

1 General information

1.1 Health and Safety

Overalls, gumboots, and disposable gloves should be worn, and double-gloving is a sensible precaution. Masks and goggles should be worn where the initial inspection of a carcass suggests an infectious disease such as tuberculosis. Any cuts or abrasions you receive during a necropsy should be cleaned immediately using an alcohol or iodine-based surgical scrub and if indicated medical attention should be sought on your return.

1.2 Sample collection notes

1.2.1 Histology samples

These should be collected into 10% formalin. Make formalin up as follows:

1 litre concentrated formalin (37-40% formaldehyde)
45g $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$
65g Na_2HPO_4
Mix well, then make up to 10 litres with stream water

Note that for optimal fixation samples should be thin (1cm thickness is fine) to enhance penetration of fixative into the tissue. Adequate fixation also requires that containers should be large enough to hold 10 times the volume of formalin relative to the volume of tissue collected (ie aim at a 1:10 tissue:formalin ratio). Once fixed, tissues can be transferred into whirlpak bags with a smaller amount of formalin for transport. Thin tissues (1cm or less in thickness) will take about 48 hours to fix, while brains will take about one week.

With the exception of brain, skull, eyes and spinal cord, all other samples can be placed into the same container. Where it is important that the location of a particular tissue is known however (for example if one particular lymph node is abnormal and several nodes have been collected), then save the tissue into a separate whirlpak bag filled with formalin and store this in the container with the other tissues from the case.

1.2.2 Frozen tissues

Collect small samples of all tissues that look abnormal. These should be placed into labelled cryovials and frozen in liquid nitrogen. These tissues can be used for bacterial and viral culture as well as for PCR to enable diagnosis of infectious agents.

1.2.3 Purulent lesions

Tissues that contain pus or fibrinous material can be sampled either by using a culture swab or by collecting a small piece into a cryovial and placing in liquid nitrogen. If collecting swabs, ensure good coating of the swab surface with infected material, then snip the top of the swab off into a labelled cryovial and freeze in liquid nitrogen.

1.2.4 Body fluids (blood, CSF, aqueous humor)

These samples should initially be collected aseptically into a plain (red or brown top) vacutainer. Samples can then be centrifuged (3000 rpm for 10 minutes) and the supernatant collected into a cryovial for storage in liquid nitrogen.

1.2.5 Parasites.

I am not aware of anyone currently doing work on parasites of NZ sea lions, but if you notice any unusual parasites, collect some into formalin, along with a piece of the tissue you find them in, and collect several into 70% ethanol in small sample bottles.

1.3 Photographs

These are invaluable for helping to interpret lesions and their significance. Try to include the case number and a scale of some sort in the image. A scale ruler and whiteboard marker are provided for this.

1.4 Preparation for sampling

To ensure a standard necropsy and collection procedure it is recommended that all containers, bags, blood tubes, vials, etc. are labelled with the case number and date before starting the carcass examination. This helps to prevent the omission of samples that can easily happen when distracted by onlookers in a field situation. Pre-labelling also minimises the risk of samples ending up unidentified. The following containers should be labelled as standard before the necropsy begins (see (A)). If an infectious disease is suspected during the course of the necropsy, additional containers will be needed (see (B)). Label each container with the case number as follows:

E (year eg 07/08) – (necropsy number) Ph

eg **E07/08-01Ph** would be the first necropsy done in the 2007/08 season

(A) Containers for standard necropsy

- White plastic 1 litre bucket for body tissues (bucket 1)
- White plastic 2 litre bucket for brain, eyes and spinal cord (bucket 2)
- White plastic 2 litre bucket for skull and dural membranes (bucket 3)
- Cryovial for serum
- Cryovial for CSF
- Cryovial for aqueous humor
- Cryovial for fresh-frozen brain tissue

(B) Additional containers for necropsy of sea lions with suspected infectious disease

- Cryovials for swabs of purulent lesions
- Cryovials for tissue samples from infectious lesions

2 Necropsy

2.1 General necropsy details

Record necropsy number (eg E07/08-01Ph), your name and the necropsy date on data sheet. Note circumstances in which body was found. Describe state of carcass, including presence or absence of rigor mortis, any scavenging that has occurred, and an indication of the degree of autolysis (eg presence of maggots, loss of fur, bloating of body etc).

2.2 Morphometrics

Record sex and tag number (if present) of pup. Weigh and record, noting if organs have been removed by scavengers. Measure total length (from tip of nose to tip of tail), girth at axilla, and blubber depth at ventral sternum. Measure girth at neck.

