

Standard Operating Procedure

BIOPSY TISSUE SAMPLE COLLECTION FOR CROCODILES

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Prepared for: Animal Ethics Committee

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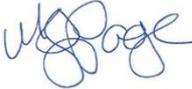
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This document has been reviewed and endorsed by the Department's Animal Ethics Committee

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1 Purpose

Department of Biodiversity, Conservation and Attractions (hereafter, Department) personnel are at times required to safely and effectively manage and handle freshwater and/or estuarine crocodiles.

This standard operating procedure (SOP) provides advice on the safe and humane treatment of free-ranging crocodiles while taking tissue samples via pole biopsy for research purposes. It describes the use of a large-bore needle mounted on an extendable pole (herein known as a biopsy pole) to collect tissue samples from crocodiles. While the instrument and its use are detailed within this document it is not intended to be a stand-alone reference for crocodile work. Appropriate and adequate training for all staff involved in crocodile work is essential.

The collection of tissue or biopsy samples from free-ranging crocodiles is usually achieved through removal of a small section of scute, cut from the tail of a captured animal prior to release or removal. Having to capture animals is time consuming and dangerous, requiring personnel to have extensive crocodile handling skills. This combination of factors makes it difficult to readily acquire desirable sample sizes (>30 individuals sampled per location) necessary for genetics analyses of crocodile populations.

Development of the biopsy pole has removed the onus on having to capture crocodiles to obtain a tissue sample. This reduces the time necessary for collecting a sample, and more importantly, minimises the time that a person has to spend within very close proximity to the crocodile being sampled.

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 when biopsy sampling of free-ranging crocodiles within tidal estuaries and other water ways across the State is required for research purposes by Department personnel. It may also be used to guide fauna monitoring and survey activities undertaken by Natural Resource Management groups, consultants, researchers and any other individuals or organisations. All Department personnel involved in collecting biopsy tissue samples of crocodiles 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.

Biopsy pole: A large-bore needle mounted on an extendable pole.

4 Approved Methods

4.1 Pole design

The biopsy pole can be made from a variety of materials but stainless steel is recommended for its resistance to corrosion in a marine environment (see Figure 1). Length of the pole should be 2.5-3.5m and, if possible, made up of 2 or 3 separate pieces that can be joined to enable total length to be adjustable.



Figure 1 A biopsy pole (top left), a protective mounting needle (bottom left) and needles (right).

Needles must be sourced from a company specialising in darts and dart delivery systems for the wildlife industry (e.g. <https://daninjectdartguns.com/>) (see Figure 1). Two systems are currently available depending on funds and preference. The more advanced system uses a heavy duty spring to control the needle action and automatically extract the tissue sample. The simpler system has a fixed needle connection with no spring, with the user controlling the effort required to take the biopsy sample. The tissue sample must then be manually extracted from the biopsy needle. The simpler version has been found to be robust for extensive biopsy sampling of crocodiles.

4.2 Technique

This method requires a minimum of three people; a sampler who takes the biopsy, a spotter who manages the spotlight, and a boat operator.

Prior to finding a crocodile for biopsy, an unused sterile biopsy needle should be affixed to the pole. This involves twisting the needle onto the syringe and inserting this into its protective cage (see Figure 1) which is screwed onto the shaft of the pole.

Once an eye-shine has been sighted, indicating a suitable crocodile (i.e. length >1.5m):

1. Boat speed should be gradually reduced to minimise any bow wave or sudden change in engine noise. This reduces the probability of the crocodile fleeing or submerging.
2. The boat should be positioned in the middle of the river until it is adjacent to the observed eye-shine, at which time a 90° turn is made directly towards the eye-shine, paying due regard to any potential obstacles.
3. The sampler should position themselves at the bow with biopsy pole at the ready, paying close attention to any signals from the spotter. Care should be taken not to move the head of the pole in front of the spotlight beam until the very last second prior to the sample being taken, or else the animal may take flight and disappear.
4. Once within striking range the pole is then jabbed firmly (but with a limited travel range or else the needle may get bent/damaged) into the meaty upper section of the tail (see Figure 2). Once the sampler has captured a biopsy sample they will indicate to the driver to head back out to midstream for the sample to be processed.



Figure 2 Tail scutes (left) are most commonly sampled for crocodile DNA, and the biopsy pole (right) enables a tissue sample to be taken from any of the well-muscled areas on the animal whilst also maintaining a safe distance.

5. The boat should not remain close to the bank in moving from one crocodile to the next as they are more easily disturbed this way. Crocodiles are less likely to move off if approached at right angles from midstream.
6. The sample is pushed from the biopsy needle with a small length of sterile stainless steel rod into a sample vial containing 70% ethanol and an appropriate identification label (see Figure 3).



Figure 3 A 7mm crocodile biopsy sample inside specimen vial. Vial contains 70% ethanol and identification label.

7. The used needle is placed into a separate container for 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 (See the Department SOP for *Tissue Sample Collection and Storage for Mammals* for further detail and other disinfection options).
8. Needles should be replaced if they are bent at the insertion point. The frequency of which needles will need to be replaced will depend on the accuracy of the sampler to avoid hitting hard scutes which cause the needles to bend. If hard scutes are avoided a needle can last more than 10 uses.

