

# MANAGEMENT OF URBAN COMMON BRUSHTAIL POSSUMS ( *TRICHOSURUS VULPECULA* )

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# Declaration

This thesis is presented for the degree of Doctor of Philosophy at Macquarie University 2006.

The work described is original and has not been submitted, in any form, for a higher degree at any other university or institution. All of the presented work was undertaken during my PhD candidature, which started in August 2002.

None of the papers in this thesis are sole-authored. The concept for this thesis is my own, and I performed the data collection, data analysis and writing for each of the chapters/manuscripts, unless otherwise stated. Hence, it is appropriate that the work be included in this thesis. However, I have obtained help in this process from various other people, and their contribution is acknowledged in the role of co-authors.

## Chapter description

**Cathy Herbert** had considerable input into every chapter. Cathy gave advice on appropriate handling of brushtail possums, discussed the design of the experiments with me, gave feedback to my interpretation of results, and helped with the editing process of all manuscripts. The particular contribution to each chapter is described below.

**Des Cooper** was my main supervisor for the first 2.5 years at Macquarie University. Hence, Des initiated some contents of this thesis, and his intellectual property is reflected in some chapters. Des also encouraged the preparation of publications.

## Chapter 1:

I reviewed the literature and wrote this chapter entirely by myself, with helpful comments from Cathy Herbert.

## **Chapter 2:**

I designed the experiments, collected all the data with assistance from AgResearch staff (see below), processed all sera samples, collected, analysed and interpreted the results and wrote the entire chapter. In this process, I received helpful feedback from Cathy Herbert as described above and the other co-authors as follows:

**Doug Eckery** supervised my research stay at AgResearch, Wallaceville, New Zealand. Doug gave helpful feedback with the design of the experiments, assisted with the data collection for the GnRH challenge, and commented on my interpretation of the results.

**Brian Thomson** made me familiar with the working procedures and handling of the brushtail possums in the breeding unit of AgResearch, Wallaceville, New Zealand. Brian assisted greatly with the serum collection for the intense 3-month trial and monitored some animals for potential offspring beyond my first stay at AgResearch.

**Tim Trigg** generously contributed the slow-release implants containing deslorelin, which are the intellectual property of Animal Peptech Health Pty Limited.

## **Chapter 3:**

I designed the experiment, organised access to private properties, collected all the field data, analysed and interpreted the results and wrote the entire chapter. I received helpful feedback from Cathy Herbert as described above.

## **Chapter 4:**

I designed the experiment, collected all the field data, initiated the collaboration with Dr JP Dubey , applied for and was awarded a PGRF grant from Macquarie University to fund my stay at the US Department of Agriculture, processed all samples in Dr JP Dubey's laboratory, analysed and interpreted

the results and wrote the entire chapter. I received helpful feedback from Cathy Herbert as described above and from Dr. JP Dubey as follows:

**J. P. Dubey** from Animal Parasitic Diseases Laboratory at the US Department of Agriculture generously offered his facilities, means and training by dedicated staff to allow the processing of brushtail possum sera samples. JP Dubey also helped with the interpretation of the results and the editing process of the manuscript for Chapter 4.

## **Chapter 5**

I designed the experiment, collected all the field data, initiated the collaboration with Lee Smythe, analysed and interpreted the results and wrote the entire chapter. The sera samples were processed at the WHO/FAO/OIE Collaborating Centre for Reference and Research on Leptospirosis, Queensland.

I received helpful feedback from Cathy Herbert as described above and from Lee Smythe as follows:

**Lee Smythe** from the WHO/FAO/OIE Collaborating Centre for Reference and Research on Leptospirosis, Queensland generously offered the processing of brushtail possum sera samples. Lee helped with the interpretation of the results and the editing process of the manuscript for Chapter 5. Meegan L. Symonds, Michael F. Dohnt and Leonie J. Barnett are staff members of the institution and were involved with the processing of the sera samples.

## **Chapter 6**

I wrote the entire final discussion to the previous chapters, with helpful feedback from Cathy Herbert.

## Appendices description

### Appendix A

I observed the adult female brushtail possum with two young when conducting the field work for Chapter 3. I collected all the data, analysed and interpreted all results, and wrote the entire manuscript. I received helpful feedback from Cathy Herbert as described above and the contribution of Mark Eldridge and Linda Neaves is described below:

**Linda Neaves** processed the brushtail possum tissue samples of the female and the two young, analysed the microsatellite and haplotype data and added the results of the genetic analysis to the manuscript.

**Mark Eldridge** extracted the DNA from the brushtail possum tissue samples of the female and the two young, helped with the interpretation of the genetic analysis, gave advice on how to present the genetic data and provided feedback to the entire chapter.

### Appendix B

I observed the problem with failure of PIT tags in individual brushtail possums when conducting the field work for Chapter 3. I collected all the data, initiated the collaboration with Lee Webley, analysed and interpreted all results, and wrote the entire manuscript. I received helpful feedback from Cathy Herbert as described above and the contribution of Lee Webley is described below:

**Lee Webley** processed part of the brushtail possum tissue samples collected (most had been already processed for Appendix C) and performed the genetic data analysis. Lee matched the identical genotypes, calculated the P(ID) and gave helpful feedback to the manuscript.

### Appendix C

Appendix C is exceptional in that it contains a manuscript that is not first-authored by me. However, I was greatly involved in the work for it: I chose the field sites, collected all the tissue samples and field

data, helped with the interpretation of the results and wrote parts of the introduction, materials and methods and discussion. I also reviewed final drafts.

**Adam Stow** is an expert in the use of genetic techniques to investigate genetic diversity and dispersal. Adam suggested the use of GenAlEx V5 and performed and interpreted major parts of the data analysis. Adam also wrote and reviewed great parts of the manuscript and hence, made the major contribution to its contents.

**Nadia Minarovic** was an honours student at Macquarie University in 2003. She processed the brushtail possum tissue samples and included the results of the genetic analysis partly into her Honours thesis which substantially differs from this publication.

**Lee Webley** was supervising the genetic work of Nadia Minarovic and performed and interpreted major parts of the data analysis. Lee also wrote parts of the manuscript and hence, substantially contributed to the contents. Lee also took care of the correspondence with the journal.

## Appendix D

This conference abstract was entirely written by me, reviewed by all other co-authors, peer-reviewed by conference participants and published in the Extended Abstracts of the XXVIIth Congress of the International Union of Game Biologists, Hannover 2005. DSV-Verlag Hamburg, pp. 78-79.

## Appendix E

I added the description of the study sites and the awards won during my candidature.

Numerous other people have made this thesis possible, and their contributions are outlined in the acknowledgements of each chapter.

Signed: .....*J. Eymann*.....

Date: .....31-March-2006.....

(Jutta Eymann)





# Abstract

The common brushtail possum (*Trichosurus vulpecula*) is indeed a common inhabitant of many Australian cities, and one of the few marsupials that has adapted well to the urban environment. Their close proximity to people provides a great opportunity to experience native wildlife in the backyard, however, their utilization of house roofs, bold behaviour and appetite for garden plants often leads to conflict with householders. Population numbers are sufficiently high to require ongoing management to minimise negative impacts for humans and brushtail possums alike in a socially acceptable manner. The aim of this thesis was to identify current management issues and address the need for improved and novel management strategies. The potential of slow-release implants, containing the GnRH agonist deslorelin, as a contraceptive agent for brushtail possums was tested on a captive population. Males appeared resistant to treatment, but deslorelin was found to inhibit reproduction in female brushtail possums for at least one breeding season, making it a promising tool to control fertility in some wild populations. A further aim was to trial deslorelin implants on a wild urban population, to collect more information about the urban biology of this species and to point out issues which have previously not been addressed. Close proximity and interaction of urban brushtail possums with humans and their domestic animals can increase the risk of disease exposure and transmission and influence the health of wild populations. Serosurveys showed that animals were readily exposed to *Leptospira* spp. and *Toxoplasma gondii*. This thesis also provides the first data on brushtail possum dispersal in urban areas, knowledge which is highly relevant to the development of management strategies such as fertility control. The findings from this research broaden our knowledge about urban brushtail possums and should assist wildlife authorities in developing alternative or improved management procedures.



# Acknowledgements

Over the last few years, I have been meeting many people who I owe a big 'thank you'. This thesis would not have been possible without their assistance, support and encouragement.

Dr. Cathy Herbert deserves the warmest thanks. Her ongoing support and enthusiasm for the project has always been there, and her advice was invaluable throughout all stages. With the help of her husband Paul, a brushtail possum expert, I was introduced to my study animal and their proper handling. Cath and I not only worked well together on many occasions, but we also had some wonderful times attending conferences and meeting native wildlife.

My other supervisor, Prof. Des Cooper, has introduced me to the world of marsupials upon my arrival in Australia. Des has been very supportive of this project and given free play to pursue different ideas. He was always generous in providing means for research and conference visits and provided me with many wonderful opportunities.

Dr. Mariella Herberstein jumped in as my official supervisor at Macquarie University, when Cath and Des changed workplace. Mariella has my deepest respect as a supervisor. She always had time for me, and has the cunning ability to understand the core of any problem immediately. Her response time was always extremely swift and apart from great advice on statistical issues, we managed to win an award for the postgraduate research fund application within the university.

The research on the effects of deslorelin on brushtail possums was conducted at the AgResearch institution in Wallaceville, New Zealand. Dr. Doug Eckery was very supportive of my visit, and I am very thankful for having been given the opportunity to work in their breeding unit and getting so close to the brushtail possums. Brian Thomson also gave great support, showing me handling procedures and helping with the data collection. Michael Beaumont did a wonderful job in looking after the animals and also helped with their handling. Dr. Janet Crawford's advice was invaluable for the hormone assay procedures, and Brigitta Mester helped as well. Leanne and Mark Phillips generously accommodated

me during my first stay and Jenny Juengel for my second stay, and both were great hosts. Many other staff of AgResearch always offered a helping hand, and overall, I had a truly great New Zealand experience.

Two weeks of my Ph.D. were also spent at the 'Animal Parasitic Diseases' Lab in Beltsville, Maryland, USA. I need to thank Dr. J. P. Dubey for welcoming me into his lab, Dr. Sam Shen for accommodating me during my stay and Dr. Oliver Kwok for showing me the lab procedures – they all made my stay truly enjoyable!

My field work back in Sydney would not have been possible without the help of many householders who allowed me to trap brushtail possums on their properties. They deserve a really huge 'thank you'. Many have been extremely supportive over the years, helping as much as they could, and spoiling me with cups of tea and biscuits. Their enthusiasm and support for the brushtail possums has driven my motivation immensely. In particular, I thank Debra Birch and Peggy and Juha Havukainen for accommodating me in their fantastic little boat sheds on Scotland Island. Special thanks to Jan Tent for taking brushtail possum pictures and even proof-reading a couple of chapters in this thesis, and also a big 'thank you' to Ron Bradner, Susan Medworth, Evelyn Hadfield, Struan Lamport, the Pulvers family, Sarah Johannson, Michelle Coady, Anne and Dave Cornford, Wendy Cousins, Izabela Konzac-Islam, Don Camble, Marianne McMillan, Maryvonne McKeyDoyle, Claire Bennet, Margo and Brian Pickering, Allison Payne, Terry and Catherine Insley, Mary Lilit, Jay Ekers, Sorbi Smith, Janet and Paul Boocock, Erica Mills, Renee Rawson, Teena Cooper, Jenny Donald and many more. A special mention to Tracey Adams, Cilla Norris, Audrey, Marjolaine, Bridget and many other students who also gave much support with the fieldwork, and to Dan Lunney from NSW National Parks and Wildlife Service (NPWS) who provided valuable advice on aspects of this work and gave his support for this project in general.

The Department of Biological Sciences, Macquarie University has been overall a very friendly place to work in, and I owe thanks to staff members and other students that have been there with me. The 'Cooper Lab' and the Behavioural Ecology lab group welcomed me warmly. Special thanks to Lee

Webley, who helped me to identify animals with PIT tag failure, and to my office-mates Jenny Kingston, Sarah Wilks and Anne Gaskett who have all been great company. Thanks also to the international student office which awarded two travel scholarships for going overseas, and the research office which generously extended my scholarship.

Lastly, my partner Pete deserves special mention for his companionship over the last few years. He has provided much support, climbing up and down the trees looking for brushtail possums, reading chapters of this thesis, and he made my Australian experience truly complete.

Just one final thank you – to the brushtail possums! They have treated me very well over the last few years, it has been a privilege to work with them and thoroughly enjoyable, and I will keep looking out for them.

# Conference Presentations and Awards

## Conference poster presentations

Eymann, J., Herbert, C.A., Lunney, D., Trigg, T., and Cooper, D.W. (2003). Researching urban management problems of brushtail possums. 49<sup>th</sup> Australian Mammal Society Meeting, Sydney, New South Wales, Australia.

Webley, L., Minarovic, N., Stow, A.J., Eymann, J., and Cooper, D.W. (2005). Male-biased dispersal in urban populations of brushtail possums (*Trichosurus vulpecula*). 18<sup>th</sup> Australasian Wildlife Management Society Conference, Hobart, Tasmania, Australia.

## Conference oral presentations

Eymann, J., Herbert, C.A., Lunney, D., Trigg, T., and Cooper, D.W. (2003). Management and conservation of brushtail possums in urban and native environments in Australia. 3<sup>rd</sup> International Wildlife Management Congress, Christchurch, New Zealand.

Eymann, J., Herbert, C.A., and Cooper, D.W. (2004). Brushtail possums in urban areas: a management challenge. 17<sup>th</sup> Australasian Wildlife Management Society Conference, Kangaroo Island, South Australia, Australia.

Eymann, J., Smythe, L., Herbert, C.A., and Cooper, D.W. (2005). Serologic survey for selected disease agents in urban brushtail possums (*Trichosurus vulpecula*) from Sydney. International Wildlife Disease Association Conference, Cairns, Queensland, Australia.

Eymann, J., Smythe, L., Herbert, C.A., and Cooper, D.W. (2005). Serologic survey for selected disease agents in urban brushtail possums (*Trichosurus vulpecula*) from Sydney. 51<sup>st</sup> Australian Mammal Society Meeting, Albany, Western Australia, Australia.

Eymann, J., Herbert, C.A., Lunney, D., Trigg, T., Eckery, D., and Cooper, D.W. (2005). Management issues of urban common brushtail possums (*Trichosurus vulpecula*) – a loved or hated neighbour. XXVII<sup>th</sup> Congress of the International Union of Game Biologists, Hannover, Germany.

### **Conference proceedings (see Appendices)**

Eymann, J., Herbert, C.A., Lunney, D., Trigg, T., Eckery, D., and Cooper, D.W. (2005). Management issues of urban common brushtail possums (*Trichosurus vulpecula*) – a loved or hated neighbour. XXVII<sup>th</sup> Congress of the International Union of Game Biologists, Hannover, Germany.

### **Awards (see Appendices)**

Macquarie University Deputy Vice Chancellor (Research) commendation for outstanding postgraduate research fund application, July 2005.

1<sup>st</sup> prize for best oral presentation in the student award competition held on the occasion of the XXVII<sup>th</sup> Congress of the International Union of Game Biologists, Hannover, Germany, September 2005.





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# Preface

## Introduction to wildlife management

Wildlife managers primarily are occupied with the management of wildlife populations (Caughley and Sinclair 1994), but may also deal with people, education, or law enforcement. Until recently, wildlife management has been a rural issue, but with a growing human population and expanding cities, some species have adapted well to urban environments (Knuth *et al.* 2001). This has led to greater human-wildlife interactions, and the management of wildlife nuisance situations, threats to health and safety, and economic damage is a growing area.

In its core, wildlife management targets manipulation or protection of a population (Caughley and Sinclair 1994). Manipulative management aims to change the population numbers, either directly or indirectly such as altering habitat, density of predators, food supply or prevalence of disease. Manipulative management is applied when a population has an unacceptably low density or, contrastingly, too high a density. Custodial management on the other hand is aimed at protecting a population and its habitat. Four management aims are available – to make a population increase or decrease or harvest it in a sustainable manner or simply leave it alone, but monitor it.

The common brushtail possum (*Trichosurus vulpecula*) is a solitary, nocturnal, arboreal marsupial native to Australia (Kerle 2001). This species is a challenge for wildlife managers. In Australia, brushtail possum populations are in decline throughout much of their natural range (Goldingay and Jackson 2004). However, their decline in some rural areas contrasts with their abundance in urban environments and on some islands e.g. Magnetic Island (Isaac 2005), Kangaroo Island and Tasmania. Ironically, while some brushtail possum populations are in decline, others are considered locally 'overabundant' and create problems. It is a dilemma if a species is in need of protection over most of its natural range, while in other native areas, such as Tasmania, they are killed to prevent damage to plantations (McArthur *et*

*al.* 2000). In New Zealand, brushtail possums are entirely out of place, having been introduced for fur trade in the late 1800s (Cowan 1990). Now, they are considered a major pest and eradication is the ultimate goal of New Zealand wildlife managers (Montague 2000).

Management of these different brushtail possum populations will differ significantly, and intervention is required if they are in decline or if they are 'overabundant'. But what does 'overabundance' really mean? Can wildlife ever be too abundant? Overpopulation may be defined as too many animals in four scenarios (Caughley 1981): a) the animals threaten human life or livelihood, b) the animals depress the densities of other favoured species, c) the animals are too numerous for their own good and d) the animals are not in equilibrium. In the case of the brushtail possum, urban animals are often viewed as a nuisance to people if they live in their roof or browse on garden vegetation; in Tasmania, they inflict commercial forestry damage by browsing on *Eucalyptus* plantations (le Mar and McArthur 2003); and in New Zealand they are a major non-native pest, spreading bovine tuberculosis, damaging native forests and preying on eggs and chicks of native birds (Montague 2000). Therefore, wildlife managers deal with quite varying problems, such as animal welfare, vegetation protection, or commercial damage.

All management actions may be restricted by the availability of funds and are directed by the urgency of the problem. In New Zealand, approximately NZ\$ 58 million was spent on research and management of brushtail possum populations in 1993/94 alone (Cullen and Bicknell 2000). The cost of knocking down a high density population was estimated at NZ\$ 20 to \$ 30/ha and for eradication of an island population more than NZ\$ 61/ha would have to be spent. However, effective management of any brushtail possum population requires knowledge of the basic biology of the species. Any attempts at managing a population may be futile if we do not know about the species life history, demography and population dynamics.

## Population ecology of the common brushtail possum

The reproduction of the brushtail possum has been extensively reviewed (Tyndale-Biscoe and Renfree 1987; Fletcher and Selwood 2000). It is a polyoestrous species, having successive oestrus cycles of about 26 days until conception occurs. The gestation period, from copulation to birth, is 17-18 days and about 8 days shorter than the oestrous cycle. Oestrus and ovulation are usually suppressed by lactation, but females can ovulate again about 8 days after loss or removal of the pouch young. Females are usually strictly monotocous (presenting one offspring at a time) and monovular with ovulation occurring spontaneously from either ovary. Brushtail possums are seasonal breeders and in Australia most females give birth in autumn, with a second minor peak of births in spring. The onset of the breeding season may vary depending on the geographical location; for the Sydney area March to May are considered the major breeding months (Bolliger 1940 in Tyndale-Biscoe 1955; Lyne and Verhagen 1957). The young exit the pouch for the first time at about 140 days and permanently leave it by 170 days (Kerle 2001). They are weaned at around 240 days and become independent.

Table 1. Summary of key definitions in population ecology.

### Key definitions (after Campbell et al. 2006)

- **Life history:** the series of events from birth through reproduction and death.
- **Demography:** the study of statistics relating to births and deaths in populations.
- **Population:** a localized group of individuals that belong to the same biological species and are capable of interbreeding and producing fertile offspring.
- **Population dynamics:** the study of how complex interactions between biotic and abiotic factors influence variations in population size
- **Population ecology:** the study of populations in relation to the environment, including environmental influences on population density and distribution, age structure, and variations in population size.

## Life history

The term 'life history' refers to the stages of life undergone by an individual from birth to death, with particular reference to strategies influencing survival and reproduction (Campbell *et al.* 2006, see Table 1). The course of life is defined by factors such as age at sexual maturity, first mating and reproduction, fecundity, inter-birth interval and age specific mortality. Life history strategies are largely determined by the species size and physiology, and by climatic conditions, habitat type and food quality (Kerle 2001). Density-dependent factors such as intra-and inter-specific competition for resources (including food and den sites) may interact with density-independent factors such as climatic and environmental pressures (Isaac 2005). The brushtail possum has an especially flexible life history strategy that is influenced by the carrying capacity and suitability of the various habitats they occupy, and populations are able to alter fecundity, behaviour and diet (Kerle 2001). Natural selection often determines which individuals survive and reproduce, but even strong selection on any trait is unlikely to make a marked difference to population dynamics in a population that is limited by the availability of resources such as nest sites (Saccheri and Hanski 2006).

## Population dynamics

By measuring life history parameters and estimating the population size it is possible to consider a range of questions (Sutherland 1996). "What determines the level of abundance? Why does the population fluctuate from year to year? How strong is density dependence and at what life stage does it operate? What are the consequences of competitors or predators on the population?" The use of observational data in answering such questions poses considerable problems; instead it requires density manipulation, for example by adding or removing animals (Sutherland 1996). The mechanistic paradigm (contrasting to the density-dependent paradigm) ignores density dependence, but focuses directly on mechanisms of regulation and addresses rather the impact of disease, predators and food shortage on population dynamics (Krebs 1995).

There have been numerous population studies of brushtail possums in Australia and New Zealand (for review see Kerle 1984, Kerle 2001; Goldingay and Jackson 2004; Efford 2000), and most studies describe basic population parameters (such as fecundity and densities) that vary considerably between different populations. However, the factors that control brushtail possum populations and what really drives their population dynamics is not clearly elucidated. Some of this variation in populations seems to be related to habitat quality (Kerle 2001). Generally, population growth is driven by key factors such as mortality rates, birth rates, migration and there are other factors which influence these. It has been suggested that resource-dependent factors are of much greater importance in the regulation of common brushtail possum populations than external factors such as predation (Kerle 2001). For example, population densities of northern brushtail possums (*T. vulpecula arnhemensis*, a subspecies) in the wet-dry tropics of Australia's Northern Territory appeared to thrive because of the ready availability of food resources that enabled females to breed continuously (Kerle 1998). On Magnetic Island, Queensland, the absence of large predators and apparently low competition for dens seemed to promote a higher density of animals than in any other reported Australian population. Food availability appeared to potentially constrain population densities (Isaac 2005). Brushtail possum populations in New Zealand reach higher densities than any recorded in their native Australia (Efford 2000). There has been great effort trying to understand the population dynamics of the brushtail possum in New Zealand because of its status as a major pest. Higher densities in New Zealand may be attributed to a combination of factors such as the absence of large predators, a lower range of parasites, higher digestibility and nutrient content of foliage and no competition with other folivorous marsupials.

Brushtail possums have adapted well to most urban areas, possibly because they provide additional resources not seen in natural habitats, such as artificial den sites and an abundant food supply (Statham and Statham 1997; Kerle 2001). The numerous studies on Australian and New Zealand populations (Kerle 1998, Isaac 2005; for reviews see Kerle 1984, 2001; Efford 2000) may not necessarily be applicable to the life history of urban brushtail possums as there is wide variation

between the different populations. The population dynamics of brushtail possum populations in general are not entirely understood to date (Efford 2000), and it remains uncertain why some populations thrive while others are in decline (Isaac 2005; Goldingay and Jackson 2004). There is also a paucity of knowledge about the biology of brushtail possums in urban areas. The few studies that involve urban brushtail possums mainly address home range and use of den sites (Harper 2005, Statham and Statham 1997). Astoundingly, urban brushtail possum population dynamics have not been investigated yet, nor are the basic urban population parameters (such as sex ratio, age structure, fecundity, mortality) readily available.

## **General thesis introduction**

The common brushtail possum (*Trichosurus vulpecula*) is indeed a common guest in Australian (sub-) urban neighbourhoods, but surprisingly little is known about its urban biology. Brushtail possums have a reputation both as ‘champions of the suburbs’ and ‘tormentors’ for taking up residence in house roofs and browsing on garden plants (Matthews *et al.* 2004). Their presence polarises the community into distinct parties, the possum-lovers, the -haters, and residents that are indifferent. This situation is a real challenge for wildlife authorities who are tested when community attitudes towards removing brushtail possums tend to conflict with management practices based on animal welfare and a policy of ‘living with wildlife’ (Matthews *et al.* 2004). In recent years, there has been increasing attention on managing overabundant native species (Garrott *et al.* 1993). However, brushtail possums may not get the same attention as large and charismatic native marsupials such as kangaroos and koalas, despite being one of the last native animals that live basically ‘next door’. Being one of the few marsupials that have adapted to our urban civilisation, brushtail possums offer the great potential to be a first hand opportunity to experience wildlife in your own backyard. However, it can not be denied that urban brushtail possums may cause undesirable situations, a fact that has been recognised by wildlife authorities (Matthews *et al.* 2004). There are also downsides to the animals living in such close contact with humans: the brushtail possum’s welfare in urban neighbourhoods is seriously threatened through



human actions such as the illegal removal of the animals from private properties, a wide-spread and common ill-practice (Eymann *et al.* 2006). This shows that current management policies may be appropriate and animal-friendly, but are either not accepted or do not reach the entire public. This situation enhances the need for research into the biology of this species in urban areas, and the need for improved and/or novel management tools. As such, this thesis has set out to address these issues and have a closer look at these ‘champions of the suburbs’.

My studies have also led me to other countries, such as New Zealand. Although the brushtail possum is not native to New Zealand, their status as a major pest has sparked huge interest into this species leading to major research projects (Cowan 2000). It seems only appropriate to utilise this knowledge and incorporate and assess if the New Zealand findings can be applied to Australia.

The central theme for this thesis is the management of urban common brushtail possums, and in pursuit of this, the key aims relevant to this topic are described as follows:

### **Main aims of this thesis**

- To review existing management practices of common brushtail possums in urban environments, outline the current situation and give an outlook on potential future management tools (**Chapter 1**)
- To investigate the potential of the slow-release GnRH agonist deslorelin as an ethical technique for controlling fertility in common brushtail possums, a captive study (**Chapter 2**)
- To observe the effects of the contraceptive deslorelin implants on a wild population and collect basic biological information on the targeted urban population, a field study (**Chapter 3**)
- To examine the health status of urban common brushtail possums (**Chapter 4 and 5**)

### **Further outcomes of this thesis (Appendices)**

- To report on a case of adoption in the urban common brushtail possum (**Appendix A**)
- To state technical problems encountered with field work and suggest potential improvements (**Appendix B**)

- To describe dispersal in this species, which is relevant to the application of fertility control  
(**Appendix C**)

These aims broadly fit together through the following rationale. The first step for this thesis was a comprehensive review, assessing and evaluating current management practices, and I have compiled this into **Chapter 1**. It was apparent that there was a clear need for improved and novel management strategies. Hence, research into a potential contraceptive agent for brushtail possums seemed a promising option.

One major focus of New Zealand research is on fertility control as a tool to control brushtail possum population numbers (Cowan 2000). Reducing the reproductive potential of a population is becoming an acceptable approach to managing 'problem' and pest wildlife (Cowan *et al.* 2003). Increasing public concern for animal welfare, together with a desire to find alternatives to culling for population control, have been major incentives for fertility control research (Rodger 2003). Although there is the desire to control brushtail possum numbers in New Zealand and in parts of Australia, these present two very different management situations. Management in Australia aims to reduce abundance to sustainable levels only in high problem areas in order to mitigate problem situations, but still aims to maintain a sufficient population size. Contrastingly, New Zealand's ultimate goal is eradication of the species (Cowan 1996, 2000). Hence, it is quite certain that fertility control techniques utilised will differ in both countries and a unique solution may not be applicable. Each unique situation requires different contraceptive attributes to achieve optimal results (Garrot 1995).

The slow-release GnRH agonist deslorelin has previously been shown to be a successful contraceptive in other marsupial species such as tammar wallabies (Herbert *et al.* 2004, 2005) and kangaroos (Herbert 2004; Herbert *et al.* 2006; Woodward *et al.* 2006). Hence, deslorelin seemed to be promising as a potential contraceptive agent for brushtail possums, and we decided to investigate its effects on the reproduction of the brushtail possum (**Chapter 2**). This research was conducted in cooperation with

AgResearch, and I was fortunate to be able to work in their brushtail possum breeding unit at Wallaceville in New Zealand.

I also initiated a field trial to test the efficacy of deslorelin implants to inhibit reproduction in wild urban brushtail possums in Sydney (**Chapter 3**). The field trial not only enabled us to compare findings with those from the captive study, but also was a unique opportunity to collect information on basic biological parameters of the targeted population such as age structure, sex-ratio, reproductive output and the health of the animals. In the past, these fundamental life history traits have been poorly recorded in urban brushtail possum populations. An appreciation of these characteristics is essential prior to any fertility control agent being applied on a large scale.

Dispersal is a fundamental issue for the success of any control operation as immigration has the potential to swamp the effects of fertility control (Cowan 2003; Ramsey 2005). In New Zealand, possums tend to disperse even at very low densities, and the potentially large distances moved by juveniles mean that buffer zones around control areas are likely to be 'leaky' (Cowan and Clout 2000). This thesis provides the first data on dispersal of brushtail possums in the urban environment and hence, will contribute towards resolving management issues for this species in urban areas (**Appendix C**).

There can be further major issues with wildlife living in urban areas (apart from residential complaints about noisy brushtail possums living in the roofs) that are important on a more widespread scale. The proximity to urban areas and contact with humans and their domestic animals can increase the risk of disease exposure for wild populations and influence their health (Deem *et al.* 2001; Riley *et al.* 2004). For example, marsupials are highly susceptible to toxoplasmosis and infection can prove fatal in captive and free-ranging populations (Canfield *et al.* 1990). **Chapter 4** shows that urban brushtail possums are readily exposed to this parasite in the urban environment. There is also concern that the close association of wildlife with humans and their domestic pets may result in 'spill over' of diseases, and

infectious diseases of wildlife can also be a threat to human health (Daszak *et al.* 2000). Hence, in **Chapter 5** I examined the notifiable zoonotic disease leptospirosis.

Working in the field has also its practical challenges, and some problems may not have been expected beforehand, such as a certain percentage of failure in the marking of the animals (**Appendix B**). Luckily, there was a way to back up and ensure the correct identity of each brushtail possum.

Additionally, the field studies on urban brushtail possum populations resulted in the surprising observation of an adult female which had adopted a second young (**Appendix A**).

Last but not least, the final Appendix gives an impression of the captive breeding unit in New Zealand and from the field sites within Sydney's North Shore suburbs (**Appendix E**).

## **Field sites**

The main part of this thesis builds on the data collection from private properties within Sydney metropolitan area that were nearby the facilities of Macquarie University in North Ryde, New South Wales (all chapters except no. 2). Householders within the different communities responded to a media campaign which asked for volunteers in a university based brushtail possum research project. The campaign involved posters on the noticeboard of the local shopping villages, a report in the local newspaper and an interview on a local radio station. Also, staff members from Macquarie University living in the particular areas volunteered and were happy to become involved in our studies.

After the establishment of the different field sites, numerous properties were visited and traps set to verify the presence of brushtail possums. Brushtail possums were investigated in the garden area of about 35 residential houses from five localities within the Sydney metropolitan area (Fig. 1). Locations included four North Shore mainland suburbs (Beecroft, Chatswood West, North Epping, and Pymble) and Scotland Island, a 52.4 ha land mass located at Pittwater (D. Van Den Bosch, GIS officer Pittwater council, pers. comm.). These areas are characterised by the presence of native trees and significant areas of remnant native bushland contributing to the landscape quality, which makes them highly

attractive not only to people, but also to brushtail possums and other native species (see Appendix E for detailed description of the field sites). In the end, trapping was conducted over 124 nights, giving a total of 1,202 trap nights and a total of 350 animals (including recaptures) caught.

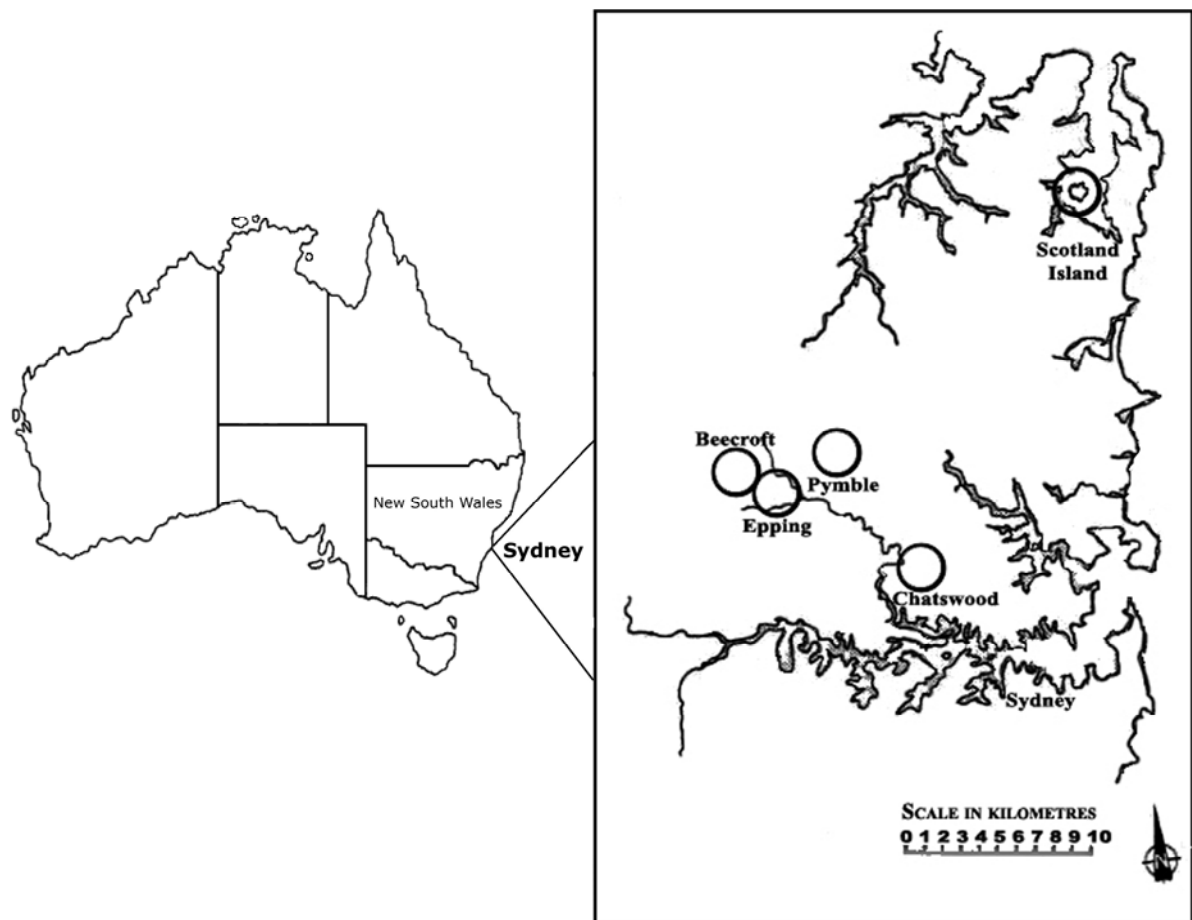


Figure 1. Map of Australia showing Sydney field sites (four metropolitan suburbs – Beecroft, Chatswood West, North Epping and Pymble; and Scotland Island) from which brushtail possums (*T. vulpecula*) were sampled.

## Structure of this thesis

This thesis has been prepared in accordance with the guidelines of the Higher Degree Research Unit (HDRU), Macquarie University, NSW, Australia (HDRU 2006). All chapters of this thesis have been written for publication in relevant journals. Hence, this thesis includes papers that are in preparation for

submission, have been submitted and/or were accepted for publication during the period of my candidature, or are now even in press (including a published conference abstract). The preface aims to give a brief and comprehensive introduction to the issues surrounding the urban brushtail possum and explains how the different chapters are interlinked. Chapter 1 reviews thoroughly the background to each following chapter and hence, not only provides an extended introduction, but also an integrative conclusion to the situation of the urban brushtail possum. Each chapter forms a coherent body of work focusing on a single project or a set of related questions. As each chapter is designed as a 'stand-alone paper', some degree of overlap has been unavoidable, particularly in the introduction and in the description of the materials and methods. Each chapter is formatted according to the requirement of the journal to which it was or will be submitted (including the references), and the English language has been adjusted to the journal's country of origin. All chapters and publications in the appendices are first-authored by me, except for the publication on sex-biased dispersal in brushtail possums, but do include co-authors to acknowledge their contribution. The specific contribution of co-authors to the preparation of each publication has been specified in the thesis certificate, and contributions of others have been recognised in the thesis acknowledgements.

Please note: the terms 'possum' and 'brushtail possum' relate to the 'common brushtail possum' throughout this thesis unless otherwise stated.

Amendment for the reader: this thesis has been mostly updated after receipt of the reviewers' comments which have been incorporated in the individual chapters. Hence, the individual chapters and appendices indicate the actual status in regard to publication upon re-submission of the thesis. Manuscripts in preparation or under review by the journal may be altered at a later point in time for publication purposes.

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## Chapter 1

# Management issues of urban common brushtail possums (*Trichosurus vulpecula*): A loved or hated neighbour

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## Abstract

The common brushtail possum (*Trichosurus vulpecula*) has readily adapted to the expanding urban settings of its native Australian environment. This has led to conflict with humans due to *T. vulpecula*'s seemingly bold behaviour in suburbia. Current management strategies encourage people to live harmoniously with possums. However, despite the cooperation of many residents, some object to this policy and illegally remove *T. vulpecula* from their properties. Wildlife managers are seeking alternative management options that aim to reduce conflict with people. These include fertility control methods which may be used to adjust possum numbers in a publicly acceptable way. Nest boxes are frequently recommended as alternative den sites for *T. vulpecula* which reside in man-made structures. The disease status of possums may alter the rationale for their management in urban areas, due to the potential consequences for humans, domestic animals and possum conservation. Understanding the biology and behaviour of *T. vulpecula* can greatly enhance the ability to select and successfully utilise adequate methods and solve outstanding issues. As such, this paper aims to review the scientific knowledge on possum populations as well as urban possum management policies and potential management tools.

## Introduction

The common brushtail possum (*Trichosurus vulpecula*) is a solitary, nocturnal, arboreal marsupial native to Australia. It is a cat-sized phalangerid generally occurring where there are trees, especially in open forests and woodlands (How and Kerle 1995). *T. vulpecula* has one of the widest distribution of any Australian mammal, but was once much more widely distributed and abundant (Gordon and Hrdina 2005). It has disappeared from large parts of the arid and semi-arid zones, tropical woodland areas, and has declined in the southeast forests (How and Hillcox 2000; Goldingay and Jackson 2004). Accordingly, *T. vulpecula* has been listed as 'rare' in South Australia and 'endangered' in the Northern Territory (Carthew 2004; Woinarski 2004). Conversely, it is very common in many Australian cities, in Tasmania, as well as on Kangaroo Island and Magnetic Island (Isaac 2005a). *T. vulpecula* is also present in New Zealand, having been introduced in the late 1800s to establish a fur trade (Cowan 1990). It has become a major invasive pest in New Zealand, not only damaging crops and native forests, but also carrying bovine tuberculosis and preying on nests of native bird species (Clout and Ericksen 2000). This creates three distinctive management issues for *T. vulpecula*: 1) their cohabitation with people in Australian urban areas, 2) their decline in Australian rural areas contrasting with their abundance on some islands, and 3) their management in New Zealand aiming to considerably reduce *T. vulpecula* numbers with the ultimate goal of complete eradication.

This paper reviews relevant literature on the biology of *T. vulpecula* populations, the policies for managing them in urban areas, and discusses potential management options to reduce conflict between possums and people in the Australian urban environment. Further management issues such as disease transmission will also be discussed.

## Urban possums

*Trichosurus vulpecula* have readily adapted to urban areas by varying their behaviour and dietary preferences and utilising human resources for both food and habitat (Kerle 1984). They can use a wide

spectrum of foods (Burchfield *et al.* 2005) and consume decorative garden plants, fruit trees and an abundance of household refuse (e.g., compost), as well as often nest within buildings (Kerle 2001; Matthews *et al.* 2004). Their density is potentially high in some urban areas. Aldred (1979 in Matthews *et al.* 2004) caught between 6 and 24 possums per 0.3 ha parkland in suburbs of Sydney, while Eyre (2004) reports up to 7 per ha for urban Brisbane. One of the few sources of information about den site use, home range, birth and mortality of urban *T. vulpecula* is a study from Launceston, Tasmania (Statham and Statham 1997). Most other research has been conducted in New Zealand or in Australian bush habitat and may not reflect the ecology of urban *T. vulpecula* in Australia. However, due to the paucity of information on urban possums, these data shall be used here to facilitate our understanding of their behaviour in urban areas.

### **Loved or hated neighbour**

In a social research study, Davies *et al.* (2004) report that 26% of people surveyed 'liked possums a lot', 35% 'a little', while 39% 'didn't like possums at all'. Moreover, the mainstream community seemed relatively unengaged with wildlife conservation and considered only those species as desirable that have a cute appearance and a good image and besides, do not cause any disturbance. Community attitudes towards *T. vulpecula* vary widely, and its behaviour in the city leads to numerous conflicts with people (Miller *et al.* 1999; Lunney and Burgin 2004b). Possums are often regarded as a pest that invades roof spaces, damages garden plants and contaminates properties with droppings (Davies *et al.* 2004; Matthews *et al.* 2004). Some believe that possums should only live in the bush where they do not cause problems. People with a positive attitude enjoy their presence and see them as a natural part of the Australian environment that we should live with.

Complaints about possum behaviour are not solely related to *T. vulpecula*. The common ringtail possum (*Pseudocheirus peregrinus*) also frequently cohabits with humans in (sub)urban areas. Miller *et al.* (1999) found that most people have problems distinguishing between ringtail and brushtail possums. *P. peregrinus* can also damage garden plants, cause faecal contamination, and although making their



own dreys, may occasionally be found in man-made structures (J. Eymann, pers. obs.). However, *T. vulpecula* usually causes the most concern, because it regularly takes up residence in roof cavities, being the primary cause of conflict.

[Note: The use of 'possum' hereafter refers only to *T. vulpecula*.]

## Feeding possums

People with positive sentiments towards possums often try to attract them by installing a nest box or by actively feeding them. A recent survey by ABC Wildwatch (2004) revealed that nearly 30% of people in urban areas feed wildlife. *T. vulpecula* is also fed by people in municipal gardens (McDonald–Madden *et al.* 2000). People with negative attitudes towards possums often have poor knowledge of the species (Miller *et al.* 1999). They can unwittingly encourage them by providing resources such as open compost bins, bird feeding stations, growing plants preferred by *T. vulpecula* and having easily accessible roofs, sheds and garages. Feeding is sometimes recommended to keep *T. vulpecula* off garden plants with people 'paying tribute' to them by providing an alternative food source (Kerle 2001). Supplementary feeding is questionable as it may not be successful at keeping possums off garden plants and likely attracts additional possums. Wildlife feeding is also discouraged by wildlife agencies, but despite this opposition, it remains a common practice (Rollinson *et al.* 2003).

## Possum repellents

Various repellents such as quassia chips are another option to keep *T. vulpecula* off plants, but Cooney (1998 in DSE 2004) suggested that only a few tested compounds show some degree of repellence. These were: White King®, Keep Off®, Camphor, Naphthalene and Scat®. Most agents were unsuccessful at stopping a hungry possum from eating the plant. Suggestions on how to keep *T. vulpecula* out of the roof include using artificial light sources at night, ultrasound devices or camphor and moth balls in the roof. However, the success rate of these measures has not been assessed objectively. Previous studies on kangaroos have shown that ultrasonic devices do not successfully alter

their vigilance responses, so they are unlikely to work as a deterrent in these species (Bender 2001). Information about what plant species *T. vulpecula* prefer and which they avoid is not readily available. Many wildlife carers have this knowledge and it may be worthwhile exploring this area to promote alternative garden plants instead of planting 'possum favourites' on properties where they are not welcome. The choice of backyard plants may be a safe and passive means of either encouraging or discouraging *T. vulpecula*.

### **Whose possum is it?**

The home range of urban possums can be as small as 0.3 ha, but data from several studies indicate that average sizes are about 1–2 ha for females, while males tend to have larger home ranges (1.2, 3.0 up to 8.6 ha) (Dunnet 1956; Statham and Statham 1997; Harper 2005). This means that *T. vulpecula* inhabits and moves across property boundaries and home ranges may overlap on a given property. A scenario might be imagined where a possum takes up residence in one householder's roof, but uses several other properties for foraging and to find a mating partner. Neighbourhood disputes arise when people encourage the presence of *T. vulpecula* against the wishes of their neighbours. Householders who feed 'their' possums may become attached to them like pets and support their protection. This may contrast with the view of neighbouring householders who regard possums as a pest and wish them to be removed. This common scenario makes it extremely difficult to formulate a general management policy that satisfies the whole community.

### **Life in the city: easy-going or dangerous?**

*Trichosurus vulpecula* may have access to abundant den sites (e.g., roofs) and food in urban areas, but do they really have an 'easier life' than possums in bushland areas? With urbanisation come various dangers, as is reflected in the number of possums taken into care by WIRES (Table 1). Causes of mortality include collision with a vehicle, dog or cat attacks and accidental (or intended) poisoning by snail bait or rat poison (Hemsley and Canfield 1993; Statham and Statham 1997). Also, high density

populations may be more prone to certain diseases such as dermatitis (see below). Illegal relocation of *T. vulpecula* is an additional risk (see below). Mortality usually goes unnoticed as deceased or relocated possums are quickly replaced by immigrants (Aldred 1979 in Matthews *et al.* 2004). The large datasets held by wildlife rescue groups have potential to illuminate the nature of interactions between humans and wildlife (Koenig *et al.* 2002), if used with caution. It may be worthwhile establishing a reporting system to make these datasets available for wildlife agencies.

Table 1. Reasons for taking *T. vulpecula* into care in 1997 (WIRES 2004).

Cause of care	Mv	HI	Uh	Ca	Da	Uk	Fn	Total
No. <i>T. vulpecula</i>	299	49	388	51	101	701	85	1,674

Mv = Motor vehicle, HI = Habitat loss, Uh = Unsuitable habitat, Ca = Cat attack, Da = Dog attack, Uk = Unknown, Fn = Fallen.

## Suburban possum management

The strategies used to manage *T. vulpecula* in Australia vary depending on their legislative status in each State or Territory (Table 2). Matthews *et al.* (2004) reviewed the New South Wales (NSW) possum management policy. Most state agencies encourage a policy of 'living with possums' (Table 2). Residents experiencing 'possum' problems will receive, as a first step, advice on how to resolve these issues. Strategies may include repairing roof entrance points, pruning adjacent branches, using tree collars, floppy fences and alternative garden plants. Nest boxes are recommended to provide *T. vulpecula* with an alternative den site, but possums have proven to be very persistent in accessing roofs. Licences can be obtained solely to remove the animals from the roof, after which they are to be released on the same property. In exceptional circumstances, some state agencies allow euthanasia of nuisance animals if release on the same property is not possible.

Table 2. Urban *T. vulpecula* management policies by Australian State and Territory government agencies.

Table 2. Urban *T. vulpecula* management policies by Australian State and Territory government agencies. All States and Territories have a 'living with possums' policy and recommend the use of nest boxes.

State	Possum status	Legislation	Authority & advice	Who can trap? (Permit holder)	Removal method	Alternative method	Notes	Reference
ACT	Protected by law; common urban areas	Nature Conservation Act 1980	Environment ACT; possum proof roof	Licensed pest control companies	Trapping, roof-repair, release on site	Not described	Severe penalties if handled without license	Environment ACT 2005
NSW	Protected by law; common urban areas	NSW National Parks and Wildlife Act 1974	DEC; possum proof roof	Licensed householders & pest controllers;	Trapping, roof-repair, release on site (50m)	Euthanasia at cost of licensee	Trapping without license illegal	DEC 2004; Matthews <i>et al.</i> 2004
NT	Protected by law; common urban, arid zone endangered	Territory Parks and Wildlife Conservation Act 2000	NRETA; possum proof roof	Licensed householders & pest controllers	Trapping, roof-repair, release on site near or in box	Relocation by pest controller into vacant areas	Trapping without license illegal; Extra take permit for relocation	NRETA 2005; L. Perry, pers. comm. EPA 2005
QLD	Protected by law; common urban areas	Nature Conservation (Wildlife) Regulation 2005	EPA, QPWS; possum proof house	Licensed pest controllers	Trapping and roof-repair	Not described	Trapping requires license	
SA	Protected by law; common urban; decline arid zone; over-abundant Kangaroo Island	National Parks and Wildlife Act 1972	DEH; possum proof house	Licensed householders & pest controllers	Trapping, roof-repair, release on site (50m)	Euthanasia (separate destruction permit)	Trapping requires license; Kangaroo Island shooting permit available	DEH 2005
TAS	Protected by law; common urban, rural (over-) abundant	Wildlife Regulations 1999 (partly protected)	DPIWE; possum proof roof & garden	Permit holder	Trapping last step; relocation undesirable	Shooting or poisoning if economic damage	Trapping or destruction requires permit	DPIWE 2005; (www.thelaw.tas.gov.au)
VIC	Unprotected (Order 2003); Common urban areas	Wildlife Act 1975; Governor in Council Order (8 July 2003)	DSE; possum proof roof & garden	Householders & licensed pest controllers	Trapping, roof-repair, release on site (50m)	Euthanasia at cost of licensee	Penalty for breach of law up to \$ 5,000	DSE 2004
WA	Common in present range	Wildlife Conservation Act 1950	CALM; possum proof roof	License holder	Trapping only as a last resort; Relocation if no habitat left	Euthanasia not permitted	Trapping and removal requires license	CALM 2005; P. Mawson, pers. comm.

ACT = Australian capital territory; NSW = New South Wales; NT = Northern Territory; Qld = Queensland; SA = South Australia; Tas = Tasmania; Vic = Victoria; WA = Western Australia; CALM = Department of Conservation and Land Management; DEC = Department of Environment and Conservation; DEH = Department for Environment and Heritage; DPIWE = Department of Primary Industries, Water and Environment; DSE = Department of Sustainability and Environment; EPA = Environment Protection Agency; NRETA = Department of Natural Resources, Environment, and the Arts; QPWS = Queensland Parks and Wildlife Service. \*

Relocation is no longer an option in most States or Territories. Studies have shown that translocation has deleterious effects on the individuals and a large percentage of animals (70%) are found to die within a week of release (Pietsch 1994). Relocated urban *T. vulpecula* experience competition with resident possums, are naïve to the forest environment and spend longer periods on the ground – making them highly susceptible to predation by the introduced red fox (*Vulpes vulpes*). Additional concerns include the impact of translocated *T. vulpecula* on habitat quality, the genetic integrity of the resident population, as well as the transmission of diseases. Some possums may display homing behaviour post-translocation, with distances up to 7 km traveled (Cowan and Clout 2000; Cowan 2001). Furthermore, they tend to disperse quickly from the release site, with the few survivors taking up residence in other buildings, indicating that the translocation procedure essentially transfers the problem elsewhere (Pietsch 1994; Statham and Statham 1997; Aldred 1979 in Matthews *et al.* 2004).

