

## Part 2. Subantarctic Pinniped Mortality Event Contingency Plan

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# 1. Introduction

This contingency plan is a result of the unusual mortality event which occurred in the New Zealand sea lion population at the Auckland Islands in January-February 1998. It was developed following a Workshop on the New Zealand sea lion Mortality Event, held in Wellington in June 1998. The plan is intended to provide Department of Conservation (DOC) staff who are working with pinnipeds with guidelines to assist in dealing with any future mortality event in the subantarctic. A mass mortality event may be caused by viral or bacterial infection, biotoxin poisoning, oil or chemical pollution, some natural imbalance in environmental conditions, or adverse human interaction that might affect sea lions, fur seals or elephant seals.

The islands of New Zealand's subantarctic present particular logistical problems for any emergency operation: they are distant from the mainland, isolated, and the weather is often inclement. There are no permanent facilities, power, or laboratories unless taken there by expeditions. The experience of the sea lion mortality event in 1998 demonstrated that advance planning for such a contingency should be part of any expedition to the subantarctic islands.

## 1.1 OBJECTIVES OF THE PLAN

To enable DOC to respond to unusual pinniped mortality events in the subantarctic in a manner that will:

- Be scientifically valuable;
- Have consideration for unaffected colonies of marine mammals; and
- Have regard to public health.

## 2. General guidelines

### 2.1 DEFINITION OF AN EVENT

An unusual mortality event is one that meets the following criteria:

- It is unexpected;
- It involves a significant die-off of sea lions or other pinnipeds;
- Mortality may be only slightly increased but large number of animals may show clinical signs of disease, and/or physical impairment.

### 2.2 RESPONSE

**Primary response** to a mortality event must:

- Adhere to standard operating procedures;
- Establish lines of communication;
- Identify spatial extent of event:
  - Number moribund or dead in total population present;
  - Distribution of dead and moribund animals;
  - Age class(es) affected;
  - Species affected if more than one involved;
- Temporal characteristics of the event:
  - When did it start?
  - Are new cases appearing?
  - How many per day?
- Identify possible cause(s) of the event:
  - Unusual environmental conditions;
  - Water quality or algal blooms;
  - Identify possible contributing factors;
- Document clinical signs of illness and gross pathology on video and/or still photography;
- Minimize spread of possible infectious agents by limiting human traffic between sites;
- Identify human health risks;
- Provide accurate and timely public information on the event.

## 3. Management of a response

### 3.1 DEPARTMENTAL NOTIFICATION

Any unusual mortality event in the subantarctic should be reported rapidly to the Southland Conservancy, which would assume the role of Local DOC Response Office. The Conservancy should assess the information with assistance from DOC Science & Research, Wellington, and a Specialist Advisory Group (SAG).

On advice from the Specialist Advisory Group, the Southland Conservator should inform the Regional General Manager, DOC Southern Regional Office, who would declare an unusual mortality event status for the area under concern. The Conservator would immediately designate a Mortality Event Response Manager (MRM) from within the Conservancy staff. That officer should discuss with the field party the designation of an On-site Response Coordinator (ORC)—normally the field team leader—and from that moment all communication between DOC and the field party would be between those two officers, unless delegated by the Mortality Event Response Manager. The MRM should receive documentation of the observations made by the On-site Response Coordinator and team as soon as possible after notification of the mortality event.

### 3.2 DELEGATION AND FUNDING

The Mortality Event Response Manager must be able to make decisions, commit funds, delegate authority, and coordinate the activities of support services. The officer should ascertain as soon as possible the approximate resources required to respond to the event, and discuss with the Southern Regional Office (SRO), Christchurch, the availability of funds and the setting up of a contingency fund for emergency expenditure. The SRO would be the DOC line management expenditure approval office.

### 3.3 SCIENCE SUPPORT

The MRM must immediately alert the Science Manager (Marine), Science & Research Unit, Wellington, who would activate an Event Science Coordinator (ESC) a Marine Group staff member previously identified to be the link between the On-site Response Coordinator and the Specialist Advisory Group of marine mammal/veterinary/public health experts. The Specialist Advisory Group would advise on scientific aspects of the response, whether additional specialist staff (i.e. pathologists, bacteriologist, epidemiologist) should be flown in, and organise the collection of samples for analysis. The Event Science Coordinator would arrange for samples to be analysed, negotiate

contracts with providers, and be the DOC reference point for receiving and reporting results of analyses.

### 3.4 MEDIA LIAISON

The Mortality Event Response Manager would also have responsibility for managing the preparation of any press releases on the event or response to it. The Conservancy Community Relations Manager would appoint or become the Event Information Officer and be largely responsible for drafting press releases, and these would be finally approved and released through the External Relations Division of DOC Head Office, Wellington. The Mortality Event Response Manager would be the main media spokesperson for DOC's response to the event.

Science issues may need to be referred to the Event Science Coordinator.

### 3.5 MINISTER AND STAKEHOLDERS

The External Relations Division would be responsible for advising the Director-General of Conservation and the Minister of Conservation on developments, and informing stakeholders: fishing industry and non-government conservation organisations (Forest & Bird Protection Society, Greenpeace, Worldwide Fund for Nature). An electronic mail loop would be set up to disseminate information rapidly as it came to hand.

## 4. Response actions

### 4.1 TRIGGER FOR NOTIFICATION OF AN UNUSUAL MORTALITY EVENT

The main criterion for notification that an unusual mortality event was most likely occurring would be an unexpected, rapid and significant die-off of sea lions/seals. “Significant” is defined as a mortality rate which is 20% greater than the maximum normal natural daily mortality rate previously documented for that species, measured over 3 days.

Secondary criteria would be widespread debilitation or physical evidence of sickness (bleeding, vomiting, diarrhoea, swellings, paralysis), associated with dying animals.

### 4.2 NOTIFYING AN EVENT

A decision about whether an unusual mortality event is underway or not would not rest with the expedition team leader or On-site Response Coordinator alone. That officer would need to relay relevant information to the Event Science Coordinator in Wellington, who would consult with the Specialist Advisory Group and advise on the probability that an event was occurring. Using this advice, and following instigation of the Primary Response procedure above, the On-site Response Coordinator would then decide whether or not to formally notify the event to the Southland Conservator.

