Standard Operating Procedure

SC25-08 SAMPLING CETACEANS USING A REMOTE BIOPSY SYSTEM (AUGUST 2025)

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 1.1

August 2025

GOVERNMENT OF WESTERN AUSTRALIA

Department of **Biodiversity, Conservation and Attractions**

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August 2025

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The recommended reference for this publication is:
Department of Biodiversity, Conservation and Attractions (2025). Standard Operating Procedure *SC25-08 Sampling Cetaceans Using a Remote Biopsy System*, Department of Biodiversity, Conservation and Attractions, Western Australia.

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Revision History

Version	Date	Details	Author/Reviewer	Approved By	Approval
1.0	24/03/2022	Document created	Dr Holly Raudino, Ellen D'Cruz & Dr Kelly Waples		
1.0	31/05/2022	Minor revision	L. Povh	M. Dziminski	August 2022
1.1	26/06/2025	Revision of content, clarification of procedures, addition of tethered biopsy darts.	M. Sheehan, A. Raycraft, Dr Holly Raudino, & Dr Kelly Waples	J. Richards	August 2025

Approvals: Version 1.1

Approved by the DBCA Animal Ethics Committee: 

Dr Jacqui Richards
Chairperson, Animal Ethics Committee
Department of Biodiversity, Conservation and Attractions

Contents

1 Acknowledgements

Advice for compiling the content of this standard operating procedure has been generously provided over the years by Delphine Chabanne, Anna Kopps, Alex Brown, Simon Allen, Daniel Barrow, Steven Busby, Jon Pridham, Glen Sutton, Tim Button, Jonnie Cobby and Paul Dyer. The procedures have been trialled by DBCA staff across the Pilbara, Kimberley, South West and South Coast Regions, including participation of joint managers and traditional owners. We would like to acknowledge the Traditional Owner's past, present and emerging of the land and sea country we work on, Noongar, Dambimangari, Murujuga, Yawuru, and it is an honour to work with these groups on their sea country. Thanks to DBCA staff and vessel crew throughout the Kimberley and Pilbara regions that have contributed to successful and safe surveys and sample collection over the years.

2 Purpose

Tissue samples collected from cetaceans can be used to investigate biological and ecological questions about individuals and populations. DNA can be extracted from these samples and used to identify species, phylogeography, gene flow and population structure and abundance through genetic analyses. Food web analyses and dietary studies can also be undertaken using stable isotope and fatty acid signature analyses and environmental and anthropogenic pollutants can be explored with contaminant/biomarker analyses. Collecting tissue samples from live, free-ranging animals can be invasive and often requires the animal to be captured. However, there are methods available that allow for remote sample collection.

This Standard Operating Procedure (SOP) describes a remote sampling technique used to collect tissue from free-ranging cetaceans using a biopsy rifle and dart or biopsy pole. While the equipment and its use are detailed within this document, it is not intended to be a stand-alone reference for cetacean work and would typically take place during other field work such as vessel-based cetacean surveys (*SOP Vessel-based Cetacean Surveys Using Photo Identification*).

Much of this information is derived from techniques that are commonly used for this type of research and on the experience of research scientists in the Department of Biodiversity, Conservation and Attractions (DBCA) Marine Science Program supplemented by work of regional DBCA staff, Murdoch University and Dr Alex Brown.

3 Scope

This SOP has been written specifically for scientific and education purposes and is approved by DBCA's Animal Ethics Committee (AEC). However, this SOP may also be appropriate for other situations.

This SOP applies to all biopsy sampling of cetaceans using a remote (pole or rifle) system 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 remote biopsy sampling of cetaceans should be familiar with the content of this document.

This SOP complements the *Australian code of practice for the care and use of animals for scientific purposes* (The Code). The Code provides the ethical framework and governing principles to guide decisions and actions of all those involved in the care and use of animals for scientific purposes 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 (https://www.nhmrc.gov.au/about-us/publications/australian-code-care-and-use-animals-scientific-purposes).

4 Animal Welfare Considerations

To reduce the level of impact 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 activities and contingencies for managing welfare issues that have been identified. All personnel involved in the project should be aware of the range of issues that they may encounter, the options that are available to reduce impacts and improve animal welfare, and the process to manage adverse events.

Department projects involving cetacean biopsy sampling require approval from the department's AEC. The key animal welfare considerations that should be considered when undertaking remote biopsy sampling of cetaceans are detailed below and highlighted throughout the document.

4.1 Injury and unexpected deaths

If adverse events including injury, unexpected deaths or unplanned requirement for euthanasia occur, it is essential to consider the possible causes and take action to prevent further issues. Adhering to the guidance in this SOP will assist to minimise the likelihood of adverse events. For projects approved by the department's AEC, adverse events must be reported in writing to the AEC Executive Officer as soon as possible after the event by completing an *Adverse Event Form*. Guidance on first aid and field euthanasia are described in the department SOPs for *First Aid for Animals*, *Euthanasia of Animals Under Field Conditions* and *Euthanasia of Small Stranded Cetaceans*. Where infectious disease is suspected, refer to the department SOP for *Managing Disease Risk and Biosecurity in Wildlife Management* for further guidance.

4.2 Level of Impact

4.2.1 Environmental

There are three main ways in which an animal may be impacted by the biopsy procedure: they may be disturbed via reaction to the actual sampling, by the close approach of the vessel, or they may be harmed by the wound created by the dart or subsequent infection.

Exposure to vessels and the associated motor noise can disturb cetaceans and disrupt critical activities such as feeding, resting, nursing and attending to calves. Vessels should be handled in a way that minimises disturbance to the targeted fauna. This may vary based on the target species as some species are more wary and reactive around vessels in close proximity than others. It is essential to have an experienced vessel master who is familiar with the species and responsive to experienced researcher advice. Minimum approach distances are prescribed under the Biodiversity Conservation Regulations 2018, however these may be amended under scientific licence conditions (see Section 8 for further information on licenses and approvals). Additional conditions may also include time limits. As standard, a research vessel will spend no more than 60 minutes within 100 m of a cetacean group, regardless of whether all group members have been photographed and/or biopsied. All time limits and approach distances prescribed in licence conditions or under legislation must be complied with. Other considerations may be around the presence of young, for example newborn calves or sampling undertaken in critical areas or at critical times such as calving and nursing. The duration of encounters should be further limited if groups contain newborns or are resting or nursing young calves, to prevent disruption of critical activities, or if individuals display evasive behaviour or appear to be disturbed.

Response to the procedure must be monitored. Darting reactions are generally mild and short-term ('flinch' or 'buck'), and most animals continue their pre-biopsy behaviours immediately post-biopsy (Krützen et al., 2002). An extreme aversive reaction would be a leap or rapid movement away from the vessel. There has only ever been one mortality from a remote biopsy attempt documented in the literature and this was thought to potentially be the result of vertebral trauma due to thin blubber layer ~7 mm (poor body condition) and/or stress (Bearzi, 2000). In the event of an unexpected death, the carcass must be retained for necropsy and the AEC notified as soon as possible, but no longer than five days of returning to the office, as per standard Adverse Event reporting.

