

## Health status and translocation success of wild and rehabilitated possums

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**ABSTRACT:** Translocation of the western ringtail possum (*Pseudocheirus occidentalis*) is currently undertaken as a management strategy in the face of habitat destruction. Translocation success has been variable over the past decade, potentially influenced by factors such as habitat quality and predation pressure. The current translocation programme incorporates an investigation of the health and disease status of western ringtail and common brushtail (*Trichosurus vulpecula*) possums, both in their natural habitat around Busselton (south-west WA) and at the translocation sites in Yalgorup National Park and Leschenault Peninsula Conservation Park 50-100km north of Busselton. We discuss the physiological parameters and diseases screened for in this programme, and the outcomes of recent translocations in relation to health status, possum dispersal and predation pressure.

### **Introduction**

The Western Ringtail Possum (*Pseudocheirus occidentalis*) is classified as vulnerable on the Threatened Species List. Populations are now restricted to the south-west corner of Western Australia from Bunbury to Albany, including both coastal and forest habitats (see maps). Populations are diminishing due to habitat loss from building development in coastal regions and from logging and fire in forest regions.

Because of continued habitat loss due to building development, particularly in the Busselton region, a translocation programme has been in operation since the mid-1990s. Displaced western ringtail possums have been translocated from building development sites in the Busselton region to national parks further north in an attempt to reestablish viable populations within their pre-European range (see maps).

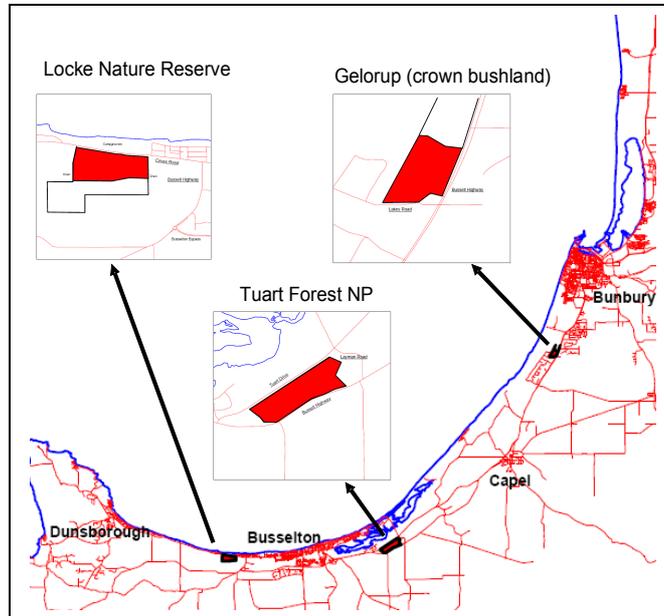
Survival and reproduction of animals translocated during the early 1990s, mainly to Leschenault Peninsula, was initially successful, but a population crash occurred at that site between 1998 and 2002 for indeterminate reasons. Possible causes of the population crash include predation (by foxes, cats, pythons, chuditch or raptors), disease, competition with the common brushtail possum (*Trichosurus vulpecula*), and habitat or climatic factors.

The current study is an extension of the work carried out by Paul de Tores and others of DEC over the past decade. It incorporates research into the health and survival of ringtail possums in their natural habitat around Busselton. The aims of the current project are to:

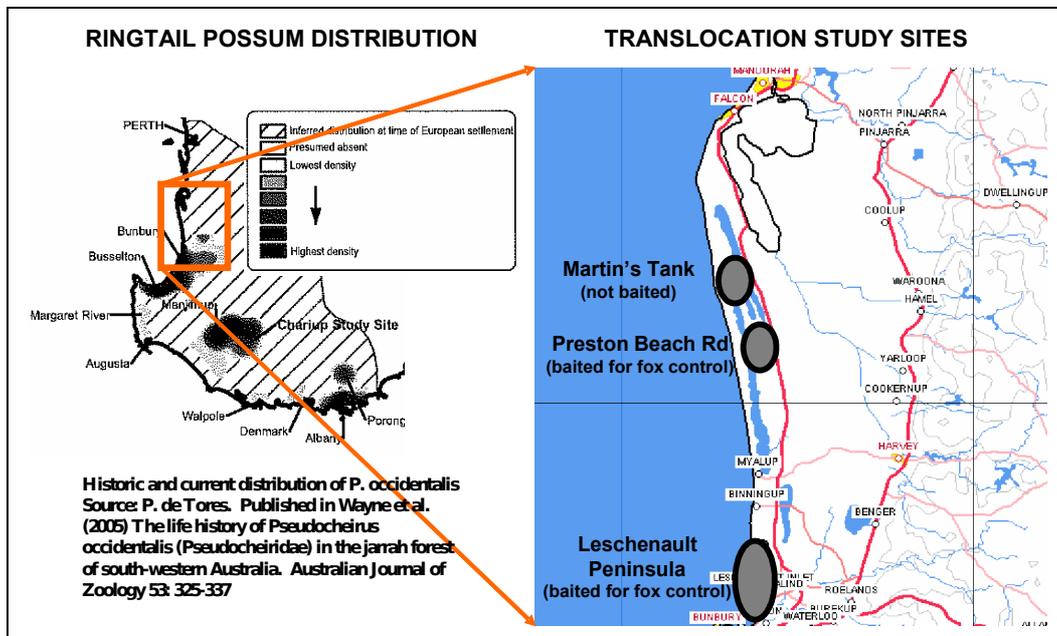
- Monitor survivorship of western ringtail and common brushtail possums, both in their natural habitat around Busselton and subsequent to translocation.
  - Examine the health status of possums at natural and translocation sites, and investigate relationships between health and survival.
  - Determine the importance of site-specific habitat variables, fox control and inter-specific competition to survival and translocation success of the western ringtail possum.
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The maps below show the sites at which western ringtail possums are being studied in their natural habitat and at the translocations sites. Health status and survival will be discussed in the following sections of this paper.

