THE UNIVERSITY OF NEW ENGLAND ANIMAL ETHICS COMMITTEE (AEC)

STANDARD OPERATING PROCEDURES FORM
(For Domestic Fowl, Native Fauna/Wildlife, Domestic Livestock & Laboratory Animals)

Tissue sampling – W20

Objective:
Tissue sampling involves collecting a small sample of tissue and storing it in a suitable manner with the aim of performing DNA testing.

Details of Procedures:
Materials:
The following equipment is needed to undertake tissue sampling:

- Surgical scissors
- 2mL plastic ring tubes with 80% ethanol
- Alcohol proof labels (Laser printed or pencil on water proof paper)
- Forceps
- A vial of 95% ethanol and cigarette lighter or portable blow torch for flaming or sterilising solution
- Paper towel
- Betadine

Animal handling:

If an animal is seriously injured during handling, seek veterinary care.

Cleaning and sterilizing

All equipment used to cut, file, or incise will be cleaned and sterilised between each animal and prior to returning the equipment for storage.

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

Flaming:

1. Clean scissors with moist paper towel to remove dirt and any leftover tissue etc.
2. Dip the equipment to be used for cutting or filing in ethanol and flame the cutting/filing part with a lighter or portable flame torch. Note: the flame from ethanol is not visible in sunlight. Ethanol is a highly flammable substance. Care will be taken to not get ethanol on anything other than the equipment needing to be flamed. Ensure a clear workspace and that the ethanol container is in a stable position and unlikely to be knocked over. Clean up any spillages immediately,

including any ethanol on hands and clothing, and if required wait until the spilt ethanol has evaporated before continuing with the procedure.
3. Allow the equipment to cool before using it on an animal.
4. DO NOT allow contact with anything else before the next animal is sampled.

Procedure:

1. Turtles will always be held immobile at the bridge between the carapace and the plastron, or held by tail end, palm on plastron and thumb on carapace. Turtles have very sharp and powerful jaws, so keep hands and fingers clear of the head region.
2. Using the sharp scissors, cut a thin sliver of skin 2mm x 4mm from the trailing flap of the vestigial toe of the rear foot.
3. The depth of the skin will depend on the size of the turtle but will aim to incorporate ~3mm of soft skin.
4. Place skin into ring tube with 95% ethanol (Hodges, Donnellan, & Georges, 2014).
5. If bleeding occurs, apply pressure with a dry gauze swab until the bleeding stops and rinse the notch with Betadine.
6. The sample label number will be recorded against the animal's identification and details
7. The sample will be kept cool and placed into -80^{o}C freezer as soon as practical.
8. A genetic sample will only be collected once from an individual animal.
9. This procedure will take a maximum of 2 minutes.

Drug, Chemicals or Biological Agents:

A betadine swab will be used to wipe an area if bleeding occurs.

Care of Animals after the Procedure:

Animals will be returned to the same body of water from which they were captured. Animals will be kept at a comfortable temperature that reflects their natural habitat, out of direct sunlight and only handled when necessary.

Qualifications, Experience, Skills or Training Necessary to Perform this Procedure:

The ability to take appropriate samples will require some skills to be developed and this will be gained through instruction from an experienced person before the procedure is performed.

Effects of Procedure on Wellbeing of Animals:

The procedure will stress the animals being tested so care will be taken to minimise this effect by handling animals as least as possible and minimising time spent taking samples.

References:

Hodges, K., Donnellan, S., & Georges, A. (2014). Significant genetic structure despite high vagility revealed through mitochondrial phytogeography of an Australian freshwater turtle (*Chelodina longicollis*). *Marine and Freshwater Research*, doi.org/10.1071/MF14102.

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