A Systematic Health Assessment of Two Dolphin Species By-caught in Shark Nets off the KwaZulu-Natal Coast, South Africa

by

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Submitted in partial fulfilment of the requirements for the degree
Master of Science
in the Faculty of Veterinary Science, University of Pretoria, South Africa

April 2013

Acknowledgements

This work was supported by the National Research Foundation (NRF) (grant number 74241 (SeaChange programme) and 70714 (DFG-NRF bilateral grant) awarded to Dr S. Plön).

I would like to acknowledge the following people:

Peter Thompson, from the Department of Production Animal Studies, Faculty of Veterinary Science, University of Pretoria, my faculty supervisor, for your statistical input and guidance, and help with necropsies.

Emily Lane, from the National Zoological Gardens of South Africa, my co-supervisor, for your tireless dedication, encouragement and mentorship, and spending many hours evaluating histology slides with me.

Stephanie Plön, from the South African Institute for Aquatic Biodiversity, c/o PE Museum/Bayworld, Port Elizabeth, my co-supervisor, for having the vision to start this project, and granting me the opportunity to do it.

Ursula Siebert, from the Institute for Terrestrial and Aquatic Wildlife Research, University of Veterinary Medicine, Hannover, Germany, my co-supervisor, for your expert teaching during necropsies in Durban as well as in Büssum, Germany.

Peter Wohlsein, from the Department of Pathology, University of Veterinary Medicine, Hannover, Germany, for your interest, enthusiasm and expert input on histopathology as well as your teaching during necropsies.

The staff of the KwaZulu-Natal Sharks Board, in particular Geremy Cliff, for providing facilities as well as help with carcass collection, handling and defrosting.

The staff and students of Bayworld, in particular Greg Hofmeyr, for help during dissections, and Dorothy Pitman for help with obtaining literature.

The laboratory staff of the Department of Pathology, Faculty of Veterinary Science, University of Pretoria, and the Department of Pathology, University of Veterinary Medicine, Hannover, Germany, for histology slide preparation and staining.

David Zimmerman for necropsy data and sampling in 2010.

Maryke Henton, from IDEXX laboratories, for culture and identification of the bacteria collected during the course of this project.

Kerstin Junker, from the Agricultural Research Council, Onderstepoort Veterinary Institute, and Kristina Lehnert, from the Veterinary University of Hannover, Germany, for parasite identification.

The Fraunhofer Institute, Hannover, Germany; the laboratory staff at the Department of Pathology, University of Veterinary Medicine, Hannover, Germany; and the staff of the EM Unit, Faculty of Veterinary Science, University of Pretoria, for electron microscopy and EDX analysis.

Susan Marsh, and the rest of the staff of the Jotello F. Soga Library, for helping me in obtaining literature.

Nic and Rina de Wet, my parents, whose encouragement and love were never ending.

And most importantly my humblest thanks and love go to my wife, Ingrid de Wet, for your help with necropsies, pictures and diagrams. Your faithful support, encouragement, strength and unyielding love allowed me to complete this project.

Abstract

Coastal dolphin populations are indicators of environmental health and may be sensitive to anthropogenic influences. An observed increase in lesions during routine necropsies of dolphins prompted the first systematic health assessment of dolphins incidentally caught in shark nets off the KwaZulu-Natal coast. A detailed standard dissecting and sampling protocol for small cetaceans was developed for use in South Africa. Thirty five Indian Ocean bottlenose dolphins (*Tursiops aduncus*) and five Indo-Pacific humpback dolphins (*Sousa chinensis*), incidentally caught between 2010 and 2012, were subsequently evaluated by full necropsy and sampling using this protocol.

All animals were considered to be in good nutritional condition, based on blubber thickness measurements and muscle condition. A large proportion of dolphins had lesions with parasitic aetiology, including pneumonia (34/40), bronchiolar epithelial mineralisation (33/40), gastroenteritis (28/40), hepatitis (24/39); endometritis (11/26), capsular inflammation of various abdominal and thoracic organs (30/40), and splenic capsular tags (18/40). Four parasite species (*Halocercus* sp., *Crassicauda* sp., *Brachycladiinae*, and *Xenobalanus globicipitis)* were recovered from six animals. Non-specific encephalomeningitis was found in 7/18 animals. Adrenal cortical hyperplasia (18/37,) possibly related to chronic stress, was also found, as well as myocardial fibrosis (10/39). Pulmonary pneumoconiosis and lymph node foreign material accumulation, possibly indicating exposure to polluted air, was seen in three animals. Lesions suggestive of morbillivirus, *Toxoplasma gondii*, or *Brucella* spp. tested negative on immunohistochemistry. The first confirmed cases of lobomycosis and sarcocystosis in South Africa were found. Most lesions were mild, although their high and apparently increasing prevalence may indicate a change in the host/parasite interface. This may be attributed to anthropogenic factors, such as stress or environmental pollution, suggesting degradation of the marine environment. This could also negatively impact human populations associated with the marine environment.

The results indicate a need for continued health monitoring of coastal dolphin populations and for further research into disease pathophysiology and anthropogenic factors affecting these populations. This standard necropsy protocol will encourage a more complete health investigation of incidentally caught and stranded cetaceans in the region and will assist in expanding the current knowledge of diseases affecting dolphin populations in southern Africa. Furthermore, we provide valuable information regarding the baseline of disease affecting these populations, which may be used to determine and monitor temporal trends.

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List of Abbreviations

APP	Amyloid precursor protein
CITES	Convention on International Trade in Endangered Species of Wild Fauna and Flora
CNS	Central nervous system
DDT	Dichlorodiphenyltrichloroethane
DNA	Deoxyribonucleic acid
EDX	Energy-dispersive X-ray spectroscopy
ELISA	Enzyme-linked immunosorbent assay
FM	Fontana Masson's
GMS	Grocott-Gomori's methenamine silver
HB	Hall's bile
HE	Haematoxylin and Eosin
IQR	Interquartile range
IUCN	International Union for the Conservation of Nature
KZNSB	KwaZulu-Natal Sharks Board
LLD	Lobomycosis-like disease
MSSB	Modified silver stain according to Bielschowsky
MT	Masson's trichrome
PAS	Periodic acid-Schiff
PCR	Polymerase chain reaction
PEM	Port Elizabeth Museum
PPB	Perl's Prussian blue
RNA	Ribonucleic acid
SEM	Scanning electron microscopy
TEM	Transmission electron microscopy
VK	Von Kossa stain
ZN	Ziehl-Neelsen

Chapter 1. General Introduction

1. Dolphins as Sentinel Species

The perception of an ecological crisis with degradation of ocean health has led to research into its possible causes and the effects that this may have on marine mammal species (Lafferty, *et al*., 2004). Coastal cetacean species, such as *Tursiops aduncus* (Indian Ocean bottlenose dolphin) and *Sousa chinensis* (Indo-Pacific humpback dolphin) in South Africa, are particularly vulnerable to the effects of human activity due to their inshore habitat. Furthermore, dolphins have long life spans, feed at a high trophic level and have extensive fat stores that can serve as deposits for chemical pollutants (Reddy, *et al*., 2001; Wells, *et al*., 2004). As a result, coastal dolphins are ideal to use as sentinel species to detect early warning signs of current or potential negative trends or impacts in our oceans, allowing better characterization and management of potential negative impacts on human and animal health associated with our oceans (Bossart, 2006).

A review of the published literature suggests a higher rate of disease outbreaks worldwide in the last four decades (Epstein, *et al*., 1998; Harvell, *et al*., 1999). Evidence suggests that the increase in disease is real, but a lack of baseline data for most marine mammals precludes a direct test of that hypothesis (Harvell, *et al*., 1999; Ward and Lafferty, 2004). This, combined with predictions of future increases of disease owing to climate change and an increase in stress factors such as pollutants, inter- and intra-specific competition (through fishing and conservation) and habitat destruction, lend new urgency to understanding the causes of marine mammal disease outbreaks (Epstein, *et al*., 1998; Harvell, *et al*., 1999; Harvell, *et al*., 2002; Lafferty, *et al*., 2004; Ward and Lafferty, 2004).

Extensive health assessments of cetaceans are currently underway in the northern (Kuiken, *et al*., 1994; Cornaglia, *et al*., 2000; Siebert, *et al*., 2001; Jauniaux, *et al*., 2002; Bossart, 2006; McFee and Lipscomb, 2009) and southern (Duignan, 2003) hemispheres, using cetaceans as indicator species to monitor the health of the marine ecosystems, and to examine any potential overlap between human activities and animal health. Standard necropsy and health assessment protocols have been developed for this purpose.

2. Shark Nets

Shark nets in the form of gill nets (each 110 m long and 10 m deep) were first deployed off the South African east coast in 1952, following incidences of shark attacks, in an attempt to

reduce the possible interaction of sharks with swimmers along the popular beaches in this area (Cockcroft, *et al*., 1990; Cockcroft, 1994). Black, multifilament, polyethylene braid nets are set in a fixed position, at staggered intervals approximately 400 – 500 m offshore, in 10 – 14 m of water (Dudley and Cliff, 2010). Currently a total length of 23.4 km of nets protects popular swimming beaches along a 320 km stretch of coastline (KwaZulu-Natal Sharks Board, 2011). An average of 42 dolphins was annually incidentally caught in the shark nets between 2006 and 2011 off the KwaZulu-Natal coast (KwaZulu-Natal Sharks Board, 2009). The majority of these dolphins were the coastal species, *T. aduncus* and *S. chinensis,* although oceanic species such as *Delphinus capensis* (long-beaked common dolphin) and the occasional tropical species such as *Stenella attenuata* (spotted dolphin) were also caught (Cockcroft and Ross, 1990a).

The geographic location of the nets; dolphin distribution, density, feeding behaviour, age and sex segregation; as well as ocean currents influencing prey movements have all been postulated as factors that influence the capture rates of *T. aduncus* off the KwaZulu-Natal coast (Cockcroft, 1992; Cockcroft, 1994). Similarly, geographic location of the nets, animal occurrence and density, and sex and age segregation within the dolphin population has been shown to influence catches of *S. chinensis*, although feeding behaviour has not been shown to have an effect (Cockcroft, 1994). Factors that contribute to *D. capensis* catches are less certain, but may be related to migrations coinciding with the annual sardine (*Sardinops ocellatus*) run (Cockcroft, *et al*., 1990; Cockcroft, 1994).

Dolphins caught in the shark nets present an opportunity to study presumed healthy individuals, and therefore can be used as representative of the overall health status of the population (Jauniaux, *et al*., 2002). In contrast, dolphins found stranded and dead along the coast, are often diseased and cannot be used to make inferences regarding the overall dolphin population health (Jauniaux, *et al*., 2002).

A long-standing agreement exists between the KwaZulu-Natal Sharks Board (KZNSB) and the Port Elizabeth Museum (PEM) under which all data and material from dolphins caught in the shark nets off the KwaZulu-Natal coast are included in the Graham Ross Marine Mammal Collection at the PEM. This agreement was formalized in a memorandum of understanding between the institutions in 2006.

3. Indian Ocean Bottlenose Dolphin (*Tursiops aduncus*)

The taxonomy of *T. aduncus* is currently under debate (Hale, *et al*., 2000; Best, 2007). The distinct species, *T. aduncus* (Indo-Pacific or Indian Ocean Bottlenose dolphin), is found in coastal waters throughout the temperate and tropical regions of the Indian Ocean and into

the south-west Pacific (Figure 1.2). In Southern Africa they have been recorded from South Africa, Mozambique, Madagascar, Tanzania, Kenya, Mauritius, Reunion and the Seychelles (Best, 2007). In South Africa, their distribution is continuous from Cape Agulhas in the south, to the Mozambican border in the north (Best, 2007). Two distinct populations are found off the KwaZulu-Natal coast: a resident population, split into two subpopulations, one north and one south of Ifafa beach; and a second, migratory population, occurring off KwaZulu-Natal only during the winter months (May to August), coincident with the annual sardine run (Peddemors, 1999; Natoli, *et al*., 2008). These migratory animals originate as far south as Plettenberg Bay, but have not been recorded north of Ifafa beach. *Tursiops aduncus* generally occurs in groups of 20 to 50 individuals, but groups of more than 1000 animals have been recorded (Cockcroft, *et al*., 1992). Both males and females attain sexual maturity between 12 and 15 years of age, although first ovulation usually occurs in females between 9.5 and 11 years of age (Cockcroft and Ross, 1990a). Births occur year round, although there is a definite peak in summer (November to February) (Cockcroft and Ross, 1990a).

Figure 1.1. *Tursiops aduncus* (photograph by S. Plön).

Tursiops aduncus feeds inshore, on at least 94 different fish and cephalopod species, with the six most important species, based on the index of relative importance, being: *Sepia* sp. (cuttlefish), *Pomadasys olivaceus* (piggy), *Trachurus delagoa* (maasbanker), *Scomber japonicus* (mackerel), *Loligo* sp. (squid) and *Pagellus bellottii natalensis* (pandora or red tjor-

tjor). No major change in prey species was found in a study comparing historic data from the early 1990's to recent data from 2000 - 2010 (Cockcroft and Ross, 1990b; Kaiser, 2012). *Tursiops aduncus* occurs almost exclusively in waters less than 30 m deep and less than 10 km from the shore (Cockcroft, *et al*., 1990) and are therefore vulnerable to anthropogenic factors, such as being caught in gill nets, boat strikes and exposure to toxins originating from nearby human settlements, either directly or by river run-off (Cockcroft and Ross, 1990a; Cockcroft, *et al*., 1990; Best, 2007).

There are at present insufficient data to classify the *T. aduncus* population status or population trend in the coastal waters of South Africa according to the criteria set by the International Union for the Conservation of Nature (IUCN) (Hammond, *et al*., 2012). They are, however, listed in Appendix II of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) (UNEP-WCMC, 2013). In the South African Red Data Book the population is generally listed as 'Vulnerable', with the migratory subpopulations listed as 'Endangered' (Peddemors and Oosthuizen, 2004).

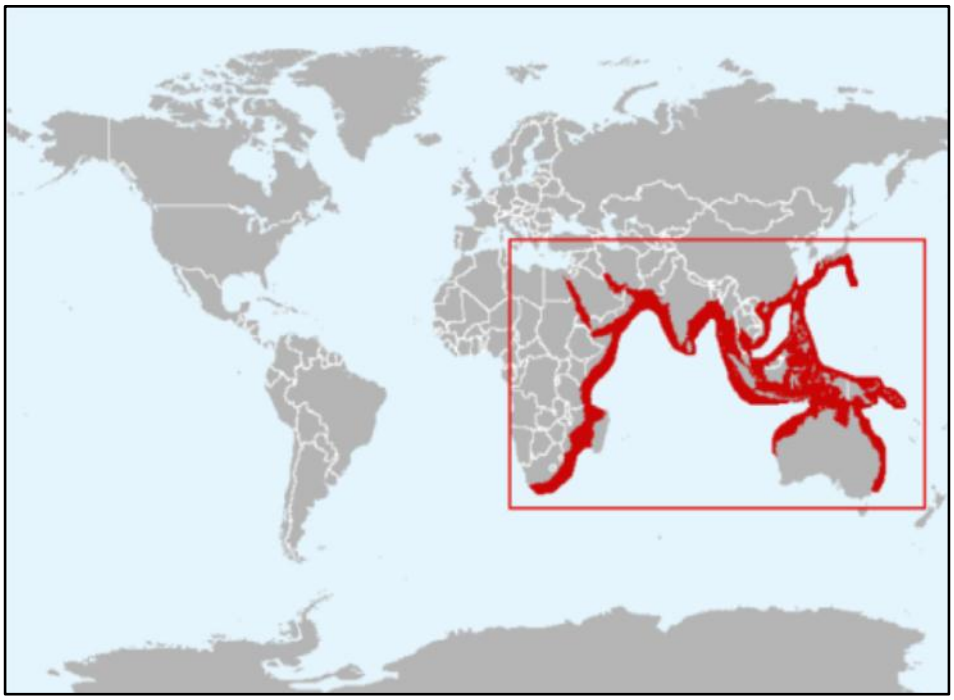

Figure 1.2. Distribution of *T. aduncus* (Hammond, *et al*., 2012; IUCN, 2012).

4. The Indo-Pacific Humpback Dolphin (*Sousa chinensis)*

Sousa chinensis is found throughout the Indian Ocean and the south-west Pacific (Figure 1.4). In the Southern African subregion, they have been recorded from South Africa, Mozambique, Madagascar, Tanzania, Kenya, and the Comoro Islands (Jefferson and Karczmarski, 2001), where they utilize a variety of habitats, including rocky reefs, sand gullies, and estuarine systems (Karczmarski, *et al*., 2000). The animals are scattered in low numbers along the coastline, most frequently as solitary animals or small groups of animals (ranging from two to 25, but most often less than ten) (Karczmarski, *et al*., 1999). Seasonal movements or migrations are not characteristic of the species, although there are increased sightings in Algoa Bay during late winter and summer (August to January) (Karczmarski, *et al*., 1999; Best, 2007).

Figure 1.3. *Sousa chinensis* (photograph by Bret Atkins/Richards Bay Humpback Dolphin Project www.arkive.org).

Sousa chinensis feeds on up to 58 fish species, which are mainly teleosts, as well as three cephalopod species (Venter, 2009). They are a predominantly shallow-water species, generally restricted to water of less than 50 m deep (Karczmarski, *et al*., 2000). As a result they are found relatively close to shore, making them susceptible to disturbances from anthropogenic factors, such as boat traffic, capture in nets, and exposure to any pollutants that enter the coastal environment, either directly or by river run-off (Best, 2007).

Research indicates that *S. chinensis* females cycle year round, although most births occur in summer (Karczmarski, 1999). Modelled population growth rates in the subregion vary from -3% to +2%, which would suggest some stability in the population (Karczmarski, 2000). But,

given their sensitivity to anthropogenic disturbances and the generally low population sizes and densities, there is considerable concern as to the future of this species in the subregion. *Sousa chinensis* is currently classified as 'Near Threatened' with a decreasing population trend by the IUCN (Hammond, *et al*., 2012), is listed under Appendix I of CITES, and is listed as 'Vulnerable' in the South African Red Data Book (Peddemors, *et al*., 2004).

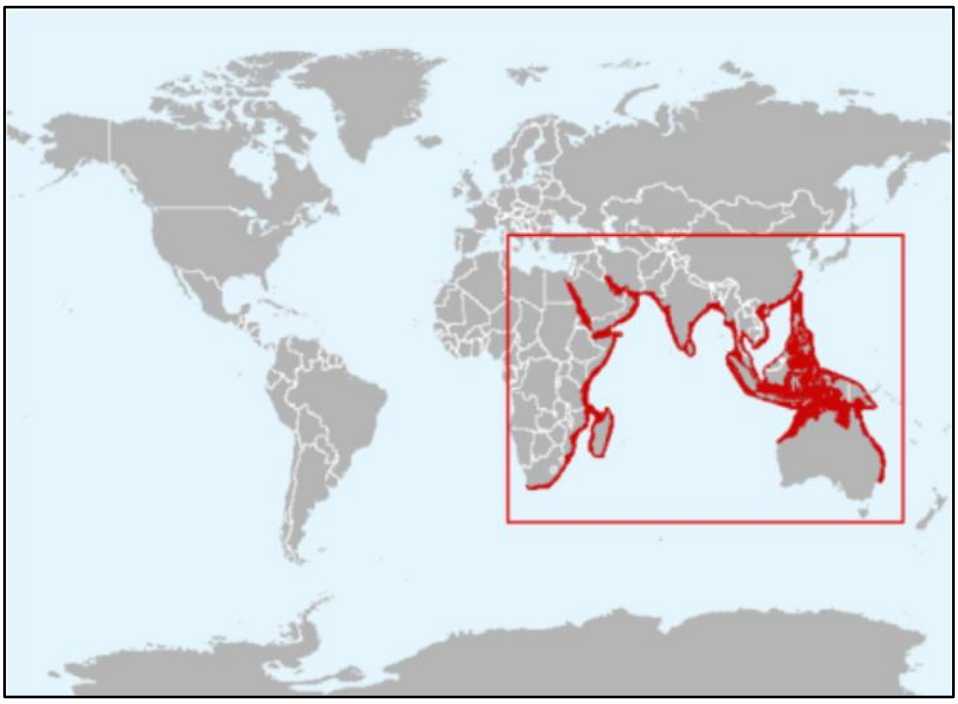

Figure 1.4. Distribution of *S. chinensis* (IUCN, 2012).

5. Infectious Diseases of Dolphins

Many organisms have been implicated in dolphin diseases, ranging from viruses (Van Bressem, *et al*., 2009a) and bacteria (Higgens, 2000) to internal and external parasites (Raga, *et al*., 2009). Infectious agents that have thus far been shown to affect dolphins are discussed below.

Owing to their entirely aquatic lifestyle, free-ranging dolphins are generally difficult to study, resulting in a lack of knowledge on diseases affecting dolphins on a population level. Literature is therefore heavily weighted towards case reports and investigation of outbreaks (Gulland and Hall, 2007) which merely document a new location or species for a disease, leaving the effects that these diseases may have on the population dynamics still largely unknown (Van Bressem, *et al*., 2009a). The increase in anthropogenic stressors affecting marine ecosystems makes it increasingly important to understand emerging marine disease

and the timing of disease outbreaks (Ward and Lafferty, 2004). Disease is also an important aspect of ecology and there is a complex interaction of disease and environmental degradation (Lafferty, *et al*., 2004). Dolphin morbillivirus has emerged as the most virulent infectious disease of dolphins, causing multiple mass mortality events (Van Bressem, *et al*., 2009a). Other pathogens that have been classified as emerging diseases in dolphins are papillomavirus, cetacean poxviruses, *Brucella ceti*, *Toxoplasma gondii* and *Lacazia loboi* (Van Bressem, *et al*., 2009a; Bossart, 2011). Pathogens that cause mass mortalities, such as cetacean morbilliviruses and *Brucella* spp. have the highest impact on populations (Van Bressem, *et al*., 2009a), although these effects may be temporary as populations are able to recover. Some concern does, however, exist as to the potential for populations of endangered species to recover (Lafferty, *et al*., 2004; Ward and Lafferty, 2004). However, many diseases act in a more subtle way, causing debility, decreased body condition, impaired immune responses and decreased fecundity in single animals (Duignan, 2003), causing many of these diseases to go undetected. This may be further complicated by the cost of detecting some of these diseases. Consequently, to determine which infectious diseases might present a risk to a population is often difficult, as populations are often understudied and information on the role that infectious diseases may play in the decline of populations is often not available (Gaydos, *et al*., 2004).

5.1. Viruses

5.1.1. Cetacean Morbillivirus

Cetacean morbilliviruses are single-stranded RNA viruses of the genus *Morbillivirus,* family *Paramyxoviridae*, and include three strains: the dolphin morbillivirus isolated in the Mediterranean *Stenella coeruleoalba* (striped dolphin) (Van Bressem, *et al*., 1991; Alex and Raga, 1993), the porpoise morbillivirus isolated from *Phocoena phocoena* (harbour porpoise) (McCullough, *et al*., 1991), and the pilot whale morbillivirus isolated from *Globicephala melas* (long-finned pilot whale) (Taugenberger, *et al*., 2000).

In recent years, morbilliviruses have emerged as the most virulent viral pathogens of pinnipeds and cetaceans, causing mass mortalities, often of thousands of animals in naïve populations, notably in *Tursiops truncatus* (common bottlenose dolphin) and *S. coeruleoalba* (Calzada, *et al*., 1994; Lipscomb, *et al*., 1994; Duignan, *et al*., 1995; Lipscomb, *et al*., 1996; Di Guardo, *et al*., 2005; Van Bressem, *et al*., 2009a). Cetacean morbillivirus is endemic in several species of cetaceans worldwide, and these are thought to be the reservoir hosts, spreading the virus to other associated species, leading to epidemics (Van Bressem, *et al*., 1998; Van Bressem, *et al*., 2001; Di Guardo, *et al*., 2005; Van Bressem, *et al*., 2009a).

Cetacean morbillivirus infection usually presents as severe, exudative bronchopneumonia with lungs that fail to collapse and multiple areas of atelactasis and consolidation, characterized by non-suppurative, subacute to chronic, bronchointerstitial pneumonia, with hyperplasia of type II pneumocytes, necrosis of bronchial and bronchiolar epithelial walls, and serofibrinous exudates, macrophages, and other mononuclear cells within bronchial, bronchiolar and alveolar lumina (Domingo, *et al*., 1992; Duignan, *et al*., 1992; Kennedy, *et al*., 1992; Lipscomb, *et al*., 1994; Di Guardo, *et al*., 2005). Prominent fibroplasia is frequently seen in the inter-alveolar septa, accompanied by oedema and hyperaemia (Di Guardo, *et al*., 2005). Variably necrotic, multinucleated syncytia often containing eosinophilic nuclear and/or cytoplasmic inclusions are sometimes present in the inflammatory lesions (Lipscomb, *et al*., 1994; Duignan, *et al*., 1995). Secondary pneumonic mycosis and bacterial colonization are often noted (Domingo, *et al*., 1992; Lipscomb, *et al*., 1994; Lipscomb, *et al*., 1996).

Lesions of the central nervous system (CNS) usually comprise non-suppurative meningoencephalitis consisting of multifocal to laminar degeneration and necrosis of neurons and glial cells in the cerebral cortex, with microglial infiltration and perivascular cuffing. Neuronal cells also frequently show intracytoplasmic and/or intranuclear acidophilic viral inclusion bodies (Domingo, *et al*., 1992; Duignan, *et al*., 1992; Di Guardo, *et al*., 2005). Syncytia are seen in both white and grey matter portions of the brain with severe astrogliosis/astrocytosis, the latter being characterized by the proliferation of peculiar astrocytes (gemistocytic astrocytes) (Di Guardo, *et al*., 2005). Chronic encephalitis has also been observed (Barrett, *et al*., 1995).

Other variable lesions include oedema of the lung-associated lymph nodes; hypoplasia and severe lymphocytolysis, with syncytia scattered throughout the lymphoid tissue of lymph nodes, spleen, thymus and gut-associated lymphoid tissue; subcutaneous oedema; ulcerative stomatitis and glossitis (Domingo, *et al*., 1992; Duignan, *et al*., 1992; Kennedy, *et al*., 1992; Di Guardo, *et al*., 2005). Animals may also become immunocompromised, with secondary infection by *Toxoplasma gondii* and *Photobacterium damselae* subsp. *damselae* reported (Domingo, *et al*., 1992; Lipscomb, *et al*., 1994; Duignan, *et al*., 1995).

Immunohistochemistry, with specific monoclonal antibodies raised against different morbilliviral epitopes, is used to confirm a diagnosis of cetacean morbillivirus infection by demonstrating viral antigen in a variety of tissues from the respiratory tract, CNS, urinary system and gastrointestinal tract (Domingo, *et al*., 1992; Duignan, *et al*., 1992; Duignan, *et al*., 1995; Kennedy, 1998; Di Guardo, *et al*., 2005). No sex predisposition has been shown; while animals aged between 11 and 20 years seem to show the highest mortality rates (Calzada, *et al*., 1994). Furthermore, high mortality rates have been observed in foetuses,

neonates and young calves, but this is probably as a secondary consequence to the death of the mothers, and not as result of being infected with morbillivirus themselves (Alex and Raga, 1993; Calzada, *et al*., 1994). Owing to the potentially devastating effect that morbillivirus infection may have on a population, it remains one of the most important infectious diseases of marine mammals (Van Bressem, *et al*., 2009a).

5.1.2. Herpesvirus

Herpesviruses are enveloped, double stranded DNA viruses divided into three subfamilies: the *Alpha-, Beta-* and *Gammaherpesvirinae* (Pellett and Roizman, 2007). Herpesviruses from the *Alpha-* and *Gammaherpesvirinae* subfamilies have been documented in *T. truncatus* and *Grampus griseus* (Risso's dolphin) (Blanchard, *et al*., 2001; Benson, *et al*., 2006), with a herpes-like virus being documented in *Lagenorhynchus obscurus* (dusky dolphin) (Van Bressem, *et al*., 1994). Herpesviruses may induce localized lesions in the skin and mucous membranes or disseminated disease (Benson, *et al*., 2006; Elk, *et al*., 2009). Localized lesions are most often associated with the genital tract (mainly the penis and the vulva) and are thought to be sexually transmitted. Pale yellow to white, irregular plaques are characterized by destruction of the epithelium, necrosis and ulceration or hyperplasia of the *lamina propria*, resembling papillomas, with prominent papillae. Disseminated, often fatal, disease has only been found in sub-adults, possibly not protected by maternal antibodies, and consists predominantly of necrotising lesions and eosinophilic intranuclear inclusion bodies in multiple organs as well as lymphoid depletion. Diagnosis is confirmed with electron microscopy and a newly developed ELISA (Elk, *et al*., 2009). The impact that localized lesions may have on reproduction, and therefore on the whole population, has not been ascertained. In contrast, disseminated disease could have a devastating effect on a naïve population, or a population consisting of immunocompromised individuals, although no such outbreak has been documented in dolphins.

5.1.3. Poxvirus

Poxviruses are enveloped, double-stranded DNA viruses and those that affect marine mammals are believed to belong to a new subfamily *Chordopoxvirinae* (family *Chordopoxviridae)* (Moss, 2007). They have been identified in 13 free-ranging species of dolphins (Geraci, *et al*., 1979; Van Bressem, *et al*., 1993; Van Bressem, *et al*., 1996; Van Bressem, *et al*., 2009b). Poxvirus infection (or tattoo skin disease) is characterized by irregular gray, black or yellowish skin discolourations anywhere on the body, consisting of cytoplasmic vacuolar degeneration of the *stratum intermedium*, which may contain eosinophilic inclusions, particularly in the superficial layer (Geraci, *et al*., 1979). Adjacent normal epithelial cells are often compressed and parakeratosis is evident in the layer

overlying the lesion. The nature of the pigmentation seen macroscopically is not well understood. Inflammatory responses are minimal (Geraci, *et al*., 1979; Van Bressem, *et al*., 1993; Van Bressem, *et al*., 2009a). In dolphins, no sex predilection has been observed; however, a strong increase in diseased individuals is usually noted around adulthood, possibly as a result of cessation of protective maternal antibodies (Van Bressem, *et al*., 1996). In endemic populations, poxviruses are not believed to induce high mortality rates, although death may occur in younger animals not protected by maternal antibodies (Van Bressem, *et al*., 2009a). Diagnosis is confirmed with electron microscopy and molecular techniques (Van Bressem, *et al*., 2009). Some evidence suggests that the severity of disease may be directly proportional to deteriorating environmental conditions, such as contamination with toxins, and/or the overall health status of the animal, and may provide a visual clue as to the health status of the individual (Geraci, *et al*., 1979; Van Bressem, *et al*., 2009).

5.1.4. Papillomavirus

Papillomaviruses are small, non-enveloped DNA viruses belonging to the family *Papovaviridae* (Van Bressem, *et al*., 1996). These have been isolated from *T. truncatus*, *D. capensis* and *L. obscurus,* and induce wart-like lesions in cetaceans, characterized by epithelial hyperplasia of the *stratum spinosum, stratum externum,* and dermal papillae of the skin with variable intra-cytoplasmic inclusion bodies (Van Bressem, *et al*., 1996). As not many warts have specifically been tested for the presence of papillomavirus, conclusive data on the prevalence of papillomavirus-induced papillomas is not available (Van Bressem, *et al*., 1996). Since warts are often seen in the genital tract of marine mammals, papillomaviruses are thought to be mainly transmitted venereally, and in some species (*T. truncatus* and *L. obscurus*) males are two to three times more often infected than females (Rehtanz, *et al*., 2010). Diagnosis is mainly by electron microscopy. Although it does not seem that it causes debilitating disease, it may indirectly impact on population dynamics by physically interfering with copulation (Van Bressem, *et al*., 1996; Van Bressem, *et al*., 2009a).

5.1.5. Enterovirus

Enteroviruses are members of the family *Picornaviridae*, within the genus *Enterovirus* and are non-enveloped, single-stranded RNA viruses (Nollens, *et al*., 2009). In terrestrial animals, enteroviruses may cause gastrointestinal disease, meningitis, myocarditis, myopathies, abortions and cutaneous blisters (Pallansch and Roos, 2007). Bottlenose dolphin enterovirus has been isolated once from a tongue erosion of *T. truncatus* (diagnosed via virus isolation and PCR) and, like enterovirus infections in other species, is thought to be

largely subclinical and probably does not pose a great risk to dolphin populations (Nollens, *et al*., 2009).

5.1.6. Parainfluenzavirus

Parainfluenzaviruses comprise a group of non-segmented, negative strand RNA viruses of the family *Paramyxoviridae*. *Tursiops truncatus* parainfluenza virus type 1, of the genus *Respirovirus*, is a suspected primary respiratory pathogen that has been isolated from a single, adult, male *T. truncatus* (Nollens, *et al*., 2008). Pathological findings are mostly confined to the respiratory tract, including pyogranulomatous bronchointerstitial pneumonia, erosive and ulcerative tracheitis and laryngitis, with secondary bacterial and yeast colonization as common sequelae. Diagnosis is confirmed by electron microscopy and PCR viral characterization (Nollens, *et al*., 2008). As only one case has been previously described from a managed population of dolphins housed in an open ocean enclosure, it is unknown what effect this virus might have on dolphin populations (Nollens, *et al*., 2008).

5.2. Bacteria

5.2.1. Brucellosis

Brucellosis is a globally occurring zoonotic disease of mammals caused by a Gram-negative, facultative intracellular bacterium of the genus *Brucella* (Ross, *et al*., 1996; Corbel, 1997; Bourg, *et al*., 2007). At least three *Brucella* spp. have been isolated from seals, porpoises and dolphins, although there still remains some debate as to nomenclature (Corbel, 1997). Suggestions include *Brucella pinnipedialis* (seals) and *B. ceti,* subdivided into *B. phocoena* (porpoises) and *B. delphini* (dolphins) (Van Bressem, *et al*., 2009a). The latter is phenotypically similar to smooth *B. abortus* and *B. melitensis,* possessing the same surface antigens (González-Barrientos, *et al*., 2010).

Brucella organisms have been isolated form *T. truncatus, D. capensis, D. delphis* (short-beaked common dolphin), *S. coeruleoalba, L. obscurus, L. acutus* (Atlantic white-sided dolphin), and *L. albirostris* (white beaked dolphin) (Ross, *et al*., 1996; Van Bressem, *et al*., 1996; Corbel, 1997; Miller, *et al*., 1999; Foster, *et al*., 2002; González, *et al*., 2002; Dawson, *et al*., 2006; Munoz, *et al*., 2006; Dagleish, *et al*., 2007; Davison, *et al*., 2009; González-Barrientos, *et al*., 2010). However, *Brucella*-associated pathology was often absent, and these animals died as result of other, unrelated causes.

Brucella ceti has been associated with a wide variety of diseases in cetaceans, including placentitis, orchitis, abortion, mastitis, septicaemia, pneumonia, subcutaneous lesions, endocarditis, arthritis, meningoencephalitis and encephalitis, hepatic and splenic coagulative

necrosis, and lymphadenitis (Ross, *et al*., 1996; Miller, *et al*., 1999; González, *et al*., 2002; Dawson, *et al*., 2006; Munoz, *et al*., 2006; Dagleish, *et al*., 2007; Davison, *et al*., 2009; Van Bressem, *et al*., 2009a). Abortion is characterized by a suppurative placentitis and necrotizing vasculitis of the placenta with variable growth of *Brucella* organisms from the placenta and organs of the foetus (Miller, *et al*., 1999; González-Barrientos, *et al*., 2010). Infection with *Brucella* spp. does not permanently affect fertility (Miller, *et al*., 1999; Dunn, *et al*., 2001). Pneumonia is characterized by chronic abscessation (Miller, *et al*., 1999). Meningoencephalitis is usually denoted by a multifocal to diffuse, lymphoplasmacytic infiltration of multiple areas of the central nervous system, including the brain, meninges, and choroid, with formation of perivascular cuffs of mononuclear cells and microgliosis often present (Munoz, *et al*., 2006; Dagleish, *et al*., 2007; Davison, *et al*., 2009; González-Barrientos, *et al*., 2010). Fibrosis has been observed as well as necrosis of white and grey matter (González, *et al*., 2002; Munoz, *et al*., 2006; González-Barrientos, *et al*., 2010). Osteoarthritis is usually fibrino-purulent in nature, with severe infiltration of the synovial fluid by macrophages and neutrophils (Dagleish, *et al*., 2007; González-Barrientos, *et al*., 2010). Endocarditis is characterized by thickening of the mitral valve, the presence of fibrin adjacent to the surface of the mitral valve, lymphoplasmacytic and histiocytic infiltration with occasional multinucleate giant cells, and mild necrosis and dystrophic calcification (González-Barrientos, *et al*., 2010). Concomitant myocardial degeneration with associated lymphocytic infiltrate has also been noted (González-Barrientos, *et al*., 2010).

Diagnosis of brucellosis is achieved by demonstration of antigen by immunofluorescence or immunohistochemistry in visible lesions, and by bacteriological culture from a variety of organs including the spleen, lymph nodes, brain, blood, and kidney (González, *et al*., 2002; Dawson, *et al*., 2006; Dagleish, *et al*., 2007; González-Barrientos, *et al*., 2010). Culture may be positive in the absence of gross and histopathological lesions in some organs (Ross, *et al*., 1996; González, *et al*., 2002). Antibodies have also been demonstrated by the Rose Bengal test on serum and pericardial fluid (Dawson, *et al*., 2006; Munoz, *et al*., 2006; Davison, *et al*., 2009).

Brucellosis is a naturally occurring infection in marine mammals and endemic in some populations, with evidence of horizontal and vertical transmission (Ross, *et al*., 1996; Van Bressem, *et al*., 2009a). Owing to the induction of abortions, as well as causing the death of neonates and sexually mature individuals, brucellosis may have a significant negative impact on populations, especially endangered cetacean populations (Ross, *et al*., 1996; Miller, *et al*., 1999; Van Bressem, *et al*., 2009a). Brucellosis is also a recognized zoonotic disease in man, with some confirmed cases of humans infected with *Brucella* spp. from marine

mammals (Ross, *et al*., 1996). The role of environmental factors in the emergence of marine mammal brucellosis is yet to be determined (Van Bressem, *et al*., 2009a).

