

Standard Operating Procedure

SC22-17 PERMANENT MARKING OF MAMMALS USING EAR NOTCHING

Animal welfare is the responsibility of all personnel involved in the care and use of animals for scientific purposes.

Personnel involved in an Animal Ethics Committee approved project should read and understand their obligations under the *Australian code for the care and use of animals for scientific purposes*.

Version 1.2

August 2022



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Approved by the DBCA Animal Ethics Committee:

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Chair, Animal Ethics Committee

Department of Biodiversity, Conservation and Attractions

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1 Acknowledgements

This standard operating procedure was originally developed by Christine Freegard and Vanessa Richter, with contributions from Stephanie Hill, Brent Johnson, Peter Orell and Neil Thomas.

2 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 using combinations of multiple notches and there is no extra weight or equipment, such as ear tags, that may hinder the animal (Mellor et al., 2004). Tissue from ear notches can also be used for DNA analysis (refer to the department SOP *Tissue Sample Collection and Storage for Mammals*).

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 application of permanent marking of mammals using ear notching.

3 Scope

This SOP has been written specifically for scientific and education purposes, and endorsed by the Department of Biodiversity, Conservation and Attractions' (DBCA) Animal Ethics Committee (AEC). 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 Western Australia by DBCA (hereafter department) personnel. It may also be used to guide fauna related activities undertaken by Natural Resource Management groups, consultants, researchers and any other individuals or organisations. All department personnel involved in fauna research and management should be familiar with the content of this document.

Projects involving wildlife may require a licence/authorisation under 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 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 all AEC approved projects. A copy of the code may be viewed by visiting the National Health and Medical Research Council website (<http://www.nhmrc.gov.au>).

4 Animal Welfare Considerations

To reduce the level of impact of ear notching on the welfare of animals, personnel must consider, address and plan for the range of welfare impacts that may be encountered. Strategies to reduce impacts should be identified during the planning stage to ensure that they can be readily implemented during ear notching, and to ensure that contingencies for managing welfare issues have been identified. Ensure that all handlers and volunteers involved in the project are aware of the range of issues that they may encounter, the options that are available for reducing impact and improving animal welfare, and the process for managing adverse events.

Department projects involving permanent marking of mammals using ear notching will require approval from the department's Animal Ethics Committee.

The key animal welfare considerations that should be considered when permanently marking mammals using ear notching are listed below and are highlighted throughout the document.

4.1 Injury and unexpected deaths

If adverse events including injury, unexpected deaths, or euthanasia occur, then it is essential to consider the possible causes and take action to prevent further issues. Adhering to the guidance in this SOP will assist in minimising the likelihood of adverse events. For projects approved by the department's Animal Ethics Committee, adverse events must be reported in writing to the AEC Executive Officer as soon as possible after the event by completing an *Adverse Events Form*. Guidance on field euthanasia procedures is described in the Department SOP for *Euthanasia of Animals Under Field Conditions*. Where disease may be suspected, refer to the Department SOP for *Managing Disease Risk and Biosecurity in Wildlife Management* for further guidance.

4.2 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. Investigators must be aware that the effects of a series of stressors, such as trapping, handling, transportation, sedation, anaesthesia and marking can be cumulative.

5 Procedure Outline

5.1 Material required

The following equipment is needed to undertake ear notching:

- 2 mm ear punch or ear notcher, in good working order (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 2 mm 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).

5.2 Cleaning and sterilising the equipment

- (a) Ear punch and 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 is advised (refer to 5.4 b).

5.3 Flaming

- (a) Dip the ear punch, notcher and the end of the tweezers into 70% ethanol. Clean with a swab to remove gross dirt and any leftover tissue. Note, ethanol is a highly flammable substance, and care should be taken to not 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 necessary, wait until the spilled ethanol has evaporated before continuing with the procedure.
- (c) Re-dip the equipment in ethanol and flame the cutting part of the ear punch or notcher, and the tips of the tweezers 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) **DO NOT** allow contact with any other biological material, including human fingers, before the animal is marked.

5.4 Cleaning and sterilising solutions

- (a) For single step sterilisation, the equipment can be dipped in a sterilising solution (e.g., 10% bleach or other commercial disinfectant, such as F10SC) for 10 minutes followed by a rinse with deionised water.
- (b) For DNA tissue collection, it is necessary to disinfect equipment between individuals to prevent cross contamination. It is also important to rinse equipment in water (preferably distilled water) after disinfecting to remove solutions that may destroy the DNA sample. Alternatively, use a new piece of equipment for each individual and disinfect all equipment at the end of the sampling session.