The biopsy 'procedure' is instantaneous and ideally occurs within the first 15 minutes of encountering the group. Sampling should be ceased if any animals show active avoidance of the vessel. Further, no more than three attempts will be made per individual. In the unusual instance that a biopsy dart sticks and does not immediately bounce off, the individual should be monitored from a distance until the dart floats free.

Biopsy darts are designed to remove a small piece of skin and blubber, typically 5mm diameter by 1-2cm depth. The size of the cutting head of the biopsy dart will be selected to suit the species i.e., the dart size used for dolphins is smaller than that used for whales. The biopsy dart leaves a very small superficial wound that should not penetrate the muscle layer. Darting wounds typically heal rapidly and without complication (Tezanos-Pinto & Baker 2012; Weller et al., 1997). Photographs collected during sampling events should be used to identify the individuals sampled and where possible, to confirm the sampling site on the animal at the time of sampling. These can then be used to ensure normal healing of the biopsy wound is occurring if the animal is re-sighted days, months and years after the procedure.

There are different stages to the biopsy wound healing process that can be monitored if repeated photographs are available. In the first stage the wound becomes covered and smooth, which generally occurs within weeks to up to three months (as a maximum time frame) (Weller et al., 1997). For example, Krutzen et al. (2002) found the first stage of healing took 23 days in individual bottlenose dolphins observed daily post-biopsy. Re-pigmentation (the second stage) takes longer; months and in some cases years in other delphinids such as pilot whales (Gimenez et al., 2011). Another study measured ten individuals from three days to seven months post biopsy and healing appeared to occur without complication; no bleeding, swelling or signs of infection were observed (Tezanos-Pinto & Baker 2012). Based on the collective literature it is expected that healing will vary between individuals but will range between two weeks and three months for the epidermis to cover the small wound created by the biopsy dart. The wound left from darting is superficial compared to large deep wounds from shark encounters that also heal relatively quickly (Corkeron et al., 1987; Orams & Deakin, 1997).

4.2.2 Social

There may be live-aboard vessels or other commercial operations occurring within the survey area. It is recommended that effort is made to contact or approach these vessels prior to conducting the survey to explain the operation, and if required, determine how to work around any commercial operations such as barramundi netting, crabbing, pearling, or tourism interactions. If commercial or recreational vessels enter the survey area, particularly if biopsy sampling is occurring, they should be contacted via radio to explain the on-going operations and instructed not to approach cetacean groups closer than 300 m while DBCA vessels are

photographing, biopsy sampling and observing. Biopsy sampling should cease when any other vessel is within 300m to avoid the risk of collision between vessels and to reduce the disturbance to targeted individuals.

Investigators must be aware that the effects of a series of stressors may be cumulative.

5 Approved methods and equipment

5.1 Biopsy rifle

There are two rifle systems that have been used by DBCA researchers to biopsy cetaceans; the PAXARMS and the Dan-Inject, as described below.

5.1.1 PAXARMS

This system is a modified 0.22 calibre rifle with Pro-Pointa red-dot sight and uses blank charges to propel a lightweight, polycarbonate plastic biopsy dart at free-ranging cetaceans. It consists of a stock, detachable barrel, and a pressure (distance) adjustable valve system (Figure 1a) that propels a biopsy dart (Figure 1b). This system (http://www.paxarms.co.nz/) is well established and has been used extensively around Australia and internationally (Krützen et al., 2002) for many years.

Figure 1 The PAXARMS biopsy system. (a) Assembled 0.22 caliber rifle with Pro-Point red-dot sight. (b) Biopsy dart and biopsy tip. Photos taken from Murdoch University Cetacean Research Unit.

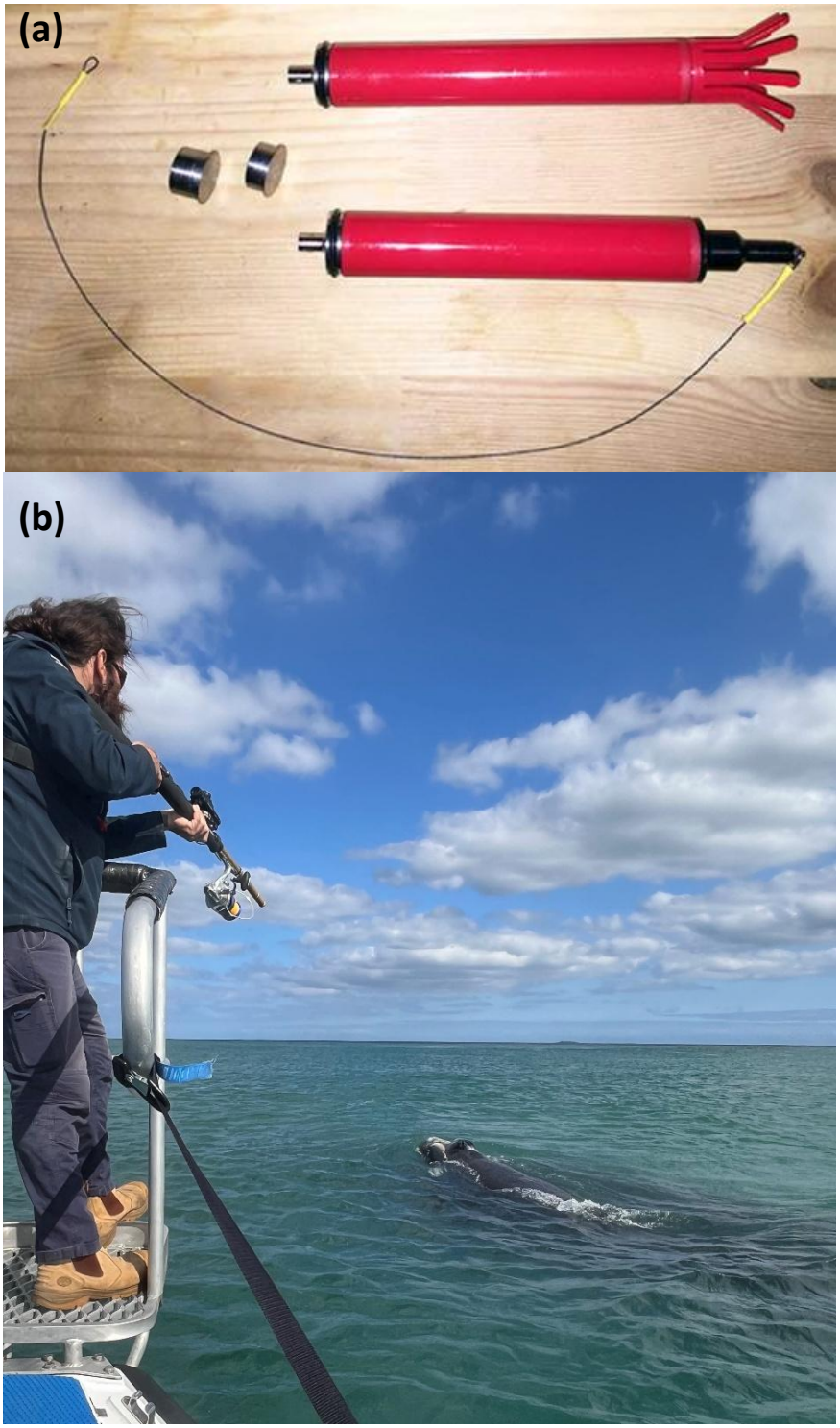


Figure 2 a) PAXARMS biopsy darts standard (top) and tethered (bottom). (b) Assembled PAXARMS with tethered dart and barrel with reel attached.

