



guidelines and conditions for
marine reptile

strandings, rehabilitation & release
in new south wales



NSW
NATIONAL
PARKS AND
WILDLIFE
SERVICE

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**Guidelines and Conditions for
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in New South Wales**

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1. Introduction

1.1 Standards for the care of marine reptiles

Marine reptile welfare incorporates issues such as rescue, care, rehabilitation and release. At times, marine reptiles may require assistance as a result of disease, injury (often associated with boat strike or fisheries activity), or marine pollution. From a welfare viewpoint, the primary aim of wildlife rehabilitation is to return each individual to the wild population with optimum chances of survival. In the case of high profile and vulnerable species like marine turtles, it is vital to set and maintain the highest standard of care. The length of time a marine reptile is held, the means by which it is held and the place of release are crucial factors. Beyond these principles, there are many other factors that need to be addressed when managing marine reptile strandings.

These guidelines then represent best practice procedures to be undertaken by NSW National Parks and Wildlife Service (NPWS) staff when assisting with, or co-ordinating the rescue, rehabilitation and release of marine reptiles.

1.2 Legislation and policy framework

The NSW National Parks and Wildlife Service (NPWS) holds statutory responsibility for all protected species under the *National Parks and Wildlife Act (NPW Act) 1974* and for all threatened species listed on Schedules I and II of the *Threatened Species Conservation Act (TSC Act) 1995*. Under these Acts it is an offence to harm any species of marine reptile found in NSW. The definition of “harm” in the legislation includes hunt, shoot, poison, net, snare, spear, pursue, capture, trap, injure or kill.

The *NPW Act* also imposes restrictions on holding protected fauna, for the purpose of rehabilitating an animal that is not capable of fending for itself. Individuals or organisations that are not licensed to rescue, hold and rehabilitate protected fauna, are required under the *NPW Act*, to notify the Director-General of NPWS, in writing, within seven (7) days, if they come into possession of a sick, injured or orphaned protected species. Such organisations or individuals are required to comply with any direction given by the NPWS.

In most situations, directions will be given by the NPWS, to immediately pass the animal to a licensed marine reptile rehabilitation institution,

where these are available. A list of institutions currently licensed (as of October 2002) to handle marine reptiles is provided in Appendix 1. The NPWS Coordinator, Wildlife Management will provide NPWS Regions with an annual update of this list.

NPWS policy on the rehabilitation of fauna (Appendix 2) further outlines the role of the NPWS in the licensing and supervision of persons and organisations involved in the rescue, care and rehabilitation of sick, injured and orphaned protected animals and their release, or retention in captivity.

The rehabilitation of marine reptiles requires specialised care and holding facilities, which are not usually available to most wildlife carer organisations or licensed individuals. The NPWS will only issue licences for the rehabilitation of marine reptiles to organisations or carer groups that can demonstrate they have the appropriate facilities and expertise for this purpose. Rehabilitation organisations can apply for endorsement of their existing licences to authorise rehabilitation of marine reptiles. Such applications will be considered on their merits, on a case by case basis. This may include an inspection of facilities by NPWS officers and an assessment of the level of care and expertise available.

Some species of marine reptiles are listed on the Schedules of the *Threatened Species Conservation Act (TSC Act) 1995*. The NPWS has specific responsibilities for the protection and recovery of threatened species, including the development of recovery plans. There are currently no approved recovery plans for threatened NSW marine reptiles. However, a draft recovery plan for marine turtles listed as Threatened under the *Commonwealth Environment Protection and Biodiversity Conservation Act (EPBC Act) 1999* has been prepared by Environment Australia (1998). The NPWS Coordinator, Wildlife Management will undertake to review these guidelines when the relevant recovery plans are prepared.

Under the *Animal Research Act 1985*, fauna in a research program is the responsibility of the researcher. It is in the interests of the researcher to notify local institutions of the nature of the program and to nominate contacts and contingency actions should an animal in the program be found sick, injured or in a dangerous

situation likely to result in injury. If a sick or injured animal is identified as a research animal, every effort should be made to contact the researcher either directly or through the local NPWS Regional or Area Manager prior to undertaking a rescue (Appendix 3).

1.3 Marine reptile species in NSW

Seventeen species of marine reptiles have been recorded from the coast or coastal waters of New South Wales (5 turtle species, 11 sea snake species and 1 sea krait) (Cogger, 2000). Four of these species may be regarded as regular visitors. They are the Green Turtle (*Chelonia mydas*), Loggerhead Turtle (*Caretta caretta*), Leathery or Leatherback Turtle (*Dermochelys coriacea*) and Hawksbill Turtle (*Eretmochelys imbricata*). The NSW populations of these species are likely to represent significant proportions of their eastern Australian stocks (Cogger, 2000). NSW records of other species, such as the Flatback Turtle (*Natator depressus*) currently appear to represent vagrant individuals which stray southward with the assistance of the eastern Australian current, especially in warmer months (Cogger, 2000, Dr Col Limpus pers. comm. Queensland Parks and Wildlife Service).

2. Marine Turtles

2.1 Marine turtle species in NSW

There are seven species of marine turtle worldwide, six of which occur in Australian waters (Appendix 4). One of these species, the Flatback (*Natator depressus*), is endemic to Australian tropical continental shelf waters while the other five have global distributions that include breeding and foraging populations within Australia (Limpus, 2000). Because of this global distribution and identified genetic variation, NSW populations of marine turtles are generally considered to belong to a single eastern Australian stock for each species.

Five marine turtle species have been recorded in NSW. Three of these species (the Loggerhead, Green and Leatherback) are listed as Threatened on the NSW *TSC Act 1995*. The Hawksbill and Flatback turtles are not threatened, but protected species in NSW waters (Table 1). All five species are listed as Threatened under the Commonwealth *EPBC Act 1999*.

2.2 Basic Biology and Ecology¹

Young marine turtles drift and feed in the open ocean before settling in inshore feeding grounds. As adults, they can migrate large distances between feeding areas and nesting sites. Animals grow slowly and may not reach sexual maturity until 30-50 years of age. Mature turtles may breed over several decades, often with two to seven years between breeding events.

Marine turtles may migrate up to 3000 km between feeding grounds and breeding sites. Nesting females typically return to the same breeding site each season and may mate with more than one male in shallow waters near the nesting beaches. After mating, males return to the feeding grounds and females congregate near nesting beaches prior to nesting.

When ready to lay, females haul out on beaches and crawl to above the high tide line typically within an hour of the night high tide. They will then excavate a pit using their flippers and lay a clutch of eggs. Each clutch may contain up to 100 white, spherical eggs. After laying, the female fills the pit with sand using its flippers and returns to sea. Females often return to the same beach to lay consecutive clutches at one to two weekly intervals.

Incubation time and sex of hatchlings is dependent on sand temperature, which can be a factor of colour of sand and beach angle. Female hatchlings occur at warmer temperatures and hatch in seven to eight weeks. Cool temperatures result in predominately male clutches that take longer to hatch. Hatchlings typically emerge from the nest at night and head for the low elevation horizon of the ocean. They can become easily disoriented and attracted to bright lights, which can be a large factor in hatchling mortality. Mortality rates for recently hatched turtles are high, as a result of predation by crabs and seabirds, on the beach and sharks and fish once at sea.

2.3 Conservation and Management

Marine turtles are recognized both nationally and internationally as species of concern. Five species found in Australian waters are listed as

¹ Information in this section has been sourced from 'Marine Turtles in Australia' published by Environment Australia, and provided by Dr Colin Limpus, Queensland Parks and Wildlife Service.

Table 1: Marine turtle species recorded in NSW and their conservation status

Common Name	Species Name	NSW Conservation Status	QLD Conservation Status	Commonwealth Conservation Status
Loggerhead Turtle	<i>Caretta caretta</i>	Endangered	Endangered	Endangered
Green Turtle	<i>Chelonia mydas</i>	Vulnerable	Vulnerable	Vulnerable
Hawksbill Turtle	<i>Eretmochelys imbricata</i>	Protected	Vulnerable	Vulnerable
Leatherback Turtle	<i>Dermochelys coriacea</i>	Vulnerable	Endangered	Vulnerable
Flatback Turtle	<i>Natator depressus</i>	Protected	Vulnerable	Vulnerable

endangered by the *International Union for the Conservation of Nature (IUCN)* in their *Red List Categories (IUCN 1994)*. In addition, all species found in Australian waters are listed under the *Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES)* and are considered a priority for conservation under the *Convention on the Conservation of Migratory Species of Wild Animals (the Bonn Convention or CMS)*.

Marine turtle face a variety of threats. These include marine pollution, habitat loss, entanglement in fishing gear, predation of eggs and hatchlings by foxes, feral pigs, dogs and goannas and over-harvesting of turtle eggs by humans. The draft recovery plan for marine turtles in Australia (Environment Australia 1998), states that there is insufficient data on abundance and distribution to assess the national conservation of species in Australian waters. It is therefore critical to gather information where possible on population parameters and threatening processes to improve our understanding and management of these species. Rescue and rehabilitation of animals and collection of information on these activities can play a vital role in developing and assessing management strategies.

2.3.1 Turtle Tagging Program

Turtle tags are commonly used as a research and monitoring tool to identify individuals over time. This can provide important ongoing information on population parameters such as population size, distribution, movement patterns, individual longevity and reproductive strategies. Tagging programs may be critical for the assessment and management of populations and species such as

marine turtles, which are migratory, long-lived and face a number of on-going threats.

The Queensland Turtle Research Project, initiated in 1968, has used various tagging methods to undertake long term ecological and migratory studies on several threatened species of marine turtle. This project has provided invaluable information to improve management strategies for these species.

The type of tag used in such studies is related to the purpose of the study. Where long term information is required on animal movement and reproductive status, standard titanium turtle tags are commonly used. These tags are non-corrosive and can effectively last for many years. Such tags have a unique alphanumeric code clearly visible on one side and a return address on the other side.

Whenever a turtle is sighted in NSW with any type of tag the following actions should be undertaken:

- Record the date and exact location of the sighting, general condition of the turtle, tag number and return address (if noted) on the tag
- Forward the information to the local NPWS Area Office (Appendix 3) with contact details of the observer
- The responding NPWS officer will enter details of the sighting into the Marine Fauna Database component of the NPWS Wildlife Atlas, and
- The responding NPWS officer will also provide the Coordinator Wildlife Management with a copy of the information

Tags should not be removed from a turtle unless the animal is dead. In this instance:

- Remove the tag and return it to the address noted on the tag with information on the location where the turtle was found and reason (where known) of its death
- Forward the information to the local NPWS Area Office with contact details of the observer
- The responding NPWS officer will enter details of the sighting into the Marine Fauna Database component of the NPWS Wildlife Atlas
- The responding NPWS officer will provide the Coordinator, Wildlife Management with a copy of the information

The NPWS Coordinator, Wildlife Management also issues standard titanium turtle tags so that all marine turtles rehabilitated in NSW are tagged prior to release. These tags have an 'NS' prefix to the tag number and a NPWS return address.

Tags are available upon request from the NPWS Coordinator Wildlife Management or from several other locations in NSW (Appendix 5). Only individuals experienced in tag application techniques will be approved to tag marine turtles. Refer to Section 2.8.4 of these guidelines for details on tagging and data collection.

The NPWS Coordinator Wildlife Management will maintain a database of information on tagged turtles within NSW, including animals tagged and released and subsequent sightings. The NPWS Coordinator Wildlife Management will provide this information annually to Dr Col Limpus managing the Queensland Turtle Research Project and to other research scientists or conservation agencies when requested.

2.4 Marine turtle nesting in NSW²

Most marine turtle nestings occur in Queensland (up to thousands each year). However, successful nestings have been recorded in NSW for Loggerhead, Green and Leatherback Turtles.

Most turtle nests recorded in NSW are Loggerhead Turtles on the far north coast. However, a number of Green Turtle nestings have also been recorded, with one successful nesting known as far south as Corindi Beach, near Coffs

Harbour. Other nesting attempts are also known from Newcastle.

Leatherback Turtles rarely nest in Australia (perhaps fewer than 40 recorded in total), but successful nestings have been recorded near Lennox Head and Ballina in 1993. A further nesting of Leatherback Turtles was recorded at Forster in 1995, but this nest did not survive, apparently because climatic conditions were too cold. Any nestings sighted for this species are considered to be very significant.

Nestings are often reported by the public and are usually identified by:

- the sighting of a nesting female
- by the tracks left shortly after nesting, or from
- the visual emergence of hatchlings

Exposed nests may also be located after storm events or heavy seas.

2.4.1 Responding to reports of nesting

Any observations of nesting marine turtles should be reported to the local NPWS Office. If a nest is reported, it is important that the nesting event be accurately recorded. This information is particularly useful in assessing the population distribution and ecology of a species.

An authorised NPWS Officer should investigate all reports of nesting and undertake the following actions:

1. Mark the nest site with a discrete marker placed immediately adjacent to (but not over) the nest site. The marker should identify the precise locality of the site (within 50 cm) and should be readily recognisable to someone searching for the nest, while discrete enough that it does not attract attention to the site.
2. Marking the nest site in such a manner is crucial because tracks left after nesting will quickly disappear with the tide and wind and finding the nest thereafter can be extremely difficult.
3. Record (wherever possible) for each nesting site data on the following parameters:
 - Species
 - Locality of nest site
 - Nesting date
 - Hatching date
 - Clutch size
 - Number eggs successfully hatched

² Information in this section was provided by Lance Tarvey, NSW NPWS.

- Number of dead or infertile eggs
 - Sand temperature.
4. Enter these details into the Marine Fauna Database component of the NPWS Wildlife Atlas.

While it may be difficult to record all of the above parameters (e.g. if the nest is discovered at, or soon after hatching), it is important to record as much information as possible.

If visual observation of a nesting female is not possible, the configuration of tracks left on a beach can assist with identifying the species of marine turtle. As a rule of thumb, Loggerhead turtles have an alternating gate on the up and down track. The track is approximately 1m-1.2m wide. Green turtles have a breast stroke style gate (flipper marks even on both sides) which is approximately 1.2m-1.5m wide. Leatherback turtles also have a breaststroke gate, which is approximately 2.5m wide.

Clutch size can only be determined by a detailed examination of the nest. However, only appropriately licensed persons should carry out this type of invasive form of examination.

Sand temperature should be measured periodically, (on an opportunistic basis where possible) throughout the incubation period. This can be done by digging a hole adjacent to the nest (not in the nest), to the level of the middle of the clutch (approximately 50cm), and inserting a digital thermometer probe. Date and time of reading and location, should be recorded along with the temperature.

Nesting sites should be monitored, where possible, for several weeks after egg laying has been noted. Females typically lay several clutches of eggs in a breeding season at two weekly intervals and will often return to the same beach to lay subsequent clutches.

2.5 Rescue³

Marine turtles come ashore for a variety of reasons, including to nest, bask on the sand, or because they have been caught on a sandbank or beach after a large tide or storm. Under these conditions, animals are likely to return to sea at next high water, if not sooner. However, in most

instances when marine turtles are found ashore in NSW, they are likely to be suffering from stress, cold water-stunning, injury, disease or entanglement.

Members of the public who find a marine turtle ashore should immediately contact their local NPWS Area Office (Appendix 3).

NPWS officers responding to reports from the general public of a marine turtle ashore (whether it is healthy, sick or injured) should complete an initial report form (MF/0 in Appendix 7) and:

- Note the animals location and condition
- Inform members of the public, or private wildlife rehabilitation (who do not possess the appropriate licence to care for marine turtles) not to attempt to capture or transport the animal
- Visit the site as soon as possible or direct another authorised organisation to attend, with proper catching, restraining and transport equipment and
- Notify the Coordinator, Wildlife Management

Figure 1 provides a quick reference guide to the procedure for marine turtle incidents.

If the incident occurs in the vicinity of a Marine Park, the appropriate Marine Park officer should also be notified (Appendix 3). Where an animal has been taken to a rehabilitation facility, aquarium or veterinarian, the local NPWS office should be contacted as soon as practicable.

The responding local NPWS officer should consider the following questions prior to undertaking any 'rescue' of a marine turtle:

- Is there a need for intervention, i.e. to take into care for veterinary assessment, treatment, rehabilitation or relocation?
- Does the animal require veterinary assessment?
- Is it able to be rehabilitated?
- Are facilities available for rehabilitation?
- Is euthanasia the best welfare option?

In situations where the animal is obviously sick or seriously injured, it is recommended that veterinary assessment be obtained to determine the probability of a successful rehabilitation attempt prior to rescue. Animals suffering from serious injuries or diseases may require euthanasia (refer to section 2.6) or intensive care and a long period of rehabilitation prior to release.

³ Much of this section has been derived from, Limpus (1998) and Kelly and Gordon (2000).

The Coordinator, Wildlife Management will assist local NPWS officers should there be a need to seek veterinary assistance from the Veterinary and Quarantine Centre at Taronga Zoo or other experts based in Sydney.

The decision to euthanase a turtle or take it into care should be made by the Area or Regional Manager or authorised delegated officer, upon advice from a veterinarian and (if practical) in consultation with the Coordinator, Wildlife Management. A record of the decision and reasons should be noted on a marine fauna incident report (MF1 and/or MF/2 Appendix 7), entered into the Marine Fauna Database and forwarded to the Coordinator Wildlife Management.

2.5.1 First Aid

In some instances there may be an extended period of time between the finding of a turtle and the arrival of an authorised authority.

Actions the public can be directed to undertake prior to the arrival of the appropriate authorities at a site is to:

- Erect shade over the animal, if it is a warm day
- Cover the turtle's head and carapace with damp towels, being careful not to obstruct the nose and mouth
- Minimise unnecessary movement around the head of the animal and
- Observe the animal for signs of breathing and movement (respiration may be slow and irregular)

These actions and information will greatly assist authorities on their arrival.

2.5.2 Turtle ashore – Considerations prior to intervention

The decision to intervene at a turtle stranding can often be made on specific physical and behavioural characteristics exhibited by the animal.

The types of behaviour that may assist the appropriate authority decide whether a marine turtle requires assistance are as follows:

- Bleeding from mouth, nose or vent
- General lethargic behaviour - a healthy turtle will vigorously try to move away from an observer, juveniles especially will slap their flippers against their carapace when

approached

- Absence of a corneal reflex – a healthy response would be retraction of eyeball when the corner of the eye is touched
- Absence of breathing – breathing is detected as intermittent movement in area of the throat, or slight raising of the head
- Absence of movement and body/limb reflex when prodded (gently). The back of the neck, forward of the carapace is sensitive area for turtles. Gently touching this area should cause the turtle to lift and/or retract its head
- Emaciation – sunken flesh around the base of the flippers, neck or plastron (belly plates); loss of stream-lined shape; sunken eye sockets (often indicates dehydration)
- Trauma – head injury, loss of limbs, lacerations or damage to the carapace as from boat strike, propeller cuts or predation attempts
- Dense cover of external parasites and commensal species - for example, barnacles, copepods, marine leeches, algae can all be found on healthy animals but can be greater in number if animal is inactive for long periods
- Entanglement – fishing lines, rope or other debris embedded in skin or around carapace
- Fibropapillomatosis (FP) – wart like growths, ranging in size from 0.1 to more than 30 cm in diameter anywhere on skin, carapace or plastron. While these growths may or may not be a cause of ill health, intervention is required due to their potentially infectious character. FP has a worldwide, circumtropical distribution and is more common in Green turtles than in other species
- Ingestion of marine debris or fishing gear – indicated by foreign materials extending from either the cloaca or mouth
- Ulceration – presence of large, open ulcers on the flippers and head
- Inability to swim properly or dive below the water's surface for prolonged periods.