Following notification, a detailed examination should be made of acutely affected and dead animals to obtain clinical data to assist the Specialist Advisory Group’s assessment of the possible cause and scientific response requirements. The Specialist Advisory Group would advise, through the Event Science Coordinator, what samples need to be collected from animals and the marine environment.

## 5. Procedures for handling diseased pinnipeds

Unusual marine mammal mortality events present risks to human health, either from injuries to field staff while undertaking their work, or from sickness or disease resulting from exposure to pathogens or toxins. Strict adherence to safety guidelines is therefore required of all staff working with diseased or dead animals.

Protective clothing should be worn, and exposed skin should be covered by disposable gloves, overalls and gumboots. A face mask should be worn, and there should be no eating or drinking in an affected area. Equipment and clothing should be disinfected with a phenolic disinfectant such as Clearsol 50 at 2% solution or Virkon at 3% solution.

### 5.1 SAMPLES

Because of the risk of shark attack, personnel should not enter the water to retrieve or examine any dead or dying seal. A grapple and line could be used to recover some floating carcasses. If moribund or apparently healthy animals are to be sampled, the standard capture, restraint and anaesthesia protocol for sea lions should be followed. The appropriate samples can be collected as directed by the Specialist Advisory Group. The most useful samples would include:

#### *Blood*

For **haematology** and **blood smear**, collect 5 mL in an EDTA tube. The blood sample should be collected in the field and later, at camp, three thin smears made on glass slides. The smears should be fixed in 70% ethanol (dipped and then air dried), packed and shipped to the laboratory for reading. Label the slide with the animal's identification code and the date. If it is possible to have samples shipped off the islands within 24 hours, the remaining unclotted blood should be stored in a cool location and shipped unfrozen to the laboratory.

For **serum**, collect 10 mL in an untreated glass tube. The serum should be separated by centrifugation and dispensed into cryovials that are pre-labelled with the sea lion's code number, the date, and contents (i.e. "serum"). Dispense the serum into at least two vials and place the vials in separate liquid nitrogen containers (-140° C) to minimise the risk of samples being lost due to evaporation of the nitrogen. The remaining blood cells at the bottom of the tube may be dispensed into cryovials for virology or toxinology. These should be labelled "blood cells" and stored in liquid nitrogen as before.

### *Bacteriology samples*

Swabs should be taken using standard cotton bacteriology swabs. Sites that may be swabbed include superficial lesions, conjunctival membranes, pharyngeal and rectal lining. At base camp, the tips of the swabs should be broken off into cryovials containing sterile glycerol broth, labelled as follows: Animal code, date, swab site (e.g. pharynx, rectum etc). The vial should then be frozen in liquid nitrogen.

Faecal samples (fresh as possible) should be collected from the sward/rookery and stored in labelled plastic screw-top pottles. These should be stored cool but unfrozen if possible for shipment to the laboratory. If there is likely to be a long delay in shipping the samples, a swab may be taken of the sample and stored in glycerol broth in a cryovial as above. Then both the frozen sample and the unfrozen sample can be shipped.

### *Milk*

Administer 1 mL oxytocin to an adult female under general anaesthesia and after 5 minutes a milk sample may be aspirated using the barrel of a 50 mL plastic syringe from which the end has been removed. The milk (1 to 2 mL) should be dispensed into two cryovials labelled “bacteriology” and “toxinology”. The samples should be stored in liquid nitrogen for shipping.

### *Biopsies*

Depending on the clinical signs/gross lesions, it may be necessary to take a biopsy of a superficial lesion. This should be conducted with the subject under general anaesthesia. The site should be prepared using surgical iodine solution and a piece of tissue at the margin of the lesion excised using a sterile scalpel and forceps. It is best to include both normal and abnormal tissue in the sample to facilitate histological interpretation. Following excision of the sample the site should be again bathed in iodine solution and allowed to heal without suturing. The sample should be fixed by immersion in 10% buffered formalin in an appropriately labelled pottle.

### *Urine*

Urine may be required to test for biotoxins or bacteria (*Leptospirosis*). A sample may be collected from an anaesthetised female by passing a lubricated sterile canine catheter through the urethra and into the bladder. The sample is collected into a labelled sterile pottle. At camp, the sample may be dispensed into two labelled cryovials and stored in liquid nitrogen.

## 5.2 PROCEDURES FOR UNDERTAKING NECROPSIES

Necropsies must be performed by trained personnel, and the protocol in Form 1 (overleaf) followed. A separate team member should act as data recorder/photographer during necropsies.



FORM 1: PINNIPED NECROPSY FORM

FIELD ID # \_\_\_\_\_ PATHOLOGY CASE # (MASSEY) \_\_\_\_\_  
..... TAG # \_\_\_\_\_

DATE: -/-/— ..... SPECIES: \_\_\_\_\_  
SEX: M / F ..... AGE: AD / SAD / JUV / P

LOCATION: \_\_\_\_\_

CARCASS CONDITION: FRESH / MILD / MODERATE / ADVANCED DECOMPOSITION.

STANDARD LENGTH (M): — ..... AXILLARY GIRTH (M): \_\_\_\_\_  
BLUBBER (MM): \_\_\_\_\_

CARCASS WEIGHT (KG): \_\_\_\_\_

PHOTOGRAPHS: HEAD / DORSAL / RT. FLIPPER / LT FLIPPER / PELVIC FLIPPER / VENTRAL / GENITAL

EXTERNAL LESIONS (DRAW DIAGRAM IF NECESSARY):

INTERNAL LESIONS. INDICATE SYSTEM OR ORGANS AFFECTED: (1) INTEGUMENT (SKIN)  
(2) ALIMENTARY (3) RESPIRATORY (3) NERVOUS (5) ENDOCRINE (6) LYMPHATIC (SPLEEN,  
THYMUS, LYMPH NODES) (7) URINARY (8) REPRODUCTIVE INCLUDING MAMMARY GLANDS.  
FOR EACH LESION NOTE THE DISTRIBUTION (FOCAL, MULTIFOCAL, DIFFUSE), SIZE,  
AMOUNT/PERCENTAGE OF THE ORGAN AFFECTED, TEXTURE (FIRM, FRIABLE ETC), COLOUR,  
ODOUR.

