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Charles Darwin University Animal Ethics Committee

Standard Operating Procedure:

DPAW SOP 19.2021 Tissue sample collection and storage for mammals

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CHARLES DARWIN UNIVERSITY AUSTRALIA

Standard Operating Procedure

TISSUE SAMPLE COLLECTION AND STORAGE FOR MAMMALS

Prepared by: Species and Communities Branch, Science and Conservation, Department of Biodiversity, Conservation and Attractions

Prepared for: Animal Ethics Committee

Version 1.1

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GOVERNMENT OF WESTERN AUSTRALIA

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This document has been reviewed and endorsed by the Department's Animal Ethics Committee

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Contents

1 Purpose

A DNA profile provides a unique and highly discriminative method for animal identification. Animal tissue yields substantial amounts of DNA and is the best biological sampling method for DNA analysis; however, it requires the animals to be captured and is invasive (Berry and Aitken, 2007).

Recent developments in molecular technology add to the effectiveness of less invasive samples (hair, scats and whiskers), which provide a source of DNA that can be used to identify not only species but also individuals providing the potential to improve the accuracy of abundance estimates and determine behavioural parameters (Piggot and Taylor, 2003). However techniques are not usually developed to a stage where they can reliably and cost effectively replace capture and sampling techniques.

Only trained and experienced personnel (or those under direct supervision) should take tissue samples from live animals.

This standard operating procedure (SOP) provides advice on the collection and storage of tissue samples from mammals for genetic studies.

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 taking biological (tissue) samples 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 with a need to take tissue samples. All Department personnel involved in survey and monitoring involving taking tissue samples from mammals 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

Adipose: Adipose tissue is the main reservoir of fat in animals.

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

Sample: a small part of tissue, intended as representative of the whole.

Cetacean: Group of marine mammals encompassing whales, dolphins and porpoises.

DNA (deoxyribonucleic acid): A macromolecule found in all living cells that contains genetic identifying information.

Tissue: an aggregate of similar cells and cell products forming a definite kind of structural material with a specific function, in a multicellular organism.

4 Procedure Outline

One major difficulty of collecting biological samples for genetic purposes is keeping the DNA from degrading and ensuring it is not contaminated. During sample storage, it is vital that the DNA degradation is minimised. This requires that the molecular environment of the DNA be inhospitable to enzymatic activity, which can be achieved by physical or chemical means (Piggot and Taylor, 2003) through chemical preservation or physically through storage and temperature control.

The purpose for collection will determine the storage techniques used.

4.1 Liaise with the laboratory

Prior to collection, it is important to know how the samples will be analysed. Each laboratory/individual may have a different approach and may prefer samples to be collected and stored/preserved in a certain way (Berry and Aitken, 2007). However, as a general approach, preservation in analytical grade ethanol (or EtOH) is the most widely used DNA preservative in most genetic laboratories.

Often the laboratory performing the analysis will provide prepared sampling tubes.

4.2 Cleaning and disinfection

Although sterile techniques are difficult in the field, cleanliness of all tissue sampling techniques is paramount to minimise the potential for infection, whilst simultaneously providing reliable genetic samples (Animal Care and Use Committee, 1998). For further guidance regarding hygiene procedures refer to the Department SOP for *Managing Disease Risk in Wildlife Management*.

All equipment used to obtain the tissue sample should be cleaned and disinfected between each animal and prior to returning the equipment for storage.

4.2.1 Flaming

(a) Dip the equipment to be used for obtaining the sample 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 that only the equipment needing to be flamed comes into contact with the ethanol).

(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) Re-dip the equipment to be used for obtaining the sample in the 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 for at least a minute before using it on an animal.

(d) DO NOT allow contact with any other biological material (including human fingers) before the next animal is sampled.

Flaming is the most common method for cleaning and disinfecting equipment but in fire risk areas it may not be possible or appropriate. Using 70% isopropyl alcohol medical swabs is a suitable alternative or solutions below.

4.2.2 Cleaning and disinfecting solutions

(a) For single step disinfection, equipment can be soaked in a disinfectant 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.

4.3 Collection

Collection of samples should never be done with bare hands as human DNA can interfere with some analysis particularly mammal sex identification (Piggot and Taylor, 2003). It is preferable to use latex or nitrile gloves (especially if analysis will involve sexing).