5.2.2. Nocardiosis

Nocardia spp. are filamentous, weakly staining Gram-positive, acid-fast bacteria, ubiquitous in the environment and found worldwide in fresh and saltwater, soil, dust, decaying vegetation, and decaying animal faecal deposits (Pier, *et al*., 1970; St. Leger, *et al*., 2009). Nocardiosis is a significant cause of mortality in captive and wild marine mammals and has been documented affecting *T. aduncus* and *T. truncatus* (Pier, *et al*., 1970; St. Leger, *et al*., 2009).

Six basic disease forms can be distinguished in terrestrial mammals: pulmonary, systemic, CNS, extrapulmonary, cutaneous (subcutaneous or lymphocutaneous), and actinomycetoma (St. Leger, *et al*., 2009). Pulmonary pyogranulomas with abscessation of the thoracic lymph nodes are the most common presentation in cetaceans (Pier, *et al*., 1970; St. Leger, *et al*., 2009). Other pathological findings include pleuritis, multiple widespread pyogranulomas or abscesses (in multiple lymph nodes, brain, aortic endothelium, spleen, adrenal, intestine, kidney and liver), pyogranulomatous dermatitis and cellulitis, and osteonecrosis (St. Leger, *et al*., 2009). Haematoxylin and eosin (HE) staining often fails to demonstrate the presence of the bacteria, but modified acid-fast stains and Gomori-Grocott methenamine silver (GMS) stain may highlight the organism, although diagnosis is confirmed on culture (St. Leger, *et al*., 2009). Some uncertainty exists as to whether infection occurs by inhalation, aspiration, inoculation and/or ingestion (Pier, *et al*., 1970; St. Leger, *et al*., 2009). The fact that nocardiosis usually affects the lungs might point to inhalation or aspiration as the main routes of entry (St. Leger, *et al*., 2009). Nocardiosis should be considered a differential diagnosis in any dolphin with central nervous system or pulmonary disease (St. Leger, *et al*., 2009).

5.2.3. Erysipelas

Erysipelothrix rhusiopathiae is a ubiquitous, small, Gram-positive bacillus, most commonly associated with disease in swine (Dunn, *et al*., 2001; Wang, *et al*., 2010), although it may persist in the marine environment for a long period of time (Wang, *et al*., 2010). Dolphins may ingest freshwater and marine fishes contaminated with *E. rhusiopathiae*, which may lead to disease (Dunn, *et al*., 2001), and has been documented in *T. truncatus, L. albirostris, L. obliquidens* (Pacific white-sided dolphin), and *G. griseus* (Higgens, 2000).

Two distinct forms of the disease have been recognized: a dermatologic form and a septicaemic form (Higgens, 2000; Dunn, *et al*., 2001). The dermatologic form is characterized by macroscopic, grey, rhomboid skin plaques over the entire body trunk (Sweeney and Ridgway, 1975; Dunn, *et al*., 2001). Septicaemia is usually fatal with animals showing non-specific signs at necropsy that include ascites, multifocal intestinal petechial and ecchymotic hemorrhages, sloughing skin, swollen lymph nodes and splenomegaly (Higgens, 2000; Dunn, *et al*., 2001). Diagnosis is based on bacteriological culture and identification of the organisms (Sweeney and Ridgway, 1975; Higgens, 2000; Dunn, *et al*., 2001).

5.2.4. Opportunistic Bacteria

Some bacteria are part of the normal flora present in marine mammals and their environment. They are opportunistic in causing disease when the animal is in some way compromised (Higgens, 2000). An example of this is morbillivirus-induced immunosuppression acting as a predisposing factor to infections with *Photobacterium damselae* subsp. *damselae* (Keck, *et al*., 2010). Opportunistic bacteria may cause a variety of lesions and various syndromes. Diagnosis is based on bacterial culture and identification. Table 1.1 contains a list of opportunistic bacteria and the reported associated organ.

5.3. Fungi and Yeasts

5.3.1. Lobomycosis

Lobomycosis is caused by the dimorphic fungus *Lacazia loboi,* formally *Loboa loboi* (Taborda, *et al*., 1999). It naturally affects *T. truncatus* and *Sotalia guianensis* (Guiana dolphin*)* as well as humans (Cowan, 1993; Paniz-Mondolfi, *et al*., 2007; Murdoch, *et al*., 2008; Durden, *et al*., 2009; Van Bressem, *et al*., 2009b) and is endemic in dolphin populations, occurring in waters of the south-eastern United States and South America (Van Bressem, *et al*., 2009a). Water, soil and vegetation appear to be ecological habitats for *Lacazia loboi*, but it has strict requirements regarding environmental conditions, such as salinity and water temperature, to become endemic in a geographic location.

Lobomycosis is a chronic disease of the skin and subcutaneous tissue, rarely involving associated lymph nodes, but no systemic distribution has been documented in animals (Reif, *et al*., 2006; Paniz-Mondolfi, *et al*., 2007; Van Bressem, *et al*., 2009a). Lesions are often present on the leading edge of the dorsal and pectoral fins, head, flukes and caudal peduncle (Cowan, 1993; Reif, *et al*., 2006; Durden, *et al*., 2009). They are grey-white to slightly pink, keloidal or verrucous, often in profound relief, and may ulcerate or form plaques

Table 1.1. Opportunistic bacteria found in cetaceans

Bacteria	Description	Animal	Target organ	Reference
Actinobacillus delphinicola	Gram-negative rod	*S. coeruleoalba*	Isolated from lungs, gastric and mandibular lymph nodes and intestinal contents,	Foster, *et al*., 1996
***Aeromona* spp.**	Gram-negative rod	*S. longirostris*	Lung	Migaki, *et al*., 1990
Escherichia coli	Gram-negative bacillus	*T. truncatus*	Liver, umbilicus, synovium of the atlanto-occipital joint and septicaemia	Elk, *et al*., 2007
Pasteurella aeruginosa	Gram-negative coccobacillus	*T. truncatus*	Lung	KyungYeon and OhDeog, 2011
Vibrio alginolyticus	Gram-negative	*T. truncatus*	skin	Schroeder, *et al*., 1985
Vibrio damsela	Gram-negative	*T. truncatus*	skin	Fujioka, *et al*., 1988
Clostridium perfringens	Gram-positive rod	*T. truncatus*	Muscle and septicaemia	Buck, *et al*., 1987
Clostridium tertius	Gram-positive rod	*S .coeruleoalba*	Skin and bone	Šeol, *et al*., 2006
Staphylococcus aureus	Gram-positive coccus	*T. truncatus*	Lung	Pier, *et al*., 1970; KyungYeon and OhDeog, 2011
Staphylococcus delphini	Gram-positive coccus	Unspecified species	Skin	Veraldo, *et al*., 1988

often coalescing to more than 30 cm in diameter (Reif, *et al*., 2006; Paniz-Mondolfi, *et al*., 2007; Durden, *et al*., 2009; Reif, *et al*., 2009). Histologically, lesions are characterized by non-necrotizing, granulomatous inflammation of the sub-epidermal layer of the skin, extending into the subcutaneous tissue. Cellular infiltrates usually include large numbers of macrophages and multinucleate giant cells, associated with abundant round to oval fungal cells, that may be free or phagocytosed in macrophages and giant cells (Cowan, 1993; Reif, *et al*., 2006; Paniz-Mondolfi, *et al*., 2007; Durden, *et al*., 2009). Lymphocyte infiltration may be minimal, although if the lesions are ulcerated, neutrophil and plasma cell infiltration is common (Paniz-Mondolfi, *et al*., 2007). Impairment of adaptive immunity was found only in endemically affected *T. truncatus* from the Indian River Lagoon, Florida (Reif, *et al*., 2009). The exact aetiology of the immunosuppression in dolphins has not yet been determined, but both environmental contaminants (such as mercury and polychlorinated biphenyls) and chronic stress as result of anthropomorphic factors have been suggested (Reif, *et al*., 2006; Reif, *et al*., 2009). *Lacazia loboi* enters the skin via penetration or accidental trauma (Reif, *et al*., 2006; Paniz-Mondolfi, *et al*., 2007). The fungus has not been cultured *in vitro*, therefore the diagnosis is mainly on histopathology and identification of yeast-like cells in skin lesions (Reif, *et al*., 2006; Durden, *et al*., 2009; Van Bressem, *et al*., 2009b).

In South America and the south-western Indian Ocean, several cases of skin disease that greatly resemble lobomycosis were observed in free-ranging, inshore *T. aduncus, T. truncatus* and *S. guianensis*. In the absence of a histological diagnosis, the disease was called lobomycosis-like disease (LLD). Similarly to lobomycosis, LLD evolves over years (Kiszka, *et al*., 2009; Van Bressem, *et al*., 2009a).

The impact of lobomycosis and LLD on dolphin populations is unknown, but the disease may have contributed to the death of some individuals (Van Bressem, *et al*., 2009a). Lobomycosis, although endemic in certain human populations residing in the Amazon basin, may be considered a zoonotic disease, but the transmission from animals to humans is believed to be very low (Taborda, *et al*., 1999; Paniz-Mondolfi, *et al*., 2007; Van Bressem, *et al*., 2009a).

5.3.2. Aspergillosis

Aspergillosis is most commonly caused by *Aspergillus fumigatus*, but *A. niger* and *A. terreus* have also been associated with disease in dolphins (Higgens, 2000). They are ubiquitous and abundant in nature, with the disease occurring worldwide, being recorded in *T. truncatus, S. coeruleoalba, Cephalorhynchus commersonii* (Commerson's dolphin) and *Lissodelphis borealis* (Northern right whale dolphin) (Howard, *et al*., 1983; Higgens, 2000; Reidarson, *et al*., 2001; Tell, 2005). Moderate to severe necro-granulomatous pneumonia is

the predominant mycosis seen (Domingo, *et al*., 1992; Higgens, 2000; Reidarson, *et al*., 2001), and may occur concurrently with other diseases such as morbillivirus infection (Domingo, *et al*., 1992). The CNS and alimentary tract have also been affected (Reidarson, *et al*., 2001). Inhalation is thought to be the main route of entry (Higgens, 2000; Reidarson, *et al*., 2001). Fungal hyphae are often present on histopathology, but culture is the usual modality for diagnosis and identification (Higgens, 2000; Reidarson, *et al*., 2001).

5.3.3. Cryptococcosis

Cryptococcus neoformans var. *neoformans* is a ubiquitous, encapsulated yeast found worldwide, and has been documented in *T. truncatus*, *S. coeruleoalba* and *L. obliquidens* (Gales, *et al*., 1985; Ellis and Pfeiffer, 1992; Higgens, 2000; Reidarson, *et al*., 2001). Lesions have been seen in the lungs, lymph nodes, spleen, gastrointestinal tract and as a systemic disease, characterized by granulomatous inflammation in these organs. Lesions show typical cryptococcal bodies, which are highlighted by Periodic Acid-Schiff stain (PAS), and rare giant cells (Gales, *et al*., 1985; Reidarson, *et al*., 2001). Free cryptococcal bodies, with minimal or no inflammatory response, have also been seen in the stomach wall (Gales, *et al*., 1985). Evidence suggests that inhalation is the most likely route of infection. Cryptococcosis in terrestrial mammals is often associated with avian colonies, although the full role of marine bird species in disseminating *C. neoformans* var. *neoformans* is yet to be determined (Gales, *et al*., 1985; Ellis and Pfeiffer, 1992; Reidarson, *et al*., 1998a; Higgens, 2000).

Cryptococcus neoformans var. *gattii* has been sporadically reported in *T. truncatus, S. coeruleoalba* and *Stenella longirostris* (Spinner dolphin) (Rotstein, *et al*., 2010). Contrary to *C. neoformans* var. *neoformans,* it is generally confined to tropical and subtropical areas of the world and believed to be associated with trees, specifically *Eucalyptus* spp. (Ellis and Pfeiffer, 1992). *Cryptococcus neoformans* var. *gattii* infection in dolphins classically involves the skin, lungs and lymph nodes, and on occasion systemic infection extending to the stomach, adrenal gland, kidney and spleen. Inflammation is granulomatous in nature, variable in severity, and associated with round to oval cryptococcal-like yeasts, similar to *C. neoformans* var. *neoformans* (Ellis and Pfeiffer, 1992; Miller, *et al*., 2002; Rotstein, *et al*., 2010). There is strong evidence to suggest that infection with *C. neoformans* var. *gattii* may only be fatal in immunocompromised animals, with subclinical disease predominating in healthy individuals (Ellis and Pfeiffer, 1992; Rotstein, *et al*., 2010). The significance of *C. neoformans* var. *gattii* infection in dolphin populations is yet to be determined (Rotstein, *et al*., 2010)

5.3.4. Opportunistic Fungi

Fungi, like bacteria, may also opportunistically affect dolphins secondary to viral infections or other causes that compromise the host's immune system. Table 1.2 summarizes opportunistic fungi affecting dolphins, with target organs.

Table 1.2. Opportunistic and endemic fungi affecting dolphins (adapted from Reidarson, *et al*., (2001) and Higgens (2000)).

	Organism	**Host species**	**Target organ**	**Reference**
Opportunistic fungi	Deuteromycetes	*T. truncatus*	Brain, trachea and cervical lymph nodes	Haubold, *et al*., 1997
	Fusarium spp.	*L. acutus*	Skin	Frasca, *et al*., 1996
	Sporothrix schenchii	*L. obliquidens*	Skin	Migaki, *et al*., 1978
	Trichosporon pullulans	*T. truncatus*	Lungs	Reidarson, *et al*., 2001
	Apophysomyces elegans (zygomycete)	*T. truncatus*	Lungs, blubber, muscle, skin, brain, and disseminated mycosis	Reidarson, *et al*., 2001
	Apophysomyces elegans (zygomycete)	*L. acutus*	Brain	Reidarson, *et al*., 2001
		L. obliquidens	Disseminated mycosis	Reidarson, *et al*., 2001
	Other zygomycetes	*T. truncatus*	Hilar lymph nodes, lungs, mandible, skin, brain, and disseminated mycosis	Sweeney, *et al*., 1976; Reidarson, *et al*., 2001
	Neoscytalidium dimidiatum	*G. griseus*	Lung and lymph nodes	Elad, *et al*., 2011

Table 1.2. (cont.)

Endemic fungi	*Blastomyces dermatitidis*	*T. truncatus*	Disseminated mycoses	Cates, *et al*., 1986
	Coccidioides immitis	*T. truncatus*	Disseminated mycoses	Reidarson, *et al*., 1998b
	Histoplasma capsulatum	*L. obliquidens*	Gastrointestinal tract	Reidarson, *et al*., 2001
	Histoplasma capsulatum	*T. truncatus*	Disseminated mycoses	Jensen, *et al*., 1998; Reidarson, *et al*., 2001
	Candida albicans, *C, rugosa,* *C. glabrata,* *C. tropicalis.*	*T. truncatus*	Blowhole, skin, bladder, or systemic disease affecting the kidneys, CNS, and heart valves.	Higgens, 2000; Reidarson, *et al*., 2001

5.4. Parasites

Parasites are an integral part of the biosphere, affecting almost every free living organism (Geraci and St. Aubin, 1987; Raga, *et al*., 2009). The effects that parasites have on their hosts are largely dependent on the type of parasite, the abundance within a host, the host's health status and other concurrent diseases (Geraci and St. Aubin, 1987; Raga, *et al*., 2009). The effects are usually minor and confined to local reactions such as irritation and inflammation, visible as predominantly eosinophilic to mixed cellular infiltrates. Some may be more damaging, such as nematodes causing ulcers and possibly haemorrhage in the intestinal tract at the site of their attachments. Others may cause substantial tissue damage, such as abscessation, necrosis and inflammatory infiltrates, which may possibly affect the function of one or more organs. This may lead to debilitating disease, inability to dive and hunt, stranding and even death, either on an individual or population basis (Geraci and St. Aubin, 1987; Kuiken, *et al*., 1994; Siebert, *et al*., 1999; Raga, *et al*., 2009). Populations may also be affected by parasites, for example when many adult female dolphins are infected with *Crassicauda* spp., which destroy mammary gland tissue, causing little to no effect on the female, but negatively impacting the quantity and quality of milk produced. This may

ultimately compromise the survival of calves and subsequently the fecundity of the herd (Geraci and St. Aubin, 1987).

Although the majority of parasites have little effect on the host, some factors (such as high mercury burdens or morbillivirus infections) may compromise the host's immune system, thereby increasing the severity and prevalence of parasitic infections (Siebert, *et al*., 1999). Parasite burdens may then be used as indicators for the overall health status of an individual (Siebert, *et al*., 1999). This assumption should, however, be used with caution, as some environmental factors, such as pollution, may also negatively affect the parasite populations (Torchin, *et al*., 2002).

5.4.1. Toxoplasmosis

Toxoplasmosis is caused by the protozoan parasite *Toxoplasma gondii* (Frenkel, 1988). It has a worldwide distribution, affecting mammals and birds, and has been described in *T. truncatus*, *S. coeruleoalba*, *S. longirostris* and *G. griseus* (Migaki, *et al*., 1990; Dubey, *et al*., 2007; Dubey, *et al*., 2009; Van Bressem, *et al*., 2009a).

Toxoplasmosis is generally asymptomatic in dolphins, with many animals testing positive for *T. gondii* antibodies in the absence of apparent disease (Dubey, *et al*., 2003; Forman, *et al*., 2007). Most dolphins that die of toxoplasmosis show below average body condition, have concurrent disease, or are for some reason immunosuppressed, e.g. as result of stress or morbillivirus infection (Migaki, *et al*., 1990; Bowater, *et al*., 2003; Dubey, *et al*., 2003; Forman, *et al*., 2007). Only four cases where *T. gondii* is believed to have acted as primary pathogen have been documented, all in *S. coeruleoalba* (Di Guardo, *et al*., 2010).

Toxoplasmosis is characterized by necrosis of any organ, but mainly the adrenals, liver, brain, spleen and myocardium, with varying degrees of mononuclear cell infiltrates and often large numbers of protozoan cysts (Frenkel, 1988; Migaki, *et al*., 1990; Bowater, *et al*., 2003; Di Guardo, *et al*., 2010). Infarction may also occur when the inflammation and necrosis is associated with blood vessels (Frenkel, 1988). Lesions in the brain are often characterized by microglial nodules and mononuclear cell infiltrates, often forming perivascular cuffs (Frenkel, 1988; Migaki, *et al*., 1990; Bowater, *et al*., 2003; Dubey, *et al*., 2009; Di Guardo, *et al*., 2010). Lymphoid hyperplasia may be seen in acute infection, but most animals that die from toxoplasmosis exhibit lymphocyte depletion (Frenkel, 1988).

In terrestrial animals, *T. gondii* has an indirect lifecycle, with felids being the definitive host, and birds and rodents classically acting as intermediate hosts (Frenkel, 1988). Oocysts are shed in faeces of the definitive host, with sporozoites that can survive for up to one year in

the environment developing in these oocysts in the presence of oxygen. The sporozoites infect the intermediate host after ingestion from the environment, contaminated food, or another intermediate host. Sporozoites are not pathogenic, but rapidly transform into tachyzoites upon entering the gut mucosa post ingestion, after which they disseminate and multiply in tissues, encysting as bradyzoites particularly in the liver, lymph nodes, lung and brain. Infections in intermediate host species are generally asymptomatic, or with transient clinical signs. The definitive host acquires the infection by ingestion of the intermediate host. Ingestion is the main route of infection, although transmission may also occur transplacentally (Frenkel, 1988).

There is still some uncertainty as to the exact manner in which dolphins may be exposed to the infective oocysts, given that dolphins mainly eat fish and marine invertebrates, and that *T. gondii* mainly infect warm blooded animals. Marine environmental contamination with feline faeces, either directly from feral cats or indirectly via river run-off, resulting in oocysts ingested by dolphins during feeding, has been postulated (Miller, *et al*., 2002; Jones and Dubey, 2010). It has also been suggested that dolphins may occasionally ingest intermediate hosts such as birds or rodents containing oocysts (Bowater, *et al*., 2003; Dubey, *et al*., 2003; Di Guardo, *et al*., 2010). Furthermore, it appears that molluscs and crustaceans may act as mechanical vectors, being able to remove oocysts from sea water, while maintaining their infectivity, and so transmit toxoplasmosis to marine mammals that prey on these molluscs and crustaceans (Lindsay, *et al*., 2001; Lindsay, *et al*., 2004).

The potential of *T. gondii* to affect dolphin populations has not yet been investigated, although it has been implicated in the slow population recovery rate of other marine mammal species such as the southern sea otter (Van Bressem, *et al*., 2009a). Furthermore, possible reactivation of latent *T. gondii* infection during concurrent disease, e.g. due to morbillivirus infection, may act synergistically to increase disease severity and mortality (Van Bressem, *et al*., 2009a). Environmental toxins that cause immunocompromise may also give rise to higher mortality rates owing to *T. gondii* infection and therefore also negatively impact populations (Van Bressem, *et al*., 2009a; Di Guardo, *et al*., 2010).

5.4.2. Sarcocystosis and Neosporosis

Protozoa belonging to the genera *Sarcocystis* and *Neospora* have been found in various marine mammals, including cetaceans (Daily and Stroud, 1978; Munday, *et al*., 1978; Resendes, *et al*., 2002; Dubey, *et al*., 2003; Raga, *et al*., 2009). Some uncertainty still exists as to the exact nature of the life cycle of these protozoan species. In terrestrial animals *Sarcocystis* species usually have a two-host predator-prey life cycle: an asexual stage forming tissue cysts is usually found in the prey species, with a sexual stage found in

carnivores (Daily and Stroud, 1978; Resendes, *et al*., 2002; Dubey, *et al*., 2003; Raga, *et al*., 2009).

Thick or thin walled tissue cysts, filled with multiple small zoites, are usually found in muscle without any inflammatory reaction, and are usually considered as incidental findings (Munday, *et al*., 1978; Dubey, *et al*., 2003). The only case of fatal *Sarcocystis*-associated hepatitis, which precipitated multiple organ failure, had mixed cellular infiltrate into the liver with a large number of protozoa schizonts, and was documented in *S. coeruleoalba* (Resendes, *et al*., 2002).

A high prevalence of *Neospora caninum* antibodies has been found in *T. truncatus* from Florida (Dubey, *et al*., 2003). Animals tested were considered clinically healthy and were part of a capture-release project on resident dolphins, further suggesting that some protozoa species may have little or no effect on the animal (Dubey, *et al*., 2003; Raga, *et al*., 2009).

Sarcocystis neurona has not been reported in dolphins (Dubey, *et al*., 2003), but has been documented to cause encephalitis in *Phoca vitulina richardsi* (Pacific harbour seal) (Lapointe, *et al*., 1998) and *Enchydra lutris nereis* (Southern sea otter) (Lindsay, *et al*., 2000; Thomas, *et al*., 2007). Marine mammals are considered to be aberrant hosts as only schizonts are found in these hosts and these are only found in the central nervous system (Resendes, *et al*., 2002), No serologic surveys are available for *S. neurona* in marine mammals (Dubey, *et al*., 2003).

5.4.3. Helminthiasis

The helminths that affect marine mammals, and in particular dolphins, are diverse, with many genera and species having been documented. The following is a concise summary of these parasites.

Three families of Trematodes (flukes), Brachycladiidae (formerly Campulidae), Pholeteridae and Brauninidae typically occur in dolphins (Geraci and St. Aubin, 1987; Cribb, 1998; Aznar, *et al*., 2006; Raga, *et al*., 2009). The family Brachycladiidae is believed to be the most diverse and cover the widest geographical range, containing 41 species, 35 of which are found in most families of cetaceans. Species of *Campula*, *Oschmarinella*, and *Brachycladium* (formerly *Zalophotrema*) live in the hepatic and pancreatic ducts; *Hadwenius* in the intestine; *Nasitrema* in the sinuses; and *Hunterotrema* in the lungs (Geraci and St. Aubin, 1987; Raga, *et al*., 2009). Their life cycles are not known, although fish and squid species, which have been found to harbour immature stages, may act as intermediate or paratenic hosts, and infect the dolphin when preyed upon (Raga, *et al*., 2009). *Pholeter*

gastrophilus (Pholeteridae) is found in many dolphin species throughout the world. They typically live in the wall of the glandular stomach, less often the duodenum, and usually cause the formation of a fibrous nodule and extensive fibrosis, with a small opening or tract to the lumen of the intestine, where eggs are released (Geraci and St. Aubin, 1987; Aznar, *et al*., 2006; Raga, *et al*., 2009). *Braunina cordiformis* (Brauninidae) is found attached to the mucosa of the stomach and the duodenal ampulla (Raga, *et al*., 2009).

Adult and larval forms of Cestodes (tapeworms) are found in dolphins (Geraci and St. Aubin, 1987; Raga, *et al*., 2009). Adult forms belonging to the families Tetrabothriidae and Diphyllobothriidae are usually found in all parts of the intestine. Zooplanktonic crustaceans and euphasiids (krill) appear to be the first intermediate host of Tetrabothriids, with fish acting as paratenic or transport hosts, while Diphyllobothriidae use copepods and fish as intermediate hosts. The larval stages (plerocercoids and merocercoids) of the family Phyllobothriidae, which affect the digestive tract, blubber and mesenteries, occur in dolphins worldwide, although the adult stages are not known (Geraci and St. Aubin, 1987; Aznar, *et al*., 2006; Raga, *et al*., 2009).

The nematodes (roundworms) of the Anisakidae, belonging to the genera *Pseudoterranova*, *Contracaecum* and *Anisakis*, are the most commonly found nematodes in dolphins. They are mainly found in the forestomach, where the larvae attach to the stomach walls, often causing ulcers. The life cycle is well documented and illustrated in Figure 1.5 (Raga, *et al*., 2009).

Nematodes of the family Crassicaudidae live in the kidneys and urogenital tract, placenta, mammary glands, muscles and pterygoid sinuses, and may cause extensive damage. Life cycles are largely unknown (Geraci and St. Aubin, 1987; Duignan, 2003; Raga, *et al*., 2009). Pseudaliids from the genera *Pseudostenurus*, *Pharurus*, *Torynurus*, *Stenurus*, *Halocercus*, *Pseudalius*, and *Skrjabinalius* are found mainly in the lungs, air sinuses and heart. Indirect transmission is thought to be the main route of infection, although some evidence suggests prenatal transmission of *Halocercus* in some dolphin species (Geraci and St. Aubin, 1987; Duignan, 2003; Raga, *et al*., 2009; Fauquier, *et al*., 2010).

Acanthocephalans (family Polymorphidae), comprising the two genera *Bolbosoma* and *Corynosome,* are found in the intestine and stomach of marine mammals, with little or no effect on the host (Geraci and St. Aubin, 1987; Raga, *et al*., 2009). Euphasiids and copepods are thought to act as intermediate hosts, with fish as transport hosts.

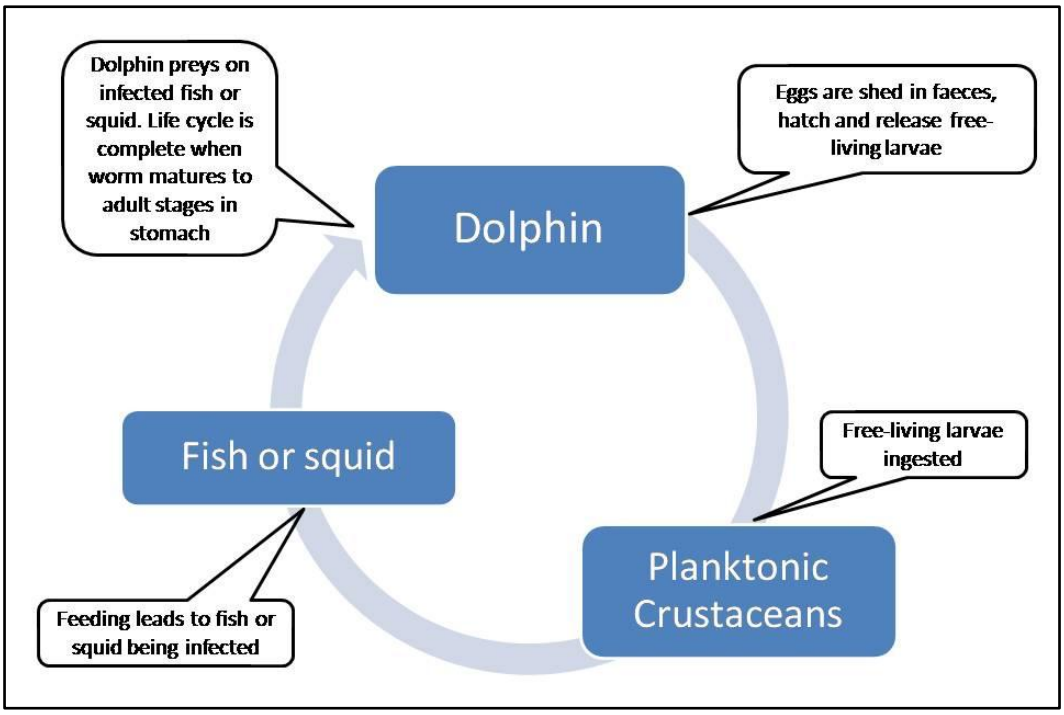

Figure 1.5. Life cycle of nematodes of the family Anisakidae.

5.4.4. External Parasites

Probably the most conspicuous external parasite of dolphins, that does not seem to have any effect on the host, is *Xenobalanus globicipitis*, a barnacle. This crustacean burrows deeply into the skin of dolphins, often along the trailing edge of fins and flukes (Geraci and St. Aubin, 1987). Smaller and less conspicuous crustaceans from the family Cyamidae can be found on the skin of dolphins, where they mostly feed on the epidermal tissue. They have a one-host life cycle and transmission is by direct contact (Geraci and St. Aubin, 1987; Raga, *et al*., 2009). Copepods of the genus *Pennella* (family Pennellidae) have successfully colonized cetaceans, through adult females burrowing into the definitive host and feeding on blood and body fluids (Raga, *et al*., 2009).

6. Non-Infectious Diseases of Dolphins

The effects of toxic substances, particularly those associated with harmful algal blooms, have become a major threat to human and ecosystem health and are a growing concern worldwide, causing mass mortalities in marine mammal communities (Flewelling, *et al*., 2005; Fire, *et al*., 2011). Very few reports of other non-infectious diseases in dolphins have been published, although persistent organohalogen compounds have been linked to increases in non-infectious diseases as well as increased susceptibility to infectious diseases (Bossart, 2011).

6.1. Toxins

A poison or toxic substance is any substance that, when ingested, inhaled, absorbed, applied to or injected, causes harm or a change in bodily function (O'Hara and O'Shea, 2001). The substances that have been shown to affect dolphins can be broadly classified as heavy metals (lead, mercury, cadmium), halogenated organics (pesticides such as DDT, polychlorinated biphenyls), biotoxins from harmful algal blooms (brevetoxins, domoic acid) and oils. Marine mammals are some of the most extensively surveyed species for contaminant levels, but very few controlled experiments have been conducted, making it difficult to interpret these survey results (O'Hara and O'Shea, 2001). Our understanding of chemical absorption, distribution, metabolism, and excretion is limited for most species. Often only a single measurement of residue levels is available, further hampering interpretation of possible effects (O'Hara and O'Shea, 2001). Few studies have proved pathology directly as result of contaminant levels, with most drawing conclusions based on correlations (O'Hara and O'Shea, 2001). Table 1.4 contains details of toxins and possible effects that have been found in dolphins.

Very high concentrations of toxic substances have been demonstrated in organs of marine mammals owing to persistence of these substances, such as halogenated organics and heavy metals, in prey fish species and subsequent bio-amplification in predator species (O'Hara and O'Shea, 2001; Duignan, 2003; Pierce, *et al*., 2008). Unfortunately, residue levels in target organs are often determined without concurrent data on histopathology or health status of the individual, limiting the ability to make conclusions regarding their effect (O'Hara and O'Shea, 2001; Duignan, 2003). Substances may accumulate in particular organs, for example lead in bone, mercury in liver and polychlorinated biphenyls in fatty tissue (blubber). In KwaZulu-Natal, the highest concentration of organochlorines in marine mammals evaluated in the area was found in *S. chinensis,* with *T. aduncus* showing concentrations twice that of other small cetaceans (Cockcroft, *et al*., 1989). Males accumulate toxins throughout life and females until sexual maturity, when levels rapidly drop as they pass materials to their offspring during pregnancy and lactation (Cockcroft, *et al*., 1989).

Dolphins appear to be very resistant to the toxic effects of some substances when compared to their terrestrial counterparts, especially in the case of heavy metals (mercury and cadmium), where very high concentrations have been recorded in dolphins without apparent pathology. The protective mechanisms are still poorly understood, with protein-binding and other chemical interactions having been proposed, such as selenium guarding against mercury toxicity (Koeman, *et al*., 1975). Dolphins are also quite resistant to the effects of oil

spills, often physically avoiding the area if possible. If dolphins do come into direct contact with oil, there seem to be minimal effects on the body, attributed to their thick epidermis (Koeman, *et al*., 1975; O'Hara and O'Shea, 2001).

Whales, porpoises, otters and mink have been shown to be sensitive to the effects of halogenated organics, with adverse effects arguably linked to an increase in susceptibility to infectious diseases (Ross, 2002; Jepson, *et al*., 2005a), immunosuppression (De Guise, 1998; Colborn and Smolen, 2003; Mori, *et al*., 2008), reproductive impairment (Colborn and Smolen, 2003; Jepson, *et al*., 2005a), endocrine disruption (Tanabe, 2002) and neoplasia (Colborn and Smolen, 2003; Newman and Smith, 2006). Data for dolphins are not available (Pierce, *et al*., 2008), which highlights the need for continued research into the effects that these chemicals may have on dolphins.

Biotoxins such as brevetoxins and domoic acid have recently emerged as the possible cause of mass strandings and mortalities in *T. truncatus* and *S. coeruleoalba* (Flewelling, *et al*., 2005; Riva, *et al*., 2009; Fire, *et al*., 2011). However, definitive evidence of biotoxins as the sole aetiological agent of disease has been scarce (Bossart, 2011; McHuron, *et al*., 2013). Brevetoxins are potent marine neurotoxins produced by the dinoflagellate *Karenia brevis*. They are either directly inhaled as aerosols or ingested in food sources (Bossart, 2011). Domoic acid is a neurotoxin produced by diatoms of the genus *Pseudo-nitzschia* (Bossart, 2011). Where biotoxins have been implicated in mass mortalities of dolphins, acute death of dolphins rather than a chronic syndrome has been seen (Flewelling, *et al*., 2005). In addition, other pathological changes such as CNS lesions and degenerative cardiac changes have been found in other marine mammals (Zabka, *et al*., 2009; Bossart, 2011; McHuron, *et al*., 2013). Therefore, marine mammals, particularly coastal species that inhabit areas close to human settlement, are good sentinels for the for the public health and ecosystem effects of harmful algal blooms and heavy metals (Bossart, 2006; Lavery, *et al*., 2008; Woshner, *et al*., 2008).

6.2. Congenital and Developmental Diseases

Few cases of developmental abnormalities have thus far been reported (Powell, *et al*., 2009), comprising mainly foetal monsters and cardiac abnormalities, which include tetralogy of Fallot, ventricular septal defect, and patent *ductus arteriosus* (Gray and Conklin, 1974; Powell, *et al*., 2009). These usually result in stillborn calves or calves that die shortly after birth. Occurrence of such conditions is extremely important as some congenital abnormalities may be linked to environmental contaminants (Dabin, *et al*., 2004). Many metal and heavy metal compounds such as mercury, aluminium, cadmium, chromium,

cobalt, indium, nickel, platinum, tellurium, thallium, ytterbium and zinc salts have been shown to be teratogenic in experimental animals (Inowve, 1989). The causes of congenital cardiac abnormalities are still unknown (Gray and Conklin, 1974; Inowve, 1989; Dabin, *et al*., 2004; Powell, *et al*., 2009).

6.3. Metabolic Disorders

Capture myopathy is a metabolic disease that has been documented in many terrestrial wildlife species, and is associated with the stress of capture, transportation and restraint, resulting in extensive rhabdomyolysis (Herráez, *et al*., 2007). This causes death via several possible mechanisms, including heart failure, volume depletion, metabolic acidosis, hypocalcaemia, hyperphosphataemia, disseminated intravascular coagulation and, most frequently, acute renal failure as a result of acute tubular necrosis. Evidence of capture myopathy has been found in a stranded *S. coeruleoalba* with pathologic changes, including acute myocardial and skeletal muscle degeneration, contraction band necrosis, and acute tubular necrosis (Herráez, *et al*., 2007).

High endogenous catecholamine concentrations associated with acute and chronic stress have been associated with pathology in cetaceans, with all stranded cetaceans showing such cardiac changes (Turnbull and Cowan, 1998). These changes were attributed to endogenous catecholamines, and the authors suggested that the lesions were related to stress owing to stranding, injury or disease. Acute histopathological lesions consist of contraction band necrosis and degenerative changes of myocytes similar to those described for capture myopathy (Turnbull and Cowan, 1998). Chronic lesions include myocardial fibrosis and adrenal cortical hyperplasia (Turnbull and Cowan, 1998; Clark, *et al*., 2006). The exact pathophysiology resulting in the myocytic changes remains unclear, although it is thought that prolonged exposure to catecholamines results in a deficient energy supply to the myocytes, leading to necrosis and degeneration (Dhalla, *et al*., 1992). An increase in adrenal corticomedullary ratio was reported in chronically stressed dolphins, and the overall mass of the adrenals was doubled when compared to acutely stressed animals (Clark, *et al*., 2006). This is thought to be related to prolonged requirements for catecholamines during the stressful period, inducing hyperplasia of particularly the adrenalin producing cells on the corticomedullary margin.