(INTERNAL FINDINGS CONTD.)

SAMPLES COLLECTED (CIRCLE AS APPROPRIATE):

BACTERIOLOGY: SWABS / FAECES / LUNG / LIVER / SPLEEN / KIDNEY / BRAIN / LYMPH  
NODE

VIROLOGY: LUNG / LIVER / SPLEEN / LYMPH NODE / THYMUS / KIDNEY / BRAIN

SEROLOGY: BLOOD/SERUM

HISTOPATHOLOGY: SKIN / BRAIN / EYE / TONGUE / TONSIL / TRACHEA / THYROID /  
LYMPH NODES / LUNGS / HEART / THYMUS / DIAPHRAGM / SKELETAL MUSCLE / LIVER /  
ADRENALS / KIDNEYS / BLADDER / STOMACH / SMALL INTESTINE / COLON / GONADS /  
MAMMARY GLAND / UTERUS. OTHER \_\_\_\_\_

TOXICOLOGY: LIVER / KIDNEY / STOMACH CONTENTS / URINE / MILK

PARASITOLOGY: (NAME ORGAN AND WHAT SAMPLES COLLECTED) \_\_\_\_\_

GENETICS: SKIN

DIET ANALYSIS: BLUBBER / MILK

AGE DETERMINATION: CANINE TOOTH / LOWER JAW:

PATHOLOGIST OR RECORDERS: \_\_\_\_\_

### 5.3 PROCEDURES FOR THE COLLECTION OF DATA AND SAMPLES

To determine the full impact of a mortality event on the population, descriptive epidemiological data will need to be collected as soon as an unusual event is suspected as outlined in Primary Response (section 2.2). These data will be required by the Scientific Advisory Group to establish a working hypothesis on the aetiology and assist in directing further investigations.

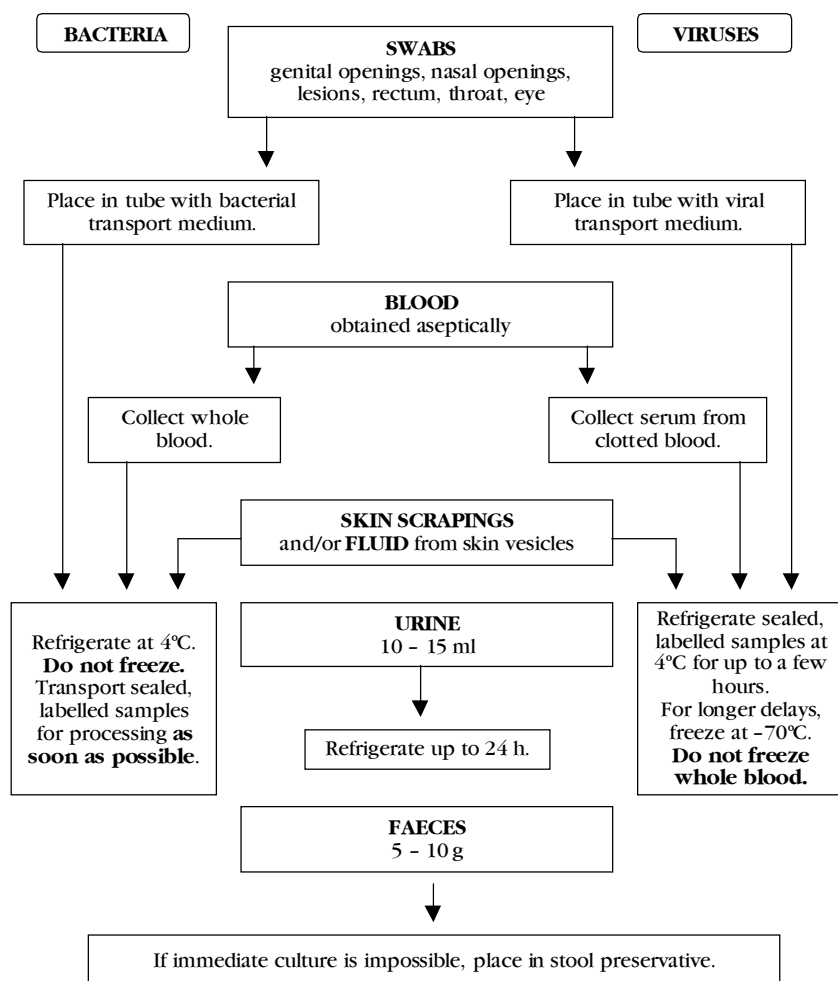
The epidemiological data should be collected periodically to monitor temporal changes in such parameters as incidence, prevalence, case fatality rates, morbidity and mortality. There may also be changes with time in the geographical extent of the event, and the age and sex of individuals affected. Following the Primary Response survey it may be possible to limit detailed repeat surveys to selected sites such as Sandy Bay to maximise the useful data that can be collected by a small team with limited resources.

The health status of living animals in the population will also need to be assessed by documenting behavioural changes, activity levels, and body condition of individuals representing both genders and all age classes. Any departure from accepted norms should be noted. As a general rule, it is better to have a complete set of samples from a few animals than an incomplete set from many individuals. As stated above, a detailed account from one site such

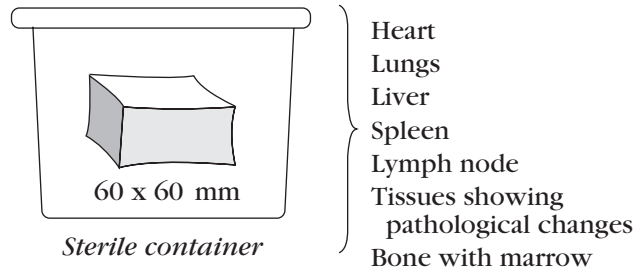
as Sandy Bay is better than incomplete records from several sites.

