Disease investigations in stranded marine mammals, 1999–2002

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ABSTRACT

This report describes a national survey of necropsy data on marine mammals stranded or beached along New Zealand coasts between June 1999 and June 2002. A total of 137 marine mammals were examined post mortem on site or at Massey University. The sample included 48 pinnipeds representing four different species and 89 cetaceans representing 12 species. The objectives were to determine the cause of death where this was not already known; to assess the health status of the animal prior to its death; to collect life history data such as size, weight, age, and reproductive status; to provide insights into the role of disease in the natural mortality of New Zealand's marine mammals; and to provide data on pathogens that could cause disease in humans or domestic animals that come in contact with beached or stranded marine mammals. Not all of these objectives were able to be realised, but the framework for a database was established which will eventually contain comprehensive information on stranding events, pathological findings, and life history data. Several new diseases were discovered, including: tuberculosis of fur seals and New Zealand sea lions, constituting a risk to people and cattle; salmonellosis of pinnipeds and cetaceans; dolphin pox in Hector's dolphins; hookworm enteritis in fur seal and sea lion pups; and *Campylobacter* septicaemia in sea lions and fur seals. Significant advances were also made in understanding the life history of several species and, in particular, the pygmy sperm whale (*Kogia breviceps*). The investigation has helped to establish protocols for collecting relevant health data from beached and stranded marine mammals, and reports on individual cases have been prepared for relevant field centres and departmental staff to provide valuable feedback on strandings. The findings also endorse the DOC policy to discourage attempts at rehabilitation and educate the public to avoid contact with potentially infected stranded animals.

Keywords: marine mammals, strandings, cetaceans, pinnipeds, necropsy, health status, disease investigations, marine mammals database, life history.

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1. Introduction

Marine mammals are the main group of native mammals in New Zealand, and as such are of great scientific, cultural, and economic value to this country. Marine mammals and strandings are familiar to most people and always generate local and national media coverage, whether it is the appearance of a southern right whale (*Eubalaena australis*) in Wellington Harbour or a pod of pilot whales on the beach at Golden Bay. Marine mammal watching is a significant feature of the tourism industry. New Zealand also has two endemic species, the New Zealand sea lion and Hector's dolphin; both are threatened species, with the sea lion now restricted to the subantarctic islands, where its existence is under threat from limited resources, fisheries competition, and disease epidemics, and the relict North Island population of Hector's dolphin in imminent danger of extinction (Russell 1999; Pichler & Baker 2000; Ferreira & Roberts 2003).

Since 1997 the Department of Conservation (DOC), Massey University, and tangata whenua have had a close working relationship in responding to marine mammal strandings. A project was devised in which it was proposed that Massey University and Te Papa would be the first call for strandings so that arrangements could be made for a pathologist or trained technician to travel to the stranded animal or for the carcass to be transported to Massey University. In some circumstances the field staff were asked to collect specific samples and have those sent to Massey University. In general, this reporting and sampling protocol has worked well, since the quality of material submitted for examination has allowed detailed investigations to be carried out. It has also allowed specific projects to develop on selected species or diseases and some of those are outlined in this report. For example, considerable advances have been made in understanding the ecology of pygmy sperm whale around New Zealand from material collected from stranded animals in Hawke's Bay, Northland, Kapiti Coast, and the Wairarapa in the North Island and Canterbury and Otago in the South Island.

A thorough investigation of a stranding should include post-mortem examination of animals to obtain information on their health status and any disease that may have caused them to strand or die. It is also necessary to determine whether the stranding or death has resulted from entanglement, trauma from boats, gunshot, dogs, or other causes. In addition, useful data on life history parameters can be collected which are of benefit to DOC in conservation management.

Disease has been recognised for some time as an important cause of marine mammal strandings overseas and is increasingly recognised as causing natural mortality of these species in New Zealand waters (Geraci & Lounsbury 1993). However, there is still incomplete understanding of disease in the dynamics of most New Zealand marine mammal populations. Devastating morbillivirus epidemics have occurred in North America, Europe and Asia and had a major impact on some pinniped and cetacean species (Duignan 1998); in a recent event in Europe, hundreds of harbour seals (*Phoca vitulina*) died. It is known that long-finned pilot whales around New Zealand have been exposed to a morbillivirus, although an epidemic has not yet occurred (van Bressem et al.

2001), but the potential for pilot whales to carry the virus and act as a reservoir and vector for infection in other species means that there is a risk of a catastrophic epidemic among Hector's dolphins, bottlenose dolphins, dusky dolphins, or common dolphins. Significant mortality of New Zealand sea lions occurred in disease outbreaks in 1998 (Baker 1999) and 2002; a new species of *Campylobacter* may be the cause of these events, but other pathogens have also been identified, such as several types of *Salmonella* and *Klebsiella pneumoniae* (Duignan et al. 2002). Further work is required on the epidemiology of these pathogens.

Pathogens that cause mass mortalities have an obvious impact on populations of animals, and complicate studies of population dynamics. However, many diseases act more subtly to cause debility, decreased body condition, impaired immune responses, and decreased fecundity. These include some parasites and bacteria which have the ability to cause infertility and abortion. In recent years these agents have been the focus of investigations into population declines among North American marine mammals such as Steller's sea lion (*Eumetopias jubatus*). Baseline data are urgently needed on the prevalence of these agents in New Zealand marine mammals in general and Hector's dolphin and the New Zealand sea lion in particular. Of great concern is the *Campylobacter* organism of sea lions and fur seals, as this genus of pathogens is associated with abortion in domestic animals and may be implicated in decreased fecundity in sea lions as happened in 2002 (Duignan et al. 2002).

Adverse environmental conditions, insufficient nutrition, and chronic stress from disturbance or competition can act through the pituitary-adrenal axis to suppress the immune response, so that otherwise benign flora can become pathogens. For example, in sea lions the prevalence of some organisms may be a reflection of the stresses of coping in a sub-optimal environment rather than of the virulence of the bacteria. Similarly, in fur seals stresses may be imposed in La Niña summers when food resources appear to be scarce and most of the animals presented for examination are malnourished. For stranded individuals of other species, too little information is available to assess what external factors may have affected the health status of individuals.

The objectives of this investigation were:

- To establish the cause of death of stranded marine mammals where possible from the carcass or specimens submitted for examination.
- To establish the occurrence and prevalence of zoonotic diseases in stranded marine mammals. The organisms of greatest concern in the New Zealand context are bacterial pathogens.
- To establish the prevalence of tuberculosis in fur seals and sea lions.
- To adapt the wildlife mortality database, Huia, for use as a database for data collected on stranded marine mammals.
- To collate stranding information and health/disease information into reports for DOC field personnel and managers that will inform them of the cause of death, presence of significant diseases, or other relevant information.
- To provide information on pathogens of beached or stranded marine mammals that could cause disease in humans or domestic animals.

Because approximately three times as many animals were submitted as had been envisaged, these objectives have been only partially fulfilled, and work remains to be completed on samples that have been preserved.

Some of the findings of completed studies have already been published (Duignan 2000a; Baker et al. 2001; Duignan et al. 2001, 2003; Clark et al. 2002). This report describes the protocols for collection and examination of samples, and outlines ongoing work and other findings of scientific and practical interest.

2. Methods

Under the terms of this contract up to 30 marine mammals were to be examined each years for three years from June 1999 to June 2002. These animals were to be obtained through DOC staff responding to strandings using the Marine Mammal Stranding Standard Operating Procedure manual produced by Rob Suisted (REF???). This directs them to contact Anton van Helden, Te Papa, and Pádraig Duignan, Massey University, when a stranded cetacean is reported or found. Pinnipeds were not covered by the notification system that was set up for cetaceans. However, the need to submit pinnipeds for examination was communicated to DOC field staff, and the joint protocol involving DOC and the Ngati Wai Trust Board also involves an agreement by Ngati Wai to contact Massey University in relation to the stranding of certain species.

When a stranding was reported to Massey University by DOC, the following information was obtained at the outset:

- The species involved.
- Whether the animal was alive or dead.
- If dead, the degree of decomposition.
- Whether the animal died under suspicious circumstances that required a forensic investigation. In these cases, either a pathologist from Massey University travelled to where the carcass was located and conducted a post mortem on site, or arrangements were made to transport the intact carcass to Massey University if logistically feasible.
- If the circumstances were not thought to be unusual, a response was determined by: species and body size; location; and degree of decomposition.
- The details were discussed and the most appropriate action decided upon to maximise return for cost and effort based on the condition of the carcass.

