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Charles Darwin University Animal Ethics Committee

Standard Operating Procedure:

DPAW SOP 17.2021 Sampling cetaceans using a remote biopsy system

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CHARLES DARWIN UNIVERSITY AUSTRALIA

Standard Operating Procedure

Sampling cetaceans using a remote biopsy system

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.*

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Prepared for: Animal Ethics Committee

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GOVERNMENT OF WESTERN AUSTRALIA

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Acknowledgement
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Contents

1 Purpose

Tissue samples collected from cetaceans can be used to investigate biological and ecological questions about the animals. DNA can be extracted from these samples and used to identify species, phylogeography, gene flow and population structure 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 Marine Science Program supplemented by work by regional staff, Murdoch University and Alex Brown.

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 remote biopsy sampling of cetaceans 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. All department personnel involved in remote biopsy sampling of cetaceans should be familiar with the content of this document.

Projects involving wildlife may require a licence under the provisions of 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 provides governing principles to guide decisions and actions of personnel involved in the care and use of animals, and contains an introduction to the ethical use of animals in wildlife studies. A copy of The Code may be viewed by visiting the National Health and Medical Research Council website (http://www.nhmrc.gov.au).

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3 Animal Welfare Considerations

To reduce the level of impact on the welfare of animals, staff 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 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 for reducing impacts and improving animal welfare, and the process for managing adverse events.

3.1 Level of Impact

Environmental

There are two main ways in which an animal may be impacted by the biopsy procedure: they may be disturbed by the actual sampling or they may be harmed by the wound created by the dart or they may be disturbed (react) to the actual sampling or the close approach of the vessel.

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 to, where possible, confirm the sampling site on the animal at time of sampling. These can then be used to ensure normal healing of the biopsy wound is occurring if the animal is re-sighted in days, months and years after.

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 is covered, smooth and absent of pink colouration which generally occurs within weeks to up to 3 months (as a maximum time frame) (Weller *et al*., 1997). For example, Krutzen et al. (2002) found wounds were healed after 23 days in individual bottlenose dolphins that they observed daily post-biopsying. Re-pigmentation takes a lot longer, months and in some cases years in other delphinids such as pilot whales (Gimenez *et al*., 2011). Another study measured 10 individuals from 3 days to 7 months post biopsying 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 we are expecting that healing will vary between individuals but will range between 2 weeks and 3 months for the epidermis to cover the small wound created by the biopsy dart which is superficial compared to large deep wounds from shark encounters that also heal relatively quickly (Corkeron *et al*., 1987; Orams & Deakin, 1997).

Response to the procedure will be monitored. Darting reactions are generally mild and short-term (‘flinch’ or ‘buck’) and most animals continue their pre-biopsy behaviours within the area (Krützen *et al*., 2002) an extreme aversive reaction would be a leap. There has only ever been one mortality from a remote biopsy attempt documented in the literature (Bearzi, 2000). In the event of an unexpected death, the carcass will need to be retained for necropsy and the Animal Ethics Committee notified within 24 hours and a comprehensive report written on the adverse event following the initial notification. 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. As a further mitigation measure, under this protocol, sampling would be ceased if any animals show

active avoidance of the vessel. Further, no more than 3 attempts will be made per individual.

Exposure to vessels and the associated motor noise can disturb cetaceans and disrupt critical activities such as feeding, resting and nursing and attending to calves. Vessels should be handled in a way that minimises disturbance to the targeted fauna. Time limits will be set and complied with. As a guide, the research vessel will spend no more than 60 minutes within 100m of a cetacean group. If all group members have not been photographed the encounter will be ended anyway. Time limits and approach distances are prescribed under the Biodiversity Conservation Regulations 2018 and further licence conditions may be in scientific licences. Other considerations may be around the presence of young e.g. newborn calves or in critical areas or at critical times i.e. calving and nursing. The duration of encounters may be limited if groups contain newborns or groups are resting or nursing young calves to prevent disruption of critical activities.

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, tourism interactions etc. If commercial or recreational vessels enter the survey area during a survey, particularly if biopsying is occurring, they should be contacted via radio communication to explain the on-going operations and told not to approach animal groups closer than 100 metres while DBCA vessels are photographing, biopsying and observing.

4 Procedure Outline

4.1 Equipment, care and maintenance

Biopsy rifle

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

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 (PAXARMS: http://www.paxarms.co.nz/) is well established and has been used extensively internationally (e.g. Krützen *et al*., 2002) as well as around Australia for a number of years.