Further, a marine turtle can become overheated if ashore for an extended period in warm air temperatures without water or shade.

2.5.3 Swim-test

If the animal displays none of the above conditions and a need to capture for assessment and treatment is not apparent, NPWS staff could, where possible, return the animal to shallow water (minimum 1m depth). If the animal swims away

rapidly in a fairly straight line, dives and stays submerged for several minutes, it can be considered 'healthy'.

If a marine turtle, once returned to water, exhibits any of the following behaviours, it is appropriate to re-capture it and seek veterinary assessment:

- appear disorientated or swims in circles
- floats near the surface
- moves only feebly or to periodically lift its head to breathe, or
- continually bobs to the surface after short dives, or lists to one side
- turtles returned to water re-beach themselves

2.5.4 Trawled turtles at sea

Marine turtles may become trapped in trawl nets. If they are alert and active, they may be able to swim away after removal from the net. If they appear weak or lifeless, the animals may simply need time to recover and may not require treatment or a period of rehabilitation.

Turtles that appear lifeless are not necessarily dead and if returned to the water before they recover may die.

Fisherman who inadvertently trap a turtle in a trawl net should notify the closest NPWS Area Office as soon as possible, whilst still at sea or after return to port.

The NPWS Officer who responds to an incident involving a trapped marine turtle in a trawl net, will provide the following advice (these procedures may take up to 24 hours):

- Land the turtle on the boat
- Monitor it for activity (breathing or movement)
- If **active** (ie. moving strongly and breathing regularly) gently return the turtle to the water with the engine in neutral where possible and without dragging or dropping the turtle on the deck, as the plastron underneath the animal can be easily damaged.
- If **not active** - keep the turtle on board and
 - ❖ raise the **rear flippers** about 20cm off the deck
 - ❖ keep it shaded and damp
 - ❖ allow to recover for up to 24 hours
 - ❖ If the turtle doesn't become active it may be moribund/weak or dead.

- Contact NPWS on return to port. NPWS will arrange veterinary assessment where appropriate or carcass retrieval.
- Acquire any information on the location of the incident and record any tag numbers that may be on the flippers of the turtle.
- A record sheet for fisherman to use when encountering marine turtles is provided in Appendix 6.

Marine turtles can survive for long periods without an oxygen supply and have been known to recover even after appearing moribund. A plastic tube may be inserted down the animal's throat to open the air passage closed by the glottis at the back of the throat. Regular blowing through the tube, followed by deflation of the turtle by depression can lead to resuscitation.

2.5.5 Hooked turtles at sea

Turtles have been recorded to take commercial drop line hooks, recreational angling hooks and long-line hooks. Ingested fishing tackle can cause perforation of the bowel or chronic bowel obstruction, which can result in death.

If a turtle is hooked, the fishing vessel should be advised to bring the animal on board and cut the line to remove any pressure.

If the hook, sinkers or swivels have been swallowed or the hook is in soft tissues in the mouth, the animal should be returned to port and the local NPWS Area Office contacted. NPWS will arrange retrieval of the animal and, where required, veterinary assessment.

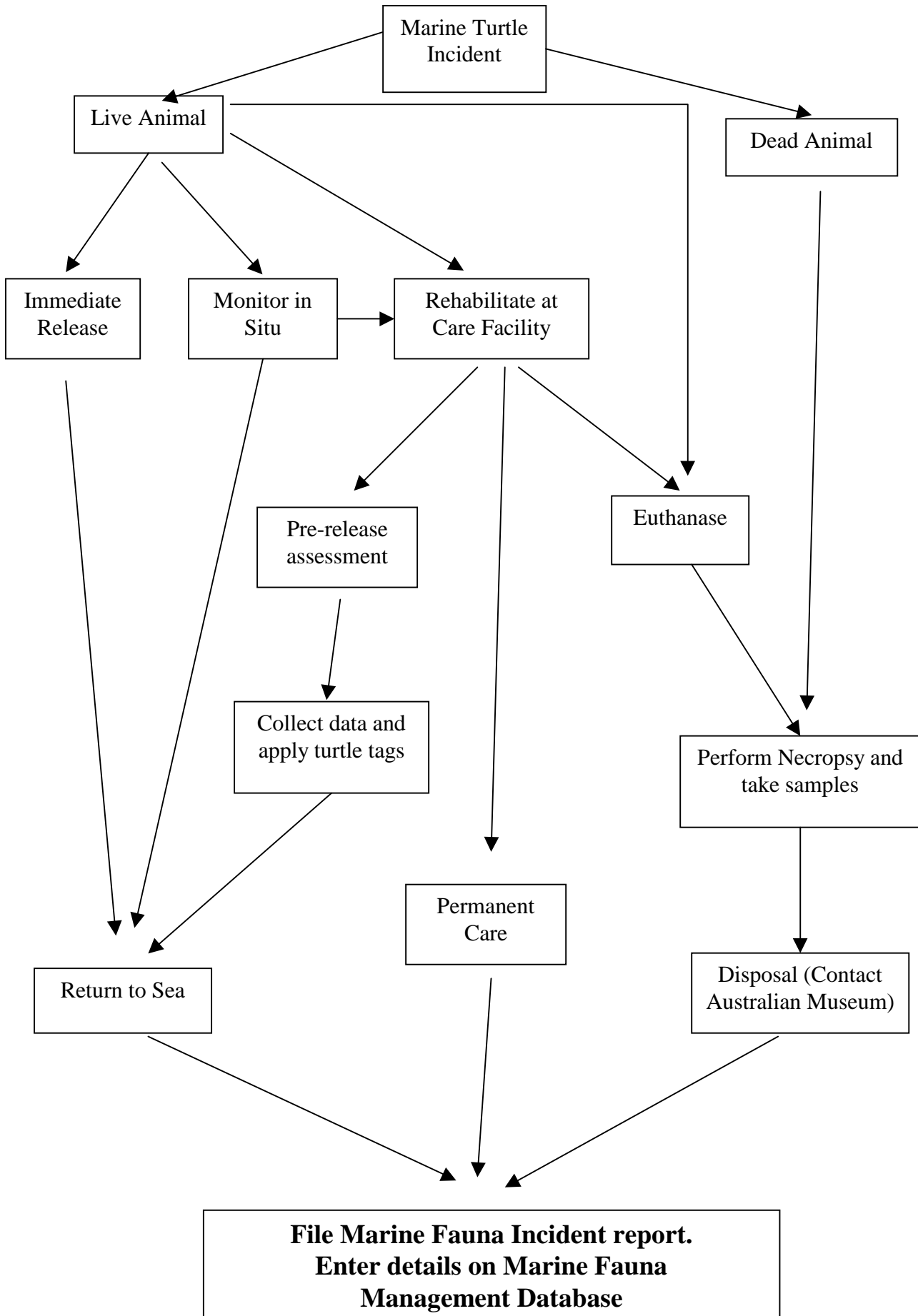
Ingested hooks are unlikely to dissolve.

Hooked animals may have also been trapped underwater for a time. If this is the case, or if the animal is not alert and moving vigorously, then the turtle recovery procedures described above for trawled turtles should be used.

2.5.6 Catching and retrieving stranded marine turtles

Marine turtles can range enormously in size and weight and as a result, catching and retrieving injured animals can either be achieved relatively simply or become a major exercise requiring special equipment.

Figure 1: A quick reference guide to the procedure for handling a marine turtle incident.



- The following actions should be undertaken by the appropriate licensed authorities when dealing with large marine turtles:
- Always assess potential danger to rescuers, e.g. environmental conditions, or handling large or aggressive animals (e.g. be aware of slapping flippers and biting, cuts from scutes and shells on carapace and animal weight when lifting)
- Ask bystanders to stand back and remain quiet. Rescuers should explain to any bystanders what is happening with the animal
- Place a light towel or cloth over the turtle's head to obscure vision – this will reduce avoidance movements and minimise risk of biting. Do not block mouth or nostrils
- The ventral surface (plastron) can be easily damaged if moved across a rough/hard surface. If turtles can not be lifted, they can be dragged by placing them on foam/soft material and a tarpaulin or strip of carpet, to minimize damage to the plastron
- Small animals (up to 10 kg approx.) can be carried by a single person using the carapace as a hand-hold, i.e. grasp carapace at base of neck with one-hand and at rear of carapace above tail; hold animal away from the body so that flapping flippers don't loosen your hold
- For larger animals ranging up to approx. 100kg at least two people will be required to lift an animal. A wheel-barrow; figure 8 rope sling; net stretcher or special purpose turtle harness can be used
- For even larger animals mechanical equipment may be needed. In such instances a cargo net may be used to lift an animal. Care should be taken to ensure the flippers do not become entangled. Animals near the water's edge may be returned to the water and floated onto a boat trailer adapted to carry an animal, rather than lifted directly off the sand
- Avoid unnecessary handling
- Be conscious of possible injuries, such as fractures of the carapace, when handling injured animals

2.5.7 Transport

Marine turtles should be transported and handled in such a way as to minimise stress. Efforts should be made to minimise transport time but, if

extended trips are unavoidable, the animal should be checked on a regular basis.

The following measures must be undertaken when transporting marine turtles, irrespective of the type of transport used:

- Turtles should always be transported in an upright position, i.e. with the dorsal surface (carapace) uppermost
- Turtles should be kept out of direct sunlight, not left in vehicles in the sun and kept away from any direct heat sources (e.g. vehicle engine, exhaust or heater, hot floors of travelling vehicles). Most species are only able to maintain their deep body temperature to within about 3°C above the ambient temperature, and are vulnerable to overheating
- Small animals can be put in a carry-box eg 6-pack cooler or similar properly secured and ventilated container with a damp foam material in the base to protect the plastron
- Larger animals can be transported in any suitable vehicle which allows the animal to lay on its ventral surface and the plastron be protected by a wet blanket/foam; movement to be restrained as best as possible; and airflow/ air-conditioning to prevent overheating
- It is difficult to restrain turtles from moving, so confinement in a box/crate is recommended
- If unable to move the animal out of direct sunlight, turtles can be kept cool by covering them with cloth and keeping them moist
- Turtles will always move toward light so covering the transport box/crate with a dark cloth is recommended
- Loggerheads and hawksbills can become aggressive (and bite) when confined with other turtles, so separation is recommended. Green turtles are not usually aggressive to other turtles

2.6 Euthanasia

Euthanasia is sometimes the most humane act we can provide a stranded or injured animal. The critical decisions for the authorised NPWS officer to consider are whether euthanasia is the most appropriate course of action, what method to use and who shall administer it.

The decision to euthanase a marine turtle will be made by the NPWS Area or Regional Manager or other delegated officer. This decision will be based on advice provided by a veterinarian or other expert individuals and the attending NPWS officer.

There are a number of indications that a marine turtle has a low probability of survival and may require euthanasia.

Indications of a low probability of survival provided by Limpus, (1998) and Kelly and Gordon, (2000) are:

- Compacted contents in gut/digestive tract, which may be associated with extensive gassing in abdominal cavity
- Fishing tackle or lengths of fishing line in digestive tract below stomach which can result in a constricted bowel
- Severed intestine
- Damage to lungs indicated by ruptured lung tissue visible or rasping/leaking noise from hole in carapace (e.g. spear head, gun shot)
- Cracked or missing section of carapace (e.g. boat strike, propeller damage) involving damage to lungs, spine or extensive bleeding
- Disease status, e.g. fibropapillomatosis (FP) and
- Nervous dysfunction – as indicated by head tilt, persistent movement of the eyeball from side to side (nystagmus) and circling or other navigational failures while swimming. Animals with suspected systemic coccidiosis are known to display these symptoms.

2.6.1 Acceptable methods of euthanasia for marine turtles.

Where euthanasia is the appropriate course of action, it *must* only be performed by a veterinarian or other suitably qualified individual. Acceptable methods of euthanasia include lethal injection and gunshot (in accordance with NPWS Firearms Policy 2002). Pentobarbitone sodium administered at an approximate dose rate of 1 ml/5kg Body Weight is a reported successful method for euthanasia of marine turtles (Kelly and Gordon 2000). An effective dose is indicated within a few minutes by spontaneous urination and loss of corneal reflex. This method should only be undertaken by a veterinarian as it can be difficult to locate appropriate injection sites in turtles. Should this method of euthanasia not be considered suitable, a veterinarian or other marine

turtle specialist should be consulted for an appropriate alternative.

The responding NPWS Area officer should record the following details when deciding to euthanase an animal:

- Condition of animal
- Decision process to euthanase
- Attending veterinarian
- Method used and administering individual
- Measurements, tissue sample collection and necropsy should be carried out wherever possible (see section 2.11 and Appendix 8).

These details should form part of the incident response (Appendix 7) and entered into the Marine Fauna Database.

2.6.2 Disposal

When a marine turtle is found dead, has died or has been euthanased, the Australian Museum should be contacted for its interest in the carcass prior to disposal. If the Museum cannot immediately be contacted a number of temporary options may be considered:

- Freeze the animal as whole
- Freeze a tissue sample of the animal (as described in Section 2.11 and bury the rest at an established 'graveyard' or suitably identifiable site in the field to be exhumed at a later date. The burial location can be entered into the Marine Fauna Database.

If the Australian Museum does not want the specimen, contact the Coordinator, Wildlife Management to see if any other authorised research institution requires the animal.

If the animal(s) has been euthanased, or has died from suspected disease like symptoms contact the Coordinator, Wildlife Management to ascertain whether additional tissue samples are required to be made available for further analysis by an organisation such as the Veterinary and Quarantine Centre at Taronga Zoo.

Ensure all relevant data is collected and entered into the Marine Fauna Database prior to disposal of the carcass.

2.7 Ex situ Assessment and Rehabilitation

The goal of long-term care is to improve the health of a marine turtle so that it can be

rehabilitated for release to the wild. Hulst (2000) provides the following information on proper assessment and holding procedures for maintaining marine turtles.

2.7.1 Assessment

- The initial assessment of a marine turtle needs to be thorough, but should be performed with as little disturbance to the animal as possible
- Marine turtles should undergo a full physical examination including an assessment of the animal's underside, inside the mouth and eyes. (Note: unpadding objects should not be used as a gag as the beak can be damaged. Also, never insert fingers into the mouth or across edge of the turtle's sharp beak. This can result in a severe injury)
- A blood sample should be collected and examined to assess hydration and consider metabolic or blood cellular components
- Wounds may be cleaned and dressed or flushed
- X-rays may be taken to assess buoyancy problems, or where there is a possibility of gastrointestinal obstruction. Radiography may or may not reveal the presence of fishing tackle, but will reveal gas and dense faecal accumulations
- Animals should be placed in a bath of freshwater maintained at 25-29°C for 24 hours. This will assist in the removal of epifauna, such as barnacles, from the carapace and marine ozobranchid leeches. The latter are often present in large numbers on turtles with fibropapillomatosis (FP)
- Barnacles should be removed from sensitive areas (e.g. around eyes and inside nostrils) or where heavy burdens may be disabling to the animal's limbs and carapace. Care should be taken to minimise trauma to skin surface. Barnacles may need to be removed progressively over time
- Marine turtles should be weighed at the time of assessment. (Note: 'normal' body weights vary across species and the marine turtle's range)
- Where faeces can be collected it should be examined by flotation to detect parasite eggs
- Marine turtles brought into a rehabilitation facility should be quarantined from other turtles wherever possible
- Where permanent quarantine is not possible, marine turtles should be quarantined until:

- ❖ they have been held in a freshwater bath for 24 hours, to kill marine leeches and other external parasites potentially capable of transmitting infectious diseases and
- ❖ it is determined whether the animal brought in for rehabilitation has, or does not have FP. Turtles without FP should not occupy the same water body as turtles with this disease.

2.7.2 Holding /Housing for Rehabilitation

Institutions licensed to hold and rehabilitate marine turtles must provide and maintain the conditions specified below.

- Turtles unable to swim, or weak turtles that cannot lift their head to breathe should be placed on moist foam pads and covered with wet towels, or placed in a shower box
- Turtles that can swim must be held in a pool that allows plenty of room to swim and dive. Marine turtles can cope well with exposure to freshwater (at the right temperatures) for up to 6 days, but long term (months) exclusion from salt water results should be avoided (Limpus, 2000). Where possible, marine turtles should be kept in salt water. Chlorine can be added at less than 1ppm to reduce bacterial and algal growth but higher levels will irritate the eyes
- Any substrate on the bottom of the tank must be of sufficient size that it cannot be ingested. Gravel should be avoided for hatchlings. Hatchlings may need to be provided with rafting material so they can trap food. This rafting material should not be ingestible. Abrasions from rough sides on cement tanks have been reported
- Water temperature must be maintained between 25-29.5°C. Even though this temperature may be higher than local waters, it is the optimum range for rehabilitation. Fluctuations in temperature should be avoided, necessary changes of more than 1-2° should take place over several days. Prior to release, turtles should be gradually acclimatised to the temperature of local waters.
- Overcrowding can lead to biting among turtles. Loggerhead and Hawksbill turtles will bite other turtles when confined, so separation is required. Green turtles are not usually aggressive to other turtles.

- Food offered in captivity should match the food eaten in the wild wherever possible.

2.7.3 Diet

Immature and adult Leatherbacks are carnivorous turtles specialising in macroplankton such as jellyfish and salps/tunicates. They have sharp edged jaws relying on suction for ingestion. The low nutritive value of the prey items means a large intake is required.

Immature and adult Loggerheads are carnivorous and consume a variety of benthic invertebrates including molluscs, crustaceans, and echinoderms, which they crush before swallowing. They also sometimes eat fish and jellyfish.

Immature and adult Hawksbills are carnivorous, primarily feeding on sponges but also other benthic invertebrates such as, bryozoans, soft corals, echinoderms, molluscs, shrimp, and jellyfish.

Flatback adults consume jellyfish, squid and soft-bodied benthic invertebrates. They have also been fed on prawns and small pieces of fish while temporarily held in captivity.

Green turtle juveniles are pelagic and appear to be omnivorous. At 35-40 cm they begin to be primarily herbivorous, feeding on seagrasses, algae and mangrove fruit. They will also eat plankton both micro and macro such as jellyfish and *Physalia*. Green turtles held in captivity, often accept a more carnivorous diet, including squid. Non-marine vegetation such as romaine lettuce, peas and cabbage has been substituted for sea grasses without apparent adverse effects.