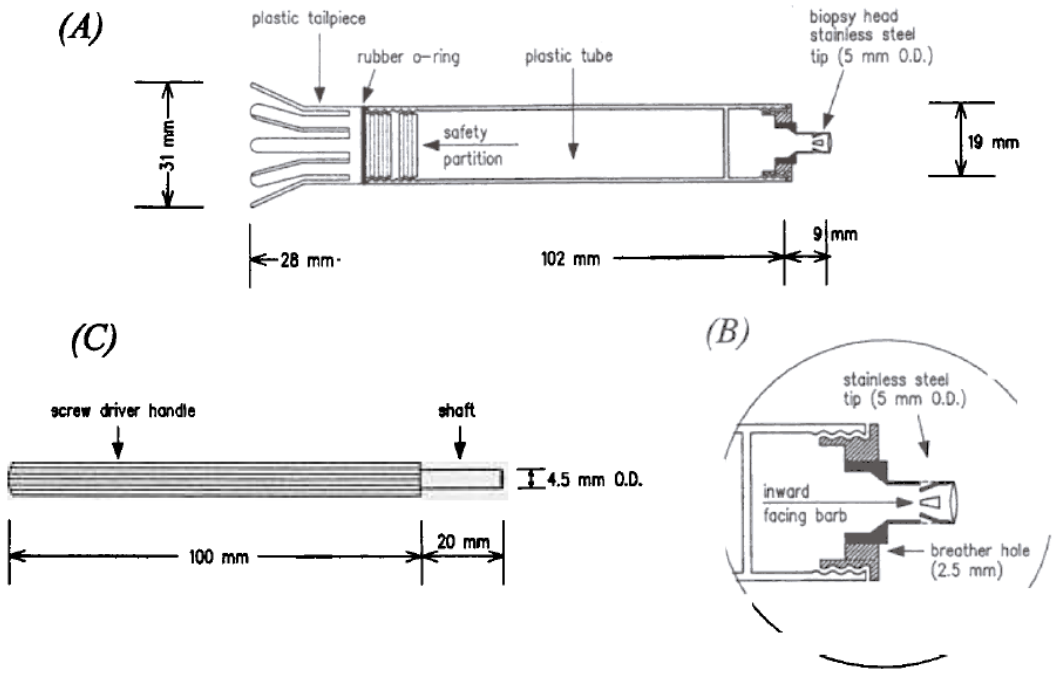


Figure 3 Biopsy dart (a) Assembled biopsy dart showing inserts of biopsy tip, safety partition, and plastic tail. (b) Detailed drawing of biopsy tip. (c) Barb resetting tool. O.D. = outer diameter (taken from Krützen et al., 2002).

The biopsy darts are positively buoyant, float in an upright position and have a total assembled weight of approximately 21.5 g. Blank charges are used to project the darts and proprietary charges can be purchased from PAXARMS. Alternatively, some brands of nail gun cartridges are suitable (see Ramset Australia Power loads (5.6 x 16mm calibre) medium (PLCYW22S) or strong (PLCRD22S) single cartridges). A dial on the side of the rifle chamber is used to adjust the pressure of the dart projection. A red-dot laser sight (Tasco) is fitted to the rifle to improve darting accuracy, as darting is faster and more accurate with this system than an open sight. For whales, a tethered biopsy dart (Figure 2a) may be preferred for recovery of the dart and biopsy sample. The biopsy darts are known to stick in whales more so than dolphins, and the reel (Figure 2b) can assist with the biopsy dart pulling free and recovery of the biopsy dart without having to reapproach the whale. This system has been successfully applied by DBCA staff to southern right whales in Western Australia. Noting the size of the reel is a careful consideration as it adds extra weight to the barrel, as well as the gauge of the monofilament line. All attempts should be made to recover monofilament line and vessel approach and communication between the shooter and vessel master is important to avoid entanglement of the whale in the line tethered to the dart.

If tagging is conducted concurrently with biopsy sampling, clear communication is needed between the tagger and biopsy dart shooter; the tag should be deployed first and the biopsy directly after. The tagger should stand next to, but forward of, the biopsy shooter and the tag should be placed forward of the biopsy dart puncture site. Ideally the tag is deployed in the same approach as a biopsy is taken to avoid multiple approaches and prolonged exposure to the vessels. Note that the distance between the vessel and the target individual being tagged can be much closer than when biopsy sampling is solely pursued. Some systems (not covered here) have a double barrel allowing for simultaneous tagging and biopsy. For further detail on

tagging refer to the National Guidelines on the use of tagging for cetaceans as tagging procedures are not covered in this SOP.

A barb-setting tool (Figure 3c) should be used to reset the barbs after repeated uses. With an unscrewed steel biopsy tip, the tool should be inserted from the underside and pushed forward to reset the barbs regularly.

5.1.2 Dan-Inject

This system is a carbon dioxide (CO_2) gas powered rifle with a red laser sight and uses CO_2 to propel the plastic syringe with biopsy dart head at a free-ranging animal (Figure 4a). The Dan-Inject has been used for skin tissue sampling on a variety of free-ranging animals (Mijele et al., 2016). The 5 mm diameter off-the-shelf dart heads were customised for use with cetaceans by reducing the shaft length of the dart head to 8 mm and adding a metal washer/stopper below this to prevent the dart from embedding into the animal.

The darts have a hollow body with a white transparent plastic tube exterior and steel biopsy bevelled tip (Figure 4b). The hollow rear chamber of the dart body (plastic syringe) causes the dart to float tail-end up and the bright pink synthetic tail makes them relatively easy to find. Two triangular barbs are located slightly in from the tip edge for sample retention. The system has a built-in CO_2 pressure gauge oriented on the rifle facing the shooter and a pressure control valve that can be monitored and adjusted while aiming. Forceps or a probe tool (or similar) can be used to push the barbs inwards to reset them regularly.