6.4. Neoplasia

There are few reports of neoplasia in dolphins (Table 1.3). This may be as a result of many deaths in marine mammals not being observed, few systematic necropsies being performed on stranded cetaceans, or that many marine mammals die before they reach old age

(Newman and Smith, 2006). The majority of reported neoplasms occur in dolphins (Newman and Smith, 2006). Viral agents, such as papillomavirus, have been implicated in tumour development (Van Bressem, *et al*., 1996). Reports of environmental toxins associated with higher prevalence of neoplasia in certain populations highlight the role of marine mammals as sentinel species for environmental health, particularly in relation to environmental exposure to carcinogens (Van Bressem, *et al*., 1996; Newman and Smith, 2006).

Table 1.3. Neoplasia recorded in dolphins, arranged by organ (adapted from (Cornaglia, *et al*., 2000; Cowan and Tajima, 2006; Newman and Smith, 2006).

Organ	**Tumour**	***T. truncatus***	***D. delphis***	***S. coeruleoalba***	***L. obscurus***	***L. acutus***	***L. obliquidens***
Intestine	Leiomyoma					X	
Liver	Adenoma	X					
	Reticuloendotheliosis	X					
Pancreas	Adenocarcinoma	X					
Tongue	Squamous cell carcinoma	X					
	Fibropapilloma					X	
Gingiva	Squamous cell carcinoma	X					
	Fibroma						X
Pharynx	Fibropapilloma					X	
Lung	Squamous cell carcinoma	X					
Kidney	Adenoma	X					
	Teratoma						X

Table 1.3. (cont.)

Ovary	Dysgerminoma				X		
	Adenocarcinoma	X					
Uterus	Adenocarcinoma	X					
	Fibroleiomyoma				X		
Testes	Leydig cell tumour		X				
Penis	Fibropapilloma					X	
Adrenal	Teratoma						
	Adenoma	X				X	
Thyroid	Adenoma	X					
Heart	Leiomyoma	X					
Lymphatics	Lymphoma	X					X
	Myelogenous leukaemia			X			
	Eosinophilic leukaemia						X
Skin	Squamous cell carcinoma	X		X			X

6.5. Physical Trauma

Coastal dolphin species live in close association with humans and have increased risk of anthropogenic disturbances, particularly incidental capture in fishing gear and shark nets (Cockcroft and Ross, 1990a; Cockcroft, *et al.*, 1990; Kuiken, *et al.*, 1994; Krogh and Reid, 1996). Dolphins that accidentally die in fishing gear or shark nets die as a result of drowning, showing pathology consistent with 'atypical drowning lung' in humans and other terrestrial mammals (Knieriem and Hartmann, 2001). Additional lesions consistent with net capture

Table 1.4. Pollutants with associated syndromes and pathology affecting dolphins.

Chemical	Species	Organ/System	Pathology	References
Oil	Various	Skin, eye and mouth	Organ failure and death, but infrequently affects cetaceans	O'Hara and O'Shea, 2001
Lead	*T. truncatus*	Liver	Haemosiderosis and hepatic fatty degeneration	Shlosberg, *et al*., 1997
		Kidney	Vascular degeneration of cortical tubular epithelium, cortical haemosiderin deposits. Death.	
		Central nervous system	Intramyelinic vacuoles of the optic nerve axons, vascular congestion on meninges, vacuolation of cerebrum and cerebellum	
Mercury	*T. truncatus*	Liver	Lipofuscin-like pigment granules in hepatocytes, with central necrosis and lymphocytic infiltration. Increased susceptibility of parasitic disease.	Rawson, *et al*., 1993; Bennett, *et al*., 2001
Organochlorines	*T. truncatus*	Immune system	Reduced immune responses (*in vitro*)	Lahvis, *et al*., 1995
Polychlorinated biphenyls	*S. coeruleoalba*	Multisystemic	Interference with endocrine processes, reproduction and the immune system. Increased susceptibility to morbillivirus.	Aguilar and Borrell, 1994; Colborn and Smolen, 1996; Kannan, *et al*., 1997; Busbee, *et al*., 1999
Domoic acid	Various	Multisystemic	Mass stranding, mortality	Riva, *et al*., 2009; Fire, *et al*., 2011

include amputation of the fins, flippers or tail flukes; circumscribing skin abrasion on the beak, flippers or tail; multiple, parallel, evenly spaced incisions in the skin; an abdominal incision, made by personnel on fishing trawlers to cause the carcass to sink; and subcutaneous, intramuscular or sub-pleural haemorrhage and skull fractures that can occur as nets are hauled on board. Animals usually contain food remnants in the stomach, which may further support the conclusion that an animal that exhibits these changes was most likely caught while feeding, and were thus incidentally caught (Kuiken, *et al*., 1994; Kirkwood, *et al*., 1997).

Blunt force trauma originating from boats, either through direct impact or propeller strike injuries, include multiple parallel and symmetrical lacerations and chop wounds, cutaneous and subcutaneous haemorrhage, skeletal fractures, amputations, as well as severe injuries to major blood vessels, underlying organs and musculature (Byard, *et al*., 2012). Animals that survive propeller strike often succumb to secondary sepsis or starvation as a result of their inability to feed (Byard, *et al*., 2012). Malicious injury to dolphins have also been reported, ranging from stabbings to gunshot wounds (Gilbert, *et al*., 2000).

6.6. Miscellaneous Disorders

Many miscellaneous conditions have been reported (Table 1.5), particularly in health status evaluations, although very little information is generally given regarding the description, severity or aetiology.

7. Purpose of the Present Study

An anecdotal increase in the frequency of macropathological changes in dolphins incidentally in the shark nets off KwaZulu-Natal was first observed in 2008 (*Pers. com.*: S. Plön). Dolphins are apex predators and therefore are important in the marine ecosystem as they often bio-accumulate toxins and may be used as sentinel species. Very little is known on the diseases present in the dolphin populations off the KwaZulu-Natal coast, and the factors that might influence the occurrence or severity of these diseases. In addition, no information is available on the health status of these dolphins, with studies mainly concentrating on taxonomy, feeding ecology, population structure and toxicology (Cockcroft, *et al*., 1989; Cockcroft and Ross, 1990a; Cockcroft and Ross, 1990b; Karczmarski, *et al*., 1999; Karczmarski, *et al*., 2000; Venter, 2009; Kaiser, 2012). The aim of the present study was to collect pathological and microbiological data from the two coastal dolphin species most frequently incidentally caught in the shark nets, *T. aduncus* and *S. chinensis*, and to relate this information to species, age, sex location and nutritional condition. The resulting data will assist in documenting diseases present in the populations, estimating their

prevalence and possibly identifying risk factors, and will represent valuable baseline data for assessing the health status of these dolphin populations and monitoring health trends over time. In addition, we aimed to develop a standard dissecting and sampling technique, taking into consideration some aspects particular to South Africa. Such a protocol will encourage the performance of more complete health investigations, which will aid in expanding the current knowledge of diseases affecting the dolphin populations.

Table 1.5. Miscellaneous conditions reported in dolphins

Condition	**Reference**
Disseminated intravascular coagulation	Turnbull and Cowan, 1998
Osteoarthritis	Turnbull and Cowan, 1999b
Degenerative joint disease	Turnbull and Cowan, 1999b
Diskospondylitis	Alexander, *et al*., 1989
Amyloidosis	Turnbull and Cowan, 1998
Gastric impaction	Turnbull and Cowan, 1998; McFee and Lipscomb, 2009
Hydrocephalus	Turnbull and Cowan, 1998
Atherosclerosis	Turnbull and Cowan, 1998
Angiomatosis (lung and lymph nodes)	Turnbull and Cowan, 1999a
Congestive heart failure	Turnbull and Cowan, 1998
Renal calculi	McFee and Osborne, 2004
Vaginal calculus	McFee and Osborne, 2004
Enterolithiasis	Burdett and Osborne, 2010
Thyroidal cysts	Cowan and Tajima, 2006

Chapter 2. Standardized Necropsy Protocol for Small Cetaceans

1. Introduction: The Importance of a Standardized Dissection Technique for Cetaceans

Cetaceans are generally difficult to study due to their entirely aquatic lifestyle, resulting in a lack of knowledge about these animals, particularly with regard to diseases and health. Therefore, carcasses of either stranded or incidentally caught animals provide a unique opportunity to collect valuable data. These data may provide information on the life history and ecology of a species, as well as help elucidate individual and population health (Norris, 1961; Geraci and Lounsbury, 1993; Rowles, *et al*., 2001; Pugliares, *et al*., 2007). In countries with long coastlines, such as South Africa, many different people may be responsible for collecting data from these carcasses. A major difficulty in using data from a number of different people or groups is that the results are seldom directly comparable (Norris, 1961). Therefore all aspects of data collection can benefit from standardized collection protocols (Rowles, *et al*., 2001). This may lead to more comparable data, which may enable more productive, coordinated research to be carried out. However, it is expected that requirements may change over time as new evidence emerges, and protocols should be adaptable to such changing circumstances (Raverty and Gaydos, 2004).

Some protocols have been developed internationally to standardize data collection from dead marine mammals (Geraci and Lounsbury, 1993; Rowles, *et al*., 2001; Raverty and Gaydos, 2004; Pugliares, *et al*., 2007).These have been developed with specific factors in mind, such as personnel, training, equipment and other resources, and these factors are not necessarily similar to those found in South Africa. No cetacean disease investigations have been conducted in South Africa, with research focusing mainly on taxonomy, population structure and feeding ecology (Cockcroft and Ross, 1990a; Cockcroft and Ross, 1990b; Cockcroft, *et al*., 1990; Venter, 2009). This may be largely owing to limited resources and inadequately trained personnel. This results in a general lack of capacity in the country when it comes to detailed cetacean necropsies and sampling, which may lead to important diseases and trends remaining unobserved. A lack of facilities able to accommodate cetacean necropsies may also be a contributing factor to the low numbers of complete necropsies being performed. Furthermore, some of the South African coastline is very

remote and inaccessible for carcass recovery. South African coastal weather conditions are generally hot and humid, causing rapid tissue decomposition, which may further hamper disease investigations. These factors prompted the need for a specific, “hands-on” South African protocol to guide and assist inexperienced or untrained field personnel and thus help to increase the number of complete necropsies performed. The objective of this study was to develop a protocol providing guidelines for researchers and their assistants to complete a comprehensive necropsy for disease testing and health assessment on cetaceans in southern Africa, focusing on dissection technique and tissue sample collection, but including information on relevant legislation, local resources and health and safety considerations.

2. Materials and Methods

This protocol was developed as part of a health assessment of dolphins incidentally caught in the shark netting program of the KZNSB. Necropsies were performed on a total of 46 *Tursiops aduncus* (Indian Ocean bottlenose dolphin) and *Sousa chinensis* (Indo-Pacific humpback dolphin) from April 2010 to April 2012. Published necropsy protocols were consulted (Kuiken and Hartmann, 1991; Geraci and Lounsbury, 1993; Rowles, *et al*., 2001; Raverty and Gaydos, 2004; Pugliares, *et al*., 2007), and this necropsy protocol established, tested and finalised during serial dissections. The protocol for this study was approved by the Animal Use and Care Committee, University of Pretoria (V011/12).

3. Results

Detailed necropsy results are presented elsewhere (Chapter 3).

This necropsy protocol does not include response plans and operating procedures for live stranded animals. An example/outline of a necropsy report and checklist for samples accompany this protocol (3.5). A glossary of terms may be found in Appendix A.

3.1. Equipment List

The equipment required to complete a necropsy on cetaceans largely depends on the individual situation, the facilities available and the type of animal. Basic equipment should include:

- Standard necropsy instruments such as scalpel handles, scalpel blades, scissors, forceps, knives, knife sharpeners, rib cutter (pruning shears) and manual saws.
- String or twine for tying off the intestine.
- Sterile instruments for collection of samples for microbiology.

- Sample bottles of various sizes.
- 10% buffered formalin for collection of histopathological samples.
- 70% alcohol for parasite collection.
- Plastic zipper storage bags, e.g. Ziploc®, for microbiology samples.
- EDTA tube or syringe for sampling of any discharges.
- Labels.
- Permanent markers and pencils for labelling.
- Data sheets for recording findings.
- Protective clothing, including waterproof aprons, gloves, boots, overalls, protective eyewear and face masks.
- Garbage bags, disinfectant, paper towels and other clean-up equipment.
- Cooler box and ice blocks if a fridge or freezer is unavailable.
- Digital camera for photographic documentation of lesions and animals.
- First aid kit.

3.2. Health and Safety Aspects

3.2.1. Zoonoses and Personnel Health

Although few zoonoses have been associated with cetaceans to date, some of the agents that may cause zoonotic diseases may be unknown (Raverty and Gaydos, 2004; Waltzek, *et al*., 2012). Persons coming into close contact with live or dead cetaceans, such as marine mammal researchers, trainers, rehabilitators and veterinarians, have an increased risk of contracting a zoonotic disease (Waltzek, *et al*., 2012). For a recent review of zoonotic diseases in marine mammals and their symptoms in humans see Waltzek *et al.* (2012), from which those diseases proven to have been transmitted from dolphins to humans are summarized in Table 2.1. To date, zoonotic diseases such as brucellosis have not been identified in cetaceans off the South African coast, which may partly be due to the lack of disease investigation, as well as a general lack of awareness of zoonotic disease by medical doctors. Other agents, such as *Salmonella* spp. (Ridgeway, 1979), *Mycobacterium* spp., (Higgens, 2000) West Nile virus (St. Leger, *et al*., 2011), influenza viruses (Ridgeway, 1979)

and *Aspergillus* spp. (Higgens, 2000), amongst others, have been isolated from cetaceans and are also known zoonoses. Therefore, caution should be taken whenever handling any organic material and the apparent absence of a disease should not result in complacency when conducting necropsy examinations.

Safety precautions consist of basic hygiene and safety when using sharp objects and should include:

- Wearing of protective clothing, including gumboots, plastic aprons and gloves.
- Face masks and goggles should be worn when the reproductive tract, foetus or brain is handled due to the potential risk of brucellosis.
- Knives and other instruments should be sharp, so that excessive force is not required during dissection, as this may result in accidents.
- Disinfection of all equipment after each necropsy examination.
- Disposal of carcasses in a manner that is safe and approved by the relevant authorities.
- Safety precautions around fixatives such as formalin and alcohol, to prevent spillage, splashing, inhalation and fires.
- Eating, drinking, smoking, and using a cell phone during a necropsy should be avoided, and persons performing the necropsy should thoroughly wash their hands and any other body parts that may have come into contact with the carcass, tissue or body fluids, before doing any of these things.

People that exhibit any symptoms potentially caused by a zoonotic disease, or noted after contact with a marine mammal, should seek immediate medical attention from a registered medical doctor.

3.2.2. Carcass Disposal

Carcass disposal should occur according to approved waste disposal practices to minimize the possibility of the spreading of diseases and environmental contamination. If animals have been stranded and have not been incidentally caught, extra caution should be taken as stranded animals may have washed ashore owing to disease. In South Africa, local municipalities have been mandated by the Constitution of the Republic of South Africa

Table 2.1. List of the most important zoonotic diseases associated with dolphins (from Waltzek *et al*., 2011)

Disease	Aetiology	Symptoms in humans
Brucellosis	*Brucella* spp.	Influenza-like symptoms, arthritis and fatigue, with rare neurological disease
Erysipeloid	*Erysipelothrix rhusiopathiae*	Localized skin infections, rarely prolonged malaise and life-threatening toxaemia
Calicivirus infection	Calicivirus	Influenza-like symptoms, rarely hepatitis
Blastomycosis	*Ajellomyces dermatitidis*	Cellulitis and lymphadenitis
Lobomycosis	*Lacazia loboi*	Localized skin infection

(No 108 of 1996) to clean public places in their municipal area of jurisdiction, including the disposal of marine mammal carcasses. As these strategies differ between local municipalities, they should be contacted for more specific information. It is also generally accepted that in remote places, where disease dissemination is unlikely to occur, carcasses may be buried on the beach or towed out to sea.

3.2.3. Legislation

The dissection, handling and research of marine mammals is controlled by the Marine Living Resources Act (Act 18 of 1998), which states that permits are required for these actions on any marine mammal, dead or alive. These permits are issued by the Department of Environmental Affairs, Branch: Oceans and Coasts (http://www.environment.gov.za/).

If a live stranded cetacean is found on the beach, a regulatory body should be contacted as soon as possible to respond. As stranded animals may be carrying diseases, it is important not to touch or interact with the cetacean. One of the following organizations may be contacted, depending on the location.

KwaZulu-Natal:

- KwaZulu-Natal Sharks Board: 031 566 0400

- Ezemvelo KZN Wildlife: 033 845 1002

Eastern Cape:

- Bayworld: 071 724 2122 or 041 584 0650

Western Cape:

- Department of Environmental Affairs: Oceans and Coasts: 021 402 3173
- Mammal Research Institute, Whale Unit: 082 570 8212 or 082 746 5579
- Dyer Island Conservation Trust: 082 907 5607

In terms of the Animal Health Act (Act 7 of 2002), brucellosis in any species is a controlled disease, as is any disease that has not previously occurred in the Republic of South Africa. Therefore, if any person should find any disease that has not previously been described or found in cetaceans in South Africa, or any known state controlled disease, (such as brucellosis), this should promptly be reported to a provincial or national state veterinary department, a list of which may be found at http://www.daff.gov.za/. Details of the relevant legislation mentioned may be found at www.info.gov.za/documents.

3.2.4. Carcass Preparation and Defrosting

Owing to a number of factors such as difficult access to animals, lack of trained personnel, and limited funding, stranded or incidentally caught cetaceans are often frozen before necropsies are performed (Siebert, *et al*., 2001; Siebert, *et al*., 2006). However, freezing commonly causes artefacts that may hinder histological interpretation of lesions and additional further decay (autolysis and putrefaction) may occur during the thawing process (Roe, *et al*., 2012b). To gain the most insight from the examination of carcasses, the following guidelines were developed during the present project, taking South African weather conditions into account:

- If a carcass cannot be immediately examined, it should be frozen as quickly as possible after death to delay autolysis and putrefaction.
- Carcasses should be defrosted slowly, indoors (in a cool place or the cool room of a freezer) or out of direct sunlight.
- On average, an adult dolphin (over 230 cm long or weighing more than 140 kg) takes three days to defrost, while a smaller animal takes two days, at an environmental temperature of 25°C.

3.3. General Comments

Before starting the necropsy, the collection of some general information on the carcass is necessary to obtain as much information as possible, and to ensure that usable samples are obtained. This section lists the type of information and measurements that should be collected, as well as sampling techniques, and should be referred to throughout the necropsy procedure.

3.3.1. Relevant History

Histopathological interpretation can be facilitated by knowledge of the circumstances surrounding the animal's death. This may be related to any recent unusual weather events or circumstances (such as storms, red tides or chemical spills) in the area where the animal was found and, in the case of a live stranding, any clinical signs exhibited by the animal prior to its death (such as uncoordinated behaviour, heavy breathing or muscle tremors), any treatments given (such as specific drugs and when they were given), time of death, and cause of death if known (such as euthanased or incidentally caught). The geographic location of the animal is also important and should be added to the history, including the geographic coordinates if available. A section is provided in the necropsy report (3.5) for the history.

3.3.2. Morphometric Measurements

Morphometric data are important for a range of biological investigations, such as age, growth rate and reproductive status determination, investigation of disease processes, and taxonomy. To ensure that the data are comparable to other studies, it is extremely important that measurements are consistently taken in the same way. The total body length (tip of upper jaw to insert of tail; measurement 1 on Figure 2.1) is important as it may be used to estimate age (Norris, 1961; Pugliares, *et al*., 2007). General guidelines, from Norris (1961) and Pugliares *et al*. (2007), for measurements include:

- All measurements, except for girths, should be straight measurements parallel to the long axis of the body, and not following the contours of the body.
- A measuring tape can be placed flat next to the animal, the starting end of the tape remaining at a constant position.
- For dolphins, straight lengths are measured from the tip of the upper jaw (maxilla).
- All measurements should be in centimetres.

A full list of measurements for morphometric studies (to be used in conjunction with Figure 2.1) can be found in Appendix B.

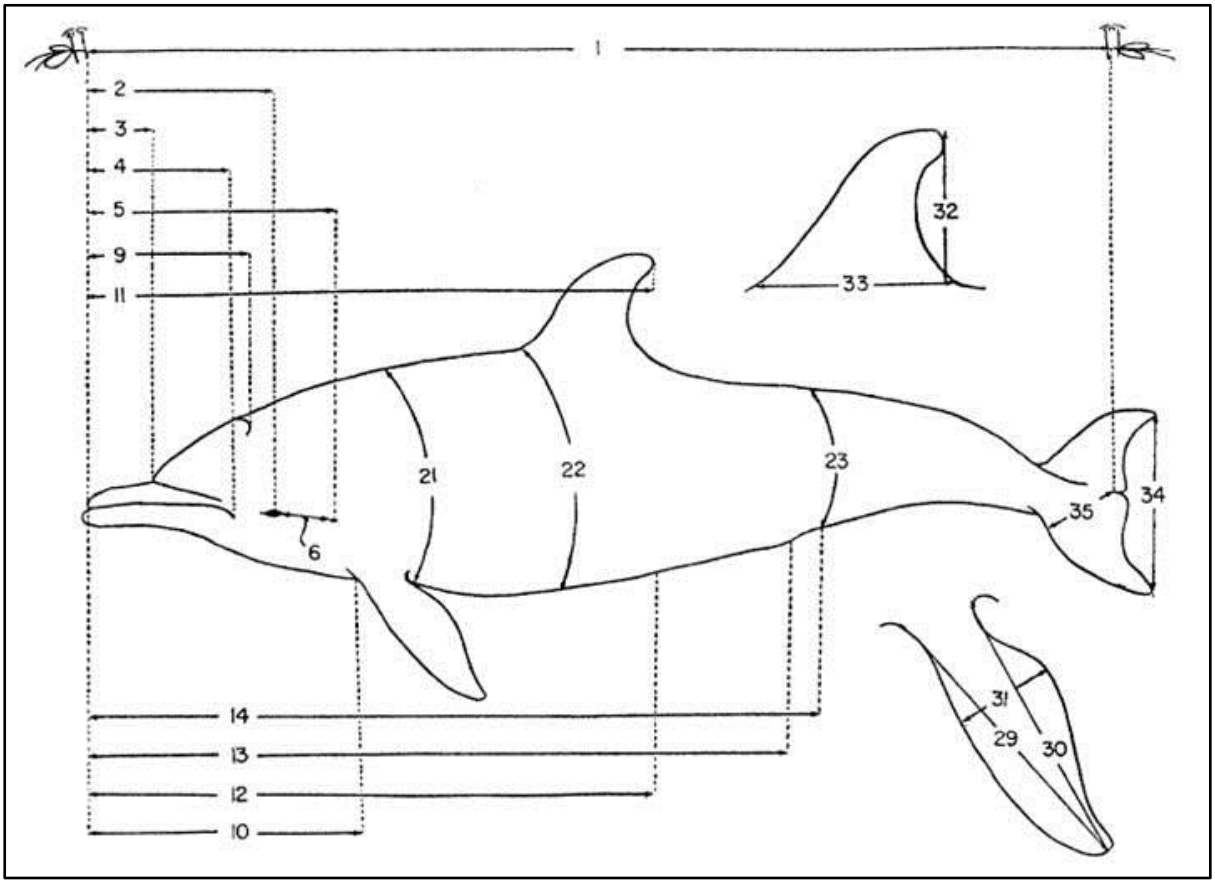

Figure 2.1. Standard measurements for morphometric studies as described by Norris (1961). For approximate age estimation only measurement 1 is required - tip of upper jaw to insert of tail. Key: see Appendix B.

3.3.3. Photography

Photographs of animals, organs, and lesions are of great value to document findings and should accompany written descriptions of lesions. Some general guidelines for photography include:

- Photographs should be taken without the use of a flash, but with sufficient natural light to see all anatomical details.
- External photographs of the whole carcass should include both left and right lateral views (Figure 2.2).

- All lesions should be photographed prior to sampling.
- Photographs of lesions should contain a label with the identification of the animal that is being dissected, and a scale if one is available, otherwise a common object, e.g. matchbox, can be used to infer scale (Figure 2.2).

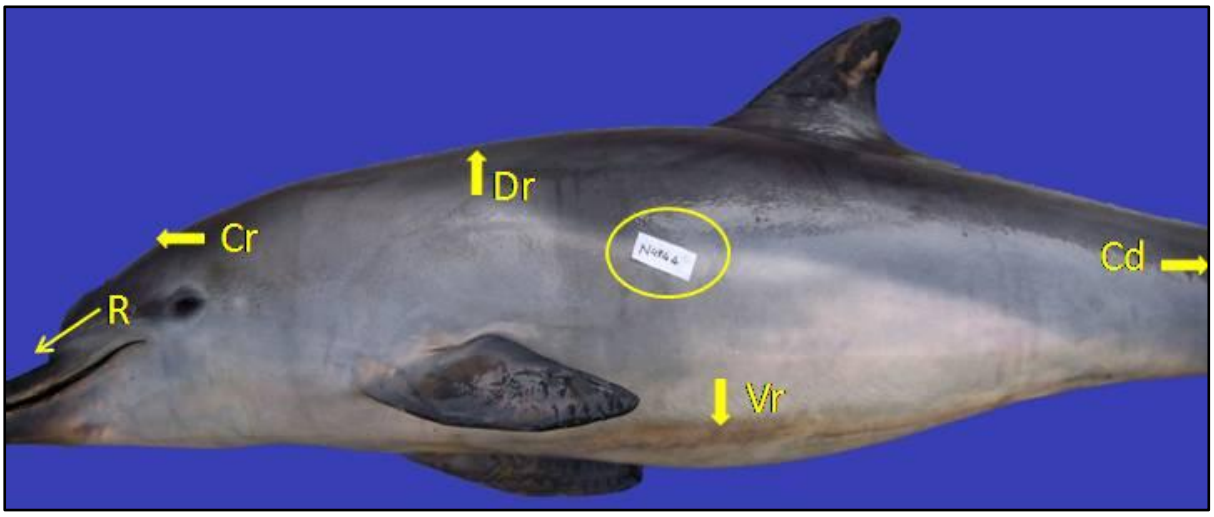

Figure 2.2. External photograph of dolphin lying on right side, with a label and scale identifying the animal (circled), and directional nomenclature indicated. Cranial (Cr): towards the head of the animal. Caudal (Cd): towards the tail of the animal. Dorsal (Dr): towards the top or back of the animal. Ventral (Vr): towards the belly of the animal. Rostral (R): Towards the tip of the beak.

3.3.4. Descriptions of Abnormalities

The most important aspect of macroscopic descriptions is to describe what is seen, without adding any interpretation or assumptions. Descriptions aid substantially in the later interpretation of histopathological results. Any description should include at least the following parameters where applicable.

- Location: exactly where the abnormality was seen and the organ affected.
- Number: single, many, approximate numbers or, in the case of liquids, the approximate volume in millilitres or litres.
- Size or extent: length x width x depth or area covered.

- Colour, consistency and opacity of liquids: thick or thin, liquid or mucoid, and cloudy or clear.
- Colour, consistency and texture of the affected tissue: firm, hard, soft, or fluctuant; gritty or rough (lesions should be sampled prior to being handled and palpated).
- Characteristics of the shape of an anomaly such as knobbly, finger-like, or wart-like.

There is no such thing as too much information; lesions and abnormalities should be described in as much detail as possible, using everyday English, and avoiding the use of jargon. If there is doubt whether something is truly abnormal, it is better to err on the side of caution and describe the possible abnormality. Examples of descriptions are given in Figure 2.3.

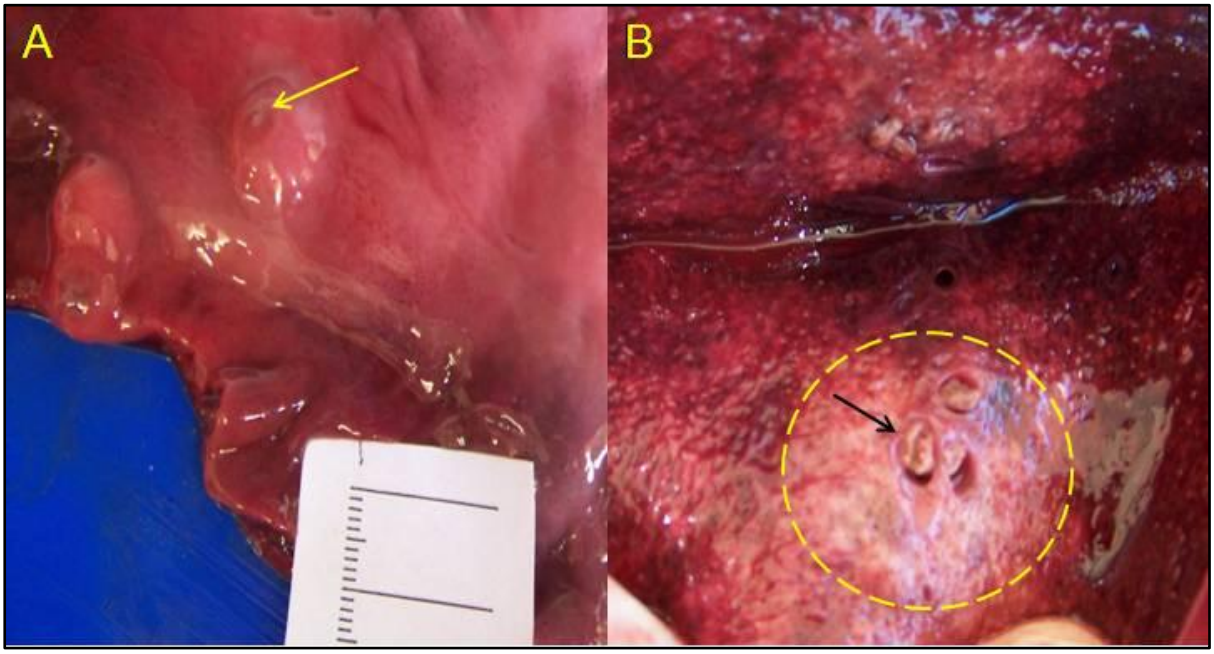

Figure 2.3. Examples of macroscopic descriptions. A: Pyloric stomach contains a small number of spherical, up to 0.7 cm diameter, firm, pink nodules extending into the lumen, with a small, *ca* 1 mm, pore (arrow) opening into the lumen. B. Lung contains a single, irregular, firm, white area (circled) around three bronchi that is filled with, and almost occluded by white, pasty material (black arrow). The rest of the lung is dark red.

3.3.5. Histopathology Sampling

- Macroscopically normal tissue should be sampled from all organs listed in the dissection guide and checklist (3.5).
- Standard samples should be taken from approximately the same area of each organ to aid in interpretations and so that samples from different cases may be more readily compared with each other.
- All samples should be less than 2 cm in diameter. If a large lesion is present, several representative samples from different portions of the lesion should be taken.
- Any lesion sampled should include macroscopically normal appearing tissue as well as abnormal tissue.
- All samples should be stored in 10% buffered formalin, with a 10:1 formalin to tissue ratio, especially for the brain, where a 10:1 formalin to tissue ratio is vital (Figure 2.4).
- All sample containers should be labelled with internal and external labels, clearly stating the identification number of the animal and, if possible, the sex, age, date of collection, and contents. Internal labels should be written in pencil, and placed inside the container. The exception being the microbiology samples (3.3.6), which should not be labelled internally as this will compromise the sterility of the sample.

Figure 2.4. Sample containers with 10% buffered formalin showing formalin:tissue ratio of 10:1.

3.3.6. Pathogen Sampling (Bacteria, Viruses, Fungi and Protozoa)

- Standard samples are listed in the checklist (3.5), and these should always be taken regardless of any visible pathology.
- Any lesion, over and above the standard samples (listed in Section 3.5), with a possible infectious aetiology should also be sampled for microbiology. If it is uncertain whether a lesion has a possible infectious aetiology, a sample for microbiology should be taken.
- Samples for microbiology should be taken with sterile instruments, and aseptic (sterile) technique should be observed.
- Equipment can be sterilised using formalin and air dried between samples being taken. This is done by dipping the instrument tip that has been used into 10% buffered formalin for a few seconds, and then allowing it to air dry in a well-ventilated area.
- Tissue samples should be placed in an empty Ziploc® bag and chilled as soon as possible.
- Fluid samples, such as discharges, should be placed in a serum (red top) blood collection tube or a sterile syringe.
- If no refrigeration facilities are available, the sample should be kept cool in a cooler box with cooler blocks or on ice.
- If bacteriological or fungal cultures will be carried out within 24 hours, the samples should be kept refrigerated until submission to the relevant laboratory. If processing will be delayed, the samples should be frozen (-25°C) as soon as possible after collection.
- All samples should be clearly labelled on the outside of the bag; for pathogen analysis no labels should be placed inside the bag as this will compromise the sterility of the sample.
- *Brucella* spp. have specific growth media requirements, and are very slow growing organisms. Therefore, if the cerebrospinal fluid and reproductive organ samples (including the placenta and the foetus) are sent to a laboratory for bacterial culture, a specific request for *Brucella* spp. culture should be made.

3.3.7. Parasitology Sampling

- Description of parasites, location, distribution, approximate number of parasites, and associated lesions should be described and recorded.
- Any parasites noted at any location should be collected in 70% ethanol (or propanol). An attempt should be made to collect as many parasites as possible.
- Samples from different locations should be kept separately, with the location clearly indicated on the label.

3.4. Necropsy Technique

3.4.1. External Measurements

Multiple measurements are routinely taken for morphometric studies by institutions such as the PEM. The length from the tip of upper jaw to insert of tail (measurement 1 on Figure 2.1) is necessary for age estimation (3.3.2.).

3.4.2. External Assessment and Sampling

- Weigh the animal if possible, and record the mass on the necropsy report.
- Determine the sex of the animal and record it on the necropsy report. In small cetacean females, the distance between the centre of the genital slit and the centre of the anus is less than 10 cm, and small mammary slits are seen on both sides, lateral to the genital slit. Small cetacean males have a distance greater than 10 cm between the centre of the genital slit and the anus, may have the penis protruding from the genital slit, and mammary slits are mostly absent, although some males may have mammary slits. Gender should be confirmed on internal examination during the necropsy (Pugliares, *et al*., 2007).
- Describe any scars, wounds, lesions or other irregularities on the outside of the animal (3.3.4).
- Describe and swab any discharge noted from orifices or external lesions for culture (3.3.4).
- Sample any external parasites that may be present for later identification (3.3.6.).
- Position the animal lying on the right hand side (right lateral recumbency).
- Take full blubber thickness measurements from the sites illustrated in Figure 2.5.

- In females, incise and evaluate the mammary glands, and sample for histopathology.
- Remove the left eye *in toto* for histopathology, with as much of the optic nerve attached as possible. Should the left eye be damaged, the right eye should be sampled instead. Using a needle and syringe, remove 0.25 ml aqueous humour (if possible) and replace with an equal volume of 10% buffered formalin to facilitate fixing.

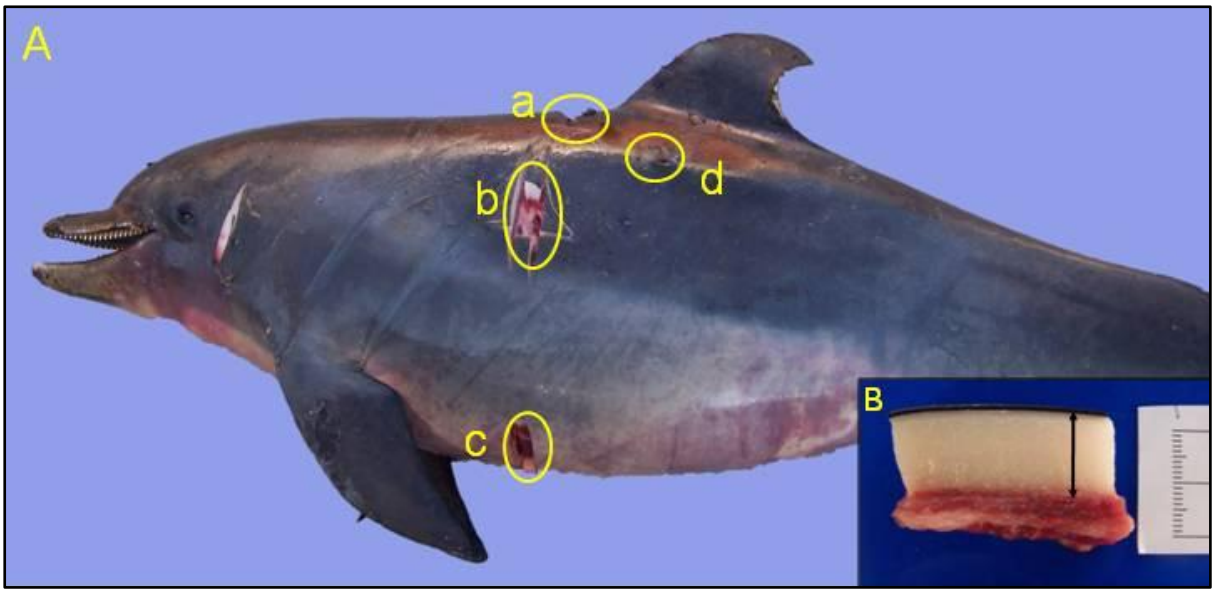

Figure 2.5. A: Location of blubber thickness measurements: (a) cranial to dorsal fin on the dorsal midline; (b) cranial to dorsal fin on the lateral midline; (c) cranial to dorsal fin on the ventral midline; (d) lateral to dorsal fin. Skin sample for histopathology is taken adjacent to site (d). B: Blubber thickness is measured from the below the skin (black) to the muscle (red). Sample the skin and full blubber thickness adjacent to the lateral blubber measurement site for histopathology (3.3.5). This location should be sampled each time as the histological appearance of the skin varies at different sites of the body.

3.4.3. Opening of the Carcass

- With the animal lying on its right side (in right lateral recumbency), make a roughly oval incision through the skin and blubber to the underlying muscle, extending from the angle of the jaw to the caudal limit of the anus, following the border of the spinal (hypaxial) muscles and the ventral midline (Figure 2.6). The lateral skin and blubber can then be divided into suitable portions and removed. Removing the left flipper and the scapula facilitates adequate exposure to the underlying thorax.