Only trained and experienced personnel (or those under direct supervision) should take tissue samples from live animals.

Note: Collection from dead animals may also be a viable source of DNA where dried material can be sampled.

4.3.1 Ear notching

The best technique to obtain a good mammal tissue sample is by ear notching, which provides both tissue and hair samples. Ear notching requires animal handling and is achieved by cutting a small piece of an individual's ear using an ear puncher. If possible, ensure that the area to be sampled is free of ecto-parasites (ticks, mites) before proceeding. The following steps have been extracted from the Department SOP for *Permanent Marking of Mammals using Ear Notching*.

(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 1).

(b) 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. Large 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. However small (<4mm) hole punches may be appropriate or necessary to avoid blood vessels.

(c) Take care positioning the ear punch/notcher so that the resulting mark is unambiguous.

Figure 1 A southern brown bandicoot positioned ready for ear notching (left) and after its ear has been notched (right). Photo: Christine Freegard/DBCA

(d) 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.

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

4.3.2 Skin off (cetaceans and dugong)

4.3.2.1 Invasive methods

Biopsy darts

Biopsy darts can be used to remotely obtain skin and blubber samples from dolphins and whales. The most useful samples contain DNA from the blubber/epidermis interface.

Prior to sampling it is recommended to thoroughly clean, sharpen and verify the darts. To do so, darts need to be disassembled, the plastic parts checked for visible cracks and cleaned individually. Latex gloves should be worn during all handling to avoid human DNA contamination. All carbonate parts should be wiped with 70% ethanol. It is important to remove tissue residues, by scrubbing the biopsy heads using a toothbrush and then boiling the heads for 20 minutes in distilled water. Once boiled, darts should be re-sharpened and edges should be smoothed using a diamond file. The biopsy head should be dipped in ethanol and flamed twice prior being stored in a clean plastic container until used again (Krützen *et. al*., 2002).

Darting should be attempted when the individual is travelling at slow to moderate speed parallel to the vessel at a distance of 4-20m at the water surface. Darting is most

successfully accomplished when the individual has reached its highest point during surfacing. The darter should aim about 10cm lateral to the base of the dorsal fin where the blubber is thickest (Krützen *et. al.*, 2002).

The dart should be retrieved immediately after darting, using a hand net; and sample removed using forceps and stored in DMSO buffer or in 80% ethanol. Latex gloves are worn when the biopsy sample is transferred from the dart to the vial for preservation.

The firing mechanism of the dart and cartridges powering the darts should be matched to the size of the cetacean. For example, cross bows are most commonly used for larger cetaceans (whales), for dolphins a modified air rifle (PAXARM) is typically used.

Sampling of cetaceans using this method should discontinue if a prolonged adverse reaction is observed by the targeted individual or group.

Biopsying is instantaneous and usually occurs within the first 15 minutes of approaching the animal. To avoid harassment of animal, darting attempts cease if animals display active avoidance of the research boat. If a dart completely misses the animal, a second attempt can be made, provided the animal shows no evidence of changed behaviour (i.e. no boat avoidance or other reaction) and remains approachable. Attempted darting generally results in mild, short-term 'flinch' or 'buck' reactions, with most cetaceans then continuing their pre-biopsy behaviour and remaining in the vicinity of the research boat (Krützen *et. al*., 2002). The behavioural response of individuals is recorded whether the sampling attempt was successful or unsuccessful. Behavioural responses can be split into five categories, following a protocol modified from Krützen *et. al*. (2002). The response categories can be scored following Bilgmann *et. al.* (2006):

- 0: no noticeable reaction and the individual continues to bow-ride
- 1: a flinch, but the individual continues to bow-ride
- 2: an individual that accelerates under water
- 3: an individual that does a single leap/porpoise
- 4: an individual that does multiple leaps and/or porpoises

Research boats are generally in the vicinity of cetaceans for between 5-60 minutes for the purposes of photo-identification, monitoring behaviour and biopsy sampling.

Note: Dolphin calves should not be targeted using this method (should be ≥1.5m).