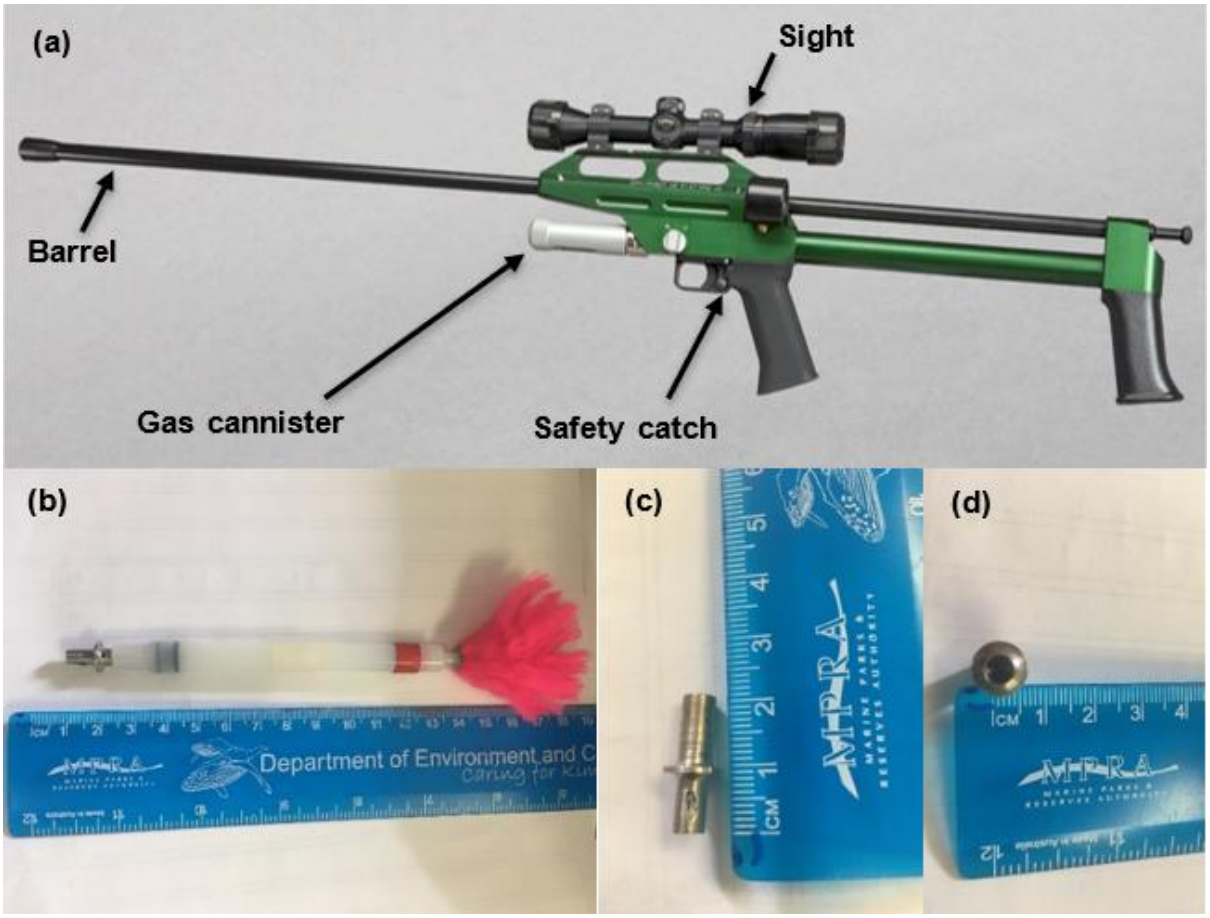


Figure 4 Dan-Inject biopsy system. (a) Assembled rifle with Pro-Point red-dot sight. (b) dart head with syringe and tail, (c) prongs and (d) slits added to the dart head in 2019 to improve tissue sample retention.

DBCA personnel with appropriate firearms training, competency and currency as a nominated person on the DBCA corporate firearms licence for Category E firearms have used both the PAXARMS and Dan-Inject system to sample cetaceans across Western Australia. The PAXARMS is tried and tested and has been successfully used to obtain tissue samples from several dolphin and whale species, whereas the Dan-inject dart heads require further modification to make them suitable for sampling small cetaceans (dolphins) successfully. Both systems described above are hereafter referred to as the biopsy rifle.

5.2 Biopsy pole

An alternative tool used for sampling is the biopsy pole, which is only suited to sampling when cetaceans closely approach the vessel (<1 metre depth or at the surface). Bow-riding cetaceans can be sampled when they are close to the water surface (<1 metre deep) and occasionally when they surface. It is a less common sampling method as the opportunities to collect such samples are fewer and dependent on the behaviour and approachability of the target species.

The biopsy pole head is similar to the biopsy dart head previously described and similarly is used to take a small plug of skin and blubber (Bilgmann et al., 2007). The dimensions of the biopsy pole are: head 30 mm width, biopsy tip 6 mm width with bevelled edge (Figure 5 taken from Bilgmann et al., 2007). The biopsy tip is a hollow cylinder that has barbs to hold the sample in place. Water that flows into the pole through the tip flushes out through a purge hole further up the pole. This prevents it from flowing back down the shaft and dislodging the sample. The biopsy pole has been used successfully to sample delphinid species that closely approach and bow ride vessels including false killer whales and bottlenose dolphins.

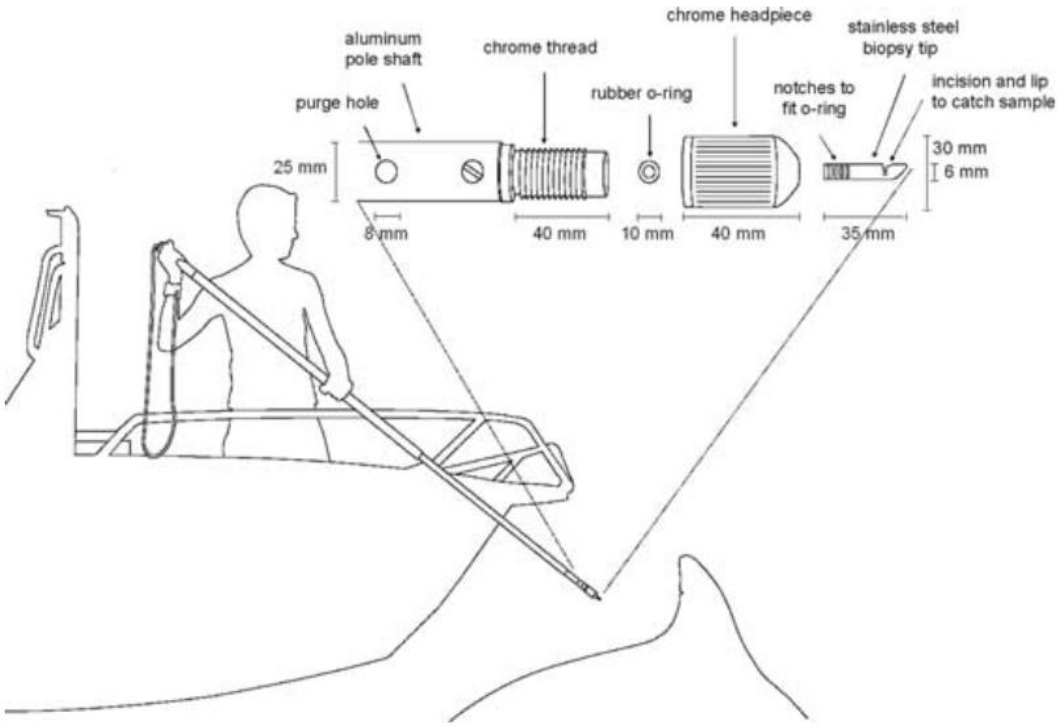


Figure 5 Lateral schematic of technique and detail of distal components of the biopsy pole system including biopsy tip (taken from Bilgmann et al., 2007).

6 Procedure Outline

6.1 Personnel

There are three essential personnel required for remote biopsy of cetaceans using either the rifle or the pole system:

- The shooter stands in the bow of the vessel, ready to biopsy.
- The observer/photographer stands behind the shooter taking identification photos of the animal at the same time as the individual is sampled (so that it can retrospectively be identified).
- The vessel master is responsible for safety and navigation.

A second observer (optional) is valuable as they can be responsible for recovering darts using a hand-net, processing samples, recording notes, and acting as a secondary photographer (if two cameras are available).

