

Standard Operating Procedure

PERMANENT MARKING OF MAMMALS USING EAR NOTCHING

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Prepared for: Animal Ethics Committee

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1 Purpose

Ear notching is a method used in fauna survey and monitoring activities to identify previously caught animals. It is a widely used method for individual identification of small mammals (Dasyurids and rodents). It should never be performed on animals with specialised ears (e.g. bats and bilbies)

The advantage of ear notching is that it is permanent, fast and inexpensive. Reasonable numbers of animals can be individually identified. Tissue from ear notches can be used to genetically identify the animal and there is no extra weight or equipment, such as ear tags, that potentially hinder the animal (Mellor *et al.*, 2004).

Ear notching requires a degree of practice, confident animal handling skills, good eyesight and a steady hand.

This standard operating procedure (SOP) provides advice on the safe administration of permanent marking of mammals using ear notching.

2 Scope

This SOP has been written specifically for scientific and education purposes, and endorsed by the Department's Animal Ethics Committee. However, this SOP may also be appropriate for other situations.

This SOP applies to all fauna survey and monitoring activities involving the use of ear notching as a permanent marking method, undertaken across the State by Department of Biodiversity, Conservation and Attractions (hereafter Department) personnel. It may also be used to guide fauna monitoring activities undertaken by Natural Resource Management groups, consultants, researchers and any other individuals or organisations. All Department personnel involved in monitoring using ear notching should be familiar with the content of this document.

Projects involving wildlife may require a licence under the provisions of the *Wildlife Conservation Act 1950* and/or the *Biodiversity Conservation Act 2016*. Personnel should consult the Department's Wildlife Licensing Section and Animal Ethics Committee Executive Officer for further guidance. In Western Australia any person using animals for scientific purposes must also be covered by a licence issued under the provisions of the *Animal Welfare Act 2002*, which is administered by the Department of Primary Industries and Regional Development. This SOP complements the *Australian code of practice for the care and use of animals for scientific purposes* (The Code). The Code contains an introduction to the ethical use of animals in wildlife studies and should be referred to for broader issues. A copy of the code may be viewed by visiting the National Health and Medical Research Council website (<http://www.nhmrc.gov.au>).

3 Definitions

Animal handler: A person listed on an application to the Department's Animal Ethics Committee who will be responsible for handling animals during the project.

Ear notching: A process that involves taking a small notch (or series of notches) with an ear notcher or punch. The combination of notches (usually using a numbering system) provides a means to identify individual animals or to simply identify previously caught animals.

Permanent marker: A marker designed to stay with an animal for its lifespan. Permanent markers tend to leave marks that are less visible and often involve tissue damage (Sharp *et al.*, 2007).

Sterilising solution: A solution that sterilises equipment in one step (e.g. Alconox®).

4 Procedure Outline

4.1 Materials required

The following equipment is needed to undertake ear notching:

- 2mm ear punch or ear notcher (see Figure 1)
- Fine tipped tweezers or jewellery forceps
- Vial of 70% ethanol and cigarette lighter or portable blow torch for flaming if in the field or sterilising solution (e.g. Alconox®)
- Topical antiseptic (e.g. Betadine®)
- Gauze swabs or tissues



Figure 1 A 2mm ear punch (left) and ear notcher (right). Flagging tape is attached to the ear punch to increase visibility to prevent it from being easily lost. Photo: Vanessa Richter/DBCA.

4.2 Animal handling

(a) Techniques for handling animals vary depending on the species of mammal involved and the experience and skills of the personnel. General advice on animal handling is contained in the Department SOP for *Hand Restraint of Wildlife*. All handling of animals should be done by (or under the guidance of) experienced personnel.

(b) Use handling bags appropriate for the species and duration of containment as advised in the Department SOP for *Animal Handling and Restraint using Soft Containment*.

(c) If an animal is injured during handling/ear notching, treat any superficial wounds with a topical antiseptic (e.g. Betadine®) (refer to the Department SOP for *First Aid for Animals*).

(d) If an animal is seriously injured, refer to the flowchart in the Department SOP for *Humane Killing of Animals under Field Conditions* to make the decision on whether or not to euthanase or seek veterinary care.