2.1 CARCASS CLASSIFICATION

This classification is based on that of Geraci & Lounsbury (1993).

A live-stranded animal is usually classified as **Code 1**.

Beached carcasses may be described as:

Code 2. *Fresh/edible,* i.e. recently dead. Although the presence of rigor mortis is generally consistent with a time since death of less than 24 hours, there are no empirical studies on marine mammals. It will vary with ambient temperature and lactic acid content of the muscle at the time of death, being longer with cool ambient temperatures. External changes such as drying of the cornea, dehydration, cracking and peeling of the skin of cetaceans can be misleading, and, on cursory examination, a reasonably fresh carcass may appear not to be so.

Code 3. *Fair*, i.e. some autolysis, but organs still intact and recognisable. The skin may be cracked and some sloughed off and there may be some scavenger damage. There may be patchy loss of the pelage of pinnipeds. The carcass may be intact but there may be some bloating. The mouth and eyes may be dry and the blubber blood-tinged and oily. The intestine may be gas-filled and the liver and kidneys very soft and mottled or discoloured.

Code 4. *Poor*, i.e. advanced decomposition with organs no longer intact. The carcass may not be intact, it is usually bloated, the skin has sloughed, the dermis is discoloured green or black, it smells rancid and the blubber is separating into oil and fibre. There may be extensive damage by scavengers. The internal organs will be gassy and very soft and almost liquefied.

Code 5. *Mummified or skeletal remains.*

From the standpoint of disease investigation and pathology, Code 2 and 3 carcasses are the most informative. Code 4 and 5 carcasses are of limited use for gross pathology (osteopathology) but may provide some data for life history studies.

2.2 BODY CONDITION

Blubber depth on a fresh carcass (Code 2) was measured at a specific site or sites. For a dolphin or whale this was at three locations: dorsal, lateral, and ventral at the level of the dorsal fin or at the level of the umbilicus, depending on the species. Any internal fat deposits such as around the heart, intestines or kidneys were also noted.

An emaciated animal may have very little blubber and atrophied muscles. Emaciation among small cetaceans manifests early on as atrophy of the neck and epaxial muscles so that a 'neck' and hollowed-out back appears. Later, more generalised loss of muscle mass imparts a ribby appearance exaggerated by blubber loss. Severely emaciated dolphins also have prominent vertebral processes. Ambient water temperature probably influences how far the process can develop, as it is unlikely that a dolphin living in colder waters in winter

could sustain as much mass and blubber loss as one in subtropical waters in summertime.

Emaciated pinnipeds appear ribby. Leopard seals (*Hydrurga leptonyx*) can be misleading as they often appear leaner than other phocids such as elephant seals (*Mirounga leonina*) and certainly lean for their length.

Dehydrated marine mammals may have sunken eyes, and dry mucous membranes, and the muscle is dull and tacky to touch on cut surface.

2.3 NECROPSY EXAMINATION ON SITE

For beached cetaceans over 3 m in length or for events where there are two or more animals, a pathologist travelled to the site to conduct a necropsy examination on the beach. A standard protocol was followed (Duignan 2000b) in which the carcass was measured and photographed and external lesions were described. Blood and external samples were collected where appropriate. Then the carcass was dissected in a systematic manner and all of the internal organs were removed intact, bagged, and returned to Massey University for detailed examination. The head was removed and, where feasible, was also taken to Massey University for examination of the brain, cranial sinuses, and teeth (if it was an odontocete). The remainder of the carcass was either boned out for the local iwi or buried on site. On completion of examination of the head, it was either stored for Te Papa or returned to the iwi.

2.4 NECROPSY AT MASSEY UNIVERSITY

The procedure was the same as above except that the internal organs were examined as they were dissected and sampled immediately for bacteriology, histopathology, virology or parasitology.

2.5 EXTERNAL EXAMINATION

Standard procedure was to confirm the identity of the species, determine the sex, and take a suite of measurements depending on the species. A small skin sample was collected in 70% ethanol for genetic analysis, particularly if the animal was likely to be a rare beaked whale. Samples were sent to Dr Scott Baker, Auckland University. The number of teeth or baleen plates and also the size, shape and colour of the latter were recorded. Photographs were taken of the head, genital area, dorsal fin and tail flukes (cetacean), baleen, and callosities (right whale), eye patches, or any other distinguishing marks (flipper tags) or scars (old tag scars, wounds, etc.).

The carcass was examined for lesions on the skin, eyes, and body orifices (mouth, blowhole, ears, genitals, and anus).

Records were taken of missing, worn or fractured teeth, and receding or ulcerated gums. If there were discharges (mucus, pus) from eyes, blowhole, or any orifice, swabs were taken for bacterial culture.

When ectoparasites were present, the approximate number was recorded and some specimens were collected. Live or fresh specimens were washed in normal saline and placed in glacial acetic acid for 24 hours, then transferred into 5% formalin or 70% ethanol. Whales or dolphins that are ill often have many more external parasites than healthy animals. Healthy seals should not have barnacles attached to their hair/fur or flippers, but if they were present, the location and approximate numbers were recorded.

2.6 INTERNAL EXAMINATION

The standard protocol followed for all animals involved systematic examination of all body systems and organs. If the carcass was recently dead, an eye was dissected out, or where the animal was small enough to manipulate, both were removed. Aqueous humour was collected from one eye for clinical chemistry if the blood was too haemolysed for analysis. The second eye was dissected free of most of the orbital muscle, fixed for 24 hours in Bouin's fixative, and transferred to 70% ethanol for histopathology.

A piece of blubber (200 g) with the skin and muscle attached was collected for toxicology and diet studies, and stored at –20°C until required for specific projects.

The carcass was flensed to remove the blubber, and any contusions in the subcutaneous tissue were noted and photographed. A piece of blubber, approximately 20% of the total mass, was selected from the abdominal flank and 5 mm serial cuts were made in it to estimate the number of parasite cysts—from which a sample was collected in 70% ethanol for taxonomic studies.

The mammary glands were examined by opening the gland through the nipple to look for parasites in the cistern and ducts and for mastitis in the parenchyma. The dissected gland was sliced in serial 5 mm sections and, if lesions were encountered, samples were collected for histology and bacteriology.

After the fore flipper and scapula were dissected free, the prescapular, axillary, and caudal scapular lymph nodes were removed and sectioned to look for tuberculous lesions. Samples were fixed in formalin for histopathology and, if warranted, some tissue was collected for culture.

2.7 EXAMINATION OF THE NECK AND THORAX

Once the flipper and muscle was removed from the chest wall of cetaceans, the ribs were disarticulated from the spine and sternum, as requested by most iwi, to avoid damage to the bone. For pinnipeds, the entire sternum was removed by cutting through the cartilage to expose the lungs, heart, diaphragm, and trachea. Bacteriology and virology samples were taken from the lungs or thorax first, before the organs could be contaminated.

If blood had not already been collected, a sample was taken from the heart or great vessels. If the animal is fresh at least 10 ml of blood was collected into plain vacutainers (for serum) and EDTA (for haematology); three blood smears were also made, air-dried, and fixed in 70% alcohol. After staining they were used for examination of blood cell morphology and a differential white cell count.

The lungs of cetaceans comprise a single lobe on each side whereas those of pinnipeds have a cranial, middle, and caudal lobe. Pinniped lungs are also more similar in consistency to those of terrestrial mammals. The lungs and heart were removed by dissecting the tongue attachments and disarticulating the hyoid bones and then dissecting back along the oesophagus, resecting the brachial vessels, and the vessels and oesophagus where they pass through the diaphragm. The oesophagus was opened along its length to check for worms or ulcers in the mucosa, and a sample was collected for histopathology (1 × 1 cm). The lungs were palpated for changes in consistency or texture.

Cetaceans have large triangular or oblong pleural lymph nodes at the caudal-lateral edge of the lungs. They also have a ring of mediastinal nodes near the base of the heart. Pinnipeds have mediastinal and bronchial nodes. Pleural and mediastinal lymph nodes were dissected and sectioned and samples were collected for culture if lesions were present. Histopathology samples were collected from the trachea and both lungs, and each lung were sampled at three locations, cranial, medial and caudal. The airways were opened using scissors to look for parasites and any present were sampled.