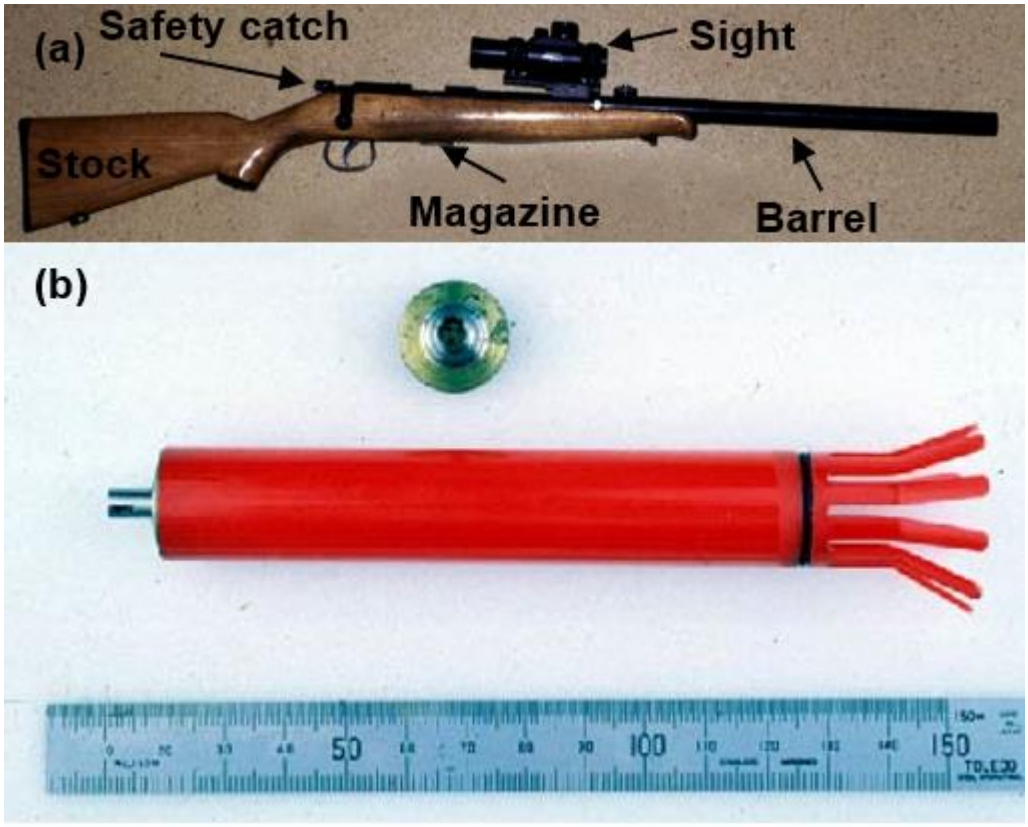

Figure 1 PAXARMS biopsy system. (a) Assembled 0.22 calibre rifle with Pro-Point red-dot sight. (b) Biopsy dart and biopsy tip. Photos taken from Murdoch University Cetacean Research Unit.

The PAXARMS darts have a hollow body with a thin, red polycarbonate plastic tube exterior and steel biopsy tips bevelled inwards (Figure 1b). Three small triangular barbs are located inside the steel tip 2 mm from the leading edge of the tip, for sample retention. A flat metal rim (flange) is welded to the base of the biopsy tip directly above where it screws into the body of the dart. The flange acts as a stop to ensure the dart does not penetrate too far into the animal. The body of the dart is a hollow plastic tube and there is an internal wall slightly below the thread for the flange to prevent flooding of the tube. A small piece of polycarbonate plastic screws into another thread at the tail of the body that acts as a safety partition if the tail separates on impact (Figure 2a). The polycarbonate plastic tailpiece has an o-ring and screws into the tail end after the partition to create a watertight seal.