(e) Captured animals must be released at point of capture (unless the purpose of the trapping is translocation or other approved reason). Animals must be released, or reach an alternate endpoint approved by the Department's Animal Ethics Committee, within 24 hours of capture. Animals should be released at a time when they are normally active.

4.3 Cleaning and sterilising

(a) Ear punch/notchers should be cleaned and sterilised between each animal and prior to returning the equipment for storage.

(b) Flaming is the most common method for cleaning and sterilising equipment but in fire risk areas, or where notches are being collected for DNA analysis, dipping the equipment in sterilising solutions (e.g. Alconox[®]) is advised.

4.3.1 Flaming

(a) Dip the ear punch/notcher and the end of the tweezers into 70% ethanol and clean with a swab to remove gross dirt and any leftover tissue etc. (Note: ethanol is a highly flammable substance; care should be taken as not to get ethanol on anything other than the equipment needing to be flamed).

(b) Clean up any spillages immediately, including any ethanol on hands and clothing, and if required wait until the spilled ethanol has evaporated before continuing with the procedure.

(c) Dip the ear punch/notcher in ethanol and flame the cutting part with a lighter or portable flame torch (Note: the flame from ethanol is not visible in sunlight). Allow the equipment to cool before using it on an animal.

(d) Ensure sterility of equipment to avoid contamination especially in the case of DNA analysis. **DO NOT** allow contact with any other biological material (including human fingers) before the animal is marked.

4.3.2 Cleaning and sterilising solutions

(a) For single step sterilisation, the ear punch or notcher can be dipped in a sterilising solution such (e.g. Alconox[®]) which does not require flaming.

(b) If in addition you are collecting tissue for DNA, it is necessary to sterilise equipment between individuals to prevent cross contamination. It is also important to rinse equipment in water (preferably distilled water) to remove solutions that may destroy the DNA sample (See the Department SOP for *Tissue Sample Collection and Storage for Mammals*).

4.4 Taking an ear notch

(a) Restrain the animal (this may be easier with two people with one person holding and the other notching, however with experience the procedure can be undertaken by one person), exposing the ears and leaving the rest of the body in the handling bag taking particular care to ensure eyes are covered (see Figure 2).

(b) As dirt/bacteria can be pushed into open wounds as the notchers move through the tissue, the ear needs to be cleaned prior to notching with dilute antiseptic (e.g. Betadine[®]) or an alcohol swab. If the tissue is being collected for DNA purposes, it is important that the handler does not touch the area where the sample will be taken from to avoid cross contamination of DNA.

- (d) Take a small (half circle) tissue notch from the margin of the ear where it is thinnest and with the least number of blood vessels (often upper outer edge of the ear). For some species a torch may be used to shine light through the ear to see where blood vessels are to be avoided. Complete holes should not be punched all the way through the ear unless it is for the fitting of an identification tag. This is to prevent a claw or vegetation catching in the hole and tearing the ear.
- (e) Take care positioning the ear punch/notcher so that the resulting mark is unambiguous.



Figure 2 A southern brown bandicoot positioned ready for ear notching (left) and after its ear has been notched (right). Photo: Christine Freegard/DBCA

- (f) Depending on the purpose of the monitoring program, the number of ear notches may vary and a numbering system may need to be followed (see Figure 3). For example an animal with notches removed at positions 2, 7 and 10 will be number 19 (i.e. 2+7+10=19).

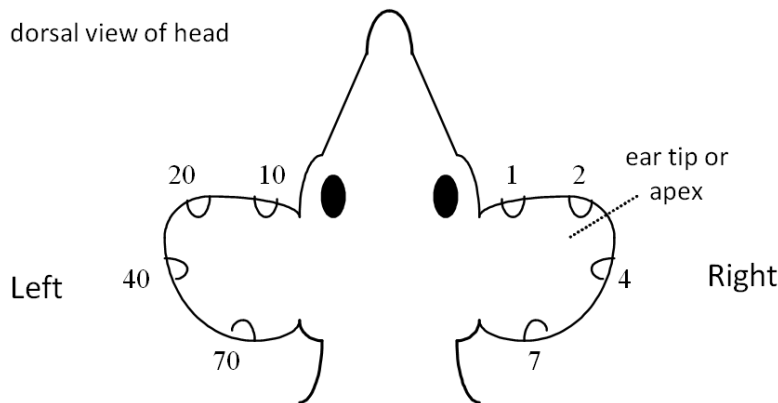


Figure 3 Diagram of ear notch numbering system (Orell, 1997)

Generally small, short-lived mammals need only be recorded as recaptures or re-traps. A single notch may be applied to identify previously caught individuals and a temporary mark (i.e. fur clip/ink) may be used to indicate re-traps during a single monitoring period.