6.2 Sterilisation

The following sterilisation procedure should be completed before starting the survey each day. This procedure MUST take place away from fuel and other flammables and close to a water supply and a suitable fire extinguisher (i.e., CO_2):

a) If facilities are available in the field, the biopsy heads should be boiled for 10-20 minutes and then, using forceps, should be submerged in 95% ethanol and then flamed (lit and extinguished) with a lighter. If boiling is not possible, the biopsy heads should be scrubbed with a clean toothbrush (or similar) and flamed and the biopsy heads allowed to cool. Biopsy heads should never be touched on the biopsy tip or cutting edge. Biopsy heads should only ever be touched with gloved hands after sterilisation (scrubbed and flamed) around the thread while being screwed onto the dart bodies. The biopsy heads can be easily grasped and manipulated with forceps and manipulated by the dart body once the heads are screwed back onto the dart body. Biopsy heads should be checked and re-sharpened and barbs reset with appropriate tool at time of cleaning process if required.
b) Sterilised biopsy heads should be stored in a clean, watertight container.
c) Sterile latex gloves must be worn when handling biopsy heads at any time, to minimise the risk of infection to both the dolphin and observer and cross-contamination by DNA/RNA from these and other samples. Gloves should be changed between sampling events/individuals.

6.3 Firearm Safety Procedures

Firearms should be stored and handled as per the *Firearms Act 2024* at all times. Safe rifle procedures while on board a vessel are outlined below, but specifications may differ between rifle types not covered by this SOP:

Rifles should be stored dissembled, where possible, in a secure case (i.e. lockable pelican case) and protected from water spray. Biopsy darts and ammunition must be stored securely in a compartment separate to any firearms.

a) When a suitable group of cetaceans has been identified the rifle can be removed from the case and assembled [Note: only a person with the appropriate Firearms Licence and experience with the firearm used can handle the firearm]:
    1. Attach barrel to biopsy rifle stock.
    2. Insert dart into barrel.
    3. Load blank cartridges into magazine and insert magazine into rifle (PAXARMS) or screw in CO_2 cannister (Dan-inject).
b) Ensure that no other vessels or people are within 300 m of the cetacean group, or likely to come within 300 m during the procedure.
c) Use biopsy rifle only from the bow of the vessel, ensure all crew remain behind the shooter at all times. As a general rule do not shoot any further back than mid-ship of the vessel or 3 and 9 o'clock on the clock face if the bow is 12 o'clock and the stern is at 6 o'clock (Figure 6).
d) Before darting, cock the rifle, and ensure the safety catch is on.
e) The shooter must always communicate with the crew to ensure that all personnel are aware that the dart is about to be fired. Once the shooter has determined that a sampling attempt is appropriate and the cetacean group has been approached, release the safety catch and line up the sight on the lateral side of the cetacean, below the dorsal fin (see Krützen et al., 2002). Only fire when safe to do so for both the target cetacean, people in the vessel, and people in any other vessel in the vicinity.
f) After a darting attempt, place the rifle in a safe, dry place (ideally a secure case) and recover biopsy dart using a hand-net. Wherever possible this should be done by someone other than the shooter.

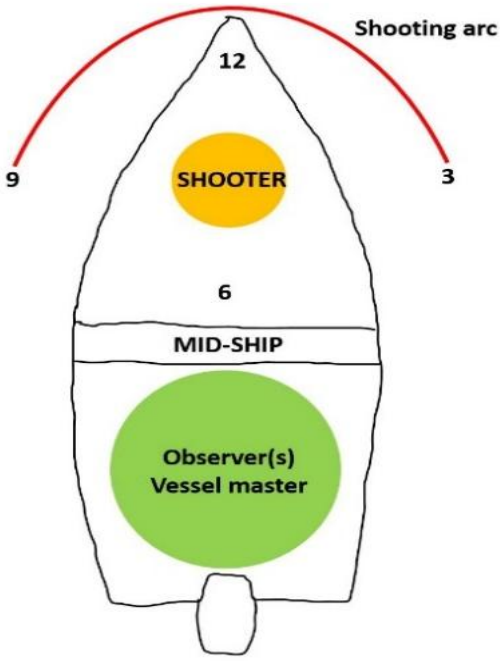


Figure 6 Overhead schematic of how personnel should be positioned on the survey vessel and the shooting arc.

g) Between sampling events ensure there is no dart in the rifle chamber.
h) If sampling more than one individual in a group, repeat from step 2 with a new sterilised biopsy dart.
i) When all sampling has been completed with the cetacean group, disassemble the rifle, and return it to its secure case in a safe, dry location on the vessel.
j) Clean and store the rifle according to manufacturer's instruction at the end of the survey day. Store the biopsy rifle in a rifle case, ensuring no blank cartridges are left in the rifle. Ensure that ammunition and rifles are stored securely (under lock and key when not in use) and separately.

A miss-shot is likely to be dissipated by the water surface. To prevent misfiring in the vessel ensure the above safety procedures are followed. If the dart completely misses, more attempts can be made if the individual (and group) shows no signs of behavioural changes (i.e., vessel avoidance or other reaction) and can still be approached.

ANIMAL WELFARE: If any individuals in the group (including sampled individuals) show signs of behavioural changes indicating disturbance (e.g., repeated rapid surfacing and dives in a direction away from the boat), sampling should be ceased and the vessel move away (Krutzen et al., 2002).

6.4 Sampling technique

6.4.1 Conditions

Biopsy sampling can only be conducted in suitable sea conditions (no rain, light winds, and seas less than 1.0 m i.e., equivalent of Beaufort Sea State [BSS] ≤ 3, Table 1) and should be

undertaken from small vessels (i.e., <10 m). Speed should be reduced to no wake or neutral, particularly when close to groups (e.g., within 50 m). The cetaceans are aware of the vessel when the motor is running, so it should not be turned on and off when interacting with a group. Sampling with a biopsy rifle should only be attempted when cetaceans are parallel to the vessel and are between approximately 5 m and 20 m from the vessel and the pressure adjusted on the rifle accordingly. Sampling with a biopsy pole should only be attempted when cetaceans closely approach the vessel and is most successful when used on individuals riding the pressure wave at the bow of the vessel. Photographs of the dorsal fin of the cetacean(s) to be sampled are taken before and during sampling for the purpose of photo-identification of individuals and to match the sample with that individual. For some large whale species, other features can be used for identification and should be photographed. This may include the underside of flukes for humpback whales, the callosity pattern on the head for southern right whales, and the lateral pigmentation pattern for blue whales. Refer to department *SOP Vessel-based Cetacean Surveys Using Photo Identification* for detailed instructions on photographing cetaceans and data collection. The shooter or person using the biopsy pole can wear a head mounted camera recording video that can later assist in individual identification and review of the biopsy process if needed.

Table 1 Beaufort Sea State scale description.

Beaufort number	Wind speed (knots)	Wave height (m)	Sea conditions
0	< 1	0	Flat
1	1-3	0 - 0.2	Ripples without crests
2	4-6	0.2 – 0.5	Small wavelets. Crests of glassy appearance, not breaking.
3	7-10	0.5 – 1	Large wavelets. Crests begin to break; **scattered whitecaps**.
4	11-16	1 - 2	Small waves with breaking crests. Fairly **frequent whitecaps**.
5	17-21	2-3	Moderate waves of some length. **Many whitecaps**. Small amounts of spray.