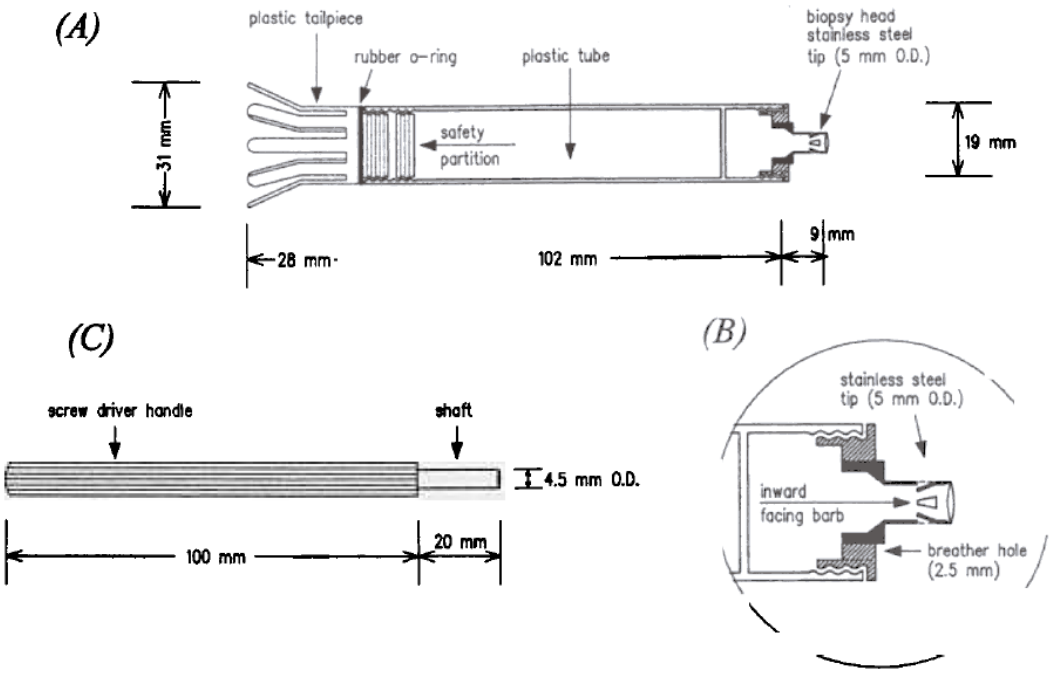

Figure 2 Biopsy dart (A) Assembled biopsy dart showing inserts of biopsy tip, safety partition, and plastic tail. (B) Detailed drawing of biopsy tip. (C) Barb resetting tool. O.D. = outer diameter (taken from Krützen *et al*., 2002).

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 or 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. A barb-setting tool (Figure 2c) should be used to reset the barbs. With an unscrewed steel biopsy tip, the tool should be inserted from the underside and pushed forward to reset the barbs regularly.

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 3). The Dan-Inject (https://dan-inject.com/ or https://daninject.com.au/) has been used for skin tissue sampling on a variety of free-ranging animals (Mijele *et al*., 2016). The 5mm diameter off-the-shelf dart heads were customised for use with cetaceans by reducing the shaft length of the dart head to 8mm 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 3). 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.