The heart was examined by first opening the pericardium and examining the external surface and coronary vessels. Then, using scissors, the chambers were opened in a sequence that followed the flow of blood: vena cava to right atrium to right ventricle to pulmonary artery. The wall of each chamber was sampled for histopathology, and the endocardium and valves were examined for lesions such as endocarditis, jet lesions, ruptured cordae. The same principle was followed on the left side by continuing the dissection from pulmonary vein to left atrium to left ventricle to aorta. Examination of the vasculature continued later by following the aorta through the abdominal cavity to the branches of the internal and external iliac arteries. Particular note was taken of any changes in the myocardium, especially that of the left ventricle and septum.

2.8 EXAMINATION OF THE ABDOMINAL CAVITY

A sample of tissue from the muscular diaphragm separating the thorax and abdomen was taken for histopathology (1 × 1 cm) to check for parasitic cysts, e.g. *Sarcocystis,* a protozoan. Once the muscles of the abdominal wall were reflected, any fluid in the abdominal cavity was noted and sampled for bacteriology if it appeared to contain pus. Bacteriology and virology samples were collected from the liver, spleen and kidney before these organs were examined in detail and then samples were taken for histopathology. The liver was removed and, using scissors, the bile ducts were opened to look for trematodes (liver flukes) and evidence of periductal fibrosis. The gall bladder of pinnipeds was also opened and examined. If trematodes were present a sample

was collected for identification. The liver was then sliced into 5–10 mm slices to examine the deeper parenchyma, and at least two areas were sampled for histopathology. A sample of liver was usually taken for toxicology (heavy metals and chemical by-products) by freezing approximately 200 g of tissue.

Stomachs of pinnipeds are a simple single chamber. In contrast, those of cetaceans are complex (especially in beaked whales), with at least three chambers. For all species the stomach contents were weighed and particulate matter was washed out, sieved, and stored in 70% alcohol for identification of prey items. Gastric parasites were also collected, washed in saline and fixed for identification of species. If parasites were numerous, a 10% sample was collected. Ulcers or erosions were described and measured, and samples were collected for histopathology.

Because decomposition sets in rapidly in the intestines, only fresh specimens were opened along their length and sampled for histopathology and bacteriology. Less preserved specimens were immersed in 10% Klots fixative and then flushed to collect the contents. These were washed and sieved, and a 10% sample of parasites was collected for identification.

The spleen was examined for size and consistency and a sample was collected for histopathology. The pancreas, located in the first bend of the duodenum, decomposes rapidly due to the digestive enzymes that it produces. If still intact, it was dissected out, the ducts were opened to check for consistency and trematodes, and a sample was taken for histopathology. Both adrenal glands were removed and sliced along their length to determine whether there was nodular hyperplasia, cysts, haemorrhage, etc. indicative of chronic or acute stress or viral infection.

The kidneys and urinary tract were examined *in situ* before the kidneys were removed. The kidneys were sectioned along their length to look for inflammatory changes, neoplasia, and cysts, tissue samples from both kidneys (1 cm^3) were fixed in formalin and samples were collected for bacteriology if necessary. Some kidney tissue (100 g) was also collected and frozen for possible analysis for toxicology (heavy metals). If there was urine in the bladder a sample was collected using a syringe and needle and placed in a sterile container for urinalysis. A 1 cm^2 piece of the bladder wall was fixed for histopathology.

The reproductive system of females consists of a Y-shaped uterus and two ovaries that may be quite large, flattened and irregular in an older animal. The entire tract was dissected out and standard measurements were taken. The weight and dimensions of each ovary was recorded along with any obvious features such as corpora albicantia and corpora lutea and their size range was recorded. Both ovaries were fixed for histology. The uterus was opened to check for pregnancy and to examine the endometrium. If a foetus was present, a small one (up to 200 mm) would be fixed entire in 10% formalin, whereas for a late-term foetus, a post-mortem examination was conducted and some of the amniotic fluid was collected aseptically for culture. The placenta was also examined for pathological changes and sampled for bacteriology and histopathology if lesions were present. The cervix and the vagina were opened and the mucosa of these and the genital slit were examined for papillomas, ulcers, erosions, etc.

In males the testes were dissected out, weighed with and without the epididymis, and measured. Samples for histology were collected and serial sections were made to be examined for abnormalities such as tumours or inflammation. The penis and prepuce were examined for papillomas, vesicles or other abnormalities and lesions were sampled as appropriate.

2.9 EXAMINATION OF THE HEAD AND BRAIN

If autolysis is moderate or advanced (carcass code 3 or 4), the brain may be too decomposed for histopathology, and if the carcass was frozen prior to necropsy, the thawed brain may be friable and unsuitable for histopathology. Where the carcass was fresh and the animal small enough, the soft tissues were dissected off the cranium and the brain was removed using a hacksaw or a Stryker® saw to open the vault of the cranium. The same approach was used for cetaceans, although the thickness of the skull made the task more difficult. The meninges were examined for signs of inflammation or subdural haemorrhage. The brain was sectioned in the sagittal plane, one half was fixed in formalin, and the other was sampled for microbiology if necessary and sectioned thinly to look for deeper lesions.

Where a skull has to be returned to iwi it is not acceptable to damage it, and in some cases the skull may simply be too big to open. In these cases an adequate sample of medulla, pons and cerebellum was obtained by collecting a core from the foramen magnum using a long fine-bladed knife. A sample of the spinal cord was dissected from the first cervical vertebra or by disarticulating the spine in the thoracic region.

The head sinuses of cetaceans were examined for inflammation and for parasites. Depending on the host, the sinuses may contain nematodes or trematodes, and these were collected for identification along with any inflamed tissues for histopathology. The auditory nerve (VIIIth cranial nerve) courses between the cranium and the tympanic bulla, and as parasitic auditory neuropathy has been documented in odontocetes (Morimitsu et al. 1987), close examination and sampling for histopathology were carried out where parasites were found.

2.10 TISSUE SAMPLING

The following sections are based on the protocols established with the various diagnostic laboratories and technical staff within Massey University.

2.10.1 Age determination

For pinnipeds and odontocetes, age was determined by cutting sections of the teeth and counting the growth layers (see Perrin & Myrick 1980; McCann 1993; Oosthuizen 1997). At least three teeth from the middle of the mandibular arcade were collected for this purpose from dolphins or other small odontocetes, while canine teeth and the first post-canine tooth were used for pinnipeds. The teeth were stored frozen or in 70% ethanol until sectioned.

Details of the method are provided in reports on bycatch (Duignan et al. 2000; Gibbs et al. 2000). If it was not possible to obtain some teeth, standard body length was used as an approximate indication of age for some species.

Baleen whales were more difficult to age, and as so few have been examined we have not developed particular age determination techniques for these species. However, an indication of age was obtained from sectioning the wax in the external auditory canal (ear plug), dissecting out and fixing and then sectioning the canal or part of it, and then counting what are considered to be growth rings. A rough indication of age of females was obtained by counting corpus albicans scars on the ovaries.

2.10.2 Bacteriology

Sampling for pathogenic bacteria was routinely undertaken where the gross lesions suggested a bacterial aetiology and where the carcass was sufficiently fresh (code 2), but, in decomposing carcasses, overgrowth of commensal bacteria occurred rapidly, making isolation of pathogens difficult. For some animals samples were collected aseptically and stored at –80°C until histopathology had been carried out. If there was inflammation consistent with bacterial infection, the pathogen was cultured from the frozen material or, if the organism was no longer viable, molecular assays such as polymerase chain reaction (PCR) was used to identify specific pathogens directly from the tissue e.g. the genome of *Campylobacter* sp. recently discovered in NZ sea lions (Stratton et al. 2001).

Generally a large piece of tissue (30 mm to 40 mm cubed) was excised, placed in a sterile 60 ml container and stored at 4°C until cultured. In the laboratory, the tissue chunk was seared with a heated spatula, incised with a sterile scalpel and swabbed. For *Salmonella* culture, faeces were extruded from the colon into a sterile container, or a segment (40 mm) of intestine was tied off, resected, and placed in a sterile container. The sample was swabbed and cultured in enrichment broth and then plated on appropriate agar. Any isolates were phage-typed at the enteric bacterial pathogen reference laboratory in Porirua and were also typed by pulsed-field gel electrophoresis (PFGE) at Massey University.

Blood was sampled if a bacteraemia was suspected. After the thorax was opened, a sample was collected aseptically from the heart or great vessels. The blood was then inoculated into a blood-culture bottle and submitted to the laboratory.