Diagnosis of the aetiology will depend on collection of samples for diagnostic tests. These may be taken from live animals (swabs, milk, blood, biopsies) or from recently-dead carcasses. Tissue and blood samples will be used for diagnostic purposes, help establish baseline values for pathogen loads, haematological parameters and serum biochemistry, and can also be archived for posterity. Samples may include skin, blubber, muscle, neural tissue, pharyngeal and rectal swabs, blood, urine, bile, faeces or internal organ samples, etc. A sampling summary and a list of tissue samples to be collected, and the means of preserving them is given in Figure 1. Samples should contain a separately bagged but attached label in pencil or

FIGURE 1: SAMPLING LIVE ANIMALS FOR INFECTIOUS AGENTS



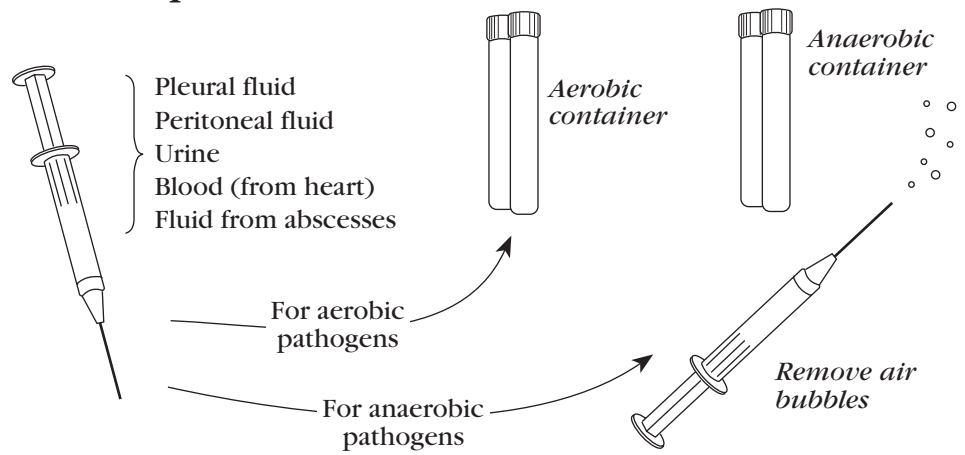
### Tissue samples



***Refrigerate, transport and process as soon as possible.***

For delays of more than a few hours, freeze at -70°C.

### Fluid samples



### Swabs

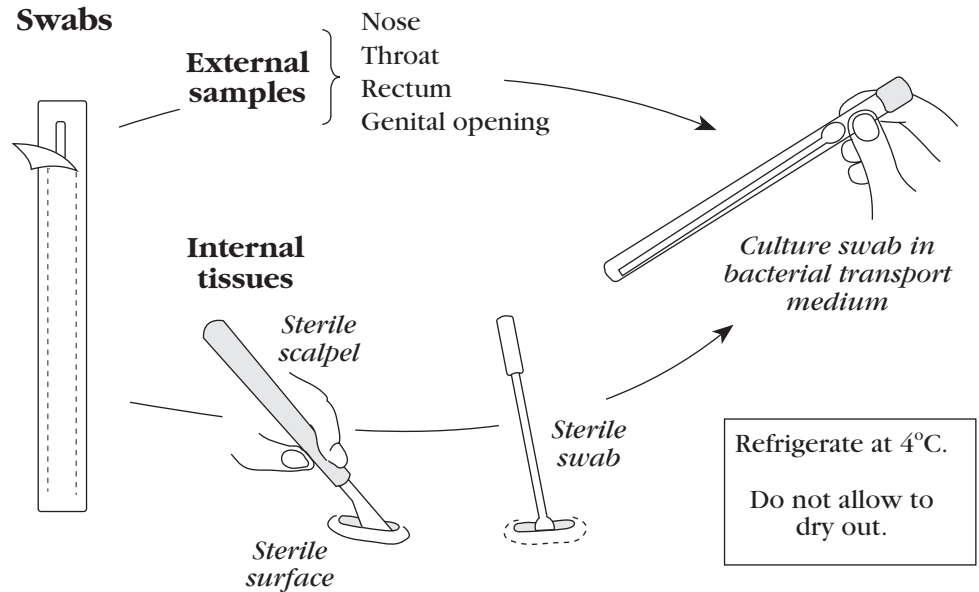
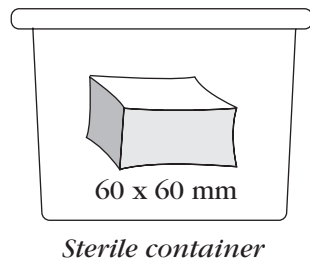


FIGURE 2: COLLECTING SAMPLES FOR VIROLOGY

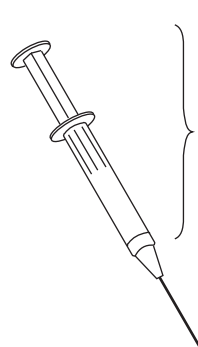
### Tissue samples



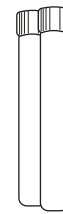
- CNS tissues
- Lungs
- Liver
- Spleen
- Kidney
- Placental/fetal tissues
- Tissues showing pathological changes
- Intestinal contents

Refrigerate sealed, labelled samples at 4°C.  
 For delays of more than a few hours, freeze at -70°C.  
 Do not freeze whole blood.

### Fluid samples

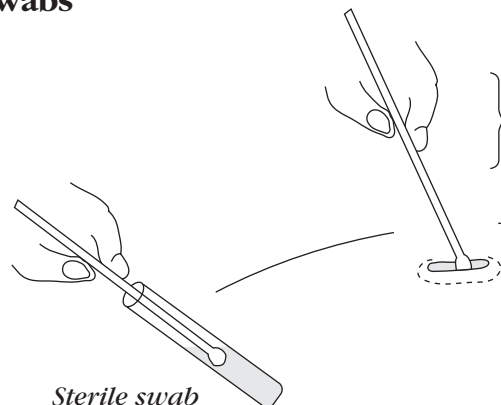


- Pleural fluid
- Peritoneal fluid
- Pericardial fluid
- Urine (10-50 ml)
- Fluid from skin vesicles
- Blood (from heart)
- Cerebrospinal fluid (10-50 ml)



*Sterile tube*

### Swabs



*Sterile swab dipped in viral transport medium*

- Nose
- Rectum
- Genital opening

*Swab broken off below point of handling and placed in viral transport medium*



*Sealed and labelled tube*

FIGURE 3: COLLECTING SAMPLES FOR BACTERIOLOGY.

indelible ink, giving the ID number of the animal, its age class and sex, the and location of collection, the name of the collector and the type of tissues collected. Similar information should be duplicated in a field note book. Procedures for collecting samples for bacteriology and virology are shown in Figures 2 and 3. Packaging instructions are given in Figure 4. Whole, recently dead specimens (i.e. pups) may be collected if adequate preservation methods are available (dry ice/ice packs) and if transport to the laboratory is imminent.

