HUSBANDRY MANUAL FOR THE NEW HOLLAND MOUSE

Pseudomys novaehollandiae

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1. Taxonomy

Class: Mammalia
Subclass: Eutheria
Order: Rodentia
Family: Muridae
Sub Family: Hydromyinae
Tribe: Conilurini
Genus species: *Pseudomys novaehollandiae*

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2. Conservation Status

Endangered: Tasmanian Threatened Species Protection Act 1995
Endangered: CNR (1995)
Threatened: Schedule 2, Flora and Fauna Guarantee Act 1988.

ASMP Category: 1A- High intensity genetic and demographic management aimed at maximising genetic diversity.

3. Natural History

3.1 General Description

The New Holland mouse is a nocturnal, burrowing rodent, very similar in appearance to the House Mouse (*Mus musculus*).
They have large eyes and ears and a long slender tail, the length of which is generally 10-15% longer than the head/body length and is bicoloured, being dusky brown above and white below.
The dorsal fur is grey-brown in colour, whilst ventrally it is grey-white and animals often have a grizzled appearance due to the presence of long dark guard hairs across the back

3.2 Distinguishing Features

The New Holland mouse can be distinguished from the House Mouse by the following features:

- larger ears and eyes
- longer tail
- possess four teats (*Mus musculus* have six teats)
- does not have the "mousey" odour commonly associated with the House mouse.
- lacks the notch on the inner surface of the upper incisors

3.3 Morphometrics

There appears to be some size variation in animals from differing locations, with Victorian and Tasmanian animals having greater bodymass than animals found in New South Wales (Table: 1).

Measure	VIC	TAS	NSW
Bodymass (g)	22-24	25-26	15-16
Head/Body length (mm)	90	85	84
Tail length (mm)	87	91-92	93-95
Pes (mm)	N/A	21-22	21
Ear (mm)	N/A	12	16

Table: 1: Average measurements of adult New Holland Mice across three states.
(Victorian values from Gainey (2000), Tasmanian from Hocking (1980) and Norton (1986), NSW from Keith and Calaby (1968) and Kemper (1976)).

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3.4 Distribution

The New Holland mouse was first described in 1843 (Waterhouse 1843), and was thought to be extinct until its rediscovery in Ku-ring-gai Chase National park, NSW in 1967 (Mahoney and Marlow 1968). The following year researchers for the CSIRO discovered a population at Port Stephens (Keith and Calaby 1968). Since this time the species has been recorded in coastal NSW, Victoria and Tasmania, and more recently in Queensland by the identification of a single animal (Van Dyck 1998).

In NSW the species has been recorded at a number of locations south of Sydney, in the Royal National Park and Kangaroo Valley (Posamentier and Recher 1974), north of Sydney, at Kuringai Chase National Park, and from Port Stephens, north of Newcastle, to Evans Head near the Queensland border, (Posamentier and Recher 1974, Keith and Calaby 1968, Fox and Fox 1978, Fox and McKay 1981, Fox and Pople 1984).

In Tasmanian populations have been observed in 7 localities in the north east of the state as well as Flinders Island (Hocking 1980; Pye 1991)

The species was first discovered in Victoria in 1970 at Tyabb on the Mornington Peninsula (Seebeck and Beste 1970), and has since been found at Loch Sport, Gippsland in the east (Braithwaite and Gullan 1978; Wilson 1996) and at Anglesea in the Otway ranges, western Victoria (Kentish 1982).
Recent surveys undertaken by Wilson (1991), Williamson (1992) and The Mammal Survey Group in 1993-4 (via Wilson 1996), indicate that the species in Victoria now only occurs in four localities: Anglesea, Loch Sport, Providence Ponds and Wilsons Promontory.

3.5 Habitat
The New Holland Mouse is found in a variety of coastal heath, heathy woodland and coastal scrub habitats.
The species prefers habitats consisting of soft substrate, usually sandy, with a heath type layer of leguminous perennials, generally less than one metre in height, and a sparse ground cover and litter. These characteristics are usually associated with early to mid stages of regeneration (Kemper via Strahan 1995), and Fox (1982) has described the species as a "disturbance enhanced species" due to this habitat preference.
It appears that the optimum habitat for the species is dry heath actively regenerating after fire (Posamentier and Recher 1974) or clearing (Kemper 1977: 1990).
Populations tend to survive fire and reach a maximum abundance after 2-3 years (Fox 1982). They are reported to recolonise an area 1 year post-fire, or 4-5 years post-sandmining (Kemper 1990)

3.6 Wild Diet and Feeding Habits.
The New Holland Mouse is considered to be omnivorous and opportunistic in its feeding habits (Wilson 1999). It has been documented to feed on stems, leaves, roots, flowers, seeds, fungi, mosses and insects, although there appears to be considerable variation in the proportion of these items in the diets of populations from differing locations. It is possible that this observation is indicative of the species opportunistic habits.
Seasonal variation is observed in all Pseudomyne rodent species studied, and it appears that the New Holland mouse is no exception. Studies undertaken by Cockburn (1980) and Thomson (1980), in Victoria and NSW respectively found that animals tended to consume a higher proportion of seed in late spring and summer, reducing consumption of

other items. Seed was found to comprise up to 97% of the diet of animals in a study carried out during spring/summer (Thomson, 1980).
Wilson (1999) observed similar trends but variation in seed consumption was not as pronounced as in the previous studies.
Other seasonal variations observed includes:

- an increase in consumption of fungi in the winter months, and
- an increase in consumption of dicotyledonous material in the summer.

Studies carried out by Wilson (1999) also indicated slight variation in the diet of the sexes, with females consuming a marginally higher proportion of seed than males, however further research is needed to confirm this.

3.7 Threats

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Potential threats to the species have being identified by Wilson (1996) and NRE (1996), are based on Victorian populations, and include:

- Alteration and fragmentation of habitat due to
    i. inappropriate fire regimes
    ii. weed invasion
    iii. land development and encroaching housing
- Infection of habitat with Cinnamon Fungus (*Phytoptherora cinnamomi*), effectively altering the floristics of available habitat

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- Predation by domestic cats and dogs and the Red Fox (*Vulpes vulpes*)
- Competition from introduced rodent species such as the Black Rat (*Rattus rattus*) and the House Mouse (*Mus musculus*)

3.8 Population dynamics

Population density has been shown to be associated with vegetation age (Wilson 1996). Studies to date indicate that the species declines as vegetation ages (Fox and Fox 1978, 1990: Wilson 1990, 1991), and may exhibit 'patch dynamics'. "Patch dynamics" describes particular population fluctuations where a population becomes extinct in some patches but re appears to colonise others.