If there were intact vesicles or pustules on the skin, the area was disinfected with 70% ethanol and allowed to dry, and material was aspirated using a sterile needle and syringe. On pinnipeds, plucking hair, collecting scab material, and scraping the skin with a scalpel blade showed whether fungal pathogens or ectoparasites were present.

Impression smears of infected internal organs were sometimes taken and examined after Gram staining.

Anaerobic bacteria which cause abscesses or other foul-smelling lesions were not found.

Granulomatous lesions in otariid seals are likely to be tuberculous and had to be handled with care due to the potential for zoonotic infection. Half of the lesion material was collected aseptically for culture and the rest was fixed for histopathology.

2.10.3 Virology

As with samples for bacteriology, the suitability of samples for virus isolation is directly related to their quality and, specifically, to the freshness of the carcass. From recently dead animals, samples were collected from any organ systems that appeared to have gross lesions, for example samples of upper respiratory tract, lungs and related lymph nodes were taken from animals that appeared to have had respiratory disease. If present, skin lesions such as papillomas, pox-like lesions, vesicles or ulcers, were sampled.

The technology for diagnosis of viral infections is a rapidly growing field. The primary options are:

- to isolate the replication-competent virus in tissue culture,
- to identify antigens of the virus in tissues or body fluids using assays such as antigen-capture enzyme immunoassay,
- to identify part of the viral genome using techniques such as polymerase chain reaction (PCR),
- to detect viral antigen using labelled antibody techniques such as immunofluorescence or immunohistochemistry,
- to use electron microscopy to detect virions in fluid samples.

Our standard procedure was to collect a pool of tissue from selected internal organs such as lung, spleen, liver, kidney, thyroid, thymus, pancreas, brain, and lymph nodes, and to store it fozen. If histopathology indicated that a viral disease was present, the stored tissue was used for either isolation or for molecular techniques such as PCR to identify the type of virus. Immunofluorescence, immunohistochemistry and electron microscopy (EM) were used for fresh or frozen samples, or samples that had been fixed in formalin or ethanol. Samples for direct EM included faeces, pathological specimens, biopsies, skin scrapings and vesicular fluid, but only specimens containing large quantities of viral particles were suitable.

Histopathology was always carried out before further application of virological techniques in order to minimise the cost of diagnostic tests. The association between suspected lesions and a particular virus was confirmed by using immunohistochemistry if antisera against the type of virus were available.

2.10.4 Serology

A blood sample was always collected from recently dead animals, and if a carcass was to be frozen prior to post-mortem examination, a blood sample for serology was collected first. The serum was stored at the lowest temperature possible until tested.

A single serum sample is useful if both IgM and IgG can be assayed, as a high IgM titre and low IgG will indicate recent infection. An IgG titre alone will only indicate exposure but will not give any indication of when it occurred. If single

samples are available from more than one animal, they may help elucidate the epidemiology of infection in a population.

2.10.5 Histopathology

All major organs were routinely sampled for histopathology. During the post mortem, entire organs or parts were removed from the carcass to a clean table for examination. The samples selected for histopathology were collected at the edge of a lesion, with some normal tissue and some of the lesion. Neutral buffered formalin (10%, at a ratio of 10 volumes fixative to one of tissue) was the fixative most commonly used. Eyes were often fixed in Bouin's fixative for 24 hours and then transferred to 70% ethanol.

2.10.6 Parasitology

Protocols developed by Richard Norman of Massey University and also based on Pritchard & Kruse (1982) and Clayton & Moore (1997) were used.

Lesions associated with parasites were normally preserved in 10% neutral-buffered formalin as for histopathology. Endoparasites were normally first washed in warm water or warm normal saline (0.9% NaCl), then fixed in alcohol-formalin-acetic acid (AFA), unless needed for genetic studies, in which case 70% ethanol was used. For isoenzyme electrophoresis on worms, at least five individuals were used. If alive, they were gently detached from mucosa, rinsed quickly in several changes 0.9% saline (or seawater), placed in a labelled Eppendorf or Nunc cryovial, and frozen immediately in liquid nitrogen. For morphometric studies at least five male and five female worms were used. These were rinsed in saline to remove adherent material, then placed in glacial acetic acid and transferred to 70% ethanol in a McCartney tube after about 10 minutes.

Nematodes constitute the largest taxonomic group of marine mammal endoparasites. Most tend to be flaccid when found in a carcass. If they are coiled, they can be straightened in glacial acetic acid and later transferred to 70% ethanol for storage; alternatively, 5% formalin can be used for fixing and storage.

Adult cestodes were usually bluntly dissected from the intestinal mucosa to remove the scolex. However, to remove cestodes intact with scolex attached, it was often necessary to dissect out part of the wall with the embedded scolex and place the worms in chilled saline or 5–10% ethanol at room temperature, then fix them in AFA. Larvae of tetraphyllidean cestodes may be found encysted in blubber or in the abdominal cavity. The cysticerci were collected in 0.9% saline or warm water, rinsed, fixed in cold AFA, and stored in 70% ethanol.

Trematodes (flukes) can be very delicate and for best results were fixed when still alive, if possible. Frequently they were so packed with eggs that internal structures were obscured but, if alive, they were induced to shed eggs by putting them in distilled water for a few minutes or by adding a few drops of glacial acetic acid, then fixing them in AFA.

Acanthocephalans (thorny-headed worms) generally have the proboscis, which is required for species identification, embedded in the enteric mucosa, so care was needed to remove it intact, usually by dissecting out a piece of the wall and

then teasing the host tissue away. Then the worms were placed in cold water to evert the proboscis, then fixed in AFA, and transferred to 70% ethanol for storage.

Arthropod ectoparasites (lice, mites, copepods and isopods) were treated in glacial acetic acid to clear the chitin and stored in 70% ethanol.

To quantify the parasite burden in the intestine or stomach, the contents or a 10% sample were collected into a bucket and washed using a 0.2 mm mesh sieve. The parasites were then collected and fixed. In the field, the entire intestine from stomach to anus was fixed in 10% formalin for later quantification of worms in the laboratory. This was used particularly for counting hookworms in intestines of fur seals and sea lion pups. Faecal samples for egg counts or identification of parasites were fixed in 70% ethanol, or 5% formalin, or frozen.

Quantification of parasites in other organs was more difficult and required a standard protocol that was easily replicated. For example, for tetraphyllidean cysticerci in cetacean blubber, 10–20% of the blubber mass was removed from the abdomen, and all cysts encountered in a series of cuts at 5 mm intervals were counted. A similar procedure is used for lungs, liver, kidney, and mammary gland.

2.10.7 Toxicology

Lipophilic chemicals

Fat-soluble chemicals such as organochlorines are deposited in blubber. These chemicals may have detrimental effects on reproduction, hormonal balance, and the immune system. A sample collection and handling protocol was obtained from Dr Paul Jones, formerly with the Environmental Science and Research Laboratory (ESR), Porirua, where samples were analysed.

Heavy metals

Analysis of tissues for heavy metals was not carried out routinely, but, when done, it was important to avoid contamination during collection and storage. Clean knives were used to collect the sample, which was then stored frozen in plastic bags. Liver and kidney (200 g) were the tissues most commonly sampled.

Biotoxins

Tests for marine algal toxins were not routinely carried out, but a protocol was developed based on information from the Toxicology Laboratory, AgResearch Ltd, Ruakura, and the Cawthron Institute, Nelson.

3. Results and discussion

3.1 OVERVIEW OF THE PROJECT

For studies of marine mammal pathology it is very important that the post mortem examination is conducted as soon as possible after the death of the animal. The effectiveness of the stranding notification process is difficult to assess but most stranding events appeared to have been reported to Massey University promptly. There were more reports from the North Island than the South Island; most within the North Island were from Hawke's Bay and East Cape, Waikanae, Auckland, Wellington, Bay of Islands, Kaitaia, and Wairarapa, with fewer calls from the Waikato, Bay of Plenty, or Coromandel.

In the period June 1999 to June 2002, post-mortem examinations were carried out on 137 marine mammals, including 48 pinnipeds representing four species, and 89 cetaceans representing 12 species (Table 1). The species most commonly submitted were pygmy sperm whales and common dolphins. Each submission was assigned an individual animal code that includes the year and an abbreviation for the scientific name of the species, and also assigned a five-digit pathology number and a Huia database number, so that the database can be searched under any of these codes and also on species. A summary of the codes, species, date found, number dead, location, submitter and organisation is presented in Appendix 1, but data entry is ongoing.