- Examine the blubber for parasites by making multiple incisions in the blubber on the sections that have been removed (Figure 2.7). Count or estimate the number of parasites found and collect as many as possible for identification. Sample any lesions for histopathology.

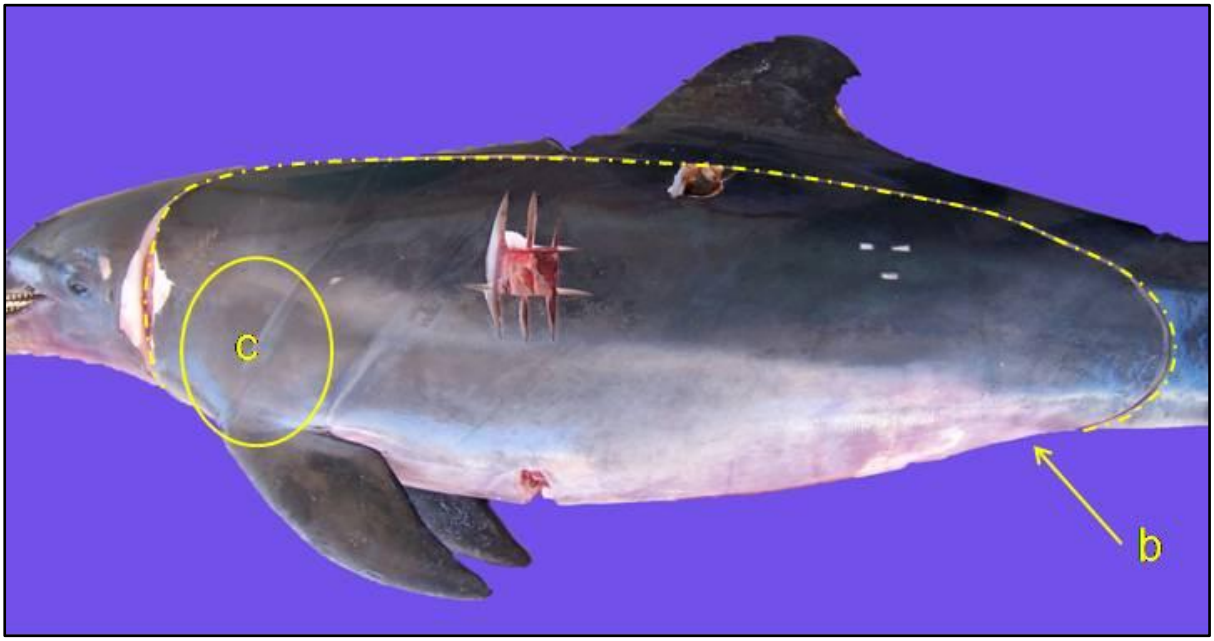

Figure 2.6. Dolphin lying on the right side. The yellow dashed line indicates the elliptical incision to follow when opening the carcass, extending from behind the head (a) (just cranial to the scapula (c)) to the caudal limit of the anus (b), following the border of the spinal muscles and the ventral midline.

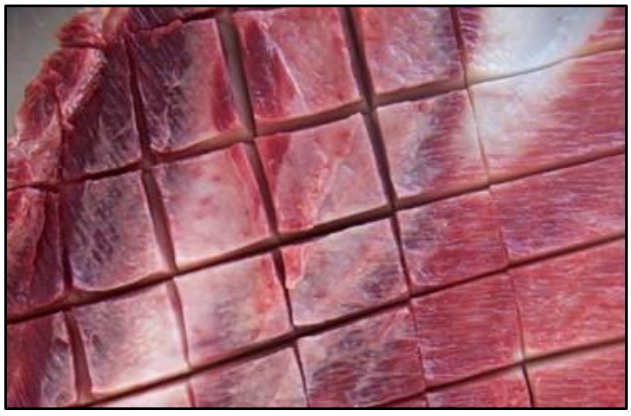

Figure 2.7. Multiple incisions are made into the blubber to evaluate for parasites.

- Evaluate muscles and subcutis for oedema, haemorrhage (bruising) or abscesses, by making multiple parallel incisions into the muscles exposed during the removal of the blubber.
- Sample dorso-lateral spinal muscle, caudo-lateral to dorsal fin for histopathology.
- Evaluate and sample the left cervical lymph node for histopathology (Figure 2.8).
- Incise the scapulo-humeral joint (shoulder) of the left flipper that was removed (see above) and evaluate for any abnormalities.

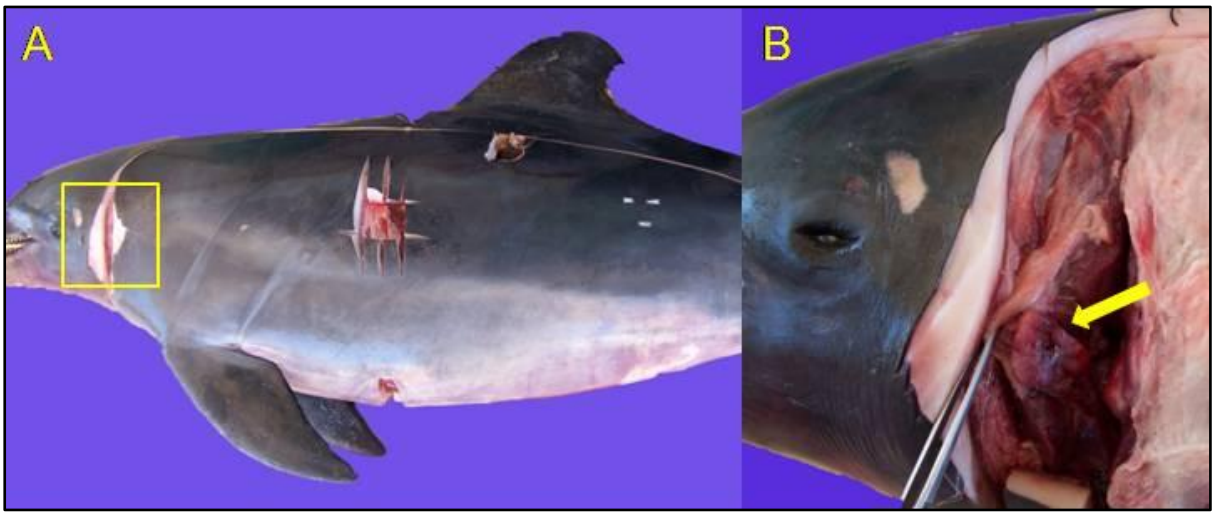

Figure 2.8. Location of the cervical lymph nodes. A: The cervical lymph nodes are located 6 – 10 cm cranial to the scapula (Cowan & Smith 1996), approximately at the level of the occipital joint (joint between the head and the first cervical vertebrae). B: Arrow indicates the appearance of the cervical lymph nodes.

- Expose the abdominal organs by making a Y-shaped incision in the body wall (Figure 2.9): make a stab incision just caudal to the last rib. Extend this dorsally up to the spinal muscles, and ventrally following the curve of the last rib. Make a cranio-caudal incision just below the spinal muscles, extending from the previous incision to just behind the anus. Care must be taken not to cut any viscera. The abdominal musculature can now be pulled down to expose the abdominal organs, and should not be cut along the ventral midline, as there is a risk of cutting the uterus.

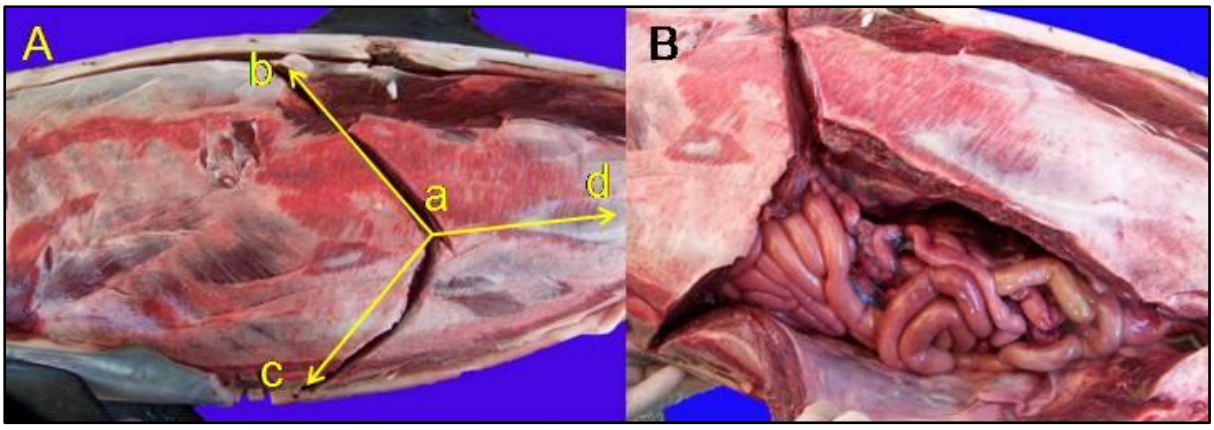

Figure 2.9. Incisions to open the abdomen. A: Make a stab incision at point (a). Extend the incision dorsally to point (b), and ventrally to point (c). The final incision is made from point (a), following the spinal muscles, cutting towards the tail to end with a Y-shaped incision (B).

- Puncture the diaphragm to evaluate the negative pressure in the thorax, by making a stab incision into the diaphragm and listening for a sudden intake of air. If there is no intake of air, there was no negative pressure in the thorax, and this should be recorded.
- Separate the ribs from the sternum (breast bone) and spine, and remove the thoracic (chest) wall. Evaluate the ribs for any lesions, which is facilitated by removing the muscle around the ribs that have been removed.
- Evaluate any fluid noted in the thoracic or abdominal cavity, and, if present, collect a sample in a serum tube or syringe for microbiology.
- Place at least five teeth from any part of the mandible for age determination in a separate, labelled vial.

3.4.4. Internal Examination

- Assess and record the stage of decomposition of the carcass, following published guidelines (Geraci and Lounsbury, 1993).
    - Code 1 – Live animal; no necropsy performed.
    - Code 2 – Carcass in good condition (fresh): normal appearance, fresh smell, minimal drying and wrinkling of skin, blubber firm and white, muscles firm and dark red, erythrocytes intact, little or no gas in intestines.

* Code 3 – Fair (decomposed, but organs basically intact): carcass intact, but bloated, skin sloughing, mild odour, mucous membranes dry, eyes sunken, blubber blood-tinged and oily, muscles soft and poorly defined, blood haemolysed, viscera soft and mottled, gut has some gas distension.

* Code 4 – Poor (advanced decomposition): carcass may be intact, but collapsing, skin sloughing, blubber soft and full of gas, muscles friable and easily torn, blood thin, viscera often identifiable, but friable and easily torn, gas filled intestines.

* Code 5 – Mummified or skeletal remains: skin draped over skeleton, indiscernible or desiccated tissues.

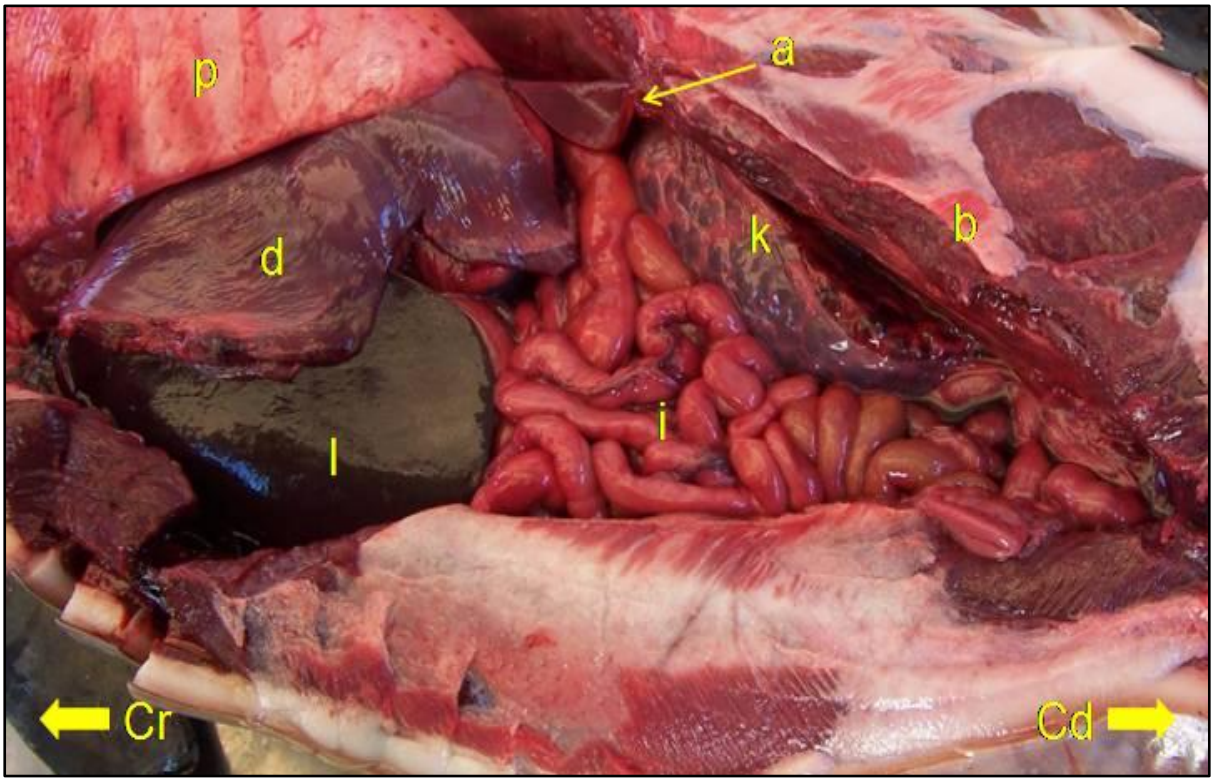

Figure 2.10. Abdominal topography. From cranial (Cr) to caudal (Cd) in the abdomen is the diaphragm (d), liver (l), intestinal loops (i) and kidney (k). (a) indicates the approximate position of the adrenal gland. The epaxial/spinal muscles that serve as guidance for the incision into the abdomen are indicated by (b). The caudal border of the left lung (p) can be seen cranially.

- Evaluate and sample any immediately visible abnormalities before handling any organs. Gently push away the intestine and locate the adrenal glands, ureters, urinary bladder, spleen, liver, reproductive tract and pancreas. For superficial topography of the abdomen see Figure 2.10. Move aside the lung to visualize the heart, bronchi and trachea (Figure 2.11). Also evaluate for any adhesions (either firm, pink or fine, soft, red abnormal attachments).

3.4.5. Abdominal Examination and Sampling

- Evaluate the diaphragm and sample for histopathology.
- Tie off the colon twice (double ligate), about 2 cm apart, as it approaches the anus and cut through the colon between the ligatures.
- Double ligate the small intestine as it exits the stomach and cut though the intestine between the ligatures.

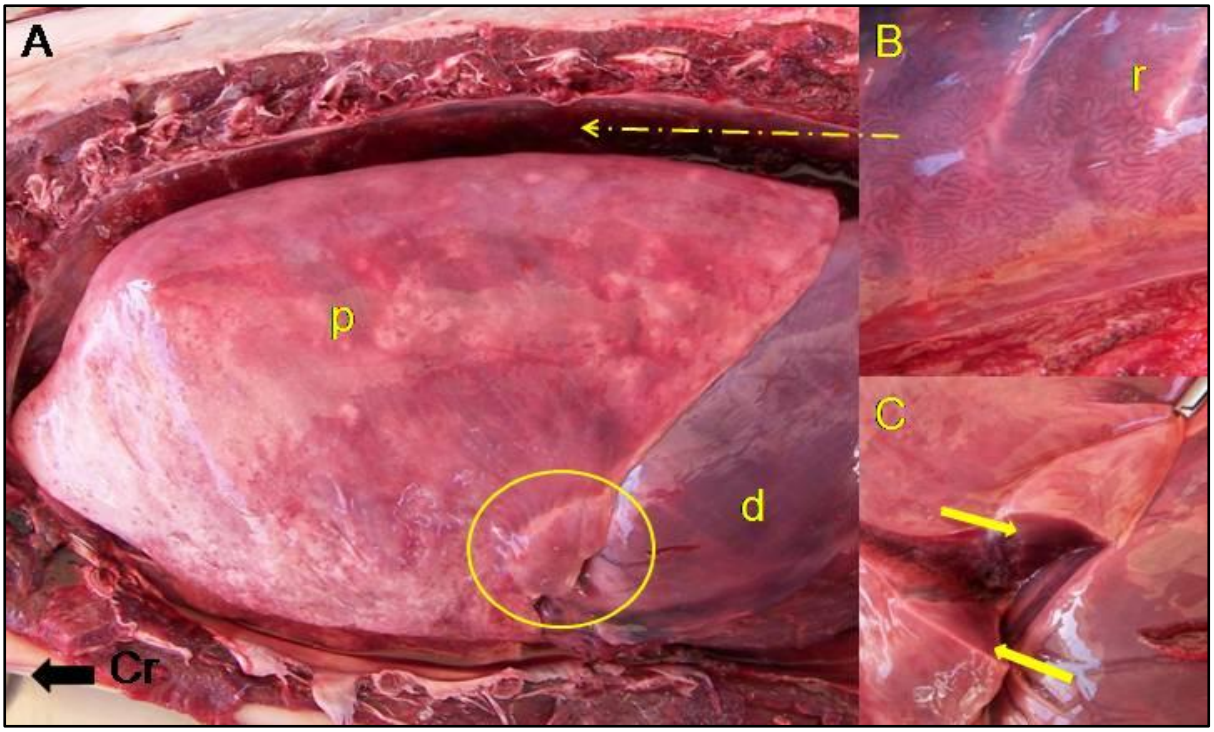

Figure 2.11. Thoracic topography. A: When the ribs are removed, the lungs (p) cover most of the thorax cranial (Cr) to the diaphragm (d). The marginal lymph node of the lung is circled, and the arrows indicate the cut surface (bottom right). B: The *rete mirabile* (r) is located mainly dorsally and dorso-laterally underneath the costal pleura. C: The marginal node of the lung on cut surface (arrows).

- Remove the intestines, starting at the colon, by cutting the membrane of the mesentery close to its intestinal attachment, thus leaving the mesentery attached to the body wall, and completely removing the intestines from the carcass. Handle the intestine by the mesenteric attachment and not by pulling directly on the intestine, to prevent damage to the tissue.

- Evaluate the intestine as follows:

    * Arrange the intestine in serial loops on a flat surface (Figure 2.12).

    * Sample one closed section from the cranial, middle and caudal parts of the intestine for histopathology and microbiology before opening the entire length.

    * Open the rest of the intestines, and evaluate and sample any parasites or abnormalities in the intestinal wall.

    * Intestinal contents should be collected in an empty sample bottle. One third of this should be frozen, and the rest diluted and preserved with 70% alcohol for parasite identification.

- Remove the mesentery with associated mesenteric lymph nodes (Figure 2.13). Evaluate and sample the lymph nodes for histopathology and microbiology. Place the lymph node on tissue paper and label the tissue paper with pencil, identifying the lymph node (i.e. mesenteric lymph node), before placing it and the tissue paper into the formalin.

- Remove the caudal oesophagus and stomach by cutting through their attachments, leaving the spleen and other organs in the abdominal cavity. Open the stomach and evaluate and sample any parasites. If the stomach contents are not processed immediately (which requires trained personnel), the contents should be kept frozen to prevent further digestion. All the stomach contents, and any contents that may be in the oesophagus, should be kept in a labelled container. Rinse the stomach wall and evaluate for ulcerations after taking a sample from each of the glandular, muscular, and pyloric sections for histopathology. In addition, describe and sample any lesions for histology. The relevant anatomy of the stomach is shown in Figure 2.14 (Harrison, *et al*., 1970).

- Evaluate and sample the liver, both left and right lobes, with bile ducts, for histopathology and microbiology.

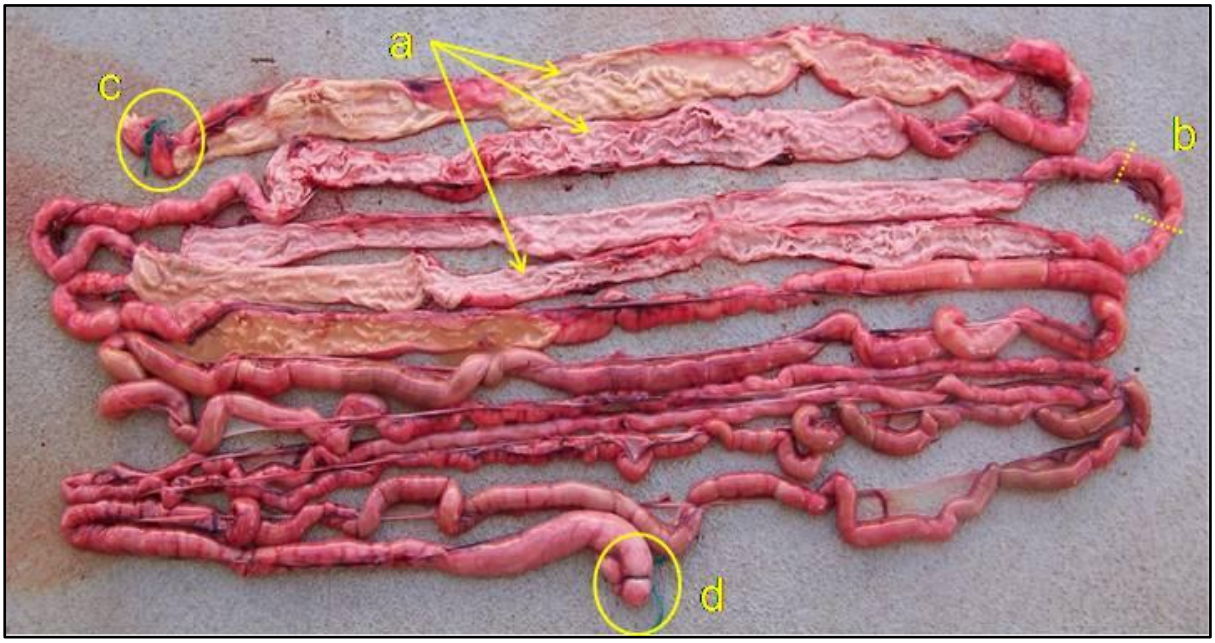

Figure 2.12: The entire intestinal tract from the duodenum (d) to the rectum (c) is arranged in parallel loops on a flat surface and then sampled by taking a closed section of intestine, as illustrated by (b), where two cuts are made along the dashed lines. The intestine is then opened (a) and evaluated. The ligatures on the colon (c) and duodenum (d) were placed when the intestine was removed to prevent faecal contamination of the carcass.

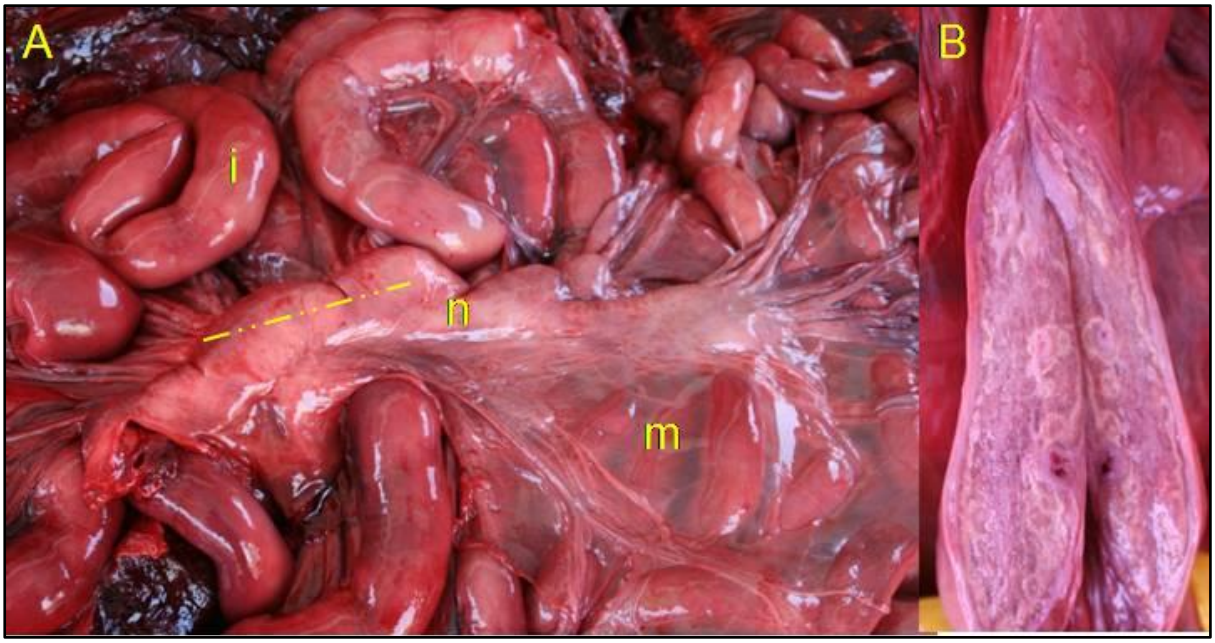

Figure 2.13. A: Mesenteric lymph nodes (n), situated in the mesentery (m) surrounded by intestine (i). B: Mesenteric lymph node on cut surface (along dashed line in A).

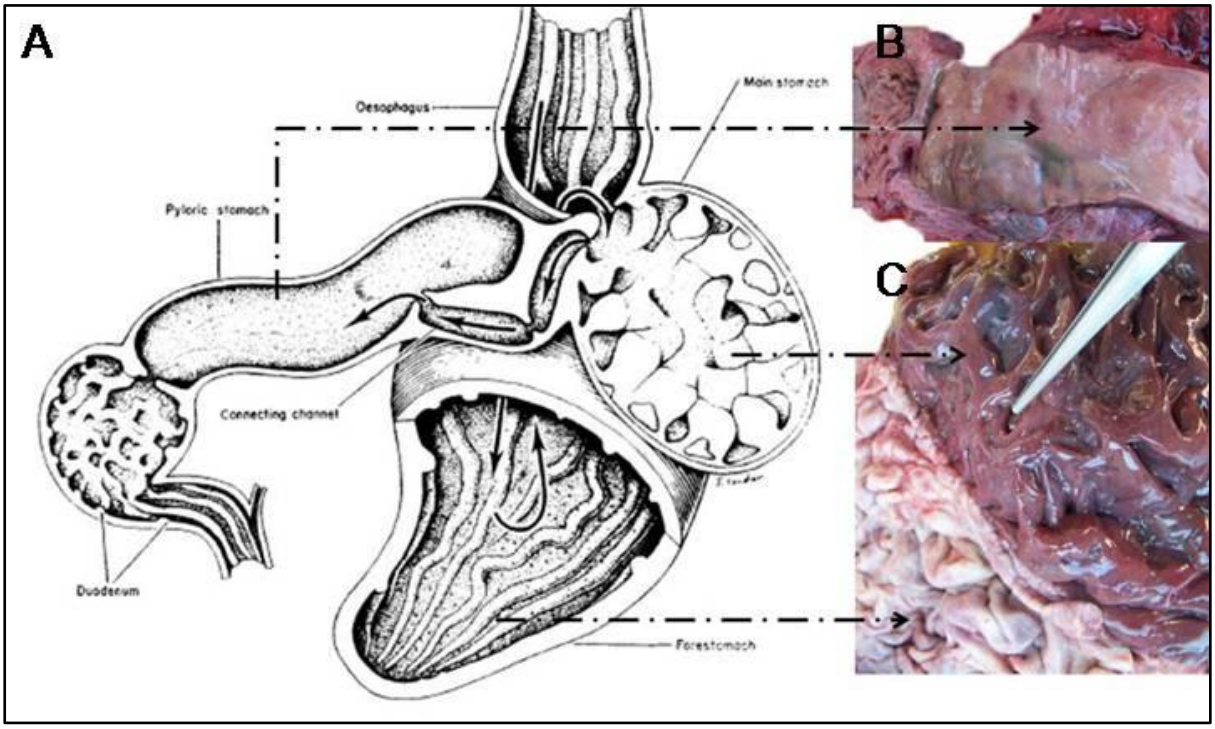

Figure 2.14. Anatomy of the dolphin stomach. A: (from Harrison, 1970) illustrates the different compartments of the dolphin stomach; the forestomach (muscular part), main stomach (glandular part), and pyloric or third compartments. B+C: The different appearances of the various compartments.

- Evaluate and sample the pancreas for histopathology. The pancreas can be found attached to the intestine as it exits the stomach. It is a light pink to tan organ, quite thin and elongated.

- Evaluate and sample the spleen for histopathology and microbiology. The spleen is typically very dark red to black in colour, approximately 5 cm in diameter. It is usually located just caudal to the stomach and liver. Dolphins usually only have one spleen, although accessory spleens are common (Cowan and Smith, 1999). The number, size and weight of the accessory spleen should be recorded on the necropsy reports, and the accessory spleens should also be sampled for histopathology.

- Evaluate and sample the adrenals, both left and right, for histopathology, including cortex and medulla (Figure 2.15). The adrenals are located just cranial to the kidneys, below the spine, and are relatively small (approximately 3 cm x 2 cm x 2 cm).

- Evaluate and sample the kidney, including the cortex, medulla and pelvis, both left and right, for histopathology and microbiology (Figure 2.15).

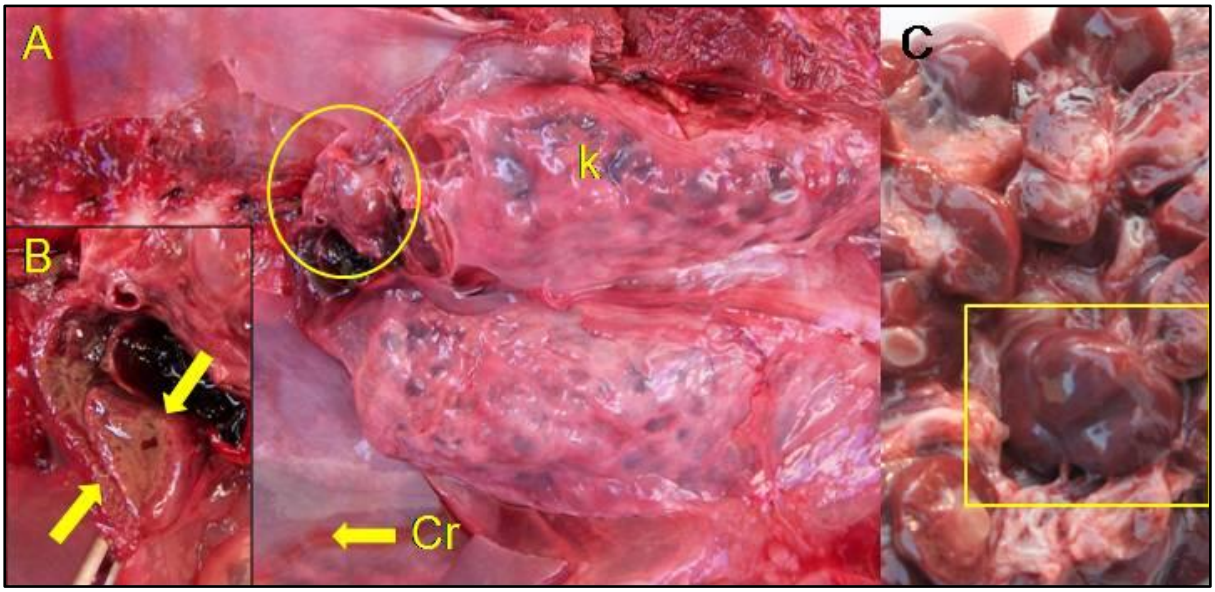

Figure 2.15. Location of the kidney and adrenal in a small cetacean. A: The adrenal glands (circled, arrows on B) are located just cranial to the kidneys (k). C: Sectioned kidney and multiple lobules (square). Pictures by David Zimmerman.

- Evaluate and sample the bladder wall for histopathology. Store urine, if present, in a sterile urine container or syringe. On fresh carcasses, a full urinalysis should be performed by trained personal either on site or in a laboratory, which consists of at least a dipstick evaluation, but should include a sediment and microbiological analysis if possible. If the urine cannot be examined on site, it may be refrigerated or stored on ice (with microbiological samples) before it is sent to the laboratory. Urine for microbiological analysis may be frozen. Urine from a previously frozen carcass may be used for microbiology, but not for dipstick and sediment analysis as freezing results in changes in parameters, rendering unreliable results.
- Remove, evaluate and sample the entire reproductive tract (Figure 2.16) as follows:
    - In juvenile animals, the reproductive tract is sampled *in toto* for later sub-sampling for histopathology.
    - In adult males both testes are kept whole if smaller than 2 cm in diameter and later sub-sampled for histopathology. If the testes are greater than 2 cm in

diameter, multiple incisions, approximately 2 cm apart, should be made to aid formalin penetration and fixation.

* In adult females the whole reproductive tract is kept in a separate sample bottle and preserved in 10% buffered formalin for reproductive studies. During sub-sampling a longitudinal section from both ovaries and transverse midsections from both uterine horns, and the body of the uterus should be sampled for routine histopathology (Figure 2.16).

* If a female is pregnant, particular attention should be paid that any investigators present wear protective clothing, as uncertainty as to the presence of *Brucella* spp. in dolphins in South Africa currently exists. The foetus should be dissected as for an adult, and the reproductive tract of the dam sampled *in toto* in the same manner as a non-gravid uterus.

* The placenta should also be evaluated and sampled for histopathology and microbiology.

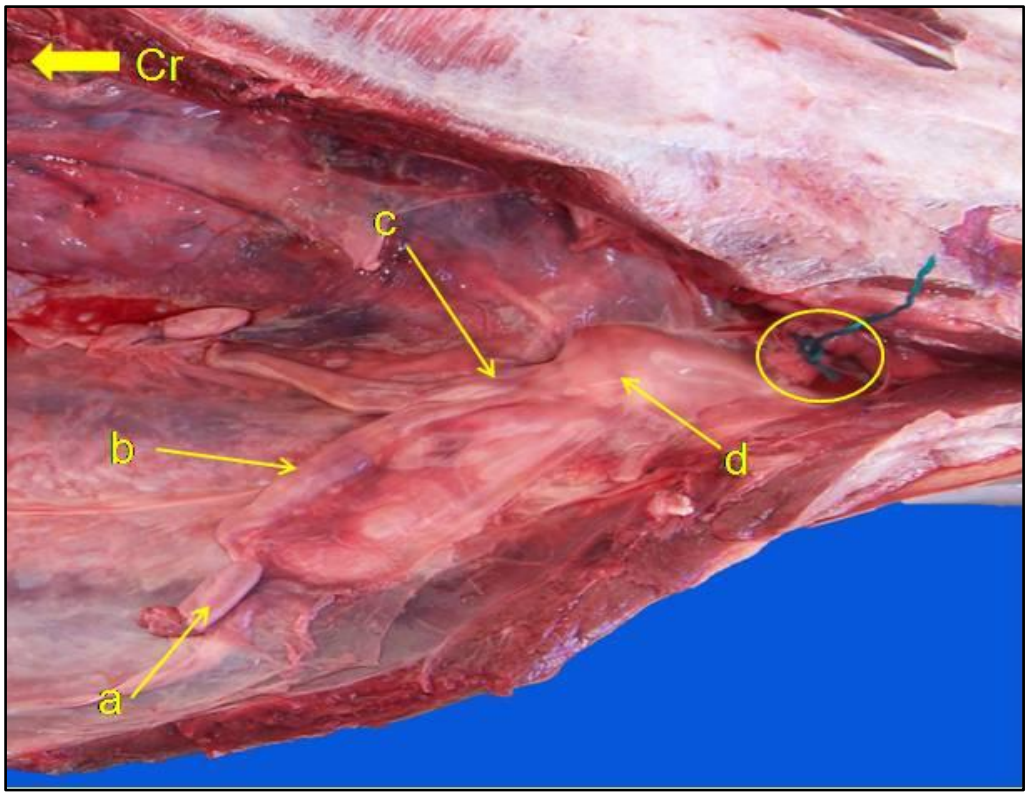

Figure 2.16. Female reproductive tract topography. The female reproductive tract in located on the ventral body wall with the ovary (a), uterine horn (b), uterine body (c) and the cervix (d). Circled is the tied off colon.

3.4.6. Thoracic Evaluation and Sampling

- To remove the pluck (heart, lungs and trachea), incise the blubber and skin on the medial aspect of both mandibles (between the lower jaw and the tongue), freeing the tongue. Extend the incisions caudally, cutting through the hyoid bones (just behind the tongue), and the pharynx that leads to the blowhole, ensuring that the larynx (goose beak) is removed together with the pluck. Free the heart, lungs, thymus and trachea all together from the thoracic cavity.
- Remove the head of one rib for histopathology.
- Evaluate and sample the *rete mirabile* (Figure 2.11).
- Evaluate the tongue and sample a section in the middle third of the tongue for histopathology (Figure 2.17).
- Open the larynx and the pharynx. Pay particular attention to the pharyngeal mucosa as the tonsils are imbedded in the mucosa (Figure 2.17). The tonsils or tonsillar area should be sampled for histopathology

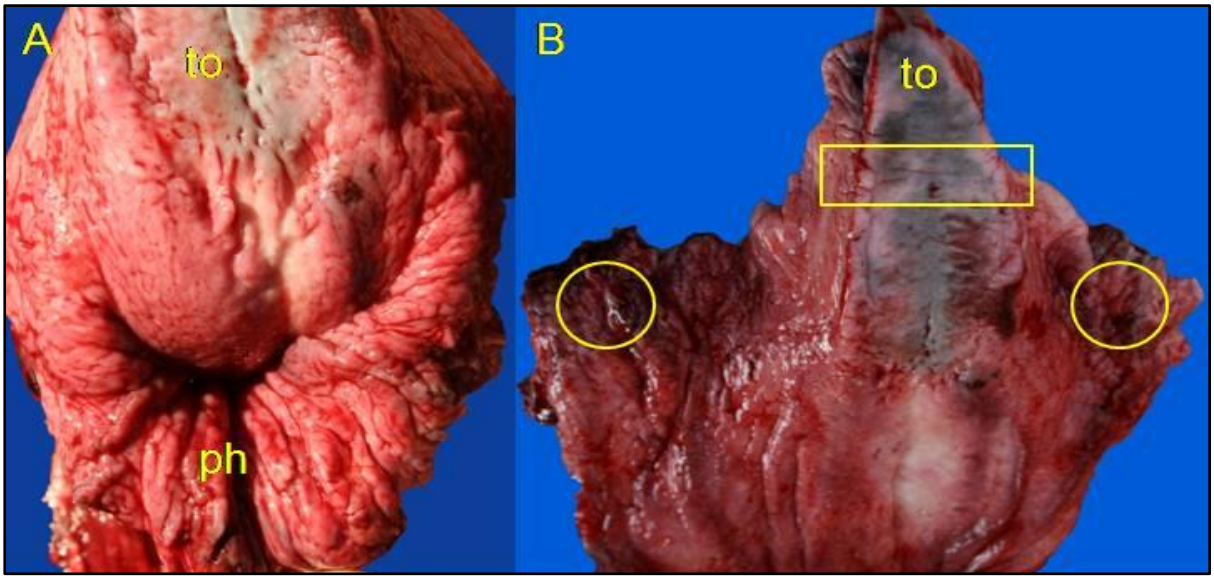

Figure 2.17. Anatomy of the pharynx and location of the tonsils. A: Pharynx (ph) before it is opened and the base of the tongue (to). B: Pharynx after it is opened containing the tonsillar area (circled). The tongue (to) sample for histopathology is taken from the middle third (square).