Where conditions are favourable, populations may reach densities of up to 17 animals per hectare, with population sizes appearing highest in autumn and lowest in spring (Kemper via Strahan 1995).
To date little is known of the dispersal habits of New Holland mice. When known, this information will aid in a greater understanding of distribution patterns and prove invaluable for future re-release programs.

3.9 Longevity

Animals live to between one and a half and two years in the wild. They may live up to three and a half to five years in captivity.

4. Housing Requirements

4.1 Indoors

When housed indoors this species has generally been kept in an environment where both lighting and temperature are controlled. Animals were maintained within a continuously controlled environment in order to facilitate continuous breeding rather than relying on seasonal breeding.

The area where the animals are housed should be well insulated against weather extremes and infiltration of wild rodents. Individuals have been housed successfully in a number of enclosures within a controlled environment.

Enclosures used at Melbourne Zoo are:

i. Autoclavable polypropylene rodent boxes, 457mm ×318mm ×165mm, with a stainless steel fitted lid, food hopper and water bottle holder have been used to house both single animals and pairs, however observations of increased aggression when housing pairs in these boxes indicate that boxes of this size are more suitable for single animals (Figure 1).

ii. Larger polypropylene boxes measuring 530×360×190mm and 600×390×240mm have been successfully used to house breeding pairs.

iii. Large glass tanks, 1850×610×500mm are also used successfully. Tanks can be halved via glass inserts and sexes housed separately until oestrus is observed (Figure 2).

Figure 1: Polypropylene rodent box, containing New Holland mouse

Figure 2: Glass tank with divider housing 1:1 New Holland mice

4.1.1 Substrate

Suitable substrates include untreated pine wood shavings as a base, with meadow hay, which provides good burrowing material. This combination is preferred in the rodent boxes.

In tanks, a substrate combination of a washed silica sand base with meadow hay for nesting material is used.

4.1.2 Enclosure Furnishings

Enclosure furnishings include bark strips from species of eucalypt and melaleuca, cardboard tubes, and PVC piping cut into small lengths, providing areas for hiding. In addition, small grass clumps (particularly *Poa* spp.), as well as foliage and branches from sheoaks and various native heaths may be provided as additional nesting material.

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4.1.3 Lighting

Light/dark cycles can be maintained on either constant spring/summer cycles, (13 hours light with 11 hours dark), to promote year round breeding, or be maintained in a cycle that mimics natural seasonal cycles.

4.1.4 Temperature

An ambient temperature range of 17-22°C (mean = 22°C) has been used when year-round breeding is required. Temperatures that mimic natural seasonal variation may be used to maintain seasonal breeding cycles (ambient temperature range 12-25 °C).

4.1.5 Spatial Requirements

This species has generally been kept singularly except when males are introduced to females for breeding. Single-sex sibling groups of 2-3 animals in a half tank situation (900×610×500mm) have been housed successfully for short periods of time at Melbourne Zoo (e.g. 2-3 months).

In addition, two groups consisting of 2:1 and 2:2 were housed at Deakin University in outside aviaries (5000×2500×6000mm) for a three month period. (Mandy Lock pers. comm.)

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4.2 Outdoors

Animals have been housed in semi-outdoor aviaries at Deakin University, however no breeding occurred in these enclosures (Mandy Lock, *pers. comm.)*.

These enclosures measured 5000mm × 2500mm × 6000mm, with substrate consisting of sandy soil to a depth of 300mm, overlain with leaf litter, sticks and logs. The enclosures were planted with native grasses, sedges and heath.

The enclosures were constructed using full-length sheet metal, (back and sides), with sheet metal on the lower 50% of the front wall and wire mesh on the upper 50%. The enclosures were subject to natural temperature fluctuations but were well protected from wind and rain. Both natural lighting and artificial lighting were used.

5. Handling and Transport

5.1 Timing of Capture and Handling

When New Holland mice are housed indoors, capture is best performed during the "night" phase of the lighting cycle. This allows capture without disturbance of animals that are sleeping in nests during "daylight" hours.

Animals housed outdoors is also best captured at night, for similar reasons. Trapping (eg. using Elliot traps) during the night-time phase of the lighting cycle is also more time efficient for personnel (as daylight handling would involve searching for individuals in dense substrates).

5.2 Catching Equipment

Clear glass or solid plastic jars are used for capture of animals, they should be small enough to allow easy manoeuvring within the animals box or tank, and provide an uninterrupted view of the animal whilst in the jar. The use of a jar is beneficial for primary observation without restraint. Handling bags used at Melbourne Zoo are onion bags or similar types which allow good observation of animals whilst in the bag.
For capture of animals in outdoor enclosures Elliot traps are used.

5.3 Capture and Restraint Techniques

Basic capture and restraint techniques for New Holland mice that are housed indoors have been developed for animals housed at Melbourne Zoo. These techniques are relatively easy to use and are effective. However New Holland mice are agile and may be nervous when handled, and handlers must take care to avoid escape/injury during the capture process.

Animals maintained indoors, in plastic tubs or glass tanks are encouraged to enter a glass jar, and then transferred to an onion bag by placing the bag over the opening of the jar and gently tipping the animal into the jar (Figures 6&7).

Next, the bag is held closed with the left hand (if right handed) and the bag is placed on a flat surface. Using the right hand, the animal is encouraged to run in the bag towards the left hand. The animal may then be scruffed using the right hand. Skin over the shoulders is held between the thumb and forefinger with firm pressure (similar to standard techniques used for restraining the domestic mouse *Mus musculus*), (Figure 8). Once firmly restrained, the bag is released and "peeled" back over the mouse. The mouse is inverted so that the ventral area is exposed for inspection (Figure 9).

Figure 6: Transferring mouse from catching jar to bag

Figure 7: New Holland mouse secured in bag for observation or manual restraint

Figure 8: Herding New Holland mouse for dorsal examination.