TABLE 1. STRANDED MARINE MAMMALS NECROPSIED AT MASSEY UNIVERSITY, JUNE 1999 TO JUNE 2002.

COMMON NAME	SCIENTIFIC NAME	1999/ 2000	2000/ 01	2001/ 02	UNKNOWN DATE	TOTAL
New Zealand fur seal	*Arctocephalus forsteri*	7	4	2	31	**44**
New Zealand sea lion	*Phocarctos hookeri*			1		**1**
Subantarctic fur seal	*Arctocephalus tropicalis*		1		1	**2**
Leopard seal	*Hydrurga leptonyx*				1	**1**
Common dolphin	*Delphinus delphis*	1	7	8	5	**21**
Bottlenose dolphin	*Tursiops truncatus*	1	2	1		**4**
Striped dolphin	*Stenella caeruleoalba*		2	1	2	**5**
Dusky dolphin	*Lagenorhynchus obscurus*	4	1	1		**6**
Pygmy sperm whale	*Kogia breviceps*	1	12	5	21	**39**
Long-finned pilot whale	*Globicephala melas*			1	1	**2**
Cuviers beaked whale	*Ziphius cavirostris*	2		1		**3**
Straptoothed whale	*Mesoplodon layardi*		1			**1**
Gray's beaked whale	*Mesoplodon grayi*			1	2	**3**
Pygmy right whale	*Caperea marginata*		1	1	1	**3**
Minke whale	*Balaenoptera acutorostrata*			1		**1**
Bryde's whale	*Balaenoptera edeni*	1				**1**

After samples were examined, a preliminary or final report was faxed or mailed as a routine procedure to the individual or field centre from where the case originated.

The greatest value of this investigation is in the collection of data on stranded marine mammals, including the cause of death, the health status of the animal, and life history statistics. The information is available to DOC for analysis and conservation management and to inform the general public or media. Because the response from field centres was greater than expected, almost twice as many animals were processed during the three-year period than was originally envisaged (and contracted for), and not all disease studies were completed. However, archives of samples from stranded animals have been established and will enable work on particular disease agents to be conducted in due course. The archive contains fixed and frozen tissues, serum, blood, bacteria, and parasites that can be used for a range of studies on disease, immune status, diet, and reproductive condition of a number of cetacean species.

3.2 INITIAL FINDINGS FROM ONGOING RESEARCH

3.2.1 Gross pathology

Stranded cetaceans frequently had abrasions caused by trauma in the surf or rubbing on rocks or gravel beaches and sand. They also sometimes had incidental abrasions and scars caused by teeth of conspecific cetaceans (rake marks) or shark bites, and these needed to be distinguished from anthropogenic trauma such as entanglement in nets or ropes, blunt trauma from boat collision, and 'high-velocity' wounds from bullets or propellers. Cetaceans occasionally had pox lesions that appeared as tiny punctate black dots often in circular patterns commonly called 'tattoo lesions'. Pox on seals was more likely to manifest as a typical proliferative skin lesion. Dermatomycosis was likely to resemble the same kind of lesion on domestic mammals, i.e. focal alopecia with hyperkeratosis.

Entanglement in fishing gear often resulted in traumatic lesions immediately apparent in the exterior of the carcass such as abrasions, amputations, penetrating wounds and fracture of limb bones, mandibles or teeth (Garcia Hartman et al. 1994; Kuiken 1994; Kuiken et al. 1994). For cetaceans, diagnosis of the aetiology was relatively simple because the sensitive hairless skin was easily damaged and characteristic net marks were often left as impression marks or lacerations around the rostrum, melon and flippers or dorsal fin. Such lesions were rarely seen on pinnipeds unless encircling fishing gear had cut deeply into tissue of the neck or a flipper. Acute blunt trauma to the body sometimes resulted in contusions, haemorrhage, and skeletal fractures that were apparent at necropsy, if only by palpation of the affected area.

Cariomyopathy occurs in stranded whales (Bossart et al. 1985) and was frequently found in marine mammals in bycatch in New Zealand waters (Duignan et al. 2003). Arteriosclerosis was not uncommon in older marine mammals stranded in New Zealand and was noted in Cuvier's beaked whales (*Ziphius cavirostris*) and a leopard seal (Duignan unpubl. data).

3.2.2 Bacterial pathogens

Detailed work has begun on characterisation of several disease agents that are primary pathogens of marine mammals. Some of these are potentially the cause of disease among people in contact with marine mammals, including a mycobacterium responsible for tuberculosis in pinnipeds. This study began with the recognition of tuberculous lesions in the lungs and lymph nodes of fur seals and a sea lion that were stranded or incidentally caught in fishing operations (Hunter et al. 1998; Cooke et al. 1999). Preliminary genetic characterisation of the fur seal tuberculosis isolates at Massey University indicated that they were different from any known species of *Mycobacterium* and most similar to the human form of this disease. This was obviously a concern for those in contact with affected animals or the organism. Further progress with characterisation of this mycobacterium was carried out in collaboration with Dr Debby Cousins, Australian Reference Laboratory for Bovine Tuberculosis, Western Australia. This study is ongoing, but results to date show that isolates that originated in pinnipeds from Australia and South America are indistinguishable but those from New Zealand fur seals are distinct and may be a new species. A second important observation is that the tuberculosis organism isolated from fur seals in New Zealand was identical to an unusual isolate, previously of unknown origin, from cattle in Hawke's Bay. It now appears that the pinniped strain of tuberculosis is a risk not only to people in contact with diseased animals but also to domestic livestock (C. Dupont et al. & D. Cousins et al. unpubl. data). Continued surveillance of stranded pinnipeds in New Zealand is required to determine the epidemiology and pathological significance of this organism for both fur seals and sea lions.

Routine culture of faeces from stranded marine mammals has resulted in the discovery of *Salmonella* infection in both pinnipeds and cetaceans. To date several *Salmonella* serotypes have been identified, including Typhimurium PT101, PT1, Cerro, Derby, Enteritidis PT4, PT8 and PT untypable, Newport, and Typhimurium PT untypable. Some such as Newport had not previously been isolated from terrestrial mammals in New Zealand or humans except for a few who had been infected overseas. A concern is that infection among marine mammals is linked to contamination of their environment by human sewage containing *Salmonella* and other organisms. There is also a risk to people who may have had contact with infected marine mammals and acquired infection from them, for example an animal carer in Otago became severely ill after contracting salmonellosis from a stranded fur seal in 2001. As with tuberculosis, continued surveillance of sick and stranded pinnipeds and cetaceans is required to better understand the epidemiology of salmonellosis in these species. The data obtained so far also provide a clear warning of the risks associated with rehabilitation of fur seals in the home environment as opposed to facilities where proper standards of hygiene and quarantine can be maintained. The findings also endorse the DOC policy to discourage attempts at rehabilitation and educate the public to avoid contact with potentially infected stranded animals.

Another bacterial pathogen of significance for New Zealand pinnipeds is a newly discovered *Campylobacter* species. This organism was first isolated from New Zealand sea lions that died from haemorrhagic septicaemia during a mass

mortality event in 1998 (Stratton et al. 2001). Subsequently the same pathology was recognised in a stranded fur seal from Otago and the same organism was identified. This pathological presentation is unlike the lesions associated with viral and biotoxin epidemics seen in pinnipeds of the northern hemisphere over the past 20 years, but appears to be similar in gross pathological presentation to epidemics among crabeater seals (*Lobodon carcinophagus*) in Antarctica during the mid-1950s (Laws & Taylor 1957), although the causative agent was not determined from those events. The 2002 epidemic among NZ sea lions further emphasises the need to better understand the bacterial pathogens of marine mammals inhabiting our waters.

3.2.3 Viral pathogens

To date the only viral disease in New Zealand stranded marine mammals that has been identified from lesions is dolphin pox in Hector's dolphins (Duignan 2000b). This virus is rarely the cause of significant disease in cetaceans and is characterised by the occurrence of black punctate tattoo lesions on the skin. It is most commonly seen in juveniles and is self-limiting. Although it has been reported from bottlenose dolphins in the Northern Hemisphere and dusky dolphins in Peru (Geraci et al. 1979; Van Bressem et al. 1993), neither of these species appears to be infected in New Zealand waters, based on animals examined at Massey University. Genital warts caused by venereally transmitted papilloma viruses have been described for dusky dolphins in Peru (Van Bressem et al. 1996) but have not been observed in New Zealand animals.