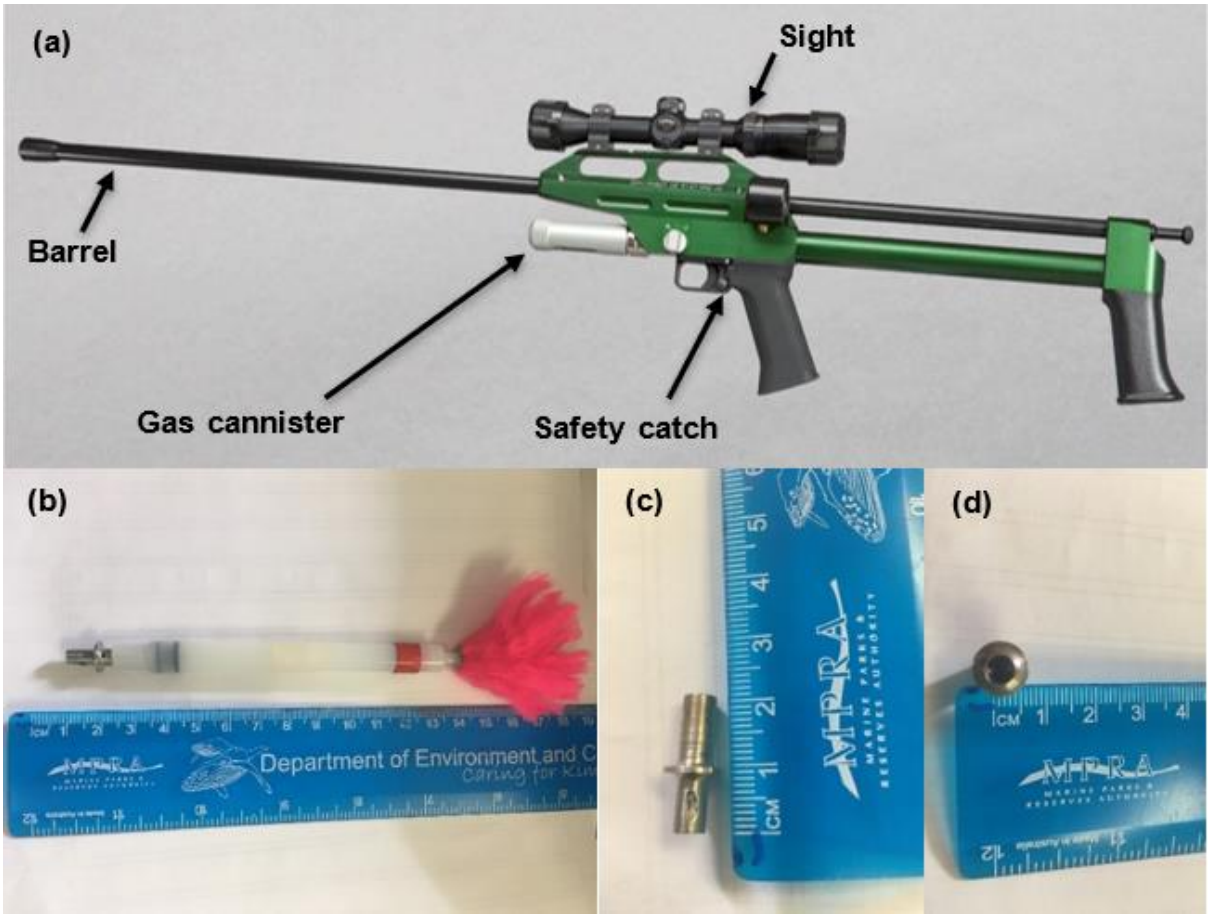

Figure 3 Dan-Inject biopsy system. (a) Assembled Dan-Inject system. (b) dart head with syringe and tail, (c) prongs and (d) slits added to the dart head in 2019 in an attempt to improve tissue sample retention.

DBCA personnel have used both the PAXARMS and Dan-Inject system to sample cetaceans across the State. The PAXARMS is tried and tested and has been successfully used to obtain tissue samples from several dolphin species, whereas the Dan-inject dart heads require further modification to make them suitable for sampling small cetaceans (dolphins) successfully. Both systems explained above are hereafter referred to as the biopsy rifle.

Biopsy pole

The 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 30mm width; biopsy tip 6mm width with bevelled edge (Figure 4 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.