Study Sites: study of naturally occurring populations



Study Sites: study of translocated populations



## Health and Disease Investigation

### *Sampling methods*

Examination and sampling is carried out under general anaesthesia, which is usually smooth and uneventful, enhances our ability to fully examine and take samples from the animals, and ameliorates welfare concerns due to the examination process. We use a purely gaseous anaesthesia, with both induction and maintenance via isoflurane in oxygen administered by mask (small cat size, with a 1L rebreathing bag). We typically use 1.5L/min oxygen throughout, with isoflurane at 5% for induction and 1.5-2% for maintenance. Animals are induced in their bags; the ringtails through their thin calico bags, and the brushtails directly but completely within their hessian bags. Induction is typically smooth and rapid, taking 1-2 minutes. There is usually minimal struggle, particularly with gentle restraint so that the animal cannot move within the bag. After processing is complete the animals are recovered back in their bags, on oxygen continued at 1.5L/min until they are fully conscious and moving voluntarily.

The use of endotracheal intubation was initially investigated for ringtails but proved to be quite difficult. We attempted intubation using a size 2.0 ET tube with a size 0 Welch-Allyn laryngoscope blade, the smallest of each readily available, but the small size and curvature of the caudal pharynx still prevented easy intubation. It would require use of a stylet in the ET tube, and possibly a different laryngoscope blade, for success. We have not pursued development of this technique, as mask anaesthesia has been simple and well tolerated, and the intubation process would probably only increase stress and processing time for each animal.

The entire anaesthesia, examination and recovery process is carried out on an electric heat pad. With such a small body weight, we have to be very conscious of hypothermia during anaesthesia. Hot water bottles or heat packs are placed in the boxes for post-recovery if necessary. Rectal temperature, as well as heart rate and respiratory rate, are monitored at least once during the anaesthesia.

Blood is collected from the lateral tail veins. We have found that the veins of ringtails are much more obvious and resilient than those of brushtails, possibly as a result of the increased demand made on the prehensile tail of ringtails. Brushtail veins do not stand up as well, and form haematomas ("blow") much more readily. Collection from the femoral vein was initially considered, but as a blind technique proved to be too risky and provide too few advantages over tail venipuncture to be worthwhile practicing. In such a small animal, we also need to ensure that we don't take more than 10% of circulating blood volume (estimated at 50ml/kg); these are both thought to be conservative figures. Therefore, for example, we can take a maximum of 5ml blood from a 1kg possum. Tail veins usually yield sufficient blood. We store a small amount of blood in EDTA anticoagulant and allow the rest to clot; this is then centrifuged and the serum pipetted off.

Urine is collected by perabdominal expression of the bladder while under anaesthesia. Scats are collected from the bag, and are therefore usually the firm scats produced by both species during the night (ringtails also produce a soft daytime scat called a caecotroph, which they re-ingest and digest).

We collect two types of swabs. One, moistened with sterile water, from the cloaca for microbiology. The second type is collected dry from mucosal surfaces – the conjunctiva bilaterally, the oropharynx and the cloaca – to collect mucosal cells for *Chlamydophila* testing.



The examination process also involves collection of body measurements and assessment of the physical condition and status of the skin, eyes, ears and teeth. A small biopsy of tissue from the edge of one ear is collected to provide DNA for genetic analysis. A simple ear tag is attached to the outer edge of the other ear and an identification microchip is applied subcutaneously between the shoulder blades. Finally, a radiocollar is fitted to allow for ongoing monitoring.

Most samples are sent for processing at the clinical pathology department of the Murdoch University Veterinary Hospital. However, the *Chlamydomphila* swabs and two sets of serum are frozen and sent interstate in batches for processing at specialist laboratories (acknowledged below).

### ***Health Screening Results***

Standard veterinary physiological parameters are evaluated by the MUVH lab haematology (complete blood count or CBC), a biochemistry panel, urinalysis with microscopy (culture not routinely carried out), faecal parasitology and microbiological culture of the cloacal swab. Occasionally external parasites are collected and subsequently identified.

We have produced a rough preliminary summary of results only, provided in these notes for the purpose of general knowledge for those working with possums. They have not been formally evaluated, however, and should not be used as a citable reference. This summary also does not involve comparison with reference ranges, due to limited preparation time and because the few published ranges available are for eastern states populations, in the case of the ringtail only for *P. peregrinus*. The final published analysis will involve more formal statistical analysis and is likely to include some comparison with published reference ranges.