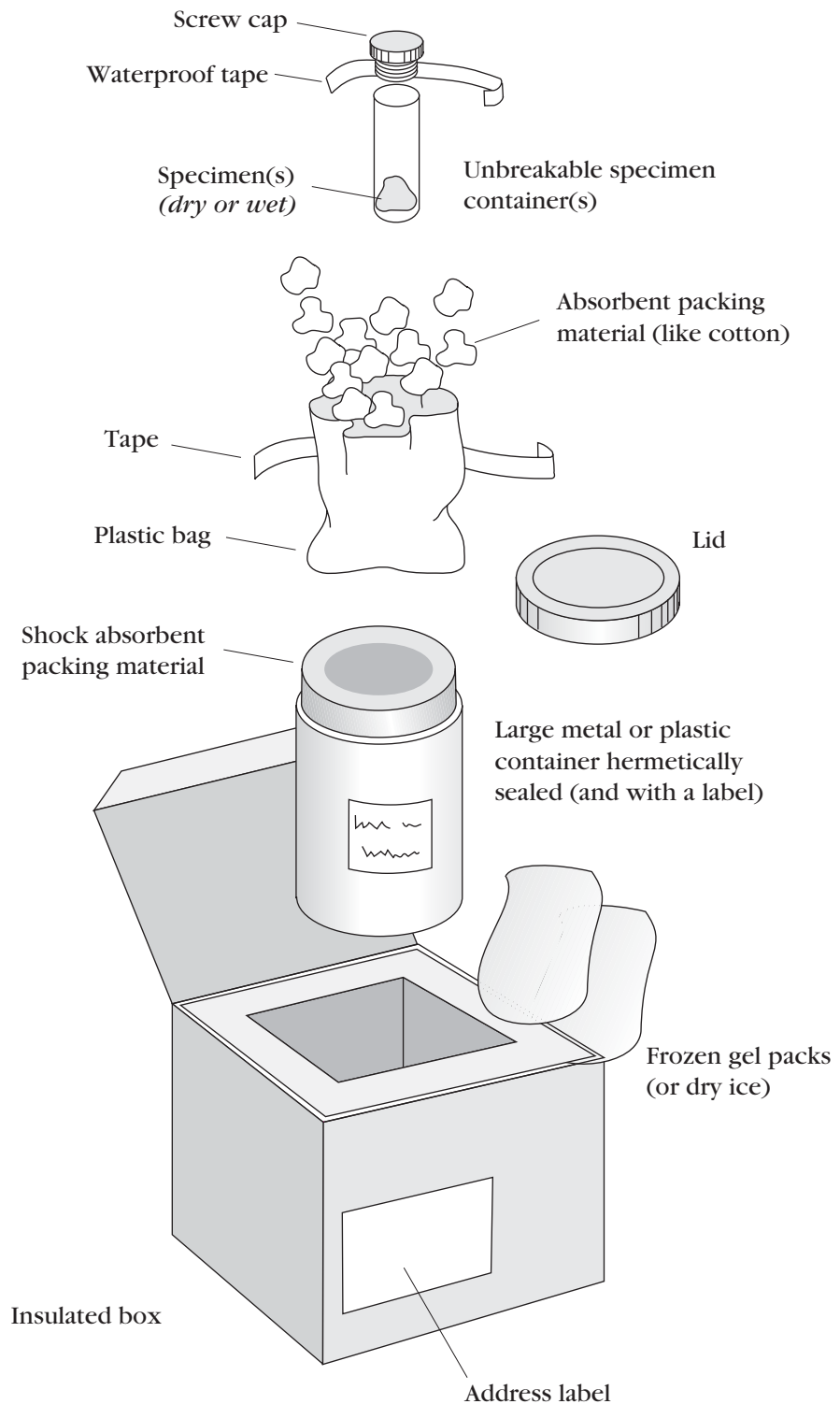


FIGURE 4: PACKAGING INSTRUCTIONS.

#### 5.4 SAMPLES TO BE OBTAINED FROM THE LOCAL MARINE ENVIRONMENT

When there is a way of returning fresh samples of sea water to the mainland for analysis (i.e. within 24 hours), the samples (250 mL) should be placed in sterile bottles wrapped in wet newspaper. Do not freeze or put on ice. If there is a delay in returning sea water samples, they should have 5 mL/L of Utermohl's solution added, which will preserve them and any algal cells for up to 6 months.

Filter-feeding fish, sea squirts and molluscs should also be collected and placed in double plastic bags and frozen in liquid nitrogen where possible. Place in dry ice for fast return to a mainland laboratory. Any already dead fish should be wrapped in aluminium foil and frozen in liquid nitrogen.

#### 5.5 PROCEDURE FOR THE DISPOSAL OF CARCASSES

Where it is possible to move carcasses, they should be accumulated in several convenient locations, to prevent double counting of dead animals and to remove decomposing and possibly contaminated bodies from rookeries and haul-out sites.

## 6. Logistics and equipment

Resources required for a rapid response to a pinniped mortality event include trained personnel on site, support staff in the Local Response Office, and equipment for necropsy, sampling, and transport of samples. A means to transport personnel and samples to and from the event site must be ascertained early in the event. Central to a successful response is a good communication system with a centralised transmission and reception site. Communication between the On-site Response Coordinator and the Local Response Office needs to be of high quality with appropriate back-up.

### 6.1 COMMUNICATION

#### ***Radiotelephone-frequencies and schedules***

**SSB:** Contact to be made with Stewart Island Field Centre (ZKSD 4) on 4619 on a daily basis as part of normal DOC communications schedule. Alternative frequencies if contact cannot be made on 4619 are 5225 and 7890. If Stewart Island FC cannot be reached then try Te Anau Base (ZKSD 5) on 3336, 5225 and 7890. If Te Anau Base cannot be reached then try Bluff Fishermans radio on 4417. Messages must be directed to Area Manager, Southern Islands (Greg Lind).