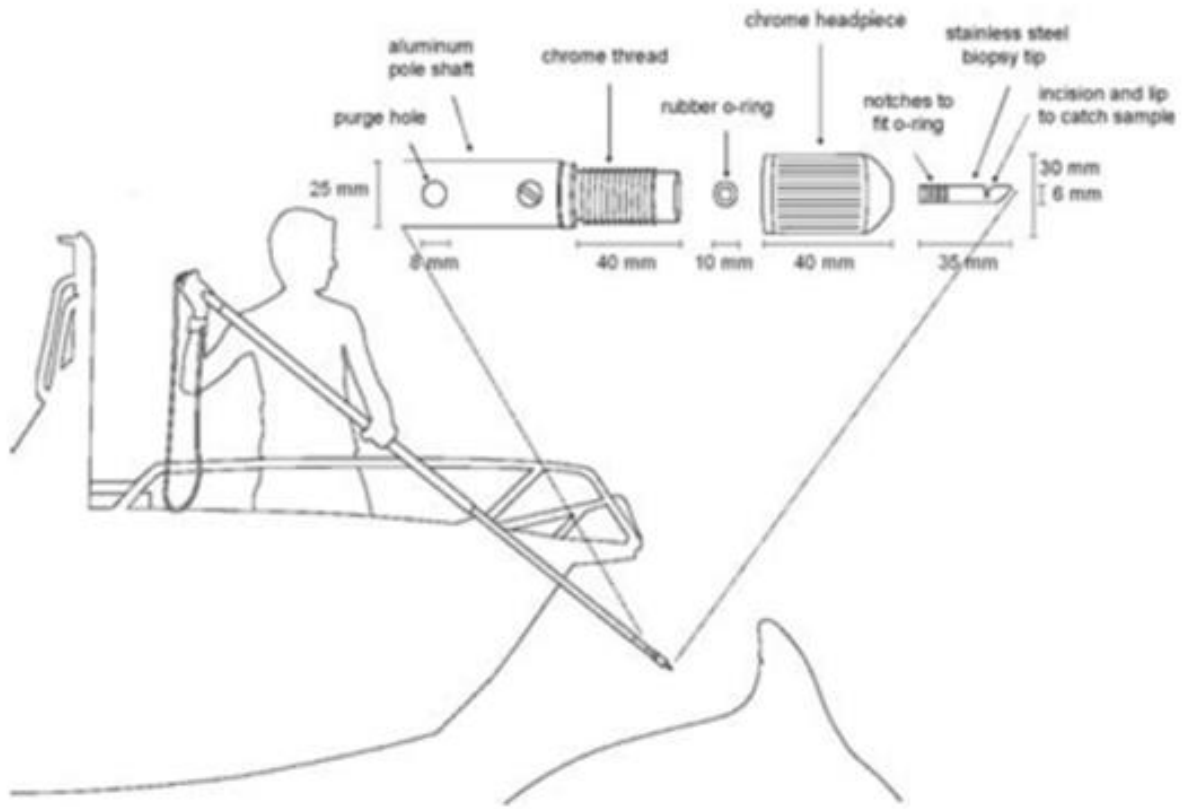

Figure 4 Lateral schematic of technique and detail of distal components of the biopsy pole system including biopsy tip (taken from Bilgmann *et al*., 2007).

4.2 Personnel

There are three essential personnel required for remote biopsying 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 being sampled (so that is can retrospectively be identified)
- The vessel master is responsible for safety and navigation.

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

4.3 Sterilisation and safety procedures

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):

1. If facilities are available in the field, the biopsy heads should be boiled for 10-20 minutes and then, using forceps, biopsy heads 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 not be touched after

sterilisation (scrubbed and flamed) the biopsy heads can be easily grasped and manipulated with forceps and manipulated by the tails once the heads are screwed back onto the tails. Biopsy heads should be checked and re-sharpened and barbs reset with appropriate tool at time of cleaning process if required.

2. Store biopsy heads in a clean, watertight container.
3. Sterile latex gloves are worn when handling biopsy heads at any time, to minimize the risk of infection to both the dolphin and observer and cross-contamination by DNA/RNA from either and other samples. Gloves should be changed between sampling events/animals. Safe rifle procedures while on board a vessel are outlined below, but specifications may differ between rifle types.

Safe rifle procedures while on board a vessel are outlined below, but specifications may differ between rifle types:

1. Place rifle into vessel in place where it is secure and protected from water spray, wash and in preferably a secure case i.e. lockable pelican case.
2. 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 being used can act as the shooter]:
   a. Attach barrel to biopsy rifle stock
   b. Insert dart into barrel
   c. Load blank cartridges into magazine and insert magazine into rifle (PAXARMS) or screw in CO_2 cannister (Dan-inject).
3. Use biopsy rifle only from the bow of the vessel, ensure crew remain behind shooter at all times. 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 5).

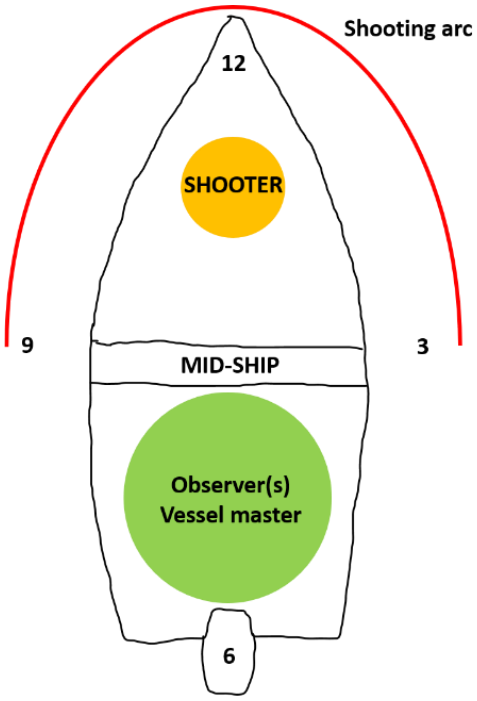

Figure 5 Overhead schematic of how personnel should be positioned on the survey vessel and the shooting arc.