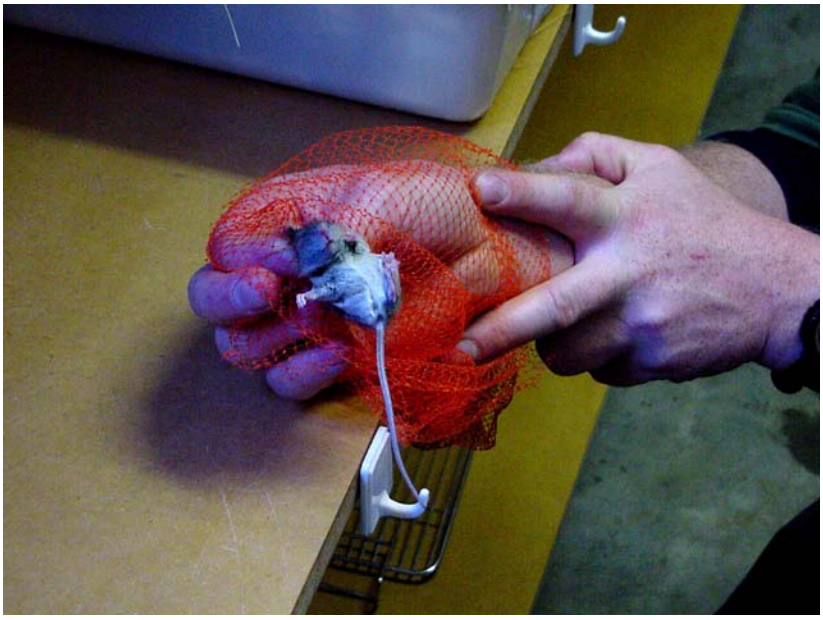

Figure 9: Restraint of mouse for inspection of ventral area

5.4 Outdoors

Elliot traps are used to capture New Holland mice housed in outdoor enclosures. Traps are set at the end of the day. They are baited with foodstuffs that form part of the animals' usual diet (eg. fruit or vegetables), and positioned on a recognised mouse pathway (if possible). Traps should be placed under cover. Nesting material should be provided in the trap, particularly if the weather is cold. Traps should be checked early the following morning. A trap that is found to contain a mouse should be opened into a suitable holding bag. The trap is gently tipped to encourage the animal into the bag. Once in the bag animals may be inspected as described previously.

5.5 Weighing and Examination

Mice can be weighed on electronic scales. They can be weighed while held in a glass jar or in a catching bag. An animal that is held in a glass jar may be weighed and visually examined with a minimum of handling stress.

For ventral examination the animal should be scruffed and inverted as described previously (see Figure 9). For dorsal examination the animal can be gently manoeuvred into the closed/blind end of an onion bag and held in place with thumb and forefinger as shown in figure 8.

5.6 Release

Animals are released from a jar or bag by laying the bag flat on the substrate of the enclosure and slightly tipping it to encourage the animal out from the open end. The opening should be facing hiding areas so that the animal has an escape route after exiting the bag.

5.7 Transport Requirements

Iata regulations outline the number of *Mus musculus* that may be housed together for transport. These figures have not been included in this manual as it is recommended that this species be transported singularly. Housing/transport of single animals will prevent aggression.

Wt of animals (g)	Space per animal (cm^2)	Box height (cm)
< 15	18	10
16-18	22	10
19-21	26	10
22-24	30	10

Table 2: IATA Spatial regulations for the transport of small rodents.

Transport boxes currently used are of 7-9mm plywood overlaid with 12 mm ×12 mm weldmesh.

Boxes measure 300× 150×150mm and have holes drilled around the upper perimeter for ventilation. Nesting material is provided in the form of shredded paper or woodwool.

7. Animal Health and Veterinary Care

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7.1 Quarantine

Introduction of new animals to an established collection can result in introduction of disease. Therefore New Holland mice that have recently arrived from another facility should undergo a period of quarantine before being housed with an established population of animals (eg. collection animals). During this period, there should be complete physical separation of the two groups (*ie.* new arrival animals and collection animals should NOT share the same air space).

At Melbourne Zoo, the standard mammal quarantine period of 30 days is used. A veterinarian examines new-arrival animals while they are in quarantine, and three faecal samples (collected 7 days apart) are examined for evidence of parasites (animals are treated for parasites if appropriate). To avoid transmission of disease via keeper clothing, new-arrival animals must be serviced after the collection animals are serviced. Separate servicing equipment should be used for each group of animals.

7.2 Physical examination

Animals can be examined under manual restraint (see previous discussion) or under anaesthesia. A routine assessment of any animal's condition will include examination for injuries affecting body and limbs (swellings, deviation, lameness), assessment of the animal's body condition and general demeanor and examination of the anogenital area for abnormalities (eg. faecal staining of coat suggestive of diarrhoea, swelling/redness affecting testes/vulva).

When examining the New Holland mouse, particular attention should be paid to the following features:

1. Examine the eyes: eyes should be examined for evidence of discharge, reddening around/inside the eye, cataract formation (where the pupil has a white appearance due to opacity in the lens) or cloudiness of the cornea (the external surface of the eye). Repetitive squinting may indicate that the eye is painful.
2. Examine the mouth: make sure the incisor teeth are not overgrown. Check for any evidence of bleeding from the mouth
3. Examine the skin of the dorsal and ventral body and the tail for evidence of wounds. Take note of any areas of hair loss/skin redness and examine the coat for moving external parasites.

7.3 Veterinary examination/ procedures

Animals that demonstrate abnormalities described above should be referred to a veterinarian for assessment. Specific veterinary procedures performed on New Holland mice at Melbourne Zoo are listed below:

- **Anaesthesia:** anaesthesia of the New Holland mouse is performed using isoflurane in oxygen, delivered by a purpose-made facemask. Animals are restrained in an

onion bag as previously described, and the facemask is placed over the nose and mouth. Isoflurane (5% in oxygen) is delivered via facemask until the animal reaches a light plane of anaesthesia. The concentration of isoflurane is then reduced so that anaesthesia is maintained (generally 2-3% isoflurane in oxygen). Particular attention must be paid to maintaining the animal's body temperature via use of supplemental heat (using warm wheat bags). Endotracheal intubation is extremely difficult and is not routinely attempted at Melbourne Zoo.

- **Faecal examination for parasite eggs:** faecal samples are examined using standard zinc sulfate flotation techniques. Faecal samples are often desiccated by the time they are collected from enclosures – moistening such samples with a small amount of saline assists when mixing for flotation.