**Satellite phone schedules:** In an emergency, contact can be made via satellite phone or fax. The sat phone will only be turned on at regular times when convenient (i.e. 2000 hours on Wednesdays and Saturdays). If sat communications are required from Mainland to the Subantarctic, then a message should be passed through Stewart Island FC stating at what time the phone should be turned on. In the first instance contact should be made with Stewart Island FC on 0064-3-214-4589 and a message directed to Area Manager, Southern Islands (Greg Lind). Other numbers include: Te Anau Base 0064-3-249-7921; DOC Invercargill 0064-3-214-4589; and Southern Lakes Helicopters 0064-3-249-7167 (Pilot: Richard Hayes 0064-3-249-7067). Southland Conservancy should confirm these details prior to each expedition.

### 6.2 TRANSPORT

Transport resources: Standard expedition transport.

Emergency transport available (helicopter, ships).

Contact in an emergency for a helicopter for travel to/from subantarctic islands can be made with Southern Lakes Helicopters 0064-3-249-7167 (Pilot - Richard Hayes 0064-3-249-7067). Contact in an emergency for a ship or travel to/from subantarctic islands can be made with Campbelltown Cargo Services Ltd (Marine Countess) on 0064-3-212-8077.



## 7. Biosecurity/Border issues

### 7.1 MAINLAND

Southland Conservancy to liaise with MAF Invercargill on border control procedures for incoming scientific samples, and personnel. Event Science Coordinator to alert the National Centre for Disease Investigation, MAF, Wellington, of an event notification.

### 7.2 SUBANTARCTIC ISLANDS

Depending on the nature and distribution of the mortality event, a quarantine may need to be placed on access to and from the locality or islands themselves, to prevent spread of a disease agent. Total island quarantine must be sanctioned by the Minister of Conservation on advice from the Regional General Manager, DOC Southern Regional Office and the Southland Conservancy.

## 8. Reporting

Weekly updates should be provided to the Mortality Event Response Manager by the On-site Response Coordinator. These would then form the basis of press releases or Ministerial briefings and be dealt with through the DOC management line. Updates would consist of information on numbers of animals found dead or sick, samples collected, analyses performed, and results to hand, plus the rationale for any other actions taken, and any recommendations for further action.

## 9. New Zealand agencies available to analyse samples

**Note: contact only through the Event Science Coordinator:**

**Massey University**, Palmerston North: Pádraig Duignan, Per Madié (bacteriology, virology samples). Phone 06-350 4163 or 06-350 6237, Fax 06-350 5636.

**AgResearch**, Ruakura: Ian Garthwaite (biotoxin analysis)

**Cawthron Institute**, Nelson: Lincoln Mackenzie (toxic algae in seawater samples).

## 10. Equipment

### 10.1 BASIC ESSENTIAL EQUIPMENT

- Anaesthesia apparatus
- Oxygen and isoflurane
- Restraint nets and weighing apparatus
- Liquid nitrogen containers (2 or 3) with cryovial straws
- Centrifuge (electric preferably) for blood tubes
- Centrifuge for capillary tubes (PCV reading)
- Refractometer (blood protein reading and urine osmolarity)
- Selection of 'chilli-bins' (cooling boxes)
- Field microscope (for haematology, parasitology)
- Selection of post-mortem knives
- Sharpening steels and whet stones
- Scalpel handles (10)
- Body bags (for collection of whole pups and adults)
- Thermometers
- Measuring cylinders
- Consumables: Plastic containers (1 litre) with screw top or snap-on lids (for formalin-fixed tissues).

## 10.2 SPECIALIZED EQUIPMENT REQUIRED BY EACH SAMPLE TYPE

### **Blood—haematology and smear**

- needles (sizes)
- syringes (sizes)
- EDTA tubes
- pipettes
- glass slides
- cover slips
- slide containers (for transport without breaking)
- 70% ethanol

### **Blood—serum**

- pipettes
- vacutainers
- centrifuge
- cryovials
- liquid nitrogen

### **Bacteriology—swabs**

- bacteriology swabs
- cryovials
- sterile glycerol broth
- liquid nitrogen

### **Bacteriology—faecal samples**

- pottles
- bacteriology swabs
- cryovials
- sterile glycerol broth
- liquid nitrogen

### **Milk samples**

- general anaesthesia gear
- oxytocin
- needles and syringes (sizes)
- 50 mL plastic syringe
- cryovials
- liquid nitrogen

### **Urine samples**

- sterile canine catheter
- pottle
- cryovials
- liquid nitrogen

### **Biopsies**

- general anaesthesia gear
- surgical iodine solution
- scalpel and blades (sizes)
- forceps
- iodine solution
- 10% buffered formalin
- pottles

### **Necropsies**

- post-mortem kits (forceps, tweezers, scissors, etc)
- post-mortem knives
- steels and whet stones
- scalpel and blades (sizes)
- pottles
- 1-L plastic jars (for formalin samples)
- formalin
- bone saws
- tape measures
- 1-L plastic containers (for formalin fixed tissues)
- labels for ease of labelling lots of pottles, jars, etc.
- waterproof paper
- zip lock bags (many sizes)
- large, strong plastic bags (for rubbish)
- sharps container
- parafilm

### 10.3 Safety gear (various sizes)

- gloves, disposable
- gloves, heavy duty
- overalls, normal and disposable
- aprons, plastic and disposable

- gumboots
- face masks/aspirators
- goggles
- phenolic disinfectant (i.e. Clearosol 50 at 2% solution or Virkon at 3% solution)
- complete first aid kit for treatment of infections, etc.