Human contact and the association between humans and food should be minimised when feeding turtles, particularly for animals that are likely to be released. This can be achieved by random placement of food in the enclosure, minimal human presence and no contact between the feeder and the turtle where possible. It has been suggested that feeding turtles at night may be useful for animals refusing food as this may limit visual disturbance and distractions.

2.7.4 Dietary Supplement

Most facilities will supplement captive diet with a commercial vitamin supplement such as Sea Tab®.

2.8 Pre-release considerations for Marine Turtles

When preparing to release a rehabilitated marine turtle, the local NPWS Area office or Marine Park Authority officer (if animal is to be released in Marine Park waters) must be contacted and provided with the following documentation:

- Pre-release assessment report (see 2.8.1 below)
- Record of the release site and environmental factors at the site such as water temperature and feeding habitat (see 2.8.2 below)
- Consideration of other factors at release site (see 2.8.3)
- Record of existing and applied tags or marks, whether temporary or permanent (see 2.8.4 below).

The responding NPWS officer should ensure this information is in order prior to release. A copy of this data should be provided to the NPWS Coordinator, Wildlife Management.

2.8.1 Pre-release assessment

There are four components to the pre-release assessment of a marine turtle. These components are:

1. Individual Record

- Date and time of arrival at rehabilitation facility
- Individual who submitted the animal, and their contact details
- Exact location where animal was found and circumstances for removal from the site
- Weight, length, apparent injuries and physical condition at time of arrival
- Weight pre-release
- Treatment, including medication, administered during rehabilitation
- Description of facilities in which animal was housed; handling and quarantine procedures during rehabilitation and
- Feeding regime during rehabilitation.

2. Pre-release health assessment

The aim of this assessment is to prevent any transmission of disease from the rehabilitated individual to wild populations and must be undertaken by a veterinarian. Investigations should include:

- parasites, e.g. digenean trematodes

- intestinal coccidiosis and
- fibropapillomatosis

Additional assessments should be undertaken at the discretion of the veterinarian. An animal, considered for release must be clinically free of disease or parasites, beyond that ‘normally’ present in wild populations.

3. *Record of prior injury*

If a marine turtle brought in for rehabilitation has some form of prior injury, careful consideration on a case-by-case basis may be required when assessing the animals’ suitability for release. This assessment should consider to what extent, if any, the prior injury may have been implicated in the circumstances that resulted in intervention.

NPWS policy on the rehabilitation of fauna (Appendix 2) states that an animal must not be returned to the wild if it is:

- Handicapped with a permanent disability which will preclude it from leading a normal life and surviving in the wild, or
- Suspected of carrying a serious disease or a disease, which is likely to be transmitted to the detriment of the habitat or population.

The QLD Parks and Wildlife Service have advised NSW NPWS, that in the instance of, for example, damage to, or loss of a flipper, that such animals are present in wild populations. Such injuries can incur both as young animals and as breeding adults. Furthermore, for known females with such injuries, there is no detectable reduction in nesting success or incubation success of the eggs.

The responding NPWS officer will, in the case of a handicapped marine turtle, consult with rehabilitation organisations and veterinarians to determine the most appropriate action prior to release of the animal. Factors to be taken into consideration include:

- Estimated age of any prior injury
- Evidence of the degree to which, if any, the current injury may have influenced the current circumstances requiring intervention
- Sex and age class of the individual and
- Conservation status of the species

4. *Behavioural assessment*

An assessment of behaviour will note the following:

- Capacity to locate and catch prey unassisted
- Capacity for the animal to swim and dive effectively, such that neutral buoyancy below the surface is readily demonstrated.

2.8.2 *Environmental factors*⁴

Prior to release of a marine turtle there are a number of environmental factors, in addition to prevailing sea and weather conditions, that must be considered when deciding upon a release site. These include water temperature, distance offshore and feeding habitat. These factors will vary according to species and age class and are summarised in Table 2.

2.8.3 *Other Release Site Factors*

In addition, there are a number of other factors to be considered prior to releasing a marine turtle. They include:

1. *Conspecifics*

While conspecifics need not be present at release sites, release should preferably occur in habitats where sightings of turtles have previously been recorded.

The NPWS Wildlife Atlas should be referred to when considering release sites. The Atlas is linked to the Marine Fauna Database and is an essential tool for identifying potentially significant feeding habitat and future release sites for rehabilitated or entangled marine turtles.

2. *NPWS Rehabilitation of Fauna Policy*

The Policy states:

In the interests of genetic integrity of native animal populations, a rehabilitated or hand-raised animal should be returned to a suitable natural environment at the locality of the original encounter. An animal should not be transported to a release point across a geographic or physical barrier it would not normally cross.

Release of a rehabilitated marine turtle in the area of the original encounter is typically the most appropriate strategy for most species of marine turtles. However, this policy currently advocates the return of Flatback turtles to Queensland waters. This species is presently regarded as a

⁴ This section is based primarily on advice from Dr Col Limpus Queensland Parks and Wildlife Service

vagrant in NSW, being generally confined to the waters north of Harvey Bay, Queensland (Col Limpus Queensland Parks and Wildlife pers. comm.). Considering the species national conservation status and current lack of knowledge regarding its ability to successfully nest in NSW waters, relocation is considered to most appropriate management response at this time.

For the purpose of this policy the release of stranded and/or rehabilitated individual(s) marine turtles does not trigger the requirement for a translocation proposal.

Release locality specifications for all marine turtles that are likely to occur in NSW species are provided in Table 2.

It should be noted that in the marine environment and for marine turtles particularly, dynamic processes such as local and large scale current systems are more important determinants of dispersal, rather than the geographic and physical barriers applicable to terrestrial organisms.

3. Interstate Release

Written authorisation from the NPWS Coordinator Wildlife Management is required prior to a marine turtle being relocated across the border into Queensland. In addition, prior to transport, QLD Parks and Wildlife Service must provide receipt of this authorisation.

4. Local Factors

In some instances, it will be necessary to release rehabilitated animals away from the original site of capture. This would be considered the appropriate management response in situations where an animal is likely to be exposed to local anthropogenic threats such as the water intake at power stations, high boat traffic areas, shark meshing, trawler operations, marine debris, or pollution incidents.

2.8.4 Identification

Identifying marks on individuals provide valuable information on animal distribution, range and abundance when resightings are reported. Turtle tagging programs have been initiated in many areas worldwide and tagged turtles are often reported.

Actions to be undertaken when finding a tagged marine turtle have been specified in section 2.3.1. Should a turtle with a tag or marking be found,

tag details should be carefully recorded and information on the sighting, data collected and outcome of the incident be reported to NPWS. The responding officer will enter the data into the marine fauna database and forward a copy of the information to the NPWS Coordinator Wildlife Management. As stated, where a turtle tag with a return address that is not NPWS is observed, the report should be sent to the address on the tag. In the case of a dead turtle with a tag, the tag should be removed and returned to the appropriate address along with details of the incident. A copy of this information should also be sent to the local NPWS office for processing.

As stated in section 2.3.1, the NPWS issues standard turtle tags with a NS prefix and return address NPWS, PO Box 1967, Hurstville NSW, so that all marine turtles rehabilitated in NSW waters may be identified. Tags are available through the NPWS Coordinator Wildlife Management and through selected Regional offices (Appendix 3). Applicators are held at several NPWS Regional offices and provided to rehabilitation facilities where required.

Pre-release tagging

All rehabilitated marine turtles should be tagged and/or marked prior to release by trained personnel. If permanent tags, or persons trained to apply such tags, are not available, temporary tags or marks should be applied. It is advised that in addition to the titanium turtle tag, an obvious temporary marking be applied to released individuals so that information on animal survival and movement may be provided by the public. Methods for applying permanent and temporary tags are specified below.

All applied tags or marks, whether permanent or temporary, should be recorded and forwarded to NPWS where they can be lodged in the Marine Fauna Management Database.

1. Permanent tagging

The standard titanium turtle tag is the preferred form of permanent tag.

- Two tags should be applied to each released turtle, one to the trailing edge of each fore flipper
- Tagging should only be conducted by an appropriately trained person, so that no injury or impairment to the animal results. NPWS will train staff to undertake tagging

Table 2 - Environmental factors for consideration prior to the release of marine reptiles.

Species	Carapace Size / Release Location	Feeding Habitat / Release Location	Water Temperature
Loggerhead (<i>Caretta caretta</i>); Green (<i>Chelonia mydas</i>); Hawksbill (<i>Eretmochelys imbricata</i>)	< 30cm release in northern NSW (ie Coffs Harbour northward) off the continental shelf; 30cm inshore in area of capture site given appropriate feeding habitat and water temperature. (above applies to all 3 species)	This will allow animal to be caught in the easterly eddies of East Australian Current (EAC) and be transported into the Pacific basin. Release over or adjacent to seagrass, coastal rock shelves or rocky reefs, preferably in habitats where turtles have been previously sighted.	Minimum 15°C on seabed or reef and rising. If falling, minimum expected temperature should not drop below 15°C for the next 2 months.
Leatherback (<i>Dermochelys coriacea</i>)	Off the continental shelf irrespective of size.	Off the continental shelf as a pelagic species.	Not critical. As has global distribution, nesting mostly in tropical areas while feeding in temperate seas; and very good thermoregulatory capabilities.
Flatback (<i>Natator depressus</i>)	Relocate to QLD waters irrespective of size.	Relocate to QLD tropical coastal non-reef waters.	Relocate to QLD preferably tropical waters.

- NPWS will acquire tags and applicators. Tags will be held centrally by the Coordinator Wildlife Management and issued to Area or Regional offices on request

2. Temporary marking

Temporary markings are a useful means for gaining information on recently rehabilitated and released animals. Obvious markings can be applied to the animals' carapace that may easily be spotted by members of the public. Road paint, sometimes known as Airpave[®] is available through the RTA and is ideal for application to turtle carapaces. A small to medium sized number or letter stripe of any colour applied to the back half of the carapace may last for several weeks. Reported sightings of turtles within this time may provide useful information on the success of rehabilitation and release effort. Where titanium tags or paint are not available animals may be marked in the following way:

- Writing a local code in lead pencil on the first large scute along the midline; and/or
- Applying a colour code using commercial stock marker

2.8.5 Checklist for release of rehabilitated marine turtles

The following checklist should be completed by an authorised NPWS officer prior to the release of a rehabilitated marine turtle:

1. Copy of individual records provided to NPWS
2. Written report provided by a veterinarian on health status noting, for example, weight on arrival and prior to release, and clearing animal of potential infectious disease
3. Record of any prior injury noted
4. Report on behaviour provided
5. Release site identified which has: water temperature above 15° C; access to feeding habitat where appropriate; and sighting records of same species
6. Logistics arranged for transport and release at nominated site
7. Permanent and/or temporary tags/marks applied and recorded
8. Summary of incident, particularly record of tags, dates and location data for capture and release sites filed and lodged in NPWS - Marine Fauna Management Database

(Note: locality data will automatically be logged into Atlas of NSW Wildlife)

2.9 Options for non-releasable marine turtles

There are three options for managers to consider when assessing the future of turtles that cannot be released.

Option 1: Euthanasia. This is the acceptable method for any suffering animal in irreversibly poor condition (refer to section 2.6).

Option 2 - Permanent retention by a Zoo, Wildlife Park or Aquaria that holds a current exhibited animals licence from the NSW Dept of Agriculture. This is the appropriate option for animals where there is written advice from a veterinarian and/or specialist that the animal is unreleasable due to health or behavioural status or, is a potential disease risk to wild stocks. Permanent retention of an animal will also require written approval from the NPWS Director-General or other delegated NPWS officer.

This option will only be considered where the potential Zoo, Park or Aquaria meets the *Exhibited Animals Protection Act 1986* (EAP Act) minimum standards for the species involved and the facility can provide an acceptable quality of life physically and nutritionally.

Option 3 - Permanent retention by an NPWS licensed individual or organisation for teaching and/or research purposes. This option will only be considered where it is an identified option under a threatened species recovery plan and has approval from an Animal Care and Ethics Committee.

2.10 Relocation of marine turtles

Factors to be taken into consideration when transporting and releasing marine turtles are covered in sections 2.5.7 and 2.8.

In summary, relocation of a healthy marine turtle should only be considered where prevailing local conditions represent a potential threat to the individual. Unless under the authority of an existing NPWS licence, NPWS must be notified of a proposal to relocate a marine turtle and will make the decision on whether to pursue relocation and at what site.

If the proposed release site is in another NPWS

Region, local staff should be notified so they have the option to attend.

2.11 Priorities for data collection

It is critical to the conservation of marine turtles that accurate information be recorded on each sighting/incident. These records prove invaluable when assessing population parameters such as range, distribution, abundance and general ecology of marine turtles in NSW.

Responding NPWS officers should make every effort to record relevant information. To assist other groups, such as local rehabilitation groups, veterinarians or other individuals (eg fisherman), who may encounter marine turtles, a basic recording sheet is provided and should be distributed by the local NPWS Area office (Appendix 6). These sheets ask for basic information, including date, species, contact details of observer, animal size and outcome of the event. Completed sheets can be sent to the local NPWS office or forwarded directly to the NPWS Coordinator Wildlife Management.

Any incident attended by a NPWS officer should be detailed in a Marine Fauna Incident Report (Appendix 7) and entered into the Marine Fauna Database, with a copy forwarded to the Coordinator Wildlife Management.

The following information is considered *Category A* data and should be collected as a minimum by NPWS staff for each marine turtle sighting/incident (alive or dead) where possible:

1. Location of sighting/incident as accurately as possible
2. Date and time
3. Species identification (photograph if possible)
4. Number of animals
5. Evidence of human interaction (photograph if possible)
6. Record of existing or applied temporary or permanent tags or markings
7. Measurements – Length measurement is the most useful and should be taken from behind the turtle's neck where skin joins the carapace, along the curved length of the carapace to the junction in two midscutes above the tail.
8. Sex of the animal (where confirmed through animal found laying eggs, necropsy or other laboratory procedure).

9. Name and contact details of person managing the incident

If the opportunity arises, the following additional *Category B* data should be collected for live marine turtles ashore or stranded:

9. Faeces – to provide to veterinarian, if necessary, for parasite investigations
10. Additional measurements: head width, measured with a straight rule at maximum width behind eyes; tail length, measured as distance the tail extends beyond carapace (+ve) or is short of the rear carapace edge (-ve)
11. If returned to sea, record of release details including date, location and conditions and any post release monitoring strategy adopted
12. Record of handling/management strategies
13. Record of veterinary assistance
14. A small skin sample to be taken for genetic studies. See procedure outlined below and in Appendix 8. Wherever possible a local anaesthetic should be applied to the sampling area for live animals.

Category B – DEAD animals

Where practicable, the responding NPWS officer should notify and offer the carcass of a dead animal to the Australian Museum (Herpetological Section, Appendix 1).

If the Museum is unable to respond, the following samples should be taken prior to disposal of the carcass:

1. A skin sample should be taken for genetic studies, according to the following procedure.
 - A small sample the size of a match-head should be taken.
 - Use tweezers to pull the loose skin away from the body between the neck and shoulder region.
 - Use a clean scalpel to collect the small piece of tissue.
 - Gloves should be worn to ensure there is no contamination of the sample with human DNA.
 - Use tweezers to place skin sample into one of the following preservatives (in order of preference):
 - Freezing
 - 70% Ethanol
 - 20% DMSO & saturated salt

- Table salt in film canister

See Appendix 8, Australian Museum - Opportunistic Collection of Tissue in the Field Manual (1999) for details.

Contact the Coordinator, Wildlife Management if any other additional samples are required. This may be the case if a suspected disease outbreak is affecting numbers of marine turtles.

NPWS staff must be trained in basic marine turtle post-mortem procedures. This will enable them to collect relevant tissue samples, which may indicate cause of death and to investigate any gross potential causes of death, such as a severely compacted gut, or presence of fish tackle or other marine debris in gastrointestinal tract. Reports of all necropsy findings should be included with the incident report (Appendix 7) and entered into the Marine Fauna Management Database.

2.12 Occupational Health and Safety

Humans may be exposed to some potential risks when undertaking to rescue marine turtles or conducting biological and veterinary investigations.

In order to minimise the risk of injury, NPWS officers and other persons handling marine turtles should follow basic occupational health and safety principles. These include:

- Handle or move potentially heavy animals such as marine turtles, in the appropriate manner
- Use rubber gloves and antibacterial soap when handling marine turtles
- Wear appropriate protective clothing, such as overalls or fishermen waders, when handling animals and when performing necropsies. Eyewear and breather masks should be also worn when performing necropsies
- Use clean and sterile equipment at all times.

3. SEA SNAKES

3.1 Sea snake species in NSW

Eleven species of sea snake and one species of sea krait have been recorded in NSW waters. Most of the sea snakes recorded from NSW are seen very infrequently and are vagrant individuals which have strayed from their core tropical populations (Cogger, 2000). The exception is the Yellow-bellied Sea Snake (*Pelamis platurus*) which is considered abundant and probably a

long-term resident in the oceanic waters off the NSW coast (Cogger, 2000). In the marine environment, sea snakes occupy a range of habitats from muddy turbid estuarine waters to clear waters of coral reefs (Heatwole 1999). Most species live in warm tropical or subtropical waters.

All species of sea snake found in NSW are protected under the *NPW Act 1974*. No sea snakes are currently listed on the Schedules of the *TSC Act 1995* or listed as Threatened under the Commonwealth EPBC Act. Table 3 lists the species that have been recorded in NSW waters and provides diagnostic characteristics to aid identification in the field. A photographic image of these species is provided in Appendix 9.

Marine snakes are readily recognisable by their small head, thick body and paddle shaped tail. As records of sea snake occurrence in NSW are limited, it is important that accurate information on species and sighting location is recorded and entered into the Marine Fauna Component of the NPWS Wildlife Atlas. Wherever possible photographs should be taken of animals to confirm identification, record injuries and other relevant details.

3.2 Rescue

Most sea snakes are entirely marine and do not leave the aquatic habitat. Sea kraits are an exception and may come ashore to lay eggs, rest or digest a meal. Thus, any sea snake found on a beach is likely to be dead, debilitated or exhausted.

Members of the public who find a live sea snake should note its location and condition and contact the local NPWS office (Appendix 3) as soon as possible.

All marine snakes are venomous with a potentially fatal bite.

Members of the public must **not** attempt to handle, capture or transport the animal. Staff of the NPWS or another approved organisation should attend as soon as possible, in particular where public safety may be of concern, with proper catching and transport equipment. Should veterinary assistance or handling assistance be required, local experts in these areas should be consulted.

If a person is bitten compression should be

applied to the area of the bite immediately to confine the venom to that area. Emergency Services should be contacted and anti-venom administered as soon as possible.