2.3 External examination

Examine the carcass for lesions on the skin, eyes, and body orifices (mouth, ears, genitals, and anus), noting any lesions likely to have been due to trauma or scavenging. Lesions caused during life will have a margin of haemorrhage, while wounds occurring post-mortem do not. Photograph lesions if present. Dehydrated carcasses will have dry tacky corneas and mucous membranes in the oral cavity. Animals with severe hookworm infestation or other causes of blood loss may have pale oral mucous membranes, while those with severe respiratory disease may be cyanotic (blue-gray discolouration of mucous membranes).

Collect a small skin sample in 70% ethanol for genetic analysis.

2.4 Internal examination

Place body in dorsal recumbency.

2.4.1 Flensing and subcutaneous lesions

Begin necropsy by removing all blubber. Make a midline incision along the ventrum, extending from the chin to the anus. Next make several incisions across the body from the ventral midline to the dorsal midline, reflecting and removing these slabs of blubber to expose the underlying muscles. Examine for puncture wounds and bruising. Photograph any lesions, and record the location, nature and size of these lesions on figure 1 on the data sheet.

2.4.2 Body condition

Subjectively assess body condition – this will require assessing blubber mass and muscle mass at this stage, and noting internal fat stores later in the necropsy.

2.4.3 Superficial lymph nodes and joints

Dissect the scapulas free on both sides to expose the rib cage. In the axillary region locate the prescapular, axillary, and caudal scapular **lymph nodes**. Incise lymph nodes. If any are enlarged or contain purulent material, cut in half and put half into formalin, and the rest into a cryovial for freezing. Open up the **shoulder joints** on both sides, noting the nature of the joint fluid. If blood-stained or purulent, collect a swab of joint fluid. Cut swab tip off into cryovial and freeze.

2.4.4 Neck and thorax

Cut along costochondral junctions on both sides of sternum and remove the sternum to expose the lungs. If the thoracic cavity appears abnormal or contains excessive amounts of fluid, take a swab of fluid and collect a piece of lung into a cryovial for freezing. (Doing this before proceeding further with the necropsy will cut down on bacterial contamination and improve the chances of culturing a causal bacterial agent from the samples collected.)

If the pup is fresh, collect a **blood sample** from the heart using a plain (red or brown top) vacutainer. This sample will need to be centrifuged later, and the serum removed into a cryovial and frozen.

Examine the **submandibular lymph nodes** for symmetry. Incise. Collect samples for histology and bacteriology if abnormal (enlarged, contain fibrinous or purulent material).

Locate and examine the **thyroid** glands (just caudal to the larynx). Collect into formalin if they appear abnormal (enlarged, nodular, discoloured).

Locate the **thymus** and collect a small piece for histology.

Remove the pluck by dissecting out the tongue and disarticulating the hyoid bones, then severing all soft tissue attachments back as far as the diaphragm. Incise the oesophagus and main vessels so that the pluck can be removed from the thoracic cavity.

Open along the **oesophagus** - check for worms or ulcers in the mucosa. Collect a piece for histopathology.

Examine and palpate the lungs for abnormalities. Collect lung samples for histology – two pieces from each lobe (one cranial and one caudal). If lesions are present, sample for histo and culture, photograph and record, taking particular note of the proportion of lung affected as this helps determine how clinically significant the lesions are. Locate and incise the **mediastinal and bronchial lymph nodes**. Collect several for histology, and save small pieces in cryovials if abnormal.

To examine the **heart**, open the **pericardium** and orient the heart with the right ventricle facing you.

Using scissors follow the flow of blood - vena cava to right atrium to right ventricle to pulmonary artery. Sample each chamber for histopathology as you go and examine the endocardium and valves for lesions such as endocarditis, jet lesions, ruptured cordae etc. Then, continue from pulmonary vein to left atrium to left ventricle to aorta and examine and sample as before. Ensure that slices of myocardium are less than 1 cm thick. Take note of any changes in consistency or colour in the muscle wall particularly the left ventricle and septum.

2.4.5 Abdominal Cavity

Collect a piece of **diaphragm** for histology.