- Locate the thyroid which is situated on the ventral aspect of the trachea, just caudal to the larynx (goosebeak) and just cranial to the lung (Figure 2.18). Evaluate and sample for histopathology.

- If the animal is young, locate the thymus, which lies between the cranial edges of the lungs, just in front of the heart (Figure 2.18), evaluate and sample for histopathology. In adult animals the thymus may resemble fatty tissue, so tissue from just cranial to the heart may be sampled as this frequently contains thymic remnants. Involution (regression) of the thymus occurs over time, but the age and rate has not been defined in cetaceans (Cowan and Smith, 1999).

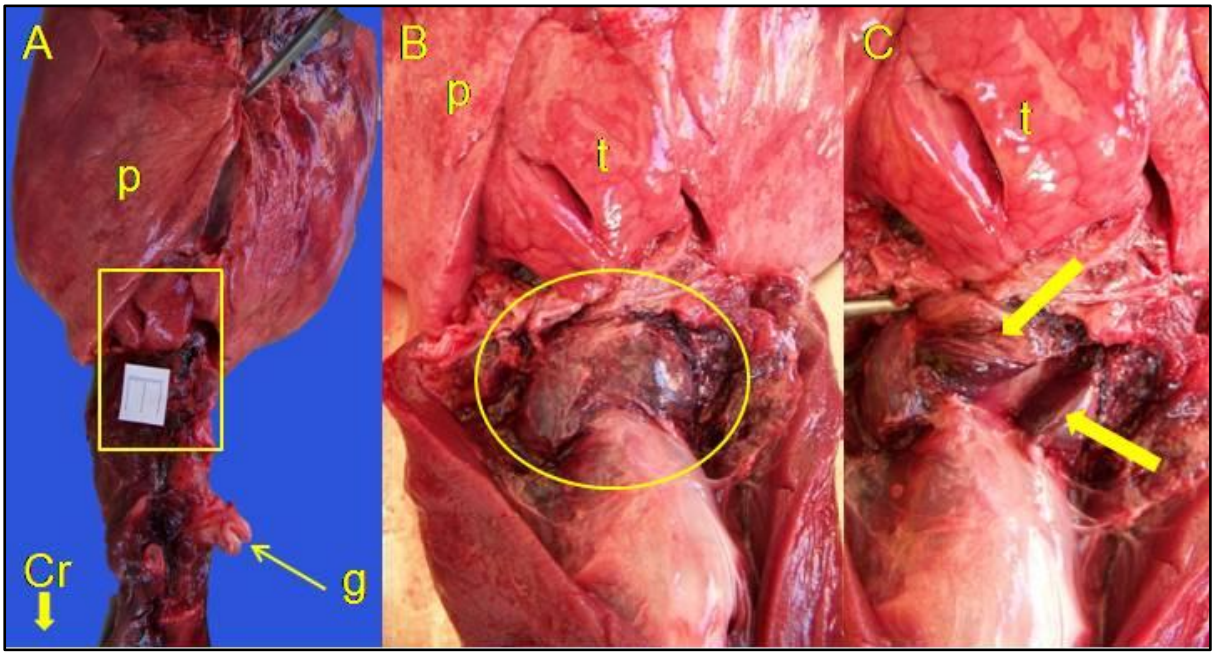

Figure 2.18. Location of the thymus (t) and thyroid (circled (B), and arrows (C)), both of which can be found cranial (Cr) to the lungs (p) and caudal to the goosebeak (larynx, g (A)) on the ventral side of the pluck (A).

- Evaluate and sample the following before opening the trachea and palpating the lungs for small lesions (Figure 2.19):
    - the cranial third of the trachea for histopathology; cranio-ventral lung lobe from both the left and right lungs for histopathology and bacteriology
    - dorsal lung lobe from both the left and right lungs for histopathology and parasites. As lung nodules may be small, the cuts should be approximately 1 cm apart.
- Open the trachea along its entire length and the bronchi as far as possible. Make multiple cuts in the lung and feel it carefully to evaluate for abnormalities
- Evaluate and sample the marginal lymph node of the lung that lies on the caudal edge of the lung (Figure 2.11) for histopathology.

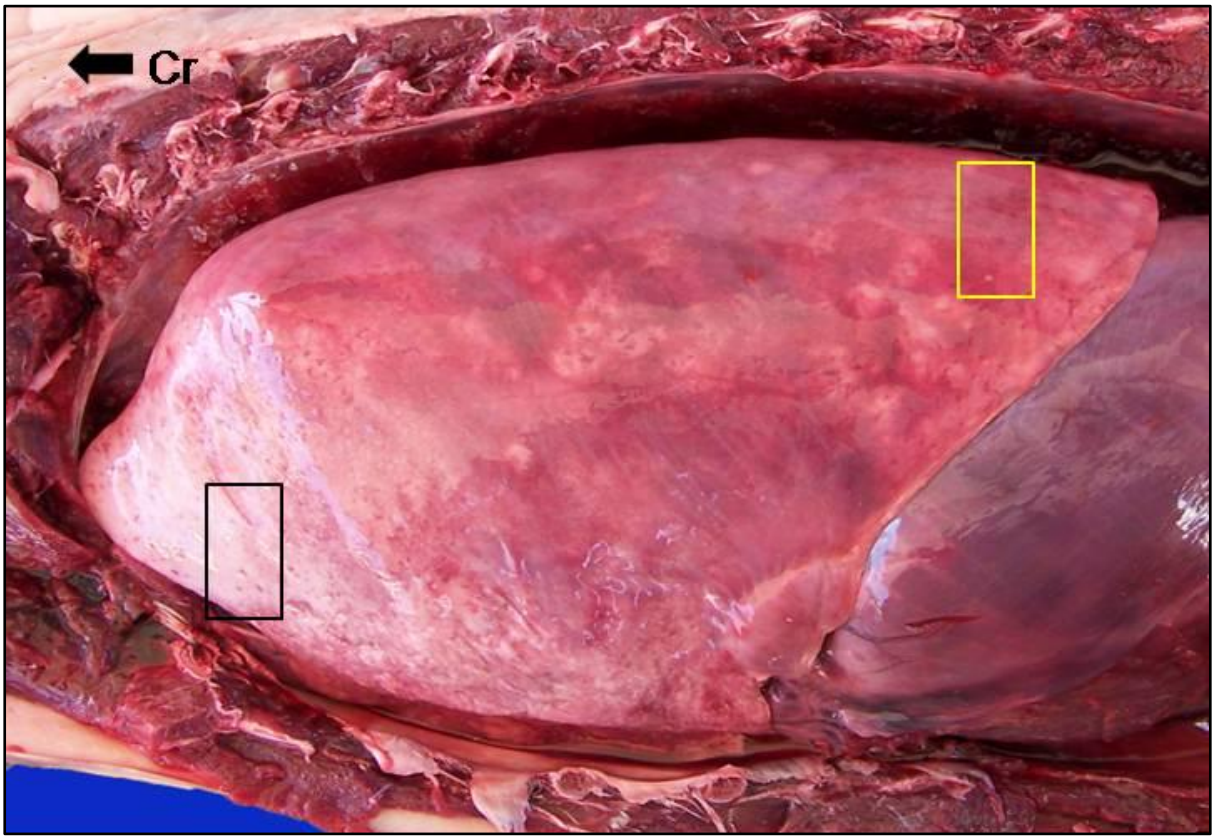

Figure 2.19. Lung with rectangles indicating the sites of sampling; black: cranio-ventral lung field, yellow: caudal lung field.

- Open the heart by incising along the *vena cava*, entering the right atrium, and cut along the right ventricle circumference, following the blood vessels in the interventricular groove, ending in the pulmonary trunk (for an overview of the anatomy of the heart see Figure 2.20). Then start an incision in the left atrium, extending straight down into the left ventricle. Open the aorta as it leaves the heart. Evaluate the heart muscle, endo- and epicardium (inner and outer lining, respectively) as well as the valves. Sample the following for histopathology:
    - right ventricle wall and pulmonary semilunar valve
    - left papillary muscle
    - interventricular septum
    - aorta

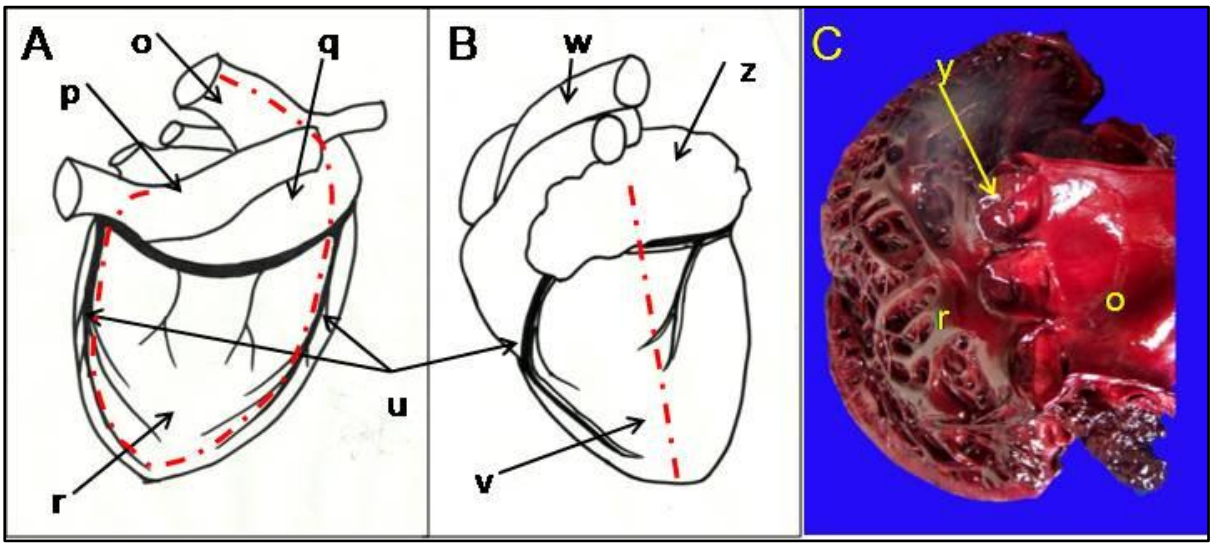

Figure 2.20. A+B: Anatomy of the heart showing the pulmonary trunk (o), vena cava (p), right atrium (q), right ventricle (r), blood vessels in interventricular groove (u), left ventricle (v), aorta (w) and left atrium (z). The red dashed line indicates the incisions made to open the heart (incisions in the right side of the heart (A), incision in the left side of the heart (B)). C: The appearance of the endocardium (right ventricle, r), the heart valves (y) and the major blood vessels (pulmonary trunk, o). Illustrations by Ingrid de Wet.

3.4.7. Skull Examination and Sampling

- Remove the head by cutting through the atlanto-occipital joint (between the spine and skull) (Figure 2.21). Evaluate the atlanto-occipital joint for any abnormalities.
- Incise and evaluate the melon (Figure 2.21).
- Evaluate and sample the auditory fat along the lateral aspect of the mandibles for histopathology. Pay close attention to any haemorrhage that may be present.
- Cut into the airsacs located under the blowhole, and further down to include the internal nares (monkey lips).
- Remove as much muscle and fat from the skull as possible.

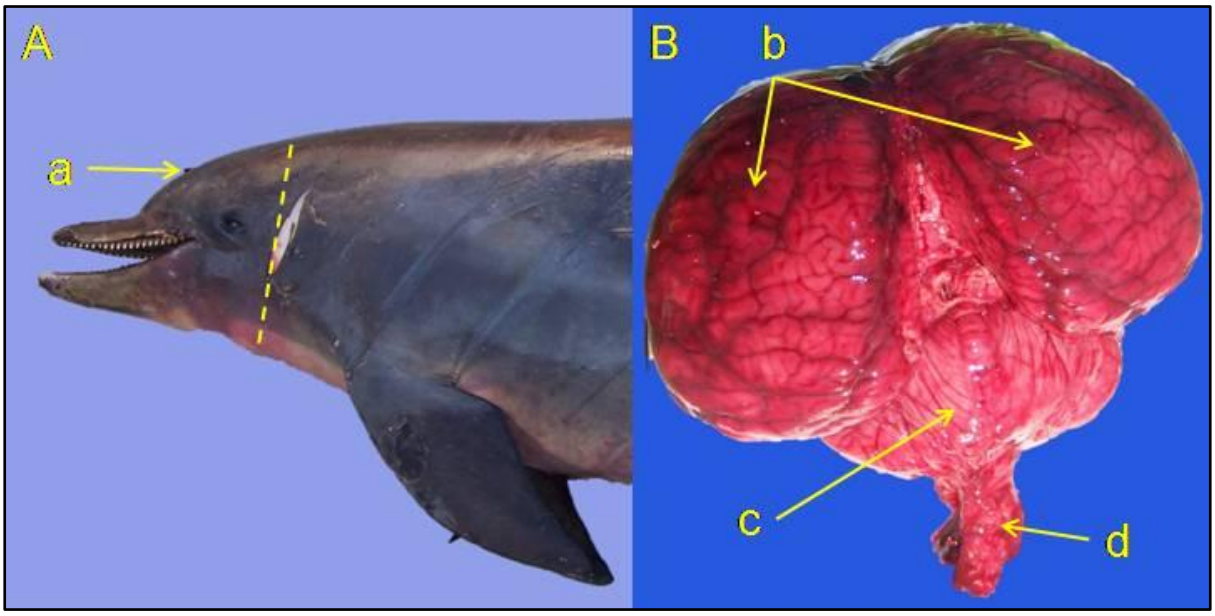

Figure 2.21. A: Dashed line shows approximate incision to separate the head from the body, and the melon (a). B: Anatomy of the brain showing the cerebral hemispheres (b), cerebellum (c) and spinal cord (d).

- The ear is located caudo-ventral to the eye, just dorsal to the temporomandibular joint. Removing the mandible (lower jaw) facilitates exposure of the ear. Place the head on the cranium and locate the temporomandibular joint and then the ear, which is located just lateral to the temporomandibular joint. Cut the attachments of the ear to the skull with a scalpel blade, evaluate and sample any parasites.

- Open the skull by making a circular incision on the caudal aspect of the skull using a saw (Figure 2.22). Once the bone has been removed, incise the *dura mater* (membrane around the brain) and *tentorium cerebelli* (membrane between the cerebrum and cerebellum) and bluntly dissect out the whole brain *in toto*. Evaluate and sample the cerebrum for microbiology and place the rest of the brain in 10% buffered formalin. If the container opening is not large enough to fit the brain without it touching the opening, it must be cut in half, separating the two hemispheres. If only small sample bottles are available, the brain can be sub-sampled, with a sample taken from the front of the cerebrum, the mid-section of the cerebrum, the cerebellum and the brain stem.

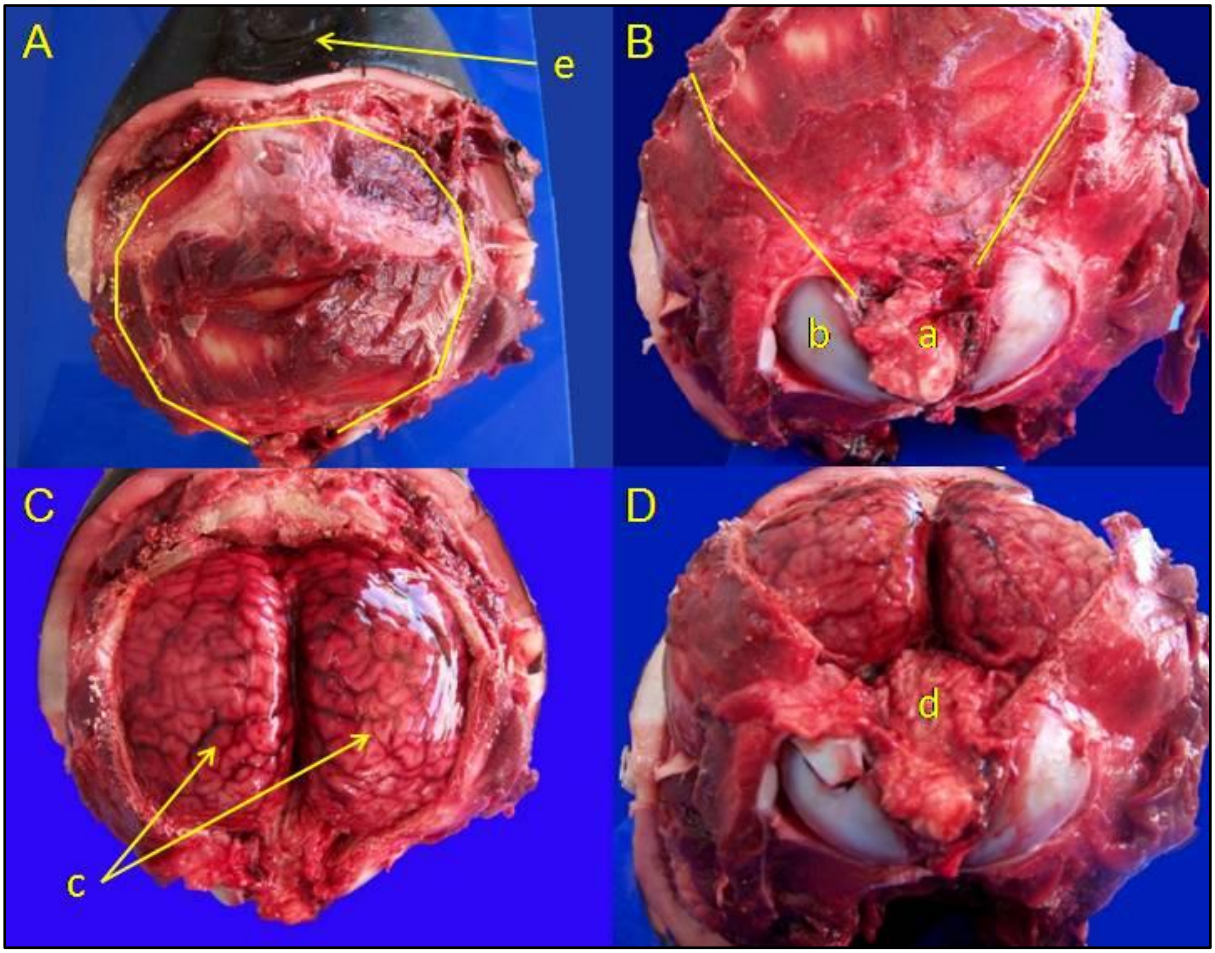

Figure 2.22. Opening the skull. Caudal view of the head with the majority of muscle removed and the blowhole (e) visible. A: Make a roughly circular incision on the dorso-caudal aspect of the skull. B: Ensure that these incisions pass through the occipital condyle (b) and exit through the foramen magnum (a). C+D: The completed cuts with the brain exposed showing the cerebrum (c) and the cerebellum (d).

- Evaluate and sample the entire hypophysis (pituitary) for histopathology by placing it on labelled tissue paper into the formalin. The hypophysis can be found in a small recess (the hypophyseal fossa*)* on the ventral midline of the floor on the cranium (skull) after the brain has been removed (Figure 2.23).

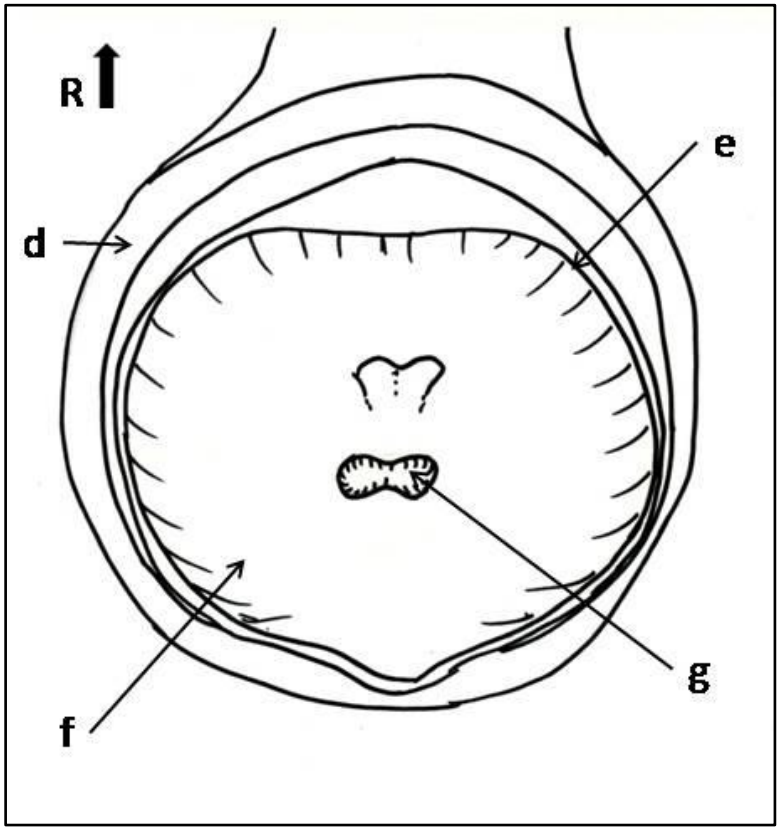

Figure 2.23: Caudal aspect of the skull (rostral, R) showing the location of the pituitary gland, located in a recess (g) in the floor of the skull (f), visible after the brain has been removed by making the cuts in the skull to expose the brain (e), and the layers of muscle and skin (d). Illustration by Ingrid de Wet.

3.5. Reporting

3.5.1. Macroscopic Pathology Report

This report has been designed specifically to follow this protocol designed for South African cetaceans. Reports should be as detailed as possible, with all information recorded (Section 3.3). If information is not available, or an organ is not evaluated, a reason should be given, to aid in further development and modification of this protocol and report.

Post Mortem Record			**Animal Identification:**	
Person conducting the PM:				
Date:				
Location:				
Species:				
Sex:				
Age:				
History:	**(circumstances leading to the animal's illness/clinical signs/death)**			
Measurements: (cm)	**Upper jaw to insert of tail:**			
Stage of decomposition		**(1=Fresh, 5=advanced decomposition)**		
Blubber thickness (cm):	**Lateral to dorsal fin**			
	Cranial to dorsal fin (dorsal midline)			
	Cranial to dorsal fin (lateral midline)			
	Cranial to dorsal fin (ventral midline)			
External:	**(Injury-scarring-bloating-discharge from orifices)**			
Ectoparasites				
Mammary Glands:	**(Evaluate-histopathology)**			

Left Eye:	
Subcutis	
Blubber:	**(parasitic cysts)**
Muscle/subcutis:	**(oedema-haemorrhage)**
Cervical Lymph Nodes:	
Teeth	**(5 teeth)**
Examination:	**(fluid-volume-clear/exudate/haemorrhage-adhesions)**
Abdominal cavity:	
Thoracic cavity:	
Abdominal cavity and organs:	**(general colour organs-fat content-blood)**
Intestines:	**(contents-amount-wet-dry-ulcers-parasites etc.)**
Mesentery and lymph nodes:	**(consistency-haemorrhage-swollen)**
Muscular stomach (first compartment)	**(contents-amount-wet-dry-ulcers-parasites etc.)**

Glandular stomach (second compartment)	**(contents-amount-wet-dry-ulcers-parasites etc.)**
Pyloric stomach (third compartment	**(contents-amount-wet-dry-ulcers-parasites etc.)**
Liver:	**(normal-swollen-colour patterns-parasites-cysts)**
Pancreas:	**(normal-swollen-colour)**
Spleen:	**(normal-swollen-lesions-accessory spleens)**
Kidneys:	**(normal-fat deposits-soft-colour-infarcts)**
Adrenal:	**(normal-swollen-haemorrhage)**
Bladder:	**(normal-wall-colour urine)**
Reproductive tract:	**pregnancy-foetus-uterus-ovaries-penis-testes-lesions)**

Thoracic cavity and organs:	
Bone marrow (head of rib)	
Rete mirabile:	
Tongue:	
Thyroid gland:	
Thymus:	**(fluid volume-clear/pus-colour)**
Oesophagus:	**(contents-amount-wet-dry-ulcers-parasites etc.)**
Marginal lymph node of the lung:	**(number-consistency-haemorrhage-swollen)**
Tonsils:	**(colour-consistency)**
Lungs:	**(collapsed-heavy-fluid or blood filled-appearance-consolidation-froth/oedema)**
Heart/pericardium:	**(fluid volume-clear/exudate-adhesions-colour)**
Diaphragm:	
Skull:	
Blowhole/airsacs:	**(abscess-parasites-blood-congestion-fluid)**
Melon:	

Ear:	**(parasites)**
Brain & hypophysis:	**(abscess-parasites-blood-congestion-fluid)**
Macroscopic diagnoses:	
Post mortem changes and incidental findings:	
Clinically significant findings:	
Cause of death:	
Signature of person who conducted the PM:	

3.5.2. Checklist

CHECKLIST		Identification							
		Smpl	√	B			Smpl	√	B
Measurements					Blood	Serum and EDTA			
Stage of decomposition					Bone marrow				
Blubber thickness						Rib	H		
Blubber sample including skin		H			Rete mirabile		H		
					Tongue	Middle third	H		
External lesions		H			Thyroid Gland		H		
		M			Thymus	If present	H		
Any discharge?		M			Oesophagus				
Ectoparasites		P				Contents	PEM		
Mammary glands		H					P		
		M				Cranial third	H		
Left eye	Include peribulbar fat	H			Marginal lymph node of the lung		H		
Blubber		P			Tonsil		H		
Skeletal muscle		H			Trachea	Cranial third	H		
	Caudolateral to dorsal fin				Lung	Cranioventral lung lobe L	H		
Cervical lymph nodes		H					M		
Teeth	Age determination	PEM				Cranioventral lung lobe R	H		
In situ examination							M		
Fluid	Abdominal/thoracic cavity	M				Dorsal lung lobe L	H		
Intestines		H				Dorsal lung lobe R	H		
	Cranial, middle and	M			Heart	R ventricle & pulmonic valve	H		
	caudal	P				L papillary muscle	H		
Mesenteric lymph nodes		H				Interventricular septum	H		
		M				Aorta	H		
Stomach					Diaphragm		H		
	Parasites	P			Blowhole				
	Stomach contents	PEM			Melon				
	Glandular stomach	H			Ear		P		
	Muscular stomach	H			Brain		H		
	Pyloric stomach	H					M		
Liver	Left and right lobes with	H			Hypophysis		H		
	bile ducts	M			Virology				
Pancreas		H							
Spleen		H							
		M							
Kidney	Left	H			Lesions				
		M							
	Right	H							
		M							
Adrenal	Left	H							
	Right	H							
Bladder	Tip of bladder	H			Key:	Sample	Smpl		
Reproductive tract						Completed task	√		
	Uterus	H				Bottle (container) number	B		
	Ovaries	H				Histopathology	H		
	Testes	H				Microbiology	M		
	Penis	H				Parasitology	P		
	Foetus	PM				Post Mortem to be done	PM		
	Placenta	H				Analysis by PE Museum	PEM		
		M							

Chapter 3. Pathological Findings in Two Dolphin Species By-caught in Shark Nets off the KwaZulu-Natal Coast, South Africa

1. Introduction

The reality of an on-going ecological crisis with degradation of ocean health has led to research into the possible causes and effects that this may have on marine mammal species (Lafferty, *et al*., 2004). Health assessments are conducted in these animals as they are indicator species for monitoring the health of the marine ecosystem, and for examining any potential overlap between human activities and animal health (Harvell, *et al*., 2002; Bossart, 2006). Standard necropsy and health assessment protocols for various species have been developed for this purpose in the northern (Kuiken and Hartmann, 1993; Cornaglia, *et al*., 2000; Siebert, *et al*., 2001; Jauniaux, *et al*., 2002; Duignan, 2003; Jepson, *et al*., 2005b; Bossart, 2006; Siebert, *et al*., 2006; McFee and Lipscomb, 2009) and southern (Duignan, 2003) hemispheres.

The rise in reports on the number and severity of diseases affecting marine mammals has raised concerns of deteriorating ocean health (Gulland and Hall, 2007). This is in part a result of more dedicated research in this field and advancement of diagnostic techniques (Gulland and Hall, 2007). However, an increase in the frequency of marine mammal mortalities over the last four decades resulting from exposure to harmful algal blooms and morbillivirus outbreaks in the North Atlantic have been found (Gulland and Hall, 2007; Raga, *et al*., 2008; Van Bressem, *et al*., 2009a). Changes in frequency of other disease outbreaks are more difficult to assess owing to a lack of baseline data for most marine communities (Harvell, *et al*., 1999; Ward and Lafferty, 2004; Gulland and Hall, 2007). This, combined with predictions of future increases in disease owing to climate change and an increase in stress factors such as pollutants, inter- and intra-specific competition and habitat destruction, lends new urgency to understanding the causes of marine mammal disease outbreaks (Epstein, *et al*., 1998; Harvell, *et al*., 1999; Harvell, *et al*., 2002; Ward and Lafferty, 2004).

The coastal distribution of some cetacean species makes them particularly vulnerable to anthropogenic impacts and trauma, which include net entanglement (Geraci and Lounsbury, 2009), boat strike (Bar and Slooten, 1999), disturbances due to boat traffic (Geraci and Lounsbury, 2009), pollution (Harvell, *et al*., 1999), nutrient enrichment (Geraci and Lounsbury, 2009), pathogen introduction and dispersal (Lafferty, *et al*., 2004), habitat

degradation (Geraci and Lounsbury, 2009) and depletion of prey (Lafferty, *et al*., 2004; Geraci and Lounsbury, 2009). Dolphins have long life spans (Lafferty, *et al*., 2004; Wells, *et al*., 2004), feed at a high trophic level (Wells, *et al*., 2004), and have extensive fat stores that accumulate chemical pollutants (Reddy, *et al*., 2001; O'Shea, *et al*., 2003; Wells, *et al*., 2004). These pollutants increase the susceptibility of marine mammals to diseases by impairing defence mechanisms (Lafferty, *et al*., 2004), as seen in increased mortalities in polluted waters during morbillivirus epidemics (Harvell, *et al*., 1999). Nutrient enrichment of the environment via sewage and fertilizer dispersal have been implicated in an increase in the occurrence of toxic algal blooms, which can have devastating effects on marine populations (Flewelling, *et al*., 2005; Riva, *et al*., 2009). The introduction of new pathogens, such as morbillivirus infections thought to be derived from canine distemper virus, has been attributed to human-related activities such as dogs transmitting canine distemper virus to marine mammals (Harvell, *et al*., 1999; Lafferty, *et al*., 2004). Furthermore, toxoplasmosis is believed to have entered the marine environment via river runoff from urban areas (Miller, *et al*., 2002; Dubey, *et al*., 2003). Habitat destruction and depletion of prey through fishing increases inter-and intra-species competition and stress that further undermine host defence mechanisms (Harvell, *et al*., 1999; Lafferty, *et al*., 2004). The susceptibility of dolphins to these factors makes them suitable sentinel species to detect early warning signs of impacts in our oceans. This allows better characterization and management of potential negative impacts on human and animal health (Bossart, 2006).

Both *Tursiops aduncus* (Indian Ocean bottlenose dolphin) and *Sousa chinensis* (Indo-Pacific humpback dolphin) occur inshore along the Southern African coast (Figure 3.1) in waters less than 30 m and 15 m deep, respectively. They both inhabit inshore areas less than 10 km from the shore and are therefore prone to anthropogenic factors (Cockcroft and Ross, 1990a; Cockcroft, *et al*., 1990; Karczmarski, *et al*., 2000; Best, 2007).

Gill nets (110 m long and 10 m deep) were first deployed off the South African east coast by the KwaZulu-Natal Sharks Board (KZNSB) in 1952 in an attempt to reduce the risk of interaction of sharks with swimmers along the popular swimming beaches in this area (Cockcroft, *et al*., 1990; Cockcroft, 1994). Currently a total of 23.4 km of nets protects beaches along a 320 km stretch of coastline (KwaZulu-Natal Sharks Board, 2011). Annually, an average of 42 dolphins has been incidentally caught in the shark nets between 2004 and 2009 (KwaZulu-Natal Sharks Board, 2009). The majority of these dolphins are the coastal species *T. aduncus* and *S. chinensis,* although pelagic *Delphinus capensis* (long-beaked common dolphin) and *Stenella attenuata* (spotted dolphin) are also caught (Cockcroft and Ross, 1990a). Since the risk of a dolphin being caught in the nets is unlikely to be

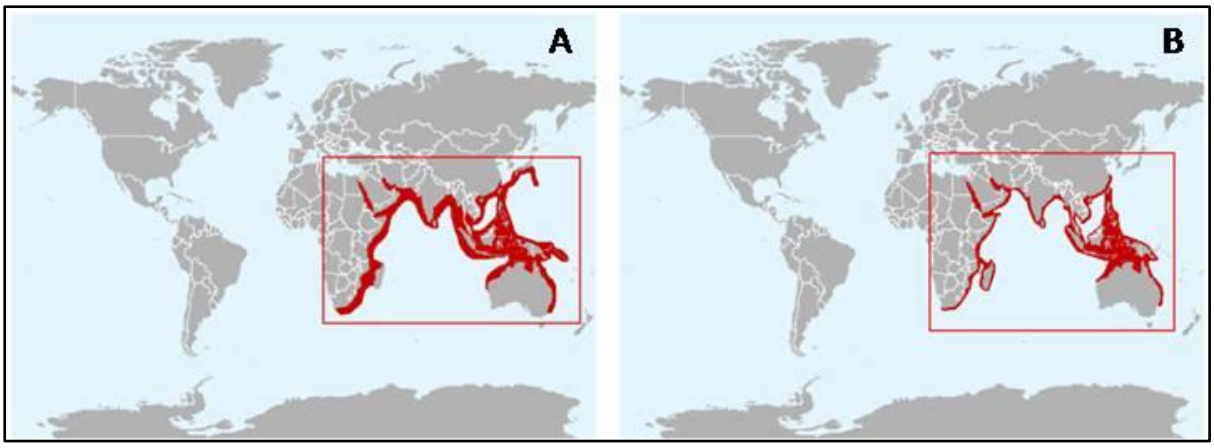

Figure 3.1. Distribution of *T. aduncus* (A) and *S. chinensis* (B) in the Indian Ocean (Hammond, *et al*., 2012).

influenced by its health status, incidentally caught dolphins are presumed to be representative of the overall health status of the population. In contrast, stranded dolphins (found dead or alive) may strand as a result of being diseased, and therefore cannot be used to make inferences regarding dolphin population health (Jauniaux, *et al*., 2002).

Since the 1970's, an agreement between the KZNSB and the Port Elizabeth Museum (PEM) allows data and samples obtained from the dolphins incidentally caught in the shark nets off the KwaZulu-Natal coast to be accessioned to the Graham Ross Marine Mammal Collection at the PEM. An observed increase in small (*ca* 5 mm in diameter), round, firm, raised foci on the peritoneal surface and abdominal organs, as well as fibrotic tags on the spleen, were found during routine dissections in 2009, and led to the initiation of this health assessment (*pers. comm*. S. Plön). No prior knowledge is available on the diseases present, overall health status or the risk factors that might influence disease in the dolphin populations off the South African coast.

For this study we collected necropsy data from *T. aduncus and S. chinensis* incidentally caught in the shark nets over a two year period, and related this to catch location, age, sex, and body condition. This allowed us for the first time to document and estimate the prevalence of diseases present in the populations, and to compile valuable baseline data for assessing the health status of these dolphin populations and monitoring health trends over time. In addition, we developed a necropsy sampling protocol for Southern African cetaceans (Chapter 2).

2. Materials and Methods

2.1. Carcass Recovery and Necropsy

A total of 46 dolphins, 39 *T. aduncus* and seven *S. chinensis*, were incidentally caught in the shark nets at various locations off KwaZulu-Natal (Figure 3.2) from April 2010 to April 2012. The shark nets were checked every weekday by the KZNSB; dead animals recovered were weighed and frozen at -20°C. Necropsies were performed every 6-8 months, depending on the number of accumulated carcasses. Carcasses were defrosted sequentially at room temperature, in the shade, starting three days prior to the planned dissections (Chapter 2); small carcasses were defrosted before larger ones to maximize freshness.