Sydney Aquarium operates a rescue rehabilitation and release programme for sea snakes found in the Sydney area. When responding to incidents in this area, the Aquarium can be contacted (Appendix 1) as they can provide expert assistance in assessing, collecting and handling sea snakes. Veterinary and Quarantine Centre, Taronga Zoo can also provide veterinarian care to sea snakes found in the Sydney area and should be notified of any sea snake brought in for rehabilitation. The Veterinary and Quarantine Centre, Taronga Zoo can also serve as a first port of call where veterinarian assessment is required (see Appendix 1 for contact details). The Coordinator, Wildlife Management should be contacted first to act as a liaison point between these agencies and the NPWS.

3.2.1 Criteria to rescue

If a live marine sea snake is found on a NSW beach, appropriate individuals with experience and suitable gear should be contacted to assess and handle the snake. If veterinarian treatment or care is required, the snake should be transported to a licensed aquaria for assessment (Appendix 1).

In situations where the animal is seriously injured immediate euthanasia may be the most humane action (see section 3.2.4).

3.2.2 Catching and retrieving injured animals

Sea snakes are venomous and should only be caught, handled and treated by persons experienced in snake handling.

Sydney Aquarium and Taronga Zoo have recommended that sea snakes may be picked up with a net or a snake hook and placed in a bucket. The bucket should be lined with moist foam and covered by a lid with ventilation holes. Special care should be taken when picking up sea snakes with tongs. An inexperienced person who grips a sea-snake too tightly near the head can do critical damage to the animal.

3.2.3 Transport

Marine snakes must be transported and handled in such a way as to minimise stress and ensure safety of handler. Should a marine snake need to be transferred to a rehabilitation facility, only an

experienced individual should attempt to capture and transport a sea snake, using the appropriate equipment.

3.2.4 Euthanasia

Euthanasia is sometimes the most humane act we can provide a stranded or injured animal. As stated for marine turtles (in section 2.6), the critical decisions to be made when considering euthanasia are; when is it appropriate, what method to use and who should administer it.

The decision to euthanase a sea snake should be made by an Area or Regional Manager or delegated NPWS officer, in consultation with an attending veterinarian. Where euthanasia is the appropriate course of action, it must be performed humanely, by a veterinarian or other suitably qualified individual.

Information regarding the stranding should be recorded including animal assessment, decision process to euthanase, method used and administering individual (as described for marine turtles in section 2.6.1). Measurements, tissue sample collection and necropsy should be carried out wherever possible (see section 2.11 and Appendix 8). The Australian Museum should be contacted for their interest in the carcass prior to disposal.

3.3 Ex situ rehabilitation and release

The only reason for advocating long-term care as a management option, is when a sea snake is likely to improve in health and be rehabilitated for release to the wild.

3.3.1 Assessment

Assessment should be undertaken by a veterinarian and/or an experienced sea snake handler.

3.3.2 Housing/holding and care

Sea snakes should only be housed in appropriate conditions at an organisation licensed for such activity (Appendix 1). Standard conditions established for sea snake care at each facility including handling, housing, husbandry and diet will apply.

3.4 Pre-release

NPWS must be contacted and the following documentation provided prior to the release of any rehabilitated sea snake:

1. Record of pre-release assessments (see 3.4.1 below)
2. Record of the release site and environmental factors at the site such as water temperature and feeding habitat (see 3.4.2)
3. Consideration of Other Factors at Release Site (see 3.4.3)
4. Record of existing and applied tags or marks, whether they are temporary or permanent (see 3.4.4).

3.4.1 Pre-release assessment

There are four components to the pre-release assessment of any animal, including marine snakes. The same pre-release assessment procedure outlined for marine turtles applies to sea snakes. See section 2.8.1 for details.

3.4.2 Environmental factors

When planning a release site, there are specific environmental conditions that must be considered in addition to prevailing sea and weather conditions. These include water temperature, distance offshore and feeding habitat. Table 4 below provides information on food habits and habitat for the sea snake species recorded in NSW. (Information taken from Table 2.1 in Heatwole 1999).

Sea snakes are reliant on water temperature and cannot maintain their body temperature above that of their surroundings. The Yellow-bellied sea snake is endemic to NSW waters and should be released in the vicinity of the capture site. Contact the Coordinator, Wildlife Management should there be a requirement to release any other species of sea snake.

3.4.3 Other Release Site Factors

1. Conspecifics

While conspecifics need not be present at release sites, release should preferably occur in habitats where sightings of sea snakes have previously been recorded (consult the NPWS Atlas of Wildlife for suitable sites).

2. NPWS Rehabilitation of Fauna Policy

The Policy states that in the interests of genetic integrity of native animal populations, a rehabilitated or hand-raised animal should be returned to a suitable natural environment at the locality of the original encounter. An animal should not be transported to a release point across a geographic or physical barrier it would not normally cross.

Release of a rehabilitated animal in the area of the original encounter is typically the most appropriate strategy. However, as stated in section 3.4.2 above, it is currently believed that most species of sea snakes are vagrants to NSW.

Geographic and physical barriers are not as relevant in the marine environment as they are in a terrestrial one. However, dynamic processes such as local and large scale current systems have a greater influence on animal movement patterns and boundaries.

3. Inter-state Release

Written authorisation from the NPWS Coordinator Wildlife Management is required prior to a sea snake being relocated across the border into Queensland. In addition, prior to transport, Queensland Parks and Wildlife Service must provide receipt of this authorisation.

4. Local Factors

In some instances it may be necessary to release rehabilitated sea snakes away from the site of capture, or to have them relocated so as to avoid re-occurrence of local incidents resulting from ongoing or periodic anthropogenic threats, such as water intake of power stations, high boat traffic areas, trawler operations, or pollution incidents. Sites experiencing these threatening processes should be avoided wherever possible.

3.4.4 Identification

Marine snakes may be permanently marked either by freeze branding or by tagging (Heatwole, 1999). Such marking should only be conducted by

an appropriately trained person so that no injury or impairment to the animal results. Photographs should be taken of all marine snakes prior to release, as it may be possible to identify individuals by marking patterns. All data collected should be provided in accordance with the requirements specified in section 3.7 below.

3.5 Options for non-releasable sea snakes

There are two options for managers to consider when assessing the future of sea snakes that cannot be released.

Option 1 - Euthanasia is acceptable for any suffering animal in irreversibly poor condition (refer to section 3.2.4).

Option 2 - Permanent retention by a Zoo, Wildlife Park or Aquaria, that holds a current exhibited animals licence from the NSW Dept. of Agriculture. This is the appropriate option for animals where there is written advice from a veterinarian and/or specialist that the animal(s) is unreleasable due for example, to health or behavioural status or is a potential disease risk to wild stocks. This will require approval from the Director-General of NPWS or delegated officer in order to relinquish Crown proprietary rights of the fauna, to the owner of the licensed facility. This option will only be considered where the potential Zoo, Wildlife Park or Aquaria meets the *Exhibited Animals Protection Act 1986* (EAP Act) minimum standards for the species involved and the facility can provide an acceptable quality of life physically and nutritionally.

Table 4: Food source and habitat of sea snake species recorded in NSW, for consideration when selecting a release site (from Table 2.1 in Heatwole 1999).

Common Name	Species Name	Food Source	Habitat
Horned Sea snake	<i>Acalyptophis peronii</i>	Gobies, eels and fish	Sandy areas on coral reefs
Reef Shallows Sea snake	<i>Aipysurus duboisii</i>	Fish	Coral reefs, up to 50 m
Olive Sea snake	<i>Aipysurus laevis</i>	Fish	Coral reefs
Stokes' Sea snake	<i>Astrotia stokesii</i>	Fish	Turbid coastal water, coral reef
Spectacled Sea snake	<i>Disteira kingii</i>	Fish	Deep water
Olive-headed Sea snake	<i>Disteira major</i>	Fish	Turbid deep water
Turtle-headed Sea snake	<i>Emydocephalus annulatus</i>	Fish eggs	Shallow water on coral reefs
Elegant Sea snake	<i>Hydrophis elegans</i>	Eels	Turbid deep water
Plain Sea snake	<i>Hydrophis inornatus</i>	Fish	
Spotted Sea snake	<i>Hydrophis ornatus/ocellatus</i>	Fish	Eurytropic, coral reefs, turbid inshore waters and estuaries
Yellow-lipped Sea Krait	<i>Laticauda colubrina</i>	Eels	Coral islands, coral reefs, mangroves and open sea
Yellow-bellied Sea snake	<i>Pelamis platurus</i>	Small pelagic fish	Pelagic, surface slicks

Option 3 - Permanent retention by an NPWS licensed individual or organisation for teaching and/or research purposes. This option will only be considered where it is an identified option under a threatened species recovery plan and has approval from an Animal Care and Ethics Committee.

3.6 Relocation of sea snakes

As previously stated, only the Yellow-bellied sea snake is endemic to NSW waters and therefore is the only species that may be released in NSW in the vicinity of the capture site. All other species are vagrants to NSW and must be released into tropical waters ie at least Coffs Harbour NSW, or northward.

Relocation of Yellow-bellied sea snakes then will only be considered when prevailing local or other conditions specified in sections 3.4.2 and 3.4.3 represent a potential threat to the individual.

Unless under the authority of an existing NPWS licence, NPWS must be notified of a proposal to relocate any species of sea snake and will make the decision on whether to pursue relocation or not and to what site.

If the proposed release site is in another NPWS Region, local staff must be consulted about the

proposed release.

All capture and transport arrangements must be made in consultation with experienced individuals

For the purpose of this policy the release of stranded and/or rehabilitated individual(s) sea snakes does not trigger the requirement for a translocation proposal.

3.7 Priorities for data collection

There are few records of sea snakes found in NSW waters. Accurate reporting and recording of information from sightings therefore is vital to improving both our understanding of these species' distribution in NSW waters and their ongoing management.

NPWS officers will encourage individuals or organisations involved in sea snake incidents to collect and provide relevant data. To this end, a basic recording sheet (Appendix 5) has been supplied for distribution by NPWS Area officers to local rehabilitation groups, veterinarians, aquaria or other individuals such as fisherman who may encounter sea snakes. These sheets request information on date, species, contact details of observer, animal size and outcome of the event. Completed sheets can be sent to the

local NPWS office or forwarded directly to the Coordinator Wildlife Management.

NPWS officers who respond to sea snake incidents must record relevant data on an Incident Report Sheet (Appendix 6) and enter this information into the Marine Fauna Management Database. A copy of the report sheet should be provided to the Coordinator Wildlife Management.

The following information is considered *Category A* data and should be collected, as a minimum, by NPWS staff for each sea snake sighting (live or dead) where possible:

1. Location of sighting/incident as accurately as possible
2. Date and time
3. Species identification
4. Number of animals
5. Evidence of human interaction (photograph)
6. Record of existing or applied temporary or permanent tags or markings and
7. Total length.

If the opportunity arises, the following additional data should be collected:

Category B - ALIVE- sea snake ashore or stranded

8. If returned to sea, record of release site, environmental conditions and any post release monitoring strategy adopted.
9. Record of handling/management strategies.
10. Record of veterinary assistance.

Category B – DEAD animals

Where practicable, NPWS will notify and offer a carcass to the Australian Museum, Herpetological Section.

If the Museum is unable to respond prior to disposal of the carcass the following details should be collected or recorded:

- location details of the site and
- a tissue and scale sample to be used for genetic studies. The sample should be lodged with the Australian Museum, Evolutionary Biology Unit. Tissue collection can be undertaken in accordance with the procedures identified in Appendix 8.

4. REFERENCES

Cogger, H. D. (2000). *The Status of Marine Reptiles in New South Wales*. A Report Prepared for the NSW National Parks and Wildlife Service.

Environment Australia (1998). Draft recovery plan for marine turtles in Australia. Prepared by Environment Australia in consultation with the marine turtle recovery team. Canberra, ACT.

Heatwole, H. (1999). *Sea Snakes*. University of New South Wales Press: Sydney.

Hulst F (2000). *Husbandry, Nutrition, Handling and Anaesthesia of Marine Turtles*. In Marine Wildlife, Proceedings Post graduate Foundation in Veterinary Science, University of Sydney.

Kelly R & Gordon A (2000). *Diseases of Marine Turtles*. In Marine Wildlife, Proceedings Post graduate Foundation in Veterinary Science, University of Sydney.

Limpus, C (1988). *Summary of the Biology of Marine Turtles in Australia*. Proceedings of Stranded Marine Wildlife Training Course, QLD Department of Environment.

Limpus C (2000). *Marine Turtles*. In Marine Wildlife, Proceedings Post graduate Foundation in Veterinary Science, University of Sydney.

Appendix 1: Organisations currently approved in NSW to rescue, hold and rehabilitate marine reptiles and/or receive tissue samples.

Organisation	Address	Contact details	Species
Australian Museum	Australian Museum William Street Sydney NSW 2010	Denis O’Meally (Tissue Collection) Ph: 02 9320 6292 Ross Sadler (Herpatology Department) Ph: 02 9320 6259	Collection of tissue samples and specimens of marine turtles and sea snakes
Australian Seabird Rescue	‘Waverley’ Pacific Highway Ballina NSW 2470	Lance Ferris Ph: 02 6686 2852 Fx: 02 6686 2852 Seabirdrescue@bigpond.com	Marine turtles and sea snakes
Manly Oceanarium	West Esplanade Manly NSW	Ph: 02 9262 2300	Marine turtles
Pet Porpoise Pool	PO Box 532 Coffs Harbour NSW 2450	Greg Pickering Ph: 02 6652 2164 Fx: 02 6650 0264	Marine turtles and sea snakes
Seaworld	PO Box 190 Surfers Paradise QLD 4217	Ph: 07 5588 2222 Fx: 07 5591 1056	Marine turtles
Sydney Aquarium	Aquarium Pier Darling Harbour NSW 2000	Chris McDonald Ph: 02 9262 2300	Marine turtles and sea snakes
Taronga Zoo (Veterinary and Quarantine Centre)	Bradley’s Head Road PO Box 20 Mosman NSW 2088	Dr. Larry Vogelnest (Live strandings) Ph (02) 9978 4618 Dr Karrie Rose (dead animals) Ph (02) 9978 4749 Note: It is recommended that liaison with Zoo staff be initiated through the Coordinator, Wildlife Management or Geoff Ross (Appendix 1) (if available).	Facilities for marine reptiles including veterinarian assistance, rehabilitation and pathology.
Native Animal Trust Fund	PO Box 1052 Toronto NSW 2283	Audrey Koosman 0500 502 294 (24hr Hotline) or 0408 640 517	Marine turtles and sea snakes (rescue and temporary hold)
Fauna (NSW)	PO Box 218 Wauchope NSW 2446	Meredith Ryan 0500 861 405 (24hr Hotline) or 02 6585 6470	Marine turtles and sea snakes (rescue and temporary hold)

Appendix 2: Rehabilitation of Fauna Policy



TITLE: REHABILITATION OF FAUNA POLICY

<i>AUTHOR</i>	<i>Mngr, Strategic Policy Division</i>
<i>DATE OF ORIGINAL ENDORSEMENT</i>	Oct 2001
<i>DATE OF EFFECT</i>	Oct 2001
<i>DATE LAST MODIFIED</i>	July 2002
<i>DUE FOR REVIEW ON</i>	June 2005

Rehabilitation of Fauna Policy

Introduction

1. Under the *National Parks and Wildlife Act 1974*, (NPW Act) the Director-General of the NSW National Parks and Wildlife Service (NPWS) has a legislative responsibility for the protection and care of protected fauna (s 92).
2. The Director-General of NPWS may also enter into arrangements for the carrying out of such works as the Director-General considers necessary for or in connection with the protection and care of protected fauna and the protection of native plants (s. 8(7)(b)). The NPWS therefore has a role in the licensing and supervision of persons and organisations involved in the rescue, care and rehabilitation of sick, injured and orphaned protected animals and the release, or retention in captivity, of those animals
3. Section 101(5)(c) of the *National Parks and Wildlife Act, 1974* and selected provisions in the *National Parks and Wildlife Regulation, 2001* relate specifically to fauna rehabilitation. Section 120 of the NPW Act provides a mechanism for the licensing of individuals or organisations to acquire and hold protected fauna for the purpose of rehabilitation. Sections 109 and 127 of the NPW Act provide for the liberation of captive or captured animals.
4. The NPWS recognises the important conservation and animal welfare role performed by wildlife rehabilitation groups. It is important that the NPWS and the rehabilitation groups maintain a partnership to achieve the optimum allocation of resources which may be available for the rehabilitation of protected fauna and to ensure compliance with the regulations is achieved.
5. This policy also applies to the treatment of protected fauna captured as a result of law enforcement processes.

Objectives

6. To provide clear guidelines which identify when it is not appropriate to return protected fauna to the wild and to establish controls which protect animal welfare and assist in meeting conservation objectives.
7. To provide clear and consistent framework for the NPWS in developing and maintaining a partnership with fauna rehabilitation groups in their approach to the rehabilitation of protected fauna.
8. To ensure that fauna rehabilitation groups and individuals are appropriately licensed and accountable for their activities, and that such groups engaged in their rehabilitation activities in the most effective and efficient manner.
9. To contribute to the maintenance of biodiversity through the successful return of temporarily disadvantaged animals to their natural environments.

Scope / Application

10. This policy applies to all of New South Wales.

Policy

Licensing

11. The NPWS will licence selected organisations for the holding of protected fauna for rehabilitation purposes with the exception of marine mammals. Marine mammals can only be held in facilities licensed under the *Exhibited Animals Protection Act 1986* in NSW, or a similar appropriately licensed facility in another State or Territory.
12. While any individual or group may approach the NPWS to operate as carers or rehabilitators of native fauna, the preferred arrangement is to licence and collaborate with a small number of key organisations. This creates a simpler, more stable and more effective administrative arrangement and enables a more efficient means of responding to animal rescue, care and rehabilitation. The NPWS will therefore only consider an application by a group for a new Section 120 licence for rehabilitation purposes if there is no currently NPWS-licensed rehabilitator or rehabilitation organisation operating in the relevant NPWS management area.
13. Licences will not be issued to individuals except in exceptional circumstances. Individuals wishing to contribute to animal rescue, care and rehabilitation will be referred to local NPWS-licensed organisations.
14. All applicants for a licence must demonstrate compliance with the following criteria:
 - Training, competency assessment, supervision and monitoring of wild animal rescuers, carers and rehabilitators with regard to the techniques of wild capture, transport, animal husbandry and housing;
 - Continuing promotion of knowledge and education about the conservation of wildlife and wildlife ecology amongst members;
 - Capacity to submit reports to the NPWS which provide information on the success of rehabilitation programs, including records of all animals from point of collection to fate;
 - Incorporation under the *Associations Incorporation Act, 1984* and/or a registered company operating under the *Prevention of Cruelty to Animals Act, 1979* (RSPCA and Animal Welfare League);
 - Evidence of public liability and personal accident insurance for members. (Incorporation under the *Associations Incorporated Act* requires associations to hold public liability insurance); and
 - Agreement to the euthanasia of animals when certain conditions are met.
15. Licences will be issued through the Wildlife Licensing Section of the Biodiversity Management Unit in NPWS Head Office. Prior to issuing a licence, the Wildlife Licensing Section will consult and seek the opinion of the relevant NPWS Regional Manager(s). Regional Managers should also liaise with the relevant regional staff who might be engaged in the regulation of the licensee. Day to day liaison with licensed carer organisations, including supervision, training and administrative tasks, will be undertaken at a Regional level.
16. Generally, new licences will not be issued unless the proposal has the support of the relevant Regional Manager (or delegated officer). Similarly, the geographic expansion of existing organisations into new localities will be subject to the support of the Regional Manager (or delegated officer) in the area affected. Any geographic restriction will be incorporated in the terms of the licence for a carer organisation.
17. Licences under Section 120 of the NPW Act which were in existence at the time of adoption of this policy, authorising private individuals to hold specific protected fauna for rehabilitation, or to care for a succession of sick, injured and orphaned protected fauna, will be renewed (subject to the continued capacity of the individual to meet licensing criteria (see 13 above)).