As a result most States and Territories now have a policy that *T. vulpecula* must be released within 50 m of the capture site (Table 2). It is often not clear to householders that a roof has to be properly sealed to prevent the possum's return. Some householders wish for their complete removal to stop nightly disturbance and damage to garden plants. The 50 m translocation rule is warranted from an animal welfare point of view, but it is unlikely to reduce the nuisance problem for many people. This often leads to non-compliance with government policies placing possum welfare at further risk. The Northern Territory is currently the only state where pest control companies may trap and relocate possums under a take-permit, and *T. vulpecula* must be released into areas of vacant native bushland of at least 20 ha, and not within 5 km of a town boundary (L Perry, NRETA, pers. comm.).

*Trichosurus vulpecula* is managed by a number of individuals and agencies in Sydney, NSW. Licensed pest controllers trapped 786 *T. vulpecula* in Sydney in 2000 (Matthews *et al.* 2004). Possums were released within 50 m of the property, but most of their clients objected to this rule and less than half of licensed pest controllers erected nest boxes as part of their service.

WIRES, a wildlife care organisation, rescued 2,916 *T. vulpecula* within the Sydney area in 1996 (WIRES 2004). Whenever possible, rescued animals are subsequently released under a 'soft-release' policy, meaning release from an aviary and a brief period of supplementary feeding (Stanvic 2004). The possum can come and go at will until it can establish and support itself in the wild. If soft-release is not available, animals are 'hard-released', meaning possums are placed in a tree in a nest box at a site deemed suitable, and no external support is given (T Adams, WIRES, pers. comm.). Although the aim is to release adults back to the point of capture, this is not always practical if the location is unsuitable or the origin of the injured animal has not been recorded (Fowler 2005). A new approach is to seek the help of the public in finding householders who are willing to support the soft-release of possums on their properties (WIRES 2005). However, no monitoring system has been established on the survival of these released animals.

Individual residents can directly obtain a license from wildlife agencies to remove nuisance possums, and traps can be hired from councils and equipment hire companies. However, there is no reporting requirement under this license and Miller *et al.* (1999) state that 90% of trap-hirers have a negative attitude towards possums. So the final fate of *T. vulpecula* is not known and release on the same property depends on the good-will of people.

### **Non-compliance with current policies**

Illegal trapping of possums is a common practice in urban areas. Traps are readily at hand, for example they are freely available as 'cat traps' in the Northern Territory (L Perry, NRETA, pers. comm.). Without acquiring the necessary permits or complying with the legislation, an unquantified number are translocated to bushland areas and national parks. Householders may not know about wildlife agency policies and are either unaware of, or ignore, the negative impacts of relocation on possums. Relocation only creates vacant 'spots' that are quickly taken over by immigrants. Therefore, the problem is only temporarily solved and continuing relocation of 'nuisance' possums is likely to occur. This shows that the agency's management practices are heavily tested when community attitudes in support of

removing possums are in conflict with management policies based on welfare and a policy of 'living with wildlife'.

Another example are the Royal Botanic Gardens in Sydney. It is believed that "possums from the inner city are being trapped and then dumped at the gardens", adding to a high population density. It also "creates fights for space, ... – what seems a benign act of relocation is actually quite cruel." (Woodford 2003). The Department of Environment and Conservation (DEC) has licensed euthanasia of *T. vulpecula* in the Gardens to manage tree damage caused by excessive possum numbers. Similar management issues occur in Melbourne and legislation has been changed to enable euthanasia of *T. vulpecula* in municipal gardens (DSE 2004).

## **The challenge of urban wildlife management**

Urban wildlife management is an emerging discipline throughout the world (Lunney and Burgin 2004a). Until recently, wildlife management has been a rural issue, but with a growing human population and expanding cities, some species have adapted well to urban environments (Knuth *et al.* 2001). This combination has led to greater human-wildlife interactions, and the management of wildlife nuisance situations, threats to health and safety, and economic damage is a growing area. Examples are various, such as martens (*Martes foina*) in central Europe that damage cars by chewing on engine parts (Gohl 1986; Lower 1989); mountain lions (*Puma concolor*) in the USA attacking urban deer as well as humans and their pets (Baron 2004); and in the Chicago region alone 18,000 'nuisance' raccoons (*Procyon lotor*) were removed in 1999 (Gehrt 2004, 2005). Another example is the rise of urban *V. vulpes* populations in European cities (Gloor *et al.* 2001). As a result, INFOX (a communication campaign) was established in conjunction with a scientific research program to ensure the peaceful coexistence of people and *V. vulpes* (Bontadina *et al.* 2001). It included communiqués for the media, a TV series, an exhibition, a children's drawing contest about urban foxes, leaflets, a telephone information line and more.

Such initiatives may be well applied to Australian 'possum education' programs. Education and communication programs need to be widespread and involve the mainstream community to be successful (Miller *et al.* 1999; Davies *et al.* 2004; Lunney and Burgin 2004b). Many urban residents want to learn about wildlife, and watching it is an important aspect of the wildlife experience for many urbanites (Knuth *et al.* 2001). Promoting the benefits of having wildlife in the backyard will make a peaceful coexistence with native wildlife more realistic (Burgin 2004). Active management, such as modifying numbers and behaviour of the wildlife, may support these measurements (Knuth *et al.* 2001). It will also require further research into the biology of urban *T. vulpecula* to assist future management decisions. Matthews *et al.* (2004) suggest potential improvements, such as changes to the 50 m relocation rule and to issuing trapping licenses. A greater understanding of possums by the broader community, and active management of the population, should help to dampen issues of conflict and prevent non-compliance with current management policies.

## **Fertility control as a management tool**

Reducing the reproductive potential of a population is becoming an acceptable approach to managing 'problem' and pest wildlife (Cowan *et al.* 2003). Increasing public concern for animal welfare, together with a desire to find alternatives to culling for population control, have been major incentives for fertility control research (Rodger 2003). In Tasmania, the main method for reducing browsing damage by *T. vulpecula* is lethal population control through poisoning with sodium monofluoroacetate (commonly known as '1080') (McArthur *et al.* 2000). This method is controversial, particularly when used against native fauna, and now non-lethal alternatives are being sought. In New Zealand, where *T. vulpecula* is considered a major invasive pest, biological control methods are currently being explored, most of them based on immunological blocks to the production of young (Cowan 1996; Cowan 2000; Landcare Research 2004). Immunocontraception has been tested extensively with variable results (Polkinghorne *et al.* 2005). Concerns lie in the design of the delivery systems and in the ability to ensure species specificity (Cowan 2000). This is of particular concern if a selfdisseminating delivery system is the



ultimate goal (e.g., using the nematode parasite *Parastrongyloides trichosuri*; Cowan *et al.* 2006), where security of the method also has to be assured on an international level (Gilna *et al.* 2005). Adaptation through natural selection of individuals that are genetically resistant to the contraceptive agent may be possible, thereby rapidly negating the effectiveness of the agent (Cooper and Herbert 2001; Magiafoglou *et al.* 2003). Although *T. vulpecula* is not formally considered a 'pest' in Australia, fertility control may be one future management tool to mitigate its minor pest status in suburbia. New Zealand control methods may not apply to the Australian situation, where control of possums is only desired in problem areas, such as (sub)urban neighbourhoods, but merely to reduce their abundance to sustainable levels and not to eradicate the animals. No control agent should be used that may potentially spread to rural populations where *T. vulpecula* is in decline. Hence, each unique management situation may require different contraceptive attributes to achieve optimal results.

Table 3 shows potential fertility control methods for *T. vulpecula*. Most methods target female reproduction as the reproductive potential of the population may not be affected as severely as expected when targeting male fertility in a polyoestrous species such as *T. vulpecula*. This phenomenon has been described for macropodids by Herbert (2004) and similarly by Garrott and Siniff (1992) who report that vasectomy in male feral horses does not have the desired effect of reducing population numbers, because the continuous cycling of the mares gives other males the opportunity to mate. In addition, most methods described in Table 3 only inhibit reproduction in females, not males (e.g. deslorelin, progestin, immunisation against sperm). The female reproductive system generally seems to be an easier and more effective target for inhibiting fertility.

Deslorelin is a promising non-steroidal, nonimmunological approach to contraception in the possum (Table 3). It is a gonadotrophin releasing hormone (GnRH) agonist formulated into implants that are administered subcutaneously (Suprelorin®, Peptech Animal Health, Macquarie Park, Australia; Herbert and Trigg 2005; Padula 2005). It temporarily inhibits the release of essential hormones and hence renders the animal infertile for a certain time. It has been successfully trialled in female tammar

wallabies (*Macropus eugenii*) (Herbert *et al.* 2004a), eastern grey kangaroos (*M. giganteus*) (Herbert *et al.* 2006) and koalas (*Phascolarctos cinereus*) (Herbert, unpubl. data). Males of macropodids are resistant to the contraceptive effects of chronic GnRH agonist treatment (Herbert *et al.* 2004b; Herbert, unpubl. data). Effects of deslorelin implants on *T. vulpecula* are currently under investigation with initial tests indicating that reproduction can be successfully inhibited in females (Eymann *et al.*, unpubl. data; see Chapter 2). GnRH agonists have been used successfully on other wildlife species with no side-effects or behavioural changes reported, giving this method much hope for success (Bertschinger *et al.* 2002; Baker *et al.* 2002, 2004; Herbert *et al.* 2005).

Table 3. Potential fertility control methods for female *T. vulpecula*.

Type of control	Method	Possum trials	Number of treatments	Success rate	Duration	Available	Side effects	References
GnRH agonist; Peptide hormone	Deslorelin	Yes	1 (implant under skin)	100%	≥16 months	Yes, veterinary product	None detected	Eymann <i>et al.</i> , unpublished data (see Chapter 2, 3)
Progestin; Steroid hormone	Levonorgestrel	No (tested in koala, tw, egk)	1 (minor surgery)	100% (koala, tw, egk)	27–48 months (egk, tw, koala)	Yes, human product	None detected	Middleton <i>et al.</i> 2003; Nave <i>et al.</i> 2002a, b;
Immuno-contraception	Sperm immunisation	Yes	1 injection + 2 booster injections	80%	≥6 months	No	None detected	Duckworth <i>et al.</i> 1998
Surgical sterilisation	Tubal ligation, ovariectomy	Yes	1 (major surgery)	100%	Lifetime (irreversible)	Yes	Undesired altered behaviour	Ji <i>et al.</i> 2000; Jolly and Spurr 1996; Ramsey in Cowan 2003

tw = tammar wallaby (*Macropus eugenii*); egk = eastern grey kangaroo (*Macropus giganteus*); koala (*Phascolarctos cinereus*).

Synthetic progestins, e.g., levonorgestrel, have not yet been trialled in *T. vulpecula*, but have proven to be successful in other marsupial species such as *P. cinereus*, *M. eugenii* and *M. giganteus* (Table 3). Levonorgestrel implants can last for up to four years in *P. cinereus*, but require minor surgery to be administered (Nave *et al.* 2002a,b; Middleton *et al.* 2003). No adverse effects were reported in these

marsupials, however, severe reproductive disorders, such as mammary adenocarcinoma, were reported in captive exotic felids after progestogen implants had been in place for 2–10 years (Raphael *et al.* 1990; Linnehan and Edwards 1991). Furthermore, the availability of levonorgestrel for use in wildlife may be limited as it is currently licensed as a human contraceptive.

Surgical sterilisation as a fertility control technique has been used in a number of high profile situations (Herbert 2004). It is probably the most well established method of fertility control, but is invasive, stressful, expensive and difficult to deliver to large populations. Surgery is irreversible and the impacts on genetic diversity need to be considered when dealing with native species. Again, targeting males may not be a feasible way to reduce population numbers if only some of the males are sterilised. Besides, sterility of adult males alters their movement patterns to resemble those of females (Ramsey in Cowan 2003). Undesirable behavioural changes may also be a problem if using this method on females (see below).

### **Positives and negatives of fertility control**

The difficulties in applying any of the above fertility control methods are various and may include undesired side effects. Behavioural changes are likely to result from methods of fertility control that block fertilization, but leave the endocrine system intact. Curtis *et al.* (2002) showed that female white-tailed deer (*Odocoileus virginianus*) vaccinated against GnRH have fewer oestrous cycles than control and porcine zona pellucida (PZP)-immunised deer. The PZP-vaccine prolonged the breeding season as females repeatedly returned to oestrus. Sterile but hormonally competent females may therefore have an increased frequency of mating contacts due to an increased frequency of oestrus. Tubal ligation of female *T. vulpecula* keeps the animals cycling and hence leads to a prolonged mating season (Ji *et al.* 2000). The presence of these oestrus females attracted additional males from surrounding areas and the body condition of these males was significantly poorer. Consort and mating behaviour may constrain foraging behaviour and is energetically exhaustive (Isaac 2005b). Additional attraction of male possums is an unsuitable scenario for Australian urban areas and declines in male body condition are an

unacceptable side-effect. A prolonged mating season may also result in increased noise disturbance for householders due to increased male-female encounters and agonistic contacts between males trying to consort and mate with females in oestrus. Furthermore, this could increase the incidence of diseases that are transmitted through social and sexual contacts. Caley and Ramsey (2001) calculated that the disease transmission rate for leptospirosis is potentially higher in populations subject to tubal ligation.

Fertility control methods that target endocrine control of reproduction (e.g., deslorelin) may cause more desirable behavioural changes such as inhibition of mating behaviour. A GnRH based contraceptive has been evaluated as a potential tool to prevent pregnancy and transmission of brucellosis in bison (*Bison bison*) (Miller *et al.* 2004). Reduced sexual contacts between *T. vulpecula* and possibly reduced agonistic contacts may reduce disease transmission and residential complaints about noisy animals. There is some concern that disruption of normal social behaviour in sterile females may hinder effective competition for resources or territories (Davis and Pech 2002). A loss of status by dominant animals may allow subordinates to breed more successfully (Jolly *et al.* 1996). However, this proposed disruption of hierarchies has not been observed after ovariectomy or vaccination of dominant female *T. vulpecula* against GnRH (Jolly and Spurr 1996).

## Management and research needs

The main disadvantage with the methods available at this point in time (Table 3) is the need to physically catch the animals to apply treatment. Remote delivery is currently not available for either deslorelin or levonorgestrel implants. However, remote delivery projectiles, termed biobullets, have been successfully used for contraception purposes on *O. virginianus* (DeNicola *et al.* 1997). Intramuscular injection of the contraceptive agent leuprolide acetate through dart delivery did suppress fertility in female elk (*Cervus elaphus nelsoni*) (Baker *et al.* 2005). These studies demonstrate the potential of remote delivery methodologies for administering contraceptives to large mammals. The efficacy of contraceptive projectiles for smaller mammals, especially a nocturnal, arboreal marsupial like *T. vulpecula*, needs further investigation. A pressurized pistol with biobullets may be helpful to quickly

deliver contraceptives like deslorelin to trapped possums, therefore eliminating the need to physically restrain or anaesthetise the animals which is currently required for administration of the implant.

If fertility control is to succeed modelling may be needed to determine the number of resident females which will have to be treated to obtain the desired result (Davis and Pech 2002). Immigration has the potential to swamp effects of fertility control, emphasising the need for application of fertility control over large areas in order to be effective (Cowan *et al.* 2003; Ramsey 2005). Juvenile *T. vulpecula*, especially males, disperse about the time of sexual maturity (Cowan and Clout 2000). Even if a population is reduced dramatically, dispersal of *T. vulpecula* may still occur as it is not strongly influenced by density-dependent factors at the natal site (Cowan *et al.* 1997), and moreover, is an integral part of male development (Efford 1998). The average dispersal distance is about 5 km for *T. vulpecula* in New Zealand (Cowan and Clout 2000), but is unknown for Australian urban populations. Also unknown are the factors that govern possum dispersal and settlement in urban environments. The tendency of possums to disperse even at very low densities and the potentially large distances moved by juveniles mean that buffer zones around control areas are likely to be 'leaky' (Cowan and Clout 2000). This issue deserves further investigation to verify its relevance to Australian cities.

Social and political issues may also restrict the use of fertility control. Applications over large scale areas will require community consensus, which may prove difficult in urban areas. The term 'overabundant' involves subjective, value-laden judgements (Garrott *et al.* 1993). This, together with the native status of *T. vulpecula*, may cause conflict within the community where attitudes towards *T. vulpecula* are quite polar. Social research will be needed to address concerns about these new approaches to controlling wildlife abundance (Lauber and Knuth 2004).

## **Future applications**

Sydney's Royal Botanic Gardens are one example where fertility control of the local *T. vulpecula* population may be an alternative to the current culling strategy. Herbert (2004) suggests that

contraception is likely to be a useful tool in parkland areas. Fertility control may work best if it goes hand in hand with other measures to reduce the availability of food and nest resources such as possum-proofing rubbish bins and fitting tree collars (DSE 2004). Illegal relocation of nuisance possums into the Gardens is a problem that should be addressed concurrently as well as the likelihood of immigration from neighbouring possum populations. As expressed by Grandy and Rutberg (2002) “Wildlife contraception should be applied judiciously, locally and in a manner that is sensitive to the needs of animals, humans and ecosystem function.”

## **Nest boxes**

### **Nest box installation**

The reasons for installation of possum nest boxes in suburbia can be manifold. The most promoted application is their use as an alternative den site for *T. vulpecula* that have been evicted from man-made structures. Possums residing in roof cavities can cause damage, noise and urine contamination of these areas, which often results in relocation of the offending animals. If there are insufficient alternative den sites, possum welfare concerns arise and an alternative den site should be provided (although this is not a legal requirement). Wildlife rescue organisations also frequently use nest boxes as part of their release strategies. Indeed, some residents install nest boxes solely to attract *T. vulpecula* to their property. For conservation purposes nest boxes may be used as a substitute for limited natural tree hollows in (sub)urban vegetation remnants (Harper *et al.* 2005b). These examples show that various parties utilise and install nest boxes. However, it is rarely monitored how successful they are at fulfilling their intended purpose. To comprehend how these nest boxes may potentially be used, a general review on den usage by *T. vulpecula* follows.

### **Den use by *T. vulpecula***

In their natural environment, usual daytime dens of *T. vulpecula* are hollows in large eucalypt trees (Kerle 2001). Ground level sites are used only when trees are absent or scarce (Allen and Bradshaw

1978; Kerle 1984). In the forests of eastern Australia *T. vulpecula* generally den alone, although a consorting male may share a den with a female in oestrus (Winter 1976) and females share with their young. However, this may vary with population density (Kerle 2001). In New Zealand, simultaneous den sharing is uncommon, unless density is high and den sites few (Cowan 1990; Ji *et al.* 2003). Many dens are used sequentially by several different possums and a few dens are preferentially used by *T. vulpecula* (Cowan 1989). In urban areas numbers of hollow-bearing trees are limited (Harper *et al.* 2005a), so *T. vulpecula* has adapted to alternative den sites. They are happy to nest in any dark recess, especially in buildings (Statham and Statham 1997; Kerle 2001). In Statham and Statham's (1997) Launceston study, 28% of all observed den sites were in trees, but they were only used occasionally. More than 40% of all den sites were associated with buildings, mainly roof cavities, and one household chimney was visited by three different possums. Den sharing was reported for mothers and their offspring and possums of different sexes. *T. vulpecula* visited several den sites, but usually up to three were regularly used. These findings indicate that urban *T. vulpecula* readily use roof cavities, and potentially, several animals may den inside the same building space. Hence, the question arises, what level of acceptance *T. vulpecula* may show towards nest boxes.

### **Nest box acceptance**

Nest box usage has mainly been studied in Australian bushland areas, with boxes being used in situations where hollow availability is limited (Menkhorst 1984a). *T. vulpecula* use nest boxes all year with no obvious seasonal variation in numbers (Ambrose 1982 in Menkhorst 1984a). Studies on optimum possum nest box design and their location show that *T. vulpecula* prefers boxes at a height of 4-5 m above ground level (Menkhorst 1984a). However, most studies report relatively limited use of nest boxes by different possum species and voice concerns over large scale application and use over long time frames (Menkhorst 1984b; Lindenmayer *et al.* 2003).

In (sub)urban vegetation remnants, *T. vulpecula* utilise boxes all year round, but more frequently in the cooler months (Harper *et al.* 2005b). But again, the utilisation of the boxes was found to be low, and the

proportion occupied at any one time varied between four and 14% (out of 120 boxes). Although about 43% (52/120) of boxes were used at some time, the majority of these were occupied only once while under observation. Their occupation was positively influenced by the abundance of *T. vulpecula* and the type of nest-box construction – thick-walled pine boxes were preferred over plywood boxes.

Harper *et al.* (2005b) also report that up to 27.5% (33/120) of boxes were occupied by the common (Indian) myna (*Acridotheres tristis*) which was difficult to deter. The European honeybee (*Apis mellifera*) not only competes with possums for tree hollows (Wood and Wallis 1998), but can also infest nest boxes (Franks and Franks 2003). Ten percent of 96 boxes in a Victorian study were occupied by feral bees and ants (Lindenmayer *et al.* 2003). Gibbons and Lindenmayer (2002) mention deterrents against feral species such as lining the ceiling of a box with carpet to deter bees, or predator shields to exclude *A. tristis*.

The seemingly low utilisation of boxes by *T. vulpecula* and the presence of feral pests in the boxes reduces their effectiveness as a management tool. It has also been questioned if nest boxes encourage an increase in population size by creating additional den sites. While this may be the desired effect in bushland areas and (sub)urban remnant vegetation, it is not welcomed by householders who do not like *T. vulpecula* on their property. This effect is unlikely if boxes are used solely as a replacement for lost den space in a sealed roof. The idea is to keep the territorial resident possum on the property to stop new immigrants from moving in. However, *T. vulpecula* may return to the roof if not all access holes are blocked (Franks and Franks 2003), or they may try to move into a neighbouring roof. Provision of nest boxes should, therefore, be coupled with adequate repairs to man-made structures which make them 'possum proof', if the goal is a reduction in human-possum conflict.

## Management and research needs

The effectiveness of nest boxes is debatable as they may encourage feral species and 'new' possums. Although nest box provision is recommended by wildlife agencies, there are concerns that possums



may not always use them. Nest box installation will also require maintenance. Longevity of nest boxes is influenced by a range of factors such as their design and the construction materials (Menkhorst 1984a). Boxes will have to be installed at a height which allows inspection and feral species removal (Franks and Franks 2003). *T. vulpecula*'s acceptance of nest boxes after their relocation from roof cavities has not been monitored, nor has the potential of boxes to 'lure' *T. vulpecula* out of man-made structures. A further issue is that sometimes several possums may use the same roof cavity – and again different individuals may make use of one and the same nest box. Targeting individual 'nuisance' possums may not be possible by using one alternative nest box. Although there is potential to assist displaced *T. vulpecula* with a new den and nest site, the use of the provided box can not be guaranteed – an issue that will require further empirical investigation.

## **Disease transmission**

Proximity to urban areas and contact with humans and their domestic animals can increase the risk of disease exposure for wild populations and influence their health (Deem *et al.* 2001; Riley *et al.* 2004). Urban *T. vulpecula* live close to humans and their pets, suggesting the possibility of three-way disease transmission. The interaction of zoonotic pathogens with wildlife, domestic animals and human populations is a factor associated with emerging infectious diseases (EIDs) (Daszak *et al.* 2000). EIDs of wildlife can be classified into three major groups: The 'spill-over' from domestic animals to wildlife populations living in close proximity; the transmission of infectious agents from wildlife to sympatric populations of susceptible domesticated animals, termed 'spill-back'; and EIDs directly related to human intervention, via host or parasite translocations. Although wild animals might be sources of infectious organisms, many infectious organisms originate from humans and their companion animals (Fayer *et al.* 2004). Wild animals may then act as a disease vector. Wildlife disease is an important issue and understanding it will help to manage wildlife more effectively.

Wildlife disease in urban areas is not just an animal welfare issue; it may also have implications for public health. *O. virginianus* have been culled in urban areas of the USA to decrease incidents of Lyme disease (Kilpatrick and LaBonte 2003). Urban *V. vulpes* in Europe are hosts of the small fox tapeworm (*Echinococcus multilocularis*), the aetiological agent of human alveolar echinococcosis, a potentially deadly infection (Deplazes and Eckert 2001; Deplazes *et al.* 2004). Anthelmintic baiting has been used to reduce urban contamination with *E. multilocularis* (Hegglin *et al.* 2003). These examples show that the health status of urban *T. vulpecula*, an area that lacks much knowledge, deserves more attention. *T. vulpecula* are known to carry a wide range of diseases (Presidente 1984), some of which may affect possum health and population numbers. Public health concerns may arise if, for example, water tanks are used by urban households. When possums move across the roof, urine and faecal contamination of water collected from the roof is possible, and adequate precautions have to be taken if tank water is used for consumption. Disease transmission involving urban *T. vulpecula* is a mostly unexplored field to date which justifies the need for investigations. A brief outline of common diseases which are currently researched follows.

Toxoplasmosis, caused by the protozoan *Toxoplasma gondii*, is found worldwide and is among the most frequently reported parasites of humans and animals (Fayer *et al.* 2004). *Tox. gondii* is a parasite of the cat and oocysts are shed in the faeces of infected cats (Viggers and Spratt 1995). Marsupials are highly susceptible to toxoplasmosis and infection can prove fatal in captive and free-ranging populations (Canfield *et al.* 1990). A recent seroprevalence study revealed that more than 6% (9/142) of urban possums had antibodies to *Tox. gondii* showing that possums are readily exposed to this parasite in urban environments (Eymann *et al.* 2006; see Chapter 4). Seropositive *T. vulpecula* were usually not recaptured after initial seroconversion, suggesting that most infected animals do not survive for long periods post-infection. Control of feral cat numbers and keeping domestic cats indoors is currently the only rational approach in preventing urban contamination with *Tox. gondii* oocysts.

Neosporosis is caused by the protozoan parasite *Neospora caninum*. This parasite is biologically similar to *Tox. gondii* and, until 1988, was misdiagnosed as *Tox. gondii* (Dubey *et al.* 1988; Dubey and Lindsay 1996). The dog has been shown to be a definitive host of *N. caninum* (McAllister *et al.* 1998). However, the seroprevalence study of Eymann *et al.* (2006) found no evidence for exposure of urban *T. vulpecula* to this parasite.

*Cryptosporidium* spp. is another zoonotic pathogenic protozoan found worldwide and a frequently reported parasite (Chilvers *et al.* 1998; Fayer *et al.* 2004). Wildlife has been recognized as a potential source of contaminating the catchments of public water supplies (Perz and Blancq 2001). Most mammalian species can be infected with *Cryptosporidium* spp. (Sturdee *et al.* 1999), with most animals recovering within 1–2 weeks of infection, but significant mortalities occur in young animals (O'Donoghue 1995). The prevalence of infection with *Cryptosporidium* spp. in wild animals was investigated in New Zealand by Chilvers *et al.* (1998). *Cryptosporidium* cysts were detected in 12.8% of 39 possums and were more prevalent in younger animals. Little is known about the potential for cross-species transmission, and it is not known if the strains of *Cryptosporidium* found in New Zealand wildlife are able to infect humans. *Cryptosporidium* spp. may be transferred to wildlife hosts through direct contact with human faeces and human contamination of waterways. Limited transfer of infection between wildlife and people was suggested for the New Zealand study. Power *et al.* (2003) showed that captive Australian *T. vulpecula* were shedding oocysts. The prevalence of cryptosporidiosis in urban possum populations and the type of *Cryptosporidium* which infects possums is currently being researched (Power and Eymann, unpubl. data). This emphasises the need for further research to investigate prevalence of *Cryptosporidium* species and cross-host infectivity.

Leptospirosis is a disease caused by bacteria called *leptospire*s (Levett 2001). It also occurs worldwide and is a notifiable zoonotic disease in Australia (Queensland Health 2004). The disease can be serious for both humans and animals and sources of infection include contact with water, soil or other materials contaminated with the urine of infected animals (Mason *et al.* 1998). *T. vulpecula* can be a carrier of

leptospirosis (Durfee and Presidente 1979a, b). Experimental *L. interrogans* infection has confirmed *T. vulpecula* as a potential maintenance host for serovar Balcanica and leptospiruria (excretion of leptospires in the urine) was observed (Hathaway 1981). Their susceptibility to leptospirosis has even been investigated for biological control purposes in New Zealand (Day *et al.* 1997). Possums not only contract leptospirosis through a contaminated environment, but also as a result of affiliative or sexual behaviour (Day *et al.* 1998). Leptospirosis was found to be prevalent in urban *T. vulpecula* from the Sydney area, with about 9% of 136 individuals tested having antibodies to *Leptospira* spp. (Eymann *et al.* unpublished data; see Chapter 5).

Dermatitis is a term used loosely for *T. vulpecula* with skin conditions and infections. It is also known as 'lumbosacral dermatitis', 'rumpiness', 'rumpwear' or 'exudative dermatitis' (Hemsley and Canfield 1994). Although dermatitis in *T. vulpecula* is a common occurrence, it has not been examined in depth. The aetiology of dermatitis in possums can be multifactorial and suggested causes of dermatitis include hypersensitivity, a variety of parasitic, bacterial and fungal agents and trauma. The condition is most common where there are dense populations of possums and amongst adult males, which may indicate an association with social stress. WIRES carers frequently report stress dermatitis in *T. vulpecula*, for example approximately 20% of 555 possums in care between 1997–2004 had stress dermatitis in various stages (L Millett, WIRES, pers. comm.).

## Management and research needs

Prevalence of diseases in *T. vulpecula* is a wide field to be investigated. There is clearly a need to understand the dynamics of infectious diseases in complex multi-host communities in order to mitigate disease threats to public health and wildlife (Cleaveland *et al.* 2001). The most important method to prevent movement of disease is by restricting translocation of wild animals (Wobeser 2002). This would be pertinent to relocation of infected urban *T. vulpecula*. However, as mentioned above, urban *T. vulpecula* are often illegally relocated by householders, which could be a source of spreading disease. Illegal relocation may pose a serious threat of transferring diseases which were picked up in

urban areas to populations in bushland areas. This issue warrants further attention by wildlife agencies. Information on intra- and inter-species disease transmission routes with regard to *T. vulpecula* in the urban environment is currently not available. The health status of possums may alter the rationale for their management in urban areas, if any diseases are identified that affect possum population size or public health. Routine monitoring and surveillance programs could be established concurrently with further research to improve our knowledge in this area, so that mitigation strategies which are sympathetic to possum conservation and human health can be developed if required.

## Conclusion

Management of *T. vulpecula* in urban areas can be a contentious issue. Wildlife agencies promote a policy of “living with possums”, which is supported by parts of the community. But not everyone welcomes wildlife into their backyard and conflicts between humans and possums continue, usually to the detriment of animal welfare. Illegal relocation of urban *T. vulpecula* reflects the lack of effective wildlife legislation and of information that reaches the public.

In our increasingly suburbanized world wildlife managers need to evaluate current management methods and continually develop new ones. Management strategies may vary depending on the given situation and often focus on individual animals in (sub)urban areas, as there is much demand for assistance with individual ‘nuisance’ possums. Nest boxes may provide alternative den sites for *T. vulpecula* when displaced from man-made structures, particularly with tree hollows being rare. However, studies indicate that their use by possums is limited which questions their potential to fulfil their supposed role. Fertility control may help to reduce possum numbers in high problem areas such as botanical and zoological gardens, and should ideally be combined with attempts to make the environment less attractive to possums. Using GnRH agonists could have the additional benefit of reducing the prevalence of diseases transmitted through social and sexual contact between *T. vulpecula*. The enhancement of existing management strategies and the development of alternative

ones will need to be accompanied by further in-depth studies on the biology of urban *T. vulpecula*, an area where knowledge is surprisingly lacking in Australia. Gaps have been pointed out throughout this paper. Some ideas for potential studies include:

- i) Efficacy of nest boxes (especially design and placement to increase usage by possums and discourage feral pests).
- ii) Feasibility of fertility control application addressing issues such as population dynamics, growth rates, abundance, dispersal and settlement, and acceptance by the community.
- iii) Survival (including possums released by wildlife care organizations) and causes of mortality (such as road kill, illegal relocation).
- iv) Dietary preferences (which native and introduced garden plants do or do not attract possums).
- v) Inter- and intra-species disease transmission (including potential risks to public health).
- vi) Development of a central database for wildlife agencies, pest controllers, councils, wildlife care organizations and trap-hiring companies (to monitor the extent of the problem).

However, to develop and utilise new strategies, wildlife managers need guidelines on how many possums can be sustained in specific areas. Without such a vision, it will be difficult to successfully implement active management strategies. Clarification is needed whether management is aiming to be active on the population level or if it is tailored to dampening conflicts with individual 'nuisance' problems.

A decisive factor will also be the design of our urban environment in the future, which is currently constructed with only the needs of the human species in mind (McKinney 2006). The increasing density of housing developments leave little to no space for native trees or possums, increasing the pressure on wildlife and resulting in conflict with people. Indeed, the major challenge of urban possum management will be the modification of people's behaviour, making social research one of the key components to achieve this goal. Reducing conflict between people and wild animals means influencing the attitudes

and beliefs of the public, and will require control of human actions and behaviour (where and how we build our homes, how we landscape our gardens, how we (do not) feed wildlife etc.). Public education needs to underpin a holistic and adaptive management framework. The 'possum problem' should be 'grabbed at its roots', meaning residents should be aware of the factors which promote possum activity within their living space. Then, householders can make informed choices about the best way to deal with any arising possum conflicts. Although some information is available, generally the public still lacks this essential knowledge. It seems rather a problem of 'getting the message through' and putting theory into practice. Efforts to promote more understanding for this native species will require ongoing support and need to address the factors promoting high population densities. Effective management methods need to be supplied to ameliorate conflict situations. Additionally, the public needs to be aware of the detrimental effects of illegal translocation if wildlife agencies hope to curb this practice.

Possums symbolize the face of Australian wildlife still vying to survive amongst the ever expanding human settlements. *T. vulpecula*'s image needs to be raised to one of the last wildlife 'icons' of suburbia. It is a privilege to experience native wildlife in the gardens of large cities. The more people we can engage to accept the presence of possums, the better the chances of peaceful coexistence with them. With a majority of people supporting possums, their long-term survival in one of their last 'native' strongholds could be secured. And the possums will be "out in the garden showing their gratitude by eating my roses and dancing on the roof in the moonlight" (Stackhouse 2005).

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## Chapter 2

# Effects of deslorelin implants on reproduction in the common brushtail possum (*Trichosurus vulpecula*)<sup>1</sup>

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### Summary sentence

The GnRH agonist deslorelin inhibited reproduction in female brushtail possums for at least one breeding season, but contrastingly not in males, although concentrations of gonadotropins declined in both sexes after prolonged exposure to the implant.

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**Key words:** gonadotropin releasing hormone, deslorelin, luteinizing hormone, follicle stimulating hormone, progesterone, testosterone, pituitary tests.

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## **Abstract**

The effect of treatment with slow release implants, containing the GnRH agonist deslorelin, on the reproductive function in common brushtail possums (*Trichosurus vulpecula*) was investigated. Female and male brushtail possums from a captive breeding unit were assigned to different treatment groups, including control (placebo implant), low dose (4.7 mg deslorelin) and high dose groups (9.4 mg deslorelin). Analysis of plasma hormone levels suggested that the mode of deslorelin action is the same in both females and males. Both sexes showed pronounced elevations in LH and males in testosterone during the acute phase of GnRH agonist treatment. This stimulatory response was transient, and continued exposure to the agonist suppressed secretion of LH, FSH and testosterone. Animals failed to respond to exogenous GnRH showing pituitary desensitization after long-term exposure to deslorelin. Inhibition of reproductive activity in females was associated with a decline in epithelial cells, leucocytes and sperm in their urine, and a reduction in progesterone levels.

Deslorelin successfully inhibited reproduction in female brushtail possums, but contrastingly males proved resistant to the contraceptive effects of chronic deslorelin treatment and continued siring offspring. Pulsatile release of LH and LH surge seemed not important for maintenance of spermatogenesis. Fertility was impaired in the female, possibly due to the inhibition of the LH surge system which suppresses ovulation. The suppressive effect of the agonist on females was reversible after cessation of agonist administration. Females remained infertile for at least one breeding season.

Hence, there is potential to control reproduction in female brushtail possums by using GnRH agonist treatment.

## Introduction

The common brushtail possum (*Trichosurus vulpecula*) is a puzzling species. It is a major introduced pest in New Zealand [1], yet it is in decline in many rural areas of its native Australia due to habitat loss, disease, drought, and predation [2]. The species' remaining strongholds in Australia are islands such as Tasmania, Kangaroo Island and Magnetic Island [3] and the suburbs of major cities such as Sydney [4]. Abundant populations in these areas require active management, but there are ethical constraints on management options especially in urbanized areas where lethal methods are deemed unacceptable. This situation is similar for other overabundant native marsupials and has resulted in research and development of new techniques to manage populations by reducing their fertility [5]. Indeed, fertility control has been applied for control of koalas (*Phascolarctos cinereus*) [6], eastern grey kangaroos (*Macropus giganteus*) [7] and tammar wallabies (*Macropus eugenii*) [8, 9] using synthetic progestins as contraceptives.

Gonadotropin releasing hormone (GnRH) has also been recognized as a potential target for the control and management of fertility [10]. The development of novel contraceptives focuses on GnRH agonists, vaccines, antagonists and GnRH-toxin conjugates, with the latter two being in an early phase of development. GnRH agonist treatment initially induces a large increase in luteinizing hormone (LH) and follicle stimulating hormone (FSH) concentrations [11]. Indeed, deslorelin implants have been used to induce ovulation in dairy cows and heifers [12]. However, chronic treatment blocks the receptor to occupancy by endogenous GnRH which causes a suppression of gonadotropin release. The GnRH superagonist deslorelin has a potency perhaps 100 times that of GnRH [13], but continued exposure impairs pituitary response to endogenous GnRH [10]. The resulting decrease in the synthesis of LH and FSH leads to a decline in the concentration of gonadal steroids, hence inhibiting follicular development

and spermatogenesis. Deslorelin is to date the only agent specifically developed for long-term fertility control of domestic animals and available as a veterinary product (in Australia and New Zealand) under the trade name Suprelorin® (Peptech Animal Health, Macquarie Park, NSW, Australia). GnRH agonists have been used successfully as contraceptives on several wildlife species including tammar wallabies [14], eastern grey kangaroos [15], wapiti (*Cervus elaphus nelsoni*) [16], mule deer (*Odocoileus hemionus*) [17] and some African wild carnivores [18], with no side-effects or behavioral changes reported.

The reproduction of the brushtail possum is well known and has been extensively described [19, 20]. Females are polyestrous and have successive cycles of about 26 days until conception occurs. The gestation period, from copulation to birth, is 17-18 days and hence about 8 days shorter than the estrous cycle. Brushtail possums are usually strictly monotocous (presenting one offspring at a time) and monovular with ovulation occurring spontaneously from either ovary. Estrus and ovulation are usually suppressed by lactation, but females can ovulate again about 8 days (up to 18 days) after loss or removal of the pouch young [21]. Brushtail possums are seasonal breeders and the onset of the breeding season may vary depending on the geographical location [22]. Most females in our captive breeding unit in New Zealand give birth in autumn, while a second minor season is notable in spring. Similar offspring records were recorded in a field study on the North Island [23], where 82% of brushtail possums were breeding in April and May, 45% in spring (Mid-October), only 13% in November and all were anestrus in January. Photoperiod seems to control onset and termination of the breeding season [24, 25].

The estrous cycle itself is under hormonal control and can be distinguished in the proliferative phase, which includes follicular growth, estrus and ovulation and the luteal phase, when the uterine epithelium develops under the influence of progesterone secreted by the corpus luteum (CL) [20]. There are preovulatory surges of both FSH and LH at a maximum level of 20 ng/ml and 30 ng/ml respectively [26] which may stimulate follicular development and ovulation. After ovulation, the ruptured Graafian follicle



transforms into a CL, and steroidogenesis is initiated [20]. Progesterone levels increase with the growth of the CL, which reaches its maximum size 7 – 10 days after estrus and begins to regress 16 days after estrus. Blood progesterone concentrations increase within two days of ovulation, ranging from 0.78 – 1.99 ng/ml [21] and reach maximum levels on days 12 – 14 after estrus, ranging from 4 – 15 ng/ml [21, 27, 28]. The CL is important for the regulation of the estrous cycle, the preparation of the epithelium in the uterus and for maintenance of pregnancy. The CL is thought to be largely autonomous, requiring very little, if any, support from the pituitary gland. However, the CL expresses receptors for both LH and prolactin, indicating that these hormones may be important in the regulation of steroidogenesis in the CL [27].

Plasma gonadotropin concentrations have been characterized in cyclic female brushtail possums. LH concentrations remain basal throughout the estrous cycle, apart from the preovulatory surge, which lasts for 7 – 9 h with maximum concentrations of 10.2 – 43.5 ng/ml [28]. The preovulatory FSH surge (occurring 1 h after the LH surge) lasts for 3 – 11 h with maximum concentrations of 1.4 – 21.4 ng/ml. It seems that, like in eutherian mammals, the final stages of follicular development are controlled by the gonadotropins and that GnRH is responsible for stimulating the surge release.

A seasonal cycle of testosterone concentrations and body weight has been described for male brushtail possums [29]. Testosterone concentrations increase in January, two months before the onset of the breeding season, peak in March and reach a nadir in September. Body weights fluctuate in a similar seasonal manner, but testis weights do not vary significantly with season [20]. Testosterone levels also vary diurnally and are higher in the morning than in the evening [30]. Individual variability in testosterone concentrations between males can be significant and is sometimes related to the presence of other males in captive situations [29].

Spermatogenesis is similar to eutherian mammals and the duration of one spermatogenic cycle is 13.5 days [31]. Several cycles are necessary before the process of spermatogenesis is completed and spermiation occurs [32]. The time to the liberation of spermatozoa is 56 days in brushtail possums [33].

Spermiogenesis in the brushtail possum is mostly similar to the process in eutherians [20]. In general, normal testicular function is controlled by the GnRH stimulated release of gonadotropins from the pituitary [32]. LH stimulates the production of testosterone in the Leydig cells, which in turn gives negative feedback on the pituitary and hypothalamus and is necessary for maintenance of spermatogenesis. Testosterone and FSH stimulate Sertoli cells into the production of factors that are required for maturation of spermatozoa [34].

This study investigated the effect of treatment with slow release implants, containing the GnRH agonist deslorelin, on reproductive function in female and male brushtail possums. Females received two different dosages of deslorelin to determine if there was a relationship between dose and duration of contraception as previously reported in other species [35, 36]. The acute response to deslorelin treatment was determined by measuring LH, FSH (males only) and testosterone (males) concentrations in plasma samples over the first 24 hours of treatment. To determine the degree of pituitary desensitization a GnRH challenge was conducted after nine weeks of deslorelin treatment. In females, the long-term effects on estrous cycles (from vaginal cell cytology and plasma progesterone concentrations), ovulation, mating and birth were measured. The reversibility of deslorelin treatment was demonstrated and the duration of the contraceptive effect determined. The long-term effects of treatment in males were assessed by analyzing testis size, fertility and plasma FSH and testosterone concentrations from samples collected at weekly intervals. In both sexes the effects on general health were determined using body weight as an indicator.

## **Materials and Methods**

### **Animal housing**

The brushtail possums used in this experiment were captured on the North Island, Wellington area (latitude 41°S) of New Zealand and housed within the breeding unit facilities of AgResearch, Upper Hutt. Newly captured animals were held in quarantine facilities for a few weeks before introduction to the

breeding unit. The unit practices a group-housing system in environmentally-enriched pens with runways [37]. In general, three females and one male share a pen to allow reproduction. Besides, the presence of males is positively correlated with the incidence of ovulation after pouch young removal [38]. A mixed diet of fresh fruit, cabbage and *Pinus radiata* branches are provided, with cereal based pellets and fresh water *ad libitum*. This housing system is successful in reducing the stress that accompanies housing wild, predominantly solitary animals in captivity.

### **GnRH agonist implant**

The GnRH agonist deslorelin (D-Trp<sup>6</sup>-Pro<sup>9</sup>-des-gly<sup>10</sup>-GnRH ethylamide) was formulated into implants that contained 4.7 mg deslorelin (Suprelorin®, Peptech Animal Health Pty. Ltd., Macquarie Park, NSW, Australia). Suprelorin has been approved for sale in male dogs in New Zealand and Australia [10]. This product releases deslorelin from a matrix consisting predominantly of low-melting point lipids and biological surfactant [35]. In a real-time dissolution system the implants release doses of > 1 µg per day for periods of > 1 year. The *in vivo* release in brushtail possum is unknown. Deslorelin was previously found to be effective at suppressing LH and FSH release in male brushtail possums [26]. The dimensions of a 4.7 mg implant were 2.3 mm in width and 12.5 mm in length. To place a 9.4 mg dose, two 4.7 mg implants were administered in a single pre-loaded device. Implants were placed subcutaneously between the shoulder blades using a single-use commercial implanting device sterilized by e-beam radiation. The injection site was sealed with a veterinary tissue adhesive (Vetbond; 3M Animal Care Products, St Paul, MN, USA).

### **Experimental design**

This experiment was conducted intensively over a period of 12 weeks between March and May 2004. Afterwards, only females with deslorelin implants removed or still in place were closely monitored to determine reversibility and the 'natural' time to expiry of the contraceptive. The majority of females and males had a record of previous breeding activity, were sexually mature and acclimatized to the breeding

unit. All animals were aged using tooth wear [39, 40] and weighed to determine two groups of low and heavy weight animals. Age, body weight and condition do influence reproductive success [41, 42], and hence, brushtail possums were randomly assigned to the different treatment groups using individuals from both weight pools. Females having pouch young (PY) or back young (BY) had their offspring removed prior to insertion of implants. Animals were weighed on each sampling occasion using hanging scales (Wedderburn SR235 6S; max. = 10 kg, d = 0.05 kg).

All experimental procedures were performed with approval of the AgResearch Animal Ethics Committee at Wallaceville Animal Research Centre under the 1987 Animal Protection (Codes of Ethical Conduct) Regulations of New Zealand.

**Female trial.** Treatment groups consisted of control (placebo implant)  $n = 10$ ; low dose (one deslorelin implant, 4.7 mg)  $n = 5$  and high dose (two deslorelin implants, 9.4 mg)  $n = 10$ . Each pen held treated and control females and one fertile male. Estrus, ovulation and mating were monitored through daily urine collections early in the morning (12 weeks). Females were caught once every week for pouch checks to examine for the presence of a neonate. Any PY found were removed, so that females continued to cycle. The age of any PY found was determined by consulting the urine data (see below) and/or calculating the age from growth tables based on the measurement of head length with vernier calipers [43]. Blood samples were collected weekly from anaesthetized females, and more intensely during the acute period (insertion of implant) and the GnRH challenge for analysis of LH and progesterone concentrations. Two baseline blood samples were taken at weekly intervals before implant administration (0 h). On 16<sup>th</sup> June 2004 the implant was removed from four females treated with a 9.4 mg dose of deslorelin to see if the suppression of the pituitary is reversible and if females subsequently return to estrus and have offspring. All other treated females were monitored until the presentation of a first PY, to determine the duration of the contraceptive effect of different dosages of deslorelin.

**Male trial.** Treatment groups consisted of control (placebo implant)  $n = 3$  and high dose (two deslorelin implants, 9.4 mg)  $n = 3$ . Each pen held one male and at least three females; therefore, it was beyond the capacity of the breeding unit to incorporate more males into this trial. Estrus, ovulation and mating of these females were monitored through daily urine collections early in the morning, allowing observations of sperm if females had mated overnight. Females were caught weekly for pouch checks to monitor the presence of neonates as an indicator of male fertility. Pouch young were aged as described above. Blood samples were collected weekly from anaesthetized males, and more intensely during the acute period (insertion of implant) and the GnRH challenge for analysis of LH, FSH and testosterone concentrations. Two baseline blood samples were taken at weekly intervals before implant administration (0 h). The length and the width (excluding the epididymis) of the left testes was measured using vernier calipers at the time of blood collection. Testes sizes declined in male dogs as a result of treatment with deslorelin and hence can be used as an indicator of gonadal activity [44]. Ejaculates of male sperm were not obtained, therefore any statement on sperm quality and/or quantity of deslorelin treated males can not be made.

### Urine sampling

Urine of females was sampled to monitor changes in the presence of epithelial cells, leucocytes and sperm as an indicator of the reproductive status of females [45, 46]. The presence of epithelial cells in the urine is a result of cyclical changes of the brushtail possum's vaginal cul-de-sac [47]. Urine was collected early in the morning from females which were trained to urinate using the method of Duckworth et al. [45]. Control females that had mated and supposedly were pregnant or that had a newborn present were no longer sampled, but urine collection was resumed after removal of pouch young (RPY). Drops of urine were placed in a hemocytometer and the cell types were identified and counted using a phase contrast microscope [46]. Pro-estrus was identified by a marked increase in the number of epithelial cells. The first day of estrus was defined as the day on which a large influx of cells appeared. In some cases, leucocytes and/or sperm cells can be observed and confirm mating. Animals

were determined to have undergone an estrous cycle if an influx of leucocytes occurred within three days of high numbers of cells.

However, epithelial cells can occur before the preovulatory LH surge, afterwards or not at all, and the numbers of cells that constitute an 'increase' vary widely between individual animals (range 40 – 4,000) [46]. Hence, urine can identify the time of estrus and ovulation only with limited accuracy and when these changes are assessed retrospectively, e.g. in association with births.

### **Blood sampling**

Blood was collected via jugular venepuncture of anaesthetized brushtail possums using 23-G needles and 3-ml syringes. Blood samples (3 ml) were immediately dispensed into 5-ml heparinized vacutainer tubes (Becton Dickinson, Vacutainer Systems, Plymouth, United Kingdom), centrifuged and plasma samples stored at -20°C until assayed.

Anesthesia was induced and maintained by inhalation of a gaseous mix of oxygen with Isoflurane (Merial New Zealand Ltd., Manukau City, New Zealand) for weekly and the acute blood sampling.

**Acute blood sampling.** This experiment was performed to monitor the immediate effects of deslorelin administration on the pituitary and the resulting changes in LH, FSH and testosterone concentrations. Blood samples were collected alternatively from left and right jugular veins at 0, 2, 6 and 24 hours relative to deslorelin implant administration.

**GnRH challenge.** This experiment was performed to determine if the pituitary of treated animals had become desensitized to exogenous GnRH as a result of long-term deslorelin treatment [48]. Brushtail possums were anaesthetized by intramuscular injection of Zoletil 100 (containing Tiletamine and Zolazepam; 12mg/kg; Virbac Laboratories (NZ) Ltd., Auckland, New Zealand) and held in hessian sacks for the duration of the challenge. Synthetic GnRH (Fertagyl; Intervet (AUS) Pty Ltd, Bendigo East, Victoria, Australia) was dosed according to body weight (2ug/kg in sterile saline) and administered into

the jugular vein at 0 minutes. Blood samples were collected at -15, 0, 15, 30, 60, 120 min relative to GnRH administration.

### **Hormone assays**

All samples from individual animals were run in the same assay to reduce variability. Samples exceeding the limits of the standard curve were diluted down to an optimum end-point within the standard curve limits. Cross-reactivity between FSH and LH has not been observed [49, 50].