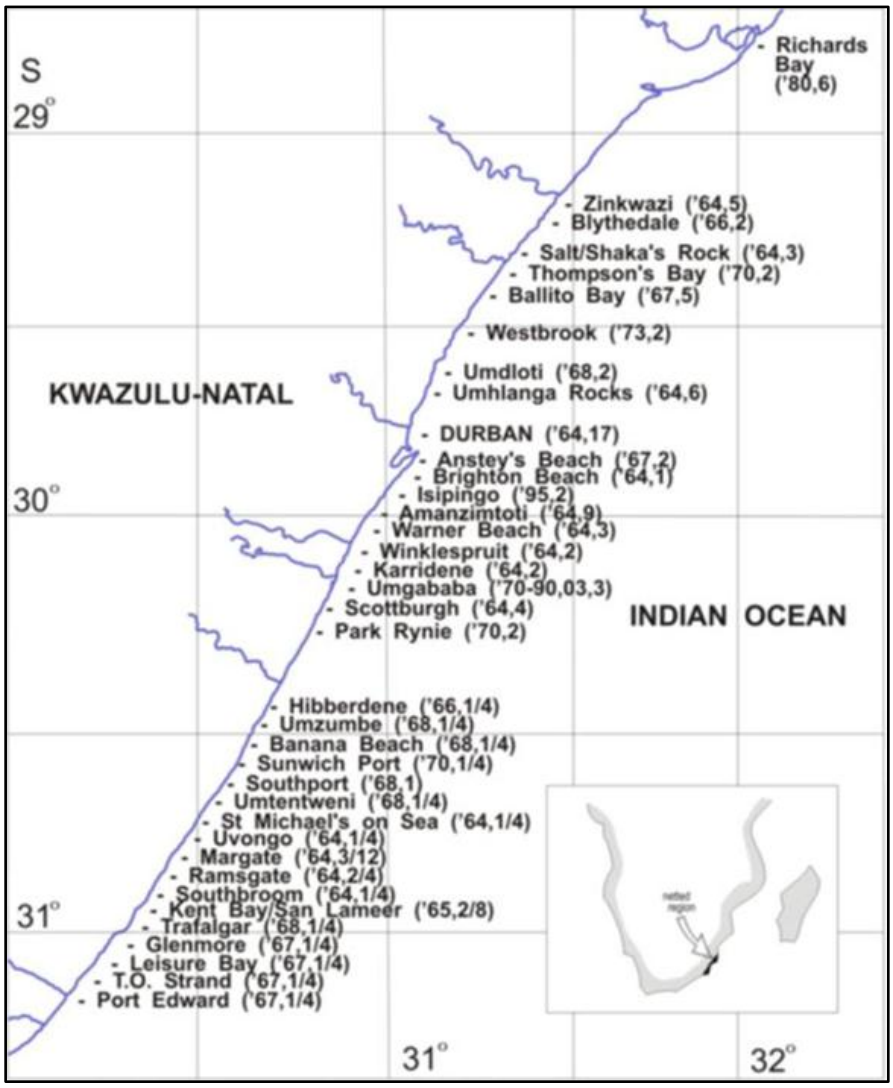

Figure 3.2. Locations of shark nets along the KwaZulu-Natal coast with the year installed and net length (km) in parentheses (KwaZulu-Natal Sharks Board, 2011).

Standard morphological measurements were taken of all the carcasses (Norris, 1961), including total length to estimate approximate age for *T. aduncus*, based on growth curves provided by Cockcroft and Ross (1990). Four mandibular teeth were sampled from all animals and were used for age determination of *S. chinensis* (Nolte, unpublished data). Necropsy examinations were performed on 46 animals; only 35 *T. aduncus* and five *S. chinensis* were sufficiently fresh for histopathological examination (Geraci and Lounsbury, 2009). Nine necropsies were performed by Dr D. Zimmerman, and 36 by the author. Necropsies were performed using a standard necropsy and sampling protocol developed for this project (Chapter 2). Animals were examined for external lesions and external parasites. Three full blubber thickness measurements were taken from all animals (ventral, lateral and dorsal midline cranial to the dorsal fin) and compared to previously published data (Young, 1998) to assess body condition. All organs were examined macroscopically and samples taken from the following tissues were fixed in 10% buffered formalin: lung, trachea, stomach (muscular, glandular and pyloric compartments), tongue, pharynx (including tonsils), oesophagus, intestine, liver, pancreas, thyroid gland, adrenal gland, kidney, urinary bladder, testis, uterus, ovary, penis, mammary gland, thymus, spleen, lymph nodes (mesenteric, cervical and marginal node of the lung), heart, aorta, *rete mirabilis*, skeletal muscle, diaphragm, skin, blubber, brain (frontal lobe, midbrain, brainstem and cerebellum), eye (including the optic nerve), bone and bone marrow. Selected lesions were documented by digital photographs.

2.2. Histopathological Analysis

Rib samples were decalcified in an aqueous mixture of 5.6% hydrochloric and 8% nitric acid (National Health Laboratory Services, South Africa) to facilitate sectioning and staining. For histopathological analysis tissues were embedded in paraffin wax. Sections (5 µm) were stained using haematoxylin and eosin and selected tissues were stained with special stains, including Gram, Von Kossa (VK), Stamp, Masson's trichrome (MT), Ziehl-Neelsen (ZN), Gomori's methenamine silver stain (GMS), Perl's Prussian blue (PPB), Hall's bile stain (HB), periodic acid-Schiff (PAS), Fontana Masson's (FM) and the modified silver stain according to Bielschowsky (MSSB) (Böck and Romeis, 1989). Immunohistochemical stains were performed on sections of known target organs where lymphoplasmacytic inflammation was suggestive of *Toxoplasma gondii* (brain, muscle and heart; Department of Pathology, University of Pretoria: VHLSOP627) and dolphin morbillivirus (brain and lung; Department of Pathology, University of Veterinary Medicine, Hannover) (Stimmer, *et al*., 2010). In addition, beta-amyloid precursor protein (APP) (Seehusen and Baumgärtner, 2010) and CD3 (Stimmer, *et al*., 2010) staining were performed on the optic nerve and brain, respectively.

Scanning electron microscopy (SEM) and transmission electron microscopy (TEM) were performed at the Department of Pathology, University of Veterinary Medicine, Hannover, Germany, and the Fraunhofer Institute (Hannover, Germany) on mineral deposits found in the lung. Post-fixing tissue samples in 2.5% glutaraldehyde and routine laboratory methods were used for SEM, with critical point drying and gold sputtering. For TEM, formalin-fixed lung samples were post-fixed in 2.5% glutaraldehyde and routinely embedded in epon resin. Tissues were routinely processed and embedded on copper grids, unstained and without contrasting, for energy-dispersive X-ray spectroscopy (EDX) analysis with TEM.

2.3. Parasitic and Microbiological Analysis.

Parasites found during necropsy were fixed in 70% ethanol. Identification was performed by Dr Junker (Onderstepoort Veterinary Institute) and Dr Kristina Lehnert (Institute of Terrestrial and Aquatic Wildlife, Veterinary University of Hannover, Germany) using standard text.

Samples of liver, kidney, spleen, lung, mesenteric lymph node, intestine and any organ with exudate or abscesses were stored at -20°C. One lung sample from each dolphin was cultured by Dr Maryke Henton (IDEXX Laboratories, Johannesburg) using standard methods for routine cultures, but excluded specific procedures for *Brucella* spp. Electron microscopy (EM) was performed on lesions with possible viral aetiology by Ms E van Wilpe (EM Unit, Faculty of Veterinary Science, University of Pretoria) using standard methods.

2.4. Statistical Analysis

Animals were divided into two groups (regions) based on capture location, north and south of Ifafa Beach (Figure 2), since population and genetic studies of *T. aduncus* have indicated that these represent different subpopulations (Peddemors, 1999; Natoli, *et al*., 2008). Animals were divided into three age categories: calves (<2 years, the approximate age of weaning) (Cockcroft and Ross, 1990a), juveniles (2-12 years), and adults (>12 years, the approximate age of sexual maturity) (Cockcroft and Ross, 1990a). Blubber thickness of *T. aduncus* was compared between age categories and between sample sites (dorsal/ventral/lateral) using a linear mixed model adjusting for sex and region and with Bonferroni's correction for multiple comparisons. For selected lesions, based on possible biological significance, occurrence of lesions was compared between species, and for *T. aduncus* between age categories, sexes and regions using Fisher's exact test. For univariable associations with $p<0.25$, adjustment for possible confounding between age category, sex and region was done using multivariable exact logistic regression models. The very small sample size for *S. chinensis* did not allow statistical analysis. Due to the exploratory nature of the analysis and the relatively small sample size, significance was

assessed at p<0.1. Statistical analysis was done using Stata 12.1 (StataCorp, College Station, TX, U.S.A.).

3. Results

3.1. Location

The dolphins sampled were caught at 18 of 36 net locations along the KwaZulu-Natal coast (Figure 3.3). The majority of animals were caught off Durban (7; 18%), followed by Richards Bay (5; 13%), with 30 animals (83% *T. aduncus* and 17% *S. chinensis*) in the northern region, and ten animals (only *T. aduncus*) in the southern region.

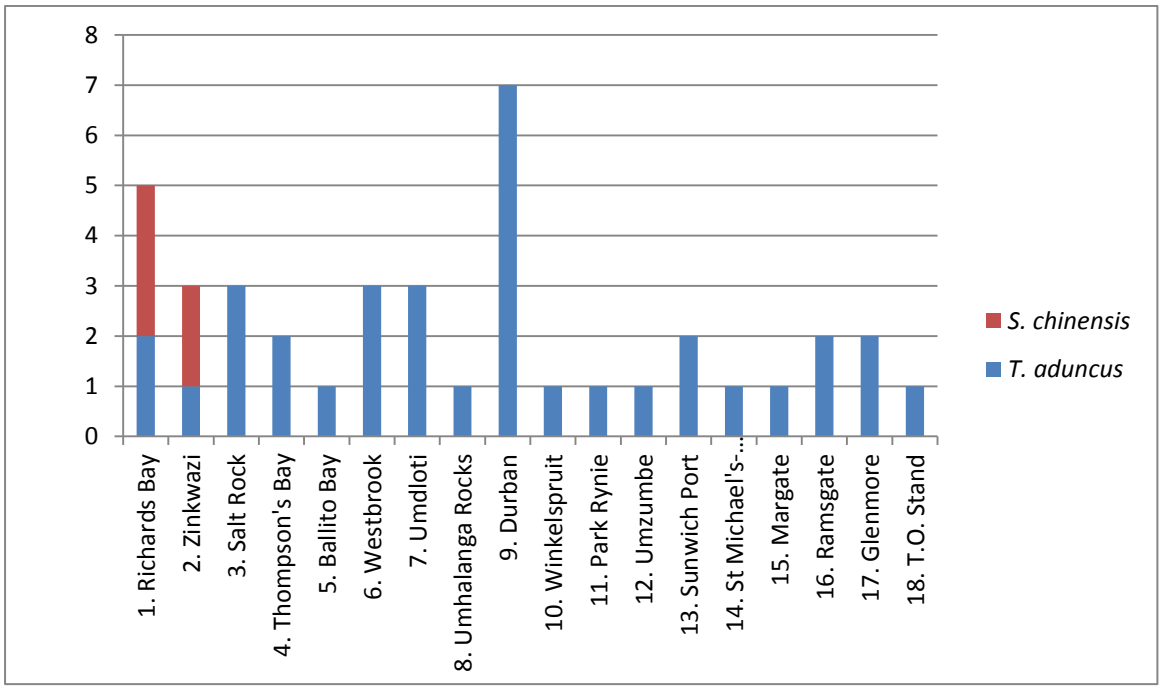

Figure 3.3. Total number of dolphins incidentally caught in the shark nets for each location (beach name) along the KwaZulu-Natal coast, (2010 - 2012). 1-11: North region; 12-18: South region.

3.2. Age and Sex Composition of the Sample

A much larger number of *T. aduncus* (35; 88%) than *S. chinensis* (5; 12%) were caught (Figure 3.4). The majority of *T. aduncus* were females (24; 69%) rather than males (11; 31%), and more juveniles (16; 46%) and calves (11; 31%) were caught than adults (8; 22%) of both sexes (Figure 3.4 A). Of the five *S. chinensis*, three were male and two were adults, two were juveniles and one was a calf (Figure 3.4 B).

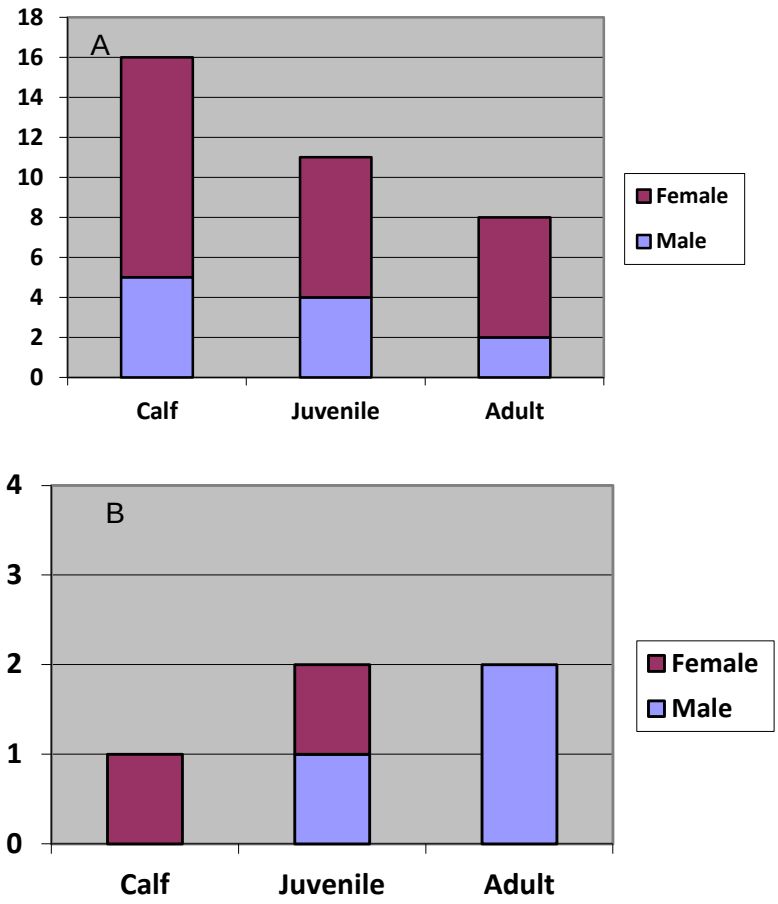

Figure 3.4. Number of *T. aduncus* (A) and *S. chinensis* (B) sampled, by age and sex.

3.3. Nutritional Condition

Blubber thickness (Figure 3.5) differed significantly between all three sites for each age category ($p<0.05$), being thickest at the dorsal and thinnest at the lateral sampling site. Blubber thickness was greater in juveniles and adults compared to calves at the dorsal ($p<0.001$) and ventral ($p<0.05$) sites, but did not differ significantly at the lateral site. There were no statistically significant effects of sex or location.

3.4. Generalised Changes

Most of the organs in all of the dolphins examined showed moderate to severe autolysis and putrefaction, and moderate to severe freezing artefact characterized by various sized fissures in tissues on histological examination. Variable numbers of round to oval, unlined vacuoles (up to 0.1 cm diameter) were found distending blood vessels or in the parenchyma of various organs (Figure D.1 B-D). These were interpreted as air supersaturation vacuoles and air emboli respectively as no bacteria were visible on HE or Gram stain. Mild to severe, acute congestion was present in most organs in all the dolphins.

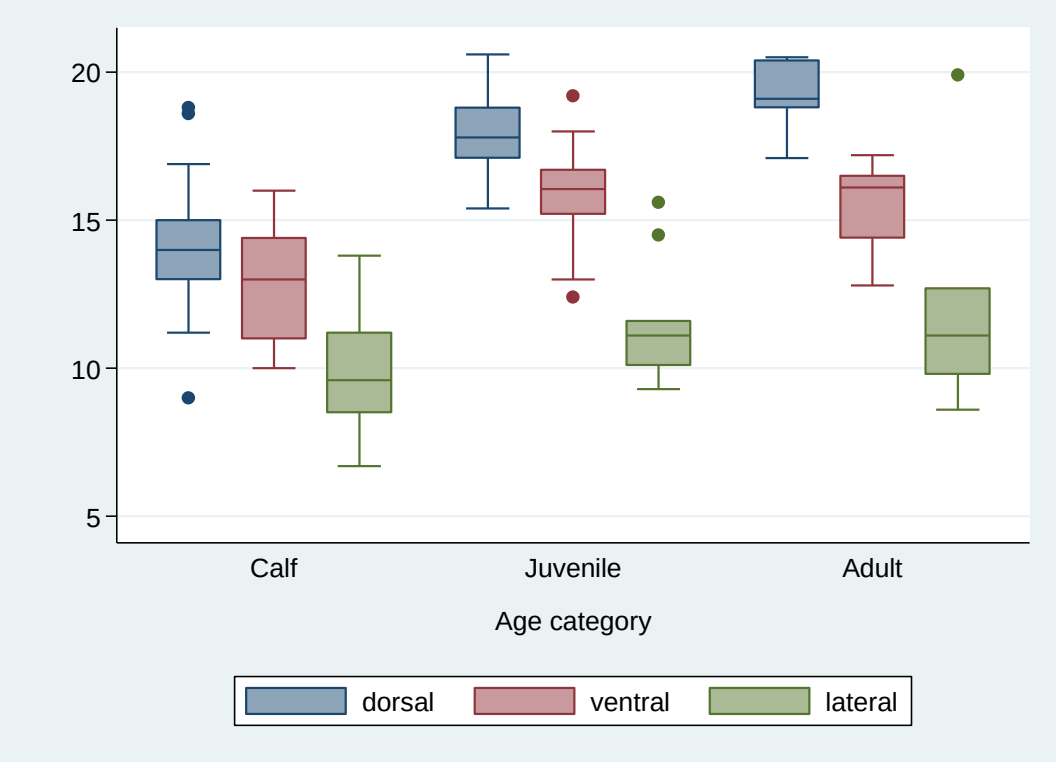

Figure 3.5. Blubber thickness measurements (mm) from *T. aduncus* arranged by age categories. Box extends from 25th to 75th percentile, horizontal line represents the median, whiskers extend to the smallest and largest observations that are >1.5 times removed from the interquartile range (IQR), dots represent outliers

Complete lists of the lesions observed are given under the respective organ systems in Appendix E. The occurrence of selected lesions in the two dolphin species is shown in Table 3.1, and for *T. aduncus* their occurrence is shown by age category, sex and region in Table 3.2. The results of the exact logistic regression models to adjust for confounding between age category, sex and region are shown in Table 3.3 for those lesions in which a significant ($p<0.1$) association was found. Further details of selected lesions in each organ system are given below.

3.5. Respiratory System

A list of the complete pathological findings from the respiratory tract, indicating occurrence per species, age group and region, is presented in Table E.1. Mild, multifocal lymphoplasmacytic and variably eosinophilic pleuritis was found in 12 (30%) animals, affecting both species, and only calves and juveniles. Mild to moderate, multifocal, subacute lymphoplasmacytic and variably eosinophilic tracheo-bronchitis was present in 12 (30%) *T.*

aduncus calves and juveniles. Variably eosinophilic and lymphoplasmacytic bronchointerstitial pneumonia was present in 37 animals (93%), in all ages and both sexes and species. Pneumonia was also frequently accompanied by follicular lymphoid hyperplasia of bronchus associated lymphoid tissue (18; 45%). Nematode adults, with (3) or without (3) microfilaria were present in the lung lesions in six (16%) animals; the adults were identified as *Halocercus* sp. (Figure D.2 A-E). Mild to moderate, multifocal to diffuse, interstitial fibrosis was more common in *S. chinensis* (60%), compared to *T. aduncus* (26%) although this was not statistically significant (p=0.149); and *T. aduncus* adults and juveniles were more affected than calves (p=0.001). In *T. aduncus* no association was found between pneumonia and pleuritis (p=0.653) or interstitial fibrosis (p=0.668).

Lung samples from all 16 animals that were tested by immunohistochemistry for dolphin morbillivirus were negative. All animals yielded a variety of bacteria on routine lung cultures, which included: *Pantoea agglomerans*, *Enterococcus solitarius*, *Enterobacter gergoviae*, *Shewanella algae*, *Shewanella putrefaciens, Photobacterium damselae*, *Aeromonas media*, *Lactococcus garviae*, *Clostridium tertium*, *Streptococcus* from the *viridians* group, and bacteria belonging to the genera *Psychrobacter, Enterococcus, Micrococcus, Lactobacillus, Brevundimonas, Bacillus, Acinetobacter* and *Proteus.*

Mild to moderate, multifocal bronchiolar mucosal mineralisation (Figure D.3) was present in 33 (83%) animals of both species, sexes and regions, and in all ages. There was no association between bronchiolar mucosal calcification and pneumonia (p=0.447). The six animals where no bronchiolar mucosal mineralisation was found originated from various beaches, and were from all age categories, of both sexes and species.

Mild to severe, multifocal to diffuse, acute alveolar oedema was present in 32 (80%) animals (Figure D.1 A). It was accompanied by marked distension of the alveoli with air, with no bacteria found on HE and Gram stain. Mild, multifocal pneumoconiosis was found in three (8%) animals: an adult female *T. aduncus* from Glenmore beach, a juvenile male *T. aduncus* from T.O. Strand (both in the southern region), and a juvenile female *T. aduncus* from Richards Bay (in the far north of the northern region).

3.6. Gastrointestinal Tract

Complete pathological findings found in the gastrointestinal tract, indicating occurrence per species and age group, is contained in Table E.2. Sarcocysts, without any associated inflammation, were found in the lingual myocytes (Figure D.4 F) in one *T. aduncus* calf caught off St. Michaels-on-Sea. Mild to severe, multifocal to diffuse, acute to chronic, variably eosinophilic and lymphoplasmacytic gastritis affecting all three gastric

compartments, was present in 26 (68%) animals of both species, all along the coast. Prevalence significantly increased with age in *T. aduncus*, affecting 10 (52%) calves, eight (89%) juveniles, and six (100%) adults (p=0.034). Moderate to severe, multifocal, chronic pyloric gastritis, associated with trematodes belonging to the family Brachycladiinae (either *Nasitrema* sp. or *Synthesium* sp.), was found in 12 (32%) *T. aduncus* and no *S. chinensis*. The pyloric mucosa overlying the nodules was calcified along the luminal margin in six animals (Figure D.5 F). In *T. aduncus*, the prevalence also increased with age, affecting five (26%) calves, five (56%) juveniles and two (33%) adults (p=0.097), although this was not statistically significant in the multivariable model (p=0.123). Adult nematodes belonging to the Anisakidae were found in the lumen of the stomach in a female *T. aduncus* calf from Sunwich Port. Mild to severe, acute to subacute, eosinophilic enteritis (Figure D.4 A-C) was found in 27 (68%) animals, affecting both species and prevalence increased significantly with age with age in *T. aduncus* (p=0.002). Adult nematodes belonging to the Anisakidae were found in the intestine of a juvenile male *T. aduncus* from Umdloti. A single female adult *T. aduncus* had moderate multifocal chronic acanthomatous epithelial hyperplasia of the hard palate.

Mild to moderate, acute to chronic, rarely necrotizing, eosinophilic and variably lymphoplasmacytic hepatitis was present in 24 (62%) *T. aduncus.* Inflammation was mostly portal (63%), but capsular inflammation (12%), or both (25%) were sometimes present. Hepatitis was not found in *S. chinensis* (p=0.037). Adults were more often affected than calves (p=0.044), although the association was not significant on multivariable analysis (p=0.112). Varying degrees of portal fibrosis (Figure D.6 A) was present in ten (26%) animals, of both species; prevalence also increased with age in *T. aduncus* (p=0.020). Green-brown, triangular trematode eggs (Figure D.6 A) were found within the portal triads in an adult female *T. aduncus* from Umdloti, a female *T. aduncus* calf from Sunwich Port and an adult male *T. aduncus* from Zinkwazi, accompanied by fibrosis and hepatitis. Mild to moderate, multifocal bile ductular hyperplasia was present in 17 (44%) animals, affecting both species equally. Portal fibrosis was positively associated with the presence of trematode eggs (p=0.013) as well as with bile ductular hyperplasia (p=0.009) in *T aduncus*. Interestingly, no association between bile ductular hyperplasia and portal hepatitis (p=0.588), bile ductular hyperplasia and the trematode eggs (p=0.401), and portal fibrosis and periportal hepatitis (p=0.468) could be demonstrated.

Evaluation of the pancreas was hampered by severe autolysis which obscured cellular detail. Mild, multifocal, subacute lymphocytic pancreatitis was detected in two female *T. aduncus* calves from Umdloti and Durban nets.

3.7. Lympho-haemopoeitic System

Complete pathological findings for the lympho-haemopoeitic system indicating occurrence per species and age group are contained in Table E.3. Splenic capsular tags (Figure D.7 A), consisting histologically of mild, multifocal, splenic capsular fibrosis (Figure D.7 B), were found in 18 (45%) animals, with a higher prevalence in *T. aduncus* (49%) compared to *S. chinensis* (20%). Capsular tags were significantly more common in dolphins from the southern coast (80%) than the northern coast (36%) (p=0.027). Mild, multifocal, lymphoplasmacytic and eosinophilic capsular splenitis (Figure D.7 C) was found in 12 (30%) *T. aduncus.* Males were more often affected than females (p=0.015); all three age classes were equally affected. Splenic tags and capsular splenitis were significantly associated (p=0.034).

Mild to severe, multifocal, subacute capsular lymphadenitis (Figure D.7 D-F), mainly eosinophilic and variably lymphoplasmacytic, was present in the cervical lymph node in ten (26%) animals, affecting only *T. aduncus (*29%), although this was not statistically significant. It was, however, associated with sex (p=0.045), affecting more males (55%) than females (17%). Identical lesions were found in the mesenteric lymph node in 18 (46%) animals, affecting both species equally. Nematode larvae (Figure D.7 F) were found associated with the capsular inflammation of the mesenteric lymph node in two *T. aduncus.* The prevalence of the mesenteric capsular lymphadenitis increased with age (p=0.009), with 26% of calves, 50% of juveniles and 100% of adults affected. Capsular lymphadenitis, similar in character to that seen in the other two lymph nodes, was also found in the marginal lymph node of the lung in 13 (43%) animals, with both species equally represented. Mild pigment accumulation was present in the mesenteric lymph node of a single adult *S. chinensis*, and in the marginal lymph node of the lung (Figure D.8) in two juvenile and one adult *T. aduncus*.

Small aggregates of cuboidal cells arranged in glandular acini were found next to the thymus (Figure D.9 A-D) in one juvenile *T. aduncus*, and one *S. chinensis* calf. These cells stained positive with chromogranin A. Mild to severe, diffuse, predominantly eosinophilic myelopoiesis was found in 22 animals of both species (75% of *T. aduncus* and 33% of *S. chinensis*) and from both regions (80% north and 63% south).

3.8. Reproductive Tract

Complete pathological findings for the reproductive tract, indicating occurrence per species and age group, are presented in Table E.4. Mild to moderate, multifocal, variably eosinophilic and lymphoplasmacytic oophoritis was found in five (19%) animals, affecting

21% of *T. aduncus* females, and 50% of *S. chinensis* females. Mild to severe, multifocal to diffuse, acute to subacute, variably lymphoplasmacytic and eosinophilic endometritis (Figure D.10 A) was found in eleven (42%) animals of both species. The endometritis was found significantly more often in adult *T. aduncus (*100%) than in calves (31%) and juveniles (29%) (p=0.044). A single adult *T. aduncus* from Thompson's Bay had a triangular trematode egg associated with the endometritis (Figure D.10 B). Mild, multifocal, subacute lymphoplasmacytic and variably eosinophilic metritis was found in six (23%) animals: five *T. aduncus* and a single *S. chinensis*. A positive association with age was found (p=0.019), although this association was not significant on multivariable analysis (p=0.107). Special stains for brucellosis were negative in all 12 cases tested.

Moderate to severe, multifocal lymphoplasmacytic mastitis associated with variable dystrophic mineralisation (Figure D.10 C) was found in two calves and one juvenile *T. aduncus* (43%). Small to large numbers of *corpora amylacea* with associated ductular ectasia (Figure D.10D), were found in two juvenile and one adult *T. aduncus* females (43%), which also had parasitic myositis in the adjacent muscle (see below).

Mild to moderate, subacute, multifocal capsular epididymitis and orchitis (Figure D.11 B-D) was found in five (38%) males, of both species and all age classes. The Stamp stain for brucellosis failed to show any organisms in these lesions.

3.9. Cardiovascular System

Complete pathological findings for the cardiovascular system, indicating occurrence per species and age group, are presented in Table E.5. Mild to moderate, focal to multifocal, acute to subacute epicarditis, endocarditis, and myocarditis (Figure D.12 A) were present in 20 (51%) in only *T. aduncus* (p=0.047). The highest prevalence was in juveniles (80%) (p=0.060), although this was not significant in the multivariable model (p=0.451). Lymphoplasmacytic myocarditis tested negative for toxoplasmosis in all cases. Mild, focal to multifocal myocardial fibrosis (Figure D.12 B) was found in 10 (51%) animals, with both species equally represented. The prevalence increased with age (p=0.001). There was no significant correlation between epi-, endo-, or myocarditis and myocardial fibrosis (p=0.393). Moderate segmental arteriosclerosis was found in a juvenile, male *S. chinensis* from Zinkwazi.

3.10. Endocrine System

Complete pathological findings for the endocrine system, indicating occurrence per species and age group, are presented in Table E.6. Mild to moderate adrenal cortical hyperplasia,

either nodular, diffuse, or a combination of these, was evident in 15 (45%) *T. aduncus* and three (75%) *S. chinensis*, of all ages and from both regions. Nodular hyperplasia was defined as cortical nodules within the medulla of the adrenal (Figure D.12 D-F). Diffuse hyperplasia was defined as a cortico-medullary ratio of more than 2:1, taking plane of section into consideration (Clark, *et al*., 2006)

3.11. Nervous System

Complete pathological findings for the nervous system, indicating occurrence per species and age group, are contained in Table E.7. Mild, multifocal, subacute meningoencephalitis (Figure D.13 B) was found only in seven (39%) *T. aduncus*. Lesions tested negative for toxoplasmosis, brucellosis and dolphin morbillivirus. Acute, focal, meningeal haemorrhage was present in one *T. aduncus* calf and one *S. chinensis* juvenile, and moderate, extensive acute haemorrhage around the cranial cervical spinal cord (Figure D.13 C-E) was found in a single female *T. aduncus* calf. Cerebellar herniation through the foramen magnum was found in another female *T. aduncus* calf.

3.12. Urinary Tract

Complete pathological findings for the urinary tract, indicating occurrence per species and age group, are presented in Table E.8. Mild to moderate, multifocal, subacute lymphoplasmacytic interstitial nephritis (Figure D.14 A-B) was found in ten (26%) animals affecting all ages, and both species, sexes and regions. Mild, multifocal, acute, eosinophilic capsular to subcapsular nephritis was present in two juvenile female *T. aduncus* and one juvenile female *S. chinensis* (8%) from the northern region. Mild, focal, cortical hypoplasia Figure D.14C) was found in a single female *T. aduncus* calf from Ramsgate. Mild, focal, chronic lymphoplasmacytic pyelonephritis (Figure D.14E-F) was found in a female *T. aduncus* calf from Westbrook. Mild, multifocal, cortical fibrosis (Figure D.14D) was found in an adult male *S. chinensis* from Richards Bay and a juvenile male *T. aduncus* from Salt Rock. Mild, multifocal, subacute lymphoplasmacytic and variably eosinophilic mural cystitis was found in ten (28%) animals of both species, sexes and regions, and all age categories.

3.13. Musculoskeletal System

Complete pathological findings for the musculoskeletal system, indicating occurrence per species and age group, are presented in Table E.9. Mild, multifocal, subacute lymphoplasmacytic skeletal myositis was present in seven (19%) animals in the northern region, affecting both species and sexes. In addition, prevalence increased with age in *T. aduncus* (p=0.007), with five calves (24%), two juveniles (20%) and three (75%) adults

affected. These areas were negative on immunohistochemistry staining for *T. gondii* in all seven cases. Mild, multifocal, subacute lymphoplasmacytic and eosinophilic serositis was present on the diaphragm in four (11%) female *T. aduncus*, a calf, two juveniles and an adult from both regions. Moderate, locally extensive, chronic eosinophilic parasitic myositis, associated with an adult *Crassicauda* sp. was found in the musculature next to the mammary gland in one *T. aduncus* adult female from Richards Bay, which also had *corpora amylacea* in the mammary gland (see above).

3.14. Skin and Subcutis

Complete pathological findings for the skin and subcutis, indicating occurrence per species and age group, are presented in Table E.10. All 40 (100%) animals had net marks (Figure D.15 G), particularly over the thorax, flippers, flukes and head. Moderate, multifocal, superficial epidermal ulcerations with no associated inflammatory reaction, were found at the attachment site of a barnacle *Xenobalanus globicipitis* (Figure D.15 A-B) in one juvenile female *T. aduncus.* Severe, locally extensive, chronic, granulomatous dermatitis and cellulitis were found in one adult male *S. chinensis*. The inflammation was associated with a large number of round fungal cells that stained positive on both GMS and PAS, consistent with lobomycosis (Figure D.15 C-D). A subcutaneous fibroma was found in a female *T. aduncus* calf from Richards Bay.

3.15. Organs of Special Senses

Complete pathological findings for the ear and eye, indicating occurrence per species, age group and region, are presented in Table E.11. Changes resembling Wallerian degeneration were present in 23 (66%) of eyes evaluated from both species, all ages, and all along the coast. Further staining of three animals with amyloid precursor protein (APP) revealed variable results. These were also stained with Bielschowsky´s stain, where all eosinophilic elements stained negative, with internal control staining positive. These structures were therefore concluded to be artefacts and of no significance.

Table 3.1. Selected pathology observed and differences in prevalence between *Tursiops aduncus* and *Sousa chinensis*

Lesion/abnormality	**Total (%)**	**Species (*n*)**		
		T. aduncus	***S. chinensis***	**p**
Pneumonia	93	32/35	5/5	1.000
Bronchopneumonia	18	7/35	0/5	0.565
Interstitial pneumonia	63	22/35	3/5	1.000
Bronchointerstitial pneumonia	30	9/35	3/5	0.149
Pulmonary parasites	15	6/35	0/5	1.000
Pleuritis	30	10/35	2/5	0.627
Pulmonary fibrosis	30	9/35	3/5	0.149
Bronchiolar mucosal calcification	83	29/35	4/5	1.000
Pulmonary anthracosis	8	2/35	1/5	0.338
Gastritis all compartments	68	24/34	2/4	0.577
Gastritis compartments 1 & 2	63	23/34	1/4	0.132
Gastritis compartment 3	65	14/21	1/2	1.000
Parasitic nodules all compartments	32	12/34	0/4	0.556
Parasitic nodules compartments 1 & 2	8	3/34	0/4	1.000
Parasitic nodules compartment 3	43	10/21	0/2	0.486
Pyloric mucosal calcification	26	5/21	1/2	0.462
Enteritis	68	25/35	2/5	0.307
Periportal hepatitis	54	21/35	0/4	**0.037**
Capsular hepatitis	23	9/35	0/4	0.556
Periportal fibrosis	26	9/35	1/4	1.000
Hepatic trematode eggs	8	3/35	0/4	1.000
Bile ductular hyperplasia	44	15/35	2/4	1.000
Splenic tag	45	17/35	1/5	0.355
Capsular splenitis	28	11/35	0/5	0.298
Cervical capsular lymphadenitis	26	10/34	0/5	0.302
Mesenteric capsular lymphadenitis	46	15/34	3/5	0.647

Table 3.1 (cont.)

Marginal capsular lymphadenitis	43	11/27	2/3	0.565
Marginal lymph node anthracosis	10	3/27	0/3	1.000
Eosinophilic myelopoiesis	71	21/28	1/3	0.195
Endometritis	42	10/24	1/2	1.000
Metritis	23	5/24	1/2	0.415
Oophoritis	19	4/24	1/2	0.354
Mastitis	43	3/7	-	-
Mammary corpora amylacea	43	3/7	-	-
Capsular orchitis	38	3/10	2/3	0.510
Endo-, myo- and epicarditis	51	20/35	0/4	**0.047**
Cardiac fibrosis	26	9/35	1/4	1.000
Adrenal cortical hyperplasia	49	15/33	3/4	0.340
Meningoencephalitis	39	7/16	0/2	0.497
Myositis	19	6/32	1/5	1.000
Combined capsular inflammation	75	26/35	4/5	1.000
Abdominal capsular lesions	60	20/35	4/5	0.631
Thoracic capsular lesions	20	18/35	2/5	1.000

Table 3.2. Selected pathology observed in *Tursiops aduncus* and bivariable associations with sex, age and region, with statistically significant results (p<0.100) in bold

Lesion/abnormality	Total (%)	Sex (*n*)			Age category (*n*)				Region (*n*)		
		Female	Male	p	<2 y	2-12 y	>12 y	p	North	South	p
Pneumonia	91	23/24	9/11	0.227	17/19	10/10	5/6	0.565	22/25	10/10	0.542
Bronchopneumonia	20	5/24	2/11	1.000	5/19	1/10	1/6	0.844	4/25	3/10	0.381
Interstitial pneumonia	63	**18/24**	**4/11**	**0.057**	10/19	8/10	4/6	0.365	14/25	8/10	0.259
Bronchointerstitial pneumonia	26	5/24	4/11	0.416	6/19	2/10	1/6	0.770	8/15	1/10	0.235
Pulmonary parasites	17	4/24	2/11	1.000	5/19	1/10	0/6	0.423	4/25	2/10	1.000
Pleuritis	29	**4/24**	**6/11**	**0.041**	6/19	4/10	0/6	0.246	7/25	3/10	1.000
Pulmonary fibrosis	26	7/24	2/11	0.685	**4/19**	**0/10**	**5/6**	**0.001**	7/25	2/10	1.000
Bronchiolar mucosal calcification	83	21/24	8/11	0.352	16/19	8/10	5/6	1.000	21/25	8/10	1.000
Pulmonary anthracosis	6	1/24	1/11	0.536	**0/19**	**0/10**	**2/6**	**0.025**	1/25	1/10	0.496
Enteritis	71	18/24	7/11	0.689	**9/19**	**10/10**	**6/6**	**0.002**	19/25	6/10	0.421
Gastritis all compartments	71	17/24	7/10	1.000	**10/19**	**8/9**	**6/6**	**0.034**	16/24	8/10	0.683
Gastritis compartments 1 & 2	68	16/24	7/10	1.000	**9/19**	**8/9**	**6/6**	**0.017**	16/24	7/10	1.000
Gastritis compartment 3	67	10/14	4/7	0.638	6/11	4/6	4/4	0.391	9/14	5/7	1.000
Parasitic nodules all compartments	29	8/24	4/10	1.000	**5/19**	**5/9**	**2/6**	**0.097**	6/24	6/10	0.431
Parasitic nodules compartments 1 & 2	6	3/24	0/10	1.000	2/19	1/9	0/6	1.000	2/24	1/10	0.508
Parasitic nodules compartment 3	48	6/14	4/7	0.659	4/11	4/6	2/4	0.620	6/14	4/7	0.659
Pyloric mucosal calcification	24	4/14	1/7	0.624	2/11	2/6	1/4	0.805	3/14	2/7	1.000
Periportal hepatitis	60	16/24	5/11	0.283	11/19	5/10	5/6	0.465	17/25	4/10	0.151
Capsular hepatitis	26	4/24	5/11	0.103	15/4	4/10	1/6	0.568	8/25	1/10	0.235

Table 3.2. (cont.)