18. A licence or authority is not required for the holding by a veterinarian of protected fauna which are sick or injured while such animals are undergoing treatment and are under the direct care of the veterinarian.

Care of animals

19. When rehabilitating protected fauna, every endeavour must be made to provide optimum levels of care and animal welfare. This involves appropriate nutrition, opportunity to express a range of natural behaviours, protection from predators and, if available, access to animals of the same species. Ideally, carers and rehabilitators of protected fauna should develop a working relationship with a veterinarian interested in native wildlife and seek advice and assistance as needed.

Private fauna parks

20. A licence under Section 120 of the NPW Act, to rehabilitate protected fauna, will be available to private fauna parks which can satisfy the Director-General that purpose-built off-exhibit facilities are available and maintained for the rehabilitation of protected fauna. These facilities must meet the standards established by the NPWS or specialist, licensed rehabilitation organisations or individuals for the species concerned and comply with the standard NPWS licensing criteria (see 13 above). This housing requirement will not apply to the rehabilitation of marine mammals or marine reptiles, which may be housed and rehabilitated within facilities, which are normally used for exhibit purposes. Licences will be processed through the Wildlife Licensing Section in consultation with the appropriate Regional Manager (or delegated officer) and the Registrar of the *Exhibited Animals Protection Act, 1986* in NSW Agriculture.
21. The rehabilitation activities of licensed private fauna parks will be monitored and/or audited by the NPWS. The NPWS may utilise spot-checks or may require parks to maintain and submit records of rehabilitation activities. NSW Agriculture administers the *Exhibited Animals Protection Act, 1986* and opportunities for collaboration with NSW Agriculture regarding the monitoring and/or auditing of private fauna parks will be explored.
22. A fauna park which cannot demonstrate the continued availability of appropriate facilities and a commitment to legal and ethically responsible rehabilitation will not be permitted to hold protected fauna for rehabilitation. Operators of such parks may accept injured or displaced animals under the terms of s101 of the NPW Act, but must report their possession to the Director-General of NPWS and then promptly pass them on to a licensed rehabilitator.

Release of protected fauna

23. In the interests of genetic integrity of native animal populations, a rehabilitated or hand-raised animal should be returned to a suitable natural environment at or near the locality of the original encounter. An animal should not be transported to a release point across a geographic or physical barrier it would not normally cross
24. Where release is proposed within a NPWS park, such actions must comply with the provisions of NPWS policies on 'Protection of Environmental Integrity' and 'Translocation of Threatened Fauna in NSW'.
25. Protected fauna must not be returned to the wild where:
 - The animal is handicapped with a permanent or long-term disability which could reasonably be assumed to preclude it from leading a normal life and surviving in the wild, or
 - The animal is reasonably suspected to carry a serious disease or a disease, which is likely to be transmitted to the detriment of populations or habitat.

Retention of protected fauna

26. Only in exceptional circumstances will the NPWS permit a privately licensed person or a rehabilitation organisation to permanently retain an unreleasable animal in captivity. Approval may be granted only if the animal will serve as an essential companion animal to others of its species which are undergoing rehabilitation, or will be used as an acceptable resource in a licensed exhibit, or an approved educational or scientific program. Consideration will also be given to the granting of approval to retain some traditional aviary and caged birds, reptiles and frogs. The NPWS may place limitations on the numbers of such animals which may be held and specify conditions under which they should be held.
27. Except in the case of threatened species covered by a captive breeding and translocation approval, unreleasable fauna may not be retained for the purpose of captive breeding and release of progeny to the wild.
28. The NPWS will consider on its merits any application from a zoo or fauna park licensed under the *Exhibited Animals Protection Act, 1986*, to recruit protected fauna which has been hand-raised or is undergoing rehabilitation, into the exhibition stock holdings of that park. Approval for the acquisition or retention of such an animal will be subject to the concurrent approval of the Registrar of the *Exhibited Animals Protection Act*.

Euthanasia

29. Euthanasia of animals is a matter of concern, contention and debate in the community. As such, decisions with respect to euthanasia should be made with due consideration of all community views. Where the extent and severity of injuries or illness in an animal mean that any treatment cannot ultimately render the animal fit to return to its natural environment, then the conservation outcome will not be positive and euthanasia should be considered. Protected fauna which cannot be released or retained under the terms of this policy (see 21 to 26) should be humanely euthanased.
30. Where euthanasia is necessary the task should be performed by a qualified veterinarian, if available. If a veterinarian is not available, the operator performing the task should be competent in such procedures and licensed by the appropriate authorities to use a firearm or restricted poisons (eg S3 and S4 poisons such as pentobarbitone sodium) for the purpose of animal welfare.
31. Where existing large collections of protected fauna are held, the NPWS will negotiate with the carer group to reduce the population consistent with the provisions of this policy.

Threatened Species

32. In some cases, it may be desirable to authorise the retention of some individual animals for educational or scientific purposes, including a translocation and captive breeding program for threatened species, in which case their progeny may be released in accordance with the approval.
33. The translocation proposal should address the handling and welfare of animals which cannot be released, and must comply with the NPWS policy on 'Translocation of Threatened Fauna in NSW'.

Vaccination of carers

34. Should it be necessary for a person who is authorised as a carer under a NPWS licence, or operating as part of a NPWS licensed carer organisation, to be vaccinated or to receive any other medical treatment for a disease or infection, or as a precaution against contracting a disease or infection, as part of those carer activities, then this shall be at no cost to the NPWS.

Definitions

NPWS parks means any area reserved or dedicated under the *National Parks and Wildlife Act, 1974* – nature reserves, national parks, Aboriginal areas, State recreation areas, historic sites,

karst conservation reserves, regional parks and State game reserves. Parks also includes Crown reserves, reserved under the Crown Lands Act, for which Director-General of NPWS has management responsibility as trustee.

Protected fauna are fauna of a species not named in Schedule 11 of the *National Parks and Wildlife Act, 1974*. Note that threatened species, as listed in Schedule 1 and 2 of the *Threatened Species Conservation Act 1995*, are by definition also considered protected fauna. Schedule 11 appears in Attachment A of this policy.

Relevant Legislation

National Parks and Wildlife Act 1974:

- Section 101(5)(c) relates specifically to the possession of protected fauna which is incapable of fending for itself.
- Section 109 prohibits the liberation of any animal which is native to NSW unless under and in accordance with a licence under Section 127 of the Act.
- Section 127 relates specifically to the licensing of a person to liberate an animal anywhere, or in a specified locality or specified localities, within NSW.
- Section 120(1)(a1) relates to the issuing of a licence authorising private individuals to hold any protected fauna for the purposes of rehabilitation.
- Section 120(5) relates to the authorisation of persons to operate under a Section 120 licence.

Threatened Species Conservation Act, 1995, Exhibited Animals Protection Act, 1986 Poisons and Therapeutic Goods Act, 1966, Firearms Act, 1996

Relevant NPWS Policies and Other Documents

Protection of Environmental Integrity - Policy

Private Holdings of Native Mammals - Policy

Firearms - Policy

Threatened Species Information Circular: Policy for Translocation of Threatened Fauna in NSW

Contacts

Wildlife Licensing Section
Biodiversity Management Unit
NPWS Head Office
(02) 9585 6481

Policy Development Group
Strategic Policy Division
NPWS Head Office
(02) 9585 6422

Appendix 3: NSW National Parks and Wildlife Service coastal offices and Marine Park Authority offices

Southern Directorate Offices

Far South Coast Region

Corner Merimbula & Sapphire Coast Drive
PO Box 656
Merimbula NSW 2548
Phone: (02) 6495 5001

Merimbula Area

Located in Regional Office

Narooma Area (Central)

Cnr Field Street and Princes Highway
PO Box 282
Narooma NSW 2546
Phone: (02) 4476 2888

Narooma Area (North)

Cnr Field Street and Princes Highway
PO Box 282
Narooma NSW 2546
Phone: (02) 4476 2888

South Coast Region

55 Graham Street
PO Box 707
Nowra NSW 2541
Phone: (02) 4423 2170

Ulladulla Area

Blackburn Estate
Coller Road
PO Box 72
Ulladulla NSW 2539
Phone: (02) 4454 9500 or
(02) 4455 5990

Nowra Area

Located in Regional Office
Phone: (02) 4428 6300

Central Directorate Offices

Wildlife Management Officer

Botany Bay National Park
Kurnell, NSW
Phone: (02) 9668 9111

Sydney South Region

Farnell Avenue
Audley NSW 2232
Phone:(02) 9542 0624

Illawarra Area (Bulli Office)

4/55 Kembla St

NSW National Parks and Wildlife Service

Wollongong NSW 2500

Phone: (02) 4225 1455

Royal Area

Royal National Park
Farnell Avenue
Audley NSW 2232
Phone: (02) 9542 0632

Botany Bay Area

Botany Bay National Park
PO Box 375
Kurnell NSW 2231
Kurnell Phone: (02) 9668 9111

Sydney Region

Sydney Harbour South Area

Greycliffe House
Greycliffe Avenue
Vaucluse NSW 2030
Phone: (02) 9337 5511

Sydney Harbour North Area

Quarantine Station
North Head Scenic Drive
Manly NSW 2095
Phone: (02) 9977 5145

Goat Island

PO Box 10
Pyrmont NSW 2009
Phone: (02) 9555 9901

Sydney North Region

Ku-ring-gai Chase National Park
Ku-ring-gai Chase Rd
Bobbin Head NSW 2074
Phone: (02) 9457 8900

Northern Beaches Area

Garigal National Park
Ferguson Street
Forrestville NSW 2087
Phone: (02) 9451 3479

Lower Hawkesbury Area

Ku-ring-gai Chase National Park
PO Box 3056
Asquith NSW 2077
Phone: (02) 9457 9006

Central Coast - Hunter Range Region

Suites 36-38, 207 Albany Street

North Gosford NSW 2250
Phone: (02) 4320 4200 (All general enquires)

Gosford Area
Located in Regional Office
Phone: (02) 4320 4280

Lakes Area
Elizabeth Bay Drive
Lake Munmorah NSW 2259
Phone: (02) 4358 0400

Northern Directorate Offices

Hunter Region
Level 1, 12 Teramby Rd
Locked Mail Bag 99
Nelson Bay NSW 2315
Phone: (02) 4984 8200

Hunter Coast Area
Located in Regional Office

Great Lakes Area
Booti Booti National Park
The Lakes Way
Pacific Palms NSW 2428
Phone: (02) 6591-0300

Mid North Coast Region
152 Horton Street
PO Box 61
Port Macquarie NSW 2444
Phone: (02) 6586 8300

Manning Area
78 Hargreaves Drive
Taree NSW 2430
Phone: (02) 6552 4097

Hastings Area
Located in Regional Office

North Coast Region
Level 3, 49 Victoria Street
PO Box 361
Grafton NSW 2460
Phone: (02) 6641 1500

Coffs Coast Area
Marina Drive
PO Box J200
Coffs Harbour NSW 2450
Phone: (02) 6652 0900

Clarence South Area
Located in Regional Office

Clarence North Area
Located in Regional Office

Northern Rivers Region
Colonial Arcade, 75 Main Street
PO Box 856
Alstonville NSW 2480
Phone: (02) 6627 0200
Office mobile: 0427 404 815
Duty Officer Pager No: 016 301 161

Richmond River Area
Located in Regional Office

Tweed Area
Cnr Alma & Pacific Highway
PO Box 5081
South Murwillumbah NSW 2484
Phone: (02) 6672 6360

Coordinator Wildlife Management
Head Office
43 Bridge Street
Hurstville NSW 2220
Phone: (02) 9585 6576
Mobile: 0419 224 905

Marine Park Authority Offices

Solitary Island Marine Park
32 Marina Drive
PO Box J297
Coffs Harbour NSW 2450
Phone: (02) 6652 3977

Jervis Bay Marine Park
PO Box 89
Huskisson NSW 2540
Phone: (02) 4441 7752

Appendix 4: Indo-Pacific Marine Turtles

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Indo-Pacific marine turtles



Dermochelys coriacea (Leatherback turtle)



Lepidochelys olivacea (Olive ridley turtle)



Eretmochelys imbricata (Hawksbill turtle)



Caretta caretta (Loggerhead turtle)



Natator depressus (Flatback turtle)



Chelonia mydas (Green turtle)

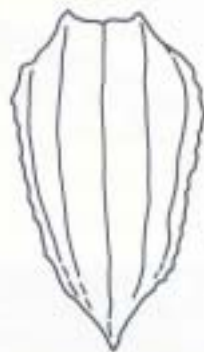


Queensland
Department of
Environment and
Heritage

Indo-Pacific marine turtles

IDENTIFICATION KEY

Carapace with
- 5 distinct ridges
- no large scales



Dermochelys coriacea
(Leatherback turtle)

Carapace with
- no continuous ridges
- large scales

4 pair costal scales



5 pair (rarely 6) costal scales
- carapace longer than wide
- colour red-brown to brown
- no pores in scales of bridge



Caretta caretta
(Loggerhead turtle)

6 pair or more costal scales
- carapace approximately circular
- colour grey green
- pores in scales of bridge



Lepidochelys olivacea
(Olive ridley turtle)

2 pair prefrontal scales
- thick overlapping carapace scales



Eretmochelys imbricata
(Hawksbill turtle)

1 pair prefrontal scales
- no thick overlapping carapace scales



- carapace low domed with upturned edges
- preocular scale
- colour olive grey



Natator depressus
(Flatback turtle)

- carapace high domed
- no preocular scales
- colour light to dark green with dark mottling



Chelonia mydas
(Green turtle)

Appendix 5: Locations of Titanium Turtle Tags and Tag Applicators held by NPWS

Directorate	Contact	Location
Policy & Science	Kelly Waples, CWM	Biodiversity Research & Management Division 43 Bridge Street Hurstville NSW 2220 Ph 02 9585 6576 Email kelly.waples@npws.nsw.gov.au
Northern	Lance Ferris	Seabird Rescue 'Waverley' Pacific Highway Ballina NSW 2470 Ph: 02 6686 2852 Seabirdrescue@bigpond.com
	Hamish Malcolm, SIMP	Solitary Island Marine Park Coffs Harbour Jetty Coffs Harbour NSW 2450 Ph: 02 6652-0912 (on loan to Greg Pickering, Pet Porpoise Pool)
Central	Khan Spokes, Field Officer	Lakes Area Workshop Munmorah NSW 2259 Ph: 02 4358 0408
	Geoff Ross, Wildlife Management Officer	Botany Bay National Park Kurnell NSW 2231 Ph: 02 9668 9111 Email:
Southern	Tim Lynch, Project Officer	Jervis Bay Marine Park Lady Denman Heritage Complex Dent St Huskisson NSW 2540 Ph: 02 4441 7752

Appendix 6: Marine Reptile Report Sheet

MARINE REPTILE REPORT

Date _____

Time _____

SPECIES: Turtle

Other

- Green
- Loggerhead
- Hawksbill
- Leatherback
- Flatback
- unidentified

- seasnake
- seabird

Notes:

SEX

MATURITY

- Female
- Male
- Unknown

- Immature
- Adult
- Unknown

Latitude ° _____ 'S

Longitude ° _____ 'E

LOCATION: (exact description of beach and specific location) _____

CONDITION

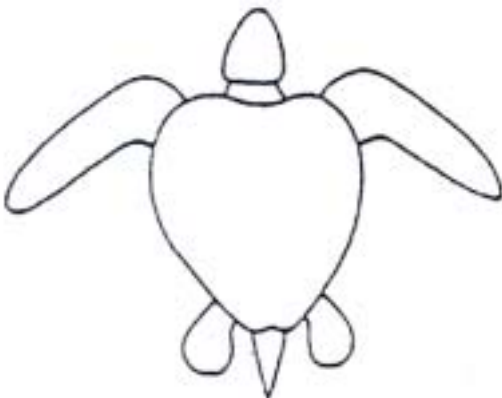
- ALIVE: Alert
- Weakly responsive
- Non-responsive
- DEAD: Carcass in good condition
- Carcass in fair condition (decomposed, organs intact)
- Carcass in poor condition (advanced decomposition)
- Mummified carcass
- Disarticulated skeletal remains

SPECIFIC INJURIES: (sketch below) _____

Identifying Marks/Tags _____

MEASUREMENTS: Curved Carapace Length _____ cm
 Tail Length (+ or -) _____ cm
 Head width _____ cm

Sketch injuries and identifying marks



RESULT

In care at: _____

Attending Vet: _____

Euthanased/Died (Time/Date): _____

Necropsy Completed by (Report Attached): _____

NPWS officer authorising: _____

Carcass disposal (location & contact): _____

Rehabilitation Report attached (treatment and assessment): _____

RELEASE By: _____ Date: _____ Location: _____

Tags: _____ NPWS Officer attending: _____

Contact: Name _____ Phone: _____
 Address _____

Appendix 7: NPWS Marine Fauna Incident Forms

The NPWS marine fauna incident management objectives are to:

1. Ensure the safety of all personnel and resources involved in the incident;
2. Ensure that the requirements and provisions of all relevant legislation are complied with;
3. Return to the sea as many healthy animals as practicable;
4. Treat humanely and minimize the suffering of all stranded, sick, injured and/or distressed marine fauna;
5. Prevent, as far as practicable, further strandings or restrandings (where applicable);
6. Collect identified priority information from living marine fauna in a benign manner wherever possible;
7. Tag/mark animals to maximize the opportunity to monitor individuals after returning to the sea, wherever possible;
8. Collect identified priority information from dead fauna;
9. Minimise health and quarantine hazards from stranded, sick, injured and/or distressed marine fauna to humans and other species;
10. Provide for the safe disposal of carcasses with regard to public health and the later collection of scientifically valuable materials, wherever possible;
11. Promote NPWS role in marine fauna conservation management; and
12. Provide community with information on key marine conservation matters and manage community expectations

Notification Protocol

1. District officer receives report
2. Area Manager notified
3. Regional Manager notified
4. Parkops notified
5. Notified as required: NPWS Wildlife Coordinator, NPWS veterinarian, local veterinarian, Marine Park Authority, relevant agencies and organizations

Forms

MF/0: This form is to be used at the time an incident is first reported to an NPWS officer. Some of the information is required for entry of an incident into the Marine Fauna Management Database but it is primarily to assist staff with standardized collection of relevant information for the purpose of local record keeping.