Biopsy pole

A biopsy pole is made of extendable sleeves that enable pole length to be adjusted from 1.5-3m. Bow-riding dolphins can be sampled when they are close to the water surface (<1m deep) and occasionally when they surface. The pole is held at an angle of between 60-90° to the water surface and depending on the depth of the dolphin and the speed of the boat, the biopsy pole is dropped or thrown lightly toward the animal, aiming at the body areas lateral to the base of the dorsal fin. The boat should be maintained at a constant speed of 2-6 knots during biopsy pole sampling (Bilgmann *et. al*., 2006).

Skin swabs

Skin swabbing is a non-injurious technique for obtaining DNA samples from cetaceans. Skin swabs can be obtained using the sampling apparatus illustrated in Figure 2.

Wooden dowels of at least 60cm long and 1cm in diameter should be filed at one end to produce a rounded tip. A rim should be fashioned on the sanded tip by cutting a shallow groove around the end of the dowel. It is advised to cover the rounded tip of the dowel with the cut finger of a clean latex glove, in order to prevent wood from water and contamination from repeated use. A new nylon scrub pad (cut into 4x4cm squares) should be attached on top of the latex covering using a plastic cable tie fastener (Wursig *et. al*., 1999).

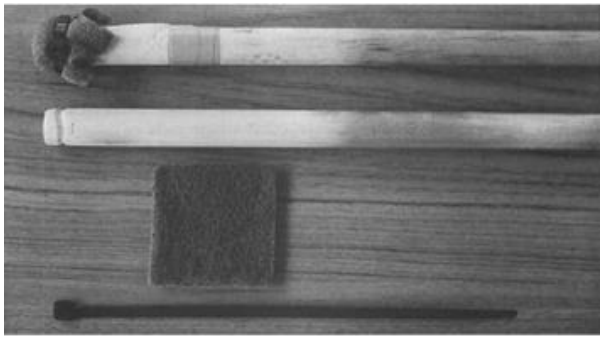

Figure 2 Assembled apparatus and components. Photo: Wursig et. al., 1999

Sample should be collected as target individual approaches the surface of the water by quickly and decidedly making contact between the sterile pad and the skin. A sample is considered successful when visible skin is present on the sampling pad.

The pad should be removed by cutting the plastic cable tie. The sampler should always wear gloves, which should be changed between each sample to avoid cross contamination. Pads can be stored at room temperature in sterile 30ml vials with DMSO buffer (Wursig *et. al.,* 1999) or in 80% ethanol.

Skin scraping

Skin scraping can be obtained from dugongs using a hand-scraper consisting of a stainless steel cylinder (about 25mm diameter, 100mm long and 1.5mm wall thickness) with a single grater-tooth (8mm wide, 8mm long, with 4mm gap height) set centrally into a 45° angled closed end (see Figure 4). The open end of the cylinder should be sealed during operation with a piece of fabric (cloth or tape) held in place with a stout rubber band. It is advised that the scraper be anchored by a wrist-strap attached to the cylinder (Lanyon *et. al*., 2010).

Note: skin scraping is not an accepted method for cetaceans

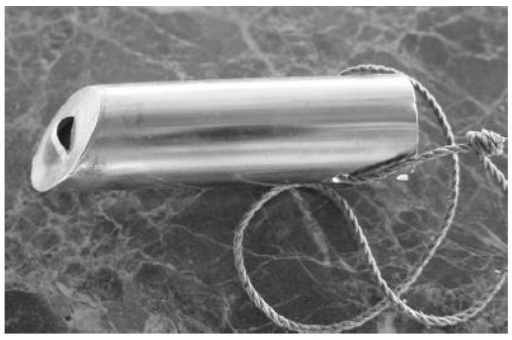

Figure 3 A hand-scraper is a 100mm long stainless steel cylinder with a single grater tooth and wrist strap. Photo: Lanyon et. al. 2010

The cylinder end with the grater tooth should be drawn firmly along the dugong's dorsum to scrape a short strip of epidermis. The sampler should be careful to target an area of the

dorsum that is clear of barnacles or heavy scar tissue. A skin sample of 3-4 mm wide and up to 80mm long should be collected.

Epidermis should be removed from the grater tooth using forceps or by gently agitating the device in a container of clean seawater after removing the fabric end.

The sample should be kept in DMSO buffer or in 80% ethanol.