Individual identification may be appropriate for longer lived species or intensively studied populations and may be achieved using a numbering system as illustrated in Figure 3.

Refer to the Department SOP for *Tissue Storage and Collection for Mammals* for details on storing the notch for genetic identification.

(f) With a gauze swab or tissue apply a topical antiseptic (e.g. Betadine®) to the area that has been notched to prevent infection. If the animal is bleeding, apply pressure with a dry gauze swab or tissue until the bleeding stops.

(g) Re-secure the animal in the handling bag and allow it to recover before releasing.

4.5 Recording data

Data should be recorded on the *Western Shield Data Sheet* and then entered into Fauna File.

5 Level of Impact

There is a high level of impact involved in ear notching animals, as it involves the removal of tissue. Potential animal welfare impacts when ear notching include:

- Distress (caused by handling, discomfort, social isolation, separation of mother and young).
- Trauma (possible injury to the animal during restraint. e.g. scratching or biting itself).
- Pain and bleeding from notching, which is usually brief.
- Infection at site of notch

Note that whilst these impacts are specifically associated with the procedure of ear notching, an animal may also experience other impacts from associated procedures such as trapping and capture.

6 Ethical Considerations

To reduce the discomfort of ear notching on animals there are a number of ethical considerations that should be addressed throughout projects involving these procedures. Department projects involving ear notching will require approval from the Department's Animal Ethics Committee.

It should be noted that whilst these ethical considerations are specifically associated with the procedure of ear notching, other ethical considerations need to be taken into account during procedures carried out prior to ear notching, such as trapping and capture.

6.1 Animal handling

To ensure minimal stress to the animals they should only be handled for as long as required to mark them and to collect any necessary measurements (usually no more than five minutes). They must be released within 24 hours of capture. Improper restraint, especially when dealing with a stressed and frightened animal can lead to major physiological disturbances (hyperthermia, stress, shock capture myopathy). It is preferable that handling be done during the cooler periods of the day (dawn/dusk).

6.2 Pain and infections

Although hygiene is difficult in the field, cleanliness of all surgical and puncture techniques is essential to minimise the potential for infection and to provide reliable DNA samples. All ear

punch and notchers should be kept extremely sharp and clean to minimise tearing, bruising, infection and transmission of disease.

Blunt instruments should be replaced. However this is not always possible in the field. Ear notchers and punches can be sharpened with a flat jeweller file, by lightly filing the cutting edge, while maintaining the same profile as the cutting face. For ear punches, it is important to maintain the flat edge of the punch and ensure that the hole is not enlarged (N. Thomas, pers. comm., 2009).

Any recaptured animals must have their ears inspected closely to assess whether any infection has occurred. Appropriate anaesthetic, antiseptic and measures of pain control must be used when/if required.

6.3 Bleeding

Care should be taken when deciding where to place the ear notch to ensure that it is in an area with few blood vessels. Ear notching and punching can result in excessive bleeding which, should it occur, needs to be controlled prior to the animal being released.

6.4 Injury and unexpected deaths

If injury, unexpected deaths or euthanasia occur then it is essential to consider the possible causes and take action to prevent further deaths. For projects approved by the Department's Animal Ethics Committee, adverse events such as injury, unexpected deaths or euthanasia must be reported in writing to the AEC Executive Officer on return to the office (as per 2.2.28 of The Code) by completing an *Adverse Events Form*. Guidance on field euthanasia procedures is described in the Department SOP for *Humane Killing of Animals under Field Conditions*. Where disease may be suspected, refer to the Department SOP for *Managing Disease Risk in Wildlife Management* for further guidance.

7 Competencies and Approvals

Department personnel, and other external parties covered by the Department's Animal Ethics Committee, undertaking projects that involve permanent marking of animals by ear notching require approval from the committee and will need to satisfy the competency requirements detailed in Table 1. This is to ensure that personnel involved have the necessary knowledge and experience to minimise the potential impacts of ear notching on the welfare of animals. Other groups, organisations or individuals using this SOP to guide their fauna monitoring activities are encouraged to also meet these competency requirements as well as their basic animal welfare legislative obligations.