Remove the abdominal wall muscles to expose the viscera. Note whether fluid is in the cavity and if so, the quantity, colour and consistency. If abnormal amounts of fluid are present, collect a sample for culture (either a swab of fluid or collect a few mls into a cryovial and freeze). If lesions are present on abdominal organs (eg liver, kidney, spleen) collect a sample of affected tissue into a cryovial before proceeding further. Examine the **umbilicus** on the peritoneal surface of the ventral abdominal wall. Incise the umbilical vein and look for evidence of infection. Collect a sample into a cryovial for culture if abnormal.

Carefully remove the **liver** by cutting through the bile duct and hepatic vessels and separating the membranous attachments to the diaphragm. Open a few bile ducts with scissors. Make serial slices part way through the liver parenchyma to check for areas of necrosis or inflammation, and to assess consistency (fibrotic livers will be firm). Collect at least two pieces of liver for histology (one from each major lobe) as well as pieces of any lesions present.

Examine the **spleen *in situ*** and collect a sample for histology. Examine the serosal surfaces of the gastrointestinal tract and note any abnormalities in position of organs, areas of congestion, haemorrhage etc. Reflect the gastrointestinal tract to one side and locate and examine the **kidneys**. If lesions are present, collect a small amount of tissue (or a swab) for culture. Leaving the kidneys in place, locate the **adrenals**. Remove each adrenal and save for histology. Examine each ureter, then remove the kidneys and incise to examine the cortical and medullary surfaces. Collect a piece from each kidney for histology.

Remove the entire gastrointestinal tract. Open the stomach and examine the mucosal surfaces for ulcers or infarcts. Note the amount and nature of contents, eg clotted milk, mucus. Collect a piece for histology. Open along the **duodenum** and collect a sample of opened duodenum with attached **pancreas**. Open the **small and large intestines** at several points and examine the contents and mucosal surfaces. Collect a piece of each for histology. If the contents appear abnormal, take a swab for culture. In the **colon** take particular note of the number of hookworms present and the presence or absence of mucosal lesions and haemorrhage into the lumen. Collect any affected areas into formalin for histology. Note that intestinal samples for histology should be opened carefully, avoiding any scraping of the mucosa which is easily damaged. Holding one end with forceps, the section of intestine can be gently 'swished' in formalin before releasing it: this washes away ingesta and bacteria coating the mucosa and enhances fixation.

Continue examining the urinary system by locating the **bladder**. Open the bladder and collect a piece of bladder wall for histology.

Locate and remove the **ovaries or testes** for histology.

2.4.6 Head, brain and cervical spinal cord (Brain study)

Using a plain vacutainer, collect a sample of **aqueous humor** from one eye by inserting a needle at the limbus (junction between the clear cornea and the white sclera) and aspirating some of the fluid from the anterior chamber.

Remove both **eyes**, leaving as much of the **optic nerve** attached as possible. Inject a small amount of formalin into the anterior chamber of the eye, inserting the needle through the limbus. Place whole eyes into formalin (bucket 2).

If not done previously, measure and record girth at neck.

Remove skin from the head, neck and jaws. Examine the **subcutaneous tissues** closely for signs of haemorrhage and oedema (bruising), including the soft tissues of the neck. If bruising is present, collect a small sample at the margin of bruised and normal tissue into formalin so that the bruises can be aged histologically. Check the skull and cervical vertebrae for evidence of fractures or dislocation.

Photograph and record any bruising, puncture wounds or fractures.

Flex and extend the neck to locate the foramen magnum in the dorsal midline. Using a long needle and a plain vacutainer, collect a sample of **CSF**. If no fluid is aspirated, collection can be attempted during removal of the head: place the body in dorsal recumbency and let the head bend backwards over the edge of the table. Begin to remove the head by cutting gradually through the soft tissues of the neck, cutting carefully once you begin to expose the dural membranes surrounding the spinal cord. Collect CSF before you cut through the dural membranes, then continue to remove the head.

Weigh head plus any removed skin and other soft tissues.

Remove the mandibles and soft tissue from the pharynx, and make sure there is very little soft tissue remaining along the midline of the skull. Bisect the head with a saw, beginning caudally at the midline between the occipital condyles and progressing cranially.

Carefully remove the **brain** halves from the skull, using curved scissors or the rounded handle end of a teaspoon to release the cranial nerves and blood vessels that attach the brain to the dural membranes and skull. Weigh both halves to give total brain weight. Examine the brain surfaces for bruising (contusions) or haematomas. Photograph any lesions. Collect a small piece of **cortex** from one of the brain halves. Include both grey and white matter, and place into a cryovial for freezing. This piece should be collected from a standardised place, approximately half-way along the cortex. Place remainder of brain in formalin (bucket 2).