Example 1: NPWS officer contacted by phone at 14:30 by member of the public to report a turtle ashore that appears to be injured or dead. The officer provides the number of, or advises he/she will contact local wildlife care group to investigate. This form allows details and NPWS management action of referral to be recorded.

MF/1: This form is to be used as a Situation Report for Marine Fauna Incidents. The majority of the fields in this report are included in the Marine Fauna Incident Database. This means that the forms can be completed manually, as is the case currently, or if the opportunity arises, information can be entered into the database at the time of the incident and the reports printed out, with only certain fields requiring manual entry. This option is available simply to minimize data entry time into the database after the incident response is completed. This form includes some Category A, B & C data.

Example 1: An NPWS officer attends a marine turtle stranded on a public beach. The turtle is in a poor condition with obvious injuries from a boat propeller and management requires a commitment of NPWS personnel and resources. This form allows details of management actions and resource allocations to be recorded.

Return immediately to Coordinator wildlife Management, NSW National Parks and Wildlife Service, PO box 1967, Hurstville, NSW, 2220. Fax (02) 9585 6544, PH: (02) 9585 6576

MF/2: This form is for Priority Data Collection for single animals and allows reporting for both Category A and B data. This form should be taken on-site by the first attending officer.

Example 1: A dead Loggerhead turtle washes ashore. An NPWS officer attends and takes measurements of the carcass. The officer arranges for collection of tissue samples, necropsy and disposal of the carcass. This form allows basic biological data to be recorded and location of tissue samples, necropsy reports and the carcass to be recorded.

Example 2: An injured green turtle hauls out on a remote beach. An NPWS officer and a local vet attend and recommend that the turtle requires transport to a care facility for rehabilitation. This form allows NPWS management responses to be recorded and the fate of the animal to be tracked.

MF/3: In the event of an incident involving a number of individuals of the same and/or different species, priority data collection from all individuals is unlikely to be possible. This form is to assist Service staff reporting on management strategies (which may or may not be the same) for different groups (be they groups of different species or groups of the same species which have been subject to for example, different management strategies, or found at different sites).

Example 1: A large toxic chemical spill occurs and impacts on a large number of sea birds, several marine turtles and one dolphin. NPWS attends the incident and assesses the condition of the wildlife. The fauna is divided into groups on the basis of condition and managed accordingly. This form allows for recording of some biological data and for tracking the management response for each group.

INITIAL REPORT OF A MARINE FAUNA INCIDENT

MF/0

Officer taking report: _____

Date: _____ Time: _____ (24 hour clock)

Name of person advising of incident: _____

Telephone number (include area code): _____ Organisation: _____

Address: _____

Will caller be available on that telephone number if further details required?: _____

Location of incident - include as much information as possible: _____

Type of animal(s) involved: _____

Number of animals involved: _____

Size of animal(s) involved: _____

Condition of animal(s): _____

Time incident began (use 24 hour clock): _____

Access to site of stranding/incident:

Vehicle access yes no

Four wheel drive only yes no

Provide details: _____

Boat access (provide details): _____

Site details (terrain, rocky, sandy etc): _____

Current state of the sea: _____

Current state of the weather: _____

Has the caller notified any other authorities? (specify): _____

Are other people present at the site?: _____

SITUATION REPORT FORM - MARINE FAUNA

MF/1

Incident Type: _____
 LGA: _____
 Sitrep No: ____ as at _____ hrs ____/____/____
 Incident Name: _____

CONTROL CENTRE DETAILS

Location: _____
 Incident Controller: _____
 Agency: _____
 Phone: _____
 Fax: _____

INCIDENT LOCATION DETAILS

Map Name: _____
 Map Scale: _____
 Grid Reference: E _____ N _____
 Accuracy: 100m 1km
 Map Attached? Yes No

INCIDENT COMMENCEMENT DETAILS

Date: _____ Time: _____

THREAT ANALYSIS (ANTHROPOGENIC OR NATURAL)

Past: _____

 Current: _____

 Potential: _____

Weather Details		Vet/ Specialist
Air Temperature		Contacted <input type="checkbox"/> Attended <input type="checkbox"/> Advice given: Yes <input type="checkbox"/> No <input type="checkbox"/> Contact details:
Sea Temperature		
Sand Temperature		
Wind Force/Wave Height		
Wind Direction		
Cloud Cover		
State of Tide		
Phase of Moon		

Category Involved	Number of Animals
<input type="checkbox"/> Cetacean	Original: _____
<input type="checkbox"/> Pinniped	Current: _____
<input type="checkbox"/> Turtle	Alive: _____
<input type="checkbox"/> Bird	Dead: _____
<input type="checkbox"/> Dugong	In Area: _____
<input type="checkbox"/> Snake	Rescued: _____
<input type="checkbox"/> Other	In Holding Area: _____
Species	name or description:
_____	_____
_____	_____
_____	_____

COMMITTED RESOURCES	NPWS	Vets	NGO	Vol.	Other / Hire
Personnel					
Vehicles					
Boats					
Heavy Plant					
Fixed Wing A/C					
Helicopters					
Other					

CONTROL DETAIL

Objectives: _____
 Strategies: _____

Agency Specific Information	Operational Period <i>From:</i>		<i>To:</i>	
Region:		District:		
Reserve:		Electorate:		

Prepared By: _____ <i>Print Name</i>	Approved By: _____ <i>Print Name</i>
Position: _____	Position: _____

INDIVIDUAL MARINE FAUNA REPORT FORM - Page 1

MF/2

Operational Period: From date: time: to date: time:	Incident Name: Incident Number:	Date Prepared:	Time Prepared:	Page
---	--	-------------------	-------------------	------

ANIMAL DETAILS

CONDITION OF ANIMAL

Animal (Event) Number : _____ Tag/Band/Brand Number: _____ Species: _____ Sex: _____ Breeding: _____ Photographs taken: _____ Photographer: _____ Comments: _____	<input type="checkbox"/> alert <input type="checkbox"/> weakly responsive <input type="checkbox"/> non-responsive <input type="checkbox"/> carcass in good condition <input type="checkbox"/> carcass in fair condition <input type="checkbox"/> carcass in poor condition <input type="checkbox"/> mummified carcass <input type="checkbox"/> disarticulated skeletal remains
--	---

MANAGEMENT RESPONSE

Evidence of Human Interaction: _____

Behavioural Condition: _____

Field Assessment of Health: _____

Results: _____

First Aid Administered: _____

Date and Location of Return to Sea: _____

Date and Location of Relocation: _____

Date of Transport and Location of Care Facility: _____

Died/Euthanased: _____

Date and Location Necropsy Conducted: _____

Contact for Necropsy Report: _____

Tissue Samples: _____

Disposal of Carcass: _____

Grave Location & Contact details: _____

Comments: _____

Prepared By:	Approved By:
<i>Print Name</i>	<i>Print Name</i>
Position:	Position:

BIOLOGICAL DATA

Cetacean	
Tooth count	
Baleen	
External Parasites	
Total Length (m)	
Maximum Girth (cm)	
Length of Pectoral Fin (cm)	
Max. width pec. flipper (cm)	
Difference between upper & Lower jaws (cm)	
Length rostrum (cm)	
Length gape (cm)	
Tip upper jaw to centre of eye (cm)	
Tip upper jaw to centre of blowhole (cm)	
Tip upper jaw to anterior insertion of pec. flipper (cm)	
Depth of notch between tail flukes (cm)	
Tip upper jaw to anus (cm)	
Tip upper jaw to tip dorsal fin (cm)	
Width of tail flukes (cm)	
Max. height of dorsal fin (cm)	
Weight (kg)	

Sea Bird	
Weight (g)	
Total length (cm)	
Wing (cm)	
Tail (cm)	
Nest/Burrow	

Pinniped	
Total Length (m)	
Weight (kg)	
External Parasites	
Tooth count	
Length pec. flipper (cm)	
Length pelvic flipper (cm)	
Girth behind pec. flippers (cm)	
Dorsal colouration	
Ventral colouration	
Earlobes	

Marine Turtle	
Carapace length (cm)	
Tail length (cm)	
Head Width (cm)	
Nest location	
Clutch size	

Sea Snake	
Body length (cm)	
Barnacles on Skin	
Distinguishing features	

Dugong	
Total length (cm)	
Length pec. flipper (cm)	
Max. width tail (cm)	
Girth behind pec. flippers (cm)	
Weight (kg)	
External parasites	
Tusks	
Teeth	

MEASUREMENTS

Cetaceans: **Total length:** measured from the tip of the upper jaw to the deepest part of the fluke notch.

Maximum girth: measured at the anterior insertion of the dorsal fin.

Length of pectoral fin: measured from anterior to tip.

Pinnipeds: **Total length:** measured from the snout to the tip of the tail.

Sea Birds: **Total length:** measured from the tip of the bill to the longest tail feather with the bird in a natural relaxed position on back.

Wing: measured along flattened wing chord - from the wrist to the longest primary.

Tail: measurement of the longest tail feather from tip to base.

Marine Turtles: **Carapace length:** measured along midline with a flexible tape from the skin shell junction at the front, to the rear edge of carapace.

Tail length: measured as the distance the tail extends beyond the carapace (+ve) or is short of the rear of the carapace (-ve).

Head width: measured with a straight rule at the maximum width behind the eyes.

Dugongs: **Total length:** measured from the tip of the snout to the tip of the fluke

Sea Snakes: **Body length:** measured from the tip of the snout to the tip of the tail.

MARINE FAUNA - SPECIES GROUP REPORT FORM

MF/3

Operational Period: from date: time: to date: time:	Incident Name: Incident Number:	Date Prepared:	Time Prepared:	Page 1
--	--	-------------------	-------------------	-----------

<p>Group name/code:</p> Animal (Event) Numbers: _____ Species: _____ Number female: _____ Number male: _____ Number immature: _____ Number pregnant: _____ Number lactating : _____	Management Action: _____ _____ Comments on Action: _____ _____ _____ _____ _____
<p>Group name/code:</p> Animal (Event) Numbers: _____ Species: _____ Number female: _____ Number male: _____ Number immature: _____ Number pregnant: _____ Number lactating : _____	Management Action: _____ _____ Comments on Action: _____ _____ _____ _____ _____
<p>Group name/code:</p> Animal (Event) Numbers: _____ Species: _____ Number female: _____ Number male: _____ Number immature: _____ Number pregnant: _____ Number lactating : _____	Management Action: _____ _____ Comments on Action: _____ _____ _____ _____ _____

Comments: _____

Prepared By: <i>Print Name</i>	Approved By: <i>Print Name</i>
Position:	Position:

Appendix 8: Opportunistic Collection of Tissue Samples in the Field
by

D. O'Meally and S. Livingston, The Australian Museum

About this Document

This document is a field manual detailing the principles and practices for taking tissues from animals found dead in the field. It describes the practical aspects of collection, methods of preservation and how to send samples to the Australian Museum. This document will be regularly revised and updated. Please contact the tissue collection manager at the address below with comments, suggestions or requests for materials or further information.

November, 1999

Contact Information

Tissue Collection Manager
Evolutionary Biology Unit
The Australian Museum
6 College St
SYDNEY NSW 2010
Ph 02 9320 6292
Fax 02 9320 6059
Email denisom@austmus.gov.au

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Background and Introduction

The Tissue Collection at the Australian Museum was established in 1988. It currently holds approximately 21,000 tissues preserved at -80°C in ultracold freezers or in ethanol. The primary use of the tissue collection is as a resource for molecular genetic studies. For the most part, these aid in issues of species/population management and provide data necessary for informed debate on biodiversity and conservation. It is also widely used in systematic studies. The existence of this resource can obviate the need for unnecessary collection and sacrifice of animals for such studies. In the case of research involving endangered or extinct species, such biochemical or genetic investigations may only be possible if material is already held in a collection of this sort. While its primary function at present is a resource for genetic studies, this may not be the case in the future. The tissue collection, like other biological collections will no doubt serve future needs that may not have been considered today.

How the collection is used

The vast majority of the collection is derived from the activities of the natural history sections within the Australian Museum. The collection contains molluscs, arthropods, fish, reptiles, amphibians, birds, and mammals. Researchers within the Museum use small sub-samples of specimens held in the collection for various projects, but the collection is basically “held for posterity”. At the discretion of the Collection Manager, the material is also available to researchers external to the Museum. This is governed by the terms and conditions of a Specimen Licence Agreement and payment of a small processing fee (we **do not** charge for curation of tissues or incorporate the cost of collection in this licensing fee). The nominal fee is \$50 for DNA preparations and listed animals (CITES, IUCN, ANZECC, ESPA, TSCA, etc) and \$25 for other samples. However, the fee is negotiable and in many cases is waived, especially for those who have contributed to the collection.

Future directions

Taronga Zoo: There is a growing component of tissues in the collection derived as a by-product of post-mortem examinations at Taronga Zoo. The aim of this arrangement is to save a valuable resource for later studies, which would otherwise be discarded. Although we are principally interested in exotics and Schedule 1 & 2 species, we also value samples of some of the more common species that may not be represented in the collection.

The National Database: Groundwork has begun for setting up a National Database of tissue collections: an initiative of the South Australian Museum. It will comprise the collections of each State’s museum, and be electronically catalogued. The South Australian, Western Australian and Northern Territory Museums’ tissue collections are already computerised, and the Australian Museum’s collections are well on the way to being computerised. Such a scheme will allow NSW government agencies better access to interstate data for biodiversity and other research projects. Along with the Australian biota, that of South-East Asia and the South-West Pacific is substantially represented. When fully implemented, it will include in excess of 100,000 specimens – probably the largest collection of its type in the world.

Genome Resource Network of Australasia (GRNA): The GRNA was established in 1997. It aims to link those collecting, storing and using biological materials for research, management and conservation. This encompasses State conservation agencies, CSIRO, Cooperative Research Centres and universities, etc. The Museum, as a major repository of such material, is in a position to be a substantial contributor to the Network.

Opportunistic collection: Only in its infancy at present, opportunistic collection should substantially enhance the collection’s breadth. As well as the NSW NPWS, we hope that this will become a routine practice of other field workers and organisations.

Opportunistic collection of tissue in the field

General collection principles

When collecting biological samples opportunistically, three things should be kept in mind:

Uses of collections change over time. Any biological collection represents an invaluable resource, the importance of which may not be realised immediately. The Australian Museum's natural history collections are the oldest in Australia, dating back to the early part of the 1830s. Since that time, the role of the collections has changed and new applications have been found. Along with traditional practices such as taxonomy, the Museum's collections also play a vital role in biodiversity research and environmental impact assessment. Likewise, tissue samples collected now will serve future needs that are yet to be considered.

Common animals may be locally rare. It should be appreciated that while some animals may be considered abundant, they may be locally rare for a particular area or the designation as common may not have been reviewed for some time and hence is out of date. Generally, information on the status of an animal is only available to a specialist researcher and is only determined after further investigation. It is important not to dismiss samples as worthless without first checking the status of the animal from which it was derived. As a general rule, collect first and ask questions later.

The temporal aspect of sampling. Any collection represents a unique event that cannot be recreated. Biological systems are dynamic and subject to continually changing circumstances. Samples taken from a population of animals ten or fifteen years apart are intrinsically different. In fact, such samples are necessary when answering questions about population decline or recovery, for example. As the collection ages, it will become more amenable to this type of research.

Practical aspects of collection

In planned collection field trips, collectors take a large canister of liquid nitrogen (-196°C) into the field. Tissue collection is carried out in clean conditions from an animal that has only just died or been euthanased. Samples are taken from the healthy liver, kidney, heart or muscle, dropped into sterile plastic tubes and then snap-frozen in the liquid. The canister is then returned to the Museum and the tissues go straight into a -80°C freezer. These tissues can be used for the greatest variety of techniques and offer the best results and most flexibility in biochemical studies. While it is unlikely that this could be a workable situation for opportunistic (as opposed to dedicated) collection situations, several alternatives are available. It may be possible to obtain dry ice (-78°C) in some regional areas, which is ideal for snap freezing of tissues from fresh carcasses. Other procedures that do not require freezing are detailed in later sections.

Biological molecules degrade as the tissue in which they are contained decomposes. Perhaps the most commonly practiced techniques use DNA and proteins. The methods described here will focus on those molecules.

Proteins degrade faster than DNA. Unless the tissue is taken from a very recently deceased animal, and promptly frozen, the protein component will probably be of limited use. While samples for protein work cannot be stored in ethanol, DNA is preserved very effectively by this method. It can also be extracted from frozen tissues.

DNA is far more robust than proteins, but it too will degrade if the tissue is:

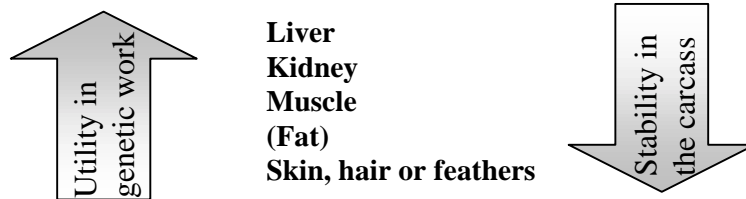
- a) already in an advanced state of decomposition.
- b) exposed to acids such as those from the bile duct or the stomach.
- c) allowed to continue to decompose because it is not preserved either by immediate cooling/freezing or dropping into ethanol (ethyl alcohol).

What to collect and where on the carcass to collect it

Almost every type of cell contains DNA: the molecule that carries all the instructions that make an organism unique. Some types of cell are better than others for extracting DNA and some contain other molecules (*eg* proteins) which can also be useful in biochemical studies. This section describes the types of tissues that we are interested in.

Types of tissue

Candidate tissues include:



They are listed in the order in which they are preferred for genetic work, but also the order in which they tend to decompose in the carcass (that is, the liver decomposes first). Liver contains many enzymes that can be used in protein studies and is a good source of DNA. It is best taken if the sample can be frozen. Other tissues are best used for DNA studies if ethanol is the only preservative available. Fat is generally not a good source of genetic material. However, it is useful for studies on bioaccumulation of pollutants such as hormones and heavy metals. While this is not at present a common use of the tissue collection, it may prove to be in the future.

Judging levels of decomposition

Tissues that are partially decomposed are still useful for some techniques (*eg* DNA work). However, the range of techniques that can be applied to a fresh sample is much greater. Therefore, the fresher the better.