We have subdivided the ringtails involved in the study into four groups for summary purposes: those sampled from naturally occurring populations (“wild” group), those translocated after being in rehabilitation care (“carer” group), those urban wild animals translocated from development sites (“development site” group), and animals retested after residence of several months at the translocation sites (“translocation retest” group).

### Haematology

Between the ringtail groups the total red blood cell numbers, haemoglobin levels and PCV (haematocrit) were very similar. Polychromasia, anisocytosis and poikilocytosis were found in many wild and carer group animals in particular; however the low levels found, the non-specificity of the changes, and the distinction between groups suggests that these findings are not of serious importance. Platelet parameters were very similar between all ringtail groups.

There was some pattern to the differences in white blood cell (WBC) parameters; however, again, until a more thorough analysis is done the biological meaning of such differences can't be determined. They are probably specific to the habitat and lifestyle of each group, and quite possibly not significant to survivorship. The average total WBC numbers were similar between all groups. However, in total neutrophil, monocyte and eosinophil numbers, and fibrinogen concentration, the wild populations were slightly higher, followed by the translocation retest, development site and carer groups. The differences could suggest an increased tendency to stress or inflammatory response in the wild populations, but the biological interpretation of this is indistinct. In total lymphocyte numbers the averages were higher in the carer group. Serum protein was slightly lower in the carer group, which may be due to dietary/metabolic differences.

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All haematological parameters between the different naturally occurring brushtail populations were very similar.

#### Biochemistry

There were differences between ringtail groups for most parameters; however they are again likely to reflect normal variation, are related to habitat and lifestyle differences, and are not likely to have pathological significance.

The creatinine kinase (CK) level was highest in the wild group, and lowest in the carer group. This is probably related to capture by darting in the former group, and associated release of enzyme from muscle. The remaining liver-related parameters (ALP, ALT, AST, total bilirubin) do not show a consistent pattern in differences. Phosphate, calcium, creatinine and cholesterol levels were similar between all groups. Urea was significantly higher in the wild ringtails; this was probably reflecting some dehydration as these animals were sampled at a time and from habitat where the ongoing drought was having significant impact. Glucose was highest and protein lowest in the carer group, again probably reflecting dietary/metabolic influences.

All biochemical parameters between the different naturally occurring brushtail populations were very similar.

#### Urinalysis

Most urinalysis results so far have been unremarkable and show variation that would be expected in normal populations. Most ringtail urine was quite turbid, especially in the naturally occurring (wild and development site) groups. These groups commonly had brown or yellow/brown urine, while carer animals more commonly had yellow urine. These differences probably indirectly relate to diet, as we find that turbidity and colour increases with increasing maturity of diet. Specific gravity and pH was similar in all groups. Most animals were negative for glucose, bilirubin, blood and SSA precipitate. All animals were negative for ketones and urobilinogen. Nearly all animals had protein in the urine at trace-2+ levels. There were no major differences between groups. Most animals had <1 WBC/hpf and <1 RBC/hpf. Epithelial cells were rare, sperm occasional, bacteria rare, and mucous, casts and fat globules very rare. Most animals had 1-3+ crystals. Most were of the following types, some animals with more than one type: calcium carbonate, calcium oxalate mono- and di-hydrate, struvite and amorphous.

Brushtail urine was commonly turbid and usually yellow, occasionally brown or amber. Specific gravity and pH was similar for all populations. All animals were negative for glucose. Most animals were negative for bilirubin, ketones, blood, protein (compare with the ringtail result), urobilinogen and SSA precipitate. One brushtail had 3+ ketones, 1+ urobilinogen but glucose in the normal range; the ketonuria was probably therefore due to weight loss. Most animals in both groups had <1 WBC/hpf, some had 2-4 WBC/hpf. Most animals in both groups had <1 RBC/hpf, a few had up to 10 RBC/hpf. Epithelial cells were rare, sperm present in most males, bacteria were rare, and mucous, casts and fat globules were very rare in both groups. Most animals had 1-2+ crystals. Most were of the following types, some animals with more than one type: calcium carbonate, calcium oxalate mono- and di-hydrate and struvite.



### Cloacal microbiology

All ringtails were negative for *Salmonella*. *Streptococcus* (non- and alpha-haemolytic) was isolated from most animals. Other common isolates included coryneform bacilli, *E. coli*, and *Staphylococcus*. *E. coli* was not isolated from any of the wild animals, and there was less incidence of coryneform bacilli. Occasional/rare isolates included *Bacillus*, *Enterococcus*, *Enterobacter*, *Micrococcus*, *Pasteurella*, *Pseudomonas*, *Serratia*, *Alcaligenes*, *Aeromonas* and *Citrobacter*. There were no major differences between carer and development site animals.

All brushtails were negative for *Salmonella*. *E. coli* produced moderate-heavy growth from most animals of both groups. Other common isolates included coryneform bacilli, *Enterococcus*, and *Streptococcus* (non- and alpha-haemolytic). Occasional/rare isolates included *Bacillus*, *Micrococcus*, *Staphylococcus*, *Serratia*, *Proteus mirabilis* and *Citrobacter*.