5.5 Animal handling

ANIMAL WELFARE: 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 10 minutes). Animals should be released as soon as possible after processing or, if additional holding is necessary, as soon as practicable allowing for animal welfare considerations. Improper restraint, especially when dealing with a stressed and frightened animal can lead to major physiological disturbances (e.g., hyperthermia, stress, shock, capture myopathy). It is preferable that handling be done during the cooler periods of the day (dawn/dusk).

- (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*.

ANIMAL WELFARE: 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 not be used. Spare instruments, in good working order, must be available in the field. In some cases, equipment 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.

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.

- (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

Euthanasia of Animals Under Field Conditions to make the decision on whether to euthanase or seek veterinary care.

ANIMAL WELFARE: 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.

5.6 Taking the 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 (Figure 2).
- (b) Clean the ear with antiseptic (e.g., Betadine®) or an alcohol swab, to prevent dirt and bacteria from the ear's surface being pushed into the open wound by the ear punch or notcher. 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.
- (c) Take a small tissue notch from the margin of the ear, where it is thinnest and where there are few blood vessels (often the upper outer edge of the ear). Shining a torch through the ear, can enable blood vessels to be seen, so that they can be avoided. A small piece of clean card placed flush against the ear can assist in achieving a clean cut.

ANIMAL WELFARE: Care should be taken for species with marginal veins, it is advisable to take a notch from further in on the ear to avoid a significant risk of bleeding.

- (d) Generally, complete holes should not be punched through the ear, unless it is for the fitting of an identification tag, to prevent a claw or vegetation catching in the hole and tearing the ear.
- (e) However, in species with marginal ear veins, notching the edge of the ear can result in heavy bleeding. For these species, using a small ear punch (diameter <2 mm) to create a complete hole, may be appropriate.
- (f) Use tweezers to remove the tissue from the ear, or ear punch, notchers, or clean card.
- (g) Take care positioning the ear punch or notcher so that the resulting mark is unambiguous.
- (h) Depending on the purpose of the monitoring program, the number of ear notches may vary



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

- (i) Generally small, short-lived mammals need only be marked with a single notch to identify them as previously caught individuals. A temporary mark (i.e., fur clip, ink) may be used to further indicate re-captures during a single monitoring period.
- (j) Individual identification may be appropriate for longer lived species or intensively studied populations and may be achieved using a numbering system (Figure 3). For example, an animal with notches removed at positions 2, 7 and 10 will be number 19 (i.e., $2+7+10=19$).

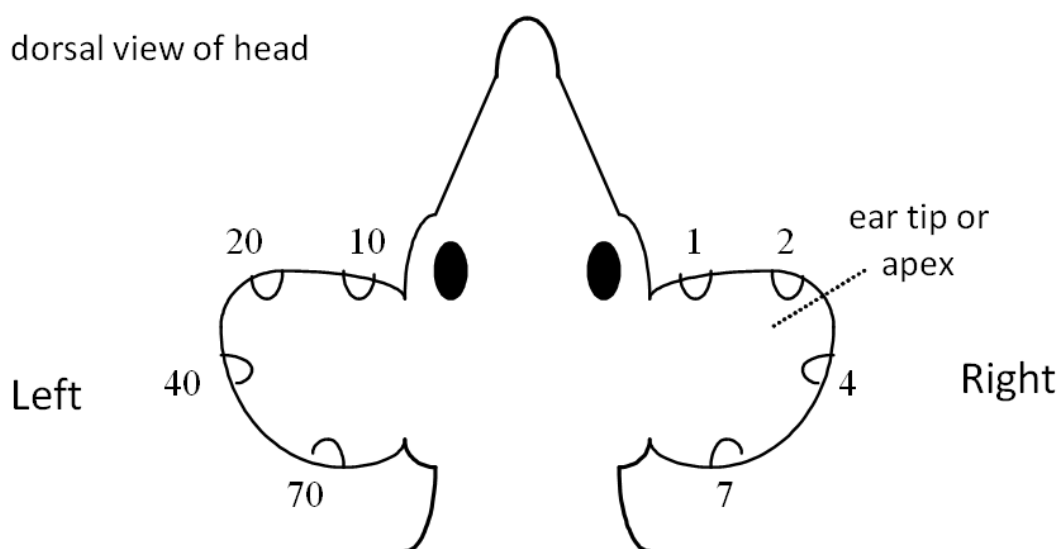


Figure 3 Diagram of ear notch numbering system (Orell, 1997)