In general, common sense is a good indicator of tissue condition. If the tissue looks soupy, slimy or green and has an incredibly bad smell it's probably no good. A slightly "off" smell is to be expected. The table below summarises some of the cues for judging the state of a tissue.

Tissue	Fresh	Decomposing
Liver	<ul style="list-style-type: none"> • Purple to red in colour • Soft to the touch • Does not "dissolve" if pinched 	<ul style="list-style-type: none"> • Brown to green in colour • Watery consistency.
Kidney	<ul style="list-style-type: none"> • Red to brown in colour • Firm to the touch 	<ul style="list-style-type: none"> • Tan to green-brown in colour • Soft to the touch
Muscle	<ul style="list-style-type: none"> • Varies in colour depending on location (white, pink or red) • Rebounds slightly to the touch. 	<ul style="list-style-type: none"> • Stays damaged/squashed when depressed • Can be slimy

Where to collect from

If possible, avoid sampling from areas of the carcass that have been exposed to the sun for long periods and/or to scavengers like flies, birds and burrowing invertebrates. Direct sunlight damages proteins and DNA, and scavengers can contribute their own proteins and DNA to the tissue, making interpretation of biochemical studies difficult.

If the collector knows that the animal has just died (*ie* within three to four hours) and feels confident in anatomy, sampling of the internal organs such as the liver or kidney would be ideal, remembering to avoid stomach/intestine and bile duct. Taking a sample of muscle and skin would also provide good material. Since the carcass is fresh, this can be taken from anywhere on the animal that appears to be in good condition. Fat (blubber) should be avoided if taking a muscle sample. Samples from a fresh carcass should be placed onto ice if available (*page 3.7-10*). If ice is not available, follow one of the other protocols (*pages 3.7-11 – 3.7-15*).

Priorities for data collection

If the carcass smells rotten from a distance or looks bloated, it is likely that significant internal decomposition has occurred. Muscle tissue may be okay from areas away from the visceral organs. Skin samples should be taken from areas where there isn't a lot of flesh rotting underneath. For marine mammals and turtles, skin and muscle samples should be taken from the back above the tail or posteriorly on the flipper. Avoid sampling near the belly on a mammal, or on the underside of a turtle. Similarly, skin, muscle or feather/fur samples from birds or mammals should be taken from areas where internal decay is least noticeable. In this case, samples should be taken from the extremities: on a limb or wing or, if it is too small, where this wing or limb joins the body. Again, when sampling from the body, avoid areas where decomposition is noticeable.

NOTE ON FEATHERS and FUR: DNA is derived from the “root” of the feather or hair. When sampling feathers, pluck a few well-attached ones, rather than feathers that have just fallen off. Fur samples should include the base of the hair, not just the shaft. Again, it is preferable to sample from areas where there's no sign of rotting flesh beneath.

Methods of Preservation

The method of preservation of biological specimens varies widely according to their intended use. The table below lists the advantages and disadvantages of some preservation methods under field conditions. They are also listed in order of preference for use in genetic studies. Freezing and/or ethanol are by far the most preferred methods, as they allow for the greatest use of the sample. However, as they may not be available to all field workers, some other methods are suggested here also. The next section gives detailed protocols for each method.

Method of Preservation	Advantages	Disadvantages	Refer to page
<p>Freezing Preferred:</p> <ol style="list-style-type: none"> 1. Liquid N₂ (-196°C) 2. Solid CO₂ “Dry Ice” (-78°C) 3. Domestic freezer (-20°C) 	<p>Can be used for almost any technique, including genetic and other studies. This is the preferred preservation method.</p>	<p>May not be readily available.</p>	<p>3.7-10</p>
<p>70% Ethanol Preferred:</p> <ol style="list-style-type: none"> 1. 70-100% pure ethanol 2. Highest proof vodka 3. Domestic methylated spirits 4. “Rubbing alcohol” (isopropanol) 	<p>Best method after freezing for preserving DNA.</p>	<p>May not be readily available. Flammable. Classed as a “dangerous good”. May cause problems posting or couriating samples if large quantities are used.</p>	<p>3.7-11</p>
<p>20% DMSO & Saturated Table Salt (dimethyl sulfoxide & NaCl)</p>	<p>Tissues are stable at room temperature or can be left in the fridge.</p>	<p>May not be readily available. Hazardous substance. DMSO acts as a universal solvent, enhancing the absorption of substances through the skin or respiratory passages. Do not use it in conjunction with other hazardous substances, <i>eg</i> formalin. Cannot be used for protein work. May limit the application of some DNA techniques.</p>	<p>3.7-12</p>
<p>Table salt in film canister</p>	<p>All materials are readily available in the field.</p>	<p>Tissues have to be well scored for salt to do the preservation work. The scoring may introduce contamination of tissue with DNA from other sources. Maximum tissue thickness is a couple of millimetres. Cannot be used for protein work.</p>	<p>3.7-13</p>
<p>10% Formalin (aqueous formaldehyde)</p>	<p>Not highly flammable like ethanol.</p>	<p>Cannot be used for protein work. Cannot be used for DNA work unless fully buffered to neutral pH. The buffering must usually be done in the lab. Formalin can be hazardous to work with. Classified as a “harmful substance”.</p>	<p>3.7-14</p>
<p>15% Trehalose</p>	<p>Sugar does not denature proteins. Therefore, this technique can be used for protein and DNA work.</p>	<p>Not widely available. Collectors would have to go prepared with solution. There can be problems with fermentation if tissues aren’t dried.</p>	<p>3.7-15</p>

Taking Samples

Which preservation method to use

The method you choose to preserve the tissue samples ultimately depends on what is available to you. You should use the method that maximises the utility of the sample, taking into account the resources you have at hand. Freezing is the preferred method for fresh samples. However, it is not likely to be available to many field workers. Ethanol is the next most preferred preservation technique. After this, any of the other preservation techniques are recommended.

Data sheets

All the information pertaining to a sample is recorded on a data sheet - it is an integral part of tissue collection. A data sheet should contain the following fields:

Field Number: Each animal is given a unique label. This usually consists of the collector's initials and a number to identify the individual animal *eg* TF105 or GH1. However, any system you use is fine.

Tissue type: The types of tissue that have been taken must also be recorded on this sheet, for example, muscle, skin or liver. It may be convenient to abbreviate these to M, S or L. If you use any abbreviations, be sure to include a key somewhere on the sheet.

Species: The type of animal (to the best of your knowledge) must be recorded. Try to be as specific as possible. If you know the scientific name, use that name or a well-known common name. It is of little help if a sample is recorded only as "whale" or "bird" - try to get a precise identification.

Location: The last piece of information that must be recorded is the location where the animal was found. Again, you should be as specific as possible and, if available, use a GPS unit to record the precise latitude and longitude (or UTM etc). If you use a GPS, give co-ordinates equivalent to "seconds" (*ie* decimal degrees to 4 decimal places and decimal minutes to 2 decimal places). When writing down locations, it is good practice to start large and finish with the specifics (*eg* "Sydney, Homebush Bay, Newington Armaments Depot" or "Lennard's Island, 7 km N of Eden, S side of island").

Comments: You should also record on the data sheet any other information that might be relevant. This might include the cause of death; the weather, tide or sea conditions; or the sex of the animal.

You will find a sample data sheet at the end of this manual. It is suggested that you make copies of this sheet. However, if you use your own sheets, make sure you include at least all the above information. An example might look like this:

DATA SHEET FOR TISSUE COLLECTED IN THE FIELD

COLLECTOR: Boris Brown **CONTACT TELEPHONE NO:** 02 9123 4567 **DATE:** 7/9/99

M=muscle, K=kidney, L=liver

Field No.	Tissue Type	Animal	Location	Time since death	Comments
BB01	M,K,L	<i>Isoodon macrourus</i> (Northern brown bandicoot)	Wyong area: Pacific Hwy outside T.T.Auto wreckers; 33°15'54" S, 151°18'12" E	probably less than 24 hours	Female, 4 enlarged teats - pouch young BB02, BB03, BB04 - one missing?. Road kill.
BB02	M,L	<i>Isoodon macrourus</i>	see BB01	see BB01	Male. Pouch young of BB01
BB03	M,L	<i>Isoodon macrourus</i>	see BB01	see BB01	Female. Pouch young of BB01
BB04	M,L	<i>Isoodon macrourus</i>	see BB01	see BB01	Male. Pouch young of BB01

Labelling

While it sounds a simple task, adequate labelling of samples is of the utmost importance. Unless a sample can be properly identified, it is close to worthless. It is disheartening to find that, after considerable effort on behalf of the collector, something as simple as poor marking of the tube renders the sample useless. Ensure that your handwriting is legible and mark the tube in at least two places with the field number in case one rubs off. Don't write on dirty, greasy or wet tubes. Labels on plastic bags, especially once frozen, are likely to rub off. Therefore, as a precaution, insert a label written with pencil inside the bag. Pencil labels are also useful for samples in ethanol as most permanent text rubs off when in contact with alcohol.

How much tissue to take

Be guided by the amount of healthy tissue available. Avoid taking decomposing tissue adjacent to healthy tissue just to make up the amount you are aiming for. Molecular methods only need match-head sized tissue samples and much better results are achieved with this amount of healthy tissue than large chunks of tissue with decomposing edges.

If sampling into ice: take larger chunks of tissue, if possible, as they defrost more slowly after being frozen (eg minimum 2-3cm³). Do not overfill the container. Expansion of the sample due to freezing may cause the container to crack.

If one of the other methods is used: a sample 1cm³ in size is more than adequate. Be guided by the size of the sample vial available. The volume of the preservative should be at least **twice** that of the sample. Taking really large amounts of tissue to be preserved in ethanol means you have to be carrying unnecessarily large amounts of the solution. This may present problems when transporting the samples (due to dangerous goods regulations). If larger samples are taken, the solution also takes longer to permeate and therefore preserve the tissue. Rather than one large sample, it is a good idea to take two small samples of ~0.5cm³ each: about the size of a frozen pea. Slicing these further will increase the surface area available for permeation of the preservative. This is very important when using methods other than freezing for preserving samples.

For all methods

Materials

For all methods of tissue collection, you will need the following:

- Data Sheet plus plastic bag for the sheet to go in (eg Zip-top sandwich bag)
- Indelible pen such as a fine texta and/or a hard-leaded pencil (HB-2H)
- Disposable gloves (available from chemist or hardware stores)
- Disposable scalpel/handle set **or** scalpel blade attached to scalpel handle **or** dissecting scissors **or** single sided razor blade (available from hardware stores) **or** clean, sharp knife if necessary. NB: Suitable sharps containers will need to be provided if using scalpels.
- Forceps or tweezers if available
- Disposable facial tissues to wipe down dissection instruments between animals
- Plastic bag to hold both the wrapped or contained specimen and the wrapped data sheet

Collection Procedure

The following procedure is common to all preservation methods. Follow the steps below, then turn to the page detailing the method you will use.

- Fill out as much of the Data Sheet as possible before commencing
- Label the bag/s or container/s in which the tissue is going to be put with Field Number, Tissue Type (eg L for liver, M for muscle, S for skin) and, if there's room, the type of animal. If possible, put the label in two places on the bag or container in case one label rubs off. The aim is to be able to link the specimen in the container with the information on the Data Sheet. Have the bag or container ready to drop the sample in as soon as it is dissected.
- Use disposable gloves. If none are available, try to avoid touching the tissue with your fingers.
- Aim not to mix your cells (from your hair or skin) with those of the animal, and also not to mix one animal's cells with that of another.

Priorities for data collection

- Remember to clean your instruments thoroughly when moving from one animal to the next. Dipping in ethanol and burning off the excess is a good way to do this. Alternately, flame the instruments quickly over a cigarette lighter or similar and wipe down afterwards. Failing this, wipe down the instruments with a clean, dry facial tissue, ensuring that no traces of blood or other material remain.

For cryogenic methods of preservation, refer to page 56.

For all other methods, refer to pages 57 - 61.

Freezing

Liquid nitrogen UN1977 NON-FLAMMABLE GAS CLASS 2	DANGEROUS GOODS	COURIER
Dry ice (CO ₂ solid) UN1845 MISCELLANEOUS CLASS 9	DANGEROUS GOODS	COURIER
Domestic Ice	See packing instructions, page 65	COURIER

Liquid nitrogen or dry ice

While liquid nitrogen and dry ice are among the best methods of preservation, each presents its own problems. Liquid nitrogen (LN₂) must be stored and transported in special 'Dewar' flasks, which are not generally available. If a large number of samples is going to be collected, the Museum can provide LN₂ and make transport arrangements. However, for small collection events, it is preferable that other methods are used.

Dry ice (solid carbon dioxide) is more widely available and easier to transport than LN₂. For couriering, more than four kilograms of dry ice is considered dangerous goods, though this amount is unlikely to be necessary. A six-pack esky holds about 500 grams which should be sufficient for an overnight service. The lid of the esky should be fastened securely, leaving somewhere for the gas to escape as it sublimates. Mark the container with the net weight of the dry ice, UN number and dangerous goods class as above. Phone the Museum for further instructions before calling the courier if either LN₂ or dry ice is used.

Domestic freezer and ice

Samples stored in a domestic freezer should not be kept longer than one week before being transported to the Museum. Protein decomposition will begin to occur even at this temperature. Try to arrange the courier before then.

Additional Materials

As well as the materials listed on page 55, you will require the following:

- Container for tissue, eg Clean plastic bag (zip-top sandwich bags are great); or Clean screw-cap plastic specimen bottle; or Plastic wrap such as Clingwrap. "Cryovials" are available from the Museum or perhaps the local university. Do not use glass, aluminium foil, or allow newspaper to come into contact with the sample.
- Dry ice or domestic ice in an insulated container such as an esky. Make sure there is sufficient ice to last until you can return to the freezer. An esky can be improvised by lining a cardboard box with towels for insulation, then plastic (eg garbage bag), then ice into this. Improvise a lid by covering with towels, wetsuit vest, etc.
- Access to a domestic freezer within a couple of hours
- Six-pack styrofoam esky to package it up for couriering

Collection Procedure

- Use whatever dissecting instrument you have to take a sample of the tissue of an appropriate size – about 2-3cm³ (depending on the type and availability of tissue).
- Try to collect a couple of samples (*eg* one muscle and one skin, or skin from two different spots on the carcass) but do not put more than one tissue-type in each container.
- Seal the sample well.
- Clean your instruments thoroughly before moving on to the next animal. Dipping in ethanol and burning off the excess is a good way to do this. Alternately, flame the instruments quickly over a cigarette lighter or similar and wipe down afterwards. Failing this, wipe down the instruments with a clean, dry facial tissue, ensuring that no traces of blood or other material remain.
- Complete the Data Sheet, place this in a sealed plastic bag and store with the sample(s) in an outer plastic bag. Bury all this in the ice. The data sheet must not be separated from the sample from now on.
- As soon as possible after sampling, put the whole bundle with samples and data sheet into a freezer. Allow the sample a few hours to freeze properly before courier pick-up (see page 65).

Non-cryogenic methods

Ethanol

Greater than 70% ETHANOL <u>or</u> more than 50mL any ALCOHOL (including methylated spirits & isopropanol)	DANGEROUS GOODS	COURIER
70% ETHANOL or less, <u>and</u> less than 50mL total volume any ALCOHOL		PARCEL POST

CAUTION: Ethanol is highly flammable. DO NOT SMOKE while handling it, AVOID splashing it in your eyes and AVOID transporting large quantities of it.

Ethanol preservation works by dehydration: water is removed from the cell and replaced by ethanol. This is also common to the other non-cryogenic methods that follow, though with some variations. Without water, few of the degradative biological processes responsible for decomposition can take place. Ethanol can directly inhibit some cellular proteins – although this is not necessarily bad. In the case of DNA, the enzymes responsible for its breakdown (nucleases) are denatured (disabled). The DNA is then left safely in the cellular structure.

Do not use 100% ethanol as it cannot be sent via Parcel Post. A 70% solution should be made by mixing 7 parts 100% ethanol with 3 parts distilled water. Measure out the parts separately. making up the volume by adding water to ethanol (or vice versa) will not result in the desired concentration. A 70% solution may also be obtained from a pharmacy. If both of these methods are unavailable, use the highest proof vodka obtainable. Undiluted methylated spirits or isopropanol (rubbing alcohol) can also be used if necessary.

Additional Materials

In addition to the materials listed on page 55, you will require the following:

- ❑ Container for tissue

NB: the smaller the container, the better. Ideally, the container should be only large enough to contain a 0.5cm³ sample plus 1mL or so of ethanol

eg Small clean screw-cap plastic specimen bottle

or sterile sample tubes (The Museum can provide these)

or, if necessary, a very clean plastic or glass food jar which will not leak ethanol when sealed. If using glass, it must be well protected from breakage.

Priorities for data collection

- ❑ OPTIONAL: Plastic Pasteur pipette, eye dropper or something to help you top up the container with the ethanol after you put the sample in.
- ❑ Solution of 70% ethanol (ethyl alcohol) or equivalent.

Collection Procedure

- Half-fill the container you are going to use with ethanol, wiping away spillage.
- Ensure that the label has not been removed by spilt ethanol. Dry and relabel if necessary, or insert a paper label with the field number and tissue type written in pencil into the container.
- Use whatever dissecting instrument you have to take a sample of the tissue of an appropriate size - about 0.5cm³ (depending on the type and availability of tissue).
- Try to collect a couple of samples (*eg* one muscle + one skin, or skin from two different spots on the carcass) but do not put more than one tissue-type in each container.
- After the sample is in the container, top it up with ethanol ensuring that the tissue is covered by at least **two** volumes of the solution (ie 1mL for a 0.5cm³ sample) and secure the lid well. If there is any possibility of leakage, keep the containers upright.
- Clean your instruments thoroughly before moving on to the next animal. Dipping in ethanol and burning off the excess is a good way to do this. Alternately, flame the instruments quickly over a cigarette lighter or similar and wipe down afterwards. Failing this, wipe down the instruments with a clean, dry facial tissue, ensuring that no traces of blood or other material remain.
- Complete the Data Sheet, place this in a plastic bag and store with the sample(s). The data sheet must not be separated from the samples from now on. To ensure this, you could put both the samples and the Data Sheet together in a zip-top plastic bag.
- Samples are now stable at room temperature, but keep them away from heat (*eg* out of hot cars, out of direct sunlight). Also, do not store samples in unventilated cupboards. Then arrange for a courier or Parcel Post (page 65).

20% DMSO & Table Salt

20% Dimethyl sulfoxide and saturated NaCl	See packing instructions, page 65	PARCEL POST
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CAUTION: Dimethyl sulfoxide is a hazardous substance. It acts as universal solvent, enhancing absorption of substances through the skin and respiratory passages. DO NOT use it in conjunction with other hazardous substances (*eg* formalin or ethanol).