It should be noted that details such as intensity of the study being undertaken will determine the level of competency required and Table 1 provides advice for basic monitoring only.

Table 1 Competency requirements for Animal Handlers of projects involving the permanent marking wildlife using ear notching

Competency category	Competency requirement	Competency assessment
Wildlife licences	Licence to take fauna for scientific purposes (Reg 17) OR Licence to take fauna for educational or public purposes (Reg 15)	Provide licence number
Formal training (Note: Suitable levels of skills/experience can substitute for formal training requirements)	Department Fauna Management Course or equivalent training	Provide course year
Animal handling and processing skills/experience	Experience in handling terrestrial mammal fauna.	Personnel should be confident in handling species being marked. This experience is best obtained under supervision of more experienced personnel. Estimated total time in field: Min 2 years involved in similar projects.
Blood, DNA and surgical skills/experience	Experience in ear clipping for DNA sampling of non-bird and non-bat fauna	Personnel should be familiar with how to operate ear notching and punching equipment. This experience is best obtained under supervision of more experienced personnel. A minimum of 5 supervised applications is required for each species. Estimated total time in field: Min 2-5 years involved in similar projects.

8 Occupational Health and Safety

Always carry a first aid kit in your vehicle and be aware of your own safety and the safety of others as well as the animals when handling.

A job safety analysis is recommended prior to undertaking any monitoring which involves hand capture. This safety analysis should include the following considerations.

8.1 Animal bites and scratches

Handling animals can result in injuries to handlers from the animals inflicting bites and scratches. All inflicted injuries (even superficial ones) should be appropriately treated as soon as possible to ameliorate possible allergic reaction, prevent infection and promote healing.

Personnel should also have up-to-date tetanus vaccinations.

If Department personnel or volunteers are injured, please refer to the Department's Health and Safety Section's 'Report a Hazard, near-miss or incident' intranet page, which can be found at http://intranet/csd/People_Services/rm/Pages/ReportingHazards,Near-MissesandIncidents.aspxZoonoses.

8.2 Zoonoses

There are a number of diseases carried by animals that can be transmitted to humans (i.e. zoonoses such as Toxoplasmosis, Leptospirosis and Salmonella). All personnel must take precautions to minimise the risk of disease transmission to protect themselves, their families and wildlife populations.

Advice on minimising disease risk is contained in the Department SOP for *Managing Disease Risk in Wildlife Management*

8.3 Allergies

Some personnel may develop allergies when they come in contact with animal materials such as hair and dander. Personnel known to develop allergies should wear gloves when handling animals and long sleeved pants/shirt.

People with severe allergies associated with animals, with immune deficiency diseases or on immunosuppressant therapy should not engage in the handling of wildlife.

8.4 Chemicals

Personnel should be aware of the dangers of the chemicals they use in the field. Refer to *Material Safety Data Sheets* (MSDS) relevant to the chemical(s) (e.g. Alconox[®], methylated spirits etc.).

8.5 Fire risk

Personnel intending to clean and sterilise equipment by flaming should be aware of the associated fire risk and take appropriate action to reduce this risk, and have adequate fire suppression equipment such as a fire blanket on hand.

9 Further Reading

The following SOPs have been mentioned in this advice and it is recommended that they are consulted when proposing to undertake ear notching of animals:

- Department SOP *Tissue Sample Collection and Storage for Mammals*
- Department SOP *Animal Handling and Restraint using Soft Containment*
- Department SOP *Hand Restraint of Wildlife*
- Department SOP *First Aid for Animals*
- Department SOP *Transport and Temporary Holding of Wildlife*
- Department SOP *Managing Disease Risk in Wildlife Management*
- Department SOP *Humane Killing of Animals under Field Conditions*

10 References

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Sharp, T., Saunders, G and Mitchell, B. (2007). *Model standard operating procedures for the humane research of pest animals in Australia*. New South Wales: Department of Primary Industries.

10.1 Personal Communication

- Neil Thomas (Animal Science, Department of Parks and Wildlife), 2009