A viral disease of greater concern is morbillivirus infection causing fatal distemper in pinnipeds and odontocetes. There have been no reports of distemper in any otariid species worldwide but many of the odontocete cetaceans in New Zealand waters are potentially susceptible. Based on gross observations and histopathology at Massey University to date there was no evidence of clinical distemper in stranded pinnipeds or cetaceans. However, a preliminary serology survey of 21 long-finned pilot whales stranded on the North Island found that 18 (86%) had antibodies against the cetacean morbillivirus, with titres ranging from 32 to 256. Adults of both sexes all tested positive. There was no between-gender variation in prevalence of seropositives among immatures (Fisher's exact test = 0.33) and hence both genders were pooled for analysis of prevalence. Variation in seroprevalence between sexually mature and immature individuals was not significant (Fisher's exact test = 0.27). The three negative-testing whales were calves, one of them a neonate. No antibodies were detected in the serum of a foetus from a positive-testing adult female whale. All animals but the negative-testing neonate and a positive-testing immature male were from a single mass stranding near Kaitaia that comprised 102 animals in total. Morbillivirus infection has previously been shown to be an endemic disease of pilot whales in the North Atlantic (Van Bressem et al. 1998). Pilot whales are also the most likely reservoir for this virus and the source of infection for other odontocete species. The significance of the findings for New Zealand is that there is now conclusive evidence that the virus is present in our waters and that species other than the pilot whale are vulnerable. Serum samples tested from a small number of Hector's dolphins were found to be negative, indicating that they are probably susceptible to infection. Further serology will be carried out to determine what species have been exposed to

the virus and to better assess which might be most at risk from an epidemic. Further investigations on the epidemiology of morbillivirus will be carried out in collaboration with the National Centre for Disease Investigation, MAF, Wallaceville.

There is serological evidence that both New Zealand sea lions and fur seals have been exposed to phocine distemper. Serology on 28 adult female sea lions sampled after the 1998 epidemic found that four animals were seropositive and three others had borderline titres. This result indicated that they had been previously exposed to infection but the titres were too low to have implicated the virus in the mass mortality event. Since then, serum has also been tested from stranded fur seals and a small number were found to be seropositive. The significance of these data is not known, and further research is required to test the significance of the results and also to get more extensive test results. Phocine distemper caused a major epidemic among harbour seals in Europe in 1988 that killed thousands of animals (Duignan 1998). In 2002, after 14 years' absence, it has returned to cause another devastating epidemic.

3.2.4 Parasites

Parasites have been implicated in strandings of several cetacean species particularly where infection affects the brain, ears, or auditory nerves, or is so overwhelming that the animal has severe pneumonia or enteritis (Geraci & St Aubin 1987; Morimitsu et al. 1987). Parasite burdens in New Zealand marine mammals are generally low compared with species in the northern hemisphere, but there are several parasites in our species that can cause significant disease. All of the adult Cuvier's beaked whales examined had a heavy burden of *Crassicauda boopis* nematodes infesting their kidneys. Species of the genera *Crassicauda* and *Placentonema* (Order Spirurida, Family Crassicaudidae, Subfamily Crassicaudinae) are among the largest nematode parasites of cetaceans and were once noted for that fact alone. However, they are now being reconsidered for their role as pathogens.

The cephalic end of the parasite is generally embedded in soft tissues of the host, frequently those of the urogenital tract, while the posterior end is free in the lumen and able to discharge fertilised eggs to the environment. Thus, *P. gigantissima*, the female of which reaches 8.5 m length, infects the uterus of sperm whales, *Physeter macrocephalus,* early in pregnancy and matures in the placenta. Various species of *Crassicauda* infect the genitals and kidneys of both baleen whales and larger odontocetes, where they induce the formation of large fibrous multidigitate masses weighing up to 5 kg and extending up the renal veins and into the vena cava (Lambertsen 1986). It has been hypothesised for fin whales (*Balaenoptera physalus*) that severe renal infection may cause death through renal failure (Lambertsen 1986). In Cuvier's beaked whales stranded in New Zealand, the parasites destroy functional renal elements and also cause physical obstruction of the urinary ducts; it seems likely that this causes renal failure. Clinical chemistry of blood collected from one whale within hours of its death indicated high serum levels for urea (44.4 mmol/L) and creatinine (141 µmol/L), suggesting azotemia. However, as azotemia is caused by several mechanisms, it would be necessary to test the concentration of the urine to confirm whether there was renal failure and that was not possible in this case.

Although material from other individuals has been examined and found to have renal crassicaudiasis, fresh blood or urine were not available for testing.

Crassicauda infection was also common in the cranial sinuses of stranded dusky dolphins in New Zealand, but inflammatory changes were rarely noted is association with the parasite. The significance of this parasite, however, is the damage it causes in the brain. Preliminary work on the heads of affected animals suggests that the parasite is embedded in brain tissue and that the part of the worm seen in the sinuses is just the caudal end with the reproductive organs. Unfortunately all specimens examined to date have been previously frozen, which renders the brain tissue unsuitable for histopathology (Section 2.9).

The most pathogenic parasite found in New Zealand pinnipeds is the hookworm, *Uncinaria* sp., in the small intestine of fur seal and sea lion pups (Morgan et al. 2000). Infection is acquired by the pups through suckling and may also be acquired from a contaminated environment through percutaneous migration through the flippers and ventral skin. Light burdens of infection are probably tolerated but heavy burdens result in anaemia, dehydration, and death. Investigations to date have found the parasite in both sea lions and fur seals, with highest burdens in the sea lions. This may reflect the different substrates favoured by these species as rookery sites: the exposed rocky sites used by fur seals are not as conducive to environmental contamination by the parasite as are the sandy beaches and soil used by sea lions.

3.2.5 Toxicology

Toxicological studies on marine mammals have not been undertaken in New Zealand in recent years. However, there is continuing research in North America and Europe on persistent organohalogen compounds because of their putative deleterious effects on the immune system and reproductive system. Collaborative studies are under way with Carlton University, Ottawa, Canada, where research on the distribution of halogenated dimethyl bipyrroles in the Pacific Basin is in progress (Tittlemier et al. 2002). Marine mammals can be good indicators of the distribution of persistent organohalogen contaminants in marine ecosystems because they tend to contain high concentrations in the blubber. Many pinniped and odontocete cetacean species occupy the upper levels of marine food webs and are thus exposed to, and subsequently accumulate relatively high levels of, organohalogens due to biomagnification (Muir et al. 1988; Nakata et al. 1997; Tanabe et al. 1997). For the present study, four halogenated dimethyl bipyrroles (HDBPs), compounds thought to be naturally produced, were quantified in blubber samples from pinnipeds and cetaceans from around the world. Hector's dolphin and NZ sea lion samples contained less HDBPs than ecologically similar species in the Northern Hemisphere. This is consistent with the few studies available concerning anthropogenic organohalogens, in which lower levels were found in species from the Southern Hemisphere (Bacon et al. 1992; Kemper et al. 1994; Connell et al. 1999). However, New Zealand fur seals contained significantly higher levels than northern seal species. At present no satisfactory explanation for this difference exists.

3.2.6 Life history of pygmy sperm whale

Another ongoing study is of stranded pygmy sperm whales, focusing on life history parameters and pathology (Stratton et al. 2000; Tuohy et al. 2001). Since 1997, detailed necropsies have been carried out where possible, on stranded whales to determine the cause of death and to collect data on morphometrics, age, reproductive status, diet, and genetics. The purpose of the latter was to determine the relatedness of individuals in multiple strandings. Age estimates, based on growth layer groups (GLGs), for 27 recently stranded whales ranged from less than one to more than 16 years. The maximum GLG age estimate was 12.5 years for a female and 16+ years for a male. This differs from preliminary data on South African pygmy sperm whale where the maximum GLG age estimate was 22 years for a female and 13 for a male (Stephanie Plön, Rhodes University, Grahamstown, South Africa, pers. comm.). The few age and length data of the small number of animals appear to show a relationship between length and age (R^2 = 0.89 for males and 0.77 for females); for animals stranded in South Africa, a similar relationship has been found (Stephanie Plön, Rhodes University, pers. comm.). The New Zealand strandings (n = 297 events) were classified into 14 categories based on number of animals involved, sex, and estimated age. The location of each event was expressed as a grid reference and analysed for temporal and spatial patterns using ArcView global positioning software. Northern Hawke's Bay had the greatest frequency of stranding events. It appears to have the greatest number of cow–calf pairs and of pregnant females, suggesting that it is a calving or nursery area. Within Hawke's Bay and its environs, the main stranding locations are Mahia and Opoutama Beaches at the Base of the Mahia Peninsula where 141 events occurred (47%), This location is close to the Hikurangi Trough and poverty Canyon, a region where there is nutrient-rich upwelling and the waters are further enriched by an abundance of methane-rich seeps that have rich faunal assemblages (K. Lewis, NIWA, Wellington, pers. comm.). These observations indicate a productive marine ecosystem that should be considered of high conservation value for pygmy sperm whales in New Zealand.