Periportal fibrosis	26	6/24	3/11	1.000	**2/19**	**3/10**	**4/6**	**0.020**	6/25	3/10	0.694
Hepatic trematode eggs	9	2/24	1/11	1.000	**0/19**	**1/10**	**2/6**	**0.044**	2/25	1/10	1.000
Bile ductule hyperplasia	43	9/24	6/11	0.467	7/19	3/10	5/6	0.109	10/25	5/10	0.712
Splenic tag	49	11/24	6/11	0.725	7/19	6/10	4/6	0.345	**9/25**	**8/10**	**0.027**
Capsular splenitis	31	**4/24**	**7/11**	**0.015**	4/19	4/10	3/6	0.328	7/25	4/10	0.689
Cervical capsular lymphadenitis	29	**4/23**	**6/11**	**0.045**	4/18	3/10	3/6	0.405	6/24	4/10	0.431
Mesenteric capsular lymphadenitis	44	9/24	6/10	0.276	**5/19**	**5/10**	**5/5**	**0.009**	11/24	4/10	1.000
Marginal capsular lymphadenitis	41	8/18	3/9	0.692	6/13	4/8	1/6	0.525	6/19	5/8	0.206
Marginal lymph node anthracosis	11	2/18	1/9	1.000	0/13	2/8	1/6	0.124	1/19	2/8	0.201
Eosinophilic myelopoiesis	75	15/20	6/8	1.000	10/16	8/9	3/3	0.353	16/20	5/8	0.371
Endometritis	42	N/A	N/A	N/A	**4/13**	**2/7**	**4/4**	**0.044**	7/16	3/8	1.000
Metritis	21	N/A	N/A	N/A	**2/13**	**0/7**	**3/4**	**0.019**	3/16	2/8	1.000
Oophoritis	17	N/A	N/A	N/A	1/13	2/7	1/4	0.344	3/16	1/8	1.000
Mastitis	43	N/A	N/A	N/A	2/3	1/2	0/2	0.657	3/6	0/1	1.000
Mammary corpora amylacea	43	N/A	N/A	N/A	0/3	2/2	1/2	0.143	2/6	1/1	0.429
Capsular orchitis	30	N/A	N/A	N/A	1/5	1/3	1/2	1.000	3/8	0/2	1.000
Endo-, myo- and epicarditis	57	14/24	6/11	1.000	**11/19**	**8/10**	**1/6**	**0.060**	14/25	6/10	1.000
Cardiac fibrosis	26	5/24	4/11	0.416	**0/19**	**4/10**	**5/6**	**0.001**	7/25	2/10	1.000
Adrenal cortical hyperplasia	46	9/23	6/10	0.448	8/18	4/10	3/5	0.795	12/24	3/9	0.458
Meningoencephalitis	44	4/11	3/5	0.596	3/9	4/6	0/1	0.302	5/8	2/8	0.315
Myositis	19	5/21	1/11	0.637	**1/18**	**2/10**	**3/4**	**0.007**	6/23	0/9	0.150
Combined capsular inflammation	74	16/24	10/11	0.217	**11/19**	**9/10**	**6/6**	**0.092**	20/25	6/10	0.393
Abdominal capsular lesions	57	12/24	8/11	0.281	**7/19**	**7/10**	**6/6**	**0.013**	15/25	5/10	0.712
Thoracic capsular lesions	51	10/24	8/11	0.146	10/19	6/10	2/6	0.665	13/25	5/10	1.000

Table 3.3. Associations of age, sex and region with presence of various lesions in *Tursiops aduncus*: results of multivariable exact logistic regression models, with statistically significant results (p<0.100) in bold. OR= Odds ratio, 95% C.I. = 95% confidence interval.

Variable and level	Pleuritis			Pulmonary fibrosis			Pulmonary anthracosis			Enteritis		
	OR	95% C.I.	p	OR	95% C.I.	p	OR	95% C.I.	p	OR	95% C.I.	p
Age category												
Calf (<2 y)	1*	–	–	1*	–	–	1*	–	–	1*	–	–
Juvenile (2-12 y)	1.54	0.19, 13.65	0.952	0.33	0.00, 2.90	0.345	1.00	0.00,∞	–	**15.26**	**1.95, ∞**	**0.006**
Adult (>12 y)	0.26	0.00, 2.53	0.270	**13.79**	**1.20, 773.5**	**0.030**	**9.52**	**0.72, ∞**	**0.085**	**6.55**	**0.82, ∞**	**0.080**
Sex (male vs. female)	**6.50**	**0.98, 59.17**	**0.053**	0.35	0.01, 4.54	0.700	1.5	0.04,∞	0.800	0.33	0.02, 3.78	0.573
Region (south vs. north)	1.17	0.00, 11.33	1.000	0.95	0.04, 14.81	1.000	3	0.08,∞	0.500	0.17	0.00, 2.48	0.303

Table 3.3 (cont.)

Variable and level	Gastritis			Gastritis compartments 1&2			Periportal fibrosis			Splenic tag		
	OR	95% C.I.	p	OR	95% C.I.	p	OR	95% C.I.	p	OR	95% C.I.	p
Age category												
Calf (<2 y)	1*	–	–	1*	–	–	1*	–	–	1*	–	–
Juvenile (2-12 y)	5.66	0.57, 291.4	0.201	7.38	0.76, 376.8	0.104	3.02	0.30, 41.55	0.482	2.20	0.29, 18.27	0.607
Adult (>12 y)	**6.21**	**0.78, ∞**	**0.090**	**7.02**	**0.89, ∞**	**0.066**	**12.64**	**1.17, 223.9**	**0.033**	4.33	0.42, 67.24	0.300
Sex (male vs. female)	1.13	0.14, 9.89	0.141	1.36	0.17, 10.91	1.000	1.21	0.13, 9.89	1.000	2.01	0.33, 14.21	0.621
Region (south vs. north)	1.97	0.23, 26.05	0.785	1.09	0.12, 10.17	1.000	1.71	0.18, 15.94	0.884	**7.75**	**1.10, 99.82**	**0.037**

Table 3.3 (cont.)

Variable and level	Capsular splenitis			Cervical capsular lymphadenitis			Mesenteric capsular lymphadenitis			Endometritis		
	OR	95% C.I.	p	OR	95% C.I.	p	OR	95% C.I.	p	OR	95% C.I.	p
Age category												
Calf (<2 y)	1*	–	–	1*	–	–	1*	–	–	1*	–	–
Juvenile (2-12 y)	2.81	0.27, 40.96	0.553	1.42	0.13, 15.20	1.000	2.85	0.42, 23.36	0.377	0.92	0.07, 9.14	1.000
Adult (>12 y)	5.41	0.37, 117.1	0.307	4.77	0.36, 90.18	0.327	**16.82**	**1.92, ∞**	**0.009**	**8.10**	**0.87, ∞**	**0.067**
Sex (male vs. female)	**11.07**	**1.51, 152.0**	**0.012**	**7.42**	**1.04, 95.28**	**0.045**	3.56	0.53, 29.82	0.247	_	_	_
Region (south vs. north)	3.30	0.36, 45.61	0.408	3.90	0.45, 54.29	0.297	0.94	0.10, 7.54	1.000	0.92	0.07, 9.14	1.000

Table 3.3 (cont.)

Variable and level	Cardiac fibrosis			Myositis			Abdominal capsular inflammation		
	OR	95% C.I.	p	OR	95% C.I.	p	OR	95% C.I.	p
Age category									
Calf (<2 y)	1*	–	–	1*	–	–	1*	–	–
Juvenile (2-12 y)	**13.97**	**1.54,∞**	**0.017**	5.73	0.20, 470.3	0.473	4.05	0.63, 35.27	0.177
Adult (>12 y)	**51.63**	**5.35,∞**	**0.001**	**14.31**	**1.31,∞**	**0.029**	**11.18**	**1.37,∞**	**0.022**
Sex (male vs. female)	4.29	0.26, 280.2	0.498	0.26	0.00, 2.31	0.246	3.00	0.44, 26.62	0.362
Region (south vs. north)	0.71	0.04, 13.09	1.000	0.33	0.00, 3.55	0.381	0.73	0.08, 5.42	1.000

4. Discussion

4.1. Location, Age, Sex and Species

The predominance of *T. aduncus* catches over *S. chinensis* likely reflects the relative population sizes of the species, with *T. aduncus* individuals numbering more than *S. chinensis* (Cockcroft and Ross, 1990a; Cockcroft, *et al*., 1990). *Tursiops aduncus* were caught all along the coast, as described previously (Kaiser, 2012). This was attributed to the widespread distribution of *T. aduncus* along the coast (Cockcroft, *et al*., 1990). The fact that young calves and juveniles are more inquisitive by nature and appear more inexperienced around the nets (Peddemors, 1995) may explain why *T. aduncus* calves and juveniles were caught more often than adults. Females with calves also feed closer to shore, around the nets, which results in higher captures rates of adult females (Cockcroft and Ross, 1990b; Cockcroft, 1992) and of calves. All five *S. chinensis* were caught in the northern region, at Richards Bay and Zinkwazi. Previously, capture rates have been found to be proportional to the relative density of *S. chinensis* along the coast, with highest numbers recorded in the northern regions of their South African distribution (Durham, 1994; Atkins, *et al*., 2013). Consistent with other studies, three of the five were juvenile males, possibly due to sex/age segregation in the population and behavioural differences, with juvenile males thought to take more risks than other age and sex categories (Atkins, *et al*., 2013).

4.2. Nutritional Condition

All blubber thickness measurements were within the range previously published for *T. aduncus* from the KwaZulu-Natal coast (Young, 1998); increasing blubber thickness with age was also recorded in that study, which did not, however, record pathology as a factor that may affect blubber thickness measurements. Severe pathology has been correlated with smaller blubber thickness measurements in *T. truncatus* and *S. coeruleoalba* from the Northern (Fauquier, *et al*., 2010) and Southern (Duignan, 2003) hemispheres. No reference ranges or prior data are available for *S. chinensis* from the KwaZulu-Natal coast. No association between lower than average blubber thickness and pathology could be demonstrated in this study. Also, parasite-associated pathology was probably not sufficient to influence the functioning of the organs affected. This is supported by stomach content analysis of *T. aduncus*, which generally indicated recent feeding. None of the animals with the thinnest blubber had major or multiple significant lesions. The majority had pneumonia and other parasitic infections, but so did the animals with the thickest blubber. One calf with no pneumonia or any other major pathology had the second thinnest blubber. Therefore, we concluded that all animals were in good nutritional condition.

4.3. Generalised Changes

Autolysis and putrefaction varied between animals and organs, and likely masked subtle histological features such as acute necrosis, particularly in the respiratory and intestinal mucosae, pancreas, brain and retina. Five of the six animals that were too decomposed to sample were caught over the weekend, and extracted from the nets on the following Monday, resulting in marked carcass decomposition before freezing. The extent of macroscopic and histological autolysis was often at variance with the carcass composition score (Geraci and Lounsbury, 1993). Freezing artefacts frequently distorted histological tissue architecture, and caused lysis of erythrocytes, which made the presence of haemorrhage difficult to differentiate from congestion. Similarly, inflammatory cell lineage was not always distinguishable; eosinophils were relatively well preserved compared to other inflammatory cells. These changes were similar to findings in *Arctocephalus forsteri* (fur seals) from New Zealand (Roe, *et al*., 2012a). Despite these drawbacks, the presence and patterns of inflammation and parasites could be confidently diagnosed. In the northern hemisphere, *P. phocoena* (harbour porpoise) are frequently frozen prior to dissection, yet still yield valuable, reliable data (Siebert, *et al*., 1999; Siebert, *et al*., 2001; Siebert, *et al*., 2006).

The clear, round vacuoles in a variety of tissues and air emboli are possibly as a result of supersaturation (Moore, *et al*., 2009), with the histological location, absence of nuclei, and variable size excluding adipocytes. Gas vacuoles, produced by saprophytic bacteria, also seem less likely as no bacteria were associated with the vacuoles on HE or Gram stain. This could not be confirmed, however, without determining the content of the bubbles. Widespread tissue congestion was likely due to terminal cardiovascular events.

4.4. Respiratory System

Halocercus spp. are commonly recovered from the lungs of many dolphin species (Jauniaux, *et al*., 2002; Raga, *et al*., 2009), although the complete life cycle remains unknown. Lesions of bronchopneumonia associated with larvae were more severe around the free larvae than around the adults, a similar finding reported for harbour porpoises (Jauniaux, *et al*., 2002). Although eosinophilic pneumonia was found in 83% of animals, parasites were seen in only 20% of animals. Pulmonary verminosis was therefore much less common than reported for *T. truncatus* (77%) and *S. coeruleoalba* (76.5%) from the Northern Hemisphere (Cornaglia, *et al*., 2000; Fauquier, *et al*., 2010). Since eosinophilic infiltrations are considered synonymous with parasitic infections in marine mammals (Bossart, *et al*., 2001; Van Dijk, *et al*., 2007) one could conclude that the eosinophilic pneumonia, even in the absence of

parasites, was caused by parasites. Differences in dolphin ages, lung sampling locations or parasite collection could account for this disparity.

Similar to a study of *T. aduncus* in southwestern Florida (Fauquier, *et al*., 2010), parasites were recovered more often from calves than from juveniles, with no parasites recovered from adults, suggesting that infestation is established *in utero* or in the early neonatal period through milk ingestion (Raga, *et al*., 2009; Fauquier, *et al*., 2010). Adult animals more often showed only chronic or resolving infections. The variable presence of lymphocytes and plasma cells associated with the eosinophilic inflammation may indicate the presence of persistent foreign antigen despite clearance of the infestation in older animals and activation of the adaptive immune response (King, *et al*., 2001). The accompanying follicular lymphoid hyperplasia may support this possibility. Parasites likely also caused the pleuritis and tracheo-bronchitis, although there was no statistically significant association between the parasitic pneumonia and pleuritis or tracheo-bronchitis.

In domestic animals, interstitial pulmonary fibrosis is usually seen as a sequel to repetitive, persistent, or severe damage to the endothelial or epithelial cells, or inflammation of the alveolar septa (Caswell and Williams, 2007). In dolphins it has commonly been reported resulting from chronic morbillivirus (Domingo, *et al*., 1992; Lipscomb, *et al*., 1996; Kennedy, 1998) and parasitic infections (Jauniaux, *et al*., 2002; Fauquier, *et al*., 2010). No association between the fibrosis and pneumonia or pulmonary verminosis could be demonstrated in our animals. Although *S. chinensis* was affected more often than *T. aduncus,* this difference was not statistically significant. Similar to previous studies (Fauquier, 2010), the fibrosis was, however, associated with age (p=0.001), since older animals showed chronic or resolving lung lesions, mostly as a result of parasitic infections. Lungworms did not appear to affect feeding, and are therefore mostly of incidental importance, as was also found in *T. truncatus* from Florida (Fauquier, *et al*., 2010).

No histopathological evidence of bacterial pneumonia was found and no pathogenic bacteria were isolated from the lung during routine lung cultures, including those listed in Higgens (2000). *Aeromonas media*, *Shewanella* spp. and *Photobacterium damselae* are classified as opportunistic bacteria; the remainder were considered post mortal contaminants or normal commensals. *Shewanella algae* is commonly isolated from marine environments, and is an opportunistic human pathogen (Tsai, *et al*., 2008). *Photobacterium damselae* has been isolated from dolphin lungs secondary to morbillivirus infections (Keck, *et al*., 2010), and *Aeromonas* spp. has also been recovered from lungs as a secondary pathogen (Migaki, *et al*., 1990).

Mineralisation of the bronchiolar epithelium has previously been reported and attributed to lungworm infection (Woodard, *et al*., 1969; Zappulli, *et al*., 2005). Unfortunately, details of the lesions were often obscured by autolytic loss of bronchiolar epithelium. The mineral deposits occurred in all areas of the bronchial tree, from the bronchus to the terminal bronchioles and stained variably positive with von Kossa stain. Deposits were birefringent on Gram stain, but the significance of this is unclear. Foreign particles accumulate in the lung due to the inability of dolphins to cough. These particles become inspissated, undergo dystrophic calcification and are later incorporated into the bronchial wall (Woodard, *et al*., 1969).

The clear distended air spaces in the lung are most likely acute pulmonary emphysema as there were no bacteria seen associated with the alveolar distension. Pulmonary oedema and emphysema were likely agonal changes associated with asphyxiation and were consistent with similar findings in other net captured cetaceans (Kuiken, *et al*., 1994; Siebert, *et al*., 2001; Duignan, 2003). In terrestrial animals pneumoconiosis is relatively rare, and due to the inhalation of polluted inorganic substances, such as carbon, silica or asbestos (Caswell and Williams, 2007; Valli, 2007). The nature of the pigment found in this study was not determined. Inhalation of smoke from burning sugar cane, farmed extensively on the KwaZulu-Natal coast, could have been responsible for the pneumoconiosis seen in some dolphins. Further research with a larger sample size and climate factors such as wind direction could shed more light on the epidemiology of this lesion and its relevance to human populations in the area. Pneumoconiosis was seen mostly in adults, presumably due to the accumulation of foreign material over time.

4.5. Gastrointestinal Tract

Sarcocysts have not previously been reported in dolphins from South African waters, although they have been reported in other cetacean populations, and are considered an incidental finding (Daily and Stroud, 1978; Munday, *et al*., 1978; Resendes, *et al*., 2002; Dubey, *et al*., 2003; Raga, *et al*., 2009; Lehnert, *et al*.). The definitive host is still unknown.

The eosinophilic nature of the gastroenteritis suggests that this was associated with parasites, and parasites from the families Brachycladiidae and Anisakidae were recovered from the intestinal tract. Parasites within the lumen of the gastrointestinal tract were recovered from only three animals; the reason for the discrepancy between recovered parasites and eosinophilic gastroenteritis is unknown, but is possibly due to sampling methodology or the degradation of the parasite as a result of autolysis, freezing and thawing. In *T. aduncus,* the gastroenteritis was significantly associated with age, with up to 100% of

juveniles and adults affected. Nematodes belonging to the family Anisakidae have an indirect life cycle, with animals ingesting infective larvae while feeding on infected fish and squid (Raga, *et al*., 2009), so calves are only infected once they start eating fish and squid. Eosinophilic gastroenteritis appeared to have no effect on the animals, since none of the animals with severe lesions had thinner blubber than less affected animals.

Parasitic nodules are common incidental findings in the stomachs of dolphins, most commonly associated with *Pholeter gastrophilus* (Geraci and St. Aubin, 1987; Aznar, *et al*., 2006; Raga, *et al*., 2009). The nodules found in the stomach in this study were associated with trematodes from the family Brachycladiinae. Identification to the genus or species level was not possible owing to advanced autolysis of the parasite. A definite predilection for the pyloric compartment was found, and it also appears that the submucosal parasite may have induced mucosal calcification. The nodules and associated parasites were only found in *T. aduncus*. The palatine hyperplasia was of unknown aetiology and probably an incidental finding.

Parasites, including trematodes, were also the most likely cause of the hepatitis and periportal hepatitis in *T. aduncus* (Geraci and St. Aubin, 1987; Jauniaux, *et al*., 2002; Raga, *et al*., 2009). The trematodes were too autolysed to be identified in this study. The trematodes *Campula*, *Oschmarinella*, and *Brachycladium* (formerly *Zalophotrema*) have been found in hepatic ducts (Geraci and St. Aubin, 1987; Cribb, 1998; Raga, *et al*., 2009). The life cycle of these brachycladiids is not known, although fish and squid species ingested by dolphins are thought to act as intermediate or paratenic hosts (Raga, *et al*., 2009). Hepatic inflammation was not sufficient in any animal to impair hepatic function.

4.6. Lympho-haemopoeitic System

Splenic tags and/or capsular splenitis were found in 45% of animals and were significantly associated in *T. aduncus*. Similar, mostly eosinophilic or fibrotic, capsular lesions were found in the mesenteric, cervical, and marginal lymph nodes of the lung; testis and epididymis; kidney and bladder; peritoneum; liver; and diaphragm and correspond to the abdominal serosal lesions that were reported to have increased in prevalence in 2009. These incidental findings may result from parasite migrations, since parasite larvae were found in one mesenteric lymph node, and necro-granulomatous inflammation was found in another mesenteric lymph node. Similar capsular lesions have been found on the liver, peritoneum and diaphragm in horses resulting from *Strongylus equinus* migrations (Brown, *et al*., 2007). In *T. aduncus* splenic tags were significantly more common in dolphins from the southern region. The southern subpopulation migrates as far south as Algoa Bay (Natoli, *et al*., 2008)

and has a different diet to the northern subpopulation (Kaiser, 2012). These results suggest altered exposure to parasites, but since the diet has remained largely unchanged in the last decade (Kaiser, 2012), the cause of this altered exposure in unknown. No association between capsular lesions and pulmonary verminosis, hepatic trematode eggs or gastric trematodes was found. Dietary preference may explain the higher prevalence in male *T. aduncus.* Although no significant resource partitioning between sexes of either *T. aduncus* or *S. chinensis* has been found (Cockcroft and Ross, 1990b; Venter, 2009; Kaiser, 2012), *T. aduncus* males eat a larger proportion of mackerel than do *T. aduncus* females (Kaiser, 2012). The complete results of the dietary analysis from these dolphins are not yet available. Research into the identity of the parasites causing these serosal lesions, and their life cycles, is needed.

The exact identification and function of the cuboidal acini next to the thymus is uncertain, although positive staining with chromogranin A indicates a neuro-endocrine origin (Charles, 2007), therefore possibly pointing towards paraganglia with the location consistent with the cervical sympathetic chain (*pers. comm. MF Sidworthy)*. Bone marrow eosinophilic haematopoiesis likely reflects the widespread parasitism in these dolphins.

4.7. Reproductive System

The eosinophilic oophoritis, endometritis and metritis were also probably caused by parasites, supported by the trematode egg present in one case. The positive association with age (up to 100 % of adult animals) suggests an indirect life cycle. Fertility would be significantly affected by oophoritis or endometritis. Since a pregnant female had mild metritis, this lesion alone may not impair fertility.

Mastitis and the *corpora amylacea* found in the dilated ducts of the mammary gland were seen mainly in calves and juveniles. This is in contrast to bovine mammary glands, where mastitis and *corpora amylacea* are often encountered, but are rare in primiparous cows and heifers (Reid, 1972; Claudon, *et al*., 1997; Claudon, *et al*., 1998).The cause of the mastitis could not be determined. *Corpora amylacea* in cows are composed of milk proteins, variable amounts of calcium and amyloid, and are similar in appearance to those reported here. The pathogenesis is unclear, although it is postulated that a protein nidus becomes calcified (Reid, 1972; Claudon, *et al*., 1998). Small numbers may be considered normal and incidental (Claudon, *et al*., 1997), although the large numbers seen in one adult *T. aduncus* may have been due to obstruction of milk outflow due to severe parasitic myositis in the adjacent muscle.

4.8. Cardiovascular System

No aetiological agent, including toxoplasmosis, could be demonstrated associated with the cardiac inflammation. Other possibilities include viral infections and resolving bacterial, fungal and parasitic infections (King, *et al*., 2001). The inflammation only affected *T. aduncus,* which may indicate an aetiological agent only present in the *T. aduncus* population, although the sample size for *S. chinensis* was very low.

Myocardial fibrosis may be a result of repair of any prior tissue damage such as inflammation or necrosis. Persistently high catecholamine levels, secondary to chronic stress and/or severe debilitating disease and very poor nutritional condition have been seen in *T. truncatus* and *S. coeruleoalba* from the Gulf of Mexico (Turnbull and Cowan, 1998). Severe debilitating disease, resulting in cachexia, was not observed in any animals in this study, and no association between cardiac fibrosis and adrenal cortical hyperplasia could be found. The association of cardiac fibrosis with age may indicate that the effects are cumulative. No association could be shown between the cardiac fibrosis and myocarditis, which may exclude myocarditis as a possible cause. However, the small sample size precludes definitive conclusions. Prior exposure to domoic acid from toxic algal blooms have been associated with cardiac fibrosis in *Phoca vitulina richardsi* (Pacific harbour seal) (McHuron, *et al*., 2013) and *Zalophus californianus* (California sea lions) (Gulland, 2000; Zabka, *et al*., 2009). However, no recent toxic algal blooms have been reported off the South African east coast. Arteriosclerosis has been previously reported in dolphins (Duignan, 2003) and does not appear to affect the functioning of the heart.

4.9. Endocrine System

Adrenal cortical hyperplasia may be the result of pituitary or adrenal neoplasia or stress, (Clark, *et al*., 2006; Newman and Smith, 2006). Dolphin adrenal glands have been shown to enlarge throughout life, but maintain a relatively constant corticomedullary ratio of 2:1 (Clark, *et al*., 2006). Although adrenal tumours have only been reported in *T. truncatus* (Newman and Smith, 2006), they are usually unilateral and presumably uncommon, which makes neoplasia unlikely in these animals since the hyperplastic changes were seen in both adrenals of affected animals and no clear neoplastic lesions were seen. Chronic stress, often associated with disease, has been shown to result in cortical hyperplasia in dolphins (Clark, *et al*., 2006). Almost half the animals (49%) in this study showed adrenal cortical hyperplasia, suggesting that a large proportion of the population may be at least mildly stressed. However, stress hormone assays, adrenal weights and objective adrenal cortico-medullary ratios by point-counting techniques (Clark, *et al*., 2006) were not done. Due to the

small numbers of *S. chinensis* sampled, the significance of higher proportion of animals with adrenal hyperplasia is uncertain. No major debilitating disease was found in these animals, but environmental stressors, such as competition for resources, and anthropogenic factors, such as boat traffic, are difficult to evaluate and may play a role.

4.10. Central Nervous, Urinary and Musculoskeletal Systems

Lymphoplasmacytic meningoencephalitis may be associated with viral infections, protozoal infections, e.g. *Toxoplasma gondii*, and resolving bacterial, fungal and parasitic infections (King, *et al*., 2001). No aetiology could be found, even though special stains and immunohistochemical testing for dolphin morbillivirus, *Toxoplasma gondii*, and *Brucella* spp. were performed. Acute haemorrhage surrounding the spinal cord and the cerebellar herniation were possibly a result of trauma associated with extreme struggling after being caught in the shark nets, although this has not been previously reported.

Apart from capsular and mural nephritis and diaphragmatic myositis, discussed above, changes in the urinary tract and musculoskeletal system were mainly non-specific.

4.11. Skin and Subcutis

The net marks evident on all animals can be expected in incidentally caught animals. The barnacle, *Xenobalanus globicipitis,* is a common incidental external parasite of dolphins (Geraci and St. Aubin, 1987; Kane, *et al*., 2008; Raga, *et al*., 2009).

This, to our knowledge, is the first confirmed report of lobomycosis in South African waters, although lobomycosis-like disease, based on macroscopic lesions, has been documented in other Indian Ocean populations of *T. aduncus* (Kiszka, *et al*., 2009). As the organism has not been cultured *in vivo*, the diagnosis was dependent on identification of the characteristic yeast like cells and granulomatous inflammation in the skin and subcutis (Higgens, 2000; Reif, *et al*., 2006; Van Bressem, *et al*., 2009a; Bossart, 2011). Impairment of adaptive immunity, possible related to chronic exposure to environmental stressors, was found only in endemically affected *T. truncatus* from the Indian River Lagoon, Florida (Reif, *et al*., 2009). Toxicological analyses are still pending for the dolphins incidentally caught in the shark nets off the KwaZulu-Natal coast. Lobomycosis is considered a zoonotic disease (Waltzek, *et al*., 2012), and the finding of it in a dolphin from the South African east coast highlights the need for safety considerations when handling any organic material. A fibroma has been reported from the mouth of a dolphin (Newman and Smith, 2006), and appears to be incidental.

4.12. Health Assessment and General Discussion

Parasites accounted for the majority of lesions seen, as expected in free-ranging wild animals. These lesions were generally mild when compared to those described in other health investigations (Siebert, *et al*., 2001; Jauniaux, *et al*., 2002; Duignan, 2003; Siebert, *et al*., 2006). However, a direct comparison may not be valid as most other health assessments evaluated stranded animals which usually show more severe pathology than incidentally caught animals (Jauniaux, *et al*., 2002). Also, parasite-associated pathology was probably not sufficient to influence the functioning of the organs affected. Stomach contents of *T. aduncus* generally indicated recent feeding, which would support this. Stomachs of *S. chinensis* are usually empty when caught as they do not really feed around the nets (Cockcroft and Ross, 1990b).

Capsular hepatitis, hepatic trematode eggs, periportal hepatitis, gastric parasitic nodules, capsular splenitis, cervical capsular lymphadenitis, pulmonary parasites and bronchopneumonia, all attributed to parasites, were only found in *T. aduncus* and not in *S. chinensis*. This may, however, be a result of the small sample size of *S. chinensis*. Alternatively, the parasites that cause these lesions could only affect *T. aduncus* and not *S. chinensis*. This may be related to the diets of the two dolphin species, since the nematodes, trematodes and cestodes reported here are thought to use fish and squid as intermediate or paratenic hosts (Raga, *et al*., 2009). The diets of *T. aduncus* and *S. chinensis* off the KwaZulu-Natal coast differ somewhat, although *Pomadasys olivaceum* (pinky grunter) makes up a significant portion of both species' diet based on the index of relative importance. Of the 94 prey species recorded in *T. aduncus* and 54 prey species in *S. chinensis*, 25 fish and squid species are eaten by both *T. aduncus* and *S. chinensis* (Venter, 2009; Kaiser, 2012). If the intermediate host is mainly or only preyed on by *T. aduncus,* it could account for the absence of these specific lesions in the *S. chinensis* population. A full parasitic analysis of dolphins off the South African coast, including the investigation of intermediate hosts, could shed more light on parasites affecting the population, and could potentially aid in elucidating life cycles and specific lesion-parasite associations.

Our results provide evidence of at least mild chronic stress in the dolphins incidentally caught in the shark nets. However, the nature of this stress is unknown. Death would have occurred too rapidly for the capture to have caused adrenal hyperplasia. The degree of disease found in these animals is unlikely to have caused stress, compared to the severity of disease seen in stressed *T. truncatus* and *S. coeruleoalba* in the Gulf of Mexico (Turnbull and Cowan, 1998; Clark, *et al*., 2006). Environmental stressors, possibly related to anthropogenic factors, may have played a role. More comprehensive testing including stress

hormone assays, adrenal weights and objective adrenal cortico-medullary ratios by point-counting techniques could confirm the adrenal hyperplasia. In addition, continued monitoring may determine temporal changes in these lesions over time, and a larger sample size may allow spatial evaluations, which could aid in elucidating the possible stress factors.

Apart from the splenic capsular tags being found more commonly in animals from the southern region, no other association between lesions and regions were found. Immunohistochemical tests for key cetacean pathogens, including cetacean morbillivirus, brucellosis or toxoplasmosis, were negative. Lack of pathogen detection may indicate that these are naïve populations. In addition, the tests used may not have been sensitive or specific enough to detect the various agents. Furthermore, as immunohistochemistry relies on the presence of antigen, the antigen could have been absent, with the resulting inflammation still present. The immunohistochemical stain used to detect morbillivirus antigen in suspected lesions has been used with success in *Phoca vitulina* (harbour seal) (Stimmer, *et al*., 2010) and *S. coeruleoalba* (as a positive control) (Siebert, *et al*., 2001). Commercially available immunohistochemical stains against *T. gondii* antibodies have been used effectively to detect *T. gondii* in associated lesions in dolphins (Di Guardo, *et al*., 2010). However, the specificity and sensitivity of the immunohistochemistry tests used is unknown. Poor fixation and degradation of tissue samples can also account for the tests being negative. Because of the widespread global prevalence of these diseases further studies are needed to clarify the status of these diseases in southern African dolphins. Reliable detection of infectious agents present at low prevalences can only be accomplished by testing larger numbers of individuals. The modified ZN (Stamp) stain is an accepted method of demonstrating *Brucella* spp. organisms in tissues (Alton, *et al*., 1975; Foster, *et al*., 2002), but only microbiological culture and biotyping would confirm the diagnosis. In future, bacterial culture of known target organs, even in the absence of macroscopic lesions, may be warranted.

Sero-epidemiological surveys of *Brucella* spp. and *T. gondii* would help to confirm the status of these diseases in the population off the KwaZulu-Natal coast. Cetacean morbillivirus antibodies were found in a stranded *D. delphinus* from East London (Van Bressem, *et al*., 2001), approximately 350 km south of the study area. Regrettably, no pathological information is available for this animal and paired serum samples could not be taken to confirm active infection. Antibody detection would confirm the naivety of the population (Van Bressem, *et al*., 2001; Dubey, *et al*., 2005) and would be a fruitful research area, although the practicalities of obtaining suitable serum from live and incidentally caught animals are significant hurdles. If these dolphin populations are in fact naïve to these pathogens, their

introduction could have devastating consequences, as has been documented previously in other populations elsewhere during morbillivirus epidemics (Domingo, *et al*., 1992; Calzada, *et al*., 1994; Lipscomb, *et al*., 1994; Lipscomb, *et al*., 1996; Di Guardo, *et al*., 2005; Van Bressem, *et al*., 2009a; Keck, *et al*., 2010). Stress and toxins, both of which were detected in the population, could further undermine the immune responses and exacerbate the situation. The results of a separate related study on toxin levels in dolphins are not yet available. We do report the first confirmed cases of lobomycosis and sarcocystosis in dolphins from the South African coast.

4.13. Conclusion

While optimum samples are not provided by frozen, incidentally caught animals, this study still yielded valuable information on the current prevalence of disease in the population, which can be used as a baseline for future monitoring projects, not only of the health status of the population, but also that of the environment. These findings further highlight the importance of disease investigation in marine mammals.

Chapter 4. Conclusions

Factors associated with degradation of the marine environment, such as pollution (Harvell, *et al*., 1999), habitat degradation (Geraci and Lounsbury, 2009), and depletion of prey (Lafferty, *et al*., 2004; Geraci and Lounsbury, 2009), as well as stress resulting from other anthropogenic impacts (Geraci and Lounsbury, 2009) may cause cetaceans to become more susceptible to disease (Harvell, *et al*., 1999; Lafferty, *et al*., 2004). In addition, dolphins have long life spans, feed at a high trophic level, and have extensive fat stores that can accumulate pollutants. They are therefore ideal species to use as sentinels for the marine environment (Reddy, *et al*., 2001; Wells, *et al*., 2004). Monitoring for disease in sentinel species can provide valuable information to monitor temporal trends of environmental change (Bossart, 2006). An increase in the prevalence of diseases has been observed worldwide in studies of cetacean populations (Epstein, *et al*., 1998; Harvell, *et al*., 1999). However, due to the lack of baseline data on the health status of coastal dolphins off South Africa, inadequately trained personnel and a general lack of facilities, diseases present in the dolphin populations have remained unidentified, and temporal trends have remained undetermined. We provide here a baseline assessment of the present health status of incidentally caught *T. aduncus* and *S. chinensis* from the KwaZulu-Natal coast, which may be used to monitor future temporal trends.

A further complicating factor to disease investigation is that many different people or research groups may be responsible for collecting data from carcasses. A major difficulty in using such data is that the results are seldom directly comparable (Norris, 1961). For this reason, a standardized necropsy protocol was developed and tested for the South African context. This protocol yielded diagnostic samples, even for dolphins frozen for up to 12 months before necropsy. Furthermore, the application of this protocol in incidentally caught animals highlighted the importance of a comprehensive necropsy and sample collection, as many lesions were only found on histopathology, with no gross abnormalities seen. It is expected that requirements may change over time as new evidence emerges, such as the detection of antibody titres to specific agents. Any protocol should therefore be adapted to such changing circumstances (Raverty and Gaydos, 2004).

We report the diagnosis of the first confirmed cases of lobomycosis and sarcocystosis in dolphins from the South African coast. Lobomycosis may be associated with significant concurrent impairment of the adaptive immune response (both humoral and cell mediated

components) in dolphins and humans, with a decrease in circulating lymphocyte counts (Reif, *et al*., 2009). The immune status of these dolphins was not determined, but both environmental contaminants (such as mercury and polychlorinated biphenyls) and chronic stress as a result of anthropogenic factors, have been suggested (Reif, *et al*., 2006; Reif, *et al*., 2009). Specific testing of the adaptive immune response as done by Reif *et al*. (2009) was not possible in our study, but should be attempted in future studies.

We present evidence indicating that the dolphins incidentally caught in the shark nets may have been stressed, with about half the population showing diffuse or nodular adreno-cortical hyperplasia. Environmental stressors related to anthropogenic factors could be related to the heavy ship traffic along the KwaZulu-Natal coast, overfishing or chemical pollutants. Continued monitoring of lesions that may be related to stress is essential to determine temporal changes, and a larger sample size may allow more detailed spatial evaluations. The determination and monitoring of blood or faecal cortisol levels, adrenal weights and objective adrenal cortico-medullary ratios by a point-counting technique (Clark, *et al*., 2006) may aid in confirming the status of stress in the population. These results, combined with the toxicological studies, would aid in elucidating possible stress factors.