**LH assay.** Plasma LH concentrations were measured using the method of Moore et al. [50]. The antiserum was raised in rabbits against ovine LH (Wa-R oLH) [51]. The reference standards were made up with purified brushtail possum LH in hypophysectomized sheep serum, and  $^{125}\text{I}$  was conjugated to purified brushtail possum LH (provided by Lloyd Moore, AgResearch, Wallaceville). The second antibody (sheep anti-rabbit gamma globulin, in-house product) and polyethylene glycol (PEG 8000) (Scharlau Chemie SA, Barcelona, Spain) were added, followed by centrifugation.

The sensitivity of the assay was 0.15 ng/ml. The intra-assay coefficients of variation, calculated for three quality control pools (low, medium and high) per assay, were 12.0%, 3.9% and 11.4% respectively. The inter-assay coefficients of variation for the same pools were 5.1%, 5.4% and 7.6% respectively.

**FSH assay.** Plasma FSH concentrations were measured using the method of Moore et al. [49]. The antiserum was raised against human FSH in a rabbit [52] and kindly supplied by Dr Alan McNeilly (University of Edinburgh). The reference standards were made up with purified brushtail possum FSH in hypophysectomized sheep serum.  $^{125}\text{I}$ -possum FSH and standards were provided by Lloyd Moore. The second antibody (sheep anti-rabbit IgG) and PEG 8000 were added, followed by centrifugation.

The sensitivity of the assay was 0.3 ng/ml. The intra-assay coefficients of variation, calculated for two quality control pools (medium and high) per assay, were 18.0% and 9.3% respectively. The inter-assay coefficients of variation for the same pools were 9.7% and 8.0% respectively.

**Progesterone assay.** Plasma progesterone concentrations were determined as previously described [21]. Progesterone was measured directly in culture medium by RIA. Standards were prepared by adding progesterone ( $\Delta^4$ -Pregnen-3,20-dione, P-0130; Sigma Chemical Company, New Zealand) to charcoal-stripped male brushtail possum plasma. The rabbit-raised antiserum for progesterone (7720-1604; Biogenesis, Poole, UK) was diluted 1:6,000 for use.  $^{125}\text{I}$ -labelled progesterone was purchased from Diagnostic Products Corporation, Los Angeles, CA, USA. The second antibody (sheep anti-rabbit IgG) and PEG 8000 were added, followed by centrifugation.

The sensitivity of the assay was 0.1 ng/ml. The intra-assay coefficients of variation, calculated for three quality control pools (low, medium and high) per assay, were 16.3%, 7.2% and 12.4% respectively. The inter-assay coefficients of variation for the same pools were 17.3%, 9.8% and 13.0% respectively.

**Testosterone assay.** Plasma testosterone concentrations were measured using the IMMULITE 2000 Total Testosterone (L2KWT2 Diagnostic Products Corporation, Los Angeles, CA, USA). The procedure is a solid-phase, competitive chemiluminescent enzyme immunoassay requiring 20  $\mu\text{l}$  sample, with an incubation cycle of 60 minutes. It takes quantitative measurements of total testosterone in serum and heparinized plasma. The solid phase is made up of a polystyrene bead enclosed within the Immulite test unit that is coated with a polyclonal rabbit anti-testosterone antibody. The Multi-Diluent 1 (L2M1Z), a human serum containing low to undetectable levels of testosterone, was used for a linearity study, and automatized dilutions of male brushtail possum plasma (1:1, 1:5 and 1:10) were linear. The antibody is highly specific, and from a panel of 21 hormones, cross-reactivity was 2% for 5 $\alpha$ -Dihydro-testosterone, and less than 0.7% for 5 $\alpha$ -Androstan-3 $\beta$ ,17 $\beta$ -diol, Androstenedione, Methyltestosterone and Progesterone. All samples were run in the same assay to reduce variability.

The analytical sensitivity of the assay was 0.2 ng/ml. The intra-assay coefficients of variation, calculated for three quality control pools (low, medium and high) per assay, were 6.3%, 2.6% and 11.0% respectively. The inter-assay coefficients of variation for the same pools were 10.8%, 10.8% and 11.5% respectively.



## Statistical analysis

Data for animal weight, LH, FSH, testosterone and progesterone concentrations were analyzed by analysis of variance (ANOVA) procedures using SPSS (SPSS Inc., Chicago, IL). We used the general linear model (GLM) repeated measures procedure, with the model being  $y = \text{treatment, time, treatment} \times \text{time}$ , with time as repeated subject. When applicable, post hoc tests were conducted using Tukey's honestly significant difference (HSD) method for multiple comparisons of group means, which is part of the GLM Post Hoc Multiple Comparisons Function of SPSS.

For GnRH challenge results, the pattern of response and the magnitude of the peak relative to the starting concentrations determined whether an individual had a positive response to the GnRH challenge [53]. Based on the magnitude of peaks in control brushtail possums, a response was considered significant when the peak value was greater than two times the mean of the two pre-GnRH samples (-15 and 0 min), and if the response occurred at the corresponding point in time (between 15, 30 and 60 min for LH, and 60 and 120 min for testosterone).

Comparisons between treated and control animals at single time points were made using the independent-samples (two-sample) t-test procedure of SPSS. Comparisons between animals of three different treatment groups (female trial) at a single point in time were made using the One-Way ANOVA procedure of SPSS. The Kruskal-Wallis and Mann-Whitney Test were used to compare offspring rates between control and treated females.

Where necessary, data were transformed to  $\log_{10}$  before analysis to overcome heterogeneity of variance. The statistical calculations for the male trial have to be interpreted with caution, due to the low number of animals in both treatment and control group. Results are presented as untransformed arithmetic means  $\pm$  standard error of the mean (S.E.M.) and reported as significant for  $P < 0.05$ .

## Results

### FEMALES

#### Live weight

The weights of animals in each group were similar at the onset of treatment (control,  $2.93 \pm 0.12$  kg; treated (low),  $2.85 \pm 0.09$  kg; treated (high),  $3.00 \pm 0.12$  kg; ANOVA,  $P > 0.7$ ). The weight of treated females seemed to decline slightly post-deslorelin treatment (Fig. 1), but there was no significant change in weight over time (GLM,  $P > 0.06$ ) nor significant treatment x time interaction (GLM,  $P > 0.2$ ) suggesting that any changes over time were unlikely to be the result of treatment. There was no significant difference between the three groups during the treatment period (GLM,  $P > 0.6$ ), although control females remained slightly heavier on average than treated animals throughout the course of deslorelin treatment (Fig. 1).

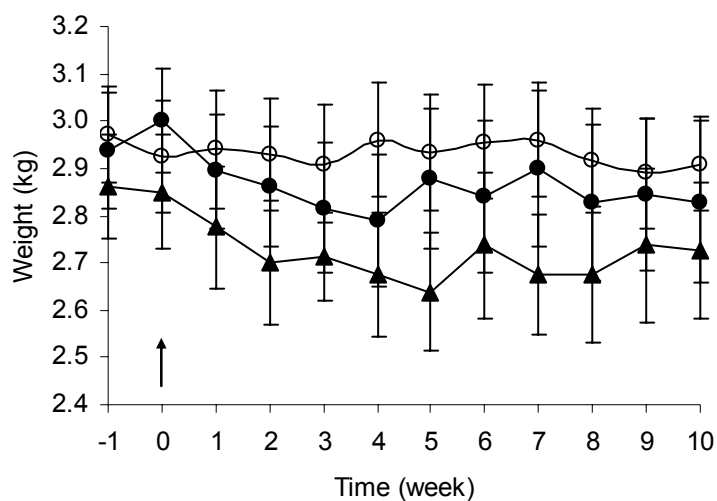


Figure 1. Weight (kg; mean  $\pm$  SEM) of control ( $\circ$ ,  $n = 10$ ) and treated females (low dose,  $\blacktriangle$ ,  $n = 4$ ; high dose,  $\bullet$ ,  $n = 9$ ) after administration of deslorelin implants ( $\uparrow$ ).

## **Mortality**

Two females were replaced at the onset of the treatment period (one placebo female which had lost condition, and one 9.4 mg female which died on day 3 post-deslorelin treatment from unknown causes), and two females died within two weeks of treatment (one 4.7 mg female died on 28-Mar-04 and the autopsy suggested an unusual kidney and hemorrhagic stomach; for the other female, with a 9.4 mg dose, the autopsy suggested chronic kidney failure and gastritis). Results from these animals have not been included in the statistical procedures due to the requirement for an equal number of observations for all animals, except for the acute response to deslorelin administration. Beyond the intense 3-month trial, some further animals were lost in all groups, including two placebo females, one 4.7 mg female and three 9.4 mg females, which reduced the data available on long-term effects of deslorelin treatment. Reasons for euthanasia or death included losing condition, chronic kidney failure, old age, and/or injuries inflicted by other brushtail possums. The degree of mortality experienced during this study was unusually high. However, of the animals that were lost over the course of the study, six were considered to be of old age and at necropsy most showed signs of kidney failure which is the most common cause of death for old brushtail possums in our colony. None of the deaths appeared to be related to deslorelin treatment.

## **Births**

Control and treated females had all pouch or back young removed prior to the time of implant administration. The majority of females had a record of previous offspring. None of the deslorelin treated females gave birth within the intense 3-month trial, although their urine and progesterone profiles suggested some cycling activity within the first 2 – 4 weeks after insertion of the deslorelin implant (Table 1). Some of these females had even mated shortly before or after GnRH agonist treatment, but again no subsequent births were observed.

Table 1. Reproductive parameters summarized for control and treated females between 1-Mar-04 and 25-May-04 (intense 3-month trial only).

ID	Mating before treatment <sup>a</sup> (Date)	Reproductive status at time of treatment <sup>b</sup>	1 <sup>st</sup> Estrus after implant insertion <sup>a</sup> (Date)	1st PY (Date)	Time to first detected PY (Days)	No. PY (1-Mar-04 – 25-May-04)	No. of P4 peaks <sup>c</sup>
<b>Control<sup>d</sup></b>							
C1	–	RBV, 11-Mar-04	23-Mar-04*	03-May-04	48	1	3
C2	–	RBV, 11-Mar-04	20-Mar-04*	07-Apr-04	22	2	2
C3	12-Mar-04	RBV, 11-Mar-04	07-Apr-04*	29-Mar-04	13	2	2
C4	–	NPY	02-Apr-04	19-Apr-04	35	2	2
C5	–	NPY	20-Apr-04	–	–	0	0
C6	–	NPY	26-Mar; 23- Apr-04*	10-May-04	54	1	2
C7	–	RPY, 15-Mar-04	26-Mar-04*	12-Apr-04	28	2	2
C8	–	NPY	02-Apr-04*	18-Apr-04	34	2	2
C9	–	NPY	21-Mar-04*	08-Apr-04	24	1	3
C10	–	NPY	20-Mar-04*	08-Apr-04	23	2	2
<b>Treated</b>							
<b>10mg</b>							
T1	–	NPY	24-Mar-04	–	–	0	0
T2	–	RPY, 15-Mar-04	17-Mar-04*	–	–	0	0
T3	–	RPY, 16-Mar-04	23-Mar-04	–	–	0	1
T4	–	NPY	19-Mar-04	–	–	0	0
T5	–	RPY, 11-Mar-04	26-Mar-04*	–	–	0	1
T6	13-Mar-04*	NPY	28-Mar-04	–	–	0	1
T7	–	RPY, 12-Mar-04	20-Mar-04	–	–	0	1
T8	2-Mar-04*	NPY	24-Mar-04	–	–	0	2
T9	–	RBV, 11-Mar-04	21-Mar-04	–	–	0	0
<b>5mg</b>							
T11	–	NPY	22-Mar-04*	–	–	0	0
T12	–	NPY	26-Mar; 09- Apr-04*	–	–	0	2
T13	–	NPY	26-Mar-04	–	–	0	0
T14	4-Mar-04*	RBV, 11-Mar-04	–	–	–	0	1

<sup>a</sup> Date represents the most likely day of mating, based on peak in cell counts, leucocytes and/or sperm observed in urine sample in association with subsequent birth (\* indicates that sperm were seen).

All implants were inserted between 15-Mar-04 and 17-Mar-04.

<sup>b</sup> RPY = Removal of pouch young; RBV = Removal of back young; NPY = No pouch young.

<sup>c</sup> The P4-peak was defined by progesterone values  $\geq 4\text{ng/ml}$  and/or a periodic pattern of 3-4 weeks.

<sup>d</sup> All pouch young were continuously removed.

The resumption of breeding was either observed after removal of the contraceptive implant or natural expiry of the contraceptive effect. In comparison, all but one placebo females had several offspring throughout the entire 3-month period of the trial (Table 1). This exceptional placebo female without any offspring showed signs of disease, was diagnosed with kidney failure and euthanized shortly after. The difference in offspring numbers between groups was found to be significant (Kruskal-Wallis,  $p < 0.001$ ), while there was no difference between the 4.7 mg and the 9.4 mg treatment group (Mann-Whitney;  $p = 1.0$ ). As a mix of control and treated females shared a pen with one male, it also demonstrated the fertility of all associated males. Hence, any observed infertility in deslorelin-treated females was due to the GnRH agonist treatment.

Four 9.4 mg females had their implants removed 93 days post treatment (Table 2). There was considerable variation in the time to resumption of breeding, with indication of previous unsuccessful cycles until parturition. Three of the females were observed to have mated within less than 30 days (12, 19 and 27 days) after removal, but only two gave birth immediately (17 d) afterwards. The remaining two females had no PY until the following year, their first newborns were present 300 and 389 days after implant removal.

Contraception was also reversible within females where the implants had been left in place (Table 2). Of the three remaining females with the 4.7 mg dose, two had offspring 259 and 432 days after implant administration, but one had not given birth by the time of the last check in March 2006 ( $\geq 734$  days). Data on the duration of the contraceptive effect of the 9.4 mg dose in females was limited as four females had the implants removed and four females were lost before resumption of breeding (two had died within four months after implant insertion, and another two were euthanized 580 and 599 days after deslorelin administration with all four animals having no PY present at their time of death). From the remaining two females with a 9.4 mg dose only one had given birth by the time of the last check. This particular female was seen to have mated in July 2005, but the first newborn was not present until 24-Sep-2005, 554 days after implant administration. The other 9.4 mg female had no PY by the time of the

last check either ( $\geq 734$  days). The two remaining deslorelin treated females, which had not resumed breeding by the time of the last check, had a record of previous breeding indicating their potential fertility. In summary, of the three females which had resumed breeding, the two 4.7 mg females had offspring earlier than the 9.4 mg female. However, due to the low numbers of females, it was not possible to determine dose-dependent differences in the resumption of breeding.

Table 2. Reproductive parameters for deslorelin treated females showing the resumption of breeding after implant removal or after 'expiry' of the implant's contraceptive effect (long term birth data until 21-Mar-06).

ID	Implants removed (Date)	1st Mating, after intense 12 week trial <sup>a</sup> (Date)	1st PY (Date)	Time to first PY, after implant removal (days)	Time to first PY, after expiry of implant (days)
10mg					
T1	16-Jun-04	—	10-Jul-05	389	n.a.
T2	16-Jun-04	28-Jun-04	15-Jul-04	29	n.a.
T3	16-Jun-04	13-Jul-04	12-Apr-05	300	n.a.
T4	16-Jun-04	05-Jul-04	22-Jul-04	36	n.a.
T5	—	—	—	n.a.	$\geq 734$ <sup>b</sup>
T6	—	28-Jul-05	24-Sep-05	n.a.	554
T7	—	—	—	n.a.	$\geq 81$ <sup>c</sup>
T8	—	—	—	n.a.	$\geq 580$ <sup>c</sup>
T9	—	—	—	n.a.	$\geq 599$ <sup>c</sup>
5mg					
T11	—	—	01-Dec-04	n.a.	259
T12	—	—	23-May-05	n.a.	432
T13	—	—	—	n.a.	$\geq 734$ <sup>b</sup>
T14	—	—	—	n.a.	$\geq 147$ <sup>c</sup>

<sup>a</sup> Date represents the most likely day of mating, based on peak in cell counts, leucocytes and/or sperm observed in urine sample in association with subsequent birth. <sup>b</sup> No PY, last check on 21-Mar-06. <sup>c</sup> Animal died/was euthanized at this point in time with no PY present. n.a.= not applicable

## Cytology

Females in this study appeared to continue cycling for the first few weeks after deslorelin administration, based on the analysis of urine data. Counts of elevated numbers of cells and leucocytes in urine samples indicated cycling activity (Fig. 2). Treated females had elevated cell counts for prolonged periods of time, and cells and leucocytes were increased on several consecutive days. Sperm was observed in the urine sample of three 4.7 mg and three 9.4 mg females generally within the first 2 – 10 days after implant insertion, except for one of the 4.7 mg females that mated exceptionally late, 23 days after deslorelin administration. The observed sperm gave evidence of mating activity, however, none of these treated females subsequently gave birth (see 'Births'). Any indication of estrous cycles, such as cell increase, leucocytes or sperm, notably declined from three weeks after implant insertion in both treatment groups, vanished approximately 4 – 6 weeks afterwards and remained nearly undetectable throughout the rest of the intense 3-month trial (Fig. 2).

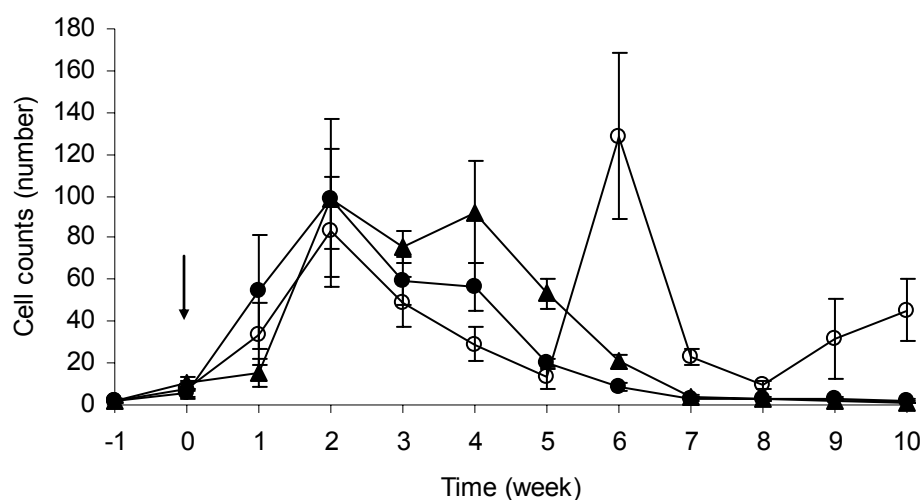


Figure 2. Mean weekly cell counts (number; mean  $\pm$  SEM) of control (○,  $n = 10$ ) and treated females (low dose, ▲,  $n = 4$ ; high dose, ●,  $n = 9$ ) after administration of deslorelin implants (↓).

However, it was not possible to observe the expected regular 26-day length of estrous cycles by means of cell and leucocyte counts alone. The observation of regular estrous cycles was only possible in control females where cell increase, leucocyte and sperm counts could be associated with subsequent

births. Control females were cycling continuously as all pouch young were consistently removed during the first 12 weeks to ensure further estrous cycles (Table 1). Interpretation of cell counts was difficult due to individual variability in cell counts. If control females were pregnant or had a pouch young, no urine was sampled, and hence, no cell count data was obtained. Besides, estrous cycles were not synchronized, therefore, individual cell rises occurred at different points in time within groups.

## Plasma LH and Progesterone

**Acute response to deslorelin (LH).** Plasma concentrations of LH increased manifold within 2 – 6 h after deslorelin administration and declined from peak concentrations by 24 h (Table 3; Fig. 3). Plasma LH concentrations were not detectable in female groups at the time of insertion (ANOVA,  $P>0.1$ ; Table 3), but were significantly elevated within 2 h for treated females (control,  $0.07 \pm 0.02$  ng/ml; treated (low),  $3.81 \pm 0.68$  ng/ml; treated (high),  $3.81 \pm 0.48$  ng/ml; ANOVA,  $P<0.001$ ). Concentrations were also significantly elevated at 6 h after implant insertion compared to control females (control,  $0.07 \pm 0.03$  ng/ml; treated (low),  $4.22 \pm 0.89$  ng/ml; treated (high),  $3.94 \pm 0.45$  ng/ml; ANOVA,  $P<0.001$ ) and peaked manifold within treated females. LH concentrations of control females remained low throughout the acute sampling period. Tukey's HSD post-hoc test (multiple comparisons) revealed that there was significant difference between control and the two treatment groups (low and high dose of deslorelin) ( $P<0.001$ ) at 2 and 6 h, but no difference between the two different treatment groups ( $P>0.9$ ). By the time of the next sample, 24 h after deslorelin administration, LH concentrations had considerably declined from peak concentrations (Fig. 3) and were no longer significantly higher than in control animals (control,  $0.25 \pm 0.18$  ng/ml; treated (low),  $0.66 \pm 0.21$  ng/ml; treated (high),  $0.70 \pm 0.17$  ng/ml; ANOVA,  $P>0.1$ ). LH concentrations of control females remained generally below or close to the detection limit of the assay throughout the 24 h acute sampling period.



Table 3. Response of females within each treatment group to a) the administration of deslorelin and b) the GnRH challenge showing the starting and peak concentrations for LH (ng/ml; mean  $\pm$  SEM) and if animals had a positive response to the treatment.

Acute sampling					GnRH challenge			
Group	n	<i>Positive response</i>	Starting concentration	Peak concentration	n	<i>Positive response</i>	Starting concentration	Peak concentration
LH females								
Control	10	0	0.07 ± 0.02	0.25 ± 0.18	10	10	0.04 ± 0.01	5.25 ± 1.40
5mg	5	5	0.06 ± 0.03	4.22 ± 0.89	4	0	0.08 ± 0.03	0.10 ± 0.03
10mg	11	11	0.03 ± 0.01	3.94 ± 0.45	9	0	0.06 ± 0.01	0.07 ± 0.02

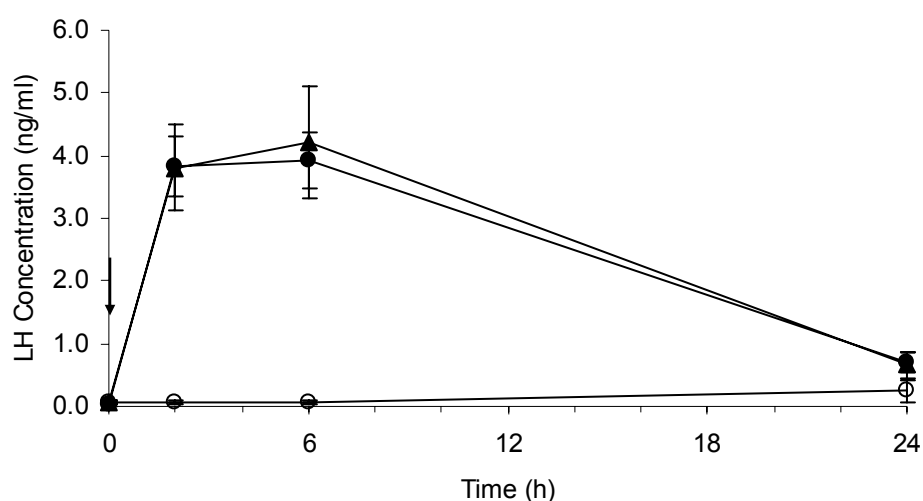


Figure 3. Plasma concentrations of LH (ng/ml; mean  $\pm$  SEM) in control (○, n = 10) and treated females (low dose, ▲, n = 5; high dose, ●, n = 11) within the first 24 h after administration of deslorelin implants (↓).

**GnRH challenge (LH).** Starting concentrations of LH, calculated as a mean of two pre-treatment samples, were below or close to the detection limit of the assay and were not significantly different between the three groups (ANOVA,  $P > 0.1$ ; Fig. 4; Table 3). Control animals responded to the synthetic GnRH with an increase in LH, and concentrations were significantly elevated within 15 min after synthetic GnRH administration (control, 5.25  $\pm$  1.40 ng/ml; treated (low), 0.07  $\pm$  0.02 ng/ml; treated

(high),  $0.06 \pm 0.01$  ng/ml; ANOVA,  $P < 0.001$ ) and 30 min afterwards (control,  $5.18 \pm 0.81$  ng/ml; treated (low),  $0.08 \pm 0.02$  ng/ml; treated (high),  $0.05 \pm 0.01$  ng/ml; ANOVA,  $P < 0.001$ ). By the time of the next sample, at 60 min, LH concentrations of the control females were declining (control,  $2.27 \pm 0.48$  ng/ml; treated (low),  $0.08 \pm 0.01$  ng/ml; treated (high),  $0.04 \pm 0.01$  ng/ml; ANOVA,  $P < 0.001$ ). By 120 min, LH concentrations of most control females had returned to pre-treatment levels (Fig. 4), but were still significantly higher than LH concentrations within the treated groups (control,  $0.37 \pm 0.16$  ng/ml; treated (low),  $0.10 \pm 0.03$  ng/ml; treated (high),  $0.07 \pm 0.02$  ng/ml; ANOVA,  $P < 0.03$ ). Tukey's HSD post-hoc test (multiple comparisons) revealed that there was significant difference between control and the two treatment groups (low and high dose of deslorelin) at 15, 30 and 60 min ( $P < 0.001$ ), and between control and the high treatment group at 120 min ( $P < 0.03$ ), but there was no significant difference between the plasma LH concentrations of the two treatment groups at all times ( $P > 0.1$ ). LH concentrations of treated females remained below or close to the detection limit of the assay at all points in time (Table 3).

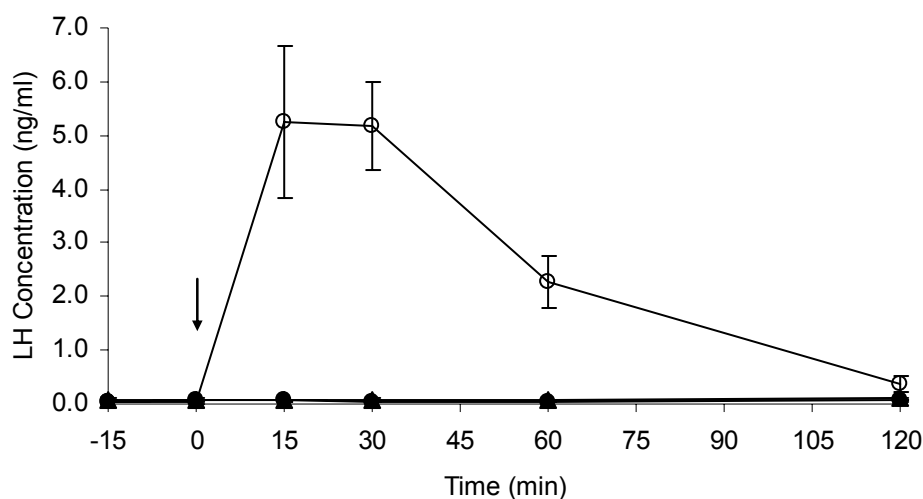


Figure 4. Plasma concentrations of LH (ng/ml; mean  $\pm$  SEM) in control (○,  $n = 10$ ) and treated females (low dose, ▲,  $n = 4$ ; high dose, ●,  $n = 9$ ) following administration of synthetic GnRH (↓).

**Long-term response to deslorelin (progesterone).** Plasma progesterone in females is highly variable as it depends on the presence and size of the corpus luteum at the time of sampling, and estrous cycles of individual females were not synchronized. The detection of elevated progesterone concentrations gave overall evidence of cycling activity. Pre-treatment samples showed that elevated progesterone concentrations were detectable in treated and control females, despite variation between individuals from 0.18 – 15.87 ng/ml (Fig. 5). There was evidence of cycling activity for all treatment groups at the onset of treatment (control,  $0.60 \pm 0.18$  ng/ml; treated (low),  $6.91 \pm 3.89$  ng/ml; treated (high),  $2.72 \pm 1.69$  ng/ml; ANOVA,  $P > 0.2$ ).

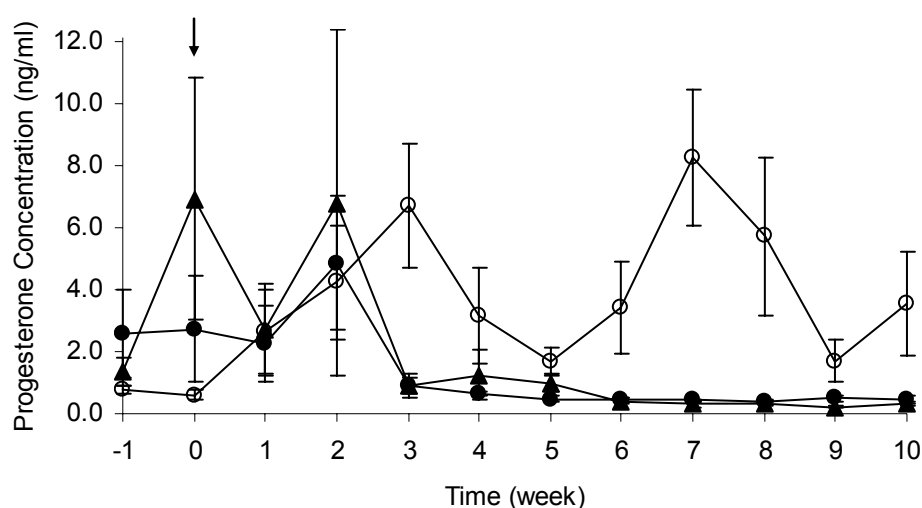


Figure 5. Plasma concentrations of progesterone (number; mean  $\pm$  SEM) in control (○,  $n = 10$ ) and treated females (low dose, ▲,  $n = 4$ ; high dose, ●,  $n = 9$ ) after administration of deslorelin implants (↓).

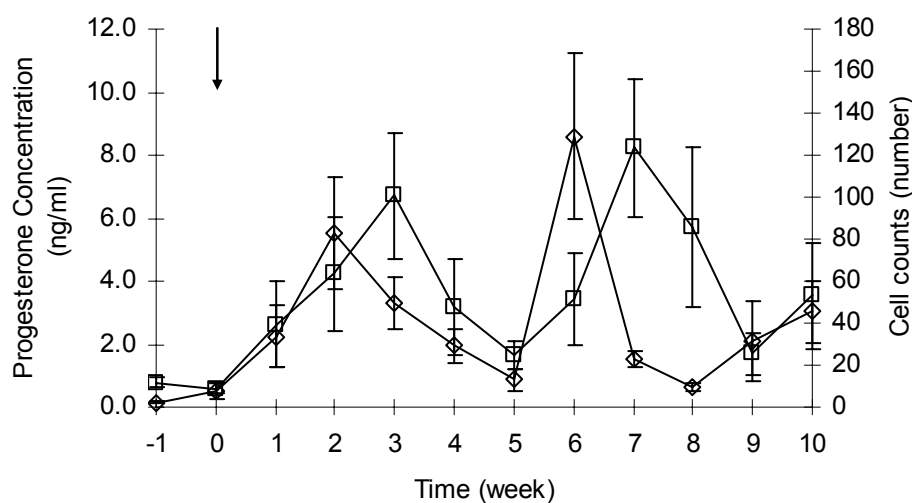
Plasma progesterone concentrations in deslorelin treated females remained markedly elevated for the first two weeks after treatment (control,  $4.23 \pm 1.82$  ng/ml; treated (low),  $6.79 \pm 5.54$  ng/ml; treated (high),  $4.85 \pm 2.18$  ng/ml; ANOVA,  $P > 0.9$ ), but decreased below values of 1 – 2 ng/ml within four weeks (control,  $3.15 \pm 1.52$  ng/ml; treated (low),  $1.25 \pm 0.78$  ng/ml; treated (high),  $0.66 \pm 0.08$  ng/ml; ANOVA,  $P > 0.1$ ) and remained low afterwards. In control animals, plasma progesterone concentrations reflected typical cyclic changes and remained elevated.

Changes in progesterone levels over time were significant (GLM,  $P < 0.01$ ), and there was significant treatment x time interaction (GLM,  $P < 0.01$ ). There was also a significant difference between groups during the treatment period (GLM,  $P < 0.01$ ), suggesting that differences were the result of the GnRH agonist treatment. Tukey's HSD post-hoc test revealed that there were significant differences between the control group and both treatment (low and high) groups ( $P < 0.05$ ), but there was no difference between the two treatment groups ( $P > 0.9$ ).

### Correlation between Cytology and Progesterone Females

There was a correlation between elevated cell counts and progesterone concentrations, the latter markedly rose 1 – 2 weeks after observed ovulation based on cell, leucocyte and sperm counts in the urine sample. Figure 6 b, c show the correlation between cell counts and progesterone concentrations for treated females which were in decline three weeks after deslorelin administration and remained low throughout the rest of the 3-month trial. Contrastingly, control females showed cyclical changes in progesterone concentrations and cell counts, but no pattern of overall declining values (Fig. 6 a).

**a**



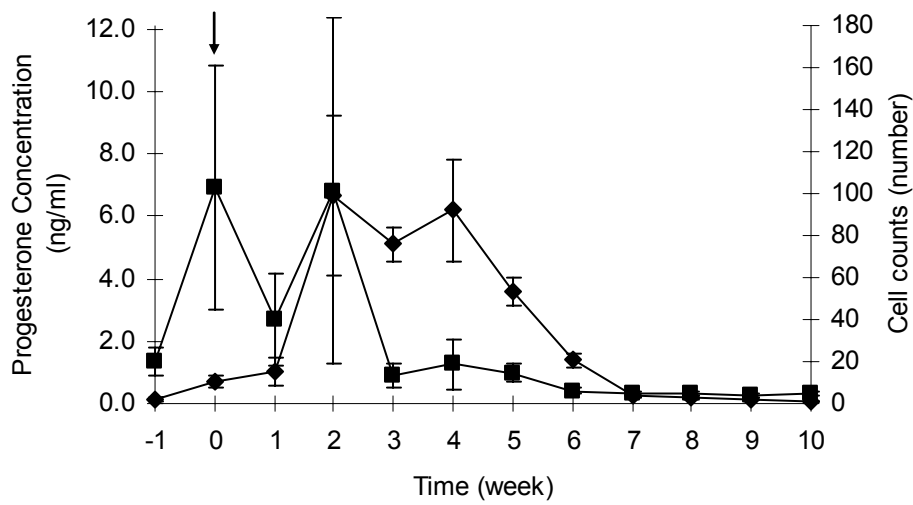
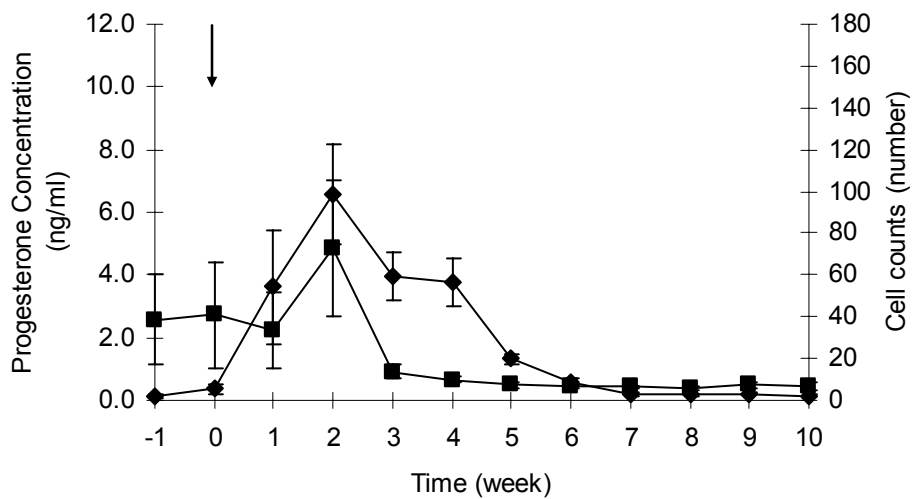
**b****c**

Figure 6. Plasma concentrations of progesterone (■; ng/ml; mean  $\pm$  SEM) in correlation to mean cell counts (◆; number; mean  $\pm$  SEM) in (a) control (□, ◇; n = 10), (b) treated (■, ◆; low dose, n = 4) and (c) treated females (■, ◆; high dose, n = 9) after administration of deslorelin implants (↓).

## MALES

### Live Weight

The weights of animals in each group were similar at the onset of treatment, but varied between individuals from 3.0 – 3.8 kg (control,  $3.32 \pm 0.19$  kg; treated,  $3.35 \pm 0.18$  kg; independent t-test,  $P>0.9$ ). Weight declined for both treated and control males during the treatment period (Fig. 7), but there was no significant change in weight over time (GLM,  $P>0.1$ ), nor was there significant treatment x time interaction (GLM,  $P>0.7$ ) or treatment effect between the two groups (GLM,  $P>0.5$ ), suggesting that these changes were not the result of treatment.

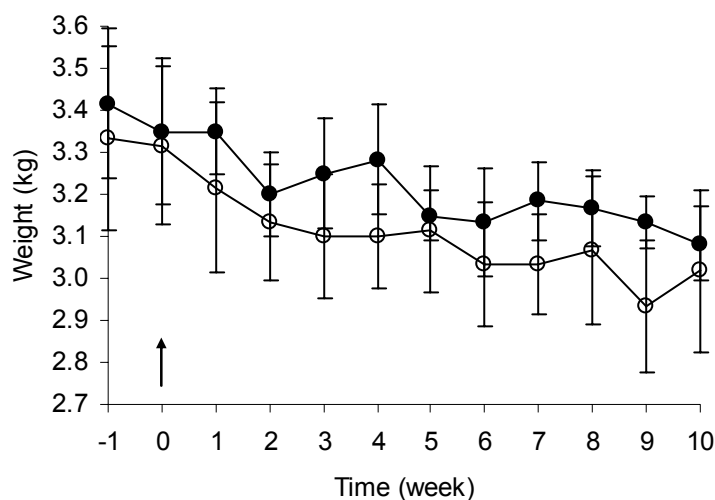


Figure 7. Weight (kg; mean  $\pm$  SEM) of control (○,  $n = 3$ ) and treated males (high dose, ●,  $n = 3$ ) after administration of deslorelin implants ( $\uparrow$ ).

### Mortalities

No mortalities occurred throughout the intense 3-month course of the experiment. Both control and treated males appeared to maintain good health, based on the weights of the animals (see 'Live Weight') and on observations of any potential fighting wounds. One treated male had a back wound inflicted by the females in his pen about two weeks after treatment, but no further fight wounds were observed.

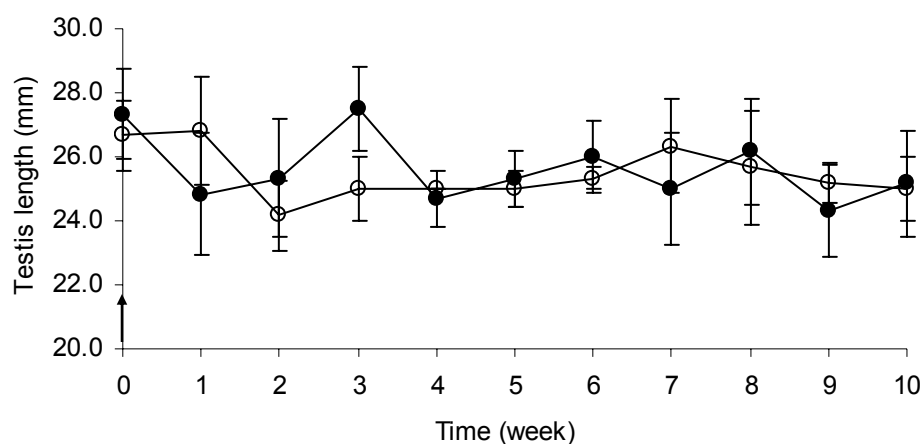
## Births in Associated Females

All control males and treated males were held in pens with 3 – 5 females. The three control males sired a total number of 14 pouch young in the 2004 breeding season. The three treated males sired a total number of 20 pouch young, with individual numbers being 4, 5 and 11 pouch young per treated male. Differences in offspring number are the result of individual male activity, frequency of pouch young removal and estrous cycles and/or number of females in the different pens. The treated males sired offspring throughout the main breeding season, including June and July 2004 (3-4 months post treatment), and again in the following year suggesting that spermatogenesis was not impaired.

## Testis Size

The testis sizes of males in each group were similar at the onset of treatment (width week 0: control,  $18.00 \pm 0.00$  mm; treated,  $19.50 \pm 1.26$  mm; independent t-test,  $P > 0.3$ ; length week 0: control,  $26.67 \pm 1.09$  mm; treated,  $27.33 \pm 1.42$  mm,  $P > 0.7$ ). There was no significant change in testis width or length over time (GLM,  $P > 0.1$ ) nor significant treatment x time interaction (GLM,  $P > 0.2$ ). There was no significant difference between the two groups during the treatment period either (GLM,  $P > 0.9$ ), suggesting that treatment did not cause any changes in testis size over time (Fig. 8 a, b).

a



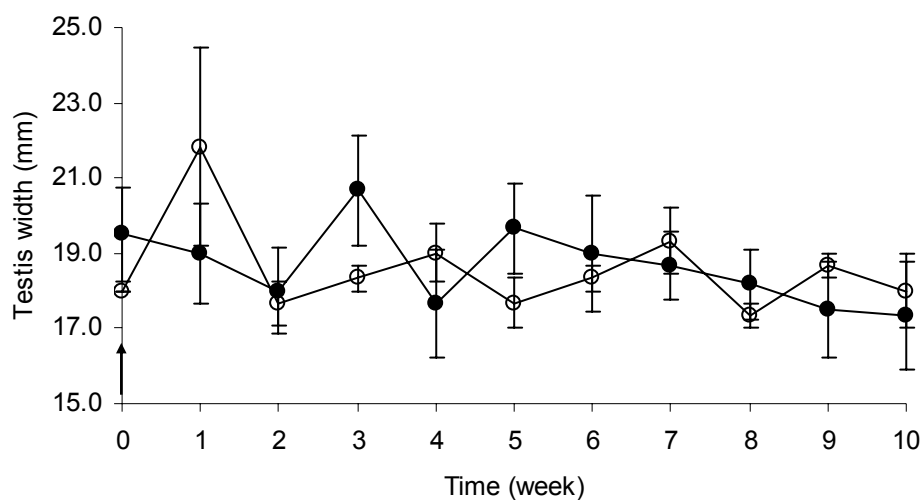
**b**

Figure 8. **a)** Testis length and **(b)** testis width (mm; mean  $\pm$  SEM) of control ( $\circ$ ,  $n = 3$ ) and treated males (high dose,  $\bullet$ ,  $n = 3$ ) after administration of deslorelin implants ( $\uparrow$ ).

## Sperm Observations

Observations of sperm, recorded from urine samples of associated females, were made in both control and treated males. From control males, sperm in associated female urine was recorded 3 – 5 times per male during the intense 3-month trial. From treated males, sperm in associated female urine was similarly noticed 3 – 4 times, with the latest observation made on 23-May-04, 67 days after insertion of the deslorelin implant. Males continued siring offspring (see 'Births'), but no further urine records were taken. Differences in the appearance of sperm between the different treatment groups were not observed, but direct samples were not taken. Therefore, observed sperm in female urine gives an indication of functional spermatogenesis, but no information on the quality or quantity of the sperm.



## Plasma LH, FSH and Testosterone Males

**Acute response to deslorelin (LH and Testosterone).** Plasma concentrations of LH and testosterone increased manifold within 2 – 6 h after deslorelin administration and declined from peak concentrations by 24 h (Table 4).

Table 4. Response of males within each treatment group to a) the administration of deslorelin and b) the GnRH challenge showing the starting and peak concentrations for LH and testosterone (ng/ml; mean  $\pm$  SEM) and if animals had a positive response to the treatment.

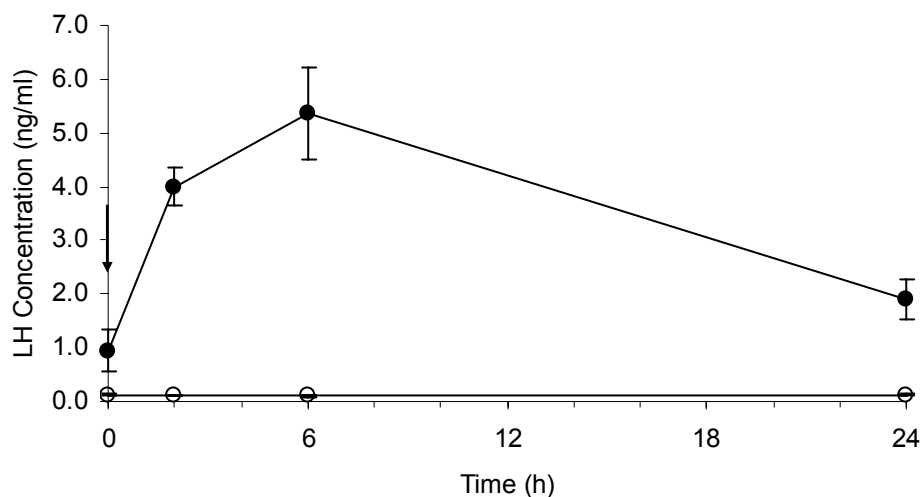
		Acute sampling			GnRH challenge			
Group	n	<i>Positive response</i>	Starting concentration	Peak concentration	n	<i>Positive response</i>	Starting concentration	Peak concentration
LH males								
Control	3	0	0.13 ± 0.02	0.13 ± 0.02	3	3	0.11 ± 0.02	2.07 ± 0.39
10mg	3	3	0.94 ± 0.40	5.36 ± 0.85	3	0	0.16 ± 0.02	0.18 ± 0.02
Testosterone males								
Control	3	0	2.57 ± 1.7	2.85 ± 0.83	3	3	2.76 ± 0.21	20.49 ± 5.71
10mg	3	3	9.31 ± 2.24	20.56 ± 1.25	3	0	1.09 ± 0.73	1.85 ± 1.18

Plasma LH concentrations were only detectable in treated males at the time of treatment (independent t-test,  $P < 0.05$ ; Fig. 9 a; Table 4) and were significantly elevated in treated animals within 2 h (control, 0.11  $\pm$  0.01 ng/ml; treated, 4.00  $\pm$  0.34 ng/ml; independent t-test,  $P < 0.001$ ) and at 6 h after implant insertion (control, 0.10  $\pm$  0.01 ng/ml; treated, 5.36  $\pm$  0.85 ng/ml; independent t-test,  $P < 0.001$ ). By 24 h, LH concentrations in treated males were still significantly higher than in control animals (control, 0.13  $\pm$  0.02 ng/ml, treated 1.89  $\pm$  0.37 ng/ml; independent t-test,  $P < 0.001$ ). LH concentrations of control males generally remained below the detection limit of the assay throughout the 24 h acute sampling period.

Plasma testosterone concentrations were variable within individuals at the time of deslorelin insertion and higher in treated than control males (independent t-test,  $P > 0.05$ ; Fig. 9 b; Table 4). Testosterone concentrations were significantly elevated within 2 h for treated males (control, 1.59  $\pm$  0.95 ng/ml;

treated,  $20.56 \pm 1.25$  ng/ml; independent t-test,  $P < 0.001$ ). By 24 h, testosterone concentrations in treated males had started to return to pre-treatment concentrations (control,  $2.85 \pm 0.83$  ng/ml; treated,  $13.65 \pm 3.82$  ng/ml; independent t-test,  $P > 0.05$ ). Testosterone concentrations of control males declined slightly throughout the 6 h acute sampling period (Fig. 9b), but had recovered by 24 h.

**a**



**b**

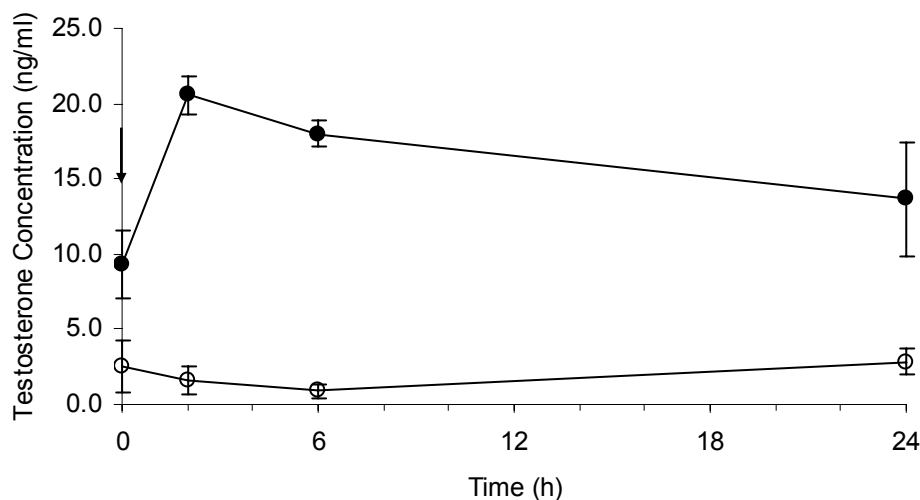


Figure 9. Plasma concentrations of (a) LH (ng/ml; mean  $\pm$  SEM) and (b) testosterone (ng/ml; mean  $\pm$  SEM) in control (○,  $n = 3$ ) and treated males (high dose, ●,  $n = 3$ ) within the first 24 h after administration of deslorelin implants (↓).

**GnRH challenge (LH and Testosterone).** Starting concentrations of LH, calculated as a mean of two pre-treatment samples, were all below or close to the detection limit of the assay and were not significantly different between the two groups (independent t-test,  $P>0.1$ ; Fig. 10 a; Table 4). Control animals responded to synthetic GnRH with a gradual increase in LH. Concentrations were significantly elevated within 15 min after synthetic GnRH administration for control males (control,  $0.91 \pm 0.15$  ng/ml; treated,  $0.18 \pm 0.07$  ng/ml; independent t-test,  $P<0.05$ ), and peaked at 60 min (control,  $2.07 \pm 0.39$  ng/ml; treated,  $0.12 \pm 0.03$  ng/ml; independent t-test,  $P<0.01$ ). By 120 min, LH concentrations of control males were in decline, but were still significantly higher than LH concentrations of treated animals (control,  $0.64 \pm 0.15$  ng/ml; treated,  $0.15 \pm 0.04$  ng/ml; independent t-test,  $P<0.05$ ). LH concentrations of treated males generally remained low throughout the entire GnRH challenge sampling period.

Plasma testosterone concentrations were variable within individuals at the onset of the GnRH challenge, but in general control males had higher testosterone levels than treated males 9 weeks after deslorelin administration (Fig. 10 b, see also 'long term response for testosterone'). Starting concentrations of testosterone, calculated as a mean of two pre-treatment samples, were not significantly different between the two groups (independent t-test,  $P>0.05$ ; Table 4). Control animals responded to the synthetic GnRH with a gradual increase in testosterone (Fig. 10 b). Concentrations were notably elevated within 60 min for control males after administration of synthetic GnRH (control,  $9.80 \pm 2.63$  ng/ml; treated,  $1.85 \pm 1.18$  ng/ml; independent t-test,  $P<0.06$ ), and peaked significantly at 120 min (control,  $20.49 \pm 5.71$  ng/ml; treated,  $1.61 \pm 1.17$  ng/ml; independent t-test,  $P<0.05$ ). Testosterone concentrations of treated males generally remained low throughout the GnRH challenge sampling period.