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Appendix 1

STRANDING DATA ON ALL MARINE MAMMALS AUTOPSIED AT MASSEY UNIVERSITY, JUNE 1999–JUNE 2002

HUIA NO.	LAB. NO.	ANIMAL NO.	DATE FOUND	NO. DEAD	LOCATION	SUBMITTER	ORGANISATION
New Zealand sea lion *Phocarctos hookeri*							
1473	32962	SS02-04Ph		1		Jim Fyfe	DOC Coastal Otago AO
New Zealand fur seal *Arctocephalus forsteri*							
	30381	SS99-04Af		1			DOC Palmerston Nth FC
	30444	SS99-07Af		1			DOC Waikanae FC
	30580	SS99-08Af					DOC Waikanae FC
	30611	SS99-09Af					DOC Hokitika AO
	30612	SS99-10Af					DOC Hokitika AO
	30613	SS99-11Af					DOC Hokitika AO
	30614	SS99-12Af					DOC Waikato CO
	30647	SS99-13Af					DOC Waikanae FC
	30659	SS99-14Af					DOC Waikanae FC
	30666	SS99-15Af					DOC Waikanae FC
	30667	SS99-16Af					DOC Waikanae FC
	30684	SS99-17Af					DOC Waikanae FC
	30689	SS99-18Af					DOC Waikanae FC
	30739	SS99-19Af					DOC Wanganui CO
		SS00-01Af					
1280	31092	SS00-12Af	23 Mar 2000	1	Unknown	Richard Gill	DOC Waikanae FC
1277	31329	SS00-38Af	10 Jun 2000	1	Mahia Peninsula	Malcolm Smith	DOC Wairoa FC
	31511	SS00-39Af					
	31514	SS00-40Af					
1276	31620	SS00-41Af	14 Sep 2000	1	Waikanae Beach	Richard Gill	DOC Waikanae FC
	31612	SS00-42Af			Open Bay Islands	Hugh Best	DOC Wellington SRU
	31613	SS00-43Af			Open Bay Islands	Hugh Best	DOC Wellington SRU
	31614	SS00-44Af			Open Bay Islands	Hugh Best	DOC Wellington SRU
	31615	SS00-45Af			Open Bay Islands	Hugh Best	DOC Wellington SRU
	31598	SS00-46Af			Open Bay Islands	Hugh Best	DOC Wellington SRU
1273	31686	SS00-49Af	Unknown	1	Te Horo Beach	Richard Gill	DOC Waikanae FC
1272	31711	SS00-50Af	10 Mar 2000	1	Foxton Beach	Vivian Nichols	DOC Palmerston Nth FC
1282	31750	SS00-51Af	08 Oct 2000	1	Tangimoana Beach	Vivian Nichols	DOC Palmerston Nth FC
1223	31915	SS00-53Af	Unknown	1	Unknown	The Manager	DOC Wellington CO
	31916	SS00-54Af				Mike Morrisey	DOC Kaikoura
	31917	SS00-55Af					
1312	32409	SS01-49Af	07 Nov 1999	1	Horrington Pt, Otago	Steve Broni	DOC Coastal Otago AO
	32667	SS01-50Af	23 Dec 1999	1	Himatangi Beach	Richard Gill	DOC Waikanae FC
	32668	SS01-51Af	27 Aug 1999	1	Hokitika Beach	Lynn Adams	DOC Hokitika AO
	32669	SS01-52Af	Unknown	1	Unknown	Reg Blezard	DOC Wellington CSL
	32670	SS01-53Af	06 Oct 2000	1	Huatai Beach, Te Araroa	Hal Hovell	DOC Te Araroa FC
	32671	SS01-54Af	Unknown	1	Unknown	Unknown	Unknown
	32753	SS01-56Af	Unknown	1	Unknown	Unknown	Unknown

HUIA NO.	LAB. NO.	ANIMAL NO.	DATE FOUND	NO. DEAD	LOCATION	SUBMITTER	ORGANISATION
New Zealand fur seal *Arctocephalus forsteri*							
	32894	SS02-01Af	Unknown	1	Hokitika	Don Neale	DOC Hokitika AO
	32946	SS02-02Af	10 Jun 1999	1	2 Mile, Hokitika	Don Neale	DOC Hokitika AO
	32947	SS02-03Af	c. Oct 99	1	Westport	Martin Abel	DOC Westport
	32995	SS02-05Af		1	Gisborne Beach	Debbie Freeman	DOC Gisborne
1508	33032	SS02-06Af	03 May 2002	1	Foxton Beach	Vivian Nichols	DOC Palmerston Nth FC
Subantarctic fur seal *Arctocephalus tropicalis*							
	30443	SS99-06At		1			DOC Waikanae FC
1283	31759	SS00-52At	10 Oct 2000	1	Unknown	Richard Gill	DOC Waikanae FC
Leopard seal *Hydrurga leptonyx*							
1274	31676	SS00-48Hl	Unknown	1	Unknown	Unknown	Unknown
Pygmy sperm whale *Kogia breviceps*							
	30351	WS99-05Kb					
	30352	WS99-06Kb					
	30353	WS99-07Kb					
	30361	WS99-08Kb				Malcolm Smith	DOC Wairoa FC
	30359	WS99-09Kb				Malcolm Smith	DOC Wairoa FC
	30360	WS99-10Kb				Malcolm Smith	DOC Wairoa FC
	30371	WS99-11Kb					
	30378	WS99-xxKb				Malcolm Smith	DOC Wairoa FC
	30379	WS99-xxKb				Malcolm Smith	DOC Wairoa FC
	30539	WS99-18Kb				Malcolm Smith	DOC Kaitaia FC
	31022	WS00-02Kb				Malcolm Smith	DOC Wairoa FC
1290	31039	WS00-03Kb				Malcolm Smith	DOC Wairoa FC
1291	31041	WS00-04Kb				Malcolm Smith	DOC Wairoa FC
	31083	WS00-07Kb				Malcolm Smith	DOC OngaOnga FC
1292	31117	WS00-12Kb				Malcolm Smith	DOC Wairoa FC
1293	32149	WS00-14Kb	14 May 2000	1	Mahia Peninsula	Malcolm Smith	DOC Wairoa FC
1294	31250	WS00-15Kb				Malcolm Smith	DOC Wairoa FC
	31327	WS00-27Kb	11 Jun 2000	1	Mahia Peninsula	Malcolm Smith	DOC Wairoa FC
	31360a	WS00-28Kb–a	15 Jun 2000	2	90 Mile Beach	Alan Macrae	DOC Kaitaia AO
	31360b	WS00-28Kb–b	15 Jun 2000	2	90 Mile Beach	Alan Macrae	DOC Kaitaia AO
1285	31384	WS00-31Kb	19 Jul 2000	1	Paraparaumu Beach	Richard Gill	DOC Waikanae FC
1298	31658	WS00-32Kb	03 Sep 2000	1	Tarawera River Mouth	Matt Cook	DOC Unknown
1300	31981	WS01-04Kb	31 Jan 2001	1	Mahia Peninsula	Malcolm Smith	DOC Wairoa FC
	31982	WS01-05Kb–a	01 Feb 2001	2	Mahia Peninsula	Malcolm Smith	DOC Wairoa FC
	31983	WS01-05Kb–b	01 Feb 2001	2	Mahia Peninsula	Malcolm Smith	DOC Wairoa FC
	32092	WS01-08Kb					DOC Gisborne AO
1303	32093	WS01-09Kb	07 Mar 2001	1	Mahia Peninsula	Jamie Quirk	DOC Gisborne AO
1304	32253	WS01-22Kb	25 Apr 2001	1	Mahia Peninsula	Jamie Quirk	DOC Gisborne AO
1305	32254	WS01-23Kb	11 May 2001	1	Mahia Peninsula	Malcolm Smith	DOC Wairoa FC
	32269	WS01-24Kb					
1306	32292	WS01-25Kb	20 May 2001	1	Mahia Peninsula	Malcolm Smith	DOC Wairoa FC
1307	32321	WS01-26Kb	Unknown	1	Mahia Peninsula	Malcolm Smith	DOC Wairoa FC
1308	32355	WS01-28Kb	Unknown	1	Mahia Peninsula	Malcolm Smith	DOC Wairoa FC
	32402	WS01-36Kb					
	32405	WS01-37Kb					
	33239	WS02-19Kb	Unknown	1	Mahia Peninsula	Malcolm Smith	DOC Wairoa FC
	33305	WS02-21Kb	Unknown	1	Mahia Peninsula	Malcolm Smith	DOC Wairoa FC