- **Urine collection for analysis:** animals will often urinate while being restrained in glass jars. A diagnostic "catch" urine sample can be collected if a clean glass jar is used.

- **Microchipping for identification:** microchips have been inserted subcutaneously in a number of New Holland mice held at Melbourne Zoo. The protocol used by Melbourne Zoo veterinarians is described in Appendix 2.

- **Blood collection**: venipuncture techniques described in standard texts for use in laboratory mice (*Mus musculus*) may be useful.

7.4 Health problems seen in New Holland mice held at Melbourne Zoo

A review of medical records and post-mortem examination findings for New Holland mice held at Melbourne Zoo between 2001 and 2004 identified four medical conditions of importance (ie. medical conditions that have been identified in ≥ three New Holland mice).

a. Parasitism:

Internal parasites: pinworm (oxyurid) eggs are frequently detected during examination of New Holland mouse faeces (using a standard zinc sulfate flotation technique). The eggs have a similar appearance to those of *Aspicularis tetraptera*, a pinworm species commonly detected in the faeces of the laboratory mouse, however the species seen in the New Holland mouse has not been identified to species level. *A. tetraptera* infections in laboratory mice do not appear to result in significant clinical illness. Similarly, pinworm infections in New Holland mice maintained at Melbourne Zoo have not resulted in detectable clinical illness.

Several therapies have been trialed in an effort to control pinworm infection in New Holland mice at Melbourne Zoo, including:

- fipronyl ("Frontline"® (Merial Australia Pty Ltd), used topically on the coat), and

- ivermectin (0.2mg/kg of ivermectin given orally; dilute 0.8g/L "Ivomec Oral Drench for Sheep"® (Merial Australia Pty Ltd) in propylene glycol to create a 0.08g/L solution for oral dosing).

Donnolly (1990) reports use of fenbendazole 50ppm in feed to control infection in laboratory mice. Reinfection frequently occurs and it is difficult to eliminate infection entirely (I. Beveridge, pers. comm.); however heavy parasite burdens may require therapy.

External parasites: during early 2003**,** infestation with *Ornithonyssus bursae* the "starling mite", became an important health issue in New Holland mice held at Melbourne Zoo. At the time of the diagnosis, New Holland mice were housed in heated rooms adjacent aviaries housing birds, and staff were managing a pest rodent infestation in the ceiling cavity of the building. Clinical signs of mite infestation included skin redness, loss of hair around the face/nose due to overgrooming (presumably a result of itchiness), and visible presence of small, moving external parasites in the coat.

Initial investigations implicated one of either:

- *Ornithonyssus bacoti* (the tropical rat mite), or
- *O. bursae* (the starling mite).

Mites were sent to Westmead Hospital Department of Medical Entomology for identification, and were speciated as *O. bursae.*

Infection was managed using topical application of topical drugs (fipronyl) and oral ivermectin, and transferring mice to a clean environment distant from aviaries used to house birds.

b. Neonatal deaths:

A relatively high rate of neonatal mortality has been observed in New Holland mice held at Melbourne Zoo. In many cases, dead offspring could not be found in the enclosure or were too decomposed for meaningful post-mortem examination, however it is assumed that deaths were the result of either traumatic injury (resulting from aggressive behaviour of dams) or maternal neglect. It is possible that New Holland mice are more likely to cannibalise/mismother their offspring than other mouse species. Therefore it is important that risk factors for this behaviour are minimised by:

- maintaining animals in a quiet environment,
- reducing handling frequency for pregnant/lactating females (during the week prior to and following parturition females are not handled at all), and
- providing generous amounts of nesting material.

c. Reproductive tract pathology affecting females:

Of 17 female New Holland mice that have been examined at post mortem at Melbourne Zoo Veterinary Department, eight have been diagnosed with disease affecting the

reproductive system. Seven of the eight New Holland mouse females diagnosed with reproductive pathology at Melbourne Zoo had uterine infections ("endometritis" or "pyometron") and one female had a tumour affecting the uterus.

Inflammation of the uterus lining ("endometritis") may occur as a result of:

- Entry of bacteria into the uterus at mating
- Progesterone stimulation of secretory activity of the glands of the uterine lining, resulting in fluid accumulation in the uterus (bacteria from the vagina can then invade the fluid-filled uterus), eg. as a result of pseudopregnancy. It is notable that when a mating is sterile (ie. male is infertile), female laboratory mice will have a pseudopregnancy lasting approximately 10-13 days. Repeated pseudopregnancies may result in increased risk of endometritis/pyometron.
- Following a difficult labour/retained placenta
- Following development of uterine tumours.

Cases of endometritis in females held at Melbourne Zoo tend to occur in elderly females. It is possible that an increased incidence of pseudopregnancies in these elderly mice, resulting from mating by sterile males (in reproductive senescence) or as a result of pheromonal influences (see discussion on page 19).

d. Traumatic injury affecting males:

Individual New Holland mice are housed separately, as they are known to be aggressive when grouped or paired. Males are periodically placed in enclosures with females to allow breeding, however there have been a number of cases of severe traumatic injury inflicted upon males by females during this breeding period. Injuries range from mild (eg. lacerating bite wounds along the tail that heal without veterinary intervention) to severe (eg. multiple, full-thickness bite wounds extending over the neck and back and resulting in severe haemorrhage, shock and death). Tail tip trauma (necessitating anaesthesia for surgical amputation of exposed bone) is common.