DMSO and saturated salt preservation works by osmotic dehydration. The mechanism is two fold: DMSO allows the salt to penetrate the tissue more readily while the salt draws water out of the cell by osmosis. Salt outside the cell is at a very much greater concentration than it is inside. To compensate for this, water moves across the cell membrane to such an extent that the cell is left dry. As with ethanol preservation, degradation is retarded in the absence of water.

A 20% DMSO/NaCl solution can be made up very easily. First, a 20% (v/v) solution of DMSO in water should be made – for 100mL, add 20mL of DMSO to 80mL of distilled water. To this, table salt (NaCl) should be added to saturation. Continue adding salt while stirring until no more will dissolve in the solution. At 20-25°C, this requires about 25g of salt. Leave a thin layer of undissolved salt in the stock solution to compensate for changes in solubility due to temperature.

Additional Materials

As well as that listed on page 55, you will require the following:

- ❑ Container for tissue

NB: the smaller the container, the better. Ideally, the container should be only large enough to contain a 0.5cm³ sample plus 1mL or so of DMSO/NaCl

eg Small clean screw-cap plastic specimen bottle

or sterile sample tubes (The Museum can provide these)

or, if necessary, a very clean plastic or glass food jar which will not leak when sealed. If using glass, it must be well protected from breakage.

- ❑ OPTIONAL: Plastic Pasteur pipette, eye dropper or something to help you top up the container with DMSO/NaCl after you put the sample in.
- ❑ Solution of 20% DMSO and saturated NaCl.

Collection Procedure

- Half-fill the container you are going to use with DMSO/NaCl, wiping away spillage.
- Ensure that spilt DMSO/NaCl has not removed the label. Dry and relabel if necessary or insert into the container a paper label with the field number and tissue type written in pencil.
- Use whatever dissecting instrument you have to take a sample of the tissue of an appropriate size – about 0.5cm³ (depending on the type and availability of tissue).
- Try to collect a couple of samples, (*eg* one muscle + one skin or skin from two different spots on the carcass) but do not put more than one tissue-type in each container.
- After the sample is in the container, top it up with DMSO/NaCl, ensuring that the tissue is covered by at least **two** volumes of the solution (ie 1mL for a 0.5cm³ sample) and secure the lid well. If there is any possibility of leakage, keep the containers upright.
- Clean your instruments thoroughly before moving on to the next animal. Dipping in ethanol and burning off the excess is a good way to do this. Alternately, flame the instruments quickly over a cigarette lighter or similar and wipe down afterwards. Failing this, wipe down the instruments with a clean, dry facial tissue, ensuring that no traces of blood or other material remain.
- Complete the Data Sheet, place this in a plastic bag and store with the sample(s). The data sheet must not be separated from the samples from now on. To ensure this, you could put both the samples and the Data Sheet together in a zip-top plastic bag
- Samples are now stable at room temperature, but keep them away from heat (*eg* out of hot cars or direct sunlight). Pack up the samples for shipment to the Museum via Parcel Post (page 67).

Table salt in Film Canister

Sodium chloride (NaCl)	See packing PARCEL POST instructions, page
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Preservation of foodstuffs by dry salting is an ancient technique. The same process is used here to preserve tissue samples for biochemical studies. Like the other methods above, salting works by dehydration. In the absence of water, most of the enzymes responsible for the breakdown of the tissue (autolysis) are destroyed or rendered inactive. However, for the technique to work effectively the tissue must be in very thin slices. This maximises the surface area on which the salt can act.

Additional Materials

As well as the materials listed on page 59, you will require the following:

Priorities for data collection

- Container for tissue (*eg* a film container; a clean screw-cap plastic specimen bottle; a very clean plastic or glass food jar; or, if necessary, a clean plastic bag). Film containers should be clean and preferably not have been used for anything but storing film.
- A package of **unused** and preferably **unopened** table salt. Non-iodised salt would be preferable to iodised, though it's not too important.

Collection Procedure

- Half-fill the container you are going to use with salt.
- Use whatever dissecting instrument you have to take a sample of the tissue of an appropriate size – about 0.2 - 0.5cm³ (depending on the type and availability of tissue). Slice the sample further so as to maximise the exposure of the sample to the salt, being careful not to introduce contamination.
- Try to collect a couple of samples (*eg* one muscle + one skin, or skin from two different spots on the carcass) but do not put more than one tissue-type in each container. However, if you have taken small samples of the same tissue put them all in the one container, noting that on the Data Sheet.
- Seal the sample well.
- Clean your instruments thoroughly before moving on to the next animal. Dipping in ethanol and burning off the excess is a good way to do this. Alternately, flame the instruments quickly over a cigarette lighter or similar and wipe down afterwards. Failing this, wipe down the instruments with a clean, dry facial tissue, ensuring that no traces of blood or other material remain.
- Complete the Data Sheet, place this in a sealed plastic bag and store with the sample(s) in an outer plastic bag. The data sheet must not be separated from the sample from now on.
- Samples are now stable at room temperature, but keep them away from heat (*eg* out of hot cars or direct sunlight). Pack up the samples for shipment to the Museum via Parcel Post (page 65).

10% Buffered Formalin

10% Phosphate buffered formalin (aqueous formaldehyde)	See packing instructions, page 65	PARCEL POST
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WARNING: Formalin is highly toxic. DO NOT use it in an enclosed space. Ensure there is adequate ventilation. AVOID breathing in fumes and AVOID contact with skin or eyes.

Formalin preservation works in a very different fashion to those methods mentioned so far. It alters the structure of the molecules that make up cells by a process called cross-linking. The components of the cells are linked together on a very small scale so they become fixed in place. Formalin preservation of tissues is not the best for DNA studies. If it is used unbuffered, formalin becomes acidic, irreversibly damaging DNA. Further, some of the cross-linking cannot be undone, leaving the DNA unusable for many biochemical studies.

There is often some confusion about the difference between formalin and formaldehyde. However, the distinction is straightforward. Formalin is aqueous formaldehyde. Formaldehyde is a gas at room temperature and can dissolve in water to a maximum concentration of about 40% (w/v). A full-strength or 100% formalin solution contains about 400g of formaldehyde per litre of water. A 10% (v/v) solution can be made by mixing one part full-strength solution with nine parts water. This can be buffered by adding 4g of sodium phosphate monobasic (NaH₂PO₄ · H₂O); and 6g of sodium phosphate dibasic (anhydrous) (Na₂HPO₄) per litre of solution. In a predicament, adding an excess of magnesium carbonate (MgCO₃) or calcium carbonate (CaCO₃) or even marble chips can achieve some buffering.

Penetration of formalin into tissue is slow (especially if the skin is intact) and decomposition can continue in unfixed areas. Samples must be no greater than 0.5cm thick in at least one dimension.

Additional Materials

In addition to the materials listed on page 55, you will require the following:

- ❑ Container for tissue

NB: the smaller the container, the better. Ideally, the container should be only large enough to contain a 0.5cm³ sample plus 1mL or so of formalin.

eg Small clean screw-cap plastic specimen bottle

or sterile sample tubes (The Museum can provide these)

or, if necessary, a very clean plastic or glass food jar which will not leak formalin when sealed. If using glass, it must be well protected from breakage.

- ❑ **OPTIONAL:** Plastic Pasteur pipette, eye dropper, or something to help you top up the container with the formalin after you put the sample in.
- ❑ Solution of 10% phosphate (or equivalent) **buffered** formalin.

Collection Procedure

- Half-fill the container you are going to use with formalin, wiping away any spillage.
- Use whatever dissecting instrument you have to take a sample of the tissue of an appropriate size (about 0.5cm³, depending type and availability of tissue).
- Try to collect a couple of samples (*eg* one muscle + one skin, or skin from two different spots on the carcass) but do not put more than one tissue-type in each container.
- After the sample is in the container, top it up with formalin ensuring that the tissue is covered by at least **two** volumes of the solution (ie 1mL for a 0.5cm³ sample) and secure the lid well. If there is any possibility of leakage, keep the containers upright.
- Clean your instruments thoroughly before moving on to the next animal. Dipping in ethanol and burning off the excess is a good way to do this. Alternately, flame the instruments quickly over a cigarette lighter or similar and wipe down afterwards. Failing this, wipe down the instruments with a clean, dry facial tissue, ensuring that no traces of blood or other material remain.
- Complete the Data Sheet, place this in a plastic bag and store with the sample(s). The data sheet must not be separated from the samples from now on. To ensure this, you could put both the samples and the Data Sheet together in a zip-top plastic bag.
- Samples are now stable at room temperature, but keep them away from heat (*eg* out of hot cars, out of direct sunlight). Also, do not store samples in unventilated cupboards. Then arrange for a courier or Parcel Post (page 65).

15% Trehalose

15% Trehalose solution	See packing PARCEL POST instructions, page 65
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Many plants and some invertebrates use trehalose to survive long periods of dehydration and even freezing. The mechanism by which it works is still not well understood, though it is thought to be by the formation of a “glass”. This fixes the cellular components in place, arresting many biological processes and preventing protein damage due to dehydration. After rehydration, most activity is restored.

A 15% (w/v) trehalose solution can be made very easily. Add 15g of trehalose per 100mL of distilled water. It is best to keep stock volumes to a minimum as the solution is prone to fermentation (*ie*. 3g trehalose in 20mL of distilled water should be ample).

Priorities for data collection

Additional Materials

In addition to the materials listed on page 55, you will require the following:

- Container for tissue

NB: the smaller the container, the better. Ideally, the container should be only large enough to contain a 0.5cm³ sample and 1.5mL or so of trehalose solution.

eg Small clean screw-cap plastic specimen bottle

or sterile sample tubes (The Museum can provide these)

or, if necessary, a very clean plastic or glass food jar

OPTIONAL: Plastic Pasteur pipette, eye dropper, or something to help you top up the container with the trehalose solution after you put the sample in.

- Solution of 15% trehalose
- Silica gel (& airtight container), drying oven or equivalent

Collection Procedure

- Half-fill the container you are going to use with trehalose solution, wiping away any spillage.
- Use whatever dissecting instrument you have to take a sample of the tissue of an appropriate size (about 0.5cm³, depending type and availability of tissue)
- Try to collect a couple of samples (*eg* one muscle + one skin, or skin from two different spots on the carcass) but do not put more than one tissue-type in each container.
- After the sample is in the container, top it up with trehalose solution ensuring that the tissue is covered by at least **three** volumes of the solution (ie 1.5mL for a 0.5cm³ sample) and secure the lid well. If there is any possibility of leakage, keep the containers upright.
- Clean your instruments thoroughly before moving on to the next animal. Dipping in ethanol and burning off the excess is a good way to do this. Alternately, flame the instruments quickly over a cigarette lighter or similar and wipe down afterwards. Failing this, wipe down the instruments with a clean, dry facial tissue, ensuring that no traces of blood or other material remain.
- Complete the Data Sheet, place this in a plastic bag and store with the sample(s). The data sheet must not be separated from the samples from now on. To ensure this, you could put both the samples and the Data Sheet together in a zip-top plastic bag.
- The samples must be stored in the trehalose solution for at least 24 hours. This should be in a cool spot, away from direct sunlight. If available, refrigerate overnight.
- The samples must now be dried: discard the trehalose solution and dry the sample using either silica gel, incubating at 35-37°C or air-drying. To use silica gel, place a couple of grams of the gel wrapped in a facial tissue in an airtight container. Place the sample in the container and seal it. Depending on the ambient conditions, it should be dry in 1-2 days. If a drying oven is used, avoid temperatures above ~40°C as some proteins may be damaged. Alternately, leaving the samples uncapped and allowing them to air dry (or sun dry) will yield reasonable results, though it will take considerably longer to dry the samples.
- Samples are now stable at room temperature. Return them to their containers and seal the lids well. Keep them away from heat and unnecessary sunlight (*eg* out of hot cars and out of direct sunlight) and arrange for shipment to the Museum via Parcel Post (page 65).

Getting your samples to the Museum

If the sample is frozen:	All other preservation methods:
<p>It must be transported in an insulated container (such as a six-pack esky) on ice.</p> <p>Avoid doing the packaging until just before the Courier is due to arrive. Make sure that the sample and data sheet are sealed together in a plastic bag to prevent melting ice getting into the sample or data sheet. Bury well in fresh ice if available.</p> <p>Seal the esky with tape and label it with the Museum's details as below and your contact details including your phone number. Add instructions such as "REFRIGERATE ON ARRIVAL"</p> <p>If the collection has occurred on a Friday, Saturday or Sunday, keep the sample frozen and ring the courier Monday. Book an overnight courier, and have them pick-up as late in the day as possible (so you can keep the parcel frozen for as long as possible). They may even be able to put the package in a cold room overnight if there is going to be a delay. If you have samples in liquid nitrogen or dry ice, please contact the tissue collection manager to make couriering arrangements.</p>	<p>Ensure that the containers are not going to leak. Check closure, and tape around it if necessary. If possible, package so the samples are sitting upright. Use padding to prevent the containers being crushed.</p> <p>Mark "FRAGILE – DO NOT CRUSH" on the package and make any additional notes <i>eg.</i> "THIS WAY UP" or "GLASS WITH CARE"</p>
<p>Label all packages with the Museum's details as below and your contact details including your phone number.</p>	

Courier

The Australian Museum uses TNT Express. Call the local branch and ask for an **overnight service** (not 'priority' as it's too expensive). If your samples constitute dangerous goods, let them know as additional paperwork must be filled in. They will send the appropriate forms. Quote account number D902460 and ask them to "charge receiver".

DELIVER TO: The Tissue Collection Manager
 Evolutionary Biology Unit
 The Australian Museum
 Gate 3, Loading Dock
 William Street, SYDNEY NSW 2010
 PHONE: (02) 9320 6292

Telephone the Tissue Collection Manager at the Australian Museum (02) 9320 6292 to alert that the package has been sent.

Parcel Post

Dangerous goods must not be posted. Check the procedures section (pages 56-65) to determine if you have a dangerous good and, if you do, use a courier. If the samples are not dangerous goods, affix the appropriate Australia Post sticker.

ADDRESS TO: The Tissue Collection Manager
 Evolutionary Biology Unit
 The Australian Museum
 6 College St, SYDNEY NSW 2010

Priorities for data collection

Telephone the Tissue Collection Manager at the Australian Museum (02) 9320 6292 to alert that the package has been sent.

Suppliers

All of the materials and chemicals that are referred to in this manual are available from the Museum by prior arrangement. You should also be able to source many of the items from regional suppliers, local universities or veterinary hospitals. Small quantities of ethanol (ethyl alcohol) may be available from your local pharmacy. Some contacts are given below.

Sigma-Aldrich Chemicals (1 800 800 097)

DMSO (Dimethyl sulfoxide)	Cat No D-5879 100mL (~\$22.00)
Trehalose	Cat No T-5251 5g (~\$17.00)
Formalin Solution 10%, Neutral Buffered	Cat No H50-1-128 4L (~\$40.00)

Delivery: ~\$15.00

Q Stores (02 9318 7888/1 800 424 613)

Dissecting Kit	Cat No 993890 (~\$20.00)
<i>or</i> Dissecting forceps	Cat No 993931 (~\$1.00)
Stainless steel surgical scissors	Cat No 841453 (~\$4.00)
Scalpel handle (N ^o 3)	Cat No 840930 (~\$4.00)
Scalpel blades (100pk) N ^o 11	Cat No 840850 (~\$19.00)
Formalin 2.5L NOT BUFFERED*	Cat No 830100 (~\$21.00)
Calcium carbonate, 500g	Cat No 811220 (~\$8.00)
Magnesium carbonate, 500g	Cat No 854680 (~\$13.00)
Silica gel, 500g	Cat No 812060 (~\$12.00)
Distilled water 5L	Cat No 203113 (~\$7.00)
Ethanol 70%, 500mL RESTRICTED**	Cat No 204268 (~\$3.00)
Sharps collector 1.4L	Cat No 841460 (~\$4.00)
Latex gloves (box of 50) Small/Medium/Large	Cat No 835224/5/6 (~\$4.00)

Delivery: Free throughout NSW

***Must be buffered** before being used for tissue samples – may be used to preserve specimens after tissue samples have been taken. Use a 10% solution for specimen fixing. See page 60 for buffering instructions.

**Requires a permit from Customs Australia.

Universities

Generally, ask to speak to someone in the biological sciences department.

Lismore

Southern Cross University 6620 3000

Armidale

University of New England 6773 3333

Newcastle

University of Newcastle 4921 5000

Sydney

Australian Museum 9320 6292/6175

Wollongong

University of Wollongong 4221 3555

ACT

Australian National University 6249 5111

Albury-Wodonga

Charles Sturt University 6051 6000

DATA SHEET FOR TISSUE COLLECTED IN THE FIELD

COLLECTOR.....CONTACT TELEPHONE NO.....DATE.....

Field No.	Tissue Type	Species	Location (give Lat/Lon or UTM if possible)	Time since death	Comments

FIELD NUMBER: A unique number assigned to an animal in the field. If no NPWS number already assigned to this animal, assign any number you wish. If more than one animal is being sampled, each must have a unique number

TISSUE TYPE: L = Liver, K = Kidney, M = Muscle, S = Skin, F=Feathers

SPECIES: Be as specific as possible, eg humpback whale, young male, loggerhead turtle, juvenile

LOCATION: Be as specific as possible, eg Sydney, Bondi Beach, or beach 1 km south of Eden, NSW. Give co-ordinates if possible. If a GPS is available, record lat/long equivalent to seconds (*ie* decimal degrees to 4 decimal places, decimal minutes to 2 places)

TIME SINCE DEATH: Actual or estimated interval between time of death of animal and time of sampling

COMMENTS: Anything else that you think might be relevant to using the tissue later on

eg Was the animal in an advanced state of decomposition?

Did you have to improvise in some way other than in the instructions?

Sample data sheet

Appendix 9: Sea snakes recorded in NSW

Photographic images have been provided by Col Limpus, Hal Cogger (Yellow-bellied Sea-snake) and Rudi Kuitert (Stokes' Sea snake).

Sea Snakes recorded in New South Wales



Acalyptophis peronii (Horned Sea snake)



Aipysurus duboisii (Reef Shallows Sea snake)



Aipysurus laevis (Olive Sea snake)



Astrotia stokesii (Stokes' Sea snake)



Disteira kingii (Spectacled Sea snake)



Disteira major (Olive-headed Sea snake)

Sea Snakes recorded in New South Wales



Emydocephalus annulatus (Turtle-headed Sea snake)



Hydrophis elegans (Elegant Sea snake)



Hydrophis ornatus/ocellatus (Spotted Sea snake)



Laticauda colubrina (Yellow-lipped Sea Krait)



Pelamis platurus (Yellow-bellied Sea snake)





NSW
NATIONAL
PARKS AND
WILDLIFE
SERVICE

General inquiries: 43 Bridge Street (PO Box 1967) Hurstville NSW 2220
Phone: 02 9585 6444 or 1300 36 1967
Web site: www.npws.nsw.gov.au