- (k) 90-100% ethanol solution is the best preservative for long term storage for DNA analysis. Refer to the department SOP for *Tissue Storage and Collection for Mammals* for details on storing an ear notch for DNA analysis.
- (l) With a gauze swab or tissue apply a topical antiseptic (e.g., Betadine®) to the area that

has been notched to prevent infection.

(m) If the animal is bleeding, apply Betadine ointment with cotton bud or gauze swab with 1% betadine solution.

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

5.7 Recording data

Data should be recorded on the *Western Shield Data Sheet* (Appendix 1) using corresponding codes (Appendix 2) and then entered into Fauna File. Email Threatened (CR, EN, VU) and Priority (P1-4) fauna records to fauna.data@dbca.wa.gov.au

6 Competencies

A person who is competent has the knowledge, skills, and experiences that allow them to capture and handle animals successfully, and appropriately manage adverse events as required. Department personnel, and other external parties covered by the department's Animal Ethics Committee, undertaking fauna-related activities require approval from the committee and will need to satisfy the competency requirements (Table 1). 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 animal welfare legislative obligations.

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

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

Competency category	Competency requirement	Competency assessment
Knowledge	Broad understanding of the framework governing, the use of animals in research, and environmental studies in Western Australia	Training (e.g., DBCA Fauna Management Course or equivalent training). In applications, provide details on the course provider, course name and year.
	Understanding species biology and ecology	Personnel should be able to correctly identify the likely species to be encountered at the site(s) being studied and understand the species' biology and ecology. This knowledge may be gained through sufficient field experience and consultation of field guides and other literature.

	Understanding environmental conditions	Personnel should be aware of the environmental and seasonal conditions, that may be expected on the project, and understand how these may impact animal welfare.
Animal handling and tagging skills/experience required	Experience handling terrestrial mammal fauna	Personnel should be experienced at hand restraint of species being marked. This experience is best obtained under supervision of more experienced personnel. In applications, provide details on the longevity, frequency & recency of experience.
	Experience in permanent marking using ear notching	Personnel should be familiar with the animal welfare principles of permanent marking using ear notching. Personnel should be familiar with how to operate ear notching and punching equipment. This experience is best obtained under supervision of more experienced personnel.
	Experience managing disease risk and biosecurity in wildlife management	Personnel should be familiar with hygiene procedures. This knowledge may be gained through sufficient field experience and consultation of literature.

In conjunction with possessing the required understanding and knowledge of the permanent marking technique and animal welfare requirements, a guide to the experience and skill requirements for an animal handler to be considered competent to permanently mark mammals using ear notching is as follows: (noting that some personnel with experience may still require initial supervision in unfamiliar locations or with species that they have not encountered previously):

- Recency of time in field: within the past 5 years.
- Minimum 10 individuals of similar species handled.

7 Approvals

A licence or authorisation may be required under the *Biodiversity Conservation Act 2016* (examples below). Contact the department’s Wildlife Licensing Section for more information. It is your responsibility to ensure you comply with the requirements of all applicable legislation.

- Fauna taking (scientific or other purposes) licence (Reg 25)

- Fauna taking (biological assessment) licence (Reg 27)
- Fauna taking (relocation) licence (Reg 28)
- Section 40 Ministerial Authorisation to take or disturb threatened species.