Measures undertaken that have reduced the incidence of traumatic injury affecting male mice have included: increasing enclosure size and available escape routes/safe retreat areas (eg: via tunnels and boxes) available for animals housed together during breeding; monitoring females for evidence of pending oestrus and only introducing males at the most appropriate time for breeding; and visualising males daily to examine for evidence of injuries (depending upon severity of injuries detected, males may be separated from females at this time).

e. Infertility:

Factors that may impact upon fertility within a breeding group of New Holland mice include:

- Nutrition: obesity has been associated with poor reproductive performance in many species. One female New Holland mouse examined at post mortem by Melbourne Zoo veterinarians displayed significant reproductive pathology associated with obesity (ovarian stromal lipidosis). Such pathology is likely to have adversely affected reproductive performance in this individual. Obesity is a well-recognised cause of reduced fertility in male laboratory mice.
- Pheromonal influences: group-housed female laboratory mice without exposure to males tend to stop cycling and display either pseudopregnancy (the "Lee-Boot" effect) or anestrus. Separation from males is likely to have a similar effect in the New Holland mouse.
- Aging/reproductive senescence: reproductive performance in female mice tends to decrease with increasing age and number of prior pregnancies. Female laboratory mice that are outbred are reported to remain fertile until they are up to 18 months of age. A number of female New Holland Mice have given birth up to the age of one year nine months. One female when given serum gonadotrophin to induce ovulation became pregnant at the age of two years nine months. It is reasonable to assume that male fertility will also decline with age.
- Stressors: stressors such as overcrowding and noise may result in infertility via altered behaviour or immunosuppression and disease.
- Pathogens: there are a number of pathogens (viral and bacterial) that have been reported as resulting in depressed fertility in laboratory mice.
- Photoperiod: see later discussion under "Breeding"

An in-house study carried out during 2003 (via examination of vaginal cells obtained in urine samples) demonstrated apparent anoestrus in a number of female New Holland mice in the Melbourne Zoo collection. Further investigation is required to fully investigate causes of infertility within the Melbourne Zoo collection, however it appears likely that the factors of most importance are:

- reproductive senescence : most of the Melbourne Zoo collection mice were over 18 months of age when the study was performed, and
- pheromonal influences: the study period was preceded by a prolonged period of separation of males from females (during a period of enforced quarantine). This may have resulted in pseudopregnancies in many females, which may in turn have resulted in a predisposition towards endometritis/pyometron (possibly post-mating), as previously discussed.

Serum gonadotrophin is frequently used to stimulate ovulation (and to maximise the number of ova released at a particular ovulation) in female laboratory mice. Techniques are described in Hogan et al. (1994). Two treatment trials were carried out using serum gonadotrophin in captive New Holland mice at Melbourne Zoo. It was hoped that treatment would result in superovulation and production of large (> 5 offspring) litters. The mice were treated using 2.5iu of serum gonadotrophin* or 5.0 iu serum gonadotrophin injected intraperitoneally. Females and males were introduced 48 hours after injection. Nine of 14 females used in two treatment trials produced offspring post-treatment, however only three females produced larger than normal litters. Further

treatment trials are required to determine the most appropriate serum gonadotrophin dose regime that will produce superovulation and production of larger than normal litter sizes in New Holland mice.

*A commercial preparation ("Folligon"®(Intervet Australia Pty Ltd) containing 1000 iu serum gonadotrophin per mL) was diluted to 40 iu per mL using sterile PBS and given intraperitoneally at 2.5 iu/mouse.

8. Behaviour

There is currently very little published information in regard to the behaviour of this species. Social behaviours such as grooming and following have been observed in paired animals at Deakin University (M. Lock, pers comm.) and at Melbourne Zoo.
Aggression is commonly seen in paired animals, with females frequently becoming very aggressive toward males soon after introduction, or during periods between copulation and parturition. Aggression during these periods can be severe, resulting in serious injury or death of males.

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9. Feeding requirements

9.1 Captive Diet

The base diet consists of seed, commercial rodent "cube" diets, and chopped fresh fruit and vegetables.

Seed used:

- Canary seed
- White millet
- Red Panicum
- Japanese Millet

Seed is feed both dry and sprouted.

Fruit and vegetables chosen from:

- Carrot
- Sweet potato
- Beetroot
- Broccoli
- Zucchini
- Sweetcorn
- Endive
- Spinach
- Apple
- Pear
- Grapes

The commercial rodent pellets used at Melbourne Zoo are "GR2 Rat and Mouse Cubes manufactured by Riddleys Agriproducts.

9.2 Presentation of food
Food is provided ad lib. Animals housed in wire topped rodent boxes can be fed via the food hoppers. Food is provided in small bowls when animals are housed in glass tanks.

9.3 Supplements
Supplementary food is offered to animals that are considered to have increased energy requirements (eg. pregnant or underweight animals) and may include:

- Mealworms
- Sunflower seed
- Sprouted seed

10. Breeding

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10.1 Litter size: 2-4 most common (1-6 range Kemper, 1980)
10.2 Gestation: 30-32 days (May be longer for post-partum matings (32-37days)
10.3 Sexual maturity: 1:0 20 weeks
0:1 13 weeks
Both sexes can reach sexual maturity when as young as 7 weeks old.

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10.4 Oestrus: 5-7 days
10.5 Parturition: Occurs during light phase of light/dark cycle.
10.6 Post partum oestrus: Occurs during dark phase following parturition
10.7 Weaning: at approximately 4-6wks of age

In the wild New Holland mice are polygamous, seasonal breeders, breeding in spring through to early summer.
At Melbourne Zoo, attempts have been made to prolong breeding seasons by manipulating light and temperature regimes (ie. altering light/dark cycles and maintaining constant ambient temperature, as previously discussed). Long-term effects of such manipulation on breeding success are not known.

10.8 Age at First and Last Breeding
Mean age for first breeding: Females 11months, 10days
Males 1year, 28 days

Mean age for final breeding: Females 1 year, 298 days
Males 2 years 140 days

10.9 **Litter Size as a percentage of total litters**

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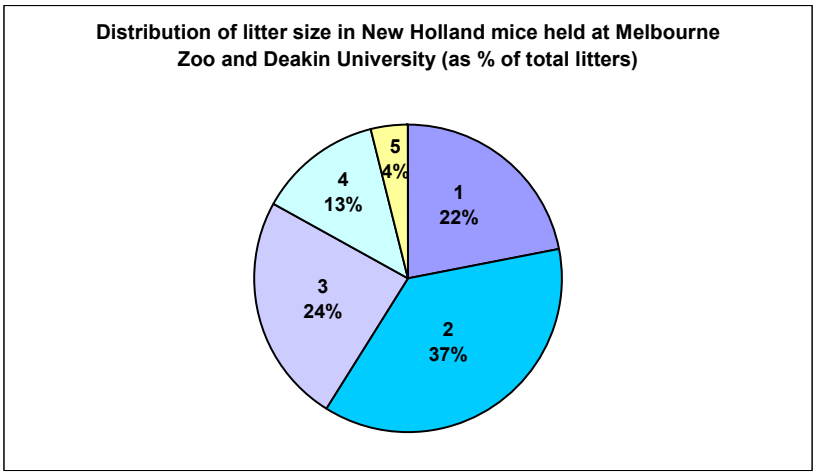
The mean litter size for both Melbourne Zoo and Deakin Uni combined is 2.4. Figure 10 shows the distribution of litter sizes seen at the two institutions.