4.3.2.2 Non-invasive methods

Collection of sloughed skin

For whales, it is possible to collect skin from an individual passively. When individuals are surface-active (tail slap, pectoral fin slap, or breach), they dislodge small pieces of skin, which can be used for genetic analysis. Such skin fragments can be collected from the water column using a net. However, this technique is not suitable for small cetacean species, such as dolphins (Wursig *et. al.*, 1999).

Note: the degradation of the DNA samples sometimes renders sloughed skin unusable and therefore collection of multiple samples is encouraged.

4.3.3 Carcasses

Although DNA is far more robust than proteins, it too will degrade if the tissue is already in an advanced state of decomposition or exposed to acids such as those from the bile duct or the stomach (O'Meally and Livingston, 2002). Therefore, it is advised to collect tissue samples from fresh carcasses. The type of tissue to be collected depends on the type of study it is needed for and the means of preservation and storage available. Tissue types are listed below (see Figure 5) in the order in which they are preferred for genetic work, but also the order in which they tend to decompose in the carcass.

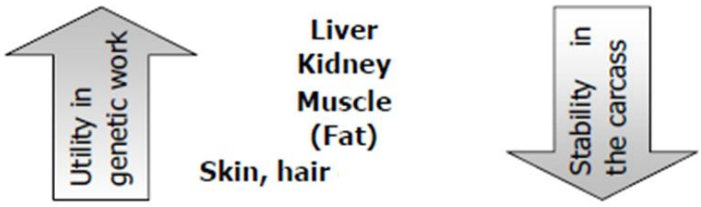

Figure 4 Ordering of tissues for genetic work preference. Image: O'Meally and Livingston, 2002

Liver contains many enzymes that can be used in protein studies and is a good source of DNA. It is best taken if the sample can be frozen. Other tissues are best used for DNA studies if ethanol is the only preservative available.

If possible, avoid sampling from areas of the carcass that have been exposed to the sun for long periods and/or to scavengers like flies, birds and burrowing invertebrates. Direct sunlight damages proteins and DNA, and scavengers can contribute their own proteins and DNA to the tissue, making interpretation of biochemical studies difficult (O'Meally and Livingston, 2002).

When collecting samples from carcasses, it is best to collect duplicate samples, one to retain and one to send for analysis (Berry and Aitken, 2007). Generally only a small amount of tissue is required (~5mm^2) for genetic analysis.

4.4 Storage

The method of storage ultimately depends on the end use of the samples and/or what the laboratory recommends (O'Meally and Livingston, 2002). Samples should be stored so they are not at risk of coming into contact with other samples or tissues from related species (Berry and Aitken 2007) and be well labelled (double tagged) with information on the date of collection, species, location and a unique identifier for the individual and sample (see Section 4.5).

4.4.1 Liquid

The most common method of storing tissue is in liquid. The volume of the preservative should be ten times that of the sample (O'Meally and Livingston, 2002).

Samples should be stored in screw-capped tubes if available. For example: Technoplas 5ml specimen tubes can be ordered from Interpath (http://www.interpath.com.au/). Ensure that the container is completely sealed to avoid leakage. Containers can be filled with a fixing solution prior to going in the field which minimises the amount of liquid needing to be transported. Ethanol is the preferred fixing solution followed by DMSO. Other less commonly used fixatives are also described below:

4.4.1.1 Ethanol

Ethanol preservation works by dehydration where the ethanol displaces water in the cells (O'Meally and Livingston, 2002).

Ethanol (analytical-grade) is the preferred tissue fixative as ethanol-fixed tissues retain excellent cell morphology for histologic examinations and ethanol-fixed tissues can be used for molecular tests, including recovery of DNA for genetic analyses (Green, 2001). Ethanol is used for other field procedures, so it makes sense to carry slightly more ethanol than another chemical.

A 70% ethanol (ethyl alcohol) solution is appropriate if tissues are to be used for histological or morphological analyses, in addition to DNA analysis (O'Meally and Livingston, 2002), or if samples are to be transported by air freight (see Section 4.6). Otherwise 90-100% ethanol solution is the best preservative for long term storage for DNA analysis. 100% ethanol is also preferred where samples of an aquatic nature are concerned as water is likely to compose a significant portion of the sample.