8 Occupational Health and Safety

The following departmental SOPs for wildlife survey and monitoring activities are relevant to occupational health and safety:

- *SOP Managing Disease Risk and Biosecurity in Wildlife Management*
- *SOP Hand Restraint of Wildlife*

Departmental personnel, contractors and volunteers have duties and responsibilities under the Occupational Safety and Health Act 1984 and Occupational Safety and Health Regulations 1996 to ensure the health and safety of all involved. Fieldwork is to be undertaken in line with the department's corporate guidelines, policies and standard operating procedures, including but not limited to, risk management and job safety analyses. Further information can be found at

<https://dpaw.sharepoint.com/Divisions/corporate/people-services/HS/SitePages/SOPs.aspx>

If department personnel or volunteers are injured, please refer to the departmental Health, Safety and Wellbeing Section's 'Reporting Hazards, Near-misses and Incidents' intranet page, which can be found at <https://dpaw.sharepoint.com/Divisions/corporate/people-services/HS/SitePages/Reporting-Hazards,-Near-Misses-and-Incidents.aspx>

9 Further Reading

The following SOPs have been mentioned in this advice and it is recommended that they are consulted when proposing permanently mark mammals using ear notching:

- Department SOP *Tissue Sample Collection and Storage for Mammals*
- Department SOP *Animal Handling and Restraint using Soft Containmentment*
- Department SOP *Hand Restraint of Wildlife*
- Department SOP *Managing Disease Risk and Biosecurity in Wildlife Management*
- Department SOP *First Aid for Animals*
- Department SOP *Euthanasia of Animals Under Field Conditions*

For further advice refer also to:

National Health and Medical Research Council (2013) *Australian code for the care and use of animals for scientific purposes*, 8th edition. Canberra: National Health and Medical Research Council.

10 References

Mellor, D.J., Beausoleil, N.J. and Stafford, K.J. (2004). *Marking amphibians, reptiles and marine mammals: animal welfare, practicalities and public perceptions in New Zealand*. New Zealand: Department of Conservation.

Orell, P. (1997). *Western Shield Operational Fauna Monitoring Protocols*. Perth, WA: Department of Conservation and Land Management.

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.

11 Glossary of Terms

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®).

Appendix 1: Western Shield Data Sheet

TRAPPING DATA SHEET	Transect/Grid/etc NAME: _____	DATE: ____/____/____
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Site	Date ____/____/____	Trap type	Small cage	Elliott trap	Pitfall trap	Bait
Area	Transect / Grid / Other:	# Traps set for this date/event				

Personnel	Animal handling (initials)	Data recording (initials)	Session Dates				
			Start time	End Time	Weather (rain/wind)	Day 1 (Date)	Last day (Date)
			__:__	__:__			

Trap No.	Trap type	Recently burnt Y/N	Species/ Trap status (OB/ON/CB/CN/ SHUT)	Total wt		Implant No or Ear Tags (record both if both present)		N/R/RT*	Sex	Age	Head L / TL	Pes S/L OR SVL	Pouch/ Repro Status	PY/ CR	Fate/Comments
				Bag wt	Animal wt (g)	L Ear	R Ear								

Appendix 2: Codes for trapping

Trap status

Trap status label	Trap status Code	Notes
Closed with bait	CB	Trap is closed, but still has bait and no animal
Closed with capture	CC	All animal captures, including captures in pitfalls
Closed with no bait	CN	Trap is shut, bait has been removed, no animal
Open with bait	OB	Open traps with bait
Open with no bait	ON	Use this code for any open trap with no bait, this includes pitfalls with no captures
Trap deliberately shut	SHUT	Deliberately closed traps (for safety, etc.) and permanent points on transect that were not set

Capture Code

Age/sex

Capture label	Capture code	Age	Age code
escaped before id checked or marked	E	Adult	A
new capture	N	Infant	I
not tagged	NT	Juvenile	J
recapture, previous trapping session	R	Subadult	S
recapture, all new tags	RN	Sex	Sex code
recapture, no prior record of tags	RP	Female	F
recapture, same trapping session	RT	Male	M

Pouch Young

Pouch Young	Description	Code
embryonic, attached	jellybean stage, attached to teat	EMBRYONC
fully furred	fully developed pelage	FUR_FULL
fur covering, short	complete but light covering of fur	FUR_SHRT
hairless, ears attached	limbs developed, no hair, ears folded onto head	HLS_EARA
hairless, ears free	limbs more developed, no hear, external ear free	HLS_EARF
hair emerged; eyes open	hair visible, protruding from skin	HR_EMERG
hair not yet emerged, closed eyes	dark skin colouration from growing hair	HR_NEMER