## 11. Contact personnel (1999)

	DOC	VPN
Southland Conservancy, Invercargill	Lou Sanson .....	5814
Southern Regional Office, Christchurch	Keith Briden.....	5593
Science & Research Unit, Wellington	Ian West .....	8237
External Relations, Wellington	Nicola Patrick .....	8117
Specialist Advisory Group members:		
Science & Research Unit, DOC	Alan Baker .....	8299
	or .....	04-471 3299
Cetacean Investigation Centre, Massey University	Pádraig Duignan	06-350 4163
National Centre for Disease Investigation, Ministry of Agriculture & Forestry	Hugh Davies ....	0800-809 966
Regulation and Implementation Group, Ministry of Health	Douglas Lush .....	04-496 2336

## 12. Acknowledgements

Pádraig Duignan of Massey University provided sampling procedure and necropsy details; Joe Geraci and Valerie Lounsbury of the National Aquarium, Baltimore, USA, assisted with information from the Florida Manatee Contingency Plan for Health-Related Events (1997), and gave permission for the use of their sampling illustrations.

Dean Wilkinson, National Marine Fisheries Service, NOAA, USA provided advice from the US National Contingency Plan for Response to Unusual Marine Mammal Mortality Events (1996).

# 13. Appendix: Conducting a Pinniped Necropsy Examination and collection of samples for health and disease monitoring

## 13.1 CARCASS SELECTION AND RECORDING

- If there are many carcasses, select the freshest and conduct a few thorough examinations rather than many partial examinations.
- Using a data sheet or field notebook, record: species, sex, approximate age, date, time, and examiner's name.
- Measure the carcass:
  - Standard length;
  - Girth behind the flippers.
- Grade the freshness of the carcass:
  - Fresh (euthanasia or died within 2 hours);
  - Mild decomposition (all organs intact and no discolouration);
  - Moderate decomposition (blubber blood-stained and slightly oily; liver and kidneys soft and friable: eyes collapsed);
  - Marked decomposition (internal organs not intact, eyes missing, etc).

## 13.2 EXTERNAL EXAMINATION

- Photograph any external lesions using a ruler to give scale.
- Look for skin lesions and for lesions around the body orifices (mouth, nostrils, eyes, genital opening, anus). Open the mouth and note missing, worn or fractured teeth, receding gums or ulcerated gums. Describe what you see and, if necessary, draw a diagram showing the location, size, shape, colour of the change.
- Collect a canine tooth (or entire lower jaw) for tooth age determination.
- If there are discharges (mucus, pus) from eyes, nose, or any orifice: take a swab for bacterial culture.
- Take samples for histopathology (sample at the edge of the lesion: some normal and some abnormal tissue and place in 10% formalin).
- Look for external parasites (e.g. lice) and collect some examples in a pottle containing 70% alcohol. Record approximately how many are present.

Healthy seals should not have many lice or other organisms such as barnacles attached to their hair/fur or flippers—if you see some, record the location and approximate numbers.

- Collect skin from the flipper web skin (approximately 5 mm x 10 mm) for genetics in a cryovial containing 70% alcohol and freeze in liquid nitrogen. The vial should be labelled on the outside and also contain a yupple paper label inside.

### 13.3 INTERNAL EXAMINATION

- (1) With the animal in dorsal recumbancy, use a sharp knife or a scalpel to incise the skin from the mandibular symphysis to the anus.
- (2) Measure the depth of the sternal blubber to assess body condition. (It is also worth noting any fat deposits internally such as around the heart, intestines or kidneys.) If the animal is emaciated it may have very little blubber and atrophied (wasted) muscles as it will have begun to use its muscle for energy. The muscle will also be sticky and the skin tightly adherent to the underlying tissue due to dehydration. Dehydrated seals may also have sunken eyes and dry mucous membranes. Note any of these changes.
- (3) Collect approximately 1 g of blubber in a cryovial and freeze in liquid nitrogen.
- (4) Record whether there is a light (up to 20), moderate (20 to 40), or heavy (more than 40) burden of tapeworm cysts in the blubber around the genital region.
- (5) Incise through the sternal and abdominal muscle layers taking care not to puncture the viscera. Sample 1 cm<sup>3</sup> of thoracic muscle for histopathology (in 10% formalin).
- (6) Open the chest by reflecting the ribs. One side of the rib cage may be removed by cutting through the costo-chondral cartilage and disarticulating the ribs at the spine.
- (7) Sample axillary and prescapular lymph nodes for bacteriology, (pottle), virology (cryovial) and histopathology (10% formalin) after reflecting one of the flippers.
- (8) If female, locate the mammary glands between the skin and abdominal muscles and sample both glands for histopathology (formalin), and one gland for virology (cryovial) and bacteriology (pottle).
- (9) Remove the abdominal muscles.
- (10) The thoracic and abdominal viscera are now exposed.
- (11) Collect a Red Top blood tube from the heart if the carcass is fresh. Separate serum and freeze later.
- (12) Collect bacteriology (pottle), virology (cryovials), and toxicology (whirly bags) samples before the viscera are contaminated:

- Lung - remove a large piece (50 g) from the apical lobe for bacteriology and place in a labelled pottle, and a small (1 g) piece from the dorsal medial lobe for virology (liquid nitrogen).
- Liver - as for lung but also collect about 500 g for toxicology in a whirly bag which should be kept as cool as possible.
- Spleen (on left side of stomach) - ditto.
- Kidney - approximately half of one kidney in a pottle for bacteriology; about 1 g for virology in a cryovial. Place remainder of kidney in a whirly bag for toxicology and keep it cool.
- Colon with faecal contents: about 6 cm piece unopened for bacteriology (not virology).

Put all the labelled pottles in a ziplock bag and then in a chilli-bin cooled with sea water if no other form of chilling available. The toxicology samples should also be stored in a cool chilli-bin. The cryovials should be frozen in liquid nitrogen.

(13) Examine all organs noting any deviations from normal in shape, size, position, texture, colour. Record any unusual findings in the note book describing:

- Anatomical location: organ(s) affected;
- Size of the affected area: what percentage of the organ is involved;
- Distribution of the lesion: focal, multi-focal, diffuse (all over);
- Nature of the change, i.e. texture (firm, friable etc), colour, smell etc.

If unsure, photograph organs and record measurements. Collect samples of all major tissues and organs. Trim samples so that pieces no larger than 1 cm x 1 cm x 0.5 cm are placed in the formalin. If the samples are bloody or sandy, rinse them off quickly in a bucket of sea water before placing in formalin.