Avoid using ink to record sample data on the container, mark in pencil or otherwise write details on a slip of (waterproof) paper or index card and place in container. This slip of paper should be clean and only handled using fresh sterile gloves, or the same gloves as when used to take the sample. Samples can be stored at room temperature for short periods of time however refrigeration is preferred (Berry and Aitken, 2007).

Upon return tissue should be stored in a -20°C freezer (or -80°C freezer if available) until DNA extraction (Gonser and Collura, 1996).

4.4.1.2 DMSO buffer

DMSO (dimethylsulfoxide) and saturated salt preservation works by osmotic dehydration. The mechanism is two fold: DMSO allows the salt to penetrate the tissue more readily while the salt draws water out of the cell by osmosis (O'Meally and Livingston, 2002).

DMSO acts as a universal solvent, enhancing the absorption of substances through the skin or respiratory passages. It is therefore advised to not be used in conjunction with other hazardous substances, such as formalin or ethanol. DMSO may limit the application of some DNA techniques, for instance, it cannot be used for protein work.

DMSO may be preferable to be used where samples are to be transported by air (as ethanol is considered hazardous goods; see Section 4.6).

20% DMSO buffer can be made up according to the following instructions (Seutin *et. al.,* 1991):

- Add about 20ml DMSO to 60ml distilled water
- 0.25M sodium-EDTA
- NaCl to saturation (about 25g of salt at 20-25°C)
- Leave a thin layer of undissolved salt in the stock solution to compensate for changes in solubility due to temperature (and it ensures the solution is saturated with NaCl).
- The final volume should be approximately 100ml.

4.4.1.3 Tissue lysis buffer

Lysis buffer is good tissue storage medium when the samples are collected for DNA analysis; lysis buffer is, however, not a suitable storage medium if the sample is collected for protein or RNA analysis (Longmire *et. al*., 1997). Lysis buffer allows greater yields of high molecular weight DNA to be obtained in comparison to other methods (Longmire *et. al.*, 1997).

The solution can be made up according to the following instructions (Longmire *et. al.,* 1997):

- 50ml of 2M Tris-HCl, pH8
- 200ml of 0.5M EDTA, pH8
- 2ml 0f 5M NaCl
- Bring to 1L with distilled water
- 25ml of 20% SDS (w/v)

4.4.1.4 Tissue preservation buffer solution

Tissue preservation buffer solution is recommended for field storage of tissue (Talbot, n.d.).

The solution can be made up according to the following instructions (Talbot, n.d.):

- 240g of 4M urea
- 11.5g of 0.2M NaCl
- 5g of 0.5% N-Lauroyl-Sarcosine
- 3.72g of 100mM Tris-HCl pH8
- Bring to 1L with distilled water

Most of these chemicals may cause irritation to skin and mucous membranes on contact. Ensure you wear appropriate PPE (personal protective equipment) (such as air mask, gloves and eye protection). Wash contacted area with plenty of water and contact physician if irritation persists. If ingested, drink copious amounts of water and call a physician.

4.4.2 Freezing

Freezing at -20°C or in liquid nitrogen preserves the integrity of DNA. However, it may not be practical in field situations as it requires quick access to a freezer once the sample has been taken. Laboratory requirements should decide the maximum time allowed from when

a sample is taken to being frozen. Samples must also remain frozen until they reach the laboratory as repeated thawing and freezing will degrade DNA (Berry and Aitken, 2007). However, once in the lab, freezing, particularly at -80°C, is recommended for long-term storage of tissue samples.

4.5 Labelling and recording data[AG1]

Labelling is of the utmost importance when taking biological samples for genetic analysis. It is important to ensure that handwriting is legible (O'Meally and Livingston, 2002).

All individual samples MUST be labelled with the following as a bare minimum:

- Date
- Species/possible species
- Location (GPS reading is preferable)
- Individual ID (to be linked to additional metadata)
- Collector's initials

Other information (metadata) can be added to the label or provided in a spreadsheet referencing the individual ID:

- Collector's name
- Sex of animal
- Other observations (age, weight, size, breeding status, etc)
- Translocation source/destination

It is advised to not write on greasy, dirty or wet tubes. Permanent markers can rub off when they come into contact with ethanol; therefore, as a precaution it is advised to insert a waterproof label written with pencil inside. If possible, label should be prepared before collecting the sample. Also inform the laboratory what liquid was used to store the sample (e.g. 100% ethanol, DMSO).