6.4.2 Suitability for sampling

The team leader makes the decision on when a cetacean is suitable for sampling. This is based on cetacean behaviour, group demographics, body condition and sea state (Table 1) and other safety considerations.

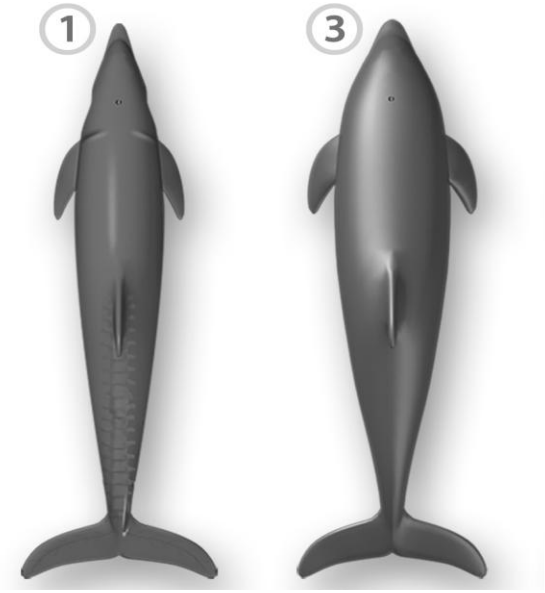
a) Only cetaceans in healthy condition (assessed by the appearance of the epaxial muscle mass on either side of the individual's dorsal fin, as in Pugliares et al., 2007 and Clegg et al., 2015) are to be sampled; individuals appearing emaciated (skinny) or in poor body condition with a body condition score (BCS) of one are not considered suitable (Table 2).
b) Calves will not be sampled and will be differentiated based on size (<2/3 the length of the mother, i.e. ~1.5m for dolphin calves) and position in relation to the mother (i.e.

dependent calves return frequently to the tailstock region of the mother (Mann & Smuts 1999).

c) Weaned calves (young juveniles) may be considered suitable to sample.

Table 2 Dolphin Body Condition Score (Taken from Clegg et al., 2015).

BCS	DESCRIPTION
1	Emaciated. *Head:* concave depression behind blowhole. Scapula bones visible. *Side view:* Ribs clearly visible; hollow area under dorsal fin above spine. *Dorsal view:* concave indent behind skull ('peanut head'), minimal fat cover.
3	Average. *Head:* fat cover around skull. Scapula bones only visible when pectoral fin is manipulated. *Side view:* ribs not visible; notable layer of fat parallel and adjacent to spine. *Dorsal view:* smooth transition from head to neck; rounded fat deposition along spine.



6.4.3 Obtaining the sample

The process for obtaining the biopsy sample varies depending on the system used and is as described below.

6.4.3.1 Biopsy rifle

a) When a sighting has been made and the group are deemed appropriate for sampling, the shooter should prepare the rifle and dart as described above, and the observer prepare the camera.
b) Photo identification can commence prior to biopsy, but once attention has been turned to the sampling event and a dart loaded, the photographer must move behind the shooter.
c) The vessel should approach the individual or group at a speed reduced to no wake or be in neutral and position the vessel to within 5-20 m of the cetacean as it surfaces, giving the shooter the best opportunity to fire the dart.

d) The photographer should be prepared (but well back from the shooter) to photograph the dorsal fin of the individual that is to be darted at the same time the dart is fired.
e) Samples should be taken from in line with and below the dorsal fin, where the blubber layer is thickest. Once fired, the dart will strike the cetacean, penetrate to a stop (generally 4-5 mm into the blubber layer), then bounce off and float at the water/sea surface for recovery. *Note: In some whales, the probability of collecting a sample including skin and blubber increases if the angle of impact of the dart is perpendicular or angular to the body surface, but this is less critical when the darts are extremely sharp (Barrett-Lennard, Smith & Ellis 1996).*
f) Once the cetaceans are clear of the vessel, recover the dart using a hand-net.

6.4.3.2 Biopsy pole

a) When the team leader deems it suitable, the researcher with the pole should position themselves in the bow of the vessel or on the side closest to the location of the cetaceans.
b) Depending on the depth of the cetacean and the speed of the vessel, the biopsy pole is held and jabbed firmly (but with limited travel range or else the needle may get bent/damaged) into the cetacean, aiming at the body areas lateral to the base of the dorsal fin.
c) The pole is held at an angle of between 60˚ and 90˚ to the water surface, depending on the sampling vessel.
d) A constant speed of 2-6 km/h is maintained during sampling (Bilgmann et al., 2007).
e) Clear communication must be maintained between the sampler and the vessel master, and the sampler must remain within the vessel, including the bow rail, during the entire sampling procedure.

6.4.4 Processing the sample

The biopsy sample is processed the same, regardless of sampling technique used and is as follows:

a) Extract the sample using sterile forceps, taking care not to touch the sample with anything other than the sterilised tool. If the sample is stuck, the biopsy head may need to be removed, and the sample carefully pulled out using forceps from the back of the biopsy head. Sterile toothpicks (or piksters) or a stainless-steel probe may be useful to extract the sample.
b) Store the sample in a small vial containing the appropriate preservative for the purposes of the research question(s). Further information on preservatives can be found in department SOP *Tissue Sample Collection and Storage for Genetic Purposes.*
c) Label the vial clearly, including the sample code (a unique identifier), ID code or name of the individual sampled if known from DolFin catalogue, species name, date, area/site being sampled (Figure 7 and Figure 8).
d) At the end of the day, all samples should be stored appropriately for the preservative used i.e., ethanol kept cool and refrigerated if possible but not frozen, DMSO should be kept cool and dry and those samples without preservative should be frozen as soon as possible.

e) All biopsy sampling equipment (dart heads, probes, forceps etc.) should be sterilised as described above (Section 6.2) and appropriately stored ready for the next day of sampling.

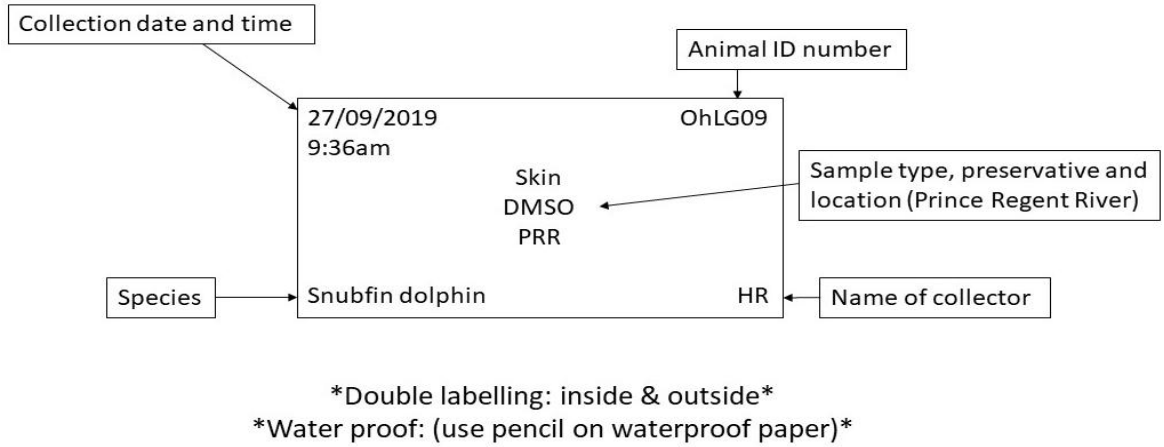


Figure 7 Example sample label showing the information to be recorded with each biopsy sample.