### Faecal parasitology

Most ringtail faecal samples were negative for parasites, from all groups. *Bertiella* sp. was identified from one of the wild animals. About half of all brushtails had either strongyle eggs and/or coccidial oocysts. There was an occasional nematode embryo or larva. *Bertiella* sp. was identified from one animal at a ringtail translocation site.

### Blood parasitology

Microfilariae were found occasionally in the blood of ringtails but not of brushtails.

### Skin parasitology

The following have been identified:

Flea *Echidnophaga myrmecobii*

Flea *Choristopsylla ochi*

Mite *Trichosuroaelaps*

Mite Trombiculid larvae

? *Amblyomma* sp.

## ***Disease Screening Results***

### Cryptococcosis

*Cryptococcus* is an encapsulated, yeast-like fungus. There are over 30 species in the genus *Cryptococcus*, but only one, *C. neoformans*, is pathogenic. Four serotypes have been identified, currently classified as *C. neoformans* var. *grubii* (serotype A), *C. neoformans* var. *gattii* (serotypes B and C) and *C. neoformans* var. *neoformans* (serotype D).

*Cryptococcus* is acquired from the environment. Animal to animal transfer has not been demonstrated, and it is not zoonotic. Infection is usually by inhalation of aerosolised yeast cells. Organisms have a predilection for the nasal cavities and lower respiratory system, and the central nervous system; disease can also be found in the skin and peripheral lymph nodes. The organism spreads through the body in blood and lymphatics. The polysaccharide capsule is the major virulence factor, as well as the source of antigen for serological testing.

*C. neoformans* var. *grubii* and var. *neoformans* have a worldwide distribution. They are ubiquitous in the environment, being found in soil, but also grow specifically in bird excreta. They generally only cause disease in immunocompromised people (such as AIDS patients) and animals. Conversely *C. neoformans* var. *gattii* has a specific association with eucalypt trees particularly the River Redgum *E. camaldulensis*, and is therefore found only in tropical and subtropical areas, either in Australia, or regions internationally where eucalypts have been

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successfully established. The specificity may result from a requirement for decaying wood as a growth substrate. *C. neoformans* var. *gattii* can cause disease in otherwise healthy, immunocompetent humans and animals .

Disease due to *C. neoformans* var. *gattii* has been reported in people, domestic animals, and wildlife including koalas , possums and dolphins . However, studies of people , dogs and cats , and koalas indicate that the organism can be isolated as a commensal from a normal upper respiratory tract. In fact, koalas are very commonly carriers, especially where the presence of particular eucalypts means a high density of *C. neoformans* var. *gattii* in the environment .

Most investigation of cryptococcosis of marsupials has been carried out in koalas. Infection may or may not progress from nasal colonization to small subclinical lesions in the respiratory system . These lesions may either be cleared or suppressed by an effective immune response, or rarely progress to severe systemic disease . Despite high rates of exposure and colonisation, the rarity of disease is presumably due to evolution of host defence mechanisms .

The tuart *Eucalyptus gomphocephala* is among the eucalypts from which *C. neoformans* var. *gattii* has been isolated . Because of this, and the opportunity our project provides to collect samples from both possum species, we are testing all animals for *Cryptococcus*.

Dr Mark Krockenburger of the Koala Infectious Diseases Research Group, Faculty of Veterinary Science, University of Sydney is providing testing of our serum samples for *Cryptococcus*. We have not yet received details on the exact assay being used, and results have not yet been returned on the first batch of samples sent over at the beginning of 2007.

#### Chlamydiosis

The Chlamydiales are gram negative, obligate intracellular bacteria . Unlike other bacteria, there are two distinct forms in the lifecycle: the ‘elementary body’, which is smaller, not metabolically active, survives in the environment for long periods in soil and faeces, is infectious and acts like a spore; and the ‘reticulate body’, which is larger, non-infectious, capable of replication and completely dependant on the metabolic processes of the host cell .

The elementary bodies, shed in faeces and secretions from infected animals, is acquired by direct contact or aerosol. They enter mucosal cells of the conjunctiva, respiratory, gastrointestinal or genitourinary tract, transform into reticulate bodies for replication, and transform back into elementary bodies before cell lysis releases them to go on to infect new cells . Members of the order Chlamydiales parasitize organisms from amoebae to higher mammals . Various Chlamydiales have been recognized as causing disease in humans, birds, pigs, ruminants, cat, rodents and koalas, infection has also been reported in invertebrates, fish, amphibians, chameleons, turtles, tortoises, crocodiles and snakes .

The taxonomy of the Chlamydiales has recently been revised and there are now two genera recognized – *Chlamydia* and *Chlamydophila* – with nine species of pathogenicity for birds, reptiles and mammals . “Chlamydial species are usually associated with specific diseases in particular hosts” ; for example, abortion in sheep (*Chlamydophila abortus*), conjunctivitis in cats (*C. felis*), encephalomyelitis in cattle (*C. pecorum*), systemic chlamydiosis in birds (*C. psittaci*). Infection does not necessarily lead to disease and overt disease is thought to be a result of several host and parasite processes . Latent infections are common, and reactivated by stress and concurrent infections .