4. Before darting, cock the rifle, and ensure safety catch is on.
5. 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 animal, below the dorsal fin (see Krützen *et al.*, 2002). Only fire when safe to do so for both the target animal, and people in the vessel.
6. After a darting attempt, place rifle in a safe, dry place and recover biopsy dart using a hand-net (ideally this is by someone other than the shooter).
7. Remove sample from dart and label clearly and store sample away from heat (if possible in esky for samples).
8. If sampling more than one animal, repeat from step 1 with sterilised biopsy dart.
9. Between sampling events ensure there is no dart in the rifle chamber.
10. When all sampling has been completed with the cetacean group, disassemble the rifle and return it to its case and a safe dry location on the vessel.
11. Clean and store rifle according to manufacturer's instruction at the end of survey day. Store biopsy rifle in rifle case, ensuring no blank cartridges are left in the rifle. 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 the following precautions are used (as described above in Section 4.3): safety catch is used, crew stand behind the person using the rifle, the rifle is stored in an appropriate case when not in use. If the dart completely misses an animal, more attempts can be made if the animal shows no signs of behavioural changes (i.e. vessel avoidance or other reaction) and can still be approached. Further, if any animals in the group (incl. 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).

4.4 Sampling technique

Biopsy sampling can only be conducted in suitable sea conditions (no rain, light winds and seas less than 1.0m i.e. equivalent of Beaufort Sea State [BSS] ≤ 3, Table 1) and should be undertaken from small vessels (generally <7m). Speeds should be reduced to no wake or neutral particularly when close to groups (e.g. within 50m). The animals are aware of the vessel when the motor is running, so it should not be turned on and off when interacting with the group. Sampling should only be attempted when animals are between approximately four and 20 metres from the vessel and the pressure adjusted on the rifle accordingly. Photographs of the dorsal fin of the animal(s) to be sampled will be taken before and during sampling for the purpose of photo-identification of individual animals 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 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 SOP No: TBA Vessel-based Cetacean Surveys Using Photo Identification for detailed instructions on photographing cetaceans and data collection.

The team leader will make the decision on when a cetacean is suitable for sampling. This will be based on cetacean behaviour, group demographics as well as sea state and other safety considerations. Only cetaceans in healthy condition (assessed by the appearance of the epaxial muscle mass on either side of the animal's dorsal fin, as in Pugliares *et al.*, 2007) are to be sampled; animals appearing emaciated (skinny) or in poor body condition are not considered suitable. Calves will not be sampled and will be differentiated based on size (<2/3 the length of the mother [i.e. 1.5m for dolphins]) and position in relation to the mother. i.e. dependent calves return frequently to the tailstock region of the mother (Mann & Smuts 1999). Weaned calves (i.e. young juveniles) may be considered suitable to sample.

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.

When a sighting has been made and the animals are deemed appropriate for sampling, the shooter should prepare the rifle and dart as described above, and the observer prepare the camera. Photo identification can commence prior to biopsying, but once attention has been turned to the sampling event, the photographer must move to behind the shooter. The vessel should approach the animal(s) at a speed reduced to no wake or be in neutral and position the vessel to within 5-20m of the cetacean as it surfaces, giving the shooter the best opportunity to fire the dart. The photographer should be prepared to photograph the dorsal fin of the animal that is to be darted at the same time the dart is fired.

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 animal, penetrate to a stop (generally 4-5mm into the blubber layer), then bounce off and float at the water/sea surface for recovery. In some whales, the probability of collecting a sample including skin and blubber increases if the angle of impact of the dart is perpendicular to the body surface, but this is less critical when the darts are extremely sharp (Barrett-Lennard, Smith & Ellis 1996). Once the animal(s) is clear of the vessel, the dart is recovered using a hand-net; the sample is extracted using sterile forceps and stored in a small vial containing the appropriate preservative for the purposes of the research question(s) and a label 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 (e.g. Prince Regent River) (Figure 6 & Figure 7). DMSO (Dimethyl sulfoxide a salt saturated solution) or 100% ethanol for genetic

molecular analysis), freezing for stable isotope analysis (if possible –80°C freezer if portable one is available) dry ice is a suitable alternative for short periods of time but decays rapidly in warm conditions. A data sheet is completed for every biopsy attempt that includes information on the date, time and location of the sighting; group composition group composition, age class and other information of interest on the group and the behavioural response of the animal(s) to the biopsy attempt (see example Appendix 1, explained further in section 4.5 Data recording). Sterile latex gloves are worn when handling the darts and biopsy heads, to minimize the risk of infection to both the dolphin and observer and cross-contamination by DNA/RNA from handling or other samples. Gloves will be changed between sampling events/animals.