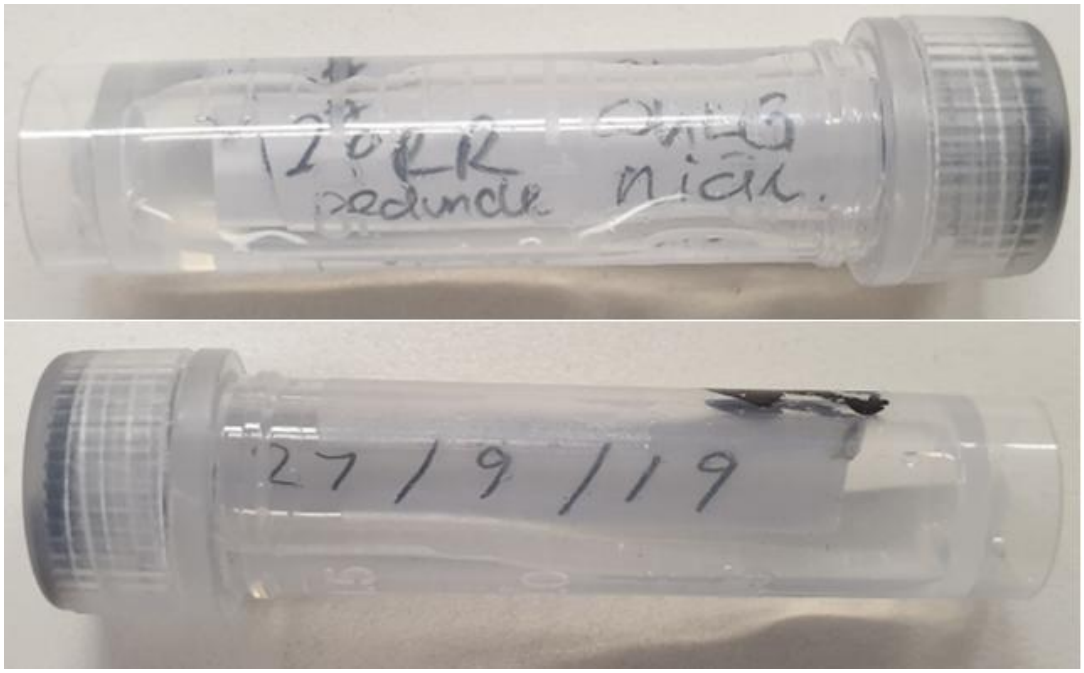


Figure 8 Small Eppendorf 2mL vial (approx. 4.5 cm length) containing biopsy sample, appropriate storage solution and label. Example label includes: sample number taken that day (#2), species (Oh = Orcaella heinsohni), area sampled (LG = Lalang-garram Marine Park, PRR = Prince Regent River), individual's identifying feature (peduncle nick), date sampled (27/9/19).

6.5 Recording data

A data sheet is completed for every biopsy attempt that includes information on the date, time and location of the sighting, group composition, age class and other information of interest on the group and the behavioural response of the individual (and group) to the biopsy attempt (see example in Appendix 1).

The observer is responsible for filling out the datasheets as the shooter and vessel driver are focussed on the cetaceans. The observer records sighting and survey information, GPS position, sample details, notes on cetacean behavioural response and all other data fields listed in the biopsy sampling datasheet (Appendix 1). GPS coordinates should be taken from the handheld GPS on board with coordinates in decimal degrees and waypoint number noted on the datasheet, or if time permits, the coordinates transcribed on the datasheet. Familiar dolphins already in the DolFin catalogue with ID codes can be listed in the catalogue ID section (should always be photographed regardless of familiarity to update the catalogue with any changes to the dorsal fin and for the sighting history), as well as sex or age (adult, sub-adult, calf, unknown) of individuals if known. Notes on distinctive dorsal fins such as unique scarring, nicks, pigmentation, deformities or 'clean fins' can be drawn or described in comments. If photos are captured of individuals, note the frame numbers on the camera using the playback display panel on the camera. The level of response of the sampled individual at the moment of sampling should be recorded (Appendix 1; 0=none, 1=flinch, 2=buck, 3=leap, 4=multiple leaps, O=other) and behaviour afterwards monitored. The observer is responsible for ensuring all data fields are filled in and taking a photo of the datasheet to indicate the end of the sighting. This also serves as a backup datasheet.

All photos from each camera used should be downloaded onto an external hard drive into the trip folder, in a new folder labelled with that day's date (YYYYMMDD). Do not clear SD cards as they will be used as backups until the photos are transferred to the server. If operating multiple vessels, the folder should include the vessel name (e.g., YYYYMMDD vessel name). Refer to department SOP SC22-28 *Vessel-based Cetacean Surveys Using Photo Identification* for detailed instructions for data processing at the end of the day.

The datasheets should be reviewed to ensure all information has been recorded on the appropriate datasheets and kept in a safe location on the mother vessel or accommodation. Once all data is copied onto the external hard drive, make a backup on the second external hard drive.

6.6 Transporting Biopsy Samples

At the end of the field trip most samples can be flown back to Perth as hand luggage. There are no restrictions on transporting samples stored in DMSO but a copy of the Safety Data Sheet (SDS) is recommended to accompany the sample. Biological samples stored in small amounts (no more than 30 ml per container and a maximum of 1 L) of flammable liquids such as ethanol can be flown in hand luggage. For frozen samples include ice bricks to keep chilled and prevent defrosting, which will compromise DNA quality. Samples that are stored on dry ice must travel as hand luggage and prior permission needs to be sought from the airline before the flight. The dry ice needs to be in an esky that can be vented to allow gas to escape. Samples should be provided to the laboratory for analysis as soon as possible after the field trip to optimise the quality of the DNA.

7 Occupational Health and Safety

Departmental SOP *SC23-06 Managing Disease Risk and Biosecurity in Wildlife Management* is relevant to occupational health and safety and should be consulted when proposing biopsy sampling.

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://dbca.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://dbca.sharepoint.com/Divisions/corporate/people-services/HS/SitePages/Reporting-Hazards,-Near-Misses-and-Incidents.aspx

Extreme care should be taken when using firearms on a vessel. When attempting to collect a biopsy sample, ensure a shoot plan and Job Safety Analysis (JSA) has been approved for the work and all personnel involved are familiar with it as well as any requirements under the *Firearms Act 2024*. Follow the safe rifle procedures for working on a vessel outlined above (Section 6.3). Ensure the layout of the chosen working vessel and positioning of personnel in the vessel allows for clear communication between shooter and vessel master at all times (i.e., centre console windscreen is not obstructing communication). It is a requirement that an experienced cetacean researcher/sampler is always present to assess the level of risk of each sample attempt.