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10.10 Birth Seasonality

Data compiled from animals held at both Melbourne Zoo and Deakin University indicate that there is an increase in production of litters in October, December and January, with the lowest number of litters in February and March.

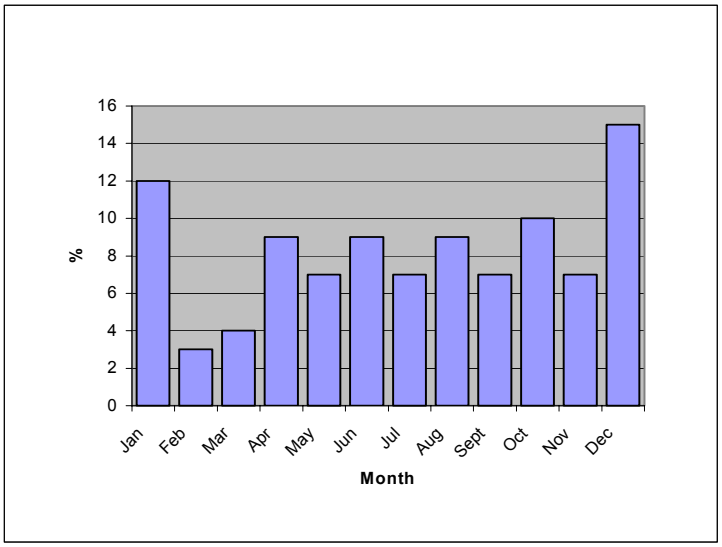

Figure 11: Percentage of litters born per month in New Holland mice held at Melbourne Zoo and Deakin University

10.11 Identification of Breeding Cycles

When animals are housed individually it is important that breeding cycles are identified and mapped. Oestrus periods must be accurately predicted in females so that males are introduced at the most appropriate time for breeding.

Oestrus

Via observation:

- Vulva generally paler in colour during oestrus and darker dioestrus.
- Vaginal lips may appear swollen pro oestrus and oestrus, decreasing during metoestrus and dioestrus.
- Although moisture is present most of the time, an increase may be seen during metoestrus.

Via vaginal smears:

The cycle of cell types found in vaginal smears from New Holland mice is similar to that of laboratory mice, *Mus musculus* (Bronson et al 1966); however large nucleated epithelial cells are not always present during pro oestrus and never during dioestrus in the New Holland mouse. Instead, smaller, less regular, partly cornified cells are seen. During the later stages of oestrus or early metoestrus smears are thick and consist of sheets of cornified cells.

During oestrus cells present are 100% cornified epithelium (Kemper 1976).

In males breeding condition will be evident by the swelling of the testes which will also take on a black colouration, and there may be an increase in activity.

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Factor	Pro oestrus	Oestrus	Met oestrus	Di oestrus
Colour	Normal	Pale	Normal	Dark
Size	Swollen	Swollen	Swelling decreasing	Normal
Moisture	Normal	Normal	Increased	Normal
Cell Type	Large nucleated epithelials/ some partly cornified	100% cornified epithelial	Mostly sheets of cornified epithelials	Cells partly cornified and irregular

Table 3: Oestrus detection in *P. novaehollandiae* via vaginal observation and smears.

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10.12 Copulation

Copulation can be detected by identification of a vaginal plug or the presence of sperm in the smear. Aggression between the pair, and signs of blood in the pairing cage, can also be used as and indication that copulation has taken place. Males should be visualised daily and checked for signs of traumatic injury.

10.13 Pregnancy

- The most obvious sign of pregnancy is an increase in bodymass, which is more pronounced in the second half of pregnancy. The average bodymass of a female at the end of pregnancy is one and a half times the original bodymass.
- Teats become more evident closer to parturition.

10.14 Parturition

Parturition occurs during the light phase of the light/dark cycle, and may be preceded by nest building behaviour.

Post-partum mating will generally occur within 9 hours of the beginning of the subsequent dark phase.

10.15 Abnormal pregnancy/parturition

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Poor nutrition, environmental stress and infection can cause abortion and stillbirth in laboratory mice. Dystocia (difficult/abnormal birth) can also occur in mice and may require veterinary intervention. Prolongation of gestation beyond published intervals (>33 days for females that were not lactating when mated; > 37 days for females mated post-partum) can be an indicator of dystocia. As an example, a female New Holland mouse held at Melbourne Zoo was observed to pass blood from the vulva 32 days after a recorded pairing. The female had been treated with serum gonadotrophin to induce ovulation, and was then paired with a male 48 hours later. On day 33, X -rays were taken and revealed the presence of at least two foetuses in the uterus. The female appeared otherwise healthy and was feeding well. As serum gonadotrophin had been administered,

the duration of gestation was believed to be most likely 32-37 days, however she had failed to give birth by day 44, therefore a caesarian was performed. Two dead pups were removed, and the female had developed a severe uterine infection and peritonitis.
This female was included in one of two trials performed at Melbourne Zoo that aimed to explore the possibilities of utilising hormones to increase fecundity. The trials included superovulation (the production of extra eggs), with cross-fostering and the use of hormones to stimulate cycling in non-cycling animals. The trials are outlined in the Veterinary care section, but for more detailed information in regards to the trials contact the Melbourne Zoo Native Mammal or Veterinary departments.

11. Growth and Development

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At birth the average weight is 1-2.4g, the eyes are closed and the ear pinnae are attached to the head. The neonates are a deep red in colour, with a faint dark dorsal pigmentation becoming evident after a few hours. Neonates are then covered with a sparse covering of fine white hair, that does not extend to the hind feet and manus until 4-5 days after birth. They are unable to maintain a firm hold on the mothers teats until the incisors have erupted (this occurs2-9 days after birth), and are unable to right themselves until 3 days old. They are crawling by the 7th day. The ear pinnae unfold after 2 days and teats are visible after 3 days. The tail hair begins to appear after 7 days, and by day ten the body pelage is complete. Animals undergo their first moult at 6 weeks of age. The eyes do not open until between 13 and 19 days. Weaning occurs at 4 - 6 weeks of age.