Two species affect koalas. *Chlamydophila pneumoniae* biovar 'koala' has been isolated from the conjunctiva and urogenital tract and may cause conjunctivitis and cystitis, but appears to have minimal virulence. The more pathogenic *Chlamydophila pecorum* causes conjunctivitis, genitourinary disease and infertility. Respiratory disease has also been reported in koalas. A survey of infection in two free-ranging koala populations in south-east Queensland suggests that infection may be transmitted from mother to young at birth or in the pouch, and due to sexual contact and other mating behaviour such as fighting.

A recent survey by Bodetti *et al.* (2003) provided the first search for Chlamydiales in any marsupial other than the koala. Five native species were sampled: the greater glider, mountain brushtail possum, western barred bandicoot, greater bilby and Gilbert's potoroo. Only the gliders and bandicoots showed evidence of clinical disease (conjunctivitis, cloacal disease, lung sounds), but at least 40% of every species were positive for Chlamydiales by PCR testing. Some of these were *C. pecorum*, others were other recognized Chlamydiales, but the majority were new, previously uncultured Chlamydiales. New Chlamydiales types were also found in western barred bandicoots from Bernier Island with significant ocular disease. Our work may be very important in extending the range of marsupials examined as host species for Chlamydiales.

The presence of chlamydial infection can be detected by cytology, serology, cell culture and direct detection of organisms by PCR assays and immunohistochemistry. Associate Professor Peter Timms of the Institute of Health and Biomedical Innovation, Queensland University of Technology is providing testing of our swabs for *Chlamydophila*. We have not yet received details on the exact method being used, and results have not yet been returned on the first batch of samples sent over at the beginning of 2007.

### Leptospirosis

*Leptospira* is an aerobic, gram negative, helical bacterium. The taxonomy of the genus is currently in a state of change and is therefore represented variably in the literature: former classification was on a serological basis, while new methods use genetic typing. In general, there are distinctly pathogenic (formerly *L. interrogans*) and nonpathogenic (formerly *L. biflexa*, mainly environmental) strains of the organism. Regardless of taxonomy, of the pathogenic strains, over 230 serovars are recognized worldwide.

The organism is fragile and therefore lasts in the environment only in warm, moist, pH neutral conditions and for several weeks. Presence is therefore maintained by animal infection. There is little true host specificity – although there are maintenance host relationships between particular serovars and animal species, humans, domestic animals and wildlife can potentially be infected with a wide variety of serovars. Rats and rodents are thought to be an important source of leptospires for people and domestic animals.

The main source of infection is in urine shed from infected animals, whether by direct or indirect contact. The organism is acquired by absorption across the mucosa or broken skin and enters the bloodstream. It then proliferates in organs including the liver, kidneys, spleen, meninges and reproductive tract. It is eventually cleared by the immune response, but can remain localized in some tissues such as the kidney, reproductive tract and brain.

Effects of infection may range from mild disease of the reproductive or urinary system to serious systemic disease. Pathogenic mechanisms are not entirely clear, but capillary damage, production of endotoxin-like substances and immune-mediated symptoms are all involved. Common features of infection in domestic animals (cattle, pigs, horses and dogs) include fever,

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anaemia and jaundice, abortion and meningoencephalitis . In people, leptospirosis presents as fever, headache, icterus and gastrointestinal symptoms .

Due to the public health significance, multiple surveys for leptospirosis have been undertaken in Australian wildlife . The organism has been detected in a variety of marsupials and rodents. Milner *et al.* (1981) concluded that in south-eastern Australia it was only likely to be common in the Common Brushtail Possum, possibly the wombat and rusa deer; and that rodents did not appear to be as important as reservoir hosts as in northern Queensland. Recent work has investigated presence in flying foxes as a potentially important natural host in Australia . Leptospiral infection in native wildlife was not thought to be a source of hazard for human or veterinary health .

Studies have shown that the Common Brushtail Possum is a maintenance host for serovar *balcanica* in Australia and New Zealand, with a very high prevalence particularly in pastoral vs. forest populations . Transmission only occurs directly between possums and infection is therefore usually acquired at the onset of sexual maturity . Infection in possums in New Zealand is significant as a potential reservoir of infection for cattle and people and as an option for biological control of the possum .

There are a variety of laboratory methods used to detect past or current leptospiral infection . The microscopic agglutination test (MAT) is commonly used to detect serovar-specific antibody, which is present from 8-10 days from the onset of illness and can last for months to years . Titres of 1:50 or higher are regarded as evidence of past or current infection .

Dr Lee Smythe of the WHO/FAO/OIE Collaborating Centre for Reference and Research on Leptospirosis, Queensland Health Scientific Services is providing testing of our serum samples for *Leptospira*. All ringtails and brushtails tested so far have been negative for all serovars tested.

### Toxoplasmosis

*Toxoplasma gondii* is an obligate intracellular coccidian parasite with a complicated lifecycle . It has a global distribution and very nonspecific host range . Domestic and wild cats are the definitive hosts, producing the oocysts capable of remaining viable in the environment, but virtually any warm blooded animal including birds and marine mammals is capable of infection as an intermediate host, and of transmitting that infection under appropriate circumstances to other intermediate hosts and to felids .