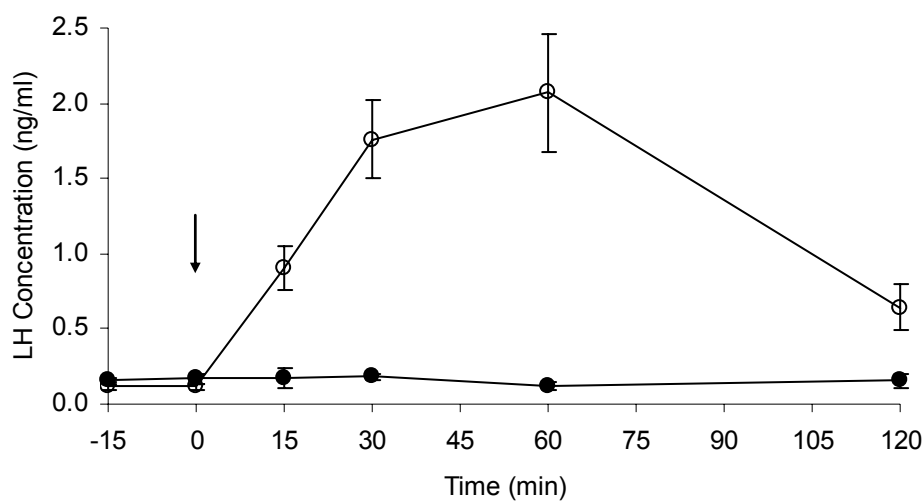
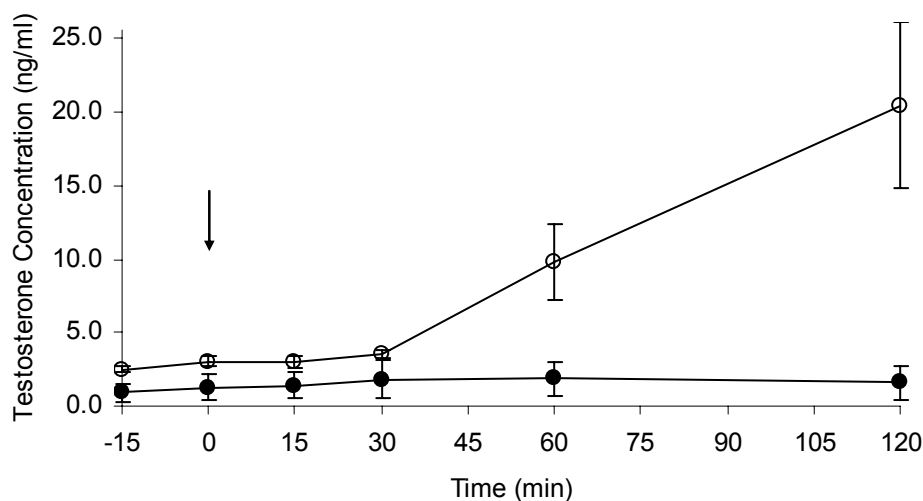
**a****b**

Figure 10. Plasma concentrations of (a) LH (ng/ml; mean  $\pm$  SEM) and (b) testosterone (ng/ml; mean  $\pm$  SEM) in control ( $\circ$ ,  $n = 3$ ) and treated males (high dose,  $\bullet$ ,  $n = 3$ ) following administration of synthetic GnRH ( $\downarrow$ ).

**Long-term response to deslorelin (FSH and Testosterone).** Pre-treatment samples showed that FSH concentrations of treated males were higher than those of control males (Fig. 11 a) and varied between individuals from 6.20 – 32.50 ng/ml. This difference was not significant at the onset of treatment (control,  $8.13 \pm 0.98$  ng/ml; treated,  $18.50 \pm 7.05$  ng/ml; independent t-test,  $P > 0.1$ ). Analysis

of weekly samples revealed a downregulation of FSH concentrations within treated males after insertion of the deslorelin implant whilst control males retained varying but similar and generally higher levels of FSH (Fig. 11a). Changes in FSH levels over time were significant (GLM,  $P < 0.05$ ), as was treatment x time interaction (GLM,  $P < 0.05$ ). There was also a significant difference between the two groups during the treatment period (GLM,  $P < 0.01$ ), suggesting that changes were the result of deslorelin treatment. However, FSH levels of the treated males remained detectable throughout the treatment period, and concentrations were within the lower limit of control males (Fig. 11 a).

The long-term response observed for testosterone concentrations was similar (Fig. 11 b). Deslorelin administration occurred at the onset of the breeding season when testosterone concentrations were already elevated in males. Pre-treatment testosterone concentrations of treated males were higher than in control males, and varied between individuals from 0.37 – 13.50 ng/ml. The difference between groups was not significant at the onset of treatment (control,  $2.57 \pm 1.70$  ng/ml; treated,  $9.31 \pm 2.24$  ng/ml; independent t-test,  $P > 0.05$ ). Analysis of weekly samples revealed a downregulation of testosterone within treated males after insertion of the deslorelin implant, whilst control males retained varying but similar and generally higher levels of testosterone concentrations (Fig. 11 b). Changes in testosterone levels over time were significant (GLM,  $P < 0.05$ ), as was treatment x time interaction (GLM,  $P < 0.05$ ). But there was no significant difference between the two groups during the treatment period (GLM,  $P > 0.5$ ), suggesting that changes over time were caused through deslorelin treatment, but that, despite significant downregulation of testosterone levels in treated males, concentrations between the two groups did not significantly differ at all times (Fig. 11 b). One treated male was exceptional as it maintained detectable testosterone levels similar to control males throughout the treatment period, despite the significant downregulation of pre-treatment testosterone levels. The pre-treatment testosterone levels in this particular male had also been higher than in any other males at the onset of deslorelin treatment (treated male 13.50 ng/ml, five other males  $4.43 \pm 1.53$  ng/ml).

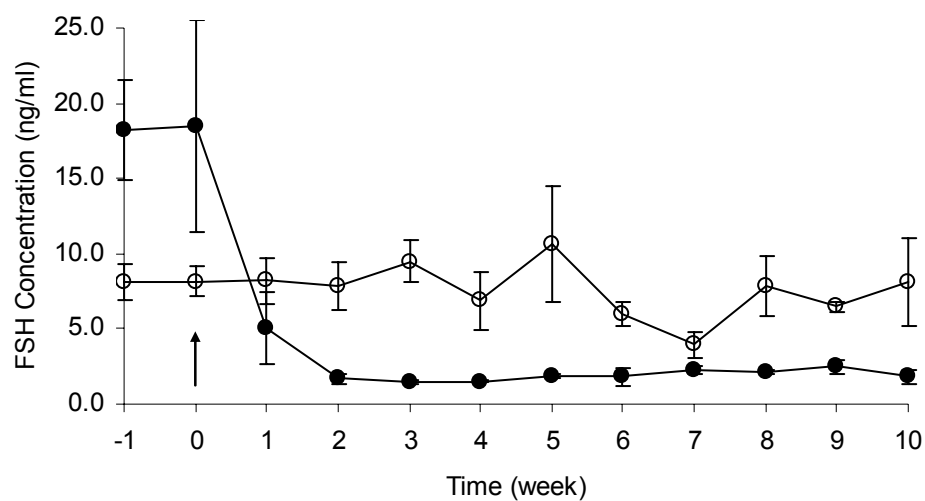
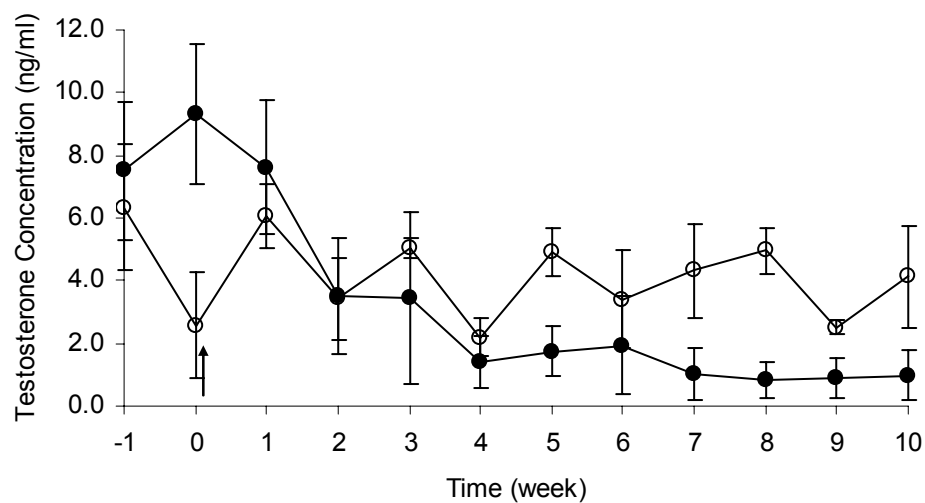
**a****b**

Figure 11. Plasma concentrations of (a) FSH (ng/ml; mean  $\pm$  SEM) and (b) testosterone (ng/ml; mean  $\pm$  SEM) in control (○,  $n = 3$ ) and treated males (high dose, ●,  $n = 3$ ) after administration of deslorelin implants (↑).

## Discussion

This study demonstrated that treatment with the GnRH agonist deslorelin temporarily inhibits reproduction in the female brushtail possum, but it has no contraceptive effect on males. In both sexes deslorelin implant administration initially stimulated a LH surge (acute phase) and in males this was accompanied by a rise in testosterone. Subsequently, long term exposure resulted in a degree of pituitary desensitization in both sexes, as evidenced by failure to respond to a GnRH challenge. Inhibition of reproductive activity in females was associated with a decline in the number of epithelial cells, leucocytes and sperm in their urine and a reduction in progesterone levels. Females remained infertile for at least one breeding season, and these effects were generally reversible. In male brushtail possums, FSH concentrations declined after initiation of deslorelin treatment, but basal levels remained detectable. Despite the reduction in FSH and testosterone concentrations and the inhibition of a LH response to a GnRH challenge, deslorelin treatment did not affect testis size and treated males continued to sire offspring.

Administration of GnRH agonist formulations can achieve long-term reversible suppression of the pituitary-ovarian axis in a wide range of species (for review see Herbert and Trigg [10]). The acute and chronic response of female brushtail possums to treatment with a GnRH agonist is comparable to females of other species such as tammar wallabies [14], mule deer [17], wapiti [16, 54] and cattle [11]. In tammar wallabies, peripheral plasma LH concentrations were significantly lower in deslorelin treated females than in controls for a period of 30 weeks [14]. In heifers, the acute phase of GnRH agonist treatment lasted several days and was characterized by an immediate large increase in plasma LH and FSH, followed by a return to basal concentrations [55, 56]. Prolonged exposure to GnRH agonists caused pituitary desensitization in cattle [11]. The use of GnRH agonists for fertility control in heifers relied on the absence of both the pulsatile secretion of LH and the pre-ovulatory surge release of LH during the chronic phase of treatment with agonist, thus blocking ovulation. The chronic phase is thought to be a consequence of the downregulation of GnRH receptors on gonadotrope cells [57], and

an uncoupling of second messenger pathways within gonadotrope cells [58]. The inhibition of the LH surge system would impair ovulation in the female brushtail possum.

Females treated with deslorelin in the present study, showed evidence of ovarian and reproductive tract activity for periods up to four weeks after insertion of the implant, as evidenced by elevated progesterone concentrations and cell counts in urine. Some females had a pouch young removed few days prior to or at the time of treatment which may have stimulated and/or enhanced a return to estrus. GnRH agonist- and RPY-induced estrus resulted in some mating. Afterwards, females ceased cycling and remained anestrous until the contraceptive effect wore off or the implants were removed. No subsequent births were recorded, although some animals had mated shortly before and after insertion of the deslorelin implant. Similar observations have been made in the eastern grey kangaroo. In this species, combined removal of pouch young and deslorelin implant placement stimulated an early behavioral estrus, but no young were produced [15]. Comparably, in tammar wallabies, follicular development and post-partum estrus were inhibited after prolonged exposure to deslorelin [59].

It is thought that gonadotropin support is required for the later stages of follicular development [56, 59]. In cattle, the early stages of follicle development are not dependent on acute support by gonadotropins. Deslorelin treatment of heifers causes a decline in the number of large follicles [60] as a result of effects at the level of the gonadotrope, rather than a direct action of the agonist at the level of the ovaries [11, 56].

Both FSH and LH are likely to be key regulators of follicular development in the brushtail possum [61]. Removal of the pituitary gland in brushtail possums one day after estrus did not alter CL development and function [20]. Pregnancies continued to term, but any fetuses died in the uterus or vaginal cul-de-sac. However, there was some suggestion that the CL may have been affected, and removal of the CL also prevented parturition [19]. In bitches, treatment with deslorelin caused failure of pregnancies at about day 40 of gestation [62]. This negative effect on pregnancy was associated with low plasma progesterone concentrations and was possibly caused by the regression of the CL due to low plasma



LH levels. No parturition occurred in any of the deslorelin treated females in this study, despite having mated before or shortly after GnRH agonist treatment. This provides further evidence, that both the pituitary gland and the corpus luteum are necessary for normal parturition.

Plasma progesterone concentrations in GnRH agonist treated females remained comparable to values found in control females within the first two weeks after treatment. This indicates that treated females were cycling for at least two weeks after treatment, before progesterone levels markedly declined. Afterwards, consistent progesterone concentrations below 1 ng/ml indicated that cycling activity was impaired as fertile females would show elevated progesterone concentrations within two days of ovulation with further increases [21]. The basal progesterone concentrations indicated the absence of a functional CL, and females remained anestrus. These results were supported by the urine data. Elevated cell counts, leucocytes and sperm were only observed for a maximum time period of four weeks; afterwards, no further observations were made. It is known that the initial stimulatory gonadotropin response to GnRH agonist treatment can induce estrus in some species [10, 15]. Our data also support previous findings that long-term GnRH agonist treatment results in a cessation of estrous cycles. In tammar wallabies, treatment with deslorelin also resulted in basal progesterone levels, and follicular development and estrus were inhibited [14, 59]. A gradual decline in progesterone and extended periods of acyclicity during prolonged GnRH agonist treatment have also previously been observed in marmoset monkeys (*Callithrix jacchus*) [63] and heifers [64]. In brushtail possums, it is thought that a single release of gonadotropins (e.g. the gonadotropin surge) is adequate to initiate steroidogenesis, and no further support from the pituitary gland is needed for the growth or function of the CL [27]. This explains why progesterone levels were still detectable up to two weeks post-deslorelin-treatment. As there is little difference between progesterone concentrations of pregnant and non-pregnant brushtail possums [19], it is not possible to determine if females had a non-pregnant cycle or if any potential pregnancy was aborted. Hence, long-term GnRH agonist treatment may have some effect on pregnancy and/or birth in the female brushtail possums, as previously observed for tammar wallabies

[14]. Negative side effects on body condition were not observed. Any weight changes were subtle and found to be not significant. Mortalities occurred in both treatment and control groups, but could not be related to GnRH agonist treatment.

All of the females in the present study that had the GnRH agonist implant removed three months post treatment subsequently had offspring, demonstrating the reversibility of the treatment. However, there was considerable individual variation in the time until complete recovery from GnRH agonist treatment (1 – 13 months), and indication of unsuccessful estrous cycles before the females gave birth. There is clearly a difference between the time to the resumption of estrous cyclicity and the time to the first birth. In tammar wallabies, some individuals underwent multiple cycles before they gave birth after treatment [14]. Animals may undergo a number of unsuccessful cycles before successfully breeding post-deslorelin treatment. Persistent suppression of ovarian function, beyond the formulated delivery period of the implant, has also been reported in mule deer and wapiti [16, 17, 54]. Crawford et al. [21] reports failure of ovulation in brushtail possums and found that preovulatory follicle development after RPY is asynchronous between animals and 33% (40/120) of females failed to ovulate after RPY. In our study, the implants were also removed outside the main breeding season, which could have reduced further mating attempts. Therefore, the extended period of time for giving birth after removal of the implant, as observed for two females, could have been a combination of factors and not necessarily solely due to the GnRH agonist treatment.

Overall, there is limited data available on recovery from long-term GnRH agonist treatment over years. Padula and Macmillan [65] report that the recovery of some cows was delayed after implant removal which had been in place for a maximum of 21 days. Failure to ovulate large, apparently estrogenic follicles prolonged complete recovery of normal ovulatory cycles in a subgroup of cows. Results of a study on the restoration of endocrine and ovarian function in goats after stopping GnRH antagonist treatment indicated that secretion of gonadotropins is restored shortly after the end of antagonist treatment, but activity of ovarian follicles is affected [66]. However, in cattle, treatment with deslorelin

did not negatively affect conception rates after termination of agonist treatment compared to control heifers [11].

Individual differences in the response to deslorelin treatment and/or a dose-response relationship are another possibility. Treated females in this study resumed breeding at different points in time, and there was some indication that a dose-dependent response may exist. The duration of temporary infertility varied widely in female tammar wallabies treated with a 5 mg dose of deslorelin, ranging from 344 to 761 days, but the strict reproductive seasonality was maintained once the implant had expired [14]. Higher doses of deslorelin resulted in longer contraceptive duration in eastern grey kangaroos [36]. The treatment of cats with deslorelin implants suppressed estradiol secretion for periods of up to 14 months, but again there was large variation in the duration of suppression between individuals [67].

In heifers, low doses of deslorelin may have been responsible for ineffective pituitary suppression [11]. Higher doses of deslorelin were associated with longer contraceptive duration in heifers [11] and dogs [35]. In a dose-response study in heifers, deslorelin suppressed ovarian follicular growth, ovulation and conception in a dose-dependent manner for up to 12 months [11]. The medium dose implant prevented conception for around 230 days, while the high dose implant was effective for approximately 330 days. This dose-response relationship is not fully understood yet [10]. It may be possible that the pituitary may take longer to recover from a higher dose. Or a higher starting dose of agonist in a slow release system could maintain the release rate above a theoretical critical threshold for down regulation for a longer period of time. There is also individual variation in the response to GnRH agonist treatment, and genetic variations in the sensitivity to GnRH agonist may affect the resumption of estrous cycles. In particular, low doses of deslorelin may be below the sub-threshold for some animals. A further hypothesis is that the implant itself may cause variability due to variation in the duration and amount of agonist released [64].

Male brushtail possums appeared to be resistant to the contraceptive effects of GnRH agonist treatment. Although there was evidence of a decline in plasma testosterone and FSH levels, testis size

remained unchanged and males continued siring offspring. The insertion of the deslorelin implant did affect the endocrine response of treated males in a comparable manner to the mode of action observed in females. In the acute phase, deslorelin caused a significant surge of LH and testosterone in males, which demonstrated its biological activity in males. In control males, there was a decline in the concentration of testosterone during the acute sampling period, which may be an artifact of stress during frequent blood sampling. Acute and chronic stress can depress plasma testosterone concentrations and such responses have been previously observed in brushtail possums [68] and other male marsupial species [53, 69].

Chronic exposure to GnRH agonist resulted in a significant decline of plasma testosterone concentrations. These males also lost the ability to respond to a GnRH challenge with a surge of LH and testosterone. Hence, prolonged exposure to deslorelin appeared to have desensitized their pituitary. Interestingly, testosterone levels in one treated male that had the highest concentrations of all males at the onset of this trial remained detectable ( $\geq 2\text{ng/ml}$ ) throughout the entire sampling period. It is known, that LH stimulates the production of testosterone in the Leydig cells [32], hence sufficient tonic levels of LH must have been maintained to stimulate testosterone production in this treated male.

The observed loss in weight during the course of the trial occurred in both control and treatment group and is most likely related to the breeding season. Males lose weight throughout the breeding season, as observed in another captive colony [29] and in a wild population on Magnetic Island [70]. Males on the island lost on average 4.2% of their body mass during the main mating season suggesting that mating and consort behavior is energetically expensive and may constrain foraging activity.

GnRH agonist treatment has shown to have contraceptive effects on some male species. Prolonged and persistent delivery of a GnRH agonist significantly reduced concentration of testosterone, testicular volume and spermatogenesis in adult male rhesus monkeys (*Macaca mulatta*) [71, 72]. Deslorelin is a successful anti-fertility agent in male dogs reducing plasma LH and testosterone concentrations to undetectable levels 27 days after administration [35, 44]. Atypical responses even in the face of

persistent exposure to a GnRH agonist have been reported from several species such as marmoset monkeys [63, 73] and red deer stags (*Cervus elaphus*) [69]. In male tammar wallabies, there was no evidence of a decline in plasma testosterone or basal LH concentrations during GnRH agonist treatment, although an underlying pituitary desensitization was found [53]. In cattle, the acute increase in LH is followed by a chronic phase during which pulsatile release of LH is blocked but basal secretion is maintained [11]. Interestingly, both bulls [74] and heifers [55] receiving GnRH agonist treatment had elevated basal concentrations of LH compared with control animals. Indeed, prolonged exposure to a potent GnRH agonist may be an effective means of enhancing steroidogenic and spermatogenic potential of developing bulls [75]. In bulls, a tonic pattern of LH release is associated with increased secretion of testosterone, which persisted for the duration of GnRH agonist treatment [11]. Aspden et al. [76] suggested that bulls treated with GnRH agonist, undergo the classical desensitization of the pituitary and downregulation of endocrine function, but that there are other testicular factors involved which maintain LH secretion. For example, the rate of transcription and translation of LH $\beta$ -subunit mRNA to LH may be increased to compensate by independent mechanisms. Increased testosterone secretion in bulls treated with deslorelin was also associated with increased testicular StAR protein and steroidogenic enzymes [75]. It is possible that an increase in testicular LH receptors allows greater gonadotropic stimulation of steroidogenesis in bulls treated with deslorelin, even though plasma LH remains typical of control bulls. Aspden et al. [77] showed that LH is involved in mediating the response of bulls to treatment with deslorelin.

The mechanism for this response in bulls has not been entirely elucidated yet, but it is thought that pulsatile release of LH is not required to stimulate the synthesis of steroidogenic enzymes that sustain elevated secretion of testosterone [11]. It was also suggested that while both LH and FSH are released from the gonadotrope in a basal manner independent of GnRH, only the release of pulses of LH is totally dependent on pulsatile GnRH input [78]. Hence, it seems likely that tonic plasma LH

concentrations in male brushtail possums are sufficient to stimulate the production of low amounts of testosterone.

The lack of contraceptive effect on males in the present study is unlikely to be a result of inefficient dose or an inefficient delivery system. The dosages given were higher than those administered to male dogs. A single slow-release implant containing 6 mg deslorelin was an efficient contraceptive in male dogs for at least one year [44]. In tammar wallabies, testosterone concentrations were not suppressed over a range of deslorelin dosages (5, 10 and 20 mg, [53]). In bulls, testicular suppression could not be induced at any dose [74]. The maintenance of testis size in deslorelin treated males in the present study and the continued evidence of births in associated females give further support that fertility was maintained during chronic exposure to the GnRH agonist. In deslorelin treated male dogs, testicular volume fell significantly to pre-treatment values and no ejaculates could be obtained after 6 weeks post-treatment [44]. These findings indicate that the regulation of testosterone secretion by the hypothalamic-pituitary-gonadal axis is not the same in all species [79].

In humans, GnRH agonist treatment in combination with testosterone did not prove useful for contraception either [80]. One explanation for the ineffectiveness of the GnRH agonist could be the escape of FSH suppression after several weeks of GnRH agonist treatment [81, 82]. Deslorelin-treated males in the current study had detectable plasma FSH concentrations throughout the entire sampling period, although there was a significant decline post-GnRH agonist treatment, and FSH levels remained significantly lower than in the control group. It may also be worth considering, that concentrations of steroid hormones in peripheral plasma samples bear little relationship to the concentrations to which the cells within the testis are exposed [83]. The complex patterns of spermatogenesis in many species, including the brushtail possum, are not entirely understood to date and require further investigation. Spermatogenesis seemed maintained in all GnRH agonist treated males in the present study throughout the treatment period, but it would be interesting to determine the amount and quality of their sperm in further studies to see if perhaps spermatogenesis was partially impaired.

In summary, deslorelin has been shown to temporarily inhibit reproduction in female brushtail possums. The mode of deslorelin action is the same in both females and males, both experienced pituitary desensitization during long-term treatment. The contrasting effects of acute (stimulation) and chronic (desensitization) exposure to GnRH or GnRH agonists leads to a biphasic pattern of gonadotropin and testosterone secretion in most adult male animals during extended treatment with GnRH agonists [84]. The stimulatory response is transient, and continued exposure to the agonist suppresses secretion of LH and testosterone. Despite this, male brushtail possums appear to be resistant to the contraceptive effects of chronic GnRH agonist treatment. It seems pulsatile release of LH and the LH surge are not important for maintenance of spermatogenesis, and tonic LH secretion is sufficient to maintain testicular function. Contrastingly, fertility was impaired in the female, possibly due to the inhibition of the LH surge system which suppresses ovulation. The contraceptive effect of the agonist on females was generally found to be reversed after cessation of agonist administration. The duration between treatment and the resumption of birth covered a period of at least one breeding season. Hence, there is potential to control reproduction in female brushtail possums using GnRH agonist treatment.

From a management point of view, this makes deslorelin a promising approach to control reproduction in some wild populations. Deslorelin is currently the only fertility control agent for female brushtail possums which is commercially available, has no undesirable side-effects and reversibly inhibits reproduction. In addition, fertility control methods that target endocrine control of reproduction may cause desirable behavioral changes, such as inhibition of mating behavior. Reduced sexual contacts between brushtail possums and possibly reduced agonistic contacts may reduce disease transmission and human residential complaints about noisy animals. There is some concern that disruption of normal social behavior in sterile females may hinder effective competition for resources or territories [85], or that a loss of status by dominant animals may allow subordinates to breed more successfully [86]. However, this proposed disruption of hierarchies has not been observed after vaccination of dominant female brushtail possums against GnRH [86].

The duration between treatment and the resumption of breeding, defined as the time to the first successful birth after treatment, is the time-frame which is of most interest to wildlife managers, who aim to control population numbers by reducing offspring. It would be worthwhile to investigate if infertility in brushtail possums can be prolonged using higher doses of agonist. For management purposes, the optimum time of treatment application in this species would be the period of anestrus (December – February), before any newborn are present and before the initiation of seasonal estrus.

However, management goals in New Zealand clearly differ from Australian ones and should not be confused. New Zealand's ultimate goal is eradication of the brushtail possums [87], which does not apply to Australia, where control operations solely aim to reduce abundance to sustainable levels in high problem areas. Fertility control techniques will most likely differ in both countries, and a common solution may not be applicable. Slow-release GnRH agonist implants could be a useful management tool in Australia for problem populations in areas such as small islands, (sub)-urban neighborhoods and/or botanical and zoological gardens [4]. In such, deslorelin implants deserve further attention to investigate their effects on natural brushtail possum populations.

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## Chapter 3

# Brushtail possums (*Trichosurus vulpecula*) in metropolitan Sydney – population biology and response to contraceptive implants

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## Abstract

The cohabitation of the common brushtail possum (*Trichosurus vulpecula*) with people in urban areas often causes conflict. Basic biological parameters are needed to evaluate potential new management options such as fertility control. This study investigated the biology of an urban brushtail possum population and the effects of deslorelin (a GnRH agonist) contraceptive implants on individual females within that population. Trapping success remained similar over time ( $2.81 \pm 0.17$  individuals per residential property), but there appeared to be a turn-over in animals during the study period between November 2002 and April 2005. The sex ratio was slightly biased towards males, and age specific survival declined earlier in males than females. Breeding was seasonal with the main peak of births in autumn. Deslorelin treatment effectively inhibited reproduction in adult females for  $519 \pm 7$  ( $n = 5$ ) and  $\geq 700 \pm 20$  ( $n = 5$ ) days after administration of one or two 4.7 mg implants respectively. Two females produced double offspring upon resumption of breeding. This study delivered essential biological knowledge on urban brushtail possum populations and provided an indication of the efficacy of using deslorelin implants as a fertility control technique for brushtail possum populations in Australian cities.

## Introduction

The common brushtail possum (*Trichosurus vulpecula*) poses challenges for wildlife managers. This solitary, nocturnal and herbivorous marsupial has one of the widest occurrences of any Australian marsupial (Kerle 2001), but has disappeared from more than half of its natural range presumably due to habitat loss, disease, drought and predation (Kerle *et al.* 1992, Goldingay and Jackson 2004). Conversely, it is abundant in Tasmania, Kangaroo Island and Magnetic Island (Isaac 2005) and indeed common in many Australian cities (Matthews *et al.* 2004). The species is also a major pest in New Zealand where it was introduced for fur trade in the late 1800s (Montague 2000). This creates distinct management scenarios. In New Zealand, the ultimate goal is eradication of the brushtail possum, and toxic baits such as 1080 (sodium monofluoroacetate) are used widely to control densities (Eason *et al.* 2000). In Australia the brushtail possum is a protected native species, placing constraints on active management options, especially in urbanised areas where lethal methods are deemed unacceptable. In most cases, control of 'problem' brushtail possum populations in Australia merely aims to reduce densities to sustainable levels and/or reduce conflict with landholders (Eymann *et al.* 2006a, see Chapter 1).

The cohabitation of the brushtail possum with people in (sub)urban areas often causes conflict because of their habit of occupying roof space and browsing garden vegetation (Eymann *et al.* 2006a, see Chapter 1). Current management practices within most states only permit relocation of brushtail possums occupying roofs and their release must be within 50m of the capture site. The effectiveness of this approach is questionable and some householders continue to illegally relocate brushtail possums to the detriment of the animals' welfare. Hence, alternative management options are being sought. Non-lethal population control methods are also being sought for other overabundant native marsupials, which has resulted in research and development of new techniques to manage populations by reducing their fertility (Adderton Herbert 2004).

Gonadotrophin releasing hormone (GnRH) agonists have long been recognised as potential agents for the control and management of fertility in mammals (Herbert *et al.* 2005). They achieve their contraceptive effects by temporarily suppressing pituitary responsiveness and inhibiting the secretion of gonadotrophic hormones, leading to an inhibition of ovulation, oestrous cycling and spermatogenesis in some species (Herbert and Trigg 2005). The formulation of GnRH agonists into long-acting depot formulations (implants) has increased the potential efficacy of their use as wildlife fertility control agents. GnRH agonists have been used successfully as contraceptives for several wildlife species including tammar wallabies (*Macropus eugenii*) (Herbert *et al.* 2005), eastern grey kangaroos (*M. giganteus*) (Herbert *et al.* 2006), giraffe (*Giraffa camelopardalis reticulata*) (Patton *et al.* 2006), wapiti (*Cervus elaphus nelsoni*) (Baker *et al.* 2002), mule deer (*Odocoileus hemionus*) (Baker *et al.* 2004) and some African wild carnivores (Bertschinger *et al.* 2002). Captive trials of the GnRH agonist deslorelin in female brushtail possums have demonstrated that fertility can be inhibited for at least nine months and potentially beyond two years after administration of deslorelin implant(s) (Eymann *et al.* 2007a, see Chapter 2). While there has been a lot of research on the effects of deslorelin implants on reproduction in captive marsupials, this is the first comprehensive account of the effect of these implants on a wild marsupial.

The reproductive biology of the female brushtail possum has been well characterised. Female brushtail possums are polyoestrous and monovular having an oestrous cycle of about 26 days (for review see Tyndale-Biscoe and Renfree 1987; Fletcher and Selwood 2000). The gestation period is 17-18 days and females are usually strictly monotocous (presenting one offspring at a time). Oestrus and ovulation are suppressed by lactation, but females can ovulate again about 8 days after loss or removal of the pouch young (Crawford 1997). Brushtail possums are seasonal breeders and the onset of the breeding season varies depending on the geographical location (Kerle 2001, 1984). In Sydney, the main breeding season occurs in autumn (peak of births in April), with a minor spring season (Lyne and Verhagen 1957).

In general, there is a paucity of knowledge about the biology of brushtail possums in urban areas. The few published studies on urban brushtail possums have investigated home range and use of den sites (Harper 2005; Statham and Statham 1997). Although there have been numerous studies on Australian and New Zealand populations in non-urban environments (for review see Kerle 1984, 2001; Efford 2000; also Kerle 1998; Isaac 2005), these may not be applicable to the life history of urban brushtail possums as there is wide variation between the different populations. As such, there is a need for baseline data from urban brushtail possum populations to enable management objectives to be set and outcomes assessed.

This study investigated the biology of an urban brushtail possum population and the effects of a long-acting contraceptive implant on individual females within that population between November 2002 and April 2005. The aims were i) to determine baseline population parameters for an urban brushtail possum population in metropolitan Sydney, including age structure, sex ratio and reproductive parameters; and ii) to determine the contraceptive success rate and duration after treatment of wild females with either one or two implants containing the GnRH agonist deslorelin (4.7 and 9.4 mg respectively). The basic population parameters reported in this study contribute towards our understanding of urban brushtail possum populations and will aid the effective evaluation of management scenarios. This study also provides an indication of the efficacy of using deslorelin implants as a fertility control technique for wild brushtail possum populations in Australian cities.

## Materials and Methods

### Study sites

Brushtail possums were studied between November 2002 and April 2005 in five urban suburbs within the Sydney metropolitan area, New South Wales, Australia. Locations included four North Shore mainland suburbs, namely Beecroft (33°45'S, 151°04'E), Chatswood West (33°47'S, 151°09'E), North Epping (33°45'S, 151°05'E), Pymble (33°45'S, 151°07'E) and Scotland Island (33°38'S, 151°17'E), a 52 ha land mass located at Pittwater (Fig. 1). These localities have high residential levels, are renowned for being 'green and leafy' and are within close proximity to bushland reserves. Animals were live-trapped in the garden area of 35 residential houses (ranging in size from 500 – 1,400 m<sup>2</sup>) which were patchily distributed within the five localities and reported to have brushtail possums present.

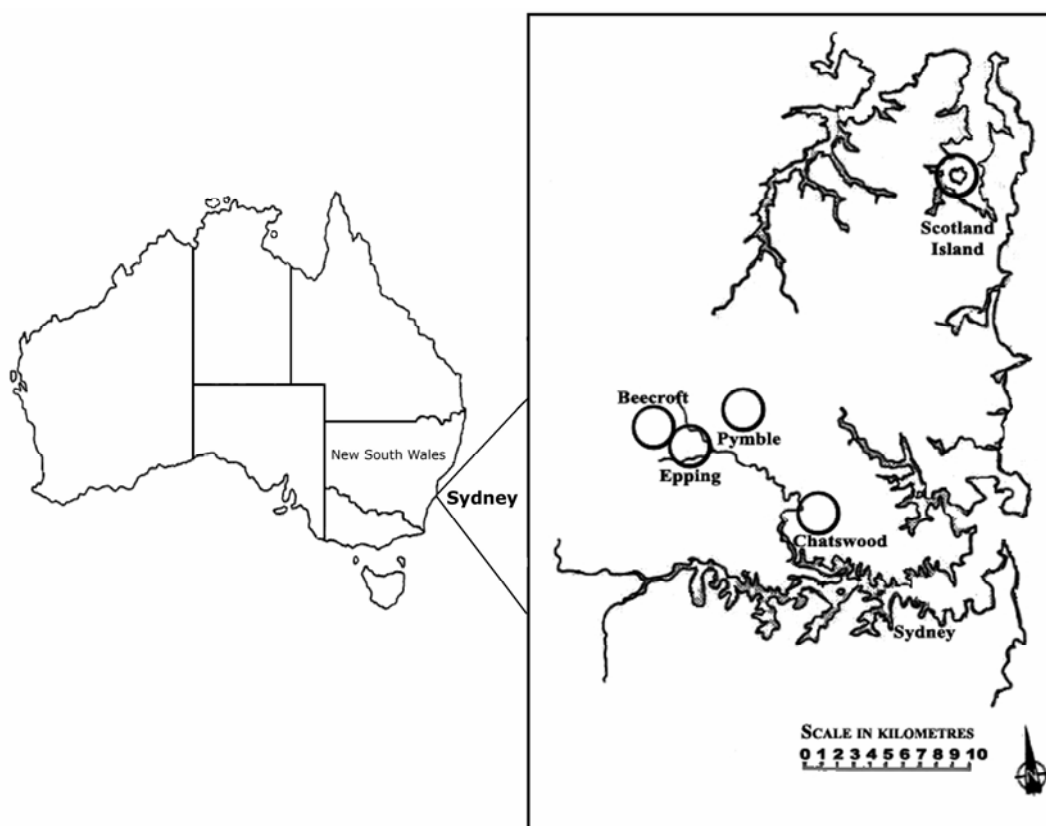


Figure 1. Map showing the location of the four Sydney metropolitan suburbs and Scotland Island from which brushtail possums were sampled (after Stow *et al.* 2006).



## Experimental design

Animals were caught overnight in metal cage traps (60 cm x 30 cm x 30 cm; Mascot Wire Works, Sydney) covered with tarpaulin and baited with apple and peanut butter, and they were examined the following morning (Duckworth and Meikle 1995). Brushtail possums were physically restrained in hessian bags while anaesthetic was administered into the gluteal muscle using a combination of ketamine (20 mg kg<sup>-1</sup>; Ilium Ketamil) and xylazine (2 mg kg<sup>-1</sup>; Ilium Xylazil-20). They were marked with a passive integrated transponder (Trovan ID-100, Microchips Australia Pty Ltd), sexed, aged and weighed to the nearest 50 g using hanging scales (Wedderburn, SA235 6S). Body measurements including head-, ear-, manus-, pes-, body- and tail-length were taken (Lyne and Verhagen 1957; Viggers *et al.* 1998). Blood and tissue samples were collected as part of other studies which describe health, performance of PIT tags, genetic diversity and dispersal of the investigated population (see Eymann *et al.* 2006b, 2007b,c,d; Stow *et al.* 2006; see Chapter 4 and 5 and Appendices A – C). Possums were held in hanging hessian bags and released at twilight on the same day at the capture site.

Trapping started in November 2002 and females were administered with deslorelin and placebo implants until March 2003, the onset of the main breeding season. Treatment groups consisted of control (placebo implant,  $n = 11$ ), low dose (one deslorelin implant, 4.7 mg,  $n = 10$ ) and high dose (two deslorelin implants, 9.4 mg,  $n = 12$ ) groups. In spring 2003, an additional four females received deslorelin implants (low dose,  $n = 1$ ; high dose,  $n = 3$ ) and four females a placebo. The duration of the contraceptive effect was estimated as the time to the first observed PY after implant insertion. Females were monitored after the main breeding season; hence, recapture attempts were made on all properties, usually for three consecutive trap nights in spring 2003 and spring 2004. Afterwards recapture attempts were only undertaken for specifically selected females in December 2004 and April 2005 which had not yet resumed breeding (i.e. data collected in this period was only used for the determination of contraceptive duration and breeding success). At each private property, initially 10 traps were set (November 2002 – April 2003) which was reduced to 5 traps on all subsequent trapping events, except

on Scotland Island. They were strategically spaced out and placed near brushtail possum 'runways' to optimise trap success. Trapping was conducted over 124 nights, giving a total of 1,202 trap nights. Trapping success was defined as captures per trap night and the average calculated from the total number of animals (including recaptures) caught in 1,202 trap nights.

## **Estimation of population parameters**

The age of brushtail possums was determined using tooth wear of the first upper molar (Winter, 1980; Cowan and White, 1989); animals were assigned into a tooth wear class from 1– 7, e.g. class 2 equals an age of 1.0- to 1.5-yr-old, class 3 equals 0.9- to 3.6-yr-old, class 4 equals 2.6- to 5.4-yr-old, etc.. Studies on brushtail possums from a range of environments have found the relationship between tooth wear class and age to be remarkably consistent (e.g. Magnetic Island, Isaac 2005; New Zealand, Cowan and White 1989; Queensland, Winter 1980). Individuals were considered adult if they were 2 kg or heavier, had class 2 or higher tooth wear and additionally males with full-sized testes and females with a fully developed pouch. All others were considered immature. Pouch and back young age was estimated from growth tables based on the measurement of head and body or crown-rump length with vernier calipers (Bach and Lodewikus 1998; Lyne and Verhagen 1957). If not attached to the teat, young were weighed using a digital scale (Tanita, KD-200; d=1g).

The reproductive status of females was determined from the presence of a pouch young (PY) or back young (BY) or the condition of the teats and mammary glands at the time of each capture. Pouch condition was classed as (1) no signs of recent activity, (2) elongated teat with associated mammary activity (young nearby or very recent loss of young), or (3) regressing teat (no milk flow; recent loss or weaning of young). Individual body weight was used as an indicator of an animal's condition over the years, and general appearance was noted.

## GnRH agonist implant

The GnRH agonist, deslorelin (D-Trp<sup>6</sup>-Pro<sup>9</sup>-des-gly<sup>10</sup>-GnRH ethylamide), was formulated into implants that contained 4.7 mg deslorelin (Suprelorin®, Peptech Animal Health Pty Ltd, Macquarie Park, NSW; Herbert and Trigg 2005). This product releases deslorelin from a matrix consisting predominantly of low-melting point lipids and biological surfactant (Trigg *et al.* 2001). The in vivo release rate in brushtail possums is unknown. The dimensions of a 4.7 mg implant were 2.3 mm in width and 12.5 mm in length. To place a 9.4 mg dose, two 4.7 mg implants were administered in a single-use pre-loaded device which was supplied sterilised by e-beam radiation. Implants were placed subcutaneously between the shoulder blades and the injection site was sealed with a veterinary tissue adhesive (Vetbond; 3M Animal Care Products, St Paul, MN, USA).

## Statistical analyses

The statistical analysis was performed using the Vassar Stats web site for statistical computation (<http://faculty.vassar.edu/lowry/VassarStats.html>) and SPSS (SPSS Inc., Chicago, IL, USA). Data for animal weight over time were analysed by ANOVA, using the general linear model (GLM) repeated measures procedure of SPSS, with the model being  $y = \text{treatment, time, treatment} \times \text{time}$ , with time as the repeated subject. Post hoc tests were conducted using Tukey's honestly significant difference (HSD) method for multiple comparisons of group means. Comparisons of the number of PY for treated versus control groups at single time points were examined using Fisher's Exact Probability Test (Vassar Stats web site). Comparisons between the duration to first offspring in control and low dose females after administration of implants were made using the independent t-test function of SPSS. Results are presented as untransformed arithmetic means  $\pm$  standard error of the mean (S.E.M.) and reported as significant at  $P < 0.05$ .

## Results

### Trapping success

Over the study period, a total of 214 individuals (including pouch young) were caught. Trapping success averaged  $29.15 \pm 2.52\%$  for the three trapping events until October 2004, ranging from 0% to 100% per individual night, and on average  $2.83 \pm 0.34$  animals were caught per night. An average of  $2.81 \pm 0.17$  individuals were trapped on each property in the mainland suburbs and  $3.66 \pm 0.60$  individuals per property on Scotland Island where the properties are generally larger. On both mainland and Scotland Island up to seven individuals were captured on particular properties. The majority of captures for each individual, whether trapped few or many times, were confined within a restricted area. Some individuals were caught alternating between a few properties, but only when houses were in close proximity (max. distance observed  $\leq 130\text{m}$  for males).

### Recapture success

Trapping success remained similar over time, but there appeared to be a turn-over in animals during the study period. Recaptured animals made up 42.4 % (36/85) of animals caught in spring 2003 and 38.8% (38/98) of animals in spring 2004. Recapture success of animals caught at the beginning of the study (Nov 02 – Apr 03) declined steadily over time and newly caught individuals replaced them. The progressive recapture rate of males that were initially caught was much lower than in females (see section entitled 'Effect of deslorelin on females'), with none of the 30 males initially captured being recaptured approximately 18 months later, between August and October 2004 (Table 3). This result was similar for all adult males captured for the first time in spring 2003, with 31% (9/29) being recaptured one year afterwards (spring 2004). Details of the progressive recapture success of the females initially captured (November 2002 – April 2003) are given below (see section entitled 'Effect of deslorelin on females').

## Sex ratio and age structure

The sex ratio remained similar during the study period, varying between 1.0 : 1.2 (females : males) and 1.0 : 1.4 (Table 1). The sex ratio of young (PY and BY) and immature animals appeared generally biased towards males. Juveniles in particular were readily trapped at the time of the first trapping event (Nov 2002 – April 2003; see Table 1).

Table 1. Age structure and sex ratio of urban brushtail possum populations.

The number of animals in each age class (and equivalent percentage) is shown. Data pooled for all sites, but separated for the successive trapping events.

Trapping period	Sex <sup>a</sup>	<i>n</i>	Sex ratio	Age class <sup>b</sup>						
				0	1	2	3	4	5	7
Nov 02 – Apr 03	Males	48	1.4	6 (12.5%)	12 (25.0%)	0 (0%)	7 (14.6%)	2 (4.2%)	0 (0%)	1 (2.1%)
	Females	37	1.0	1 (2.7%)	6 (16.2%)	0 (0%)	9 (24.3%)	9 (24.3%)	3 (8.1%)	1 (2.7%)
	Unknown	0								
Aug 03 – Oct 03	Males	49	1.3	3 (6.1%)	1 (2.0%)	15 (30.6%)	22 (44.9%)	5 (10.2%)	2 (4.1%)	1 (2.0%)
	Females	38	1.0	5 (13.2%)	2 (5.3%)	7 (18.4%)	9 (23.7%)	10 (26.3%)	4 (10.5%)	1 (2.6%)
	Unknown	3		3						
Aug 04– Oct 04	Males	45	1.2	11 (24.4%)	1 (2.2%)	8 (17.8%)	14 (31.1%)	8 (17.8%)	3 (6.7%)	0 (0%)
	Females	39	1.0	5 (12.8%)	1 (2.6%)	1 (2.6%)	14 (35.9%)	14 (35.9%)	3 (7.7%)	1 (2.6%)
	Unknown	2		2						

<sup>a</sup> Pouch young recently born could not be sexed.

<sup>b</sup> 32.9% (28/85) of animals were not aged in Nov 02 – Apr 03; age class 0 = pouch/back young, age class 1 = immature animals (0.5 – 1.33 yrs, mean true age 0.9 yrs), age class ≥ 2 = adults; age group classifications (after Winter 1980; Cowan and White 1989): class 2 = 1.0 – 1.5 (1.2 yrs), class 3 = 0.9 – 3.6 (1.7 yrs), class 4 = 2.6 – 5.4 (3.7 yrs), class 5 = 4.2 – 9.3 (6.2 yrs), class 7 = 7.3 – 11.5 (9.6 yrs), no animals were observed in age class 6.

Generally, males were more frequently trapped in the younger age groups, peaking at age class 3 and declining notably in the following age classes (Fig. 2). On the other hand, the majority of females were within age classes 3 and 4, but thereafter also declined markedly. For both sexes, older animals (age class 5 to 7) represented a small minority of the population. Hence, survival appeared to decline notably beyond a mean true age of 3.7 years (age class 4 = 2.6 – 5.4 years).

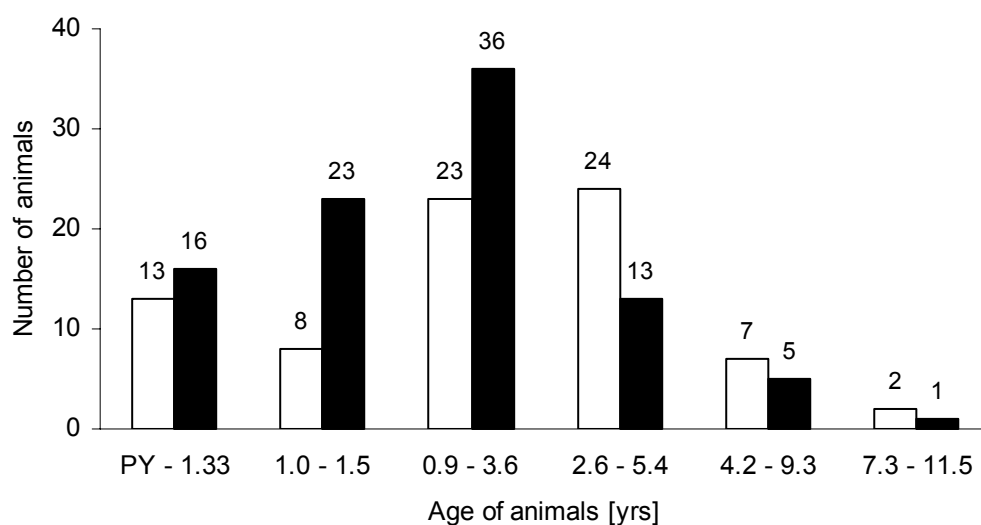


Figure 2. Age distribution of female and male brushtail possums, pooled for both trapping events in spring 2003 and 2004.

Open bars denote females, black bars males, numbers above bars are sample sizes. Age class of young and immature was pooled (PY – 1.33 yrs), for all other age classes refer to Table 3.

## Reproduction in untreated females

During the study period, most births occurred in autumn, with a peak in April (Table 2). A second smaller birth peak was noted in October, which is thought of as the minor spring breeding season. Some females ( $n=3$ ) had offspring in both breeding seasons – all were observed with a new offspring within 7 – 8 months time, but it is unknown if their first offspring survived (see Table 5a, supplement data; nos. C4, C7 and C9). On the other hand, some untreated adult females did not breed at all (nos. 80, 82). No females that were both  $\leq$  age class 2 and  $\leq$  2.0 kg weight had offspring, with the following exception: three females (out of 14) below 2.0 kg (weight was  $\geq$  1.9kg, but their age class was 3) and four females in age class 2 (but their weight was  $\geq$  2.25kg).

Few juvenile females were recaptured in adulthood, but the time to first offspring appeared variable. One individual was observed with a back young at 19 months of age while another female was still not breeding at 26 months of age (see Table 6, supplement data). Some of the young/juvenile females that were subsequently recaptured as adults remained at their original location throughout the study period.

Table 2. Summary of monthly births for all breeding females during the study period.

The table does not include data from deslorelin-treated females.

Females	Jan	Feb	Mar	Apr	May	June	July	Aug	Sep	Oct	Nov	Dec
( $n = 38$ )	1	0	4	10	7	4	2	1	2	6	1	0

## **Effect of deslorelin on females**

### **Weight**

The weights of animals in each group were similar at the onset of treatment (control ( $n = 15$ ),  $2.44 \pm 0.07$  kg; treated (low dose,  $n = 11$ ),  $2.60 \pm 0.11$  kg; treated (high dose,  $n = 15$ ),  $2.51 \pm 0.09$  kg; ANOVA,  $P > 0.5$ ). The majority of treated females gained weight over time until resumption of breeding, but this was equally the case for some control females. For females which were recaptured on two and three consecutive trap seasons the GLM showed some change in weight over time, but no significant treatment  $\times$  time interaction and no significant difference between the three groups during the treatment period either ( $P > 0.05$  for both). This suggests that any changes over time were unlikely to be the result of treatment.

### **Recapture of treated and untreated females**

Recapture rates were very similar for all three groups of females (control, low and high dose) that were initially captured between November 2002 and March 2003 (Table 3) suggesting that survival rates did not vary between the groups. Of the females which received deslorelin ( $n = 4$ ) and placebo ( $n = 4$ ) implants in spring 2003, only 2 females with a high dose of deslorelin were recaptured in spring 2004. Recapture attempts of all deslorelin treated females which had not resumed breeding by spring 2004 were undertaken (Dec 2004, April 2005), with 3 high dose individuals being recaptured.