Check the inner surfaces of the cranium for fractures and for bleeding into the dura. Photograph. Place skull halves and attached dura into bucket 3.

Return to the remainder of the body. Remove all remaining soft tissue from the dorsal part of the neck. With rongeurs, remove the dorsal parts of the cranial cervical vertebrae to expose the spinal cord as far back as C4/C5 if possible. Remove this section of spinal cord and save in formalin in brain bucket.

Finally, open a few more joints to check for evidence of infectious arthritis. Collect swabs of joint fluid if it appears abnormal, noting which joints are affected.

3 Provisional diagnosis

In many cases a tentative diagnosis as to cause of death can be made from the gross necropsy. Record all disease processes you find: it is quite likely that some pups will have several causes of morbidity, and it may not be possible to determine which one is responsible for the death.

The main diagnostic categories seen in previous years are:

1. Stillbirth
2. Trauma (note which body systems are involved, eg head, thorax etc)
3. Bacterial infection
4. Starvation
5. Hookworm infection
6. Other (eg congenital anomalies)

NZ sea lion necropsy template 2007/08.

Accession No: E07/08-

Location Details:

Necropsy date:

Necropsy performed by:

Morphology:

Sex

Tag number

Weight

Length

Girth @ axilla

Girth @ neck

Blubber depth (ventral sternum)

Liver weight

Carcass classification:

fresh / mild decomposition / moderate decomp / severe decomp

no scavenging / superficial scavenging / scavenging of internal organs

External examination:

Skin:

(puncture wounds, bite wounds, scars, laceration, abscesses, vesicles, ectoparasites, dermatitis, alopecia)

Traumatic wounds/bruises:

(Note location and extent on diagram)

Nasal or ocular discharge etc:

(amount, colour, consistency)

Umbilicus:

(fresh or dried stump present? incise to check for abscessation)

Photographs: Y / N

Internal examination:

Body condition:

(Blubber depths, internal fat stores, muscle condition)

Subcutaneous lesions:

(Bruising, abscesses, cestodes)

Head and neck:

Lymph nodes (colour, consistency, abscesses, parasites); thyroid glands; oral cavity (ulcers, mucous membrane colour, hydration status; soft tissue trauma or abscesses)

Joints:

(Colour and consistency of joint fluid; joint surfaces)

Thoracic cavity:

(Amount and nature of thoracic fluid; lung colour, consistency, fluid/pus/mucus/parasites/ingesta in airways; pericardium (fluid/blood in sac, fibrosis of pericardium); heart; thymus; lymph nodes)

Abdominal cavity:

(Abdominal fluid (colour, amount, consistency); peritoneum; liver (fibrosis, abscesses, necrosis); gall bladder; spleen; kidneys, pancreas; adrenals; diaphragm; stomach (serosal and mucosal surface, contents (colour, amount, consistency), ulcers, nematodes); small intestine (serosal and mucosal surface, contents (colour, amount, consistency), parasites); large intestine (serosal and mucosal surface, contents (colour, amount, consistency), hookworms, other parasites); gonads; bladder)

Head, neck, brain and cervical spinal cord

Head weight:

Eyes:

(scavenging, haemorrhage, discharge (amount and nature), ulcers, opacity)

Subcutaneous tissues: (indicate on diagram)
(bruising, puncture wounds, skull fractures, cervical dislocation)

CSF:

(amount, colour)

Brain:

(contusions, lacerations, purulent exudate, congenital anomalies, meningitis, meningeal haemorrhage)

brain weight:

Calvarium and dura:

(fractures, haematomas above or in dural membranes)

Cervical spinal cord:

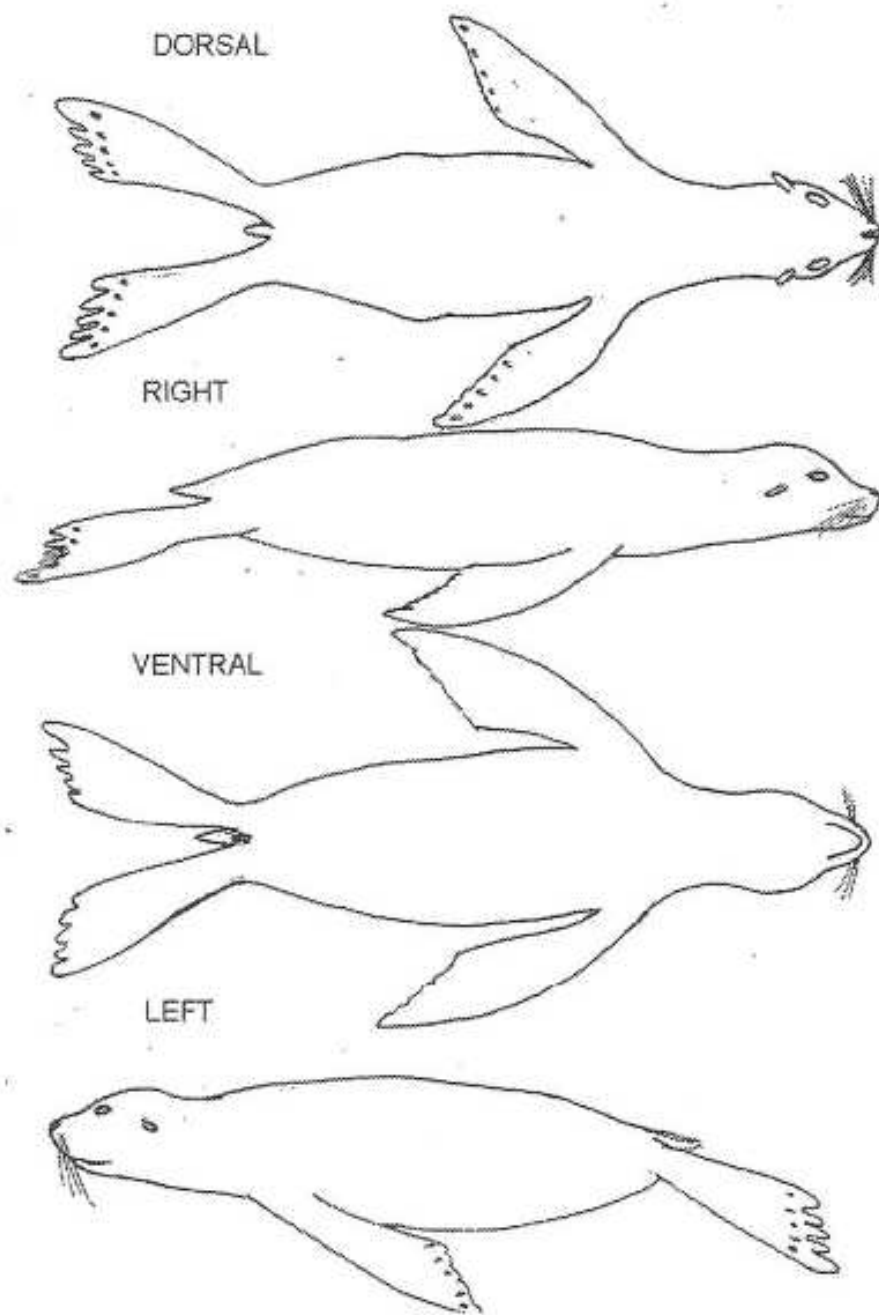
(subdural or epidural haemorrhage; vertebral fractures or dislocations; CSF)

Sample checklist:

Vacutainers		
aqueous humor	serum	CSF
Cryovials		
tissues sampled: swab tips	brain (cortex)	
Alcohol vials		
parasites	skin (genetics)	
Histology		
Bucket 1		
lymph nodes (list):		
thyroid	thymus	oesophagus
heart ventricles	heart atria	diaphragm
umbilicus (if infected)	liver	gall bladder
spleen	kidneys	adrenals
stomach	duodenum + pancreas	small intestine
colon	bladder	gonad
Bucket 2		
eyes + optic nerve	soft tissue bruises	brain
Bucket 3		
calvarium + attached dura		
Photographs		

Provisional diagnosis:

Comment:



3.1.1 External lesions

3.1.2 Head trauma – soft tissue lesions

3.1.3

3.1.4

3.1.5

3.1.6

3.1.7

3.1.8

3.1.9

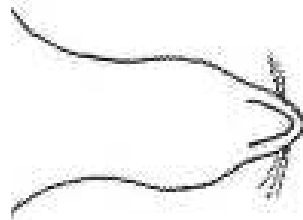
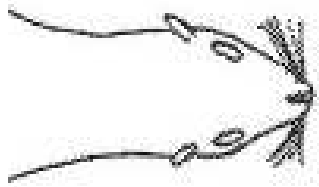
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3.1.15 Skull fractures