Herbivorous intermediate hosts most likely acquire infection by ingestion of oocysts, shed by infected cats, which subsequently contaminate a food or water source . Carnivorous intermediate hosts (as well as cats) can also acquire infection by consumption of organs (usually brain, skeletal muscle and liver) of infected animals that contain infective tissue cysts . Disease is caused by rapid multiplication and dissemination of the organism throughout the body, affecting initially the intestinal tract and related lymph nodes, then spreading through all body tissues within cells in the blood and lymphatics . Acute pathology can include enteritis, myositis, myocarditis, hepatitis and meningoencephalitis .

Toxoplasmosis is an important zoonosis, acquired by contact with oocysts shed by infected cats, or by consumption of inadequately cooked infected meat or offal. Serious disease usually only occurs in the aged, infants and immuno-deficient people, or in previously non-exposed pregnant women .



Species susceptibility to toxoplasmosis varies. Marsupials appear to be very sensitive to infection and disease, and the clinical course of toxoplasmosis in marsupials is often very rapid. The high susceptibility is possibly due to a history of evolution in isolation from felids, and therefore of exposure to *T. gondii*. Relative weakness in the immune system of marsupials may also contribute to a high susceptibility – a full immune defence results in minimal clinical signs and lifetime encystment in the tissues of the host, while a delayed immune response can result in a rapid and fatal systemic infection. Neurological signs are often noted in marsupial toxoplasmosis; the loss of agility and altered nocturnal behaviour may then make animals more susceptible to predation. Studies of the Eastern barred bandicoot indicate that although most animals are likely to die within weeks of infection, some appear to survive and host latent infection.

Evidence of infection has been reported in wild kowari (Brush Tailed Marsupial Rat), Bennett's wallabies and Tasmanian pademelons and Eastern barred bandicoots. Evidence of infection has been reported in captive Bennett's wallabies, Black-faced kangaroos, Tasmanian pademelons, Eastern barred bandicoots and several dasyurid species. Obendorf *et al.* also mentioned an unpublished case of acutely fatal toxoplasmosis in a common wombat. Despite the arboreal nature of ringtails seemingly limiting exposure to infective oocysts, the infection has been detected in two animals in an earlier stage of our translocation program (Paul de Tores, unpublished data).

Serology is commonly used, as exemplified by all the cases just mentioned, to survey wildlife for toxoplasmosis by detection of antibody that would indicate recent or past infection. Usually the direct agglutination test (DAT) and modified direct agglutination test (MAT) are both run on samples; in combination the results indicate whether infection is acute or latent. Pat Statham of the Animal Health Laboratory, Department of Primary Industry, Water and Environment, Tasmania is providing testing of our serum samples for *Toxoplasma gondii*. All ringtails and brushtails tested so far have been negative on DAT and MAT for toxoplasmosis, with the exception of one wild ringtail at Ludlow with an ambiguous result (Pat Statham, pers. comm.; DAT and MAT titres both 1:16).

### **Possum Survivorship and Translocation Outcomes**

The initial stages of the translocation programme have been described in de Tores *et al.*. Briefly, translocation of western ringtail possums into Leschenault Peninsula commenced in 1991 following instigation of a 1080 fox-baiting programme. By 1996 the translocated population was increasing in numbers through recruitment of locally born young. Increases continued until 1998, but by 2002 the population had crashed. Western ringtail possums were also translocated into two baited sites within Yalgorup National Park in the late 1990's, and, unlike the case at Leschenault, these populations were still increasing in number by 2002. The translocation research project re-commenced in 2004 with particular focus on Leschenault Peninsula (baited for fox control) and two sites at Yalgorup: Martin's Tank (unbaited) and Preston Beach Road (baited). Most of the western ringtail possums translocated in 2004 were animals displaced from their habitat in the Busselton region due to the ongoing demand for building development. By mid 2005 results of this work demonstrated a high rate of mortality at Leschenault (attributed mainly to python and cat predation), and better survival statistics at Yalgorup, at both the baited site and the unbaited site.

In 2006 the translocation programme was expanded to include a health-screening component. Furthermore, comparative studies were initiated to investigate the health, survival and home range of western ringtail possums in their natural habitats around Busselton. The work is being carried out in conjunction with a larger study into the relationships between various predator

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species (fox, cat, python, chuditch, varanid spp) and their prey at a variety of baited and unbaited study sites. Here we provide an overview of interim results for the most recent stage of the western ringtail possum research in which we have been directly involved (2006-07).

### ***Methods***

Western ringtail possums are captured using a dart gun and injectable anaesthetic (Zoletil). This species of possum does not readily enter traps, so darting is the most effective method of capture. Captured possums are transported in calico bags to field stations where they are health-screened and radio-collared prior to release (see health and disease section of this manuscript). Survival of radio-collared possums is monitored at least weekly by radio-telemetry. The collars incorporate movement sensors which enable differentiation between live and dead signals, thus permitting prompt determination of cause of death. Animals are also regularly radio-tracked during both the day and at night to determine home range size and habitat characteristics of diurnal rest sites and nocturnal foraging locations.