4.6 Transport

Samples stored in DMSO buffer, tissue lysis buffer, tissue preservation buffer solution and in less than 70% ethanol or less than 50ml of total volume of alcohol can be transported via Parcel Post (O'Meally and Livingston, 2002), providing that they are adequately sealed with parafilm wrapped around the cap and with sufficient absorbent material (e.g. cotton wool) to contain the liquid if spilt. Tissue samples stored in >70% ethanol can be transferred to DMSO or other liquid preservative for transport if required. In particular cases this may not however be appropriate. If so, samples stored in more than 70% ethanol or more than 50ml of total volume of alcohol

5 Level of Impact

There is a varied level of impact involved in taking tissue samples for genetic purposes. Invasive procedures have more impact as it involves the removal of tissue. Potential animal welfare impacts when taking a tissue sample include:

- Distress caused by handling, discomfort, social isolation, separation of mother and young

- Trauma from possible injury to the animal during restraint (e.g. scratching and/or biting itself)
- Pain and bleeding
- Infection at site of tissue sample

It should be noted that whilst these impacts are specifically associated with the procedure of taking tissue samples for genetic identification, an animal may also experience other impacts from associated procedures such as trapping and capture.

6 Ethical Considerations

To reduce the level of impact of tissue sampling procedures on the welfare of animals there are a number of ethical considerations that should be addressed throughout projects involving these procedures. Department projects involving the use of tissue sampling for genetic purposes 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 collection and storage of tissue samples, other ethical considerations need to be taken into account during procedures carried out prior, such as trapping and capture.

6.1 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 sampling equipment should be kept extremely sharp and clean to minimise tearing, bruising, infection and transmission of disease.

6.2 Bleeding

Invasive tissue sampling can result in excessive bleeding which, should it occur, needs to be controlled prior to the animal being released. Refer to the Department SOP for *First Aid for Animals* for further guidance.

6.3 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.

6.4 Spread of disease or parasites

Personnel must be aware of the possibilities of transferring disease or parasites from animal to animal as well as from one location to another if handling animals at multiple sites.

Good hygiene practices should be maintained to reduce the risk of spreading pathogens between animals and sites. Refer to the Department SOP for *Managing Disease Risk in Wildlife Management* for further advice.

7 Competencies and Approvals

Department personnel, and other external parties covered by the Department's Animal Ethics Committee, undertaking projects that involve taking tissue samples for genetic purposes 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 tissue sampling on the welfare of the animals. Other groups, organisations or individuals using this SOP to guide their survey and 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 taking tissue sampling for genetic identification

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 study species.	Personnel should be confident in handling those species having biopsies taken. This experience is best obtained under supervision of more experienced personnel. Estimated total time in field: Min 2+ years involved in similar projects with study species (or similar).
Blood, DNA and surgical skills/experience	Experience in collecting tissue for DNA analysis	Personnel should be familiar with how to operate equipment. This experience is best obtained under supervision of more experienced personnel. Estimated total time in field: Min 2+ years involved in similar projects with study species (or similar).

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 tissue sampling. 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.

To improve safety, field personnel should be aware of the treatment for snakebite and carry appropriate pressure bandages. Personnel should also have up-to-date tetanus vaccinations. Department personnel must not capture bats unless fully vaccinated against Australian Bat Lyssavirus.

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, 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 the *Material Safety Data Sheets* (MSDS) relevant to the chemical(s) they may be using (e.g. Alconox, methylated spirits, ethanol etc.). Many of the chemicals described in the procedures above may cause irritation to skin and mucous membranes on contact.

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.

9 Further Reading

The following SOPs have been mentioned in this advice and it is recommended that they are consulted when proposing to undertake tissue sample collection for mammals.

- Department SOP *Animal Handling and Restraint using Soft Containment*
- Department SOP *Hand Restraint of Wildlife*
- Department SOP *Permanent Marking of Mammals using Ear Notching*
- Department SOP *Permanent Marking of Reptiles by Scale Marking*
- Department SOP *First Aid for Animals*
- Department SOP *Managing Disease Risk in Wildlife Management*
- Department SOP *Humane Killing of Animals under Field Conditions*

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