5 Level of Impact

Standard biopsy needles are 3mm in diameter and 25mm in length. They retrieve an average sized core sample of 7mm long by 2mm thick. The sample should, where possible, always be taken from the thickest section of the tail. Minimum length of crocodiles for sampling with this method is 1.3m as animals smaller than this can be captured easily by hand with minimal risks. While the needle does leave a minor wound, crocodiles are often involved in fighting with other individuals, resulting in much more significant wounds than the biopsy needle inflicts. Despite this the natural mortality rates of adult crocodiles are very low (Grigg and Kirshner, 2015).

Spotlighting does appear to affect crocodiles and other wildlife, but only for a short duration. It is important that if other wildlife are accidentally spotted the light must be removed from them as quickly as possible to minimise unnecessary disturbance. It is recommended that once a crocodile's eye-shine has been sighted, that the light beam be turned down on approach or held just below or above the reflecting eye to minimise impact.

Where there is a chance that live-aboard vessels or other commercial operations are taking place on the same stretch of water to be surveyed, it is recommended that efforts are made to contact or approach these vessels prior to conducting the survey to explain the operation, and if necessary how to work in with commercial operators such as barramundi netters, crabbers, pearlers, etc.

6 Ethical Considerations

To reduce the level of impact of hand capture of wildlife on the welfare of animals there are a number of ethical considerations that should be addressed. Department projects involving biopsy of crocodiles will require approval from the Department's Animal Ethics Committee.

- Minimise the amount of disturbance caused by shining a spotlight on a crocodile.
- Extract only a single sample from each crocodile.
- Only extract samples from crocodiles greater than 1.3m in length.
- Only extract samples from the thickest section of the tail.

6.1 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 crocodile biopsy sampling 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 biopsy sampling on the welfare of the crocodiles, and reduce the risk of injury and damage to people and property. 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 spotlighting and biopsy sampling of crocodiles

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 and licensing requirements for different roles	Spotter/Observer: Prior training	Have observed and assisted for at least three nights of spotlighting and sampling under the direction of an experienced supervisor, until the supervisor is satisfied that a required level of expertise has been achieved.
	Boat Operator: Coxswain 2 Near Coastal (NC) Certificate	Department of Transport approved assessment. Note there may be limitations to the area of operation for various qualifications.
	Biopsy Sampler: Trainee prior training	Have observed and assisted for at least three nights of sampling under the direction of an experienced

Competency category	Competency requirement	Competency assessment
		supervisor, until the supervisor is satisfied that a required level of expertise has been achieved.
	Marksman (at least one of the other roles also requires this competency): suitable training, authorisation and documentation for use of firearm	Written verification of completion of nationally recognised training, copy of a nominated person's authorisation from WA Police, and listed on Department's Corporate Firearms Licence.

8 Occupational Health and Safety

Always carry a first aid kit, satellite phone and VHF radio on your vessel and be aware of your own safety and the safety of others as well as the animals when conducting crocodile interactive work

A job safety analysis is recommended prior to undertaking a crocodile interactive work. This safety analysis should include the following considerations.

8.1 Animal bites, stings and scratches

Extreme care should be taken when working in tidal estuaries to avoid exposure to disease carrying insects, like mosquitos and sand-flies. All inflicted injuries (even superficial ones) should be appropriately treated as soon as possible to ameliorate possible allergic reaction, prevent infection and promote healing.

Not all crocodiles display the same behavioural traits. Each animal must be assessed prior to encroaching on its personal space when attempting to extract a biopsy sample. If a crocodile is showing aggressive behaviour on approach, the biopsy team should avoid any further interaction with the crocodile and move on to another animal. It is recommended that an experienced crocodile handler is always present to assess the level of risk for each sample attempt.

In the case of a bite from a crocodile, a spike hammer or suitable firearm (308 or shotgun), must be readily available to swiftly euthanise the crocodile by trauma to the brain - to prevent further injury to the handler. Particular caution must be taken when using a firearm near people and boats.

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 Personal Protective Equipment

Safety gear should be worn when required. Such equipment may include leather/latex gloves, goggles/face shields and safety clothing (long sleeved shirt, pants and boat shoes). This shall be determined by the project specific job safety analysis.

8.4 Precautions when working near/on water

Take care to avoid slipping into the water from the vessel, river banks and boat ramps. Life jackets must be worn by those working on vessels. Refer to the Department's boating policy.

9 Further Reading

It is recommended that the following Department documents are consulted when proposing to conduct crocodile interactive work:

- Department SOP *Managing Disease Risk in Wildlife Management*
- Department SOP *Tissue Sample Collection and Storage for Mammals*
- Department SOP *Humane Killing of Animals under Field Conditions*
- Department Corporate Policy Statement *Boating*

For further advice refer to:

Fukuda, Y., Saalfield, K., Webb, G., Manolis, C. and Risk, R. (2013). Standardised method of spotlight surveys for crocodiles in the tidal rivers of the Northern Territory, Australia. *Northern Territory Naturalist* 24: 14-32.

10 References

Grigg, G. and Kirshner, D. (2015) *Biology and Evolution of Crocodylians*. Australia: CSIRO Publishing.

NHMRC (2004). *Australian code of practice for the care and use of animals for scientific purposes* (7th ed.). Canberra: National Health and Medical Research Council.