A first aid kit, satellite phone and VHF radio should be carried in the vessel at all times. You must be aware of your own safety and the safety of others. Extreme care should be taken when working in tidal estuaries to avoid exposure to disease carrying insects, such as mosquitos and sand-flies. All injuries (even superficial ones) and bites should be appropriately treated as soon as possible to prevent infection and promote healing and reported through the Health & Safety Incident Report Form accessed via the link above.

7.1 Personal Protective Equipment

Protective gear required shall be determined by the project specific JSA. This may include but is not limited to adequate sun protection (i.e., long-sleeved clothing, sunscreen, hat, face buff, hand gloves, boat shoes, polarised sunglasses), protection from wind chill and other changes in weather (raincoat may be required), and latex gloves. The person shooting is required to wear long sleeved clothing, long pants, enclosed boots, and eye protection (ideally polarised sunglasses rather than safety glasses). Hearing protection that would typically be worn during shooting operations is not required for the two firearms (PAXARMS and Daninject) outlined in this SOP as it will prohibit communication with vessel master and other personnel and the sound is not considered to pose the same risk as other calibres or classes of firearm.

7.2 Working around the water's edge and from vessels

Personal flotation device (PFD) Type 1 Life jackets must be worn by those working on vessels. Care must be exercised to avoid slipping into the water from the vessel or when working on riverbanks and boat ramps. All limbs must be kept within the vessel at all times.

8 Competencies

A person who is competent has the knowledge, skills, and experiences that allow them to undertake cetacean biopsy sampling and appropriately manage adverse events as required. Department personnel, and other external parties covered by the department's AEC, undertaking projects involving biopsy sampling require approval from the committee and will need to satisfy the competency requirements (Table 3). Other groups, organisations or individuals using this SOP to guide their 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 3 provides advice for standard monitoring only.

Table 3 Competencies for personnel involved in cetacean biopsy sampling.

Competency Category	Competency Requirement	Competency Assessment
Shooter*	Minimum current corporate firearm licence (Category E)	Hold a DBCA corporate firearms licence. Trained in category A, B and C3 firearms. Have observed and assisted under the direction of an experienced shooting mentor, until the mentor is satisfied that the mentee is competent in biopsy sampling free-ranging cetaceans. Assessment can be made on target on water prior to sampling.
Photographer/Observer	Prior experience – competency requirement	Have observed and assisted under the direction of an experienced supervisor as a photographer, until the supervisor is satisfied that a required level of expertise has been achieved.
Vessel operator	Minimum of Coxswain 2 Near Coastal certificate	AMSA approved assessment. Note there may be limitations to the area of operation.
Secondary observer/Data recorder	Prior training, experience ideal – competency requirement	Have observed and assisted under the direction of an experienced supervisor, until the supervisor is satisfied that a required level of expertise has been achieved.

*The *Firearms Act 1973* was repealed in March 2025 and replaced by the *Firearms Act 2024*. Transitional arrangements are in place while arrangements under the new Act are

implemented. Requirements for firearm operators are therefore subject to change, and it remains the responsibility of individuals to ensure they comply with all applicable legislation.

In conjunction with possessing the required understanding and knowledge of sampling cetaceans using a remote biopsy system and animal welfare requirements, a guide to the experience and skill requirements for personnel to be considered competent to biopsy sampling is as follows (note 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 10 years.
- Minimum two individuals of a variety of species handled.

9 Approvals

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. Projects involving wildlife may require a licence/authorisation under the *Biodiversity Conservation Act 2016* (examples below).

- 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.

Personnel should consult the department's Wildlife Licensing Section for further guidance 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.

10 Further Reading

The following SOPs have been mentioned in this advice, and it is recommended that they are consulted when proposing sampling of Cetaceans Using a Remote Biopsy System:

- 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*
- Department SOP *Euthanasia of Small Stranded Cetaceans*
- Department SOP *Vessel-based Cetacean Surveys Using Photo Identification*

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
- National Guidelines on the use of tagging for cetaceans https://www.dcceew.gov.au/sites/default/files/documents/national-guidelines-tagging-cetaceans.pdf

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- Department of Parks and Wildlife. (2016). Boating Code of Practice.
- *Firearms Act 2024*
- Firearms Regulations 2024
- *Occupational Safety and Health Act 1984*
- Occupational Safety and Health Regulations 1996
- *Biodiversity Conservation Act 2016*
- Biodiversity Conservation Regulations 2018.

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12 Glossary of Terms

Cetacean: marine mammals commonly known as whales, dolphins and porpoises.

Beaufort Sea State (BSS): scale for estimating wind strengths based on appearance of the sea surface. Often used by researchers as it affects the probability of sighting cetaceans.

Delphinids: small body sized toothed marine mammal (whale, dolphin or porpoise) from the family Delphinidae i.e. largest species killer whale (*Orca orcinus*).

DNA (deoxyribonucleic acid): a macromolecule found in all living cells that contains genetic identifying information of organisms

Dorsal fin: the fin located on the top side of an animal, commonly used to identify species and sometimes individuals.

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

Tissue: groups of cells that have similar structure with a specific function, in a multicellular organism. For the purposes of this SOP, tissue will most often refer to the skin and underlying blubber layer of a cetacean.

Appendix 1

Cetacean Biopsy Sampling Data Sheet

DATE ____/____/____ TIME _____ SIGHTING # _____ SHOT # _____

SAMPLE ID _____ CATALOGUE ID _____ RECORDER _____

SAMPLER _____ DRIVER _____ PLATFORM _____

GUN SET / DISTANCE ____/____ POLE _____ DEPTH _____

SPECIES _____ *LOCATION _____

LATITUDE _____ LONGITUDE _____ WPT _____

SAMPLE TAKEN? YES ☐ NO ☐ SEX OBSERVED (blank if unknown) M F _____

FULL SAMPLE? YES ☐ NO ☐ DOLPHIN SIZE C SA A U

MARK LOCATION HIT

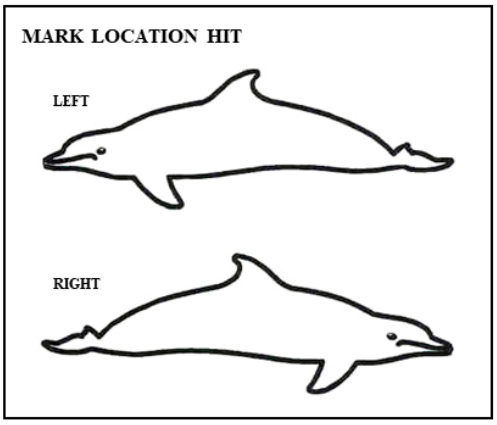

PHOTO: ☐ FOLDER # _____ PICTURES: ID _____ (frame #(s)) HIT _____ (frame #)

LEVEL OF RESPONSE: 0 1 2 3 4 O BEHAVIOUR: PRE-BIOPSY _____ POST-BIOPSY _____

(RESPONSE CODES: 0=none, 1=flinch/accel, 2=buck, 3=1 leap, 4=mult leaps, O=OTHER:_____)

GROUP SIZE _____ IF SAMPLE FROM SUBGRP, THEN SUBGRP SIZE: _____

*MULT SAMPLES FROM THIS GROUP? YES NO (circle one) IF YES, OTHER SAMPLE #S & IDS: _____

COMMENTS: _____