Standard Operating Procedure

SC22-24 BIOPSY TISSUE SAMPLE COLLECTION FOR CROCODILES

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

GOVERNMENT OF WESTERN AUSTRALIA

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Approved by the DBCA Animal Ethics Committee:

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1 Acknowledgements

This standard operating procedure was originally developed by Daniel Barrow and Andrew Halford.

2 Purpose

Department of Biodiversity, Conservation and Attractions (DBCA, 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.

3 Scope

This SOP has been written specifically for scientific and education purposes and endorsed by the 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 taking biological (tissue) samples undertaken across Western Australia by 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 tissue sample collection from crocodiles 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 biopsy on the welfare of crocodiles, 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 tissue sampling, 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 tissue sampling for genetic purposes will require approval from the department's AEC. The key animal welfare considerations that should be considered when performing tissue biopsies on crocodiles 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 Biodiversity in Wildlife Management* for further guidance.

4.2 Level of impact

Standard biopsy needles are 3 mm in diameter and 25 mm in length. They retrieve an average sized core sample of 7 mm long by 2 mm thick. Minimum length of crocodiles for sampling with standard biopsy is 1.3 m. The modified biopsy needles are 1.5 mm x 8 mm, retrieve an average sized core sample of 5 mm by 2 mm and suitable for sampling crocodiles >0.9 m (Table 1). The sample should, where possible, always be taken from the thickest section of the tail. Animals smaller than this, should be captured easily by hand with minimal risks to avoid potential for injury with the pole (Barrow & Halford, 2019). 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 (Fukuda et al., 2013).

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.

5 Approved Methods

5.1 Biopsy Pole

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. Length of the pole should be 2.5-3.5 m and, if possible, made up of 2 or 3 separate pieces that can be joined to enable total length to be adjustable (Figure 1A and 1B)

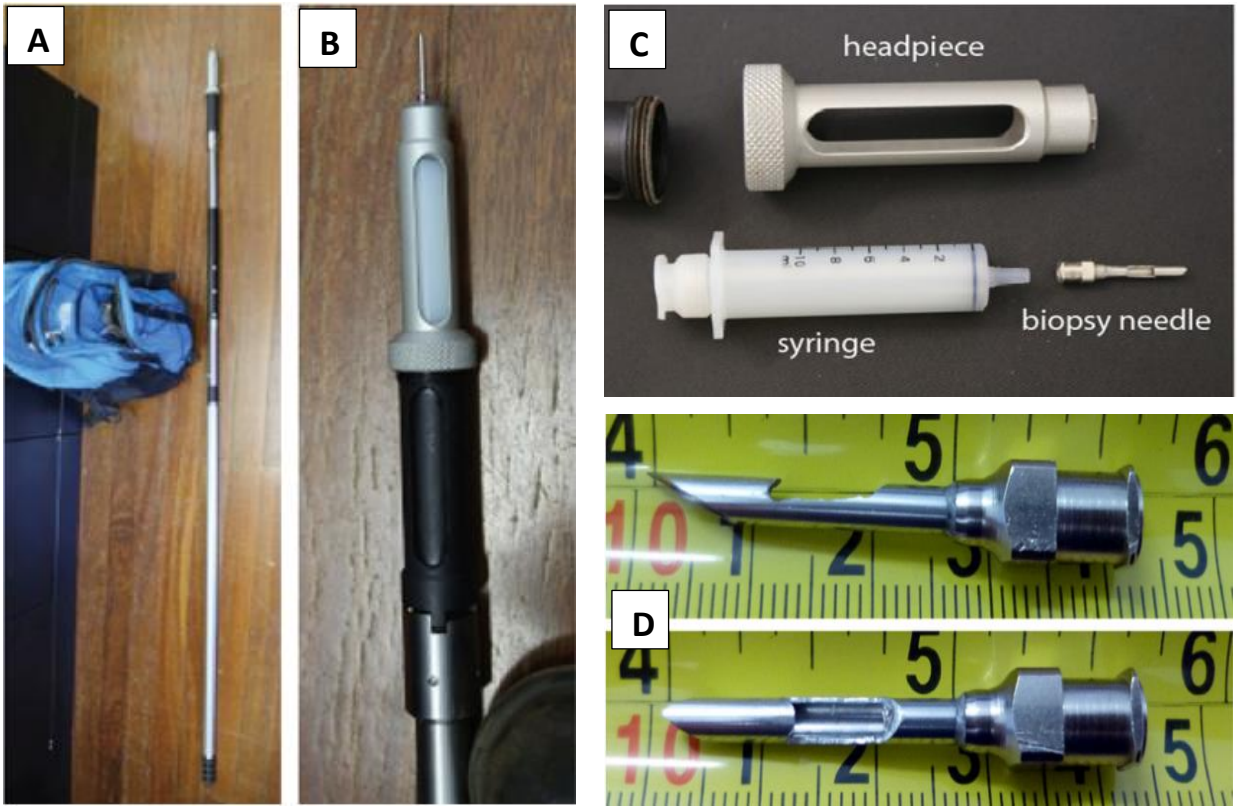

Figure 1 A) The biopsy pole. B) Biopsy pole with the delivery system attached. C) the biopsy delivery system, consists of a protective headpiece for attachment to the pole, syringe, and biopsy needle. D) A modified standard 3mm x 25mm biopsy needle. Photo: Daniel Barrow (DBCA).