A number of common brushtail possums have also been captured, health-screened and radio-collared at each of the study sites at which the ringtail work is being carried out. This will enable us to compare health status, survival and habitat use between the two possum species, and will also allow determination of differences between Busselton and the translocation sites in terms of health status and behaviour of the local brushtail possum populations. Brushtail possums are captured using cage traps baited with rolled oats, peanut butter and honey. After capture they are transferred into hessian sacks and transported to the field stations for health-screening and radio-collaring. Animals are released during the evening, back at the sites of capture. Survival and habitat use are monitored weekly by radio-telemetry.

### ***Survival of translocated western ringtail possums***

Survival and habitat use of 46 translocated western ringtail possums have been studied between early 2006 and mid 2007. Approximately half of the possums were displaced animals from building development sites and the remainder were orphaned or rehabilitated animals obtained from wildlife carers. Ages of translocated possums were categorised as adult or sub-adult (testicles or pouch not fully developed). All possums carried radio-collars to enable regular monitoring of dispersal, home range, habitat use and survival. Possums were translocated to one of three field sites: Leschenault Peninsula, Martins' Tank and Preston Beach Road (see maps). Roughly half the translocations occurred during the winter months (May-Oct) and half during summer (Nov-April).

Of the 46 translocated possums, 29 died, 12 collars had gone off the air and five animals were still alive by mid-June 2007. Collar reliability has been an ongoing problem, with some collar batteries lasting only 2-4 months instead of the expected 6-7 months. Because ringtail possums do not enter traps it has not been possible to determine translocation outcomes for animals beyond the functional life of their collars. Possums have been routinely recaptured four months post-release for collar replacement, but most of those whose collars failed unexpectedly before this time were unable to be relocated for collar replacement and have thus been omitted from survival statistics (Table 1).

Possum deaths have occurred at all three sites from a variety of causes. Leschenault Peninsula has been the site of highest mortality, and cat predation has been implicated in the majority of cases. Carpet pythons (endemic to the region) are the second most common cause of mortality at this site. Possum mortality rate has also been high at Preston Beach Road with a number of deaths attributed to raptor predation of animals choosing rest sites in *Melaleuca* trees close to the edge of Lake Preston. Martin's Tank (the unbaited site) has had the best survival rate of



possums, although a few deaths have occurred due to python predation; and others due to poor body condition and unidentified causes. Possums that dispersed most widely following release were the least likely to survive.

Table 1. Mean survival rates for translocated western ringtail possums in the current study. Mean days alive was calculated up to 18 June 07. (Animals whose collars have gone off the air are not included.)

Site	N released	N died	Mean days to death	Mean days since release for animals currently alive
Leschenault	16	16	80	
Martin's Tank	13	8	98	240
Preston Beach Rd	6	6	40	

There was no obvious difference in survival between possums released as adults versus those released as sub-adults. It was, however, found that rehabilitated/orphaned animals were more likely to die in the first month post-release than animals derived from development sites. Survival of translocated animals after this first month was independent of their origin. The longest-lived currently alive possum was one that was originally translocated in April 2005 by Paul de Tores. It had gone off the air due to collar battery failure but has recently been recaptured. The high mean survival rate at Martin's Tank in Table 1 is largely due to this individual which has now been alive for 786 days since release.

All western ringtail possums were released into areas dominated by *Agonis flexuosa* (peppermint), their preferred habitat in Busselton region. Not all released animals remained close to their release location, however. Those that moved into lightly vegetated areas necessitating ground travel were most likely to die. Widest dispersal occurred in summer, related perhaps to drought-related foliage quality or to competition with previously translocated animals. The possums surviving longest have adopted home ranges within densely vegetated areas, particularly sites with a dense under-story. Although night sightings of possums in peppermint trees are common, they are also frequently observed in *Melaleuca* spp, *Acacia* spp and Tuart trees, the leaves of all of which they have been seen eating.

***Survival of western ringtail possums in their natural habitat in the Busselton region***

Survival of western ringtail possums is currently being studied at Ludlow Tuart Forest National Park (Mill and Bullock Blocks) and at Lakes Rd bushland near Gelorup. Possums have been captured, health-screened, radio-collared and re-released at these sites in the months since January 2007. To date, 14 western ringtail possums have been captured at Ludlow and nine at Gelorup. Of these, eight have died, two have gone off the air and 13 remain alive. Seven of the eight deaths occurred during the summer months at Ludlow and the majority of these showed moderate to high levels of emaciation, indicating that nutritional conditions were poor at the time. Since the winter rains began fewer deaths have occurred. Predation appears to be less of a problem at these two sites than at the translocation sites; however, the duration of time that these animals have been on the air is shorter than that of many of the translocated animals. Table 2 provides survival statistics for animals of known fate.

A number of the animals that died in poor body condition at both the Busselton and the translocation field sites showed moderate to high levels of endo- and/or ecto-parasitism. Parasites identified include tapeworms (*Bertiella* spp), nematodes, fleas (*Echidnophaga myrmecobii*, *Choristopsylla ochi*) and mites (*Trichosuroaelaps* spp, trombiculid larvae).