Table 3. Percentage of brushtail possums (caught during the first trapping session) known to be alive during the study period, shown for adult treated females in each group, adult males and summarised for all individuals caught.

'Total animals' includes tagged PY and BY, but these are not included within the other groups.

Sex (& treatment group)	<i>n</i>	Nov 02 – Apr 03	Aug 03 – Oct 03	Aug 04 – Oct 04
Females: Control	11	100%	63.6% (7/11)	54.5% (6/11)
F: Deslorelin 4.7 mg	10	100%	70.0% (7/10)	70.0% (7/10)
F: Deslorelin 9.4 mg	12	100%	58.3% (7/12)	50.0% (6/12)
Males	30	100%	33.3% (10/30)	0.0% (0/30)
Total animals	85	100%	42.4% (36/85)	25.9% (22/85)

## Births

At the onset of the study (December 2002 – March 2003) few females had offspring (30.3 %, 10/33; Table 4) with some showing signs of having recently weaned a young (see Table 5, supplement data). Most of these animals were caught before the onset of the major breeding season in April (Table 2). Females treated with a low or high dose of deslorelin before April 2003 had no offspring upon recapture approx. 5 – 8 months later (spring 2003) compared to 43% of the control group with offspring ( $p < 0.05$ ; see Table 4 and Table 5, supplement data) and 89% of newly caught females (Table 4). The pouches of deslorelin treated females appeared small and there was no sign of recent activity. One year later, in spring 2004, females treated with a high dose of deslorelin still had no offspring, but the majority of females treated with a low dose implant (5 out of 7 recaptured) had resumed breeding.

Two of these females (low dose of deslorelin) each had two offspring and were lactating from both mammary glands. One female carried two young of the same age (estimated age 140 days); the other female had two pouch young of differing ages (estimated ages 15 and 60 days).

The contraceptive duration calculated for the low dose animals who had resumed breeding was  $519.60 \pm 6.83$  days ( $n = 5$ ), and this was significantly longer than the time between administration of placebo implants and births in three control females (controls,  $157.67 \pm 31.87$  days,  $n = 3$ ; t-test,  $P < 0.05$ ). The duration of the contraceptive effect could not be determined for females treated with a high dose implant as none of them had resumed breeding by the time of last recapture (high dose female,  $n=5$ ,  $\geq 700.00 \pm 19.83$  days). The maximum duration of contraception observed for a high dose female was  $\geq 875$  days.

Table 4. Percentage of females breeding at time of treatment (Nov 02 - Apr 03) and at each subsequent trapping event.

Treatment group	<i>n</i>	Nov 02 – Apr 03	Aug – Oct 03	Aug – Oct 04
Control	11	36.4% (4/11)	42.9% (3/7)	66.7% (4/6)
Deslorelin 4.7 mg	10	30.0% (3/10)	0.0% (0/7)	71.4% (5/7)
Deslorelin 9.4 mg	12	25.0% (3/12)	0.0% (0/7)	0.0% (0/6)
Newly caught	9	—	88.9% (8/9)	66.7% (6/9)

## Discussion

This study has described basic population parameters for brushtail possums in the urban environs of Sydney and has demonstrated that deslorelin implants effectively inhibit reproduction for extended periods in free-living female brushtail possums, with no apparent negative side effects. Brushtail possums were readily captured on residential properties supporting the notion that they are common in urban areas (Matthews *et al.* 2004). On average, approximately three individuals were trapped per residential property at any one point in time, suggesting that they have overlapping home ranges. However, these values can not be extrapolated to the broader urban environment, as we were strategically trapping on selected 'possum-friendly' properties, not along transects, and the home range of these animals would probably cover several properties. Home range estimates for urban female brushtail possums range from 1 – 2 ha (but can be as small as 0.3 ha) while males tend to have larger home ranges (Dunnet 1956; Statham and Statham 1997; Harper 2005). Our results give an indication of the potential extent that properties are visited by brushtail possums. As management of urban brushtail possums to date operates at the individual property level (by advising householders about dealing with 'nuisance' animals), this data gives wildlife managers an idea of how many individuals may need attention on a particular property. It is difficult to calculate density figures in this environment because of the patchy nature of the landscape, issues with access to properties and associated difficulties conducting standard population indices, such as spotlighting transects or stratified trapping, in an urban area. For urban Brisbane, densities of up to 7 individuals per ha have been observed (Eyre 2004). High densities have also been reported for brushtail possum populations in New Zealand (between 3 ha<sup>-1</sup> up to extreme densities of 25 ha<sup>-1</sup> depending on the habitat; Efford 2000) and 5 ha<sup>-1</sup> on Magnetic Island in Queensland (Isaac 2005). Population densities in Sydney are likely to be within the range of these other high density populations.

The age structure of brushtail possum populations in Sydney resembled that of the high density population on Magnetic Island, where animals reached a plateau at age three for males and age four for

females and age-specific differences in survival occurred (Isaac 2005). Male survival declined earlier and faster than female survival, as in our study, and annual probability of survival declined from age five in both sexes. Contrastingly, the adult sex ratio was significantly female-biased on Magnetic Island (Isaac 2005) whilst we observed a male-biased sex ratio, particularly for immature animals. It has been suggested that the sex ratio of offspring can be adjusted in response to the relative competition for resources (Johnson *et al.* 2001). Females tend to produce more male offspring to avoid competition when resources such as den sites and food are limited, particularly in high-density populations. First time mothers on Magnetic Island also produced a male-biased sex ratio, possibly to avoid life-long competition with philopatric daughters (Isaac 2005; Isaac *et al.* 2005). Adult sex ratios may reach parity as the population stabilises (Kerle 1984) or perhaps due to lower survival rates of immature males. How (1981) reported from a rural population in north-eastern New South Wales that males appear to have lower survival in the first 16 months of independent life than females. However, it is possible that the male-biased sex-ratio is influenced by a trap bias – males are generally easier to catch than females (Kerle 1998).

Male biased sex ratios have also been previously reported as typical for disturbed populations. Strong adult male bias and a greater proportion of immature animals appear related to re-colonising or increasing populations due to the differences in dispersal patterns of immature males and females (Kerle 1984; Efford 2000). In general, males disperse from their natal area and gradually shift their ranges away from their mother's whilst the females remain and inherit their mother's home range (Cowan and Clout 2000; Clinchy *et al.* 2004). Similarly in this study, the few juvenile females that were recaptured as adults did not move properties while juvenile males were generally not recaptured (or were caught on another property). Using genetic techniques, Stow *et al.* (2006) showed that dispersal in this study area was male-biased and apparent out to distances of 900 m.

Recapture success in this study progressively declined and this was particularly apparent for males. This is likely to be related to mortality, although we cannot rule out other behavioural responses to

capture, such as animals becoming trap-shy (Kerle 1998) and potential handling-stress of individuals (Clinchy *et al.* 2001), or seasonal differences in the ability to trap brushtail possums (How 1981). In a suburban environment, mortality may result from collision with a vehicle, dog or cat attacks and exposure to certain diseases (Hemsley and Canfield 1993). Brushtail possums may be more prone to stress-related dermatitis (Hemsley and Canfield 1994) and pathogens originating from domestic pets, such as toxoplasmosis (Eymann *et al.* 2006b). Illegal relocation of 'nuisance' animals by annoyed householders is a common practice in Sydney (Eymann *et al.* 2006a) and adds to the disappearance of individuals. In our study, some householders reported relocation of brushtail possums by 'non-possum-friendly' neighbours between trapping intervals. This usually resulted in the inability to recapture marked animals, but an influx of new individuals in the particular trapping area. Aldred (1979 in Matthews *et al.* 2004) also reported that mortality in urban areas usually goes unnoticed as deceased or relocated brushtail possums are quickly replaced by immigrants. In New Zealand, removal of animals by control operations resulted in some rearrangement of the home ranges of immediately adjacent brushtail possums (Cowan and Clout 2000; Ji *et al.* 2001).

Most females gave birth in the autumn season, as was expected from previous research (Lyne and Verhagen 1957), with a peak of births in April (major autumn breeding season) and a secondary peak in October (minor spring breeding season). The majority of untreated adult females bred at some time during the study period. Long-term studies in New Zealand found that more than 80% of adult females breed in most years (Ramsey *et al.* 2002). There was not enough data to make a conclusive statement about the number of females breeding in both the primary and secondary breeding seasons or on the age of first reproduction, but there was an indication that some females bred in both seasons. Breeding in both seasons is thought to be caused by the resumption of oestrous cycling in females which had either lost their young in one season or successfully weaned their first young (Kerle 1984). The average age for commencement of breeding is extremely variable between populations. In some populations, most females begin reproducing at the end of their first year, while in others they are 2 – 3 years old

before breeding. Body condition and population densities influence reproduction and food availability appears to be a major factor determining reproductive success (Ramsey *et al.* 2002). It has been reported that abundant food supply enables females to breed continuously (Kerle 1998). Generally, it is thought that the abundant food supply (and artificial den sites, such as roofs) in Australian cities enable urban brushtail possum populations to reach high densities (Kerle 2001). However, the age at which females reach sexual maturity may be a trade-off between the apparent abundance of food and the high population densities which may promote competition for resources.

Reducing the reproductive potential of a population is becoming a popular approach to managing 'problem' wildlife. In our study, implants containing the GnRH agonist deslorelin were shown to be an effective contraceptive for female brushtail possums in a wild population. There was no difference in the recapture success of treated versus control females suggesting that survival rates were similar and that the treatment had no negative side-effects. All treated females were recaptured on the same properties after administration of deslorelin indicating that they kept their territory and that no loss of status occurred. A study that vaccinated dominant female brushtail possums against GnRH found no evidence of loss of status either (Jolly *et al.* 1996). In this study, body weight of both treated and some control females increased slightly over time suggesting no significant effect of treatment on body condition. These results were similar in a study on captive brushtail possums (Eymann *et al.* 2007a, see Chapter 2) and indicate that deslorelin is a safe contraceptive for females.

The fact that we observed double offspring in two females from our field study when they resumed breeding about 1.5 years after deslorelin treatment was interesting. It is unclear if this was related to previous deslorelin treatment, or a chance event. This phenomenon was not observed in control females during the study period, or after deslorelin treatment in a captive brushtail possum trial (Eymann *et al.* 2007a, see Chapter 2). It is possible that it may be related to some form of compensatory mechanism or perhaps to 'irregularities' of the endocrine system regulating the oestrous cycle once the females resumed breeding. From cattle it is known that recovery from GnRH agonist treatment can be

delayed and return to normal ovulatory cycles can be prolonged (Padula and Macmillan 2005). Overall, there is limited data available on recovery from long-term GnRH agonist treatment. In women, twinning was more frequent among births of infants conceived soon after cessation of oral contraceptive use (Rothman 1977). It was suggested that a mechanism involving the rebound increase of pituitary gonadotrophins may be responsible for increased twinning (Bortolus *et al.* 1999). Further research is required to see if this phenomenon also applies to contraceptive agents using GnRH agonist treatment as oral contraceptives for women are steroid-based.

The duration between treatment and the resumption of breeding, defined as the time to the first successful birth after treatment, is the time-frame which is of most interest to wildlife managers, who aim to control population numbers by reducing offspring. Both the field and the captive study showed that deslorelin can inhibit reproduction for at least one breeding season, while some females remained infertile for at least two years. Individual differences in response to deslorelin may be responsible for this variation, but there was evidence from both studies that a higher dose (9.4 mg deslorelin, 2 implants) may delay the resumption of breeding further compared to a low dose implant. A dose-response relationship has previously been observed with deslorelin treatment in cattle (D'Occhio *et al.* 2000) and dogs (Trigg *et al.* 2001) and this apparent relationship in brushtail possums warrants further investigation.

Given the age structure of this population, deslorelin implants would probably have to be administered two to three times to inhibit reproduction over the lifetime of a female. Females tended to live longer than males and peaked in age class 3 – 4, indicating a mean life span of at least four years before survival declines notably. The effectiveness of deslorelin implants could be greatly advanced if the duration of the contraceptive effect could be extended. It would considerably increase the cost-effectiveness of deslorelin implants as a population management tool if females needed fewer treatments within their lifetime.

The cost of any fertility control operation will depend very much on both the cost of the contraceptive agent and the cost of delivering it to the animals. At this point in time, the use of deslorelin implants requires physically catching the animals to apply treatment. This makes the implant's application labour-intensive and questions the feasibility for large-scale treatment of populations. In our study, it additionally required anaesthesia of females to reduce stress in the animals (they tended to be aggressive if not sedated) and to enable application by a single person. Once the animals were sedated the implants could be very quickly administered subcutaneously. To overcome the need of sedatives, remote administration may be a possibility in the future. Remote delivery is currently not available for deslorelin implants, but research in regard to dart delivery is under way for macropod species and koalas (Herbert, pers. comm.). However, the efficacy of contraceptive projectiles for smaller mammals, especially a nocturnal, arboreal marsupial like the brushtail possum, needs further investigation. A pressurised pistol with bio-bullets may be helpful to quickly deliver contraceptives like deslorelin to trapped possums, therefore eliminating the need to physically restrain or anaesthetise the animals.

At present, deslorelin implants are the only readily available contraceptive for female brushtail possums. The use of a GnRH agonist implant that targets endocrine control of reproduction has additional benefits. Apart from reducing birth rates it also causes desirable behavioural changes such as the inhibition of mating behaviour. Reduced sexual contacts between brushtail possums and possibly reduced agonistic contacts may reduce residential complaints about noisy animals. Inhibition of mating behaviour also helps to manage sexually transmitted diseases such as leptospirosis. Brushtail possums are a maintenance host for *Leptospira interrogans* serovar Balcanica and transmission appears to occur mainly with sexual activity in the breeding season (Day *et al.* 1998; Cowan *et al.* 1991). Individuals may possibly carry this serovar within the Sydney metropolitan area (Eymann *et al.* 2007c, see Chapter 5). Therefore, deslorelin implants could be used to reduce abundance and disease transmission concurrently.



Public opinion can often drive wildlife management practices, particularly in urban areas. The public acceptability of a particular fertility control technique may determine if it is really useful in the current management context. Some householders in this study did not want 'their' brushtail possums treated with contraceptives as they encourage and enjoy their presence. However, if fertility control is to succeed, it will require applying contraceptive agents at the population level, not at the level of individual properties. If fertility control is feasible for (sub)urban neighbourhoods will require thorough debate.

The scenario may be different for small localised areas, and deslorelin implants appear most suitable for small-scale local usage, such as small islands or botanical and zoological gardens. In this respect, deslorelin implants deserve further attention to investigate their effects on natural brushtail possum populations. Their potential to have an effect on the local population level could be addressed by studying a population confined to a small area, such as a botanical garden. This approach should ideally be combined with attempts to make the environment less attractive to the animals. For management purposes, the optimum time of treatment application in this species would be the period of anestrus (December – February), before any newborn are present and before the initiation of seasonal oestrus (main breeding season autumn). If the majority of females within a defined area were treated, it could be assessed how long it takes for the population to actually decline and if the effects of fertility control can be swamped by immigration from neighbouring brushtail possum populations. In New Zealand, immigration by yearling-aged brushtail possums compensated for reductions in local recruitment and it was suggested that fertility control would need to be applied at large scales to reduce this effect (Ramsey *et al.* 2005).

Knowledge of the social structure and mating system of a specific population is crucial before population control is contemplated or even attempted (Caughley *et al.* 1992). It is also essential to know the dynamics of the targeted population and to model the potential impacts of fertility control. Modelling should utilise population parameters site-specific to the target population as brushtail possum populations vary widely in age structure, reproduction and mortality across the different locations. Our

field study documented population parameters for brushtail possums within Sydney metropolitan area – for the first time. Long-term data will be required to elucidate the complex patterns of population dynamics within any brushtail possum population, but the data presented in this paper could form the basis of a population model to predict the outcomes of any potential fertility control operations.

In summary, deslorelin implants are an effective contraceptive in female brushtail possums and treated females appeared to retain their site-fidelity. However, wide-scale application of this form of fertility control would most likely require increased longevity of contraception to ensure cost-effectiveness and modifications to the implant administration procedure to negate the need for animal sedation. The clear seasonality of reproduction in this (and other) populations means that large numbers of animals can be treated during the non breeding season with confidence that very few would already be expecting or having offspring. Although the management goals for brushtail possums differ between Australia and New Zealand (Cowan 2000; McDowell *et al.* 2006; Eymann *et al.* 2006a), results from this study provide evidence that reproduction in females can be inhibited through disruption of GnRH. Slow-release GnRH agonist implants could be a useful management tool in Australia for ‘problem’ populations in small localised areas, such as small islands or botanical and zoological gardens. As such, deslorelin implants deserve further attention to investigate their effects on such populations.

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## Supplemental data

Table 5. Reproductive parameters summarised for control (a) and deslorelin treated females with (b) low dose, 5mg, and (c) high dose, 10mg, and additionally captured adult females during the study period. In treated females, the resumption of breeding after natural 'expiry' of the implant's contraceptive effect is shown.

a)

Female control group (n=15)	Implant insertion (Date)	Reproductive status at implant insertion (estimated PY date of birth) <sup>a</sup>	Recapture dates <sup>b</sup> (Aug 03 – Oct 04)	Reproductive status at recapture (after implant insertion; estimated PY date of birth)	Time to first/second PY (after implant insertion) (Days)	No. PY (after implant insertion)
C1 (10)	15-Jan-03	NY, RT	–	–	–	–
C2 (15)	16-Jan-03	NY, RT	23-Aug-03	<b>PY</b> (20-Apr-03)	94	1
C3 (21)	20-Jan-03	<b>PY</b> (12-Oct-02)	22-Aug-03 04-Nov-04	NY ET	– –	(0)
C4 (34)	13-Feb-03	<b>PY</b> (14-Jan-03)	08-Oct-03 15-Sep-04	<b>PY</b> (19-Aug-03) <b>PY</b> (23-Apr-04)	187 (435)	2
C5 (57)	07-Mar-03	NY, NA	–	–	–	–
C6 (59)	14-Mar-03	<b>PY</b> (04-Nov-02)	–	–	–	–
C7 (70)	25-Mar-03	NY, ET	14-Oct-03 07-Sep-04	<b>PY</b> (03-Oct-03) <b>PY</b> (12-May-04)	192 (414)	2 <sup>c</sup>
C8 (77)	28-Mar-03	NY, NA	18-Sep-03 10-Aug-04	NY, ET <b>PY</b> (27-May-04)	– (426)	1
C9 (79)	28-Mar-03	<b>PY</b> (16-Jul-02)	– 12-Aug-04	– <b>PY</b> (15-Mar-04)	– (353)	1
C10 (80)	02-Apr-03	NY, NA	09-Sep-03	NY, NA	–	0
C11 (82)	02-Apr-03	NY, NA	09-Sep-03 11-Aug-04	NY, NA NY, NA	– –	0
C12 (99)	26-Aug-03	<b>PY</b> (28-Apr-03)	–	–	–	–
C13 (107)	05-Sep-03	<b>PY</b> (28-Apr-03)	–	–	–	–
C14 (127)	23-Sep-03	<b>PY</b> (13-Sep-03)	–	–	–	–
C15 (143)	23-Oct-03	<b>PY</b> (28-Sep-03)	–	–	–	–

<sup>a</sup> NY= no young; PY=pouch young; BY=back young; NA= no signs of recent activity; (ET) elongated teat with associated mammary activity (young nearby or very recent loss of young), or (RT) regressing teat (no milk flow; recent loss or weaning of young).

<sup>b</sup> Control no. C12 – C15 were added to the control group at the second trapping event.

<sup>c</sup> This female had a second young adopted in September 2004, see appendix A.

b)

Female deslorelin treated group (5mg dose, <i>n</i> =11)	Implant insertion (Date)	Reproductive status (at implant insertion) <sup>a</sup>	Recapture dates (Nov 02 – Apr 05) <sup>e</sup>	Reproductive status at recapture (after implant insertion; estimated PY date of birth)	Time to first PY (after implant insertion) (Days)	Contra- ception duration (days)	No. PY (after implant insertion)
T16 (2)	06-Nov-02	<b>PY</b> (07-Oct-02)	–	–	–	–	–
T17 (9)	15-Jan-03	NY, RT	–	–	–	–	–
T18 (19)	19-Jan-03	NY, RT	21-Aug-03 30-Oct-04	NY, NA <b>BY</b> (12-Jun-04; 12-Jun-04)	– 510	– 510	<b>2<sup>f</sup></b>
T19 (25)	21-Jan-03	NY, NA	28-Aug-03 04-Nov-04	NY, NA NY, NA	– –	– ≥ 653	0
T20 (29)	10-Feb-03	NY, NA	08-Oct-03 16-Sep-04	NY, NA <b>PY</b> (01-Sep-04)	– 569	– 569	1
T21 (45)	25-Feb-03	NY, NA	23-Sep-03 20-Aug-04	NY, NA NY, NA	– –	– ≥ 542	0
T22 (48)	26-Feb-03	NY, NA	31-Oct-03 31-Aug-04	NY, NA <b>PY</b> (02-Jul-04; 16-Aug-04)	– 492; 537	– 492	<b>2<sup>f</sup></b>
T23 (53)	04-Mar-03	NY, NA	19-Sep-03 25-Aug-04	NY, NA <b>PY</b> (04-Jul-04)	– 488	– 488	1
T24 (66)	19-Mar-03	<b>BY</b> (05-Oct-02)	22-Oct-03 06-Oct-04	NY, NA <b>PY</b> (08-Sep-04)	– 539	– 539	1
T25 (72)	25-Mar-03	<b>BY</b> (06-Oct-02)	–	–	–	–	–
T26 (129)	25-Sep-03	<b>PY</b> (08-May-03)	–	–	–	–	–

<sup>e</sup> Female no. T26 was added to the deslorelin low dose treatment group at the second trapping event.

<sup>f</sup> These two females had double offspring.

c)

Female deslorelin treated group (10mg dose, <i>n</i> = 15)	Implant insertion (Date)	Reproductive status at implant insertion <sup>a</sup> (estimated PY date of birth)	Recapture dates (Nov 02 – Apr 05) <sup>d</sup>	Reproductive status at recapture (after implant insertion; estimated PY date of birth)	Contra- ception duration (Days)	No. PY (after implant insertion)
T1 (4)	07-Nov-02	<b>PY</b> (15-Jul-02)	– 05-Aug-04	– NY, NA	– ≥ 637	0
T2 (6)	12-Nov-02	NY, RT	– 06-Aug-04 21-Dec-04 05-Apr-05	– NY, NA NY, NA NY, NA	– – – ≥ 875	0
T3 (7)	15-Jan-03	NY, RT	19-Aug-03	NY, NA	≥ 216	0
T4 (14)	16-Jan-03	NY, NA	21-Aug-03 30-Oct-04	NY, NA NY, NA	– ≥ 653	0
T5 (23)	21-Jan-03	NY, RT	27-Aug-03 04-Nov-04	NY, NA NY, NA	– ≥ 653	0
T6 (28)	10-Feb-03	NY, RT	08-Oct-03 14-Sep-04 23-Dec-04	NY, NA NY, NA NY, NA	– – ≥ 682	0
T7 (39)	20-Feb-03	<b>BY</b> (15-Jun-03)	03-Sep-03	NY, NA	≥ 195	0
T8 (43)	21-Feb-03	NY, NA	–	–	–	–
T9 (46)	25-Feb-03	<b>PY</b> (23-Oct-02)	–	–	–	–
T10 (51)	27-Feb-03	NY, NA	– 03-Aug-04	– NY, NA	– ≥ 523	0
T11 (54)	06-Mar-03	NY, NA	03-Sep-03	NY, NA	≥ 181	0
T12 (62)	19-Mar-03	NY, NA	15-Oct-03	NY, NA	≥ 210	0
T13 (92)	20-Aug-03	<b>PY</b> (16-Jun-03)	30-Oct-04	NY, NA	≥ 437	0
T14 (112)	11-Sep-03	NY, ET	04-Aug-04 13-Apr-05	NY, NA NY, NA	– ≥ 580	0
T15 (121)	18-Sep-03	<b>PY</b> (11-May-03)	–	–	–	–

<sup>d</sup> Females no. T13 – T15 were added to the deslorelin high dose treatment group at the second trapping event.

## d) Additionally captured adult females

Females additionally caught ( <i>n</i> =17)	First capture (Date)	Reproductive status at first capture (PY date of birth) <sup>a</sup>	Recapture dates (Aug 03 – Apr 05)	Reproductive status at recapture (after implant insertion; estimated PY date of birth)	No. PY
A1 (86)	15-Aug-03	<b>PY</b> (27-Apr-03)	–		1
A2 (153)	10-Aug-04	<b>PY</b> (02-May-04)	–		1
A3 (156)	11-Aug-04	<b>PY</b> (03-Apr-04)	–		1
A4 (162)	19-Aug-04	<b>PY</b> (11-May-04)	–		1
A5 (168)	25-Aug-04	<b>PY</b> (07-Apr-04)	–		1
A6 (171)	26-Aug-04	NY, NA	–		0
A7 (177)	07-Sep-04	NY, ET	–		(0)
A8 (182)	14-Sep-04	<b>PY</b> (17-May-04)	21-Dec-04 06-Apr-05	Not examined <b>PY</b> (03-Apr-05)	2
A9 (189)	29-Sep-04	NY, ET	–		(0)
A10 (194)	06-Oct-04	<b>PY</b> (18-Jun-04)	–		1
A11 (197)	21-Dec-04	<b>BY</b> (04-Jun-04)	–		1
A12 (210)	06-Apr-05	<b>PY</b> (18-Mar-05)	–		1
A14 (217)	13-Apr-05	<b>PY</b> (25-Mar-05)	–		1
A15 (219)	20-Apr-05	NY, NA	–		0
A16 (220)	20-Apr-05	<b>PY</b> (08-Apr-05)	–		1
A17 (222)	20-Apr-05	<b>PY</b> (10-Apr-05)	–		1
A18 (225)	22-Apr-05	<b>PY</b> (31-Mar-05)	–		1

Table 6. Reproductive parameters of juvenile females that were captured in adulthood.

Juvenile females recaptured in adulthood * ( <i>n</i> = 3)	Captures (Date)	Estimated date of birth, age [months] and weight [kg] at capture	Reproductive status at capture <sup>a</sup> (estimated PY date of birth)	No. PY
J1 (24)	21-Jan-03	26-May-02; 7 mths, 1.1 kg	NY, NA	0
	27-Aug-03	15 mths, 1.75 kg	NY, NA	0
	05-Nov-04	30 mths, 2.2 kg	ET	(1)
J2 (40)	20-Feb-03	15-Jun-02; 8.5 mths, 1.35 kg	NY, NA	0
	03-Sep-03	15 mths, 1.85 kg	NY, NA	0
	06-Aug-04	26 mths, 2.1 kg	NY, NA	0
J3 (96)	23-Aug-03	20-Apr-03; 4 mths, 0.25 kg	NY, NA	0
	04-Nov-04	19 mths, 1.95 kg	ET, observed with back young	1

\* All females were recaptured on the same residential properties throughout the study period.



## Chapter 4

# **Serologic survey for *Toxoplasma gondii* and *Neospora caninum* in the common brushtail possum (*Trichosurus vulpecula*) from urban Sydney, Australia**

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## Abstract

The common brushtail possum (*Trichosurus vulpecula*) has adapted well to increasing urbanization, resulting in greater interaction with humans and their domestic pets. Wildlife species in urban areas face a higher risk of exposure to zoonotic pathogens and may be affected by parasites hosted by cats (*Toxoplasma gondii*) or dogs (*Neospora caninum*), yet it is unknown to what extent urban brushtail possums are exposed to these parasites. Antibodies to *T. gondii* and *N. caninum* were assayed in sera of 142 adult brushtail possums from the city of Sydney, Australia. Using the modified agglutination test, antibodies to *T. gondii* were found in 9 (6.3%) of the 142 animals in titers of 1:25 (4), 1:50 (1), 1:100 (1), 1:800 (1), 1:3,200 (1), 1:6,400 (1), and 1:12,800 (1). For some brushtail possums multiple sera samples within a 2-yr frame could be collected, but seropositive animals in general were not recaptured after initial seroconversion. One animal had a high *T. gondii* titer on 2 consecutive bleedings, 14 mo apart, and seropositive animals appeared normal when captured. Sex seemed not to have an effect on antibody prevalence, but age and location may play a role. Antibodies to *N. caninum* were not detected in 1:25 dilution of sera in the *N. caninum* agglutination test, indicating that brushtail possums may not have been exposed to this parasite. This is the first serological survey for *T. gondii* and *N. caninum* infections in urban brushtail possums.

## Introduction

The common brushtail possum (*Trichosurus vulpecula*) is a solitary, nocturnal, general herbivorous, arboreal marsupial in its native Australian environment (Kerle, 2001). This cat-sized phalanger generally occurs where there are trees, but has disappeared from large parts of its former natural habitat, including all of arid Australia (Goldingay and Jackson, 2004). Declines are attributed to a combination of factors, including habitat loss and disturbance, disease, drought, and impact of predators on depleted populations. Conversely, the brushtail possum is quite common in many Australian cities, and it is a major introduced pest in New Zealand, damaging native forests and spreading bovine tuberculosis



(Montague, 2000). This creates distinctive management issues for brushtail possums. Our research focuses on Australian urban areas where they cohabitate with people and their domestic pets, taking up residence in house roofs and browsing on garden plants (Eymann et al., 2006). Wildlife species that live in urban areas are increasingly likely to come into contact with both domestic pets and humans, suggesting the possibility of disease transmission. Emerging infectious diseases are associated with a range of factors, including the interaction of zoonotic pathogens with wildlife, domestic animals, and human populations (Daszak et al., 2000; Brown, 2004). Emerging infectious diseases of wildlife include “spill-over” from domestic animals to wildlife populations living in close proximity and also may be directly related to human intervention, via host or parasite translocations. Many infectious organisms originate from humans and their companion animals (Fayer et al., 2004), and Australians keep at least 1.25 million cats and 1.5 million dogs (English, 1982).

*Toxoplasma gondii* is among the most frequently reported parasites of humans and animals worldwide (Dubey and Beattie, 1988; Tenter et al., 2000; Dubey and Odening, 2001). This coccidium uses felids as the definitive host and warm-blooded animals as intermediate hosts. Oocysts are shed only in the feces of infected cats. Australian marsupials have evolved in the absence of *T. gondii* and have only recently been exposed to the parasite as there were no cats in Australia before European settlement (Dubey and Beattie, 1988). This makes marsupials highly susceptible for toxoplasmosis, and infection can prove fatal in captive and free-ranging populations. It has serious implications for zoological gardens exhibiting susceptible animals, such as kangaroos, in close proximity to felids (Spencer et al., 2003), and there are numerous reports of deaths in zoos (reviewed in Dubey and Odening, 2001). Toxoplasmosis may remain clinically inapparent, causing sudden death without clinical signs in animals that often have good body condition (Canfield et al., 1990; Obendorf and Munday, 1990). If stress reduces immunocompetence, the parasite may multiply and cause a range of symptoms, including lethargy, unnatural daytime activity, inappetence, respiratory distress, and neurological disturbances. Nutritional and weather stresses are considered possible factors causing latent *T. gondii* infection to become

clinically obvious and subsequently fatal (Obendorf and Munday, 1983). *Toxoplasma gondii* infection has been found in a number of wild Australian marsupials, including macropods (Johnson et al., 1988, 1989), eastern barred bandicoots (*Perameles gunnii*) (Obendorf et al., 1996), quokkas (*Setonix brachurus*) (Gibb et al., 1966), dasyurids (*Dasyuroides byrnei*) (Attwood et al., 1975), and wombats (*Vombatus ursinus*) (Hartley and English, 2005). The brushtail possum previously has been diagnosed with toxoplasmosis (Cook and Pope, 1959; Presidente, 1984; Canfield et al., 1990; Viggers and Spratt, 1995), including animals from the Sydney metropolitan area (Obendorf et al., 1998); Hartley (1993) found *T. gondii* encephalitis in an unknown number of brushtail possums held at Taronga Zoo Pathology, Sydney. However, the prevalence of *T. gondii* in brushtail possum populations remains unknown. A serological survey of brushtail possums from Kangaroo Island, South Australia, detected no antibodies to *T. gondii* in 30 brushtail possum sera tested (Callaghan and Moore, 1986). Cats may contaminate urban areas, which places brushtail possums at risk, as they commonly feed on the ground (MacLennan, 1984). Brushtail possums may be infected by accidental ingestion of food or water contaminated with oocysts from infected cat feces.

*Neospora caninum* is a recently recognized coccidian biologically similar to *T. gondii* (Dubey et al., 1988; Dubey, 2003). The dog (*Canis domesticus*) and the coyote (*Canis latrans*) are its definitive hosts (McAllister et al., 1998; Gondim et al., 2004). Although there are reports of *N. caninum* infections in many species of animals (for review see Dubey, 2003), including the South American opossum (*Didelphis marsupialis*) (Yai et al., 2003), it has not been reported in brushtail possums yet. The objective of this study was to investigate seroprevalence of *T. gondii* and *N. caninum* in urban brushtail possums and to analyze any association between seroprevalence and sex, age, body condition, and geographic location.

## Materials and Methods

### Collection of samples

Brushtail possum blood samples were collected in the garden area of 35 residential houses from five localities within the Sydney metropolitan area, New South Wales, Australia (Fig. 1). Locations included four North Shore mainland suburbs (Beecroft, Chatswood West, North Epping and Pymble) and Scotland Island, a 52 ha land mass located in Pittwater. The environment of Scotland Island is similar to that of the sampled mainland suburbs, with high residential levels and dense surrounding vegetation including many trees. Attempts were made to recapture brushtail possums in consecutive trap nights on four different occasions between November 2002 and April 2005 to allow repetitive monitoring. Seven samples from an opportunistic field trip to Myall Lakes National Park, NSW also were included in this study. In total, 195 blood samples from 142 individuals of both sexes and varying ages were collected.

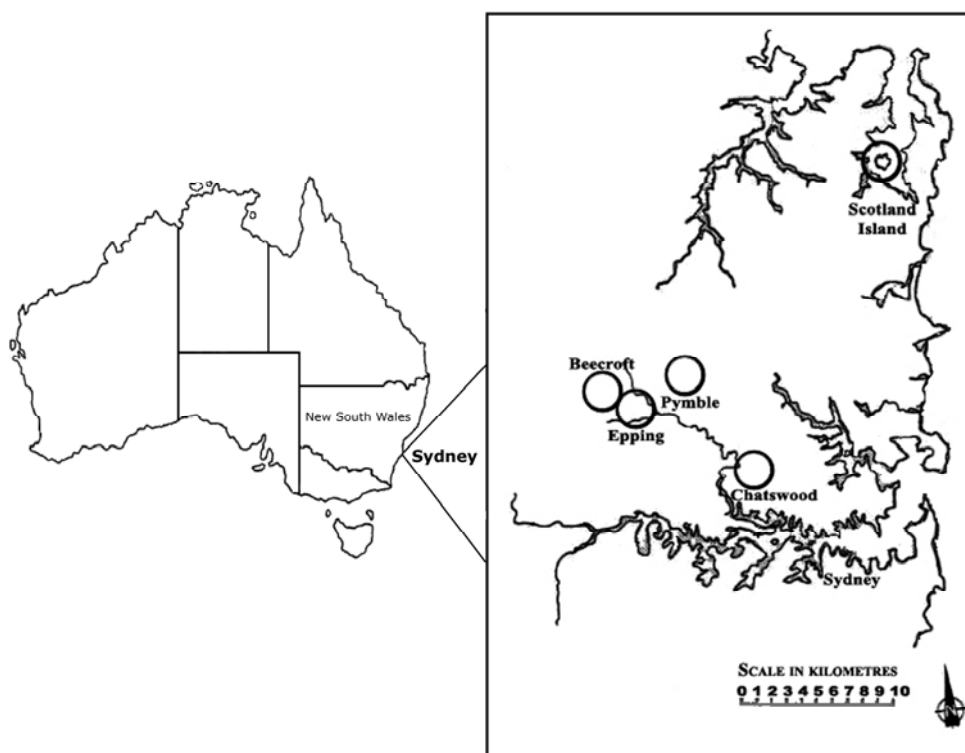


Figure 1. Map of Australia showing Scotland Island and 4 Sydney metropolitan suburbs in New South Wales – Beecroft, Chatswood West, North Epping and Pymble – from which brushtail possums (*Trichosurus vulpecula*) were sampled.

## Brushtail possum handling

Animals were caught overnight in brushtail possum cage traps (0.6 m x 0.3 m x 0.3 m) baited with apple and peanut butter and were examined the following morning (Duckworth and Meikle, 1995). They were physically restrained in hessian bags while anaesthetic was administered into the gluteal muscle using a combination of ketamine (20 mg/kg) and xylazine (2 mg/kg). Animals were microchipped, sexed, aged, and weighed, and general appearance was noted. Individuals were classified as adult if they were 2 kg or higher and had class 2 or higher teeth wear (Winter, 1980; Cowan and White, 1989). Tooth wear gives only an indication of the brushtail possum's age; for example, class 2 equals an age of 1.0- to 1.5-yr-old, class 3 equals 0.9- to 3.6-yr-old, class 4 equals 2.6- to 5.4-yr-old, etc., and brushtail possums may live up to 14 yrs in captivity (Kerle, 2001), but annual survival of wild animals declines from age 5 (Efford 2000; Isaac 2005). Individual body weight was used as an indicator of a brushtail possum's condition over the years. Blood (3 ml) was collected from either the ventral tail vein or preferably from a jugular vein of each adult animal. Serum was separated and stored frozen at -20 °C until processed. Animals were held in hanging hessian bags and released after sunset (when dark) the same day, allowing sufficient time to recover from sedation and to minimize handling-induced stress in this nocturnal mammal.

## Serological examinations

All serological examinations were conducted at the Animal Parasitic Diseases Laboratory, Beltsville, Maryland. Antibodies against *T. gondii* and *N. caninum* were detected using agglutination tests, which included the parasite specific antigens. For *T. gondii* the modified agglutination test (MAT) as described by Dubey and Desmonts (1987) was used, and sera were diluted 2-fold starting at the 1:25 dilution. For *N. caninum*, the test was performed as described by Romand et al. (1998), and sera were screened at 1:25 serum dilution. These agglutination tests measure only IgG antibodies; therefore, acute infections may not be detected. Although there is no information on the sensitivity and specificity of MAT for diagnosis of toxoplasmosis in brushtail possums, based on a validation study of the MAT in pigs

naturally and experimentally infected with *T. gondii* (Dubey, 1997), a MAT titer of  $\geq 1:25$  was considered an indicator of *T. gondii* exposure and termed “seropositive”.

## Statistical analysis

The statistical analysis was performed using the Vassar Stats web site for statistical computation (<http://faculty.vassar.edu/lowry/VassarStats.html>). Seroprevalence was analyzed considering the variables of sex, age and location (sampling sites). The effect of sex on infection was analyzed by binomial probabilities. For comparison of age groups and different locations the chi-square test was used. As animals were trapped over yrs, the estimated age group of brushtail possums used for statistical analysis was for seropositive animals when they first tested positive and for the seronegative ones when they were last trapped. Differences were considered statistically significant when  $P \leq 0.05$ . However, sample sizes for infected brushtail possums were low; therefore, statistical results should be interpreted with caution.

## Results

Antibodies to *T. gondii* were found in 9 (6.3%) of 142 individual brushtail possums and in 10 (5.1%) of 195 serum samples. Seropositive animals were observed in nearly all suburbs: 0 (0%) of the 4 from Beecroft, 2 (14.3%) of the 14 from Chatswood West, 4 (18.2%) of the 22 in North Epping, 1 (1.6%) of the 61 in Pymble, and 2 (5.9%) of the 34 on Scotland Island, but not in Myall Lakes National Park (0 [0%] of the 7). The variation in seroprevalence of *T. gondii* within the different suburbs, from 0% (Beecroft) up to 18.2% (North Epping), was not significant ( $\chi^2 = 7.83$ ,  $P = 0.1$ ,  $df = 4$ ). Seropositivity ranged from a titer of 1:25 (Brushtail possum nos. 48, 96, 139, and 140), 1:50 (no. 184), 1:100 (no. 29), 1:800 (no. 141), 1:3,200 (no. 72), and 1:6,400 (no. 101) up to a titer of 1:12,800 (no. 101) (Table 1).

Table 1. Individual *Toxoplasma gondii* antibody titers in sera of 142 brushtail possums.

Antibody titer	No. of positive sera (%)	Animal ID
1:25	4 (2.8)	48, 96, 139, 140
1:50	1 (0.7)	184
1:100	1 (0.7)	29
1:800	1 (0.7)	141
1:3,200	1 (0.7)	72
1:6,400	1 (0.7)	101
1:12,800	1 (0.7)	101
Total positive	9 (6.3)	

Table 2 presents the trapping and serum sampling record of each positive tested animal. There was no apparent sex-biased seropositivity; both females (4) and males (5) had been exposed (binomial  $p = 1.0$ ). Seropositive brushtail possums were found mainly in the age group 3 (7 of 9 = 77.8%), which indicates an age of 0.9- to 3.6-yr-old. The majority of seronegative animals were also found in the age group 3 (43 of 133 = 32.3%) or age group 4 (32 of 133 = 24.1%). Few animals were older (16 of 142 = 11.3%), 13.4% (19 of 142) were younger (age group 2) and 16.9% (24 of 142) of adults from initial trapping were not aged. The differences between age groups were not significant ( $\chi^2 = 8.11$ ,  $P = 0.09$ ,  $df = 4$ ). Body weight of seropositive converted brushtail possums usually did not decrease compared to their previous weight when seronegative (apart from no. 29), suggesting there was no loss in body condition, and they remained within the normal adult brushtail possum weight range. Five animals were not recaptured after exposure to *T. gondii* was detected (nos. 29, 72, 139, 140, 184), 1 tested negative on the 3<sup>rd</sup> time caught (no. 48), although seropositivity of 1:25 was found on the 2<sup>nd</sup> time caught. The other brushtail possums (nos. 96, 101, 141) were found to have *T. gondii* antibodies at the end of the sampling period, so their fate remains unknown. However, animal no. 101 was found seropositive on 27 August 2003 (1:12,800) and 4 November 2004 (1:6,400), indicating that it survived exposure to *T. gondii* for more than 14 mo.

Table 2. *Toxoplasma gondii* antibody titers of 9 affected brushtail possums including their capture records, sex, age, weight and location.

Animal ID	MAT titre	Date of (re)capture	Sex	Age group	Weight [kg]	Location (suburb, house)
29	No sample	10 Feb 03	Female	2	1.65	Chatswood 6
	Negative	08 Oct 03		3	2.4	As above
	1:100	16 Sep 04		3	2.2	As above
	—	Not recaptured (Dec 04; Apr 05)		—	—	As above
48	Negative	26 Feb 03	Female	3	2.75	Pymble 22
	1:25	31 Oct 03		3	3.1	As above
	Negative	31 Aug 04		4	2.95	As above
72	1:3,200	25 Mar 03	Female	Not aged	2.55	Epping 5
	—	Not recaptured (Oct 03; Sep 04)		—	—	As above
96	No sample	23 Aug 03	Female	130 days	0.25	Scotland Isl. 35
	1:25	04 Nov 04		2–3	1.95	Scotland Isl. 32
101	1:12,800	27 Aug 03	Male	3	2.3	Scotland Isl. 34
	1:6,400	04 Nov 04		3	2.5	As above
139	1:25	15 Oct 03	Male	3	2.6	Epping 3
	—	Not recaptured (Oct 04; Apr 05)		—	—	As above
140	1:25	15 Oct 03	Male	3	2.8	Epping 3
140	—	Not recaptured (Oct 04; Apr 05)		—	—	As above
141	Negative	21 Oct 03	Male	3	2.5	Epping 2
	1:800	07 Oct 04		3	2.6	As above
184	1:50	15 Sep 04	Male	4	2.75	Chatswood 6
	—	Not recaptured (Dec 04; Apr 05)		—	—	As above

By comparison, 57–35% of the seronegative brushtail possums were retrapped on subsequent trapping events (declining recapture success with increasing time). Seropositive animals sometimes were caught on the same property, observed for nos. 29 and 184 (Chatswood 6) and nos. 139 and 140 (North Epping 3), perhaps indicating a common source of infection (Table 2).

Nine householders (25.7%) owned cat(s), of which 5 had their cat(s) free-ranging in the backyard during daytime including house 2 North Epping and house 35 on Scotland Island.

Antibodies to *N. caninum* were not found in any of the 195 samples tested. Seven householders (20%) owned dog(s); some of these allowed their dogs to defecate frequently in the backyard.

## Discussion

The results of this study show that urban brushtail possums are likely to be exposed to *T. gondii* in their environment, but no evidence for exposure to *N. caninum* was found. Similar or higher levels of exposure to *T. gondii* were found in studies on other Australian marsupials. A study on wild bandicoots revealed that 6.7% of trapped animals (150) had antibodies to *T. gondii* (Obendorf et al., 1996), whereas infection was found in more than 21% of 150 quokkas living near the settlement on Rottnest Island (Gibb et al., 1966). The high titers recorded from some of our samples indicated current or recent infection with *T. gondii* (Table 1). Exposure to *T. gondii* was linked to particular backyards for several brushtail possums (Table 2). Animals were found seropositive in the same backyards at both Chatswood and North Epping, yet not all brushtail possums trapped in these backyards tested seropositive. It is difficult to locate the exact source of infection as presence of free-range domestic cats and seropositive animals are not necessarily linked. Householders may have free-range cats, yet brushtail possums on these properties were seronegative, and vice versa. Brushtail possums may become infected on a neighboring property contaminated with *T. gondii* oocysts, or stray and feral cats may have shed oocysts in the affected backyard. Therefore, the exact location of infection cannot be determined as brushtail possums and cats may move through several properties having extended home



ranges. The home range area for urban brushtail possums from Launceston, Tasmania was found to be sex-dependent with females usually having an average home range of 2 ha, although males may move within a range of up to 10.9 ha (Statham and Statham, 1997). Diurnal home range areas of suburban domestic cats in Canberra ranged from 0.02 to 17.19 ha with a mean of 2.73 ha (Barratt, 1997). In general it can be presumed that opportunities for exposure to *T. gondii* are available and depend on the presence of oocysts in a particular area reflected by the varying extent of seropositive brushtail possums in different suburbs.

There was no evidence that seropositivity may be sex-linked either, although males perhaps have a greater chance of encountering oocysts because of their larger home ranges compared to females. The majority of seropositive brushtail possums were found in age group 3 (0.9- to 3.6-yr-old), however the majority of adults trapped also fell into age group 3–4 (up to 5.4 yrs old), and few older animals were caught. It is a common pattern for many host species and disease agents that, as an animal ages, its cumulative likelihood for exposure increases (Zarnke et al., 2000). In wild brushtail possum populations in New Zealand (Efford, 2000) and on Magnetic Island, Australia (Isaac, 2005) annual survival peaked for animals age 2 – 5 yrs. This corresponds with our age data and a sound knowledge of the average longevity of the animals helps to interpret potential impacts of the disease on population structure.

Seropositive brushtail possums were usually not recaptured; only 1 animal (no. 101) had antibodies to *T. gondii* on 2 bleedings 14 mo apart. Recapture success for seronegative brushtail possums declined over the sampling period from 57–35% and is influenced by further factors such as exposure to other diseases, illegal relocation of brushtail possums, road kill and/or trap shyness (Eymann et al., 2006). One brushtail possum (no. 48) seroconverted from a positive titer of 1:25 to a negative titer; whether this change was related to technique, passively or actively acquired antibodies, or a result of the passage of time was not known. These findings are similar to those reported in bandicoots (Obendorf et al., 1996) and suggest that most animals exposed to *T. gondii* do not survive for long periods post-infection. Occasionally they may remain asymptotic. Some animals may not even survive initial infection and die

of acute toxoplasmosis before IgG antibodies can be detected (Johnson et al., 1989; Canfield et al., 1990; Lynch et al., 1993; Skerratt et al., 1997).

Antibodies to *N. caninum* were not found in brushtail possums. The exposure of brushtail possums may depend on the presence of *N. caninum* oocysts, which may be less widespread in the environment. It is known that the dog sheds relatively few oocysts when infected (McAllister et al., 1998), but little is known at present regarding the frequency of shedding of *N. caninum* oocysts by canids, the resistance of the oocysts, and whether dogs shed oocysts more than once (Dubey, 2003). Reichel (2000) reported that 5–15 % of Australian dogs had antibodies to *N. caninum*.

The difference in seroprevalence between *T. gondii* and *N. caninum* found in this study may be due to the fact that *T. gondii* is a very successful parasite, and its definitive host, the cat, can shed very large numbers of oocysts in its feces, producing substantial environmental contamination. Oocysts in the soil can be spread mechanically by flies, cockroaches and earthworms and a single live oocyst is enough to infect a pig orally (Dubey et al., 1996). Survival of *T. gondii* oocysts in the environment (and thus the potential reservoir of infection) may be an influencing factor on the differing seroprevalence: *T. gondii* oocysts can survive for 1 yr or more (Yilmaz and Hopkins, 1972), although at present it is not known how long *N. caninum* oocysts persist in the environment. A seroprevalence study on a population of domestic cats in Melbourne showed that more than 38% of 103 tested cats had *T. gondii* positive IgG titers (Sumner and Ackland, 1999) and in a Sydney study 50% of 80 domestic cats were seropositive (Watson et al., 1982). Cats are infectious only for a short period before acquiring immunity, but millions of oocysts may be released in the feces in a single day. Although only few cats may be shedding *T. gondii* oocysts at any given time, the enormous numbers produced and their resistance to destruction ensure widespread contamination (Dubey, 2004). Little is known of treatment and prophylaxis, and at present there is no vaccine to control toxoplasmosis in humans, cats, or wild animals (Lynch et al., 1993; Reddacliff et al., 1993; Dubey and Odening, 2001; Bhopale, 2003). Therefore control of feral cat numbers and keeping domestic cats indoors would be the only rational approach to prevent urban

contamination with *T. gondii* oocysts – an approach that would also protect native urban wildlife from preying cats (Grayson and Calver, 2004).

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## Chapter 5

# Leptospirosis serology in the common brushtail possum (*Trichosurus vulpecula*) from urban Sydney, Australia

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**Abstract:**

The common brushtail possum (*Trichosurus vulpecula*) is a familiar species in major cities of Australia. There is concern that abundant wildlife in close proximity to people and their domestic pets may face an increased risk of disease exposure, hence facilitating the transmission of certain pathogenic agents. Brushtail possums are known to be susceptible to leptospirosis and are considered a maintenance host for *L. interrogans* serovar Balcanica. A total of 192 brushtail possum blood samples was collected from suburban areas of metropolitan Sydney from November 2002 through to November 2004. The sera of 136 individuals were screened against a reference panel of 21 *Leptospira* spp. using the microscopic agglutination test (MAT). Leptospiral antibodies were detected in 9.6% (13/136) of the sera and represented two serovars, of which *L. interrogans* serovar Hardjo was most frequently identified (11/136). A representative of the serogroup Ballum was found in (2/136) of brushtail possums and regarded as exotic to Australia. Exposure to leptospirosis seemed to be linked to age with older animals having a higher incidence, but there was no distinction in relation to gender. The seroprevalence varied between the different sampling sites and several seropositive brushtail possums emerged from very few backyards only. Our results support that transmission of leptospirosis not only occurs through a contaminated environment alone, but may also be the result of affiliate and sexual behavior. The potential of brushtail possums as a maintenance host in urban environments and the likelihood of impacts on public health, if any, will be discussed.