5.2 Biopsy Needles

Needles must be sourced from a company specialising in darts and dart delivery systems for the wildlife industry (e.g., https://daninjectdartguns.com/). 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 (Figure 1B). The tissue sample must then be manually extracted from the biopsy needle (Figure 1C, 1D). The simpler version has been found to be robust for extensive biopsy sampling of crocodiles.

a) Standard Needle

The standard biopsy needle for sampling crocodiles >1.3 m (Table 1), consists of a 3 mm in diameter and 25 mm in length needle, and retrieves an average sized core sample of 7 mm long by 2 mm thick.

b) Modified Needle

The modified biopsy needle by Barrow & Halford (2019), for sampling crocodiles >0.9 m (Table 1), consists of an 8 mm-long × 1.5-mm cut deep section from the middle of the standard needle shaft, with the section ends cut at a 45° angle in the direction of the needle base to create a barb near the needle tip (Figure 2D). The modified needle retrieves an average sized core sample of 5 mm by 2 mm is ultimately retained when the needle is removed from the crocodile.

Table 1 Appropriated technique and equipment to collect tissue sample according to the length of crocodile

Length of crocodile	Equipment and technique
<0.9 m	Manual restraint
0.9 - 1.3 m	Modified needle affixed to the pole
>1.3 m	Standard needle affixed to the pole

5.3 Technique

This method requires a minimum of three people; a sampler who takes the biopsy, a spotter who manages the spotlight (Fukuda et al., 2013), and a boat operator.

ANIMAL WELFARE: Minimise the amount of disturbance caused by shining a spotlight on a crocodile.

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 which is screwed onto the shaft of the pole.

Once an eye-shine has been sighted, indicating a suitable crocodile (i.e. length >0.9 m):

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.

ANIMAL WELFARE: Extract only a single sample from each crocodile.

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 (Figure 2A and B). 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.

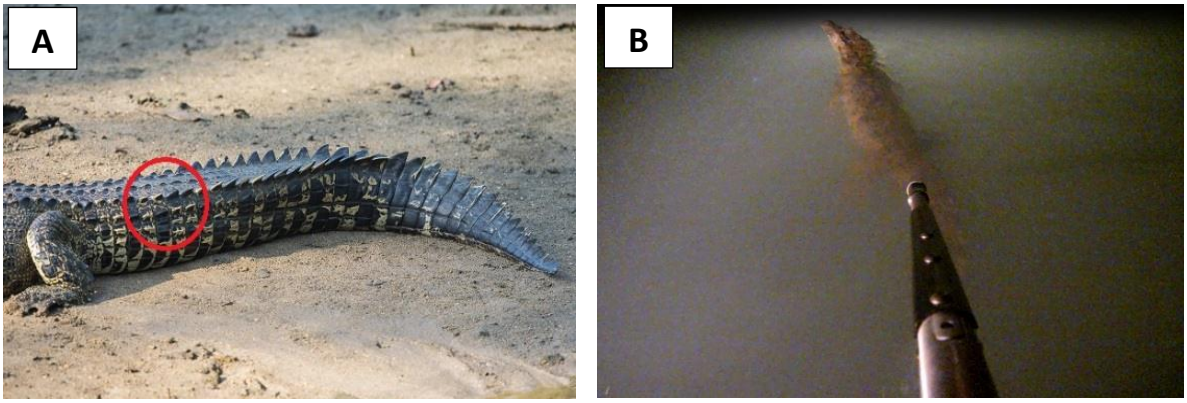

Figure 2 A) Tail scutes are most commonly sampled for crocodile DNA. B) the biopsy pole enables a tissue sample to be taken from any of the well-muscled areas on the animal whilst also maintaing a safe distance. Photo: Daniel Barrow (DBCA).

ANIMAL WELFARE: Only extract samples from the thickest section of the tail.

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 90-100% ethanol solution, and an appropriate identification label (Figure 3).
7. 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 biopsy tissue sample for DNA analysis.
8. 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 sterilisation options).

Figure 3 A 7 mm crocodile biopsy sample inside specimen vial. Vial contains 90% ethanol and identification label. Photo: Daniel Barrow (DBCA).

9. 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.

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

6 Competencies

A person who is competent has the knowledge, skills, and experiences that allow them to work with 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 2). 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, as well as to reduce the risk of injury and damage to people and property. Other groups, organisations or individuals using this SOP to guide their tissue sampling 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 2 provides advice for standard monitoring only.

Table 2 Competency requirements for Animal Handlers of projects involving spotlighting and biopsy sampling of crocodiles

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 or experience). 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.
Skills/experience required	Experience spotlighting for crocodiles	Personnel should be experienced at spotlighting crocodiles from a boat. This experience is best obtained under supervision of more experienced personnel. In applications, provide details on the longevity, frequency & recency of experience.
	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.
	Experience in collecting tissue from crocodiles via biopsy for DNA analysis	Personnel should be familiar with the animal welfare principles of tissue biopsy for DNA analysis. Personnel should be familiar with how to operate tissue sampling 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 tissue sampling technique and animal welfare requirements, a guide to the experience and skill

requirements for an animal handler to be considered competent to collect and store crocodile tissue samples are as follows:

- Recency of crocodile tissue collection and storage experience: within the past 10 years.
- Minimum 2 individuals of similar species sampled.

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

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.

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

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.

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

- SOP Managing Disease and Biosecurity Risk 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 *Managing Disease Risk and Biosecurity in Wildlife Management*
- Department SOP *Tissue Sample Collection and Storage for Mammals*
- Department SOP *Euthanasia of Animals Under Field Conditions*
- Department Corporate Policy Statement: *Boating*

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

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.

Barrow, D., & Halford, A. R. (2019). A modified biopsy needle with pole for repeatable tissue extraction from free-ranging crocodiles. *Wildlife Society Bulletin*, 43(2), 308-312.

Gonser, R.A. and Collua, R.V. (1996). Waste not, want not: toe clips as a source of DNA. *Journal of Herpetology* 30 (3):445-447.

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

11 Glossary of Terms

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.

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