(14) Starting at the tongue, dissect out the respiratory system and heart together. Sample the following:

- Tongue;
- Tonsils (in wall of pharynx);
- Thyroids (paired bean-shaped purple organs caudal and lateral to the larynx on the wall of the trachea);
- Trachea (1 cm<sup>2</sup>);
- Bronchial lymph nodes between forks of bronchi;
- Using scissors, open along the major airways looking for discharges in the lumen or for lung parasites. If parasites are present, collect some (10) and fix for 10 minutes in glacial acetic acid and then transfer to 70% alcohol. If there is pus or thick mucus in the bronchi, swab it for bacteriology. Break the tip of the swab into a cryovial of glycerol broth and freeze;
- Both lung fields cranially, medially, and caudally (6 pieces);



- If it is a pup, sample the thymus (diffuse pink organ at base of heart) for virology and histopathology;
- Using scissors, open the heart in direction of blood flow: right atrium - right ventricle - pulmonary artery - left atrium - left ventricle - aorta. Sample each area for histopathology;
- Sample the diaphragm for histopathology;
- Dissect out the liver and sample it for histopathology at three sites;
- Dissect out both adrenals and section them lengthwise. Place both in formalin;
- Remove stomach and intestines. Open the stomach. If it has contents save them in a ziplock bag for toxicology (store as cool as possible). Sample for histopathology;
- Open along intestine using scissors, stopping periodically to examine the mucosa and to look for worms (refer to the hookworm protocol if it is a pup). If worms are present, collect some (10), wash off in sea water then place in glacial acetic acid for 10 minutes. Then transfer to pottle with 70% alcohol;
- Take a few pieces of intestine for histopathology (2 cm segments opened along their length without damaging the mucosa);
- Sample the pancreas for histopathology (attached to upper small intestine);
- Sample mesenteric lymph nodes (attached to membrane of intestine);
- Dissect out remaining kidney and section it lengthwise. Remove piece using scalpel and forceps for histopathology but handle carefully as it damages easily;
- Open bladder and note colour or urine. If it is blood-stained collect some in a cryovial and freeze. Otherwise just collect some of the bladder wall for histopathology;
- Collect gonads and section lengthwise (one testicle, both ovaries) and place whole organ in formalin;
- Examine accessory reproductive tract for lesions. If female, note if pregnant. If the fetus is at or near full term, treat it as if it were a pup and do a post-mortem. If it is less than 15 cm long, open the abdomen and chest and fix the entire fetus in formalin. (Code it in reference to the adult, e.g. label the female A and the fetus B under the same case number);
- Dissect the head off and if possible remove the brain. Section it from front to back just to one side of the midline. Place the bigger section in formalin (use a separate container from other tissues until it is fixed, c. 5 days). Collect 1 gram of remaining part in cryovial and freeze for virology. Also collect a swab from inside the base of the cranium and freeze in glycerol broth for bacteriology.

## 13.4 COLLECTION PROTOCOLS FOR SPECIFIC STUDIES

### 13.4.1 Hookworm study

#### *Worm handling protocols*

- 1. Worms for isoenzyme electrophoresis.** Need at least 5 individual worms. If worms are alive (worm body is turgid and may move, and the carcass was very fresh) gently detach from mucosa and rinse quickly in several changes of sea water in a dish or pottle by agitation. Some material may need to be dislodged by gentle manipulation with forceps. The worm must be clean. Place worm in labelled cryovial and freeze immediately in liquid nitrogen. Speed is important, to minimise enzyme degradation. Freeze/thaw must be avoided after collection.
- 2. Worms for morphometric study.** Need 5 male and 5 female worms, but prefer 10 males and 10 females. Should be as fresh as possible. Rinse in sea water to remove adherent material. Drop in glacial acetic acid for 10 min. Transfer to 70% ethanol in mcartney (glass) tube and label with small slip of yupple paper **inside** specimen bottle. Screw down lid tightly to avoid leaks or evaporation of ethanol.
- 3. Worms for DNA study.** Need about 5 worms, not necessarily fresh. Rinse to remove all adherent material. Worm needs to be clean. Drop in 70% ethanol.

#### Collection protocol 1

Animal selection: 10 fresh pups (< 12 hours dead) up to 1 month of age.

Necropsy: Do the same as above, but in addition do the following:

Intestine (from pylorus to anus) dissect out entire intestine and place in a ziplock bag with 10% formalin (sufficient to submerge the entire gut). Store bags in a plastic bucket.

#### Collection protocol 2

Animal selection: 5 fresh pups as before.

Necropsy as before but this time open the intestine along its length on the side opposite the mesenteric attachment. Stop every 5 to 10 cm to search the mucosa for hookworms (they will be from 5 to 25 mm long). They may be attached to the mucosa. Deal with collected worms as described above.

#### Collection protocol 3

Animal selection: as available - post-yearling and adult females, especially 3+ years old

Necropsy: Standard necropsy. Identify external mammary teats and attempt to dissect out mammary gland tissue. Fix in 10% formalin in ziplock bag. If tissue is bulky, make multiple deep parallel incisions into it to allow formalin penetration, but try to keep each gland more or less together. If glands are very bulky, then obtain and fix only one gland from each cow.

#### Collection protocol 4

Live animal examination. Animal selection: pups older than about 2 weeks of age, 10-15 individuals.

Procedure: Fill out data sheet incl. species, identification code, sex, date, location and collector.

Weigh and measure standard length and girth.

(1) Obtain 10 mL blood sample from gluteal vein and immediately make 2 blood smears with drop of fresh blood. Later fix the smears by dipping in alcohol. Put remaining blood in Red Top tube. Back at base, separate serum and freeze in cryovial in liquid nitrogen.

(2) Collect faecal specimen by inserting gloved digit in rectum (of seal). Transfer any faecal sample to a small amount of 10% formalin in a 100 mL pottle for later identification of hookworm eggs at Massey University.

#### 13.4.2 Faecal steroid study

If possible, observe 10 females **before and after pupping** (if possible paint mark them or stain them with vegetable dye so that repeat samples can be collected from the same individuals) and collect a fresh faecal sample from the beach or sward in a small whirly bag, label and freeze in liquid nitrogen.