## Introduction

The common brushtail possum (*Trichosurus vulpecula*) is the most familiar of the Australian possums as it often cohabits with people (Kerle, 2001). This species has a reputation both as 'champions of the suburbs' and 'tormentors' for taking up residence in house roofs and browsing on garden plants (Matthews et al., 2004). There is concern that the close association of wildlife with humans and their domestic pets may result in 'spill over' of diseases (Daszak et al., 2000). An example is Toxoplasmosis, a parasitic disease hosted by cats, which is prevalent in urban brushtail possum populations (Eymann et al., 2006). No further studies, however, have assessed the health and condition of these animals in urban areas. Monitoring the health of brushtail possums is important to analyze the impact of diseases on urban populations. Surveillance could verify if they act as reservoirs and sources of infection, and will help to understand the three-dimensional relationship and potential for disease transmission between brushtail possums, pets and people. Such measurements would enable an appropriate understanding of the health of populations (Moerner et al., 2002).

Leptospirosis is a zoonotic bacterial disease of worldwide importance that can affect humans, domestic and wild animals (Levett, 2001). These bacteria are spread through the urine of infected animals. Transmission occurs directly from a mammalian host or through indirect transmission via contact with *Leptospira* contaminated water or soil. Leptospire shed in urine may remain infective in water and soil for more than two months under favorable conditions (Faine et al., 1999). Leptospirosis has also been reported from urban environments (Bharti et al., 2003). Dogs and rats are a recognized source of infection for humans (Jansen et al., 2005). Feral rats carrying pathogenic strains were identified as the source of infection for inner-city Baltimore residents and leptospirosis is considered to become increasingly problematic in deteriorating inner cities with expanding rat populations (Vinetz et al., 1996).

Although wildlife species are often reported to be potentially important reservoirs of *Leptospira* serovars, few surveys have investigated the potential of wildlife maintenance hosts in urban and recreational

areas. Leptospirosis was present in raccoons from Quebec (Mikaelian et al., 1997), coyotes in Tucson, Arizona (Grinder and Krausman, 2001) and in Connecticut peridomestic wildlife including raccoons, skunks and squirrels (Richardson and Gauthier, 2003). Some *Leptospira* serovars are indigenous to Australia and native animals need to be recognized as a source of infection (Slack et al., 2006). Contact with kangaroos, wallabies, native rats, bandicoots and possums was reported in some human cases of leptospirosis.

The brushtail possum is a major introduced pest to New Zealand spreading bovine tuberculosis (Montague, 2000). The relatively high numbers of leptospiral infections in domestic stock and humans (Hathaway, 1981b; Butcher, 2000) sparked a series of investigations into the disease status of New Zealand's wildlife, including brushtail possums. The first isolates of leptospires from brushtail possums were thought to be *L. interrogans* serovar Hardjo (Brockie, 1975; De Lisle et al., 1975). Further studies isolated and identified them as serovar Balcanica which was found to be widespread in New Zealand brushtail possum populations (Marshall et al., 1976; Hathaway et al., 1978). The serovars Hardjo and Balcanica have a tendency for cross reaction in the microscopic agglutination test (MAT) making differentiation of the infecting serovar by serological methods quite difficult. However, isolation studies showed the brushtail possum to be a reservoir host for Balcanica (Hathaway et al., 1978).

Subsequent studies in Australia were also able to demonstrate serovar Balcanica in brushtail possums (Durfee and Presidente 1977). A study of 352 brushtail possums from different populations in Victoria, found that the prevalence of antibodies to serovar Hardjo varied between 14% and 66%, but it appeared that Balcanica is widespread in some Victorian populations (Durfee and Presidente, 1979a, b). Focal interstitial nephritis was found in kidneys of brushtail possums and associated with leptospiral infection. Serovar Balcanica has not been detected in Tasmanian brushtail possums (Munday, 1978 in Durfee and Presidente, 1979b). Experimental infection of brushtail possums with *Leptospira* serovars showed that brushtail possums are highly susceptible to serovar Balcanica and develop antibodies to Hardjo antigen, but are less susceptible to inoculation with serovar Hardjo (Durfee and Presidente, 1979c). In

1981 Milner et al. found antibodies to serovar Hardjo in 20% (5/25) of brushtail possum sera sampled from southeastern Australia.

Serovar Balcanica was originally isolated from cattle, pigs and humans in Eastern Europe, but the brushtail possum strain found in New Zealand and Australia is genetically different from the Bulgarian strain (Biosecurity Australia, 2000). Serovar Balcanica was probably introduced to New Zealand when brushtail possums were imported from Australia last century to establish a fur trade (Presidente, 1984). Nowadays, infection in New Zealand brushtail possums is widespread on the North Island (up to 80% of adults), but appears absent from the South Island (Horner et al., 1996). The characteristics of experimental *L. interrogans* infection have confirmed brushtail possums as a potential maintenance host for serovar Balcanica (Hathaway, 1981a). Leptospiral infection in a maintenance host is characterized by (1) high susceptibility to infection, (2) low pathogenicity of the serovar to the host, (3) long-term leptospiuria, (4) temporary sub-clinical effects, and (5) natural transmission within the host species (Hathaway et al., 1981a; Day et al., 1997a). All animals in Hathaway's study (1981a) maintained serum agglutination titers for longer than a year, leptospiuria (excretion of leptospires in the urine) was present and its pathogenicity on brushtail possums was low. The paradoxical reaction to Hardjo antigen was found again in their sera. Brushtail possums were not susceptible to challenge with Hardjo, but infection with Ballum showed characteristics of leptospiral infection in an accidental host.

The characteristics of leptospiral infection in an accidental host are different to those in a maintenance host (Hathaway, 1981b): Animals have a low susceptibility to the disease, however, once infection is established, the pathogenic effects can be severe. Infections with pathogenic *Leptospira* serovars can range from asymptomatic or subclinical to acute and fatal (Biosecurity Australia, 2000). In animals, symptoms of acute leptospirosis include sudden agalactia in the lactating female, icterus and haemoglobinuria in the young, meningitis, and nephritis and hepatitis in dogs. Chronic leptospirosis can cause abortion, stillbirth, runting, and infertility. In brushtail possums, infections with serovar Ballum have been described as sporadic and intraspecies transmission as not efficient (Hathaway, 1981b).

Sporadic Ballum infections are probably the consequence of indirect contact with free-living maintenance host species such as the ship rat (*Rattus rattus*) and house mouse (*Mus musculus*). Infection with Balcanica seems to be maintained in the brushtail possum population through direct contact and transmission may occur with sexual behavior. These findings were confirmed by Day et al. (1997b), who concluded that brushtail possums are unlikely to contract leptospirosis through a contaminated environment alone. Infection in captive animals was due to affiliative or sexual behavior in the breeding season (Day et al., 1998).

The objective of this study was to investigate the seroprevalence of *Leptospira* spp. in urban brushtail possums and to analyze any association between seroprevalence and geographic location, sex, age and body condition. Their potential as a reservoir host of leptospirosis in urban areas and the likelihood of posing a source of environmental contamination and infection for humans (and pets) shall be discussed.

## Materials and Methods

The collection of samples, handling of brushtail possums and statistical procedures are similarly described in Eymann et al. (2006), and the methods used for serological examination are found in Smythe et al. (2002a), but are herewith reiterated to facilitate the reader's understanding.

### Collection of samples

Brushtail possum blood samples were collected in the garden area of 35 residential houses from five localities within the Sydney metropolitan area, N.S.W., Australia (Fig. 1). Locations included four North Shore mainland suburbs: Beecroft (33°45'S, 151°04'E), Chatswood West (33°47'S, 151°09'E), North Epping (33°45'S, 151°05'E) and Pymble (33°45'S, 151°07'E). Brushtail possums were also sampled at Scotland Island (33°38'S, 151°17'E), a 52.4 hectare island located at Pittwater (Albani and Johnson, 1974). The environment of Scotland Island is similar to that of the sampled mainland suburbs, with high residential densities and dense surrounding vegetation including many trees.

Attempts were made to recapture brushtail possums in consecutive trap nights on three different occasions between November 2002 and December 2004 to allow repetitive monitoring. A total of 192 blood samples from 136 individuals of both sexes and varying ages was collected.

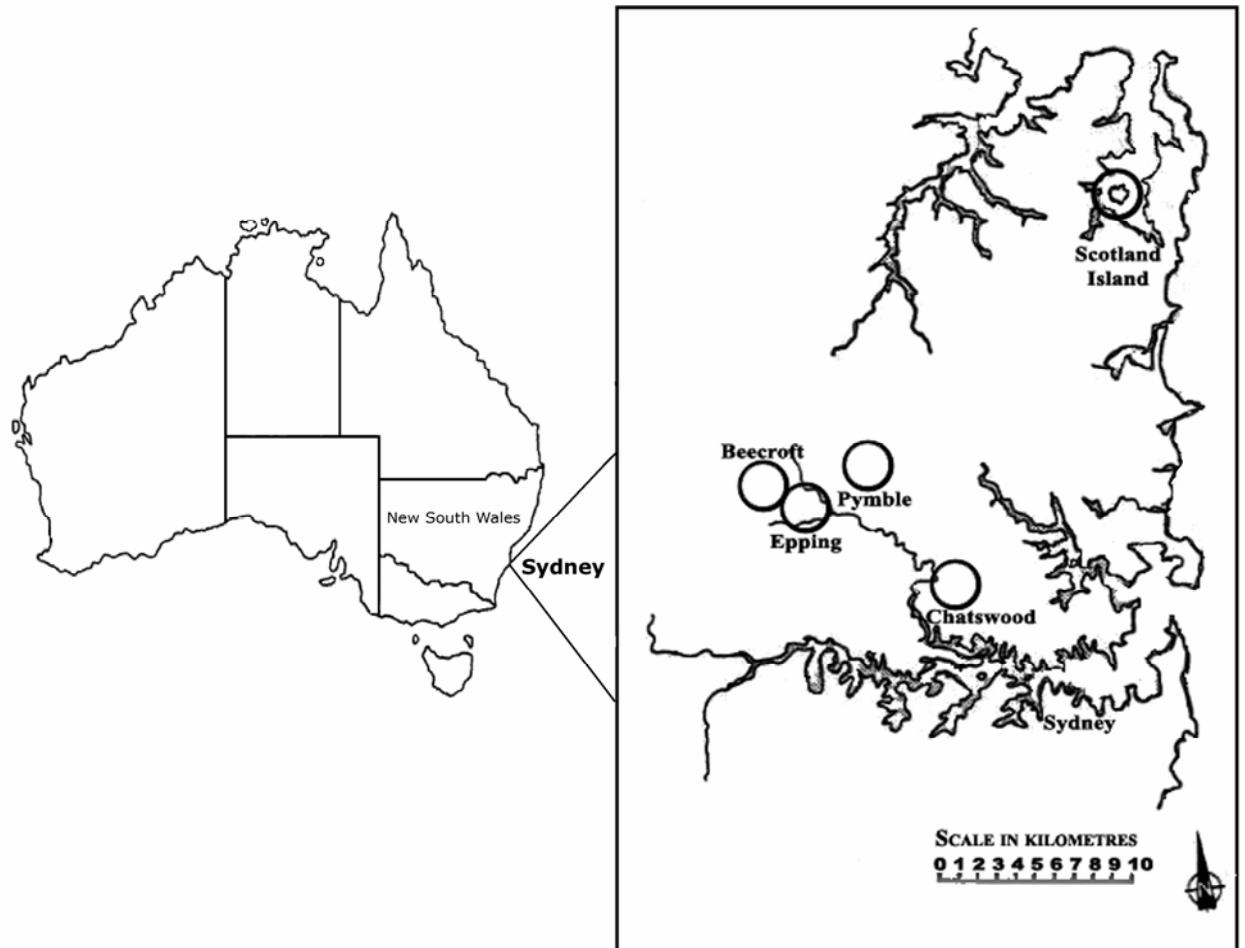


Figure 1. Map of Australia showing four Sydney metropolitan suburbs (Beecroft, Chatswood West, North Epping and Pymble) and Scotland Island from which brushtail possums (*T. vulpecula*) were sampled.

### Brushtail possum handling

Animals were caught overnight in brushtail possum cage traps (0.6 m x 0.3 m x 0.3 m) baited with apple and peanut butter. The traps were checked and cleared early the following morning (Duckworth and Meikle, 1995). Brushtail possums were physically restrained in hessian bags, weighed using hanging

scales (Wedderburn, SA235 6S) and anaesthetized using a combination of ketamine (20mg/kg i.m.; Ilium Ketamil, containing 100mg/ml ketamine HCl, Parnell Laboratories Pty. Ltd., Alexandria, NSW, Australia) and xylazine (2mg/kg i.m.; Ilium Xylazil-20, containing 20mg/ml xylazine HCl, Troy Laboratories Pty. Ltd., Smithfield, NSW, Australia) to facilitate sample collection. They were microchipped, sexed, aged (based on tooth wear) and their general appearance was noted. Individuals were classified as adult if they were 2 kg or over and had class 2 or higher teeth wear (Winter, 1980; Cowan and White, 1989). Tooth wear only gives an indication of the brushtail possum's age and class 2 equals an age of 1.0-1.5 (mean true age 1.2yr), class 3 = 0.9-3.6 (1.7yr), class 4 = 2.6-5.4 (3.7yr), class 5a = 4.2-7.3 (5.6yr), class 5b = 5.3-9.3 (6.8yr), class 6 = 7.2-8.3 (7.5yr) and class 7 = 7.3-11.5 (9.6yr) etc. and brushtail possums may live up to 14 yr in captivity (Kerle, 2001). Individual body weight was used as an indicator of a brushtail possum's condition over the years. Blood (3 ml) was collected from either the ventral tail or jugular vein of each adult animal. Serum was separated and stored frozen at -20°C until processed. Brushtail possums were held in hanging hessian bags and released after sunset the same day, allowing sufficient time to recover from sedation and to minimize handling-induced stress in this nocturnal marsupial.

## Serological examinations

The sera were screened against a reference panel of 22 leptospiral serovars using the microscopic agglutination test (MAT) (Stallman, 1982). The MAT is the most appropriate test to employ in epidemiological serosurveys, since it can be applied to sera from any animal species, and the range of antigens used can be expanded or decreased as required (Levett, 2001). It is a serovar-specific reaction, with each serogroup represented in the test panel by the serovar(s) most prevalent in the region under investigation (Slack et al., 2006).

The panel represented those serovars of *Leptospira interrogans*, *L. borgpetersenii*, *L. weilii*, and *L. kirschneri* genospecies previously isolated in mainland Australia: Australis (strain Ballico), Balcanica,



bulgarica (Nicolaevo), Canicola (Hond Utrecht IV), Celledoni (Celledoni), Copenhageni (M 20), Grippotyphosa (Moskva V), Hardjo (Hardjoprajitno), Kremastos (Kremastos), Medanensis (Hond HC), Pomona (Pomona), Robinsoni (Robinson), Szwajizak (Szwajizak), Tarassovi (Perepelitsin), Zandoni (Zandoni) and the exotic serovars, of genospecies *L. borgpeterseni*, *L. interrogans*, *L. kirschneri*, *L. noguchii*, and *L. santarosai*, Ballum (Mus 127), Bataviae (Swart), Cynopteri (3522 C), Djasiman (Djasiman), Javanica (Veldrat Batavia 46), Panama (CZ 214), and Shermani (1342 K). 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. This approach is consistent with that of previous studies of leptospiral infections in wild animal populations in Australia (Emanuel et al., 1964; Durfee and Presidente, 1979b; Mason et al., 1998; Smythe et al., 2002a), and of unpublished findings of the reference laboratory. Sera with titers to more than one serovar were assessed for known cross-reactions. On the basis of these cross-reactions, a positive result was recorded against only the likely infecting serovars. Only most likely infecting serovars are listed and not the cross-reactions.

### Statistical analysis

The statistical analysis was performed using the Vassar Stats web site for statistical computation (<http://faculty.vassar.edu/lowry/VassarStats.html>). Seroprevalence was analyzed considering the variables of sex, age and location (sampling sites). The effect of sex on infection was analyzed by binomial probabilities. For comparison of age groups and different locations the chi-square test was used. As brushtail possums were trapped over years, the estimated age group of brushtail possums used for statistical analysis was for seropositive animals when they first tested positive and for the seronegative ones when they were last trapped. Differences were considered statistically significant when  $P \leq 0.05$ . However, due to the low numbers of positive samples any statistical results have to be interpreted with caution.

## Results

Antibodies to *Leptospira* spp. were found in 9.6% (13/136) of individual brushtail possums and in 8.9% (17/192) of all serum samples. Table 1 presents a complete record of each positive animal, including data of location, sampling date, sex, age, weight and reactivity to different serovars.

Table 1. Titers of *Leptospira* spp. serovars in 13 brushtail possums (*T. vulpecula*) from different locations in Sydney, Australia that tested positive to one or more serovars (by MAT) including dates of capture, gender, age class and weight.

Host no. <sup>d</sup>	Location <sup>e</sup>	Date <sup>d</sup>	Sex	Age class	Weight [kg]	<i>Leptospira</i> spp. serovar (titer) <sup>a, b, c</sup>			
						Hardjo	Balcanica	Ballum	Szwajizak
1	Beecroft 1	02 Nov 02	male	—	2.30	800	200	—	100
3	Beecroft 1	06 Nov 02	male	—	2.40	200	—	—	—
3	Beecroft 1	15 Aug 03	male	7	2.65	400	—	—	—
39	Pymble 21	20 Feb 03	female	—	2.25	—	—	50	—
39	Pymble 21	03 Sep 03	female	5b	2.35	—	—	100	—
54	Pymble 24	06 Mar 03	female	—	2.40	1600	200	—	—
54	Pymble 24	03 Sep 03	female	4	2.60	3200	100	—	—
55	Pymble 24	06 Mar 03	male	—	2.75	3200	200	—	—
55	Pymble 24	03 Sep 03	male	4	2.90	3200	50	—	400
110	Pymble 19	09 Sep 03	male	4	2.45	—	—	50	—
115	Pymble 16	16 Sep 03	male	3	2.40	—	—	—	—
115	Pymble 13	11 Aug 04	male	3	2.60	100	—	—	50
123	Pymble 13	28 Mar 03	female	—	2.20	—	—	—	—
123	Pymble 13	18 Sep 03	female	3	2.20	—	—	—	—
123	Pymble 13	10 Aug 04	female	3	2.55	50	—	—	—
124	Pymble 13	18 Sep 03	male	5a	3.50	—	—	—	—
124	Pymble 13	10 Aug 04	male	5b	3.05	100	—	—	100
126	Pymble 13	19 Sep 03	male	3	2.70	400	—	—	—
161	Pymble 16	18 Aug 04	male	2	2.15	1600	400	—	100
162	Pymble 16	19 Aug 04	female	3	2.55	200	100	—	—
164	Pymble 16	24 Aug 04	male	5a	2.90	100	50	—	100

<sup>a</sup> All likely infecting serovars are listed and/or potential cross-reactions are included, except for serovar Medanensis.

<sup>b</sup> Microscopic agglutination test titers  $\geq 50$  were classified as positive.

<sup>c</sup> Sera from five brushtail possums reacted to one serovar (nos. 3, 39, 110, 123, and 126), five reacted to two serovars (nos. 54, 55, 115, 124, and 162), and four reacted to three serovars (nos. 1, 55, 161, and 164). Two animals had a single-reaction to serogroup Ballum (nos. 39 and 110).

<sup>d</sup> Brushtail possums no. 3, 39, 54 and 55 had serovar reactions on subsequent occasions, with the time frame between recaptures being 6–9 months.

<sup>e</sup> Individuals from location Pymble 13 and 16 are possibly in direct contact.

## Serology

The most common infecting serovar was Hardjo, with 84.6% (11/13) of the seropositive brushtail possums showing reactivity (Table 1). Seropositivity for serovar Hardjo ranged from titers of 1:50 up to 1:3,200 (Table 1). Serologic reactivity with one or more leptospiral serovars was identified in these individuals. Cross-reactions occurred with the serovars Balcanica, Szwajizak and Medanensis and the sera reacted with up to four different serovars. Cross-reaction with Medanensis was observed in all samples that had a reaction to serovar Hardjo, except for one case. MAT reactions of serovar Medanensis are not shown in Table 1 as titers are the result of antigen sensitivities and are simple cross-reactions, while the other serovar reactions warrant retention.

After discounting Medanensis, sera from five brushtail possums reacted to one serovar (nos. 3, 39, 110, 123, and 126), five reacted to two serovars (nos. 54, 55, 115, 124, and 162), and four reacted to three serovars (nos. 1, 55, 161, and 164). Two animals had a single-reaction to serogroup Ballum (nos. 39 and 110). Double or triple-infections can not be definitely excluded, but multiple reactions are most likely the result of cross-reactions. In the majority of cases, serovar Hardjo scored the highest titer. After discounting further known and observed potential serovar cross reactions (Wolff, 1954), serovar Hardjo was identified as the most likely infecting serovar. Reactivity to the exotic serovar Ballum, serogroup Ballum, was found in 15.4% (2/13) of animals and no cross-reactions occurred. From other studies conducted in Australia by the WHO/FAO/OIE Collaborating Center for Reference and Research on Leptospirosis, Brisbane, we suggest that these two animals are likely to have been exposed to the serovar Arborea. This Ballum serogroup representative appears newly established in Australia and an emerging source of leptospirosis in humans (Slack et al., 2006). Isolation studies are needed to confirm the identity of the serovar(s) in the Ballum serogroup found in brushtail possums. In the absence of this information, future references for the serovar Ballum identified in this study will describe only the serogroup.

## Location, sex, age and health

Seropositive animals were observed in two of the sample locations: 2/4 (50%) from Beecroft and 11/62 (17.7%) in Pymble whilst all samples from other locations were seronegative (Fig. 2). The variation in seroprevalence of *Leptospira* spp. within the different suburbs, from 0% (Chatswood West, North Epping, Scotland Island) up to 50% in Beecroft, was significant ( $\chi^2 = 19.8$ ,  $P = 0.0006$ ,  $df = 4$ ).

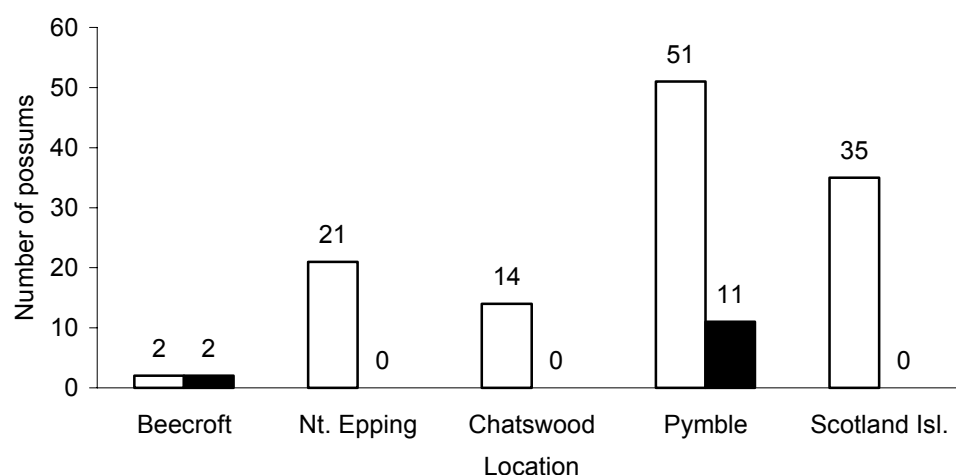


Figure 2. Location distribution of sampled brushtail possums (*T. vulpecula*).

<sup>a</sup> Black bars show seropositive brushtail possums, open bars denote seronegative animals, absolute numbers included.

There was no apparent sex-biased seropositivity, both females, 4/52 (7.7%) and males, 9/84 (10.7%) had been exposed (binomial  $p = 0.27$ ). Age seemed to be associated with leptospiral antibody status. Although seropositive brushtail possums were found in all age groups, there was an indication that older animals have a higher incidence of leptospirosis (Fig. 3). 19.9% (27/136) of adult brushtail possums from initial trapping were not aged. However, the differences between age groups were not significant ( $\chi^2 = 5.6$ ,  $P = 0.24$ ,  $df = 4$ ). Body weight of seropositive converted brushtail possums did not usually decrease compared with their previous weight when seronegative (apart from no. 124), suggesting there was no loss in body condition and they remained within the normal adult brushtail possum weight range.

However, two elder animals which were both seropositive for serogroup Ballum (no. 39, 110) showed severe signs of Dermatitis, a condition known to affect stressed urban brushtail possums (Hemsley and Canfield, 1994).

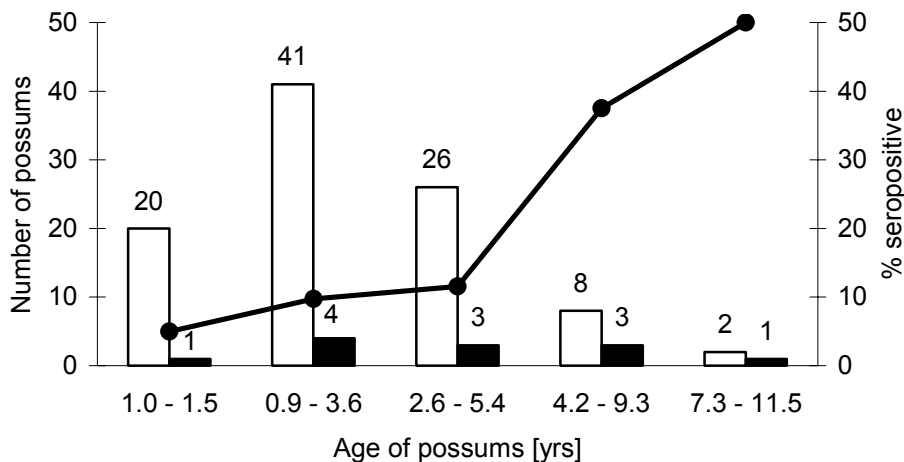


Figure 3. Age distribution of sampled brushtail possums (*T. vulpecula*).

<sup>a</sup> Black bars show seropositive brushtail possums, open bars seronegative animals, absolute numbers included.

<sup>b</sup> Line graph shows the proportion of seropositive brushtail possums within each age group.

<sup>c</sup> Age group classifications: class 2 = 1.0-1.5 (mean true age 1.2yr), class 3 = 0.9-3.6 (1.7yr), class 4 = 2.6-5.4 (3.7yr), class 5 = 4.2-9.3 (6.2yr) and class 7 = 7.3-11.5 (9.6yr).

## Recapture success

Most seropositive brushtail possums were initially recaptured after exposure to *Leptospira* spp. was detected (no. 3, 39, 54, 55), but later capture attempts proved unsuccessful (Table 1). The recapture data indicate that seropositive animals can demonstrate MAT titers for a long time (up to nine months recorded; no. 3). Some brushtail possums were found to have *Leptospira* spp. antibodies at the end of the sampling period; therefore, a multiple sera record could not be obtained. By comparison, 55% to 33% of the seronegative brushtail possums were recaptured on subsequent trapping events (declining recapture success with increasing time).

We observed local 'hot spots' meaning that seropositive animals were caught on few properties, and additionally, more than one seropositive animal usually originated from the same property. This phenomenon occurred in both Beecroft and Pymble (Table 1). For example it was very apparent on two Pymble properties (Pymble 13, 16) within close proximity (120m) where a total of seven seropositive brushtail possums originated from (Fig. 4). Between March and September 2003 a total of 11 brushtail possums had been caught there, of which one (no. 126) tested seropositive. Yet one year later in August 2004 three individuals were found to be positive by seroconversion (no. 115, 123, 124) and an additional three brushtail possums (no. 161, 162, 164) tested positive. So at the end of the two year sampling period seven of a total of 16 (43.8%) animals had antibodies to *Leptospira* spp. (Note: the first 'generation' of brushtail possums seemed partly replaced by newcomers then). Brushtail possum no. 115 was caught alternating between both these properties showing a linkage between these two sites.

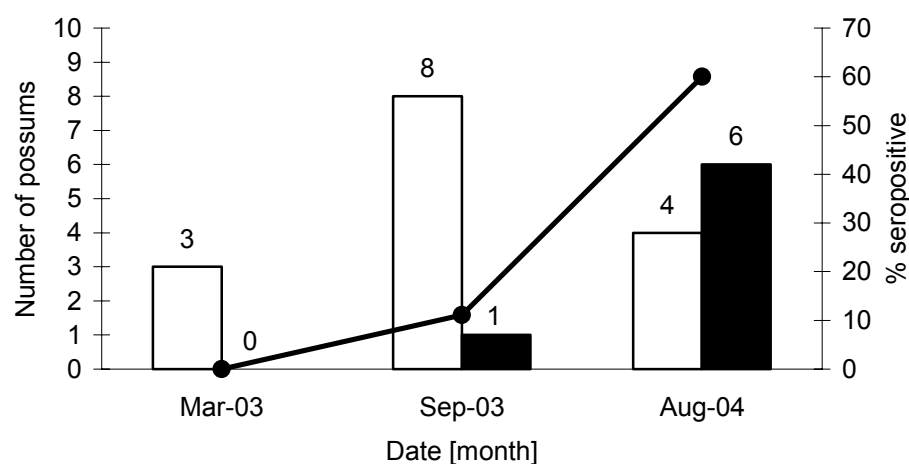


Figure 4. Total number of brushtail possums (*T. vulpecula*) including recaptures caught on two properties in close proximity (120m), showing the increase in seropositive animals over time and suggesting focal sources of leptospirosis infection.

<sup>a</sup> Black bars show seropositive brushtail possums, open bars denote seronegative animals, absolute numbers included.

<sup>b</sup> Line graph shows the proportion of seropositive brushtail possums for each trapping occasion.

## Rodent prevalence

In the 113 trap nights of this survey (November 2002 – November 2004), a total of 1,102 traps were set, with a by catch of 38 rats. They were identified as black or ship rats (*Rattus rattus*) due to their size, morphological features and behavior (Strahan, 1995), but none were collected, examined or marked. Recaptures may have been possible, but the numbers of rodents occasionally escaping through the wired mesh of the traps are not included either.

## Discussion

### Serology

Our results show that the brushtail possum is readily exposed to *Leptospira* spp. infection in an urban environment. More than 9% of animals had antibodies for leptospirosis, although previous studies found higher prevalence in other populations. In a serological survey of brushtail possums in Victoria, Australia the overall prevalence of antibodies to Hardjo varied from 14% in the Melbourne Zoological Gardens and 37% in a peridomestic population to 66% in rural and more typical brushtail possum habitat (Durfee and Presidente, 1979a). Most brushtail possums had antibodies against *L. interrogans* serovar Hardjo, but there was some cross-reaction with other serovars including Balcanica (Table 1). Cross-reactions between different serogroups are common, and the highest titer detected is not necessarily the infective serovar (Richardson and Gauthier, 2003). Indeed, in Hathaway's (1981a) captive study, brushtail possums infected with Balcanica showed paradoxical reactions and had Hardjo titers that were higher than or equal to Balcanica titers in all but one case. Therefore, the interpretation of the MAT is complicated by these cross-reactions, especially in acute-phase samples (Levett, 2001). The detection of both IgM and IgG antibodies in the MAT and the presence of several common antigens shared among the leptospires may cause cross-reactivity. This is only to some extent predictable, and samples often have similar titers to all serovars of an individual serogroup. Therefore, conclusions about infecting serovars cannot be drawn without isolates or a sound knowledge of local serovars endemic to the

region under investigation. Without these, the MAT data can only identify which serogroups are present within a population.

Isolation of the causative leptospire from its host and identification through cross agglutinin-absorption tests (Durfee and Presidente, 1979a) may be the only way to distinguish between infection with serovar Hardjo or Balcanica. New diagnostic techniques may help in this process, for example using PCR based technologies (O’Keefe, 2002; Levett et al., 2005); however, currently available tests merely distinguish between pathogenic and non-pathogenic species (Smythe et al., 2002b), but do not allow identification of the isolate. The isolation of *Leptospira* from urine in any animal is difficult and generally results in a high contamination rate of the culture media and stress for the animal (Lee Smythe, pers. comm.). More success is gained from culture of actual kidney samples; however, this would involve killing the animals and would not be ethically acceptable in many cases and impact on further investigations of tagged or recaptured animals. Therefore, these methods were beyond our study, and there remains some uncertainty as to which serovar some of the brushtail possums were actually infected with. Previous agglutinin-absorption studies suggested that some brushtail possums with antibodies to Hardjo may be indeed infected with serovar Hardjo (Durfee and Presidente 1979a).

Two brushtail possums had antibodies to the serogroup Ballum, and no cross-reactions occurred. This more likely represents an infection with a local strain from the serogroup Ballum. Serovar Arborea from the Ballum serogroup has recently been isolated in Australia and may represent the infecting serovar identified in this study (Slack et al., 2006). Serovar Arborea is one of the five known members of the Ballum serogroup and was first isolated in Europe (Kmety and Dikken, 1993 in Slack et al., 2006). Prior to 1998 it had not been isolated from humans in Australia, and it is now regarded as an emerging serovar (Slack et al., 2006). However, identification of serovar Arborea is based upon the lack of evidence that *L. borgpetersenii* serovar Ballum exists in Australia as it has never been isolated. More research is needed to clarify the identity of the infecting serovar(s) in brushtail possums in the Ballum serogroup by isolation studies.



The level of antibody titers in individuals varied from 1:50 to 1:3,200 (Table 1), and we did not observe a large increase in antibody titers between serum samples of the same animal. Experimental studies of serovar Balcanica infection in captive brushtail possums showed a peak in antibody titers within the first two weeks of infection (Hathaway, 1981a). The maximum titers recorded were 1:384 to serogroup Ballum, 1:12,288 to Hardjo and 1:768 to Balcanica with the majority of Hardjo titers being higher or equal to the Balcanica titers. Titers fell rapidly from the peak levels until reaching a plateau after about two months post-inoculation which was maintained at almost constant levels for the remaining 11 months of the experiment. Therefore there is no certain indication of recent (re-) exposure to the infection in our study, and the brushtail possums were likely chronically infected with leptospirosis. A transient serological response to serovar Balcanica has been observed in captive brushtail possums which converted seronegative after some time, but leptospires were still recovered from kidneys or observed in urine (Hathaway, 1981a; Day et al., 1998). It is known that animals can be seronegative carriers (Levett, 2001). Our data suggest that *Leptospira* infection is endemic in urban brushtail possum populations.

### **Location, sex, age and health**

Exposure to *Leptospira* was linked to particular backyards for most brushtail possums in our study (Table 1). Most seropositive animals were found on just a few properties, although not necessarily all brushtail possums trapped in these backyards tested seropositive. Home ranges for brushtail possums in urban areas can be small, especially for females, often less than 2ha (Statham and Statham, 1997; Harper, 2005), so the density of brushtail possums is potentially high in some urban areas (Matthews et al., 2004). Within two years a total of 17 brushtail possums were caught on two properties in close proximity (Fig. 4), indicating overlapping home ranges and more than 50% of all seropositive animals had emerged from these two properties by the end of our study. It seemed that animals were infecting each other successively over time with *L. interrogans* serovar Hardjo. After the major breeding season in 2004, numbers of seropositive brushtail possums in these two backyards had sharply increased,

probably due to social contacts between them. New Zealand research suggests that transmission occurs with sexual activity in the breeding season (Day et al., 1998) and that seroprevalence may be elevated in the breeding season months (Cowan et al., 1991). A recent study showed that contacts between wild brushtail possums are related to mating and associated behavior and therefore mainly occur in the peak mating season (Ji et al., 2005). Based on the proposition that serovar Balcanica is transmitted predominantly by sexual contact, a model-selection approach determined that density-dependent transmission was the most appropriate form of transmission of leptospiral infection in brushtail possums (rather than frequency-dependent transmission) (Caley et al., 2001).

Age and serological evidence of infection are also closely linked (Durfee and Presidente, 1979a; Cowan et al., 1991). Primary infections are thought to be acquired with sexual maturity and serovar Balcanica infection is predominantly found in brushtail possums over 18 months of age. The prevalence of antibodies increases with age, for example in the study of Cowan et al. (1991) 30% of younger animals (2–3 years of age) were seropositive, while by the age of eight nearly 70% of brushtail possums had antibodies for leptospirosis. Accordingly most seropositive brushtail possums in our study were found in older age groups and with increasing age most brushtail possums had antibodies for leptospires (Fig. 3). Two brushtail possums had antibodies against serogroup Ballum, and while infection through a contaminated environment alone is unlikely for serovar Balcanica (Day et al., 1997b), it is likely the cause for infection with serovar Ballum (Hathaway, 1981a, b). The black or ship rat (*Rattus rattus*) was occasionally found in the traps, and is considered a maintenance host for serovar Ballum (Hathaway, 1981a) indicating a likely source of environmental exposure. Commensal rodents such as the House mouse (*Mus musculus*), the Norway or Brown rat (*Rattus norvegicus*) and the Black or Ship rat (*Rattus rattus*) are carriers of leptospirosis, but a limited survey of 26 *R. norvegicus* in Metropolitan Sydney did not find any evidence of leptospirosis (Singleton et al., 2002).

The prevalence of infection does not appear to differ between sexes (Durfee and Presidente, 1979a; Cowan et al., 1991), and we did not find any significant differences for sexes either. Transmission

between the same sexes has only been observed for females in captivity and was a result of affiliative behavior in the breeding season (Day et al., 1998).

Several studies suggested that leptospirosis has low pathogenicity to brushtail possums infected with serovar Balcanica due to the mild nature of kidney lesions (Hathaway, 1981a), the lack of clinical signs (Cowan et al., 1991; Day et al., 1997b) and a transient loss of body weight (Day et al., 1997a). In animals, the extent of renal lesions caused by leptospires is dependent on the serovar-host relationship (Mikaelian et al., 1997). Cowan et al. (1991) did not find any association between body condition and infection, or between genotype and susceptibility to infection, and clinical signs of disease were absent. We found no association between body weight and infection either. The brushtail possums appeared healthy, apart from the two individuals with serology for serogroup Ballum infection, which had dermatitis. The causes for dermatitis in brushtail possums are multifactorial and defects of immune competency may facilitate the development of skin lesions (Hemsley and Canfield, 1994). Ballum infection in brushtail possums is characteristic of infection in an accidental host and may cause illness (Hathaway, 1981a), although one female (no. 39) persisted for at least six months with the serogroup Ballum infection.

### **Potential risk of disease transmission**

Contamination of the urban environment through brushtail possums may be possible as captive brushtail possums infected with serovar Balcanica shed leptospires in their urine (leptospiuria) and leptospiuria can be of high intensity and persist for more than 12 months (Hathaway, 1981a; Day et al., 1997b). It is thought that leptospiuria in accidental hosts is of low intensity and short duration and leptospire shedding was not observed in brushtail possums within three weeks of infection with Ballum (Hathaway, 1981a). Leptospiuria of long duration ensures the transmission of infection between successive generations of a maintenance host population and the survival of the infecting serovar. One potential source of human infection can be the use of water tanks which are for example the main source of water collection on Scotland Island. Human cases of leptospirosis have been linked with

household use of rainwater catchment systems contaminated by rat urine in Hawaii (Sasaki et al., 1993).

Reports of human cases of leptospirosis from Metropolitan Sydney are not available, hence annual incidence rates, if given, are unknown. Epidemiologic characterization has been limited by difficulties with both case detection and confirmation, and leptospirosis is frequently misdiagnosed as a result of its nonspecific flu-like illness presentation (Katz et al., 2001). Pyrexia of unknown origin in patients may not be associated with leptospirosis due to the urban setting and hence it is not part of the differential diagnosis. The great majority of infections caused by leptospires are either subclinical or of very mild severity, and patients may not seek medical attention (Levett, 2001). Occupation is a major risk factor, direct contact with infected livestock and animals accounts for infections and affects for example dairy farmers, veterinarians or rodent-control workers (Levett, 2001). However, in high risk groups such as brushtail possum trappers in New Zealand, where brushtail possums are mainly infected with serovar Balcanica, leptospirosis seems not to be readily transmitted (Cowan et al., 2000). High risk groups within Sydney Metropolitan area may include pest controllers, which specialize in removal of nuisance brushtail possums from buildings, and wildlife carers.

Little data is available to support the role of wildlife in transmission of leptospires to domestic animals or people (Higgins, 2004). Studies on the nidality of leptospirosis revealed no evidence of interspecies transmission despite high prevalences of endemic infection in livestock and wildlife (Hathaway, 1981b). Accidental infections in domestic animals and men are the consequence of ecological associations with maintenance host populations, but infection with serovars maintained by wildlife are considered a rare occurrence and favorable environmental conditions are important for transmission, if no direct contact is established. Cases of wildlife-to-human transmission have rarely been reported, for example infection occurred due to direct contact of a man with raccoon urine (Falk, 1985). The arrival of exotic serovars in Australia, as present in serogroup Ballum, could have a potential impact on both human and animal health. This would depend on the ability of new serovars to become native, feral or domestic animal

adapted and the subsequent impact of these increased levels of exposure to humans or domestic animal populations.

### **Management implications**

Infection with leptospires can be reduced through hygienic measurements and vaccines have been developed for humans (Martinez et al., 2004) and dogs (Schreiber et al., 2005). Several problems confront the use of these vaccines (Bharti, 2003), and vaccines are not in use in Australia or many other countries. Vaccines tend to be serovar specific. Marshall and Manktelow (2002) suggest aggressive reduction of hosts such as rodents combined with possible vaccination of at-risk persons as the best approach to control human leptospirosis, while control of the disease in wildlife may not be feasible. However, vaccination of at risk-groups is not a likely scenario in Australia, but rather a strategy focusing on awareness and prevention education to reduce the risk of exposure to the disease agent (L. Smythe, pers. comm.).

Strategic lethal control operations would not be an option for brushtail possum populations as they are a protected species by law and people are encouraged to live with wildlife (Matthews et al., 2004). One potential future tool to help manage sexually transmitted disease in wildlife is fertility control using agents that target endocrine control of reproduction (Caley et al., 2001). Fertility control agents, such as long-acting contraceptive implants containing deslorelin (Herbert et al., 2005), inhibit mating behavior and reduce birth rates, and hence could be used to reduce abundance and thereby reduce disease transmission.

Potentially the brushtail possum may act as a source of infection, but its role, if any, for interspecies transmission is unclear. Effective control of diseases will require estimating the extent of disease transmission between and within species (Caley and Hone, 2004). Although the seroprevalence in the current study was low compared with other studies, there were definitely focal areas of infection, where leptospirosis is perhaps maintained within the brushtail possum population. Brushtail possums in these

focal areas of infection may pose a disease transmission risk to humans, particularly in urban areas where brushtail possum population densities are high, with numerous animals having overlapping home ranges and a high density of people. Flying foxes (*Pteropus* spp.) were also identified as carriers of pathogenic *Leptospira* spp., but there was no evidence that flying foxes pose a significant risk of leptospirosis to the wider community or that humans who are in regular, close contact with flying foxes are at risk of leptospirosis (Cox et al., 2005). It is likely that a critical point of serovar 'load' in an animal carrier population has to be reached, before the serovar becomes a serious public health issue. To determine the potential risk of brushtail possums acting as an important reservoir of leptospiral infection, information will be needed about the extent to which infected brushtail possums shed leptospires in their urine, the degree and longevity of environmental contamination and the potential for humans/pets to come into contact with leptospires shed by brushtail possums. Further research may include isolation studies of leptospirosis in urban brushtail possum populations to enable studies on the role of brushtail possums, if any, in interspecies transmission of leptospirosis. Routine monitoring and surveillance programs could be established, so that mitigation strategies which are sympathetic to brushtail possum conservation and human health can be developed if required.

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## Chapter 6

# Conclusions

This chapter reviews the overall findings from each previously presented chapter (no. 1 – 5). The original research questions, the materials and methods used, results and conclusions specific to each chapter are briefly summarised, followed by a synthesis of conclusions. Each chapter is assessed for its relevance and potential application to the management of urban brushtail possum populations. The conclusions made may either support and/or provide an impetus to change existing management practices. Limitations inherent in the data and suggestions for overcoming these are given. As such, the synthesis of management implications includes an outlook on future research requirements for brushtail possums in urban environments.

## Summary

### Chapter 1

- The literature on common brushtail possums (*T. vulpecula*) was reviewed, with a focus on existing management practices for urban populations and typical nuisance situations between people and brushtail possums.
- The challenge of urban wildlife management was highlighted and potential problems with the current brushtail possum policies (e.g. non-compliance by some householders and illegal relocation of animals) were identified.
- In particular, fertility control methods for brushtail possums, the use of nest boxes in urban backyards, and disease transmission in the urban environment were reviewed and further management and research requirements were recognised.

- The conclusions summarised an outlook on potential future management tools and future research objectives.

## **Chapter 2**

- The potential of implants containing deslorelin (a slow-release GnRH agonist) to inhibit reproduction in brushtail possums (both sexes) were investigated in a captive breeding colony at AgResearch, Wallaceville, New Zealand.
- Blood and urine samples were taken, a GnRH challenge and hormone assays employed (LH, FSH, Progesterone, Testosterone) and females were frequently monitored for offspring.
- Acute effects of deslorelin treatment at the level of the pituitary gland were similar between the two sexes. Treated females became anoestrus, and remained infertile for at least one breeding season. The effects of treatment were also reversible in a subset of females that had their implants removed. Paradoxically, male brushtail possums remained fertile during chronic deslorelin exposure and there was no evidence of testicular regression despite significant declines in basal FSH and testosterone concentrations.
- In conclusion, there is potential to control reproduction in female brushtail possums by using chronic GnRH agonist treatment.

## **Chapter 3**

- In a field trial, some females received the contraceptive implant deslorelin to monitor its effect on wild individuals compared to a placebo group. Basic population parameters such as age structure, sex ratio and reproduction were also recorded.
- Brushtail possums from suburban locations within Sydney metropolitan area were trapped overnight on private properties, sedated, examined and released at twilight at the capture spot.



- Trapping success remained similar over time ( $2.81 \pm 0.17$  individuals per residential property), but there appeared to be a turn-over in animals during the study period between November 2002 and April 2005. The sex ratio was slightly biased towards males, and male survival declined earlier compared to females. Breeding was seasonal with the main peak of births in autumn. Deslorelin treatment effectively inhibited reproduction in adult females for  $519 \pm 7$  ( $n = 5$ ) and  $\geq 700 \pm 20$  ( $n = 5$ ) days after administration of one or two 4.7 mg implants respectively. Two females produced double offspring upon resumption of breeding.
- This study delivered essential biological knowledge on urban brushtail possum populations and provided an indication of the efficacy of using deslorelin implants as a fertility control technique for brushtail possum populations in Australian cities.

## Chapter 4

- The prevalence of *Toxoplasma gondii* and *Neospora caninum* in the urban brushtail possum population was investigated as part of the health assessment.
- Sera from 142 individuals inhabiting the Sydney metropolitan area were collected and analysed using a modified agglutination test (MAT) for detecting antibodies to *T. gondii* and *N. caninum*. Laboratory tests were performed during a visit to the Animal Parasitic Diseases Laboratory of Dr. JP Dubey, US Department of Agriculture.
- Antibodies to *T. gondii* were found in 6.3% (9/142) of individuals tested, with age and location possibly playing a role in the exposure risk; seropositive animals were generally not recaptured. Antibodies to *N. caninum* were not found.
- At present there is no vaccine to control toxoplasmosis in marsupials (nor in humans); the only way to prevent urban contamination with *T. gondii* oocysts – and thus, exposure of brushtail possums to this parasite – is to control feral cat populations and encourage residents to keep domestic cats indoors.

## Chapter 5

- The prevalence of *Leptospira* spp. in the urban brushtail possum population was researched as another part of the health assessment.
- Sera from 136 individuals were screened against a reference panel of 21 *Leptospira* spp. using the microscopic agglutination test; it was performed by the WHO/FAO/OIE Collaborating Centre for Reference & Research on Leptospirosis, Queensland Health Scientific Services.
- 9.6% (13/136) of brushtail possums had antibodies to *Leptospira* spp. Most infected animals had antibodies to *Leptospira interrogans* serovar Hardjo (11/136), but some cross-reaction with serovar Balcanica occurred. Two animals had been exposed to *L. borgpetersenii* serovar Arborea (2/136) which is a new arrival to Australia and has been emerging in recent years. Exposure to leptospirosis seemed to be associated with age, and seropositive animals emerged from only a few local 'hot spots'.
- The impact of serovar Arborea on brushtail possum population numbers is unknown; the potential role, if any, that brushtail possums could have in leptospirosis disease transmission to humans and domestic pets in urban Australia is not clear either – it will depend on the serovars they are exposed to and if they are a maintenance host for a particular serovar; these issues could be addressed in future investigations.

## Concluding remarks

Management of brushtail possums in urban areas can be a contentious issue. While wildlife agencies promote a policy of "living with possums", not everyone welcomes wildlife into their backyard (Eymann *et al.* 2006; see Chapter 1). There is high demand for assistance with individual 'nuisance' possums and hence, management strategies focus on individual animals in (sub)urban areas, rather than acting on the population level. The 50m rule of relocating animals which occupy a roof is not satisfactory to some members of the community (Matthews *et al.* 2004). Conflicts between humans and possums continue,

usually to the detriment of animal welfare. Illegal relocation of urban brushtail possums by annoyed property owners is a common practice in urban areas – and reflects the lack of effective wildlife legislation and of information that reaches the public (Eymann *et al.* 2006, see Chapter 1). Botanical and zoological gardens also report problems with ‘overabundant’ brushtail possums e.g. the Royal Botanical Gardens in Sydney have tried to mitigate this problem by euthanising animals in the past. However, strategic lethal control operations are not an option for urban brushtail possum populations as the animals are supported by many members of the community and protected by law.

One potential promising new management tool could be fertility control. Until recently, surgical sterilisation has been the only fertility control option available. This method is very invasive and expensive, and therefore it is not considered a viable alternative to lethal control techniques in the majority of management situations. At present, predominantly 1080 (sodium monofluoroacetate) and other toxic baits are used to control ‘problem’ brushtail possum populations both in New Zealand (Eason *et al.* 2000) and Tasmania (McArthur *et al.* 2000; le Mar and McArthur 2003). In New Zealand, there is great interest to develop fertility control technologies for brushtail possum populations because of their status as a major pest (Cowan 2000). The focus lies on the development of orally delivered target-specific contraceptive baits and/or a self-disseminating system and research is ongoing (McDowell *et al.* 2006). It is important at this point to remember that management aims differ significantly between Australia and New Zealand. New Zealand control methods may not apply to the Australian situation, where control of possums is only desired in problem areas, such as (sub)urban neighbourhoods, and where the goal is to reduce their abundance to sustainable levels rather than to eradicate them. A self-disseminating contraceptive agent is out of question in the species’ native Australia as it could spread to rural populations which are already in decline (Kerle 2004). Each management situation is unique and may require different contraceptive attributes to achieve optimal results. The evaluation of a variety of fertility control techniques provides wildlife managers with greater flexibility when managing problem brushtail possum populations.