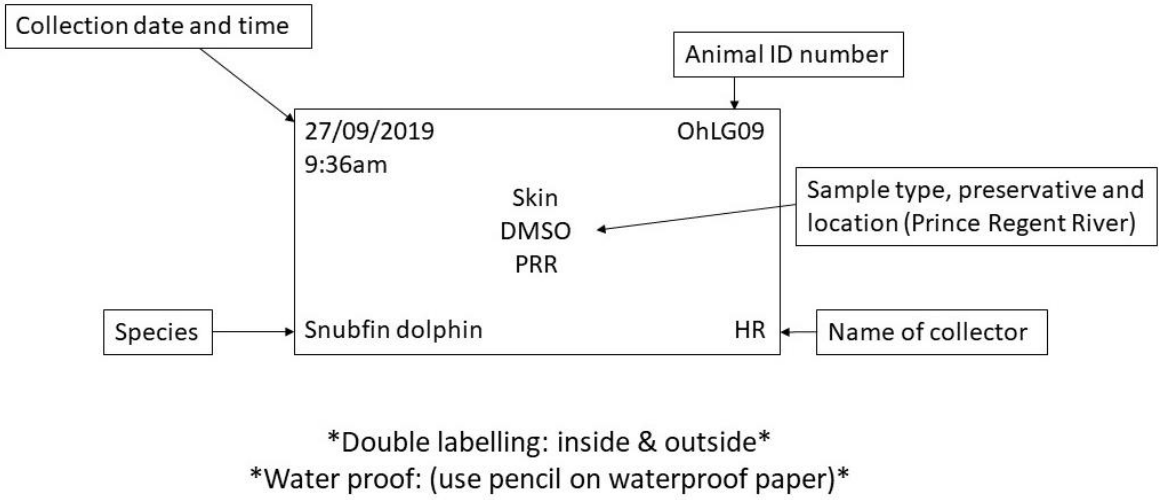

Figure 6 Example sample label showing the information to be recorded with each biopsy sample.

