**Chair of AEC, Macquarie University**

23 August 2007

Mr Michael Roberts
7 Penterong Way
Haywards Bay
NSW 2530

**Reference: 2007/020**

Dear Mr Roberts,

**FINAL APPROVAL LETTER**

**Title of Project: Collection of radio collars from grey kangaroos from expired protocol (2003/016)**

The Animal Ethics Committee considered and approved the above application at its meeting of 16 August 2007. Animal Research Authority Reference **2007/020** has been attached to this letter.

The approval is subject to the following condition/s:

1. *Approved is granted for a period of one year to encourage the safe removal of collars and to allow for unforeseen delays and unfavourable weather conditions.*

Please note the following standard requirements of approval:

1. If the project has been completed, abandoned, discontinued or not commenced for any reason you are required to submit a Final Report on the project. If you complete the work earlier than you had planned you must submit a Final Report as soon as the work is completed. The Final Report is available at http://www.ro.mq.edu.au/ethics/animal/forms/.

2. Alternately, at the end of each 12-month period from the original approval date if the project is still current you should submit a Progress Report (if the project has run for less than three (3) years). This form is available at http://www.ro.mq.edu.au/ethics/animal/forms/. If the project has run for more than three (3) years you cannot renew approval for the project by submitting a satisfactory Progress Report. You will need to complete and submit a Final Report (see Point 1 above) and submit a new application for the project. (The three year limit on ethical

clearance allows the Committee to fully re-review research in an environment where legislation, guidelines and requirements are continually changing.)

3. Please remember you must notify the Committee in writing regarding any alteration to the project.

4. You must notify the Committee immediately in the event of any adverse effects, unexpected animal deaths or any unforeseen events that might affect animal welfare and/or the continued ethical acceptability of the project.

5. At all times you are responsible for the ethical conduct of your research in accordance with the guidelines established by Commonwealth and State bodies and the University (http://www.ro.mq.edu.au/ethics/animal/).

If you will be applying for or have applied for internal or external funding for the above project it is your responsibility to provide Macquarie University's Grants Officer with a copy of this Final Approval letter as soon as possible.

Yours sincerely,

**Associate Professor R Harcourt**
**Chair, Animal Ethics Committee**

ANIMAL ETHICS COMMITTEE
RESEARCH OFFICE (C5C)
MACQUARIE UNIVERSITY

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