## **Fertility control using deslorelin implants**

Implants containing the GnRH agonist deslorelin were shown to be an effective contraceptive for female brushtail possums (see Chapter 2 and 3). We demonstrated that deslorelin is a safe contraceptive for females and can inhibit reproduction for at least one breeding season, while some females remained infertile for at least two years. Paradoxically, males appeared to be resistant to the contraceptive effects of GnRH agonist treatment (despite the same mode of action on the level of pituitary as in females), and the underlying reasons are unclear. The complex patterns of spermatogenesis are not entirely understood to date and require further investigation. For wildlife managers, nonetheless, it only matters that the implants are effective in one sex, in this case the female.

At present, deslorelin implants are the only readily available contraceptive for female brushtail possums. Deslorelin targets the endocrine control of reproduction. This has the advantage that in addition to reducing birth rates, it causes desirable behavioural changes such as the inhibition of mating behaviour. One potential future tool to help manage sexually transmitted disease in wildlife is fertility control using agents that target endocrine control of reproduction (Caley *et al.* 2001). Reduced sexual contacts between brushtail possums and possibly reduced agonistic contacts could reduce transmission of certain diseases (see further below, 'leptospirosis'). Therefore, deslorelin implants could be used not only to reduce animal abundance but to control disease transmission at the same time. Furthermore, the reduction of mating activities should lower the number of residential complaints about noisy animals. Additionally, all deslorelin treated females in our field study were recaptured on the same properties indicating that no loss of territory occurred. Similarly, no loss of status was observed after vaccination of dominant female brushtail possums against GnRH (Jolly *et al.* 1996).

The main disadvantage with the deslorelin implants available at this point in time is the need to physically catch the animals to apply the treatment. This makes the implant's application labour-intensive and questions the feasibility for large-scale treatment of populations. In our study, it additionally required anaesthesia of females in order to reduce stress in the animals (they tended to be

aggressive if not sedated) and to enable application by a single person. Once the animals were sedated the implants could be very quickly administered subcutaneously. To overcome the need of sedatives, remote administration may be a possibility in the future. Remote delivery is currently not available for deslorelin implants, but research in regard to dart delivery is under way for macropod species and koalas (Herbert, pers. comm.). However, the efficacy of contraceptive projectiles for smaller mammals, especially a nocturnal, arboreal marsupial like the brushtail possum, needs further investigation. A pressurised pistol with bio-bullets may be helpful to quickly deliver contraceptives like deslorelin to trapped possums, therefore eliminating the need to physically restrain or anaesthetise the animals.

An additional limitation in their application is the duration of the contraceptive effect at present. In our studies, a single implant (4.7 mg dose deslorelin) inhibited reproduction for at least one main breeding season (average of 519 days in the field study). Urban females tended to live longer than males in our study and peaked in age class 3 – 4, indicating a mean life span of at least four years before survival declines notably. Therefore, deslorelin implants would probably have to be administered two to three times to inhibit reproduction over the lifetime of a female. If the contraceptive duration of the deslorelin implants could be increased this would contribute to a major improvement of this technique. Either the implant's formula itself could be modified or an extension of the contraceptive duration may perhaps be achieved by administering higher doses. There was evidence in both our captive and field study that a higher dose (9.4 mg deslorelin, 2 implants) increased the duration of contraception. Females remained infertile for periods longer than 700 days, up to 875 days. This has important implications to the cost of fertility control. The cost of any fertility control operation will depend very much on the cost of physically capturing the animals as well as the cost of the contraceptive agent. It would considerably increase the cost-effectiveness of deslorelin implants if females needed fewer treatments within their lifetime.

The fact that we observed double offspring in two females from our field study when they resumed breeding about 1.5 years after deslorelin treatment was interesting. It is unclear if this was related to previous deslorelin treatment, or just a chance event. It is possible that it may be related to some form of

compensatory mechanism or perhaps to 'irregularities' of the endocrine system regulating the oestrous cycle once the females resumed breeding. However, there was no indication from our captive study that offspring numbers are affected after expiry of the implant's contraceptive effect – we observed only single offspring in the captive environment. Control females in our field trial never gave birth to two young – the only case of double offspring in one control female was actually a case of adopting a second young (see Appendix A).

At present, deslorelin implants would be most suitable for small-scale local usage, such as small islands (e.g. the size of Scotland Island, 52ha), (sub)urban neighbourhoods, botanical and zoological gardens and of course in captive environments (e.g. research facilities). It is clear, that deslorelin implants are not an option for control of New Zealand brushtail possum populations. However, the results from our studies provide evidence that in general, reproduction in females can be inhibited through disruption of GnRH.

Before any fertility control agent is applied to a particular population, knowledge of the social structure and mating system is crucial before population control is contemplated or even attempted (Caughley *et al.* 1992). It is also essential to know the dynamics of the targeted population and to model the potential impacts of fertility control. Modelling should utilise population parameters site-specific to the target population as brushtail possum populations vary widely in age structure, reproduction and mortality across the different locations. Our field study documented population parameters for brushtail possums within Sydney metropolitan area – for the first time. Only by acquiring long-term data the complex patterns of population dynamics within a particular brushtail possum population can be elucidated.

If fertility control is to succeed, modelling is needed to determine the number of resident females which will have to be treated to obtain the desired result. Generally, fertility control will require that a large number of females are treated to achieve any decline in population number. New Zealand models suggest that up to 80% of females need contraceptive treatment to achieve a decline in numbers, and this will be time-delayed if not coupled with initial culling (Barlow 2000). As the response of a population

to fertility control is inevitably delayed it is difficult to monitor the changing population densities. To 'fine-tune' the level of control is an important issue when dealing with a species that one wishes not to eradicate or to decrease to very low levels. This enhances the need for modelling and continued monitoring of the target population. It also needs to be remembered that some factors, such as immigration, have the potential to swamp the effects of fertility control (Cowan *et al.* 2003; Ramsey 2005).

Deslorelin implants deserve further attention to investigate their effects on natural brushtail possum populations. Their potential to have an effect on the population level could be addressed by studying a population confined to a small area, such as a botanical or zoological garden. This approach should ideally be combined with attempts to make the environment less attractive to the animals. For management purposes, the optimum time of treatment application in this species would be the period of anestrus (December – February), before any newborn are present and before the initiation of seasonal oestrus (main breeding season autumn). If the majority of females within a defined area were treated, it could be assessed how long it takes for the population to actually decline and if the effects of fertility control can be swamped by immigration from neighbouring brushtail possum populations.

One important question remains to be answered – when should fertility control be applied to a particular brushtail possum population? If fertility control is recommended for (sub)urban neighbourhoods it will require thorough debate. Public opinion can often drive wildlife management practices, particularly in urban areas. The public's acceptability of a particular fertility control technique may determine if it is really useful in the current management context. Significant parts of the community welcome brushtail possums on their properties and do not wish the populations to decrease in number. It needs to be seriously considered that there is a very real chance that fertility control may never be applied in (sub)urban areas. However, the scenario may be different for small localised areas, such as botanical and zoological gardens.

## Health status

Wildlife disease is an important issue and understanding it is another essential part of monitoring and managing populations. Proximity of wildlife to urban areas and contact with humans and their domestic animals is known to influence the health of animal populations (Daszak *et al.* 2000; Deem *et al.* 2001). Wildlife diseases in urban areas may have implications both to animal welfare and public health. Given this, it is surprising that there does not seem to be any publications available which assess the health of urban brushtail possums and their exposure to disease agents. The only exception is a brief report on brushtail possums suffering from dermatitis (Hemsley and Canfield 1994). Hence, there is potential for a wide range of investigations, and the data presented in this thesis has added to our knowledge on the health status of urban brushtail possums. In particular, toxoplasmosis and leptospirosis were investigated:

*Toxoplasma gondii* is among the most frequently reported parasites of humans and animals worldwide (Dubey and Beattie 1988). Marsupials are highly susceptible to toxoplasmosis and infection can prove fatal. In our study, urban brushtail possums were found to have been exposed to this parasite hosted by cats. Our results give an indication on the prevalence of the disease in the urban population of brushtail possums. However, the ultimate fate of individuals from our study which had been exposed to *T. gondii* remains unknown and it is unclear what percentage of animals within the population die of toxoplasmosis. Hence, it remains unknown how and if this parasite impacts on urban population levels, an issue that could be addressed in future studies. At present, little is known of treatment and prophylaxis, and there is no vaccine available to control toxoplasmosis in marsupials, nor in humans (Lynch *et al.* 1993; Dubey and Odening, 2001; Bhopale 2003). To prevent exposure of brushtail possums to infective *T. gondii* oocysts (shed only by cats) would require preventing or decreasing oocyst contamination of their urban environment. At present, the only available management option is the control of feral cat numbers and keeping domestic cats indoors at all times. Such an approach would at the same time protect native wildlife from cat predation (Grayson and Calver 2004). It is also in the



best interest of the general public health, as humans like brushtail possums can become infected with toxoplasmosis by accidentally ingesting *T. gondii* oocysts. The confinement of domestic cats indoors is already widely promoted and practised by some cat owners. However, to reach the entire community ongoing information programs are necessary to further the understanding of these issues and hence increase cooperation of the public in keeping cats indoors.

Leptospirosis is a zoonotic bacterial disease which is also of worldwide importance (Levett 2001). These bacteria are spread through the urine of infected animals and infections range from asymptomatic or sub-clinical to acute and fatal. An understanding of whether the bacteria cause serious disease in infected animals has important management consequences. For example if there is a substantial impact on health, this may affect population growth and therefore have management implications that are quite different to those that would arise if the species was an asymptomatic carrier that could potentially transmit to other susceptible species. In Australia, leptospirosis in humans is a notifiable disease, and in temperate regions, such as Sydney, serovar Hardjo is the predominant serovar infecting humans (Biosecurity Australia 2000). Some *Leptospira* serovars are indigenous to Australia and native animals need to be recognised as a potential source of infection (Slack *et al.* 2006). Contact with kangaroos, wallabies, native rats, bandicoots and possums was reported in some human cases of leptospirosis.

Our study showed that urban brushtail possums are readily exposed to leptospires. We found a representative of the exotic serogroup Ballum, most likely *Leptospira borgpetersenii* serovar Arborea, which identified brushtail possums as a previously unknown and potential source of serovar Arborea. It also shows that serovar Arborea is present in metropolitan Sydney. Arborea is a new serovar in Australia and knowledge of any sources holds both public health and epidemiological value. To date, the WHO/FAO/OIE Collaborating Centre for Reference & Research on Leptospirosis, Queensland has identified rodents as the principal carriers. Brushtail possums are considered an accidental host for serovar Ballum (serogroup Ballum) and exposure to it could potentially have severe effects on an animal's health. The individuals whom we detected to have been exposed to serogroup Ballum both

suffered from severe dermatitis, but it was unclear if this was related to exposure with this serogroup. The causes for dermatitis in brushtail possums are multi-factorial and defects of immune competency may facilitate the development of skin lesions (Hemsley and Canfield 1994). The clinical features of brushtail possums infected with serovar Ballum have not been described by Hathaway (1981), and the impact of serogroup Ballum on brushtail possum population numbers remains unknown. However, as leptospires are not shed by brushtail possums infected with serovar Ballum (Hathaway 1981) no concerns in regard to human health arise.

All other seropositive brushtail possums in the study had been exposed to *Leptospira interrogans* serovar Hardjo, but some cross-reaction with serovar Balcanica occurred. This caused some uncertainties in regard to which serovar individuals had actually been exposed to. This is a crucial point as brushtail possums are known to be a maintenance host for serovar Balcanica (Hathaway 1981). As a maintenance host, infected animals only show sub-clinical effects, but they are capable of leptospiruria (excretion of leptospires in the urine). Hence, infected animals represent a potential source for contaminating the urban environment with leptospires. To isolate the causative serovar would require further investigation, but this was beyond the scope of the current study because of difficulties with sample collection and technical difficulties with sample analysis. Urine can be used for isolation studies, but sample collection is stressful for the animal. There is also a high contamination rate of the culture media and the associated cost is high (Lee Smythe, pers. comm.). Additionally, leptospires can be shed intermittently (Day *et al.* 1997), thus requiring several urine samples to obtain a trustworthy result. The use of PCR (polymerase chain reaction) for detection of the organism or DNA in urine or body fluids is also an alternative, but this does not allow identification of the isolate either. More success is gained from the culturing of kidney samples (Lee Smythe, pers. comm.). However, this would involve killing seropositive animals, which was incompatible with the capture-recapture studies being conducted, and has ethical implications.

It also remains unclear if brushtail possums can potentially transmit serovar Balcanica to other species, particularly domestic species (pets) and humans. In Hawaii, human cases of leptospirosis have been linked with household use of rainwater catchment systems contaminated by rat urine (Sasaki *et al.* 1993). Hence, rainwater tanks may enable interspecies transmission under the assumption that brushtail possums were indeed infected with *L. interrogans* serovar Balcanica. In Australia, the use of rainwater tanks is increasing with water shortages, and tank water is generally collected from roof tops which are frequently used by urban brushtail possums as runways. The potential role that possums could have in leptospirosis disease transmission to humans in urban Australia requires further investigation.

Interestingly, we did find evidence of intra-species transmission. We identified local 'hot spots' and demonstrated the increase in seropositive brushtail possums over time in these focal areas. Our results support New Zealand research that transmission of leptospirosis not only occurs through a contaminated environment alone, but may also be the result of affiliate and sexual behaviour. Transmission may occur with sexual activity in the breeding season (Day *et al.* 1998) and seroprevalence may be elevated in the breeding season months (Cowan *et al.* 1991). As mentioned previously, one potential future tool to manage sexually transmitted disease in wildlife is using fertility control agents that inhibit mating behaviour. Therefore, deslorelin implants could be used to reduce abundance and disease transmission at the same time (see further above, 'fertility control using deslorelin implants').

## **Suggestions for future research**

Fertility control may help to reduce possum numbers in high profile problem areas such as botanical and zoological gardens, and should ideally be combined with attempts to make the environment less attractive to possums. Using GnRH agonists could have the additional benefit of reducing the prevalence of diseases transmitted through social and sexual contact between brushtail possums. The

enhancement of existing management strategies and the development of alternative ones will need to be accompanied by further in-depth studies on the biology of urban brushtail possums, an area where knowledge is surprisingly lacking in Australia. Some previously mentioned ideas for future studies are herewith briefly summarised (more can be found in Chapter 1):

- Investigating why deslorelin does not inhibit fertility in male brushtail possums and why some females produced double offspring after 'natural' expiry of the implant's contraceptive effect.
- Development of a more practical delivery method for deslorelin.
- Researching if the contraceptive duration of deslorelin implants in female brushtail possums can be prolonged.
- Long-term research on the population ecology of urban brushtail possums – addressing life history parameters such as survival and mortality and ultimately growth rates and population dynamics.
- Feasibility of fertility control with currently available agents for broad-scale application.
- Modelling of fertility control scenarios specific to the urban brushtail possum population and using site-specific population parameters.
- Investigating public opinion towards fertility control and if such a management action would receive support by the broader community.
- Answering a key fundamental question – is fertility control actually required for (sub)urban brushtail possum populations? Or should the population be left alone and just be monitored?
- Determine the impact of certain diseases e.g. toxoplasmosis, leptospirosis (serovar Arborea) at the population level.
- Carry out isolation studies for leptospirosis seropositive brushtail possums to clarify the identity of the infecting serovar.
- Investigate the potential of inter-species disease transmission within the urban environment.

As described in the conclusions of Chapter 1:

“Wildlife managers need guidelines on how many possums can be sustained in specific areas to develop and utilise new strategies. Without such a vision, it will be difficult to successfully implement active management strategies. Clarification is needed whether management is aiming to be active on the population level or if it is tailored to dampening conflicts with individual ‘nuisance’ problems.

A decisive factor will also be the design of our urban environment in the future, which is currently constructed with only the needs of the human species in mind (McKinney 2006). The increasing density of housing developments leave little to no space for native trees or brushtail possums – increasing the pressure on wildlife and resulting in conflict with people. Indeed, the major challenge of urban brushtail possum management will be the modification of people’s behaviour, making social research one of the key components to achieve this goal. Reducing conflict between people and wild animals means influencing the attitudes and beliefs of the public and will require control of human actions and behaviour (where and how we build our homes, how we landscape our gardens, how we (do not) feed wildlife etc.). Public education needs to underpin a holistic and adaptive management framework. The ‘possum problem’ should be ‘grabbed at its roots’, meaning residents should be aware of the factors which promote possum activity within their living space. Then householders can make informed choices about the best way to deal with any arising possum conflicts. Although some information is available, generally the public still lacks this essential knowledge. It seems rather a problem of ‘getting the message through’ and putting theory into practice. Efforts to promote more understanding for this native species will require ongoing support and need to address the factors promoting high population densities. Effective management methods need to be put in place to ameliorate conflict situations. Additionally, the public needs to be aware of the detrimental effects of illegal translocation if wildlife agencies hope to curb this practice.

Possums symbolise the face of Australian wildlife still vying to survive amongst the ever expanding human settlements. The brushtail possum’s image needs to be raised to one of the last wildlife ‘icons’ of

suburbia. It is a privilege to experience native wildlife in the gardens of large cities. The more people we can engage to accept the presence of possums, the better the chances of peaceful coexistence with them. With a majority of people supporting possums, their long-term survival in one of their last 'native' strongholds could be secured. And the possums will be "out in the garden showing their gratitude by eating my roses and dancing on the roof in the moonlight" (Stackhouse 2005)."

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# APPENDICES

## Appendix A

### **Surprise in the pouch – a case of adoption in the common brushtail possum (*Trichosurus vulpecula*)**

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## Abstract

The common brushtail possum (*Trichosurus vulpecula*) is usually a strictly monotocous species, presenting one offspring at a time. Two concurrent offspring is a rare occurrence, but twin pouch young or young spaced one oestrous cycle apart have been reported. We investigated the biology of urban *T. vulpecula* populations within the Sydney metropolitan area between November 2002 and April 2005. As part of these studies, we trapped one adult female *T. vulpecula* with two young. Analysis of morphological data from the young and development of the female's mammary gland suggest that only one young was her offspring. The results of genetic analysis support this conclusion. Herewith, we describe an apparent case of adoption in *T. vulpecula*.

## Introduction

Reproduction in the common brushtail possum (*Trichosurus vulpecula*) is well known and has been extensively reviewed (Tyndale-Biscoe and Renfree 1987; Fletcher and Selwood 2000). *T. vulpecula* are seasonal breeders and in Australia most females give birth in autumn, with a second minor peak in spring. The onset of the breeding season may vary depending on the geographical location; for the Sydney area March to May are considered the major breeding months (Bolliger 1940 in Tyndale-Biscoe 1955; Lyne and Verhagen 1957). *T. vulpecula* is a polyoestrous species, having successive cycles of about 26 days until conception occurs. The gestation period, from copulation to birth, is 17-18 days and about 8 days shorter than the oestrous cycle. Oestrus and ovulation are usually suppressed by lactation, but females can ovulate again about 8 days after loss or removal of the pouch young. *T. vulpecula* have two mammary glands which functionally develop with the progression of the oestrous cycle (Sharman 1962). After parturition only the gland to which the newborn attaches, enlarges and lactates (Tyndale-Biscoe and Renfree 1987). Mammary development is independent of whether the female is pregnant or not, so even a non-pregnant female at the same post-oestrus stage can sustain a newborn transferred to her pouch.

*Trichosurus vulpecula* is usually strictly monotocous (presenting one offspring at a time) and monovular with ovulation occurring spontaneously from either ovary (Fletcher and Selwood 2000). Two concurrent offspring is rare, but has been observed (Kerle 2001). There may be a number of reasons underlying double-offspring. On rare occasions post-partum ovulation does occur and the two pouch young will differ in age by about 24 days (Tyndale-Biscoe and Renfree 1987). Twin pouch young are found when a female gives birth to two offspring on the same day. There are two types of twins in vertebrates, dizygotic (two eggs, fraternal twins) or monozygotic (one egg, identical twins) which is less common (van Oorschot and Cooper 1989). The third reason for double-offspring is adoption which has been observed in captivity. Gemmell (1995) transferred a newborn *T. vulpecula* to the pouch of a female that had recently given birth and both pouch young were successfully raised. Jones *et al.* (2004) transferred

successfully two pouch young of black-footed rock-wallabies (*Petrogale lateralis*) to surrogate tammar wallaby mothers (*Macropus eugenii*) for cross-fostering purposes, and some time later the two young even shared the same foster mother. In addition, young of *Macropus* species, after first emergence from the pouch, can be adopted by other females already carrying a pouch young of similar age (van Oorschot and Cooper 1989).

Double-offspring in *T. vulpecula* has been reported from wild and captive populations both in New Zealand and Australia, but is rare. A New Zealand study of several hundred *T. vulpecula* revealed one female with a pouch young and an embryo in the uterus, spaced 24 days apart, while in another female twin embryos at the same stage of development were found in one uterus (Tyndale-Biscoe 1955). Brockie (1992) reports that of 8,500 pouches examined between 1946-75 only one contained true twins. From a breeding colony in Queensland, one twin birth to 259 single newborns (0.4%) was recorded (Gemmell 1995). A double-ovulation was observed in two of 64 females (3.1%) collected in the vicinity of Sydney, but was not further reported upon (Hughes and Hall 1984). The incidence of double-offspring in *T. vulpecula* may be similar to that reported from macropods which varies between 0.1 and 0.7% of births (Inns 1980; Norbury 1987).

Survival of double-offspring to weaning is rare. One of the twins reported by Gemmell (1995) died, while the other one developed normally. However, a female which was experimentally given a second young, successfully sustained the twins throughout lactation. In captive tammar wallabies (*Macropus eugenii*) one pair out of three sets of twins survived to maturity (van Oorschot and Cooper 1989). In a wild population of *M. eugenii* two pouch young, separated 31 days in age, survived the full term of pouch life (Inns 1980). Anecdotal observations in New Zealand suggested that two *T. vulpecula* pouch young of different ages rarely survive together (Tyndale-Biscoe 1955). A critical point in time may be the change from early- to late-lactation milk at about 140 days after birth (Cowan 1989; Crisp *et al.* 1989). Late-lactation milk is concentrated and rich in lipids and during this last quarter of lactation production and

growth rate of the young are at a maximum. The young exit the pouch for the first time around this time and permanently leave it by 170 days. They are weaned at around 240 days and become independent.

The purpose of this paper is to report what we believe is a case of adoption by a female *T. vulpecula* carrying two pouch young of similar age in an urban population.

## Materials and methods

The study site was in the metropolitan suburb North Epping (33°45'S, 151°05'E), Sydney, New South Wales. The animals were observed on several occasions between March 2003 and April 2005. The methods of live-trapping, marking and examination are described in Eymann *et al.* (2006; see Chapter 4). The age of the pouch young was estimated using birth date determination (Bach and Lodewikus 1998) relying on measurements such as head length (Lyne and Verhagen 1957) and weight. A tissue sample was obtained from the ear of each animal and stored in 90% ethanol for genetic analysis. As a general rule, biopsies from females were taken from the left ear and males sampled from the right ear.

Genetic analysis was undertaken to determine the relationship between the female and her two potential offspring. Tissue samples were subjected to DNA extraction by the 'salting out' procedure described in Sunnucks and Hales (1996). Six microsatellite loci, Tv16, Tv19, Tv53, Tv58, Tv64 (Taylor and Cooper 1998) and TvM1 (Lam *et al.* 2000) were examined. The PCR conditions were as described by Taylor and Cooper (1998), and the products were resolved on a 6% denaturing polyacrylamide gel and visualised by autoradiograph as described by Taylor *et al.* (1994). A region of the maternally inherited mitochondrial DNA (mtDNA) control region was also investigated using the marsupial specific primers described in Fumagalli *et al.* (1997). The resultant PCR products were purified and sequenced using BigDye termination and resolved on an ABI 3730xl automated sequencer (Applied Biosystems; performed at the Macrogen Sequencing Facility, South Korea). The resultant sequences were deposited in GenBank under accession numbers (see Table 2).

## Results

An adult female *T. vulpecula* (no. 70) was first caught in the backyard of a householder in March 2003 (Table 1). The female had no offspring in her pouch or at foot, but had an enlarged mammary gland associated with an elongated teat. The female was lactating suggesting that she was still sustaining a back young (young out of pouch). About six months later, female no. 70 was recaptured in the same backyard, and a recently born pouch young was present (no. 138).

Table 1. Capture dates of an adult female *T. vulpecula* and her offspring between March 2003 and April 2005 at North Epping, Sydney, NSW including morphological data.

Date	ID no.	Sex	Age	Weight	Notes
25-Mar-03	70	Female	–	2.4kg	Lactating (one elongated teat and milk); back young not caught
14-Oct-03	70	Female	0.9 – 3.6 yr (class 3)	2.35kg	One PY, no. 138
14-Oct-03	138	Unknown	10 days	–	HL=10mm; CRL=25mm
07-Sep-04	70	Female	0.9 – 5.4 yr (class 3–4)	2.6kg	Two PY: nos. 175, 176 Lactating (one elongated teat and one enlarged mammary gland)
07-Sep-04	175	Female	130 days	270g	HL=57mm; TBL=210mm; Tail=175mm; appears in good condition
07-Sep-04	176	Male	118 days	155g	HL=52mm; TBL=170mm; Tail=140mm; appears emaciated
20-Apr-05	176	Male	343 days (class 1)	1.6kg	Moved 250m to another property; appears in good condition

\* Age estimated, with class 1 = 0.5 – 1.33 yr (mean true age 0.9 yr); class 3 = 0.9 – 3.6 yr (1.7 yr); class 4 = 2.6 – 5.4 yr (3.7 yr) (Winter 1980; Cowan and White 1989).

\*\* HL=Head length; CRL=Crown-rump length; TBL=Total body length; PY=Pouch young.



Nearly one year later, in September 2004, the same female (no. 70) was again recaptured in the same location, and she carried two pouch young (Fig. 1). One young was a female which was estimated to be between 130 days (head length) to a maximum of 144 days (weight) old. The estimated birth date was 30-April-2004, and the female young appeared in good condition, alert and there were no abrasions evident. The other smaller young was a male, estimated to be a maximum of 120 days old (head length). His weight placed him between 110 – 122 days old, but the male seemed in poorer condition than the female young. He had abrasions on his right arm (Fig. 1) and other body locations and seemed emaciated. The male's estimated birth date was 12-May 2004. Inspection of the mother's enlarged pouch revealed that only one mammary gland was enlarged, producing milk and its teat elongated, while the other gland was not enlarged and its associated teat only everted a couple of millimetres.



Figure 1. The two *T. vulpecula* young on 7-Sep-2004, estimated age 130 days for the female and 118 days for the male. Female young on right hand side, male young on left (photographer Jutta Eymann).

Microsatellite genotyping of the female and her two potential offspring revealed that the male young shared alleles with his potential mother at all examined loci. In contrast the female young did not share alleles with her potential mother at one locus (Tv 64) (Table 2), identifying her as the adopted young. However, the mother and both offspring shared an identical mtDNA control region haplotype (Table 2) with no differences present in 564 bp of sequence.

Table 2. Genetic data (microsatellite and mitochondrial DNA) of the adult female *T. vulpecula* (no. 70) and her two back young (nos. 175, 176). For microsatellite loci, allele sizes are given in base pairs as measured relative to a standard M13 sequencing ladder. For both offspring, potential maternally derived alleles are shown in bold. Only young no. 176 has a maternally derived allele at each locus and is therefore the natural offspring of female no. 70.

Animal	ID	Loci						mtDNA
	Number	Tv16	Tv19	Tv54	Tv58	Tv64	TvM1	Haplotype
Mother	70	92, 92	218, 222	218, 222	110, 110	122, 126	204, 196	Tv1
Female young*	175	<b>92</b> , 92	<b>218</b> , 234	<b>222</b> , 222	<b>110</b> , 110	104, 114*	<b>196</b> , 198	Tv1
Male young	176	<b>92</b> , 96	<b>218</b> , 234	<b>218</b> , <b>222</b>	102, <b>110</b>	104, <b>122</b>	<b>204</b> , 198	Tv1

\* Locus identifying the adopted offspring.

GenBank accession number EF166067.

Some time later, the same adult female (no. 70) with her two back young was observed on the same property by the householder (Fig. 2). Identification was possible as the ear marks are easily visible on the (enlarged) picture, and both the mother and the female young (on the right side of mother's back) have marks in the left ear whilst the male young (on the left side) has its mark in the right ear. No other

female with two young was marked in the area and animals are usually encountered on the same few properties.



Figure 2. Female *T. vulpecula* with two back young (female young on the right side, see ear mark) on the same property some time later (photographer Jan Tent).

The fate of the mother and her adopted young remain unknown, as no further trapping was conducted on this particular property. However, the male young was recaptured 225 days later on another property, approximately 250 m away from its original location (distance determined using GPS). The immature male was then approximately 343 days old and appeared in good condition. His weight was within the normal range of immature males and no abrasions or evidence of ill-health were visible.

## Discussion

We believe that this observation is the first genetically authenticated record of a female *T. vulpecula* in a wild population adopting a second young of similar age to her own offspring. The only other report on possible adoption in wild *T. vulpecula* is from New Zealand where Brockie (1992) reports that 'rarely, a young abandons or is lost from its mother's pouch, and installs itself in another female's pouch, sharing it with her young'. In a case of 'true' twins both pouch young would be expected to be of nearly the same size and weight (Gemmell 1995). If double-offspring is the result of a second ovulation after birth of the first young, the pouch young would be spaced apart at least 24 days. These potential explanations for double-offspring can not adequately explain the two young we have observed. Although we acknowledge that there can be some variability in age for a given head-length or weight (Cowan 1990, in Fletcher and Selwood 2000; Bach and Lodewikus 1998), the estimated age difference of 10 – 20 days was not within the length of the post-partum ovulation cycle of about 26 days.

However, the most striking evidence was that only one mammary gland was well-enlarged and its associated teat elongated, whilst the second gland was not enlarged, did not produce milk and its teat was only a couple of millimetres everted. The suckled teat and its associated mammary gland grow considerably during lactation (Smith *et al.* 1969; Tyndale-Biscoe and Renfree 1987). In a true case of double-offspring, either by twins or a second ovulation, both mammary glands and teats would be enlarged. In captive *Macropus* species adoption has been observed when young are of similar age, and examination of the pouch revealed that only one teat and associated mammary are enlarged (van Oorschot and Cooper 1989).

Genetic analysis revealed that only one of the two young was the natural offspring of the female. The male young, sharing alleles at all examined loci with the mother, is her natural offspring. However, the female young, although genetically similar and potentially related, seems adopted by the adult female failing to share an allele with her 'mother' at one locus.

The smaller male young had sore spots on his body and seemed less alert than the female young when they were first encountered (Fig. 1). Similarly, a cross-fostered female *P. lateralis* vacated her *M. eugenii* foster mother's pouch and joined another surrogate mother with a male young (Jones *et al.* 2004); subsequently, the male young lost condition and had to be removed from his foster mother while the female was left with her new surrogate foster mother. Gemmell (1995) reports that a female with twins lost one of her young due to a severe wound, although it was not clear what had caused it. Another experiment showed that a female is able to successfully sustain two pouch young of the same age throughout the period of lactation. In a captive breeding colony in New Zealand, females have been observed to closely associate with each other (J. Eymann, pers. obs.). For example, two adult females occasionally shared one nest box at a time with both their daughters and accepted the presence of each others back young after weaning had occurred. The fact that the mother and her two young in this study were again observed some time later after the initial observation (Fig. 2) indicates that the observed relationship was more than just a temporary brief association.

Important in a case of adoption may be that the young are of similar age, as the composition of the milk changes to late-lactation milk at about 140 days after birth (Cowan 1989). Furthermore, the pouch young first detach from the teat at about 80 days of age (Kerle 2001) and exit the pouch of their mother for the first time no earlier than day 118 (Gemmell 1995; Fletcher and Selwood 2000). Hence, our observed case of adoption probably occurred very recently. Density of *T. vulpecula* can be potentially high in some urban areas (Matthews *et al.* 2004), and especially females have small home ranges in urban areas (Harper 2005). Several animals were trapped on this particular property (unpubl. data). Thus, there is opportunity for females to encounter others with young – perhaps the female young lost her own mother and was subsequently adopted.

The presence of two young that were estimated to be born around May 2004 and the presence of a newborn in October the previous year also indicate that female no. 70 was breeding in both seasons, autumn and spring. Survivorship of the newborn observed in October 2003 is unknown, but it could

potentially have been weaned by May or June 2004, and ovulation may have occurred after the young left the pouch. Females can simultaneously carry a large young on their back and have a newborn in their pouch (Tyndale-Biscoe 1955). A minority of females breed twice a year when conditions are favourable or after loss of a young (Kerle 1984).

The fate of the adult mother and her female young was not known. However, the male young was recaptured several months later on a nearby property. In New Zealand, most juveniles disperse before they are one year old (Cowan and Rhodes 1993; Cowan *et al.* 1996, 1997). Some animals may disperse later, but dispersal is an integral part of male development (Efford 1998). In general, males disperse from their natal area and gradually shift their ranges away from their mother's whilst the females remain and inherit their mother's home range (Cowan and Clout 2000). A study on a population inhabiting old-growth eucalypt forest in north-eastern New South Wales, Australia showed similar results (Clinchy *et al.* 2004). Almost all daughters settled on or beside their mother's home range, and sons settled two or three home ranges away. It appears then that *T. vulpecula* follow a matrilineal descent system. In our study, the sharing of mtDNA haplotypes suggests that the adopted female is from the same maternal lineage as the female which adopted her. Given the tendency for females to remain within the natal area, the likely high level of relatedness between the female and her adopted offspring is not surprising, and may perhaps suggest some form of kin-recognition.

One general question that arises is – why do lactating females nurse alien offspring (Roulin 2002)? Various hypotheses have been put forward, including that females nurse related juveniles for inclusive fitness benefits. For example, allosuckling in wild fallow deer (*Dama dama*) has been described as a possible result of kin selection and/or reciprocal altruism (Ekvall 1998). In common gulls (*Larus canus*), kin altruism was also considered as a factor in adoptions (Bukacinski *et al.* 2000). However, in ring-billed gulls (*Larus delawarensis*), the apparent costs associated with a behaviour that appears to enhance the fitness of others, have not been attributed to kin selection, nor reciprocal altruism (Brown 1998). It was suggested that selection has merely failed to eliminate adoption because the long-term

reproductive cost of an occasional adoption may be offset by the relatively higher costs associated with stricter kin discrimination mechanisms such as parental infanticide. Interestingly, in Hawaiian monk seals (*Monachus schauinslandi*) high female density does appear to increase fostering frequency as it also increases the likelihood that separated females and pups will encounter another potential partner before reuniting (Boness *et al.* 1998). However, in the present case of adoption in *T. vulpecula*, it remains unknown what ultimately caused the adult female to adopt a second young.

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## Appendix B

# Performance of passive integrated transponders (PIT tags) as a marking system for brushtail possums (*Trichosurus vulpecula*) – a cautionary tale

J. Eymann, L.S. Webley, D.W. Cooper, and C.A. Herbert

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**Key words:** identification, mark-recapture, marsupial, microchip failure, probability of identity.

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## Introduction

Permanent individual identification is important to the success of any long-term study of both wild and captive populations (Elbin and Burger 1994). In the past, several types of marking systems have been used for identification purposes depending on the desired outcome. Identification of common brushtail possums (*Trichosurus vulpecula*) has been achieved using fur dyeing, numbered metal fingerling ear tags with different colours of reflective tape, ear clipping and tattooing, but all have limitations (Winter 1976). Fur dyeing only allows identification of individuals for distances up to a 100m and for a very limited time; ear tags can be lost or pulled out soon after being applied; while ear notches are intrusive, can grow out or result in tears that are hard to differentiate from a natural tear. Tattooing appears to be the most successful long-term identification method in *T. vulpecula*. Some tattoos may become thick and blurry, but remain readable, with the notable exception of growing young (Winter 1976).

Passive integrated transponders (PIT tags) were first used in the mid-1980s and are a great improvement to traditional approaches of externally marking animals for identification (Gibbons and Andrews 2004). A PIT tag is composed of a small microchip and an electromagnetic coil enclosed in a glass case that is administered internally (Feldheim *et al.* 2002; Rogers *et al.* 2002). Each PIT tag has a unique alpha-numeric identification code that can be activated and displayed on an electromagnetic scanner. Several companies manufacture PIT tags, but their systems are not compatible.

PIT tags are harmless to the animal, unobtrusive, aesthetically acceptable, easy to apply and theoretically permanent (Rogers *et al.* 2002). The survivorship or behaviour of the animal is not affected and transponders are easy, quick and accurate to read (Germano and Williams 1993; Freeland and Fry 1995). They have been applied to a wide variety of mammal species (Fagerstone and Johns 1987; Freeland and Fry 1995; Lambooi *et al.* 1995; Wright *et al.* 1998; Morley 2002; Rogers *et al.* 2002; Fokidis *et al.* 2006) and other animals such as blunt-nosed leopard lizards (*Gambelia sila*; Germano and Williams 1993) and seahorses (*Hippocampus abdominalis*; Woods 2005). PIT tags have even been used to determine the consumption of tagged salmonids (*Oncorhynchus* spp.) by piscivorous birds

(Ryan *et al.* 2001). Limitations of PIT tags include a detection range of only about 5 – 6 cm (Elbin and Burger 1994; Freeland and Fry 1995) and problems with environmental interference, particularly metallic barriers (Freeland and Fry 1995).

Although PIT tags have been reported as being reliable (Morley 2002; Bubb *et al.* 2002; Woods 2005) and are generally assumed to work permanently, there are reports of problems with PIT tag loss and/or malfunctioning (Germano and Williams 1993; Elbin and Burger 1994; Freeland and Fry 1995; Lambooij *et al.* 1995; Feldheim *et al.* 2002; Rogers *et al.* 2002; Fokidis *et al.* 2006; Hill *et al.* 2006). The retention of the tag over the lifetime of an individual is an important criterion for any tagging method (Feldheim *et al.* 2002). For example, if a recaptured animal has lost its tag it will most likely be classed as a new individual. Consequently, this will impact the recording of life history parameters – for example survivorship may be underestimated while abundance may be overestimated. Hence, it is important to calculate the reliability of the marking technique in the targeted species.

We used PIT tags to identify individual *T. vulpecula*, which were part of a long term study. The objective of this paper is to report on the performance of the PIT tags used in *T. vulpecula* and to show how genetic techniques were employed to overcome problems encountered with PIT tag identification.

## Methods

*Trichosurus vulpecula* is a solitary, nocturnal, arboreal Australian marsupial (Kerle 2001). Animals were trapped using possum cage traps (0.6 m x 0.3 m x 0.3 m; Mascot Wire Works, Sydney) on private properties within the Sydney metropolitan area, NSW, Australia between November 2002 and April 2005 (Eymann *et al.* 2006). Recapture attempts of PIT tagged individuals took place in consecutive trap nights on four different occasions. Trapping was conducted over 124 nights, giving a total of 1202 trap nights. Trapped animals were transferred from the metal cage trap to a hessian bag to enable reading of the PIT tag and further examination.

196 animals received a pre-sterilised Trovan ID-100 implantable transponder (Microchips Australia Pty Ltd, Keysborough, Victoria) whilst anaesthetised with a combination of ketamine (20mg kg<sup>-1</sup> i.m.; Ilium Ketamil, Parnell Laboratories Pty Ltd, Alexandria, NSW) and xylazine (2mg kg<sup>-1</sup> i.m.; Ilium Xylazil-20, Troy Laboratories Pty Ltd, Smithfield, NSW). The injection site was cleaned and disinfected and the PIT tag inserted subcutaneously between the shoulder blades using an implantation needle with insertion depth indicator and Trovan Deluxe Implanter (IM-100G). The implantation site was sealed with a veterinary tissue adhesive (Vetbond; 3M Animal Care Products, St Paul, MN, USA). Each PIT tag was verified for functionality with a LID-500G conventional hand-held reader after insertion (Microchips Australia Pty Ltd, Keysborough, Victoria). Only furless pouch young were not administered a PIT tag.

Individuals were sexed and aged based on tooth wear (Winter 1980; Cowan and White 1989), and information on distinct individual characteristics such as a white tipped tail, broken tooth or deformed tail bone were noted. Additionally, ear tissue biopsies were obtained from each individual using a leather hole punch for genetic analysis. As a general rule, samples from females were taken from the left ear, while males were sampled from the right ear. The biopsy resulted in a small round hole, approximately 3.5 mm in diameter for adults and 2 mm for pouch young, which remained visible as a scar or small hole for the duration of this study. On the same day, all animals were released at dark, at the capture site.

DNA was extracted from 148 tissue samples using the salting out method of Sunnucks and Hales (1996). This included samples from 128 animals trapped between November 2002 and September 2003, with five of these suspected of PIT tag failure, plus 20 animals trapped on subsequent occasions, between October 2003 and November 2004. Of these 20 individuals, 12 were suspected to have lost their PIT tag, while the others were previously caught animals and thought to be possible matches for individuals with PIT tag failure based on sex, age, locality and distinct characteristics. Individuals were genotyped at five highly polymorphic species specific microsatellite loci: Tv19, Tv53, Tv58, Tv64 (Taylor and Cooper 1998) and TvM1 (Lam *et al.* 2000). PCR conditions were essentially as described by Taylor and Cooper (1998). GeneCap version 1.2.1 (Wilberg and Dreher 2004) was used to calculate the



probability of identity ( $P_{(ID)HW}$ ), being the probability of two individuals in the population sharing the same genotype. Since *T. vulpecula* show localised genetic structure (Stow *et al.* 2006), the more conservative measure of probability of identity amongst siblings ( $P_{(ID)SIB}$ ) (Waits *et al.* 2001) was also calculated using GeneCap. In addition to GeneCap identifying matching genotypes, it also shows individuals that differ by only one or two alleles. These individuals can then be checked for genotyping errors, which may exclude them as recaptured animals.

## Results

Of 196 *T. vulpecula* fitted with a PIT tag at the time of first capture, not all individuals were recaptured and recapture success rate varied over time. We suspected PIT tag failure in 17 of these animals because a puncture hole, a scar and/or a tear in the ear were noted, presumably from previous tissue sampling. These animals also corresponded with other characteristics of previously caught ones, such as sex, age and trap location. Rare features were also important in identifying suspected PIT tag failure individuals. The cause of PIT tag failure could not be determined by routine inspection of animals in the field. Recovery of faulty PIT tags or taking x-rays for fault diagnosis was impractical. However, in some individuals ( $n = 6$ ) a PIT tag appeared palpable under the skin at the place of insertion, but was not detected by the scanner (Table 1). The PIT tags of two *T. vulpecula* (ID no. 48 and 51) felt broken, whilst for the remaining suspects the PIT tag could not be felt beneath the skin. In three animals, the PIT tag malfunctioned after a prolonged period of time (5 – 16 months), despite successfully working at previous recaptures (ID no. 14, 48 and 82; Table 1). For those animals that had readable PIT tags, none appeared to move internally as readings were always obtained from the insertion area, and no adverse side effects of the PIT tags, such as abscesses, were apparent.

Table 1. Recapture records of individual *T. vulpecula* with PIT tag failure including the initial date of PIT tag administration and the date of re-application.

ID no.	Sex	Age class <sup>a</sup>	Date of marking	Date(s) of recapture 2003	Date(s) of recapture 2004
14 <sup>b</sup>	Female	4	16-Jan-03	21-Aug-03	30-Oct-04 <sup>c,d</sup> (31-Oct, 4-Nov)
17	Male	4	18-Jan-03	20-Aug-03 <sup>d</sup> (26-Aug)	—
19	Female	4	19-Jan-03	21-Aug-03 <sup>c,d</sup> (26-Aug)	30-Oct-04 (31-Oct)
33	Male	3	13-Feb-03	08-Oct-03 <sup>d</sup>	—
34	Female	4	13-Feb-03	08-Oct-03 <sup>d</sup>	15-Sep-04 (16/17-Sep)
37	Male	3	20-Feb-03	02-Sep-03 <sup>c,d,e</sup> (3/4/5/9-Sep)	—
38	Male	2	20-Feb-03	02-Sep-03 <sup>d</sup>	—
48 <sup>b</sup>	Female	4	26-Feb-03	31-Oct-03	31-Aug-04 <sup>c,d</sup>
51	Female	3	27-Feb-03	—	03-Aug-04 <sup>c,d</sup> (5-Aug)
67	Male	2	19-Mar-03	23-Oct-03 <sup>d</sup>	—
77	Female	3	28-Mar-03	18-Sep-03 <sup>d</sup> (19-Sep)	10-Aug-04 (12-Aug)
82 <sup>b</sup>	Female	4	02-Apr-03	09-Sep-03 (11-Sep)	11-Aug-04 <sup>d</sup> (13-Aug)
141	Male	3	21-Oct-03	n.a.	07-Oct-04 <sup>c,d</sup>

<sup>a</sup> Age class was calculated when last time trapped; age class 2 = 1.0 – 1.5 yr (mean true age 1.2 yr); class 3 = 0.9 – 3.6 yr (1.7 yr); class 4 = 2.6 – 5.4 yr (3.7 yr)

<sup>b</sup> The 1st PIT tag was still readable several months after administration

<sup>c</sup> Former 'old' PIT tag felt between the shoulder blades, but no signal read

<sup>d</sup> Date of 2nd PIT tag administered

<sup>e</sup> This male died on 19-Nov-03 (autopsy suggested pyothorax as cause of death)

n.a. = not applicable

From 17 animals suspected of having PIT tag failure, 13 could be verified as recaptured individuals through genotypic matching. For unrelated individuals  $P_{(ID)HW}$  was  $2.85 \times 10^{-8}$ , while for potentially related individuals  $P_{(ID)SIB}$  was  $3.88 \times 10^{-3}$ , which is acceptably low (Waits *et al.* 2001). Using the reciprocal of the sample size as a logical cut off point (Peakall *et al.* 2006) suggests that  $P_{(ID)}$  values  $< 0.006$  (1/148) are sufficient to conclude that matching genotypes are multiple samples from the same individual. All matching genotypes were congruent with suspected PIT tag failures and with location information, morphological data and, if present, rare or unique characteristics.

The remaining four *T. vulpecula* which were trapped between August and November 2004 did not match any of the other genotyped animals and were treated as newly captured ones. These four animals were suspected of being recaptured animals with PIT tag failure due to the presence of a scar and/or tear in the ear, a characteristic that may also have been obtained from processes unrelated to capture and samples collection such as fighting or misadventure.

With 13 individuals being identified as recaptured individuals based on genotypic matches, the total number of animals trapped was adjusted from 196 to 183 individuals. This indicates that 7.1 % (13/183) of PIT tagged *T. vulpecula* in this study either received a PIT tag that malfunctioned or lost their PIT tag. However, many PIT tagged individuals were not recaptured ( $n = 101$ ); adjusting the data to include only the 82 individuals recaptured at least once, the PIT tag failure rate increases to 15.9% (13/82) for *T. vulpecula*.

## Discussion

The majority of the administered PIT tags worked successfully and numerous readings were obtained over the 2.5 yr study period; however, we did observe a relatively high failure rate. Studies testing the performance of PIT tags on a wide range of species report varying success, and although the majority of PIT tags seem to work well, problems have been recognized in some species. Occasional PIT tag failure seems to range from 6.9% (12/174) in badgers (*Meles meles*; Rogers *et al.* 2002), 3.6 – 7.2 % in

different rodent species (Fokidis *et al.* 2006) and 7.3% in pigs (*Sus scrofa domesticus*; Lambooij *et al.* 1995) up to 12.1% (47/388) in lemon sharks (*Negaprion brevirostris*; Feldheim *et al.* 2002). PIT tags inserted into 14 different species of Australian mammals worked effectively, with the notable exception of flying and arboreal species (Freeland and Fry 1995). PIT tag loss occurred in one black flying fox (*Pteropus alecto*), one ghost bat (*Macroderma gigas*) and in sugar gliders (*Petaurus breviceps*), which had an exceptional high loss rate, with 50% of eight tagged individuals losing their PIT tags.

Potential reasons for PIT tag failure include broken glass coverings and expulsion during inflammation (Lambooij *et al.* 1995). Germano and Williams (1993) reported that PIT tag malfunctioning in lizards resulted from PIT tags breaking through regions of tight skin or being broken during aggressive encounters, but the failure rate of 8.4% could be reduced by changing the PIT tag injection site. An aggressive encounter was also believed to be the reason for PIT tag failure in a black-footed ferret (*Mustela putorius furo*) which had a wound on its neck (Fagerstone and Johns 1987). Aggressive encounters between individuals have the potential to crack the glass capsule of the microchip. Agonistic behaviours between *T. vulpecula* do occur and animals may 'wrestle fight', bite or hit each other, and mating without a consort period can be aggressive (Winter 1976).

Incorrect usage of PIT tags can also bring about malfunction, such as insufficient insertion of the PIT tag, repenetration of the skin or a lack of concentration. For these PIT tags, the majority appear to fail shortly after implantation (Lambooij *et al.* 1995; Fokidis *et al.* 2006). PIT tag loss in steelhead trout (*Oncorhynchus mykiss*) was estimated to be 7.2% and peaked four weeks after implantation (Hill *et al.* 2006). Also, Feldheim *et al.* (2002) reported that considerable PIT tag loss (23.4%) occurred in *N. brevirostris* before the insertion wound healed fully. Additionally, two animals 'shed' their PIT tags after 1 – 2 years, while two other *N. brevirostris* lost their PIT tags even twice.

In our study we observed PIT tag loss and/or malfunctioning in recaptured *T. vulpecula* with a tear in the ear, puncture hole and/or a biopsy scar, a recapture feature previously used by Feldheim *et al.* (2002). For some animals a PIT tag could be felt beneath the skin, but no reading of the PIT tag could be

obtained, which was similarly the case with *M. meles* (Rogers *et al.* 2002). However, a malfunctioning PIT tag was not always palpable, suggesting that PIT tag loss also occurred, although the use of a skin adhesive at the insertion site should have limited short-term PIT tag rejection in the current study. Fokidis *et al.* (2006) suggested that some small mammals may actually be able to open wounds sealed by adhesive. The ultimate cause of failure of the PIT tags that remain *in situ* was not determined.

We used matching genotypes to alleviate problems with identification of individuals that were suspected of PIT tag loss/failure. This method of identifying individuals by their genetic tag has also been successfully applied to *N. brevirostris* (Feldheim *et al.* 2002). Whilst genetic tagging can provide a definitive result about an individual's identity, it may be a relatively expensive method of identifying recaptured individuals, particularly for species for which genotyping techniques such as microsatellites are unavailable. Applying genetic tagging for purposes of identification alone may be impractical. However, obtaining a genetic match for a 'suspicious' individual can be narrowed down by using location and phenotypic clues.

It has been questioned previously if PIT tags are a worthwhile method of identification given the relatively high failure rate. Following a study on *M. meles*, Rogers *et al.* (2002) concluded that PIT tags were less effective than tattooing which remained the preferred technique for *M. meles* due to greater reliability and affordability (total cost of PIT tags was £3,301.75 versus £285.88 for tattooing). If PIT tags are used in *T. vulpecula*, it would be advisable to have a 'back-up method' of identifying recaptured individuals, such as the genetic methods used in this study or another reliable method such as tattooing (Winter 1976). In addition, the administration of PIT tags itself should be investigated to determine if there is a more suitable position that will avoid potential damage through fights between animals. Recently, implantable transponders with lancet have been developed, which allow visual verification of PIT tag insertion (Trovan Ltd). These may assist in ensuring sufficient PIT tag insertion, and may limit the possibility that PIT tags work their way 'back-out' through the puncture site and are lost.