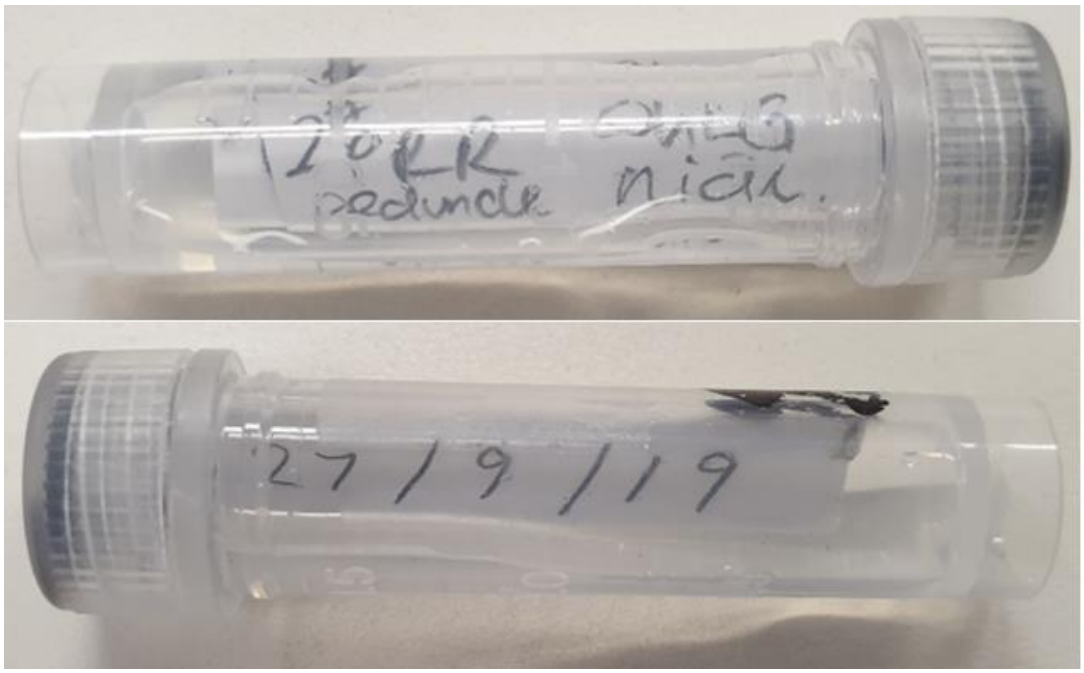

Figure 7 Small vial (approx. 4.5 cm length) containing biopsy sample, appropriate storage solution and label. Label includes; sample number taken that day (#2), species (Oh = *Orcaella heinsohni*), area being sampled (LG = Lalang-garram Marine Park, PRR = Prince Regent River), individual's identifying feature (peduncle nick), date sampled (27/9/19).

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. Sampling will not be attempted on calves (must be >1.5 metres for dolphins and not swimming in baby position at the tailstock region of the mother). 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 animals. 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 animal, aiming at the body areas lateral to the base of the dorsal fin. The pole is held at an angle of between 60˚ and 90˚ to the water surface, depending on the sampling vessel. A constant speed of 2-6 kn will be maintained during sampling (Bilgmann *et al*., 2006).

The biopsy pole is most successful when used on individuals riding the pressure wave at the bow of the vessel. 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.

4.5 Data recording

The observer is responsible for filling out the datasheets as the shooter and vessel driver are focussed on the animals. 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 animals 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 animals, note the frame numbers on the camera using the playback display panel on the camera. The level of response of the sampled animal 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 good back up of the datasheet.

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. 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 on to the server. If operating multiple vessels, the folder should include the vessel name (e.g. YYYYMMDD_vessel name). Refer to SOP No: TBA 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 then 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. All biopsy sampling equipment (dart heads, probes, forceps etc.) should be sterilised as described above (Section 4.3) and appropriately stored ready for the next day of sampling.

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 (Dimethyl sulfoxide) but a copy of the MSDS is recommended. Biological samples stored in small amounts (no more than 30ml 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.

5 Competencies and Approvals

DBCA personnel undertaking cetacean biopsy sampling will need to satisfy the competency requirements (Table 2) to ensure they have the knowledge and experience required to minimise any potential risk of injury to people, animals and property.

Table 2 Competencies for personnel involved in cetacean biopsy sampling

Competency Category	Competency Requirement	Competency Assessment
Shooter	Minimum current corporate firearm licence (Category A, B and C3)	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 biopsying 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.

6 Occupational Health and Safety

Extreme care should be taken when using firearms on a vessel. When attempting to biopsy

sample, ensure a job safety analysis has been approved for the work and all personnel involved are familiar with it. Follow the safe rifle procedures for working on a vessel outlined above (Section 4.3). 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 recommended 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, like 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.

If DBCA personnel or volunteers are injured, a First Aid Slip and "Incident and Near Hit Notification" form must be completed within 24 hours and forwarded to the employees' manager, appointed district safety officer and Health and Safety Section.

6.1 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), latex gloves.

6.2 Manual handling and working with ropes

Manual handling and working with ropes can be an integral part of working with small boats with detachable outboards and there is a high risk of injury (muscular or back). Further advice is available to DBCA personnel by accessing Departmental guidance notes on manual handling.

6.3 Working around the water's edge and from vessels

PDF type 1 Life jackets must be worn by those working on vessels. Refer to Department of Biodiversity, Conservation and Attractions Corporate Policy Statement No. 84 Boating.

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.

7 Further Reading

The following documents have been mentioned in the advice regarding cetacean biopsy surveys. It is recommended these documents and others listed below are also considered when proposing to undertake remote biopsy sampling.

- SOP:Vessel-based Cetacean Surveys Using Photo Identification (In prep).
- Murdoch University Animal Ethics Committee Standard Operating Procedure SOP (0100 - 03): Wildlife (Marine) - Remote biopsy sampling of cetaceans available https://www.environment.gov.au/system/files/consultations/78209f29-2e46-

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- Department of Parks and Wildlife Corporate Policy Statement No. 84 Boating
- Department of Parks and Wildlife Boating Code of Practice 2016
- Department of Environment and Conservation Boating Guidelines 2012
- *Firearms Act 1973*
- *Firearms Regulations 1974*
- *Occupational Safety and Health Act 1984*
- *Occupational Safety and Health Regulations 1996*
- *Biodiversity Conservation Act 2016*
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9 Glossary of Terms

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

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.

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

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.

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.