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Developmental stages for various characteristics are summarised below in Table 4:

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Factor	Time period
Eyes	Open 13-20 days
Ears	Pinnae free 1-3 days Open 12-14 days
Upper incisors	Erupt 2-6 days
Lower incisors	Erupt 4-9 days
Teats	Visible at 3 days, covered by fur by 19 days
Umbilical mark	Visible up to 13 days
Pelage	Complete by day 10, guard hairs 6mm, underfur< 3mm By 19 days guard hairs are 11mm and underfur 6-8mm

Table 4: Stages of development for New Holland mouse young (from Kemper, 1976)

12. References

Braithwaite, R.W. and Gullan, P.K. (1978) Habitat selection by small mammals in a Victorian Heathland. Australian Journal of Ecology 3: 109-127

Cockburn, A. (1980). The Diet of the New Holland Mouse (*Pseudomys novaehollandiae*) and the House Mouse (Mus musculus) in Victorian Coastal heathland. Australian Mammalogy 3: 31-4

Fox, B.J. and Fox, M.D. (1978) Recolonization of coastal heath by *Pseudomys novaehollandiae* (Muridae) following sand mining. Australian Journal of Ecology 3: 447-465.

Fox, B.J and McKay, G.M., 1981. Small mammals responses to pyric successional changes in eucalypt forest. Australian Journal of Ecology 6: 29-41.

Fox, B.J., 1982. Fire and mammalian secondary succession in an Australian coastal Heath. Ecology 63: 1332-41.

Fox, B.J. and Pople, A.R., 1984. Experimental confirmation of interspecific competition between native and introduced mice. Australian Journal of Ecology 9: 323-34.

Gainey, S.A. (2000). Population monitoring, burrow location, and habitat vegetation analysis of *Pseudomys novaehollandiae*, New Holland Mouse, on Yanakie Isthmus, Wilsons Promontory National Park, Victoria. Melbourne, The University of Melbourne, Department of Zoology.

Hocking, G.J., 1980. The occurrence of the new Holland mouse *Pseudomys novaehollandiae* (Waterhouse) in Tasmania. Australian Wildlife research 7: 71-78.

IATA, 2003. Live Animal Regulations. International Air Transport Association, Montreal

Keith, K. and Calaby, J.H. (1968). The New Holland Mouse, *Pseudomys novaehollandiae* (Waterhouse), In the Port Stephens district, New South Wales. CSIRO Wildlife Research 13, 45-58.

Kemper, C.M. (1976) Reproduction of *Pseudomys novaehollandiae* (Muridae) in the laboratory. Australian Journal of Zoology 24: 159-67.

Kemper, C.M. (1980) Reproduction of *Pseudomys novaehollandiae* (Muridae) in the wild. Australian Wildlife Research 7: 385-402

Kemper, C.M. (1995) New Holland Mouse *Pseudomys novaehollandiae* (Waterhouse, 1843). The Mammals of Australia. R. Strahan. New South Wales, The Australian Museum and Reed Books: 611-612.

Kemper, C.M. (1990) Small mammals and habitat disturbance in open forest of coastal New South Wales. I. Population Parameters. Australian Wildlife Research 17: 195-206.

Lock, M.L. and Wilson, B.A. (1999). The distribution of the New Holland Mouse (*Psuedomys novaehollandiae*) with respect to vegetation near Anglesea, Victoria. Wildlife Research 26: 565-77.

Mahoney, J. A and Marlow, B. J. (1968). The rediscovery of the New Holland Mouse. Australian journal of Science 31, 221-3.

Norton, T.W. (1986) Notes on the size of *Pseudomys novaehollandiae* (Waterhouse) (Rodentia: Muridae) in Tasmania. Australian Mammalogy 9 (1&2): 61-63

Posamentier, H.G. and Recher, H.F., (1974) The status of *Pseudomys novaehollandiae* (the New Holland Mouse). Australian Journal of Zoology 18 (2): 66-71

NRE (1996) New Holland Mouse, *Pseudomys novaehollandiae*, Action Statement 74.

Posamentier, H.G. and Recher, H.F. (1974) The status of *Pseudomys novaehollandiae* (the New Holland Mouse). Australian Journal of Zoology 18:66-71

Pye, T., 1991. The New Holland Mouse (*Pseudomys novaehollandiae*) (Rodentia: Muridae) in Tasmania: a field study. Wildlife Research 18:525-32

Van Dyck, S. (1998) New Holland Mouse. Nature Australia, Summer 1998-99: 48-53.

Wilson, B.A. (1991) The ecology of *Pseudomys novaehollandiae* (Waterhouse 1843) in the eastern Otway Ranges, Victoria. Wildlife Research 18: 233-47.

Wilson, B. A. (1996). The distribution and status of the New Holland Mouse, *Pseudomys novaehollandiae* (Waterhouse 1843) in Victoria. Australian Mammology 19: 31-46.

Wilson, B.A., and Bradtke, E. (1999) The diet of the New Holland Mouse, *Psuedomys novaehollandiae* (Waterhouse) in Victoria. Wildlife Research 26: 439-451.

13. Bibliography

Atkin, B.W. (1996). New Holland Mouse *(Pseudomys novaehollandiae)* trapping program at Yanakie Isthmus, Wilsons Promontory National Park; 6th-9th May, 1996. Yarram, Flora, Fauna & Fisheries Unit. Department of Natural Resources and Environment.

Atkin, B. W. and Quin, B.R. (1999). New Holland Mouse *Pseudomys novaehollandiae* (Rodentia: Muridae): Further finding at Yanakie Isthmus, Wilsons Promontory National Park. The Victorian Naturalist 116 (5): 169-72

Batrouney, A., Lees, K. and Nguyen, P. (2001). Anglesea Community Knowledge and Values of the New Holland mouse, *Pseudomys novaehollandiae*. Melbourne, RMIT School of Social Science & Planning: 46.

Bradtke, E.L. (1995). The Diet of the New Holland Mouse (*Psuedomys novaehollandiae*) in Victoria. Biological and Chemical Sciences. Deakin University, Geelong.

Fox, B.J., Higgs, P. and Lou, J. (1993). Extension of the breeding season of the New Holland Mouse: a response to above-average rainfall. Wildlife Research 20: 599-605.