Despite some current limitations, PIT tags offer novel opportunities for studying free-living animals and answering questions that could not be addressed previously (Gibbons and Andrews 2004). They remain an effective tool for identifying individuals for a large variety of species. As this technology becomes cheaper and more advanced, the range of potential uses will increase; for example, remote-monitoring experiments where PIT tags are a useful tool to record individual *T. vulpecula* through fixed-mounted readers (e.g. when returning to their nest box). However, a 'back-up method', such as tattooing or genetic tagging, should be considered when life-history parameters are recorded using mark-recapture techniques.

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## Appendix C

# Genetic structure infers generally high philopatry and male biased dispersal of common brushtail possums (*Trichosurus vulpecula*) in urban Australia.

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## Abstract

The common brushtail possum (*Trichosurus vulpecula*) is in decline throughout much of its natural range with the notable exception of urban areas and a few islands. In some urban areas, their density can be high enough to cause conflict with householders. We provide the first genetic-based study of dispersal for *T. vulpecula* in the urban environment. Seven microsatellite markers were used to investigate genetic structure of adult male (n = 53) and female (n = 39) possums sampled from mainland suburbs of Sydney, and on nearby Scotland Island. Samples from Scotland Island also provide an opportunity to assess the effect of isolation on genetic variability, which as theory would predict, was significantly lower than observed in mainland samples. Male-biased dispersal was inferred from patterns of relatedness between individuals of each sex. Average relatedness was significantly higher between adult female possums than between adult male possums sampled within the same garden area. In addition, males were genotypically more similar to one another at substantially greater geographic distances than females. Along with male-biased dispersal, strong localised genetic structure for both sexes infers generally high philopatry. Dispersal distances were greatest for adult male possums sampled from mainland locations. However, even for these males, genotypic similarity between possums separated by distances further than approximately 900 meters was lower than the sample average, suggesting infrequent dispersal beyond this distance. Knowledge of dispersal patterns are important to managing the density levels of overabundant *T. vulpecula* in urban areas.

## Introduction

The common brushtail possum (*Trichosurus vulpecula*) is a predominately solitary, nocturnal and herbivorous marsupial that formerly had one of the widest occurrences of any Australian marsupial (Kerle 2001). Although *T. vulpecula* is still commonly observed in its natural habitat on many islands (Isaac 2005) it has disappeared from more than half of its natural range, including all of arid Australia (Goldingay and Jackson 2004). In many natural areas where they remain, including coastal forests of southeast NSW, *T. vulpecula* is found only in low numbers (Kerle 2004; Matthews *et al.* 2004; Paull and Kerle 2004). Declines are attributed to a combination of factors including habitat loss and disturbance, disease and introduced predators (Kerle *et al.* 1992; Goldingay and Jackson 2004). Despite population declines in their natural habitats they have adapted well to most urban areas where they can occur in high densities (Kerle *et al.* 1992; Kerle 2001). Urban areas may be one of the last endemic strongholds for *T. vulpecula*, possibly because they provide additional resources not seen in natural habitats, such as man-made den sites and an abundant food supply (Statham and Statham 1997; Kerle 2001).

Dispersal of *T. vulpecula* has been primarily examined using traditional ecological techniques such as capture-mark-recapture and radiotelemetry (Clout and Efford 1984; Cowan *et al.* 1996; Clinchy *et al.* 2001), and more recently using molecular approaches (e.g. Ji *et al.* 2001; Taylor *et al.* 2004). Most dispersal studies have been carried out in New Zealand where they are regarded a pest, destroying natural habitat and spreading bovine tuberculosis (Montague 2000). In New Zealand, non-urban, male-biased dispersal has been identified in *T. vulpecula* (Clout and Efford 1984; Ward 1985; Cowan *et al.* 1996; Efford 1998; Cowan and Clout 2000; Taylor *et al.* 2000; Ji *et al.* 2001), with juveniles beginning to disperse before they reach one year of age, coinciding with the time of sexual maturity (Cowan and Rhodes 1993; Cowan *et al.* 1997). Comparatively, little research has been carried out on dispersal of *T. vulpecula* in urban areas, notwithstanding the substantial management and conservation issues involving this species. For example, it is not clear *a priori* whether urbanisation will influence dispersal.

Certain anthropogenic impacts, such as land clearing, have been associated with lower dispersal for some species and may also result in sex-specific impacts (e.g. Voles; Jon and Rolf 1999, Skinks; Stow *et al.* 2001).

Although urban areas may provide an important refuge for *T. vulpecula*, population numbers are sufficiently high that management strategies are required to minimise negative impacts for humans and possums alike. The habit of *T. vulpecula* to occupy roof spaces and browse garden vegetation has led to conflict with affected householders (Eymann *et al.* 2006a). Relocation to distant areas has been eliminated as a management option in NSW since 1998, as most translocated animals do not survive (Pietsch 1994). In addition, areas in New Zealand that underwent possum removal rapidly became occupied again (Ji *et al.* 2004), suggesting translocation may not be an effective long-term management tool in the urban environment. Current management policy within NSW only permits the relocation of possums occupying roofs and their release must be within 50m of the capture site (Matthews *et al.* 2004). The effectiveness of this approach is questionable and alternative management options are being sought (Eymann *et al.* 2006a). To effectively manage possum density, knowledge of dispersal characteristics is clearly important. The relevance of dispersal is highlighted by concern over potential disease transmission in the urban environment, particularly as possums in New Zealand are a vector for the commercially threatening diseases bovine tuberculosis (Coleman and Caley 2000). Whilst this disease may not currently be of a concern in Australian populations, other diseases such as leptospirosis may require management (Cowan *et al.* 2000; Eymann *et al.* 2006a).

Our sampling strategy included *T. vulpecula* from Scotland Island which allowed for comparison of genetic structure and diversity in island and mainland *T. vulpecula*. Given the rapid decline of *T. vulpecula* populations throughout much of mainland Australia, knowledge of the genetic integrity of individuals on island 'refuges' could be important. Many species that were previously widely distributed are now predominately confined to islands (Sinclair 2001; Eldridge *et al.* 2004), therefore, islands can be of high conservation value. Knowledge of genetic variability in island populations is valuable as it has

been established that levels of genetic variation provide a good predictor of population persistence (e.g. Spielman *et al.* 2004). Further, low genetic variation in island populations suggests that they may be a less than ideal source of founders for reintroduction purposes (Eldridge *et al.* 1999; Mills *et al.* 2004).

This study on dispersal patterns of female and male *T. vulpecula* should contribute towards management of one of the last strongholds of this species, the urban environment. More generally, our comparison of mainland *T. vulpecula* with those on Scotland Island contributes further data towards understanding the effects of isolation on population dynamics and genetic diversity.

## Materials and Methods

### Sampling and genetic assays

*Trichosurus vulpecula* were sampled from five urban suburbs within the Sydney metropolitan area, NSW, Australia (Fig. 1). Collections were made at four mainland suburbs; Beecroft, North Epping, Chatswood West and Pymble, which are separated from each other by distances ranging from 2.3 km to 11 km. Samples were also collected from Scotland Island, which is 52 hectares in size and located in Pittwater. Scotland Island is approximately 400 meters from the mainland at the closest point, from which it has been separated for about 6,000 years (Albani and Johnson 1974). The environment of Scotland Island is similar to that of the sampled mainland suburbs, with high residential levels and dense surrounding vegetation. On the mainland and Scotland Island, animals were trapped and released at the garden area of residential houses (ranging in size from 500 – 1,400m<sup>2</sup>; Eymann *et al.* 2006b).

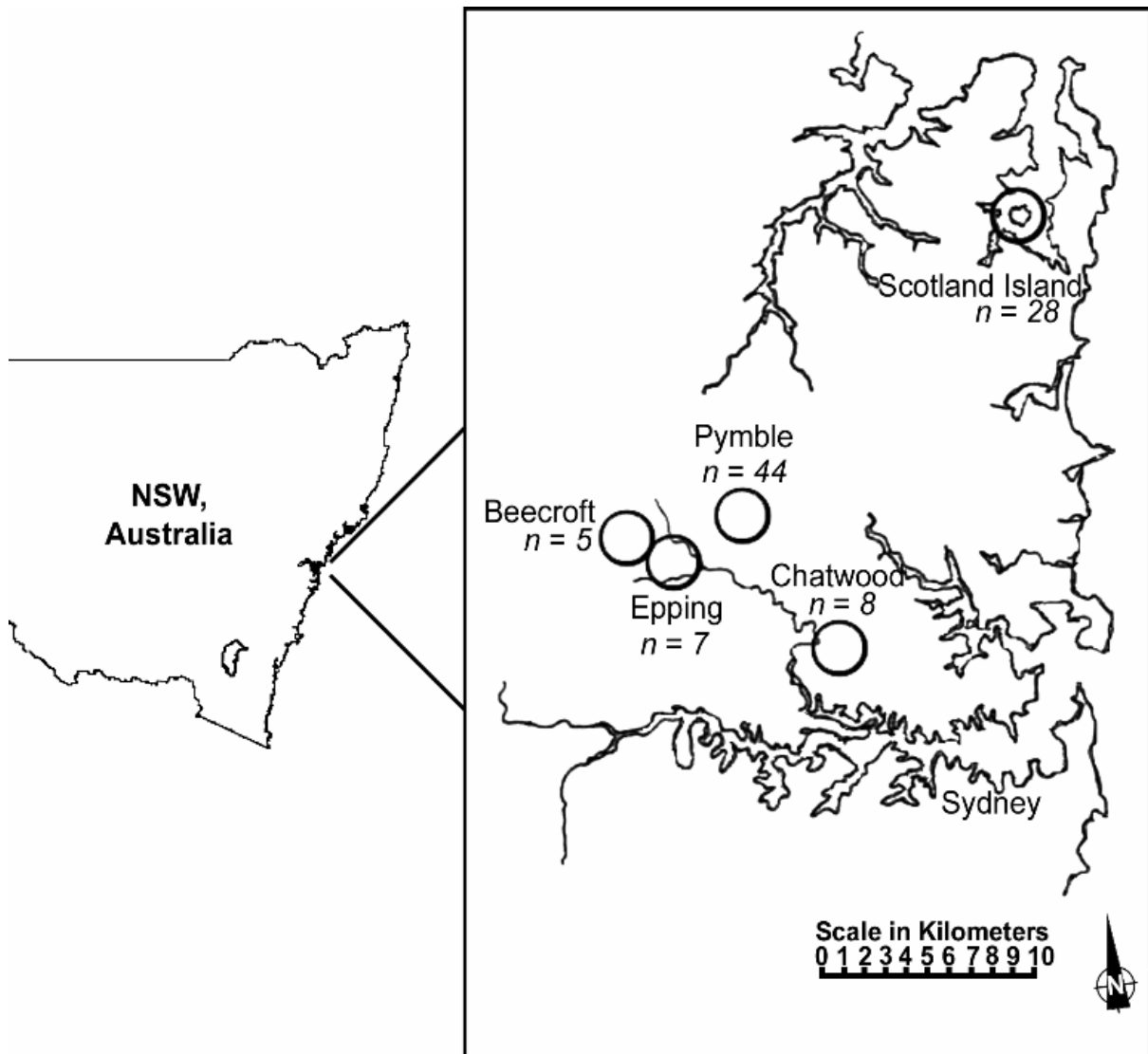


Figure 1. *Trichosurus vulpecula* was sampled from Scotland Island and four Sydney metropolitan suburbs: North Epping, Chatswood West, Pymble and Beecroft.

Tissue biopsies were obtained from the ears of adult individuals; 64 samples were collected from the mainland (28 females and 36 males) and 28 samples from Scotland Island (11 females and 17 males). Because dispersal in brushtail possums mainly occurs about the time of sexual maturity (Cowan and Clout 2000) only adults were included in these analyses. Individuals were sexed and classified as adult if they were 2 kg or over and had class 2 or higher teeth wear (Winter 1980; Cowan and White 1989).



Tissue was subjected to DNA extraction by salting out (Sunnucks and Hales 1996) and genotyped at seven microsatellite loci, Tv16, Tv19, Tv27, Tv53, Tv58, Tv64 (Taylor and Cooper 1998) and TvM1 (Lam *et al.* 2000). PCR conditions were essentially as described by Taylor and Cooper (1998) and length polymorphisms were visualised by autoradiography.

### Summary genetic data

Observed heterozygosity ( $H_o$ ) and expected heterozygosity ( $H_E$ ) from both mainland and Scotland Island possums were calculated using the program GenAlEx V5 (Peakall and Smouse 2001). Any deviation from Hardy-Weinberg equilibrium for each of the loci was tested using GENEPOP version 3.3 (Raymond and Rousset 1995). In order to compare levels of genetic diversity among groups of varying sample size a measure of allelic richness (which accounts for sample size) was estimated using FSTAT version 2.9.3 (Goudet 2001). The mean level of allelic richness for each locus, averaged from each of the mainland sampling locations, was compared with values obtained from Scotland Island with a Wilcoxon sign rank test.

### Dispersal inferred from genetic structure

Patterns of dispersal can be inferred by examining relatedness structure. For example with sex-biased dispersal, individuals of the philopatric sex will have higher pairwise relatedness in close proximity to one another and a more pronounced decline in pairwise relatedness with distance (Taylor *et al.* 1997; Stow *et al.* 2001; Peakall *et al.* 2003). Pairwise relatedness among individuals of each sex was calculated using KINSHIP 1.2 (Goodnight *et al.* 1998). For individuals sampled at the same garden area the mean relatedness between male possums was compared with mean relatedness between female possums. The significance of any differences in means was determined using randomisation as executed by software POPTOOLS 2.6.2 (Hood, 2005).

To examine the relationship between genotypic similarity and geographic distance spatial autocorrelation was measured. A coefficient of genotypic similarity ( $r$ ) was calculated for adults of each

sex and analysed using the program GenAlEx V5 (Peakall and Smouse 2001). Values obtained have been found to be similar to those calculated using softwares such as KINSHIP (Banks *et al.* 2005). Genotypic similarity was calculated for several predefined distance classes and the 95% confidence intervals around these estimates determined by bootstrapping (999 replicates). The 95% confidence intervals around the null hypothesis of no spatial genetic structure ( $r = 0$ ) were generated by 999 permutations (Peakall and Smouse 2001). Individuals from the different mainland suburbs were pooled (i.e. individuals sampled  $> 2$  km apart) to enable spatial genetic structure to be examined to distances greater than 10 km.

To further examine the geographic extent to which sex-biased dispersal can be detected, adjusted assignment indices (Alc values; Favre *et al.* 1997) were calculated and male and female possums within each of the four mainland suburbs (i.e. individuals sampled at distances  $< 2$  km from each other) compared using the randomisation procedure implemented by FSTAT version 2.9.3 (Goudet 2001). A lower Alc value is expected for the dispersing sex. Small sample sizes prohibited us from analysing Alc values for adults of each sex at geographic distances less than this.

## Results

### Male-biased dispersal in urban populations

Male-biased dispersal for *T. vulpecula* was clearly apparent on both the mainland and Scotland Island. Relatedness between individuals that were sampled at the same residential garden was significantly lower for males than for females (Fig. 2). In addition, genotypic similarity ( $r$ ) calculated in GenAlEx was lower for males than females sampled within the same garden area (distance class zero; Fig. 3). Male-biased dispersal was also illustrated by the correlograms of  $r$  where positive genetic structure was present at substantially greater geographic distances for male than female possums (see the intercept of  $r$  with the x- axis; Fig 3). However, even for male possums, the extent of positive structure did not extend beyond approximately 900 m indicating generally high levels of philopatry. Therefore, sex-biased

dispersal will be most apparent at distances less than 900meters. Indeed, on the basis of individual genotypes, female possums were not significantly more 'likely to belong' to the suburb from which they were sampled than male possums (Mean Alc  $\pm$  S.E, Male =  $-0.117 \pm 0.239$ ; Female =  $0.094 \pm 0.213$ ,  $P = 0.279$ ).

Figure 2. Mean relatedness  $\pm$  S.E for adults of each sex sampled from the same garden area from mainland localities (MF = female; MM = male) and Scotland Island (SIF = female and SIM = male). Relatedness was significantly higher among females than among males on the mainland (mean  $\pm$  S.E; male =  $0.088 \pm 0.045$ ; female =  $0.297 \pm 0.042$ ,  $P = 0.033$ ) and on Scotland Island (mean  $\pm$  S.E; male =  $0.103 \pm 0.0360$ ; female =  $0.315 \pm 0.065$ ,  $P = 0.002$ ).

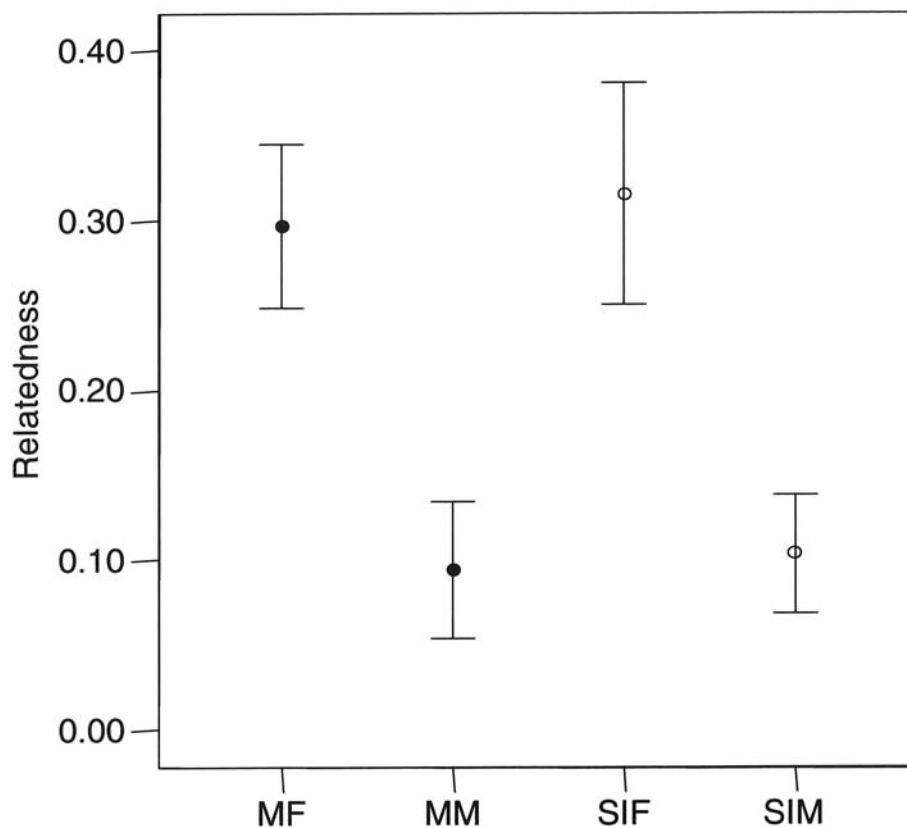
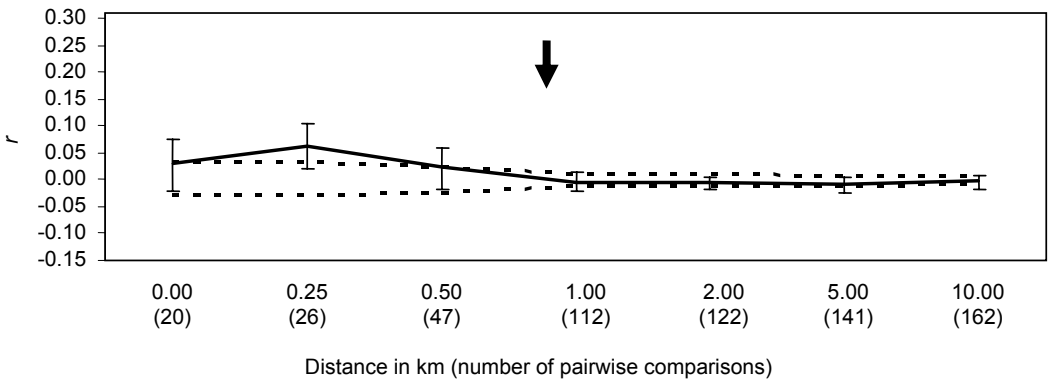
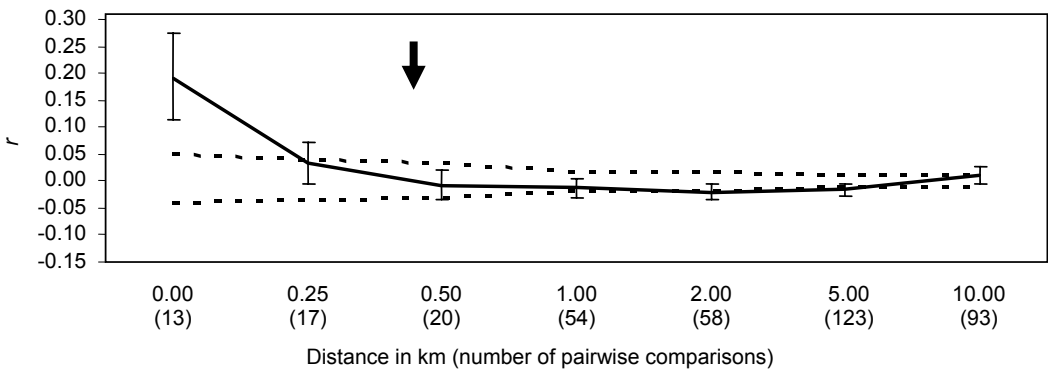


Figure 3. Spatial autocorrelation correlograms for *Trichosurus vulpecula* located in the mainland Sydney metropolitan area and Scotland Island. Correlograms are of genotypic similarity ( $r$ ) with geographic distance at seven distance categories, measured in kilometres. The solid line tracks observed relationship, dashed lines represent the upper and lower 95% confidence interval around random expectations while bars around  $r$  show the 95% confidence determined by bootstrapping. The arrows indicate the x-axis intercept (in kilometres) for adult male (0.896; a) and female (0.454; b) possums from the mainland and adult male (0.413; c) and female (0.309; d) possums from Scotland Island.

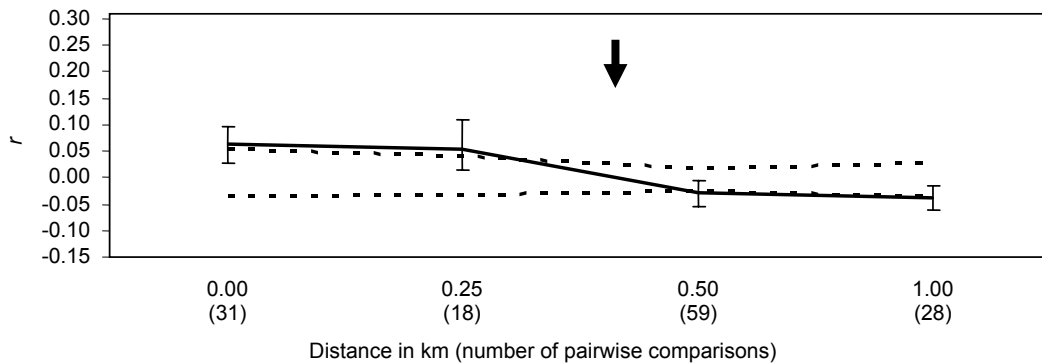
(a) Mainland adult male possums



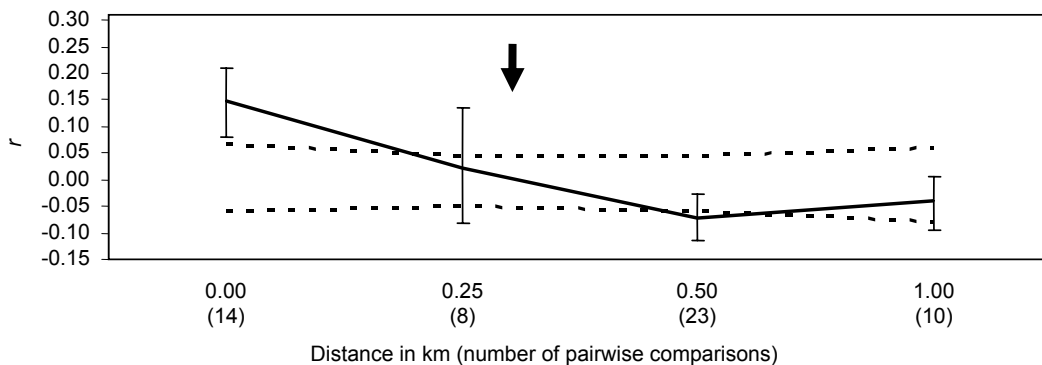
(b) Mainland adult female possums



## (c) Scotland Island adult male possums



## (d) Scotland Island adult female possums



### Lower levels of dispersal for possums on Scotland Island than on the mainland

The level at which *T. vulpecula* dispersed was generally less on Scotland Island than the mainland (Fig 3 a - d). In particular, the spatial extent to which male possums show positive genetic structure is less on Scotland Island than the mainland (413 m *versus* 896 m respectively). The distribution of  $r$  with geographic distance also indicated that female dispersal may be slightly lower on Scotland Island than the mainland (extent of positive genetic structure 309 m *versus* 454 m respectively).

## Genetic diversity in island and mainland populations

Genetic variability for *T. vulpecula* was significantly higher in the mainland than Scotland Island samples (Table 1). Greater allelic richness was estimated in the mainland than the Scotland Island individuals (Wilcoxon sign rank test,  $Z = -2.028$ ,  $P = 0.043$ ).

Table 1. For seven polymorphic loci in *T. vulpecula*, the number of alleles ( $k$ ), allelic richness ( $Ar$ ), observed ( $H_O$ ) and expected ( $H_E$ ) heterozygosity and probability of Hardy-Weinberg equilibrium (HWE). All of the loci were in HW equilibrium after sequential Bonferroni corrections for multiple tests (Rice 1989). Wahlund effect, resulting from genetic subdivision, may explain the mild homozygote excess in the mainland data.

Locus	Mainland Suburbs					Scotland Island				
	$k$	$Ar$	$H_E$	$H_O$	HWE	$k$	$Ar$	$H_E$	$H_O$	HWE
<b>Tv16</b>	14	5.945	0.809	0.659	0.051	9	4.857	0.875	0.802	0.719
<b>Tv19</b>	13	5.432	0.873	0.823	0.919	10	5.780	0.808	0.837	0.931
<b>Tv27</b>	11	6.056	0.875	0.861	0.871	9	5.422	0.885	0.820	0.612
<b>Tv53</b>	17	7.071	0.911	0.873	0.046	8	4.475	0.692	0.734	0.094
<b>Tv58</b>	10	5.096	0.748	0.756	0.854	6	3.870	0.615	0.678	0.189
<b>Tv64</b>	23	7.275	0.910	0.902	0.621	11	5.753	0.885	0.841	0.378
<b>TvM1</b>	11	5.852	0.856	0.828	0.560	9	5.519	0.885	0.829	0.334
<b>Mean</b>	14.143	6.104	0.854	0.814	-	8.857	5.097	0.806	0.792	-

## Discussion

Our analysis of dispersal in urban *T. vulpecula* reveals generally high philopatry and male-biased dispersal. Male-biased dispersal was inferred by significantly lower male than female relatedness for possums sampled within the same garden area. In addition male possums showed higher levels of genotypic similarity at substantially greater geographic distances than females. However, significant male-biased dispersal was only apparent out to distances of approximately 900 meters. Beyond this distance our data suggests that dispersal of *T. vulpecula* is relatively infrequent.

Our genetic-based inference of especially low female dispersal is congruent with ecological studies, carried out in non urban environments, where females were shown to be philopatric. Daughters typically reside beside their mothers (Clinchy 1999) and/or take up home ranges that largely overlap with their mothers (Cowan and Clout 2000). Female *T. vulpecula* from urban areas have average home range sizes of approximately 1-2 ha (Statham and Statham 1997; Harper 2005). In natural environments sons have been shown to disperse and settle two or three home ranges away from their mother (Clinchy *et al.* 2004). Interestingly, these distances for male dispersal are in agreement with our data showing relatedness among adult males to be highest when individuals are separated by 250 to 500 meters (Figure 3a). It is therefore apparent that urban development has not dramatically altered male dispersal or sex-based differences in dispersal. Male-biased dispersal has been shown in previous studies on *T. vulpecula* from non-urban areas in New Zealand where males disperse from their natal area and females remain (Cowan and Clout 2000; Ji *et al.* 2001).

Lower adult dispersal on Scotland Island coupled with reduced genetic variation suggests that this population is largely isolated. The most likely explanation for significantly lower genetic diversity on Scotland Island was isolation and loss of diversity through drift (Frankham *et al.* 2002). Indeed, many species have been found to have lower genetic diversity on islands compared to their mainland counterparts (Frankham 1997). Reduced genetic diversity for island-dwelling *T. vulpecula* was also

shown for the Tasmanian population, which showed reduced genetic variability when compared to the mainland (Triggs and Green 1989; Taylor *et al.* 2004). Similarly in New Zealand possums introduced to offshore island generally had reduced genetic variability when compared to both the north and south islands of New Zealand (Taylor *et al.* 2004). Loss of genetic diversity as a consequence of being confined to an island has also been seen in other native Australian species such as the dibbler (Mills *et al.* 2004), quokka (Sinclair 2001) and several macropod species (Eldridge *et al.* 1999; Eldridge *et al.* 2004).

Lower levels of dispersal apparent for possums on Scotland Island compared with those sampled on the mainland were possibly a consequence of isolation, small island size (52ha) and resource availability. With resource availability typically higher in insular systems, island species are expected to have smaller home-range sizes than their mainland counterparts (Stamps and Buechner 1985). Reduced dispersal rates in island populations have also been observed in rodents (Adler and Levins 1994), foxes (Roemer *et al.* 2001), voles (Tamarin 1977) and deer mice (Sullivan 1977). In future studies it may be of interest to investigate whether reduced dispersal is associated with a higher population density and perhaps higher levels of aggressive interaction (e.g. Peacock and Smith 1997).

Dispersal is undoubtedly one of the most important parameters influencing the recovery of possums after they have vacated an area (Cowan and Clout 2000). Even after a severe population reduction, possums continue to disperse, albeit in reduced numbers, showing that density reduction does not affect the normal dispersal behaviour of possums (Cowan *et al.* 1997; Cowan and Clout 2000). Therefore, irrespective of any management that reduces the density of *T. vulpecula* in urban environments, it seems likely that ongoing management will be necessary. Our data indicate that dispersal across distances of more than around 900 meters is infrequent. Knowledge of the typical extent of dispersal in the urban environments will assist with designing strategies to reduce population densities. Moreover, information on dispersal for male and female *T. vulpecula* could be particularly relevant to future management strategies using fertility control (Eymann *et al.* 2006). For example, with



male-biased dispersal reducing local population densities may be best achieved by targeting female fertility (Zenger *et al.* 2003)

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## Appendix D

# Management issues of urban common brushtail possums (*Trichosurus vulpecula*) – a loved or hated neighbour

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## Introduction

The common brushtail possum (*Trichosurus vulpecula*) is a solitary, nocturnal, arboreal marsupial in its native Australian environment (Kerle 2001). It is a cat-sized phalanger generally occurring where there are trees, especially in open forests and woodlands. It has disappeared from large parts of its former natural habitat, including all of arid Australia (Goldingay and Jackson 2004). Declines are attributed to a combination of factors including habitat loss and disturbance, disease, drought and impact of predators on depleted populations. Conversely possums are very common in many Australian cities. *T. vulpecula* is also a major invasive pest in New Zealand damaging native forests and spreading bovine tuberculosis (Montague 2000). This creates distinctive management issues for *T. vulpecula* and our research focuses on the cohabitation with people in Australian urban areas (Eymann *et al.* 2006). *Trichosurus vulpecula* has readily adapted to urban areas by varying its behaviour and dietary preferences and can occur in high densities (Kerle 2001). Conflicts with residents arise when *T. vulpecula* invades roof spaces and damages garden plants and community attitudes towards this species vary widely. Some enjoy seeing possums on their property, while others regard them as pests and wish them to be removed (Lunney and Burgin 2004).

The strategies used by wildlife agencies to manage *T. vulpecula* in Australia vary depending on their legislative status in each state. Possums are a protected species and most state agencies encourage a policy of “living with the possum” (Matthews *et al.* 2004). Licences can be obtained solely to remove them from the roof, and nest boxes are recommended to provide *T. vulpecula* with an alternative den site. *Trichosurus vulpecula* must be released within 50m of the capture site as relocation has deleterious effects on the animals. In exceptional circumstances some state agencies may allow euthanasia of nuisance animals. The 50m translocation rule is warranted from an animal welfare point of view, but it is unlikely to reduce the nuisance problem for many people. The possum can return or new individuals immigrate into the vacant space. This often leads to non-compliance with government policies resulting in illegal trapping and removal of possums from private properties, and “desperate people will do

anything, and the kitchen stove is within the 50m possum zone of most suburban blocks” (The Daily Telegraph, 21/02/01). The issues arising from urban possum management are manifold and require further comprehensive research aiming to help in the development of alternative strategies. Our research focuses on specific management issues such as humane control of abundant possum populations and transmission of disease in an urban environment:

a) Possum control:

Fertility control may be a publicly acceptable way to reduce possum numbers to mitigate their minor pest status in urban areas. Reducing the reproductive potential of a population is increasingly becoming an accepted approach to managing wildlife (Cowan *et al.* 2003). Growing public concern for animal welfare, together with a desire to find non-lethal alternatives to culling methods have been major incentives for our research. Deslorelin is a promising non-steroidal, non-immunological approach to contraception in the possum. It is a gonadotrophin releasing hormone (GnRH) agonist formulated into implants that are administered subcutaneously. It inhibits production of sexual hormones and renders the animal temporarily infertile. It has been successfully trialled in females of *Macropus eugenii* (tammar wallabies; Herbert *et al.* 2004), *M. giganteus* (eastern grey kangaroos) and *Phascolarctos cinereus* (koalas); males of these species are resistant to the contraceptive effects of chronic GnRH agonist treatment. We are currently investigating contraceptive effects on *T. vulpecula*.

b) Possum disease:

A further management issue that has been hardly addressed to date is disease transmission in the urban environment. Proximity to urban areas or contact with humans and their domestic animals can increase the risk of disease exposure for wild populations and influence their health (Deem *et al.* 2001). Urban *T. vulpecula* live in close proximity to humans and their pets and interact to varying levels with them, suggesting the possibility of transmission of diseases in all directions. Our seroprevalence study

focuses on diseases that can occur between humans, pets and possums such as leptospirosis and toxoplasmosis.

## Methods

A captive study in New Zealand included animals of both sexes and various methods were used to investigate the effects of deslorelin on possums. A field trial with deslorelin was started in Sydney's suburbs in 2003 which included live trapping of *T. vulpecula* in householders' backyards. Trapped possums were microchipped, aged, weighed and some females received a deslorelin implant. Offspring numbers of treated and control females were checked over the last 2 years. Serum samples for the seroprevalence study were obtained from more than 200 adult possums. To date, we have tested for leptospirosis, a bacterial disease. Serology for the different *Leptospira* serovars, was carried out using the microscopic agglutination test (MAT) at Queensland Health Scientific Services.

## Findings to date

### a) Possum control using Deslorelin:

Our results show that Deslorelin can successfully inhibit reproduction in female possums. Males have proven to be resistant to the treatment. The field trial in Sydney testing the long-term contraceptive effects of Deslorelin confirms this result: it inhibits fertility in females for at least one breeding season and no negative side-effects were observed. Deslorelin has been used successfully on other wildlife species with no side-effects or behavioural changes reported (Bertschinger *et al.* 2002). This gives this method hope for success, and we have delivered a humane option for control. Further research will be needed to evaluate a model for control operations and to assess the extent of application within an urban environment.

## b) Possum disease:

Antibodies to leptospirosis were found in less than 10% of all samples. The main agent was identified as serovar Hardjo, with 2 possums demonstrating exposure to serovar Ballum. This serovar appears to be new to Australia but is endemic to New Zealand. Seroprevalence varied from 0 to less than 12% in the different suburbs. Seroprevalence for leptospirosis was slightly related with age, with older possums seeming to be more affected. Seroprevalence did not show a significant distinction in relation to gender and was connected to a few backyards only. This data support New Zealand research findings that possums are unlikely to contract leptospirosis through a contaminated environment alone (Day *et al.* 1998). Transmission also probably occurs as a result of affiliative or sexual behaviour. The present study demonstrates for the first time the presence of antibodies to leptospirosis in urban brushtail possums.

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## Appendix E

# Description of study sites

### **AgResearch possum breeding unit, Wallaceville, New Zealand**

The brushtail possums used for the experiment in Chapter 2 were captured on the North Island, Wellington area (latitude 41°S) of New Zealand and housed within the breeding unit facilities of AgResearch Wallaceville, Upper Hutt. Newly captured animals were held in quarantine facilities for a few weeks before introduction to the breeding unit. The breeding unit practices a group-housing system in environmentally-enriched pens with runways (McLeod *et al.* 1997). In general, three females and one male share a pen to allow reproduction. Besides, the presence of males is positively correlated with the incidence of ovulation after pouch young removal (Crawford *et al.* 1998). A mixed diet of fresh fruit, cabbage and *Pinus radiata* branches are provided, with cereal based pellets and fresh water ad libitum (Fig. 1). This housing system successfully reduces the stress that accompanies housing wild, predominantly solitary animals in captivity.



Figure 1. The possum breeding unit pens at AgResearch with an animal feeding a) on fresh fruit and cabbage and b) from a *Pinus radiata* branch (photos, Jutta Eymann).

## Field sites within Sydney Metropolitan area (North Shore suburbs and Scotland Island)

Beecroft (33°45'S, 151°04'E) and North Epping (33°45'S, 151°05'E) are both part of the Hornsby Shire which is also known as the Bushland Shire. It covers a total of 51,000 hectares of which approximately 67% is bushland, “making the opportunity for natural experiences enormous” (Hornsby Shire Council 2006). Hornsby Shire Council is responsible for the management of over 5,750 hectares of public bushland and the conservation of a diverse array of native fauna and flora within the Shire that is currently serving the needs of about 150,000 residents.

Chatswood West (33°47'S, 151°09'E) is part of Willoughby City Council which is located only 8.5 km from the Sydney CBD. Willoughby has a population of about 63,000 people and estimated 27,000 households. Since European settlement, 87% of bushland in the Willoughby Local Government Area

(LGA) has been cleared for development (Willoughby City Council 2006). With this huge clearance of bushland, an unknown number of native trees, shrubs, grasses, vines, moss, fungi and other species have become extinct from the LGA. However, one of the principal characteristics of the Willoughby LGA is the presence of significant areas of remnant native bushland, particularly along the Lane Cove River. Willoughby contains approximately 300 hectares of bushland under the care, control and management of the Council. Nowadays, it is well known as a green and leafy area, and street trees play a central role in contributing to its landscape quality and can not be pruned or removed without council approval (Fig. 2).



Figure 2. 'Chatswood seen through the trees' (photo courtesy of Willoughby council).

Pymble and West Pymble (33°45'S, 151°07'E) are part of the Ku-ring-gai Local Council area which is fortunate to retain a large proportion of natural areas close to urban and commercial zones. It has approximately 1,100 hectares of remnant bushland in Council reserves (Ku-ring-gai Council 2006a). Ku-ring-gai is located about sixteen kilometres from Sydney and is on average 152 metres above sea level. The area has a moderate climate with an average maximum temperature in the hottest month of 25.8°C, an average minimum in the coldest month of 7.7°C, and the average rainfall is 1,118 millimetres per annum (Ku-ring-gai Council 2006b). The Ku-ring-gai local government area covers



approximately 8,446 hectares and displays a wide variation in landscape and wildlife with significant areas of urban bushland identified as having high conservation status. Of the residential area, 95% is occupied by low-density housing (Fig. 3), and only 5% of land use is designated to business and other usage. The residential area is bounded by Garigal National Park in the east, Lane Cove National Park in the west and Ku-ring-gai Chase National Park in the north. The area is part of the eastern rim of the Cumberland Basin, elevated during the Kosciusko uplift, and consists of Hawkesbury Sandstone and Wianamatta Group Rocks of the Triassic Period. Natural vegetation varies from Sydney Blue Gums and Blackbutt in the West Forest area of south-west Wahroonga and Turramurra to Stringy Bark, Grey Iron Bark and Bloodwood types in the Dry Forest areas.



Figure 3. Example of a backyard in West Pymble where possum traps were set (photo, J. Eymann).

It is noteworthy that the Lane Cove National Park is interwoven with all the described suburbs. The Lane Cove River winds through a bushland valley within easy reach of the city centre (Fig. 4), extending from East Ryde to Wahroonga/Pennant Hills (National Parks and Wildlife Service 2006). Woodland and shrubland communities grow on the upper slopes and ridges where the poorer soils derived from sandstone have low nutrient levels and poor water-holding capacity. Scribbly gum, Angophora, old man

Banksia and acacias are also found. Eucalypt forests are located in sheltered gullies, and the park's gallery forest communities have black wattle, blueberry ash, sweet pittosporum, lilly-pilly, coachwood, watergum and other rainforest species.



Figure 4. Sydney CBD viewed from Lane Cove National Park (photo, J. Eymann).

Scotland Island is situated in an estuary called Pittwater, which is part of Broken Bay about 25 km from Macquarie University (Fig. 5). The environment of Scotland Island is similar to that of the sampled mainland suburbs, with high residential densities and dense surrounding vegetation (Fig. 5). There are still significant areas of bushland in the Pittwater Local Government Area (Pittwater Council 2006). Residents are encouraged to care for bushland and to enhance planting in the garden by using locally native species of plants. Pittwater Council holds the view that native birds and other animals can not live in the bushland reserves alone, but need to rely on the vegetation occurring on private land to survive. Scotland Island has a special and active community life, which includes for example a regular e-newsletter where any issues (even possums...) can be published ([www.scotlandisland.org.au](http://www.scotlandisland.org.au)).

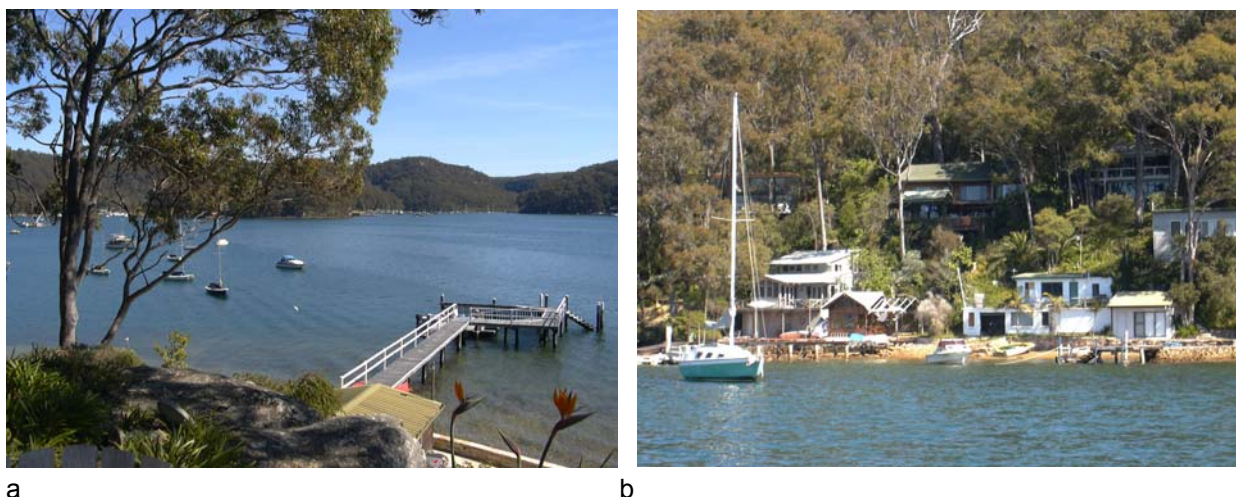


Figure 5. The view from Scotland Island, situated in Pittwater, to Ku-ring-gai Chase National Park (a) and residential houses on the waterfront (b) (photos, J. Eymann).

### Scientific Field Station Smiths Lake, Myall Lakes National Park

Macquarie University has access to a field station at Smiths Lake which lies adjacent to Myall Lakes National Park (Fig. 6). The opportunity arose to conduct possum trapping at the site, and the results from this field trip have been incorporated in Chapter 4 (Note: Serological survey of possum sera from Smith Lake revealed that none of the animals ( $n=7$ ) had been exposed to toxoplasmosis nor to leptospirosis). Smith Lake is approximately 240 km north of Sydney ( $32^{\circ}24'S$ ,  $152^{\circ}28'E$ ), and the dominant feature of the park is the lake system (National Parks and Wildlife Service 2006). Three interconnected lakes - Bombah Broadwater, Boolambayte Lake and Myall Lake - occupy an ancient river basin bounded by high sand dunes on the coast and much older flatter dunes in the west. The dominant geology within the park is sedimentary rock consisting of sandstones, silt-stones and mudstones. Average summer temperatures range from  $18-28^{\circ}C$ , winter temperatures from  $5-17^{\circ}C$ , average annually rainfall is 1342.4 mm, and the highest point of elevation in the park measures 496 metres. The native plant communities are diverse within the park, and rainforest is found at Mungo Brush hill, swamps between Mungo and Seal Rocks road and eucalypt forests in the western section of the park. More than 20 per cent of the vegetation in the park is open woodlands and heath. The park supports a



diverse range of animals typical of this coastal, forested environment with 41 species of mammals recorded.

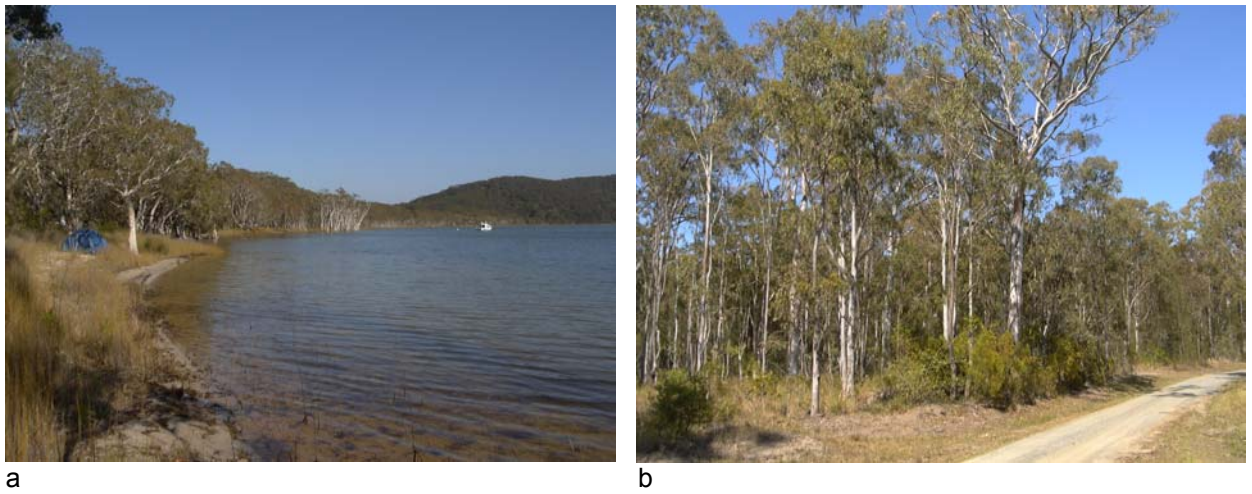


Figure 6. Smiths Lake (a) and nearby forest area (b) where *T. vulpecula* were caught (photos, J. Eymann).


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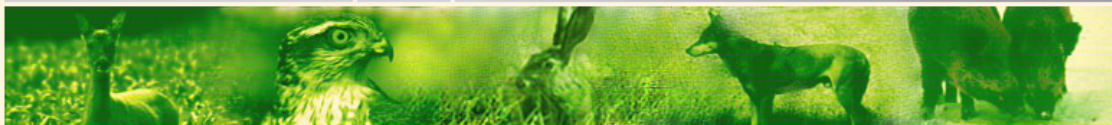
# Awards

## Student award for best oral presentation

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### Student Award

In the Student Award competition, prizes were awarded to both the best oral presentations and the best posters presented by students.

Out of 30 **oral presentations** submitted for the Student Award the jury, consisting of Wilfried Bützler (Myanmar), Horst Kierdorf (Germany) and Paul Krausmann (USA), elected the following winners:

1st Prize **Jutta Eymann** (Macquarie University, Department of Biological Sciences, Sydney NSW 2109 Australia)  
Management issues of urban common brushtail possums (*Trichosurus vulpecula*) - a loved or hated neighbour?

2nd Prize **Eduardo Ferreira** (Department of Biology, University of Aveiro, Campus Universitário de Santiago, 3810-193, Aveiro, Portugal)  
Portuguese wild boar (*Sus scrofa*) population structuring: results from a microsatellite analysis.

3rd Prize **Joanna Olko-Pieczkolan** (Institute of Environmental Sciences, Jagiellonian University, 7 Gronostajowa Str., 30-387 Krakow, Poland)  
The home ranges of domestic cats (*Felis catus*) in the Ojcow National Park, southern Poland.

Out of 28 **posters** submitted for the Student Award the following authors were nominated for their presentations:

1st Prize **Michaela Nováková** (Institute of Vertebrate Biology AS CR, Kvetná 8, 603 65, Brno, Czech Republic)  
Is stone marten (*Martes foina*) food competitor of western polecat (*Mustela putorius*) in the Czech Republic?

2nd Prize **Jarmila Prokešová** (Institute of Vertebrate Biology AS CR, Kvetná 8, 603 65, Brno, Czech Republic)  
Roe (*Capreolus capreolus*) and red (*Cervus elaphus*) deer foraging habits under snow conditions.

3rd Prize **Sandra Cellina** (Department of Biology & Environmental Science, University of Sussex, Brighton BN1 9QG, UK)  
Supplemental feeding of wild boar *Sus scrofa* in Luxembourg.

Congratulations for their excellent work!

We would especially like to thank the Publishing House M & H Schaper and the Deutsche Landwirtschaftsverlag for sponsoring this year's prizes – books, calendars are thermos flasks. Some of the winners could not attend the Closing Ceremony, in these cases we will send the respective prizes as soon as possible.

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## **DVC (Research) Commendations awarded for Outstanding Postgraduate Research Fund Applications**

Round 2 of the 2005 offering of the Postgraduate Research Fund attracted a total of 84 applications, the highest number for this Scheme in recent years. Of these, four were singled out to receive the Deputy Vice Chancellor (Research) Commendation.

### **Congratulations to:**



**Aaron McKay**  
(Physics, ICS)



**Jutta Eymann**  
(Biological Sciences, ELS)



**Valeria Murgulov**  
(Earth & Planetary Sci, ELS)



**Joanna Hoyle**  
(Physical Geography, ELS)

These students not only submitted applications of a high standard, they were deemed to exhibit a true commitment to and a deep understanding of their chosen research field, and communicated this with skill and professionalism in their interview. From this round forward, the award also comprises a further \$500.00 of funding for the students' research projects.

By Karyn Knowles; Pictures of A McKay, J Eymann and V Murgulov by Allyn Sayers

Source: <http://www.ro.mq.edu.au/news/news3.htm>