Table 2. Mean survival rates for western ringtail possums in the Busselton study sites. Mean days alive was calculated up to 18 June 07. (Animals whose collars have gone off the air are not included.)

Site	N captured	N died	Mean days to death	Mean days since collaring for animals currently alive
Ludlow	12	7	41	73
Gelorup	9	1	41	56

### ***Survival of common brushtail possums at all sites***

Survival of a number of common brushtail possums has been studied at both the Busselton field sites and at the translocation sites. Table 3 summarises the survival statistics for these animals. Survival rates of the naturally-occurring brushtail possums at the translocation sites have been greater than those of the translocated ringtail possums. At the Busselton sites the survival rates of both species have been similar. Causes of death are variable. Fox predation appears to be the main cause of death at the translocation sites while a mixture of causes including raptor predation, motor vehicle injury and predation/scavenging have been identified at Ludlow.

Table 3. Mean survival rates for common brushtail possums at all study sites. Mean days alive was calculated up to 18 June 07. (Animals whose collars have gone off the air are not included.)

Site	N captured	N died	Mean days to death	Mean days since collaring for animals currently alive
Leschenault	12	2	112	109
Martin's Tank	5	2	78	188
Preston Beach Rd	4	1	79	196
Ludlow	14	9	93	148
Gelorup	8	0	-	81

### ***Where to from here?***

These interim results raise a number of questions that we hope will be able to be answered as further data is collected. Issues that we hope to clarify include:

- Is cat predation limiting translocation success at Leschenault? At present this seems to be the case, but we have limited direct evidence of cat abundance at any of the field sites. Determination of relative predator (fox/cat/chuditch) abundance at all three translocation sites is planned for the upcoming year, along with ongoing quantitative research into relationships between predator species. Ultimately, cat control measures are likely to be pre-requisite for western ringtail possum translocation success.
- How important is foliar nutrient quality for survival of western ringtail possums, both in their natural habitat and after translocation? Our results to date are suggestive of a nutritional factor affecting survival of both groups of possums. Although foliage quality research is beyond the scope of our projects, we are liaising with botanists regarding this topic and hope that some specific research will soon commence.
- Are translocated possums more likely to disperse widely in summer than in winter? If so, and if survival is reduced due to dispersal into sub-optimal habitat, then it might be wise to limit future translocations to the winter months. Translocating animals in winter will also reduce the risk of python predation during the period of initial acclimation of the animals to their new habitat.
- Is the apparently highest survival rate at Martin's Tank (where no fox-baiting currently occurs) due to habitat/foliage quality or to low exotic predator numbers? Analysis of data in relation to home-range and habitat characteristics as well as investigation of

predator abundance will help answer this question. Similar analyses may elucidate the reasons for variation in possum survival amongst sites in the Busselton region.

### **Addendum: Hard vs Soft Release and Predator Aversion Training**

All western ringtail possum translocations in the current study have been carried out using hard release techniques. This approach has been driven mainly by logistic and resource limitations. Focused research incorporating both hard and soft methods and the effect of predator awareness training on translocation success would be a major project in its own right (and ideally should one day be carried out). The closest we have come is to initiate a nest-box release trial which currently about to commence as an Honours level project (Tracey Moore, supervised by Paul de Tores and Trish Fleming (DEC/Murdoch)).

Research into predator aversion training of marsupials is currently in its infancy. A limited number of reported studies on captive macropods have shown that it is possible to train naïve marsupials to recognise models of predator species and to associate these models with adverse experiences. However, habituation to repeated stimuli must be avoided if training is to remain effective. Social learning amongst individuals of a macropod population has also been demonstrated. No directed predator aversion training has been carried out on possum species and the effects of anti-predator training on post-release survival have not been experimentally evaluated on any marsupial species. It has been suggested that the historical lack of eutherian predators in Australia has limited the evolutionary capacity of marsupials to recognise such threats. Predator naiveté amongst Australian marsupials has been demonstrated in some field studies. The topic obviously requires further directed research; this is again beyond the scope of our current projects.

### **BIOGRAPHY:**

Helen graduated from the veterinary school at Murdoch University in 2000. She worked for 3 years in mixed practice, and then for 2 years part-time in small animal practice while completing a Bachelor of Science in Conservation Biology. Helen commenced candidature towards a PhD with Murdoch University early in 2005, studying the ecology and health status of the wild Western Ringtail and Common Brushtail possum populations around the Busselton area.

Judy graduated from the veterinary program at the University of Sydney in 1985. After a brief period in veterinary practice and 2 years working in molecular biology research, she joined the Australian Antarctic Division in Hobart. Judy was involved in long term monitoring of the Adélie Penguin, studying feeding ecology relating to the krill fishery, and breeding biology. She obtained a Masters in zoology in 1999. Judy came to Western Australia at the beginning of 2006 to commence postgraduate research into the health and translocation success of the Western Ringtail Possum with the Western Australian Department of Environment and Conservation (DEC) and Murdoch University.

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**Health status and translocation success of wild and rehabilitated possums,  
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