Hocking, G. J. (1996) Rodents of Tasmania. The Australian Mammal Society 42nd Meeting and Biology and Conservation of Rodents in Australia Symposium, The University of Melbourne, The Australian Mammal Society.

Kemper, C. (1977) The Biology of the New Holland Mouse *Pseudomys novaehollandiae*. Ph.D. thesis, Macquarie University: Sydney.

Hogan, B., Beddington, R., Costantini, F and Lacy, E. (1994) Manipulating the Mouse embryo. Cold Spring Harbor Laboratory Press. Pp:130-132.

Kentish, K.M. (1981) The New Holland Mouse, a new record from Anglesea. Geelong Naturalist 18(1): 3-7.

Kentish, K.M.(1982) A new record of the New Holland Mouse (*Pseudomys novaehollandiae*) from Anglesea, Victoria. Victorian Naturalist 99: 128-29.

Lazenby, B. (1999). Vegetation associations and spatial relations in the New Holland Mouse, *Pseudomys novaehollandiae* (Rodentia: Muridae) in Tasmania. Zoology Department. Hobart, University of Tasmania: 146.

Lock, M.L. (1995) The distribution and abundance of *Psuedomys novaehollandiae* (New Holland Mouse), within the Forest Road Flora Reserve, Anglesea, Victoria. Biological and Chemical Sciences. Geelong, Deakin University.

Mills, D. (1992). Habitat use and movements of New Holland Mouse at Anglesea. Geelong, Deakin University.

Myroniuk, P and Wilson, B.A. (1992). A preliminary population viability analysis (PVA) of *Psuedomys novaehollandiae* (New Holland Mouse) in Victoria. Melbourne, Department of Conservation and Environment, Victoria.

Myroniuk, P. (1997). Conservation genetics of the New Holland Mouse *Pseudomys novaehollandiae,* Waterhouse, 1843 (Rodentia: Muridae): and analysis of mitochondrial DNA control region variation. Geelong, Deakin University.

Myronuik, P. (1997). Conservation Genetica and Biodiversity Management: the New Holland Mouse and Pilliga Mouse as examples. Melbourne, Royal Melbourne Zoological Gardens.

Myronuik, P., Wilson, B. and Seebeck, J. (1997). Recovery of the New Holland Mouse (*Pseudomys novaehollandiae*) in Victoria: A proposal to the Zoological Parks and Gardens Board to establish a captive breeding research program. Melbourne, Zoological Parks and Gardens Board, Deakin University, Department of Natural Resources and Environment.

Norton, T.W. (1987). The ecology of small mammals in north-eastern Tasmania. II. *Pseudomys novaehollandiae* and the introduced *Mus musculus*. Australian Wildlife Research. 14: 435-41.

Quin, B.R. (1996). New Holland Mouse *Pseudomys novaehollandiae* (Rodentia: Muridae) in South Gippsland, Southern Victoria. Part One- Distribution and Status. The Victorian Naturalist 113 (5): 236-246

Quin, B.R. and R.C. Williamson (1996). New Holland Mouse *Pseudomys novaehollandiae* (Rodentia: Muridae) in South Gippsland, Southern Victoria. Part two- Conservation and Management. The Victorian Naturalist 113 (6): 281-288.

Rounsevell, D.E., R.J. Taylor and Hocking, G.J. (1991). Distribution Records of native terrestrial mammals in Tasmania. Wildlife Research 18 (6): 699-717.

Seebeck, J.H. and Beste, H.J. (1970). First Record of New Holland Mouse (*Pseudomys novaehollandiae* (Waterhouse, 1843)) in Victoria. Victorian Naturalist 87: 280-287.

Seebeck, J.H., Menkhorst, P.W., Wilson, B.A., and Lowe, K.W. (1996) Action Statement No. 74- New Holland Mouse, *Pseudomys novaehollandiae*. Victoria, Department of Natural Resources and Environment.

Seebeck, J.H., Menkhorst, P.W., Wilson, B.A., and Lowe, K.W. (1996). New Holland Mouse *Pseudomys novaehollandiae*. Melbourne, Department of Natural resources and Environment and Deakin University.

Vance, A, and Ward, S. (2001). Population Dynamics and habitat selection of the New Holland Mouse *Pseudomys novaehollandiae* in the Gippsland Lakes Coastal Park, Victoria. The 47th Scientific Meeting and Possum and Glider Symposium. Queensland Museum, South Brisbane, Australian Mammal Society.

Wilson, B.A., Robertson, D., Moloney, D.J.,Newell, G.R., and Laidlaw, W.S. (1990) Factors affecting small mammal distribution and abundance in the eastern Otway Ranges, Victoria. Proceedings of the Ecological Society of Australia 16: 379-96.

Wilson, B.A. (1993). Management and conservation of habitat for the New Holland Mouse in Victoria. Melbourne, National Estates Grants Program Report.

Wilson, B.A. (1994) The distribution of the New Holland Mouse *Pseudomys novaehollandiae* (Waterhouse 1843) in the Eastern Otways, Victoria. The Victorian Naturalist 111(2): 46-53.

Wilson, B.A. (1996) The distribution of the New Holland Mouse *Pseudomys novaehollandiae* (Waterhouse 1843) in Victoria. Australian Mammalogy 19: 31- 46

APPENDIX : NEW HOLLAND MOUSE *(Pseudomys novaehollandiae):*
MELBOURNE ZOO MICROCHIPPING PROTOCOL

Anaesthesia:

- Mask down using 5% isoflurane/oxygen; maintain anaesthesia using 2% - 3.5% isoflurane/oxygen
- Maintain on supplemental heat (eg: a warmed wheat bag)

Microchipping:

- Microchips are to be placed subcutaneously in the interscapular region
- New Holland Mice have groomed out microchips when the chips are placed close to the insertion wound. The skin wound should be at least 1cm from the final location of the microchip (*eg.* in the left flank)
- Microchips should be inserted using the small Trovan® syringe applicator.

- **Technique:**
    1. routine skin disinfection at the left flank insertion site
    2. make a small skin incision in the skin of the left flank using a sterile scalpel blade
    3. insert microchip applicator needle and advance the needle subcutaneously so that the microchip is located in the interscapular region following injection
    4. inject the microchip, remove the needle and close the skin incision using a drop of Vetbond® (3M Animal Care Products) tissue glue
    5. give 0.0.1mL of long-acting penicillin (Norocillin LA) subcutaneously.