HUIA NO.	LAB. NO.	ANIMAL NO.	DATE FOUND	NO. DEAD	LOCATION	SUBMITTER	ORGANISATION
Pygmy sperm whale *Kogia breviceps*							
	33319	WS02-23Kb	11 May 2002	2	Waitarere Beach, S Foxton	Richard Gill	DOC Waikanae FC
	33341	WS02-26Kb	13 Feb 2002	2	Waitarere Beach, S Foxton	Richard Gill	DOC Waikanae FC
	33350	WS02-27Kb	14 May 2002	1	Te Kopi, Palliser Bay	Bruce Dix	DOC Wairapa FC
Common dolphin *Delphinus delphis*							
	30447A	WS99-14Dd	Unknown	1	Unknown	Bruce Dix	DOC Wellington
1059	30890	WS00-01Dd	17 Dec 1999	4	Motutura Rock, Cape Brett	Alan Fleming	DOC Russell AO
1278	31313	WS00-26Dd	Unknown	1	Unknown	Unknown	Unknown
1286	31675	WS00-33Dd	Unknown		Unknown	Unknown	Unknown
1147	31751	WS00-34Dd	09 Oct 2000	2	Kawau River, Warkworth	Thelma Wilson	DOC Auckland
1148	31752	WS00-35Dd	09 Oct 2000	2	Kawau River, Warkworth	Thelma Wilson	DOC Auckland
1155	31799	WS00-39Dd	05 Oct 2000	1	90 Mile Beach	Alan Macrae	DOC Kaitaia AO
1158	31808	WS00-41Dd	22 Oct 2000	1	Warkworth	Bill Walsh	DOC Auckland
1157	31809	WS00-42Dd	15 Oct 2000	1	Waikaretu Beach	Gary Hickman	DOC Waikato AO
1164	31819	WS00-43Dd	29 Oct 2000	1	Langs Beach, Waipu	Unknown	DOC Whangarei AO
1174	31848	WS00-44Dd	10 Nov 2000	1	Mathesons Bch, Warkworth	Thelma Wilson	DOC Auckland
1310	32375	WS01-30Dd	12 Jun 2001	1	Anaura Bay, Gisborne	Jamie Quirk	DOC Gisborne AO
	32435	WS01-39Dd	Unknown	1	Unknown	Unknown	Unknown
1523	32618	WS01-43Dd	Unknown	1	Unknown	Simon Mowbray	DOC Auckland CO
1418	32933	WS02-03Dd	20 Jan 2002	6	French Pass Area	Bill Cash	DOC Sounds AO
1419	32934	WS02-04Dd	20 Jan 2002	6	French Pass Area	Bill Cash	DOC Sounds AO
1424	32935	WS02-05Dd	20 Jan 2002	6	French Pass Area	Bill Cash	DOC Sounds AO
1425	32936	WS02-06Dd	20 Jan 2002	6	French Pass Area	Bill Cash	DOC Sounds AO
1426	32937	WS02-07Dd	20 Jan 2002	6	French Pass Area	Bill Cash	DOC Sounds AO
1454	32938	WS02-08Dd	20 Jan 2002	6	French Pass Area	Bill Cash	DOC Sounds AO
1531	33100	WS02-14Dd	14 Mar 2002	1	Unknown	Richard Gill	DOC Waikanae FC
Striped dolphin *Stenella caeruleoalba*							
1153	31797	WS00-37Sc	13 Oct 2000	4	Rarawa Beach	Alan Macrae	DOC Kaitaia AO
1154	31798	WS00-38Sc	13 Oct 2000	4	Rarawa Beach	Alan Macrae	DOC Kaitaia AO
1311	32384	WS01-31Sc	Unknown	1	Auckland	Unknown	DOC Auckland
	32434	WS01-38Sc	Unknown	1	Unknown	Unknown	Unknown
1493	33051	WS02-13Sca+b	28 Feb 2002	1	Bland Bay, Whangaruru	Alan Fleming	DOC Russell
Bottlenose dolphin *Tursiops truncatus*							
	30475	WS99-16Tt	19 Jul 1999	1	Onahau Bay, Marlboro' Sds	Mike Avis	DOC Sounds AO
1163	31806	WS00-40Tt	19 Oct 2000	1	Leigh Wharf, Warkworth	Sharon Dunning	DOC Auckland
1284	32088	WS01-07Tt	11 Mar 2001	1	Blueskin Bay, Otago	Jim Fyfe	DOC Coastal Otago AO
	32695	WS01-45Tt	23 Sep 2001	1	Omakiwi Cove, B. of Islands	Alan Fleming	DOC Russell AO
Dusky dolphin *Lagenorhynchus obscurus*							
	30445	WS99-12Lo	12 Jul 1999	1	Ruakaka Bay, Queen Charlotte Sds	Mike Avis	DOC Sounds AO
	30446	WS99-13Lo	16 Jul 1999	1	Parapara Inlet, Collingwood	Kay Stark	DOC Golden Bay
	30465	WS99-15Lo	20 Jul 1999	1	Ruakaka Bay, Queen Charlotte Sds	Mike Avis	DOC Sounds AO
	30753	WS99-23Lo	21 Jul 1999	1	Sandfly Bay, Otago Peninsula	Jim Fyfe	DOC Coastal Otago AO
1309	32387	WS01-32Lo	02 Nov 2000	1	Ocean View Beach, Kaikorai	Jim Fyfe	DOC Coastal Otago AO
	33386	WS02-29Lo	Feb	1	Unknown	Jim Lilley	Marine Watch Chch

HUIA NO.	LAB. NO.	ANIMAL NO.	DATE FOUND	NO. DEAD	LOCATION	SUBMITTER	ORGANISATION
Long-finned pilot whale *Globicephala melas*							
	30593	WS99-21Gm				Anton van Helden	Te Papa Tongarewa
	33331	WS02-25Gm	c. Apr 2002	1	Unknown	Don Neale	DOC Hokitika AO
Cuvier's beaked whale *Ziphius cavirostris*							
	30487	WS99-17Zc	20 Jun 1999	1	Kiritehere Beach, Marokopa	Philip Bradfield	DOC Maniapoto AO
1279	31113	WS00-08Zc	15 Mar 2000	1	Purakanui Bay, N of Dunedin	Jim Fyfe	DOC Coastal Otago AO
	33306	WS02-22Zc	06 May 2002	1	Mahia Peninsula	Helen Jonas	DOC Wairoa FC
Straptoothed whale *Mesoplodon layardi*							
1258	32182	WS01-10Ml	07 Apr 2001	1	Gisborne	Jamie Quirk	DOC Gisborne AO
Gray's beaked whale *Mesoplodon grayi*							
	30544	WS99-19Mg				Anton van Helden	Te Papa Tongarewa
1224	31985	-	Unknown	1	Unknown	Anton van Helden	Te Papa Tongarewa
	33251	WS02-20Mg	04 Jul 2002	1	Karamea	Marty Thomson	DOC Karamea
Pygmy right whale *Caperea marginata*							
1149	31766	WS00-36Cm	12 Oct 2000	1	Fitzroy Beach	Bryan Williams	DOC New Plymouth
1358	32579	WS01-42Cm	Unknown	1	Muriwai Beach	Chris Roberts	DOC Auckland CO
	32741	WS01-46Cm	17 Oct 2001	1	Pekapeka Beach	Clinton Durches	DOC Kapiti AO
Bryde's whale *Balaenoptera edeni*							
	30573	WS99-20Be	13 Aug 1999	1	Westend Beach, Tawharanui	Thelma Wilson	DOC Warkworth
Minke whale *Balaenoptera acutorostrata*							
	33402	WS02-30Ba	27 May 2002	1	Foxton Beach	Don Levine	DOC Palmerston Nth FC