Reproductive activity

Reproductive condition	Description	Code
abdominal testes	testes not visible	ABD_TEST
Active pouch (stained, Moist)	pouch empty but ready for young	ACTV_PCH

elongated teat, not lactating	teat elongated but not lactating	ELO_TEAT
inactive or dormant	no signs of reproductive activity	INACTIVE
lactating, dets of YAH separate rec	lactating, with young at heel present, details of young at heel recorded as a separate record	LAC_INDV
lactating, offspring absent	lactating with offspring absent	LAC_NOO
Lactating with offspring present	lactating with offspring present	LAC_OSP
uncertain if PY or YAH, dets sep rec	uncertain whether offspring is pouch young or young at heel (fully furred), offspring details recorded as a separate record	LAC_UNC
lactating	female with lactating teats - any mammal	LACTATNG
repro status not recorded	reproductive status was not recorded	NOT_REC
oestrus	oestrus, female reproductively active or receptive	OESTRUS
parous, not active (stained, dry)	marsupial female of breeding age, no pouch activity	PAR_NOAC
scrotal testes	testes visible in scrotal sac	SCR_TEST
suckling, dets of PY separate rec	suckling, with pouch young, details of PY recorded as a separate record	SUC_INDV
suckling PY attchd, lact teat, YAH absnt	suckling young attached, spare lactating teat, young at heel absent	SUC_LACA
suckling PY attchd, lact teat, YAH prsnt	suckling and lactating with pouch young and young at heel present	SUC_LACP
suckling, lact, YAH prsnt, YAH sep rec	suckling and lactating with pouch young and young at heel present, details of young at heel recorded as a separate record	SUC_LACY
suckling PY attached	young attached to mothers teat(s)	SUCKLING
suckling, lact, YAH absnt, PY sep rec	suckling and lactating with pouch young present and young at heel absent, details of pouch young recorded as a separate record	SUCP_LAC
undefined pouch activity	cannot determine pouch activity or activity does not fit any standard codes	UNDEFIND
undeveloped	female not yet reproductively mature	UNDEVLPD
developed pouch, no signs of use virginal	developed pouch but no signs of use - virgin mammal	VIRGINAL

ID method (insert in comments)

Identification method	DESCRIPTION	ID CODE
capture marked	For temporary marks or non-unique marks - Marked to identify recaptures for this session only	CPMARKED
individual id marked	Use the animals body to provide the id by physically marking it (i.e. ear notch, digit removal, scale clip)	IDMARKED
microchip implant, tags added	Animal already has a microchip at capture, external tags have been added in this event	IMPL_TAG
microchip implant	No previous unique mark, implanted with microchip in this event	IMPLANT
none	No identification is taking place	NONE
tagged, microchip implanted	Animal already has an external tag at capture a microchip was implanted in this event	TAG_IMPL
tagged (metal/plastic)	Physically affix metal/plastic tag	TAGGED

All fate codes to be listed in comments section of data sheet

Fate released*

Fate	DESCRIPTION	Data sheet code
released	released	RELEASED
minor casualty, released	minor casualty, released	REL_CSLT

Fate — Unexpected death*

Fate	DESCRIPTION	Data sheet code
died during handling procedures	died during handling procedures	DIED_HND
deceased in trap	deceased in trap	DIED_TRP
 euthanised, welfare, sharp blow to head	euthanised for welfare reasons by sharp blow to back of head	EUW_BLOW

Fate — implants radio tags*

Fate	DESCRIPTION	Data sheet code
i-device surgically implanted, released	i-device surgically implanted, released	REL_IDVS
radio tag removed, released	radio tag removed, released	REL_RTXR

Fate — Pouch Young*

Fate	DESCRIPTION	Data sheet code
mother + PY left in bag, failed	mother + PY left in bag, failed	MPY_BAGF
mother + PY left in bag, successful	mother + PY left in bag, successful	MPY_BAGS
PY taped in pouch, released, failed	PY taped in pouch and released, failed	PCH_TAPF
PY taped in pouch, released, successful	PY taped in pouch and released, successful	PCH_TAPS
PY taped + bagged, failed	PY taped + bagged, failed	TP_BAG_F
PY taped + bagged, successful	PY taped + bagged, successful	TP_BAG_S

Fate — translocations*

Fate	DESCRIPTION	Data sheet code
translocated	translocated	TLOC_STD
translocated, after held in captivity	translocated, after held in captivity	TLOC_CAP
translocated, fitted with radio tag	translocated, fitted with radio tag	TLOC_RTX