The southern Indian Ocean has traditionally been thought to contain relatively low contaminant levels, although recent studies have shown very high concentrations of chemical pollutants in dolphins off the coast of Brazil (Lailson-Brito, *et al*., 2012). Current contaminant levels are unknown for cetaceans off the coast of South Africa, although *T. aduncus* and *S. chinensis* have historically been shown to contain high contaminant levels (Cockcroft, *et al*., 1991; Cockcroft, 1999). Furthermore, recent studies off Durban indicated high concentrations of pathogenic organisms related to sewage entering the marine environment (Mardon and Stretch, 2004). Examining the coastal dolphin populations for lobomycosis can be a valuable monitoring tool, as an increase in the prevalence of lobomycosis could be an indication of environmental degradation or an indication of stress (Van Bressem *et al.,* 2009a). Monitoring should strive to determine a definitive diagnosis of all skin lesions by histology, as macroscopic lesions, even if documented by photographs, cannot be used to make definitive diagnoses.

All 40 animals evaluated had lesions that were related to parasites, and the lesions that were observed to be on the increase in 2009 were also of parasitic origin. This observed increase may be attributed to better systematic evaluation, although neither the dissecting protocol nor the personnel performing the dissections changed during this time. Alternatively, the increase may be due to changing prey composition and parasite exposure of the populations. The majority of parasites have little effect on the host, causing mainly minor

parasite associated inflammation and not severe disease, but growing evidence suggests that compromise of the host's immune system leads to an increase in the severity and prevalence of parasite associated diseases (Siebert, *et al*., 1999). Parasite burdens may then be used as indicators of the overall health status of the individual (Siebert, *et al*., 1999; Torchin, *et al*., 2002; Raga, *et al*., 2009). This assumption should, however, be used with caution, as some environmental factors, in particular pollution, may also negatively affect the parasite populations (Torchin, *et al*., 2002). Health assessments should therefore monitor multiple variables in the population and not focus on a single indicator.

Further research into parasite identification and quantification as well as into the cause of death in stranded as well as incidentally caught dolphins is essential to develop valuable information regarding parasite burdens, and the effect of these on the host animal.

Bronchiolar mucosal calcification has been described associated with lungworm infections (Woodard, *et al*., 1969), although mineralisation was not a common feature of verminous pneumonia in other studies (Siebert, *et al*., 2001; Jauniaux, *et al*., 2002; Fauquier, *et al*., 2010).

Testing for brucellosis, toxoplasmosis, and cetacean morbillivirus by immunohistochemistry was negative in suspected lesions, which may indicate the absence of these diseases. Alternatively, the testing may not have been sensitive enough to detect these agents or the prevalence was too low to detect with the tests used and the small sample size. Routine testing for specific antibodies against *Brucella* spp., *Toxoplasma gondii* and cetacean morbillivirus would be necessary to confirm the naivety of the population (Van Bressem, *et al*., 2001).

Brucellosis and toxoplasmosis are zoonotic diseases, posing a risk to animals and humans alike (Waltzek, *et al*., 2012). Brucellosis is a naturally occurring infection in marine mammals and endemic in some populations, with pathology often absent (Ross, *et al*., 1996; Van Bressem, *et al*., 2009a). The role of environmental factors in the emergence of marine mammal brucellosis is yet to be determined (Van Bressem, *et al*., 2009a). Toxoplasmosis is generally asymptomatic in dolphins (Dubey, *et al*., 2003; Forman, *et al*., 2007). Most dolphins that die of toxoplasmosis show below average body condition, have concurrent disease, or are for some reason immunosuppressed, potentially as a result of stress or morbillivirus infection (Migaki, *et al*., 1990; Bowater, *et al*., 2003; Dubey, *et al*., 2003; Forman, *et al*., 2007). Therefore, the prevalence of clinical toxoplasmosis may be useful in monitoring population health trends. Although stress may have been present in these dolphins, they did not have confirmed evidence of disease including morbillivirus infection and were in good nutritional condition. Cetacean morbillivirus can have a devastating effect

on naïve populations (Van Bressem *et al.*, 2009a), and can be of particular concern for endangered species.

Although identification of the material causing the pneumoconiosis was not possible, inhalation is the likely source of the foreign material, which could indicate air pollution. Although it did not appear to affect the health of the affected individual animals, it does suggest that these animals had been exposed to environmental air pollution, the origin and extent of which is currently unknown.

Although aspects of nutritional condition have previously been explored in dolphin species off the South African coast (Young, 1998), concurrent health investigation was not undertaken. Therefore, the degree to which animals may be affected by disease, and the effect that this may have on the nutritional condition, has not been determined. Further determination of blubber thickness measurements in incidentally caught animals as well as in stranded animals, with concurrent pathological investigations, will elucidate associations between disease and nutritional condition, and will greatly aid in interpretation of blubber thickness measurements and the assessment of nutritional condition.

In conclusion, this project yielded valuable information on the current health status of the incidentally caught dolphins off the KwaZulu-Natal coast. Furthermore, these dolphins provide a unique opportunity to evaluate individuals thought to be representative of the general population, whereas stranded dolphins may present a biased view of population health. This will be useful in monitoring the temporal and spatial changes in the health status of the population and may indicate changes in the health of the environment. These findings highlight the importance of a standardized necropsy and sampling protocol for disease investigation in marine mammals.

References

Aguilar, A., Borrell, A., 1994. Abnormally high polychlorinated biphenyl levels in striped dolphins (*Stenella coeruleoalba*) affected by the 1990–1992 Mediterranean epizootic. Sci. Total Environ. 154, 237-247.

Alex, A., Raga, J.A., 1993. The striped dolphin epizootic in the Mediterranean Sea. Ambio 22, 524-528.

Alexander, J.W., Solangi, M.A., Riegel, L.S., 1989. Vertebral osteomyelitis and suspected diskospondylitis in an Atlantic bottlenose dolphin (*Tursiops truncatus*). J. Wildl. Dis. 25, 118-121.

Alton, G.G., Jones, L.M., Pietz, D.E., 1975. Laboratory techniques in brucellosis. World Health Organization, Geneva. pp 1-80.

Atkins, S., Cliff, G., Pillay, N., 2013. Humpback dolphin by-catch in the shark nets in KwaZulu-Natal, South Africa. Biol. Conserv. 159, 442-449.

Aznar, F.J., Fognani, P., Balbuena, J.A., Pietrobelli, M., Raga, J.A., 2006. Distribution of *Pholeter gastrophilus* (digenea) within the stomach of four odontocete species: The role of the diet and digestive physiology of hosts. Parasitology 133, 369-380.

Bar, K., Slooten, E., 1999. Effects of tourism on dusky dolphins at Kaikoura. Conservation Advisory Science Notes 229, 5-10.

Barrett, T., Blixenkrone-Moller, M., Di Guardo, G., Domingo, M., Duignan, P., Hall, A.J., Hall, A.J., Mavaev, L., Osterhaus, A.D.M.E., 1995. Morbilliviruses in aquatic mammals: Report on round table discussion. Vet. Microbiol. 44, 261-265.

Bennett, P.M., Jepson, P.D., Law, R.J., Jones, B.R., Kuiken, T., Baker, J.R., Rogan, E., Kirkwood, J.K., 2001. Exposure to heavy metals and infectious disease mortality in harbour porpoises from England and Wales. Environ. Pollut. 11, 33-40.

Benson, K.A.S., Manire, C.A., Ewing, R.Y., Saliki, J.T., Townsend, F.I., Ehlers, B., Romero, C.H., 2006. Identification of novel alpha- and gammaherpesviruses from cutaneous and mucosal lesions of dolphins and whales. J. Virol. Methods 136, 261-266.

Best, P.B. (Ed.), 2007. Whales and Dolphins of the Southern African Subregion. Cambridge University Press, Cape Town. pp 167-187.

Blanchard, T.W., Santiago, N.T., Lipscomb, T.P., Garber, R.L., McFee, W.E., Knowles, S., 2001. Two novel alphaherpesviruses associated with fatal disseminated infections in Atlantic bottlenose dolphins. J. Wildl. Dis. 37, 297-305.

Böck, P., Romeis, B., 1989. Romeis Mikroskopische Technik. Urban and Schwarzenberg, Munchen, Germany. pp 1-439.

Bossart, G.D., 2006. Marine mammals as sentinel species for oceans and human health. Oceanography 19, 134-137.

Bossart, G.D., 2011. Marine mammals as sentinel species for oceans and human health. Vet. Pathol. 48, 676-690.

Bossart, G.D., Reidarson, T.H., Dierauf, L.A., Duffield, D.A., 2001. Clinical Pathology. In: Dierauf, L.A., Gulland, F.M.D. (Eds.), CRC Handbook of Marine Mammal Medicine. CRC Press Inc, Boca Raton, pp. 383-436.

Bourg, G., O'Callaghan, D., Boschiroli, M.L., 2007. The genomic structure of *Brucella* strains isolated from marine mammals gives clues to evolutionary history within the genus. Vet. Microbiol. 125, 375-380.

Bowater, R.O., Norton, J., Johnson, S., Hill, B., O'Donoghue, P., Prior, H., 2003. Toxoplasmosis in Indo-Pacific humpback dolphins (*Sousa chinensis*), from Queensland. Aust. Vet. J. 81, 627-632.

Brown, C.C., Baker, D.C., Barker, I.K., 2007. Peritoneum and Retroperitoneum. In: Maxie, M.G. (Ed.), Jubb, Kennedy & Palmer's Pathology of Domestic Animals (Fifth Edition). Elsevier, Edinburgh, pp. 279-296.

Buck, J., Shepard, L., Spotte, S., 1987. *Clostridium perfringens* as the cause of death of a captive Atlantic bottlenose dolphin (*Tursiops truncatus*). J. Wildl. Dis. 23, 488-491.

Burdett, L.G., Osborne, C.A., 2010. Enterolith with a stingray spine nidus in an Atlantic bottlenose dolphin (*Tursiops truncatus*). J. Wildl. Dis. 46, 311-315.

Busbee, D., Tizard, I., Stott, J., Ferrick, D., 1999. Environmental pollutants and marine mammal health: The potential impact of hydrocarbons and halogenated hydrocarbons on immune system dysfunction. J. Cetac. Res. Manage. 1, 223-248.

Byard, R., Winskog, C., Machado, A., Boardman, W., 2012. The assessment of lethal propeller strike injuries in sea mammals. J. Forensic Leg. Med. 19, 158-161.

Calzada, N., Lockyer, C., Aguilar, A., 1994. Age and sex composition of the striped dolphin die-off in the western Mediterranean. Mar. Mammal Sci. 10, 299-310.

Caswell, J.L., Williams, K.J., 2007. Lungs. In: Maxie, M.G. (Ed.), Jubb, Kennedy & Palmer's Pathology of Domestic Animals (Fifth Edition). Elsevier, Edinburgh, pp. 540-575.

Cates, M.B., Kaufman, L., Grabau, J.H., Pletcher, J.H., Schroeder, J.P., 1986. Blastomycosis in an Atlantic bottlenose dolphin. J. Am. Vet. Med. Assoc. 189, 1148-1150.

Charles, J.A., 2007. Pancreas. In: Maxie, M.G. (Ed.), Jubb, Kennedy & Palmer's Pathology of Domestic Animals (Fifth Edition). Elsevier, Edinburgh, pp. 389-424.

Clark, L.S., Cowan, D.F., Pfeiffer, D.C., 2006. Morphological changes in the Atlantic bottlenose dolphin (*Tursiops truncatus*) adrenal gland associated with chronic stress. J. Comp. Pathol. 135, 208-216.

Claudon, C., Francin, M., Delabarre, S., Laurent, F., 1997. Structure and composition of *corpora amylacea* in the bovine mammary gland. Livest. Prod. Sci. 50, 173-174.

Claudon, C., Francin, M., Marchal, E., Straczeck, J., Laurent, F., Nabet, P., 1998. Proteic composition of *corpora amylacea* in the bovine mammary gland. Tissue Cell 30, 589-595.

Cockcroft, V.G., De Kock, A.C., Lord, D.A., Ross, G.J.B., 1989. Organochlorines in bottlenose dolphins *Tursiops truncatus* from the east coast of South Africa. S. Afr. J. Mar. Sci. 8, 207-217.

Cockcroft, V.G., Ross, G.J.B., Peddemors, V.M., Borchers, D., 1992. Estimates of density and undercounting of bottlenose dolphins off northern Natal, South Africa. S. Afr. J. Wildl. Res. 22, 102-109.

Cockcroft, V.G., 1992. Incidental capture of bottlenose dolphins (*Tursiops truncatus*) in shark nets: An assessment of some possible causes. J. Zool. 226, 123-134.

Cockcroft, V.G., 1994. Is there common cause for dolphin captures? A review of dolphin catches in shark nets off Natal, South Africa. Report of the international whaling commission. Special issue 15, 541-547.

Cockcroft, V.G., 1999. Organochlorine levels in cetaceans from South Africa: A review. J. Cetacean Res. Manage. Special Issue 1, 169-176.

Cockcroft, V.G., Ross, G.B.J., Connell, A.D., Gardner, B.D., Butler, A.C., 1991. Occurrence of Organochlorines in Stranded Cetaceans and Seals from the East Coast of Southern

Africa. In: Leatherwood, S., Donovan, G.P. (Eds.), Cetaceans and Cetacean Research in the Indian Ocean Sanctuary. UNEP Marine Mammal Technical Report, pp. 272-276.

Cockcroft, V.G., Ross, G.J.B., 1990a. Age, growth and reproduction in bottlenose dolphins (*Tursiops truncates*) from the east coast of Southern Africa. Fish. B-NOAA 88, 289-302.

Cockcroft, V.G., Ross, G.J.B., 1990b. Food and Feeding of the Indian Ocean Bottlenose Dolphin off Southern Natal, South Africa. In: Leatherwood, S., Reeves, R.R. (Eds.), The Bottlenose Dolphin. Academic Press, San Diego, pp. 295-308.

Cockcroft, V.G., Ross, G.J.B., Peddemors, V.M., 1990. Bottlenose dolphin *Tursiops truncatus* distribution in Natal's coastal waters. S. Afr. J. Mar. Sci. 9, 1-10.

Colborn, T., Smolen, M.J., 1996. Epidemiological analysis of persistent organochlorine contaminants in cetaceans. Rev. Environ. Contam. T. 146, 92-172.

Colborn, T., Smolen, M.J., 2003. Cetaceans and Contaminants. In: Vos, J.G., Bossart, G.D., Fournier, M., O'Shea, T.J. (Eds.), Toxicology of Marine Mammals. Taylor & Francis, London, pp. 291-232.

Corbel, M.J., 1997. Brucellosis: An overview. Emerg. Infect. Dis. 3, 213-221.

Cornaglia, E., Rebora, L., Gili, C., Guardo, G., 2000. Histopathological and immunohistochemical studies on cetaceans found stranded on the coast of Italy between 1990 and 1997. J. Vet. Med. A 47, 129-142.

Cowan, D.F., 1993. Lobo's disease in a bottlenose dolphin (*Tursiops truncatus*) from Matagorda Bay, Texas. J. Wildl. Dis. 29, 488-489.

Cowan, D.F., Smith, T.L., 1999. Morphology of the lymphoid organs of the bottlenose dolphin, *Tursiops truncatus.* J. Anat. 194, 505-517.

Cowan, D.F., Tajima, Y., 2006. The thyroid gland in bottlenose dolphins (*Tursiops truncatus*) from the Texas coast of the Gulf of Mexico: Normal structure and pathological changes. J. Comp. Pathol. 135, 217-225.

Cribb, T.H., 1998. The diversity of the digenea of Australian animals. Int. J. Parasitol. 28, 899-911.

Dabin, W., Cesarini, C., Clemenceau, I., Dhermain, F., Jauniaux, T., Canneyt, O., Ridoux, V., 2004. Double-faced monster in the bottlenose dolphin (*Tursiops truncatus*) found in the Mediterranean Sea. Vet. Rec. 154, 306-308.

Dagleish, M.P., Barley, J., Howie, F.E., Reid, R.J., Herman, J., Foster, G., 2007. Isolation of *Brucella* species from a diseased atlanto-occipital joint of an Atlantic white-sided dolphin (*Lagenorhynchus acutus*). Vet. Rec. 160, 876-878.

Daily, M., Stroud, R., 1978. Parasites and associated pathology observed in cetaceans stranded along the Oregon coast. J. Wildl. Dis. 14, 503-511.

Davison, N.J., Cranwell, M.P., Perrett, L.L., Dawson, C.E., Deaville, R., Stubberfield, E.J., Jarvis, D.S., Jepson, P.D., 2009. Meningoencephalitis associated with *Brucella* species in a live-stranded striped dolphin (*Stenella coeruleoalba*) in south-west England. Vet. Rec. 165, 86-89.

Dawson, C.E., Perrett, L.L., Young, E.J., Davison, N.J., Monies, R.J., 2006. Isolation of *Brucella* species from a bottlenose dolphin (*Tursiops truncatus*). Vet. Rec. 158, 831-832.

De Guise, S., 1998. Effects of in vitro exposure of beluga whale leukocytes to selected organochlorines. J. Toxicol. Environ. Health A 55, 479-493.

Dhalla, N.S., Yates, J.C., Naimark, B., Dhalla, K.S., Beamish, R.E., Ostadal, B., 1992. Cardiotoxicity of Catecholamines and Related Agents. In: Acosta, D. (Ed.), Cardiovascular Toxicology. Raven Press, New York, pp. 239-282.

Di Guardo, G., Marruchella, G., Agrimi, U., Kennedy, S., 2005. Morbillivirus infections in aquatic mammals: A brief overview. J. Vet. Med. A 52, 88-93.

Di Guardo, G., Proietto, U., Francesco, C.E., Marsilio, F., Zaccaroni, A., Scaravelli, D., Mignone, W., Garibaldi, F., Kennedy, S., Forster, F., Iulini, B., Bozzetta, E., Casalone, C., 2010. Cerebral toxoplasmosis in striped dolphins (*Stenella coeruleoalba*) stranded along the Ligurian Sea coast of Italy. Vet. Pathol. 47, 245-253.

Domingo, M., Visa, J., Pumarola, M., Marco, A.J., Ferrer, L., Rabanal, R., Kennedy, S., 1992. Pathologic and immunocytochemical studies of morbillivirus infection in striped dolphins (*Stenella coeruleoalba*). Vet. Pathol. 29, 1-10.

Dubey, J.P., Fair, P.A., Bossart, G.D., Hill, D.F.R., Sreekumar, C., Kwok, O.C.H., Thulliez, P., 2005. A comparison of several serologic tests to detect antibodies to *Toxoplasma gondii* in naturally exposed bottlenose dolphins (*Tursiops truncatus*). J. Parasitol. 91, 1074-1081.

Dubey, J.P., Mergl, J., Gehring, E., Sundar, N., Velmurugan, G.V., Kwok, O.C.H., Grigg, M.E., Su, C., Martineau, D., 2009. Toxoplasmosis in captive dolphins (*Tursiops truncatus*) and walrus (*Odobenus rosmarus*). J. Parasitol. 95, 82-85.

Dubey, J.P., Morales, J.A., Sundar, N., Velmurugan, G.V., González-Barrientos, C.R., Hernández-Mora, G., Su, C., 2007. Isolation and genetic characterization of *Toxoplasma gondii* from striped dolphin (*Stenella coeruleoalba*) from Costa Rica. J. Parasitol. 93, 710-711.

Dubey, J.P., Zarnke, R., Thomas, N.J., Wong, S.K., Bonn, W.V., Briggs, M., Davis, J.W., Ewing, R., Mense, M., Kwok, O.C.H., Romand, S., Thulliez, P., 2003. *Toxoplasma gondii*, *Neospora caninum*, *Sarcocystis neurona*, and *Sarcocystis canis*-like infections in marine mammals. Vet. Parasitol. 116, 275-296.

Dudley, S.F.J., Cliff, G., 2010. Shark Control: Methods, Efficacy, and Ecological Impact. In: Carrier, J.C., Musick, J.A., Heithaus, M.R. (Eds.), Sharks and their Relatives II: Biodiversity, Adaptive Physiology, and Conservation. CRC Press, Boca Raton, pp. 567-592.

Duignan, P.J., 2003. Disease investigations in stranded marine mammals, 1999–2002. Department of Conservation, Science Internal Series 1-32.

Duignan, P.J., Geraci, J.R., Raga, J.A., Calzada, N., 1992. Pathology of morbillivirus infection in striped dolphins (*Stenella coeruleoalba*) from Valencia and Murcia, Spain. Can. J. Vet. Res. 56, 242-248.

Duignan, P.J., House, C., Geraci, J.R., Duffy, N., Rima, B.K., Walsh, M.T., Early, G., Aubin, D.J.S., Sadove, S., Koopman, H., Rhinehart, H., 1995. Morbillivirus infection in cetaceans of the western Atlantic. Vet. Microbiol. 44, 241-249.

Dunn, J.L., Buck, J.D., Robeck, T.R., 2001. Bacterial Diseases of Cetaceans and Pinnipeds. In: Dierauf, L.A., Gulland, F.M.D. (Eds.), CRC Handbook of Marine Mammal Medicine. CRC Press Inc, Boca Raton, pp. 309-337.

Durden, W.N., St. Leger, J., Stolen, M., Mazza, T., Londono, C., 2009. Lacaziosis in bottlenose dolphins (*Tursiops truncatus*) in the Indian River lagoon, Florida, USA. J. Wildl. Dis. 45, 849-856.

Durham, B., 1994. The distribution and abundance of humpback dolphin (*Sousa chinensis*) along the Natal coast, South Africa. Unpublished MSc thesis, University of Natal. pp 1-32.

Elad, D., Morick, D., David, D., Scheinin, A., Yamin, G., Blum, S., Goffman, O., 2011. Pulmonary fungal infection caused by *Neoscytalidium dimidiatum* in a Risso's dolphin (*grampus griseus*). Med. Mycol. 49, 424-426.

Elk, C.E., Bildt, M.W.G., Jong, A.A.W., Osterhaus, A.D.M.E., Kuiken, T., 2009. Herpesvirus in bottlenose dolphins (*Tursiops truncatus*): Cultivation, epidemiology, and associated pathology. J. Wildl. Dis. 45, 895-906.

Elk, C.E., Bildt, M.W.G., Martina, B.E.E., Osterhaus, A.D.M.E., Kuiken, T., 2007. *Escherichia coli* septicemia associated with lack of maternally acquired immunity in a bottlenose dolphin calf. Vet. Pathol. 44, 88-92.

Ellis, D., Pfeiffer, T., 1992. The ecology of *Cryptococcus neoformans.* Eur. J. Epidemiol. 8, 321-325.

Epstein, R.P., Sherman, D., Spanger-Siegfried, E., Langston, A., Prasad, S., 1998. Marine ecosystems: Emerging diseases as indicators of change. Harvard Medical School, Boston, MA. pp 1-85.

Fauquier, D.A., Kinsel, M.J., Dailey, M.D., Sutton, G.E., Stolen, M.K., Wells, R.S., Gulland, F.M.D., 2010. Prevalence and pathology of lungworm infection in bottlenose dolphins *Tursiops truncatus* from southwest Florida. Dis. Aquat. Org. 88, 85-90.

Fire, S.E., Wang, Z., Byrd, M., Whitehead, H.R., Paternoster, J., Morton, S.L., 2011. Co-occurrence of multiple classes of harmful algal toxins in bottlenose dolphins (*Tursiops truncatus*) stranding during an unusual mortality event in Texas, USA. Harmful Algae 10, 330-336.

Flewelling, L.J., Naar, J.P., Abbott, J.P., Baden, G.D., Barros, N.B., Bossart, G.D., Bottein, M.D., Hammond, D.G., Haubold, E.M., Heil, C.A., Henry, M.S., Jacocks, H.M., Leighfield, T.A., Pierce, R.H., Pitchford, T.D., Rommel, S.A., Scott, P.S., Steidinger, K.A., Truby, E.W., Van Dolah, F.M., Landsberg, J.H., 2005. Red tides and marine mammal mortalities. Nature 435, 755-756.

Forman, D., West, N., Powell, M., Francis, J., Guy, E., 2007. *Toxoplasma* in cetaceans around the British Isles. Vet. Rec. 161, 279.

Foster, G., Jahans, K.L., Reid, R.J., Ross, H.M., 1996. Isolation of *Brucella* species from cetaceans, seals and an otter. Vet. Rec. 138, 583-586.

Foster, G., MacMillan, A.P., Godfroid, J., Howie, F., Ross, H.M., Cloeckaert, A., Reid, R.J., Brew, S., Patterson, I.A.P., 2002. A review of *Brucella* sp. infection of sea mammals with particular emphasis on isolates from Scotland. Vet. Microbiol. 90, 563-580.

Frasca, S., Dunn, J.L., Cooke, J.C., Buck, J.D., 1996. Mycotic dermatitis in an Atlantic white-sided dolphin, a pygmy sperm whale, and two harbor seals. J. Am. Vet. Med. Assoc. 208, 727-729.

Frenkel, J.K., 1988. Pathophysiology of toxoplasmosis. Parasitol. Today 4, 273-278.

Fujioka, R.S., Greco, S.B., Cates, M.B., Schroeder, J.P., 1988. *Vibrio damsela* from wounds in bottlenose dolphins, *Tursiops truncatus*. Dis. Aquat. Org. 4, 1-8.

Gales, N., Wallace, G., Dickson, J., 1985. Pulmonary cryptococcosis in a striped dolphin (*Stenella coeruleoalba*). J. Wildl. Dis. 21, 443-446.

Gaydos, J.K., Balcomb, K.C., Osborne, R.W., Dierauf, L., 2004. Evaluating potential infectious disease threats for southern resident killer whales, *Orcinus orca*: A model for endangered species. Biol. Conserv. 117, 253-262.

Geraci, J.R., Lounsbury, V.J., 2009. Health. In: Perrin, W.F., Würsig, B., Thewissen, J.G.M. (Eds.), Encyclopedia of Marine Mammals (Second Edition). Academic Press, London, pp. 546-553.

Geraci, J.R., Hicks, B.D., St. Aubin, D.J., 1979. Dolphin pox: A skin disease of cetaceans. Can. J. Comparat. Med. 43, 399-404.

Geraci, J.R., Lounsbury, V.J., 1993. Specimen and Data Collection. In: Geraci, J.R., Lounsbury, V.J. (Eds.), Marine Mammals Ashore: A Field Guide for Strandings. Texas A&M Sea Grant Publication, Galveston, Texas, pp. 167-251.

Geraci, J.R., St. Aubin, D.J., 1987. Effects of parasites on marine mammals. Int. J. Parasitol. 17, 407-414.

Gilbert, J.D., Kemper, C.M., Hill, M., Byard, R.W., 2000. Forensic studies of a stabbed infant bottlenose dolphin. J. Clin. Forensic Med. 7, 150-152.

González, L., Patterson, I.A., Reid, R.J., Foster, G., Barberán, M., Blasco, J.M., Kennedy, S., Howie, F.E., Godfroid, J., MacMillan, A.P., Schock, A., Buxton, D., 2002. Chronic meningoencephalitis associated with *Brucella* sp. infection in live-stranded striped dolphins (*Stenella coeruleoalba*). J. Comp. Pathol. 126, 147-152.

González-Barrientos, R., Morales, J.-., Hernández-Mora, G., Barquero-Calvo, E., Guzmán-Verri, C., Chaves-Olarte, E., Moreno, E., 2010. Pathology of striped dolphins (*Stenella coeruleoalba*) infected with *Brucella ceti.* J. Comp. Pathol. 142, 347-352.

Gray, K.N., Conklin, R.H., 1974. Multiple births and cardiac anomalies in the bottlenose dolphin. J. Wildl. Dis. 10, 155-157.

Gulland, F., 2000. Domoic acid toxicity in California sea lions (*Zalophus californianus*) stranded along the central California coast, May-October 1998. Report to the national marine fisheries service working group on unusual marine mammal mortality events. US department of Commerce, NOAA Technical Memmo NMFS-OPR-17, 1-45.

Gulland, F.M.D., Hall, A.J., 2007. Is marine mammal health deteriorating? Trends in the global reporting of marine mammal disease. Ecohealth 4, 135-150.

Hale, P.T., Barreto, A.S., Ross, G.J.B., 2000. Comparative morphology and distribution of the *aduncus* and *truncatus* forms of bottlenose dolphin *Tursiops* in the Indian and western Pacific oceans. Aquat. Mammals 26, 101-110.

Hammond, P.S., Bearzi, G., Bjørge, A., Forney, K.A., Karkzmarski, L., Kasuya, T., Perrin, W.F., Scott, M.D., Wang, J.Y., Wells, R.S., Wilson, B., 2012. *Tursiops aduncus*. in: IUCN 2012. IUCN red list of threatened species. <www. iucnredlist. org> .

Harrison, R.J., Johnson, F.R., Young, B.A., 1970. The oesophagus and stomach of dolphins (*Tursiops*, *Delphinus*, *Stenella*). J. Zool. 160, 377-390.

Harvell, C.D., Kim, K., Burkholder, J.M., Colwell, R.R., Epstein, P.R., Grimes, D.J., Hofmann, E.E., Lipp, E.K., Osterhaus, A.D.M.E., Overstreet, R.M., Porter, J.W., Smith, G.W., Vasta, G.R., 1999. Emerging marine diseases - climate links and anthropogenic factors. Science 285, 1505-1510.

Harvell, C.D., Mitchell, C.E., Ward, J.R., Altizer, S., Dobson, A.P., Ostfeld, R.S., Samuel, M.D., 2002. Climate warming and disease risks for terrestrial and marine biota. Science 296, 2158-2162.

Haubold, E.M., Cowan, D.F., Cooper, C.R., McGinnis, M.R., 1997. Unusual mycotic infections in stranded western Gulf of Mexico dolphins: Paeohyphomycosis and lobomycosis, abstr. 28th Annual International Association for Aquatic Animal Medicine Conference, Harderwijk, the Netherlands, 26–27.

Herráez, P., Sierra, E., Arbelo, M., Jaber, J.R., Espinosa de, l.M., Fernández, A., 2007. Rhabdomyolysis and myoglobinuric nephrosis (capture myopathy) in a striped dolphin. J. Wildl. Dis. 43, 770-774.

Higgens, R., 2000. Bacteria and fungi of marine mammals: A review. Canadian Vet. J. 41, 105-116.

Howard, E.B., Britt, J.O., Marsumoto, G.K., Ithara, R., Nagano, C.N., 1983. Bacterial Diseases. In: Howard, E.B. (Ed.), Pathobiology of Marine Mammal Diseases. Vol. I. CRC Press, Florida, pp. 69-118.

Inowve, M., 1989. Teratology of heavy metals: Mercury and other contaminants. Teratology 40, 652.

IUCN, 2012. IUCN red list of threatened species. <www. iucnredlist. org> Last accessed 1 March 2013.

Jauniaux, T., Petitjean, D., Brenez, C., Borrens, M., Borrens, L., Haelters, J., Tavernir, T., Coignoul, F., 2002. Postmortem findings and causes of death of harbour porpoises (*Phocoena phocoena*) stranded from 1990 to 2000 along the coastlines of Belgium and northern France. J. Comp. Pathol. 126, 243-253.

Jefferson, T.A., Karczmarski, L., 2001. Sousa chinensis. Mammalian Species 1-9.

Jensen, E.D., Lipscomb, T., Bonn, B., Miller, G., Fradkin, J.M., Ridgway, S.H., 1998. Disseminated histoplasmosis in an Atlantic bottlenose dolphin (*Tursiops truncatus*). J. Zoo Wildlife Med. 29, 456-460.

Jepson, P.D., Bennett, P.M., Deaville, R., Allchin, C.R., Baker, J.R., Law, R.J., 2005a. Relationships between polychlorinated biphenyls and health status in harbor porpoises (*Phocoena phocoena*) stranded in the United Kingdom. Environ. Toxicol. Chem. 24, 238-248.

Jepson, P.D., Deaville, R., Patterson, I.A.P., Pocknell, A.M., Ross, H.M., Baker, J.R., Howie, F.E., Reid, R.J., Colloff, A., Cunningham, A.A., 2005b. Acute and chronic gas bubble lesions in cetaceans stranded in the United Kingdom. Vet. Pathol. 42, 291-305.

Jones, J.L., Dubey, J.P., 2010. Waterborne toxoplasmosis - recent developments. Exp. Parasitol. 124, 10-25.

Kaiser, S.M.L., 2012. Feeding ecology and dietary patterns of the Indo-pacific Bottlenose dolphin (*Tursiops aduncus*) incidentally caught in the shark nets off KwaZulu-Natal, South Africa. Unpublished MSc thesis, Nelson Mandela Metropolitan University. pp 1-68.

Kane, E.A., Olson, P.A., Gerrodette, T., Fiedler, P.C., 2008. Prevalence of the commensal barnacle *Xenobalanus globicipitis* on cetacean species in the eastern tropical Pacific Ocean, and a review of global occurrence. Fish. Bull. 106, 395-404.

Kannan, K., Senthilkumar, K., Loganathan, B.G., Odell, D.K., Tanabe, S., 1997. Elevated accumulation of tributyltin and its breakdown products in bottlenose dolphins (*Tursiops truncatus*) found stranded along the U.S. Atlantic and Gulf coasts. Environ. Sci. Technol. 31, 296-301.

Karczmarski, L., 2000. Conservation and management of humpback dolphins: The south African perspective. Oryx 34, 207-216.

Karczmarski, L., Cockcroft, V.G., McLachlan, A., 1999. Group size and seasonal pattern of occurrence of humpback dolphins *Sousa chinensis* in Algoa Bay, South Africa. S. Afr. J. Mar. Sci. 21, 89-97.

Karczmarski, L., 1999. Group dynamics of humpback dolphins (*Sousa chinensis*) in the Algoa Bay region, South Africa. J. Zool. 249, 283-293.

Karczmarski, L., Cockcroft, V.G., Mclachlan, A., 2000. Habitat use and preferences of Indo-Pacific humpback dolphins *Sousa chinensis* in Algoa Bay, South Africa. Mar. Mammal Sci. 16, 65-79.

Keck, N., Kwiatek, O., Dhermain, F., Dupraz, F., Boulet, H., Danes, C., Laprie, C., Perrin, A., Godenir, J., Micout, L., Libeau, G., 2010. Resurgence of morbillivirus infection in Mediterranean dolphins off the French coast. Vet. Rec. 166, 654-655.

Kennedy, S., 1998. Morbillivirus infections in aquatic mammals. J. Comp. Pathol. 119, 201-225.

Kennedy, S., Kuiken, T., Ross, H.M., McAliskey, M., Moffett, D., McNiven, C.M., Carole, M., 1992. Morbillivirus infection in two common porpoises *(Phocoena phocoena*) from the coasts of England and Scotland. Vet. Rec. 131, 286-290.

King, D.P., Aldridge, B.M., Kennedy-Stoskopf, S., Scott, J.T., 2001. Immunology. In: Dierauf, L.A., Gulland, F.M.D. (Eds.), CRC Handbook of Marine Mammal Medicine. CRC Press Inc, Boca Raton, pp. 237-252.

Kirkwood, J.K., Bennett, P.M., Jepson, P.D., Kuiken, T., Simpson, V.R., Baker, J.R., 1997. Entanglement in fishing gear and other causes of death in cetaceans stranded on the coasts of England and Wales. Vet. Rec. 141, 94-98.

Kiszka, J., Van Bressem, M.F., Pusineri, C., 2009. Lobomycosis-like disease and other skin conditions in indo-pacific bottlenose dolphins, *Tursiops aduncus,* from the Indian Ocean. Dis. Aquat. Org. 84, 151-157.

Knieriem, A., Hartmann, M.C., 2001. Comparative histopathology of lungs from by-caught Atlantic white-sided dolphins (*Leucopleurus acutus*). Aquat. Mammals 27, 73-81.

Koeman, J.H., van de Ven, W.S.M., de Goeij, J.J.M., Tjioe, P.S., van Haaften, J.L., 1975. Mercury and selenium in marine mammals and birds. Sci. Total Environ. 3, 279-287.

Krogh, M., Reid, D., 1996. By-catch in the protective shark meshing programme off south-eastern New South Wales, Australia. Biol. Conserv. 11, 219-226.

Kuiken, T., Hartmann, M.G., 1991. Proceedings of the first European cetacean society workshop on cetacean pathology: Dissection techniques and tissue sampling. European Cetacean Society Newsletter 17 Special issue, 1-39.

Kuiken, T., Hartmann, M.G., 1993. Dissection Techniques and Tissue Sampling. .In: Proceedings of the European Cetacean Society Workshop. Leiden, pp. 1-39.

Kuiken, T., Simpson, V.R., Allchin, C.R., Bennett, P.M., Codd, G.A., Harris, E.A., Howes, G.J., Kennedy, S., Kirkwood, J.K., Law, R.J., Merrett, N.R., Phillips, S., 1994. Mass mortality of common dolphins (*Delphinus delphis*) in south west England due to incidental capture in fishing gear. Vet. Rec. 134, 81-89.

KwaZulu-Natal Sharks Board, 2009. Catch statistics, http://www.shark.co.za, Last accessed 15 January 2013.

KwaZulu-Natal Sharks Board, 2011. Shark nets, drumlines and safe swimming, http://www.shark.co.za, last accessed 15 January 2013.

KyungYeon, E., OhDeog, K., 2011. Two cases of bacterial pneumonia in bottlenose dolphins (*Tursiops gillii*) at the Seoul zoo, Korea. Pak. Vet. J. 31, 260-262.

Lafferty, K.D., Porter, J.W., Ford, S.E., 2004. Are diseases increasing in the ocean? Annu. Rev. Ecol. Evol. Syst. 35, 31-54.

Lahvis, G.P., Wells, R.S., Kuehl, D.W., Stewart, J.L., Rhinehart, H.L., Via, C.S., 1995. Decreased lymphocyte responses in free-ranging bottlenose dolphins (*Tursiops truncatus*) are associated with increased concentrations of PCBs and DDT in peripheral blood. Environ. Health Perspect. 103, pp. 67-72.

Lailson-Brito, J., Dorneles, P.R., Azevedo-Silva, C.E., Bisi, T.L., Vidal, L.G., Legat, L.N., Azevedo, A.F., Torres, J.P.M., Malm, O., 2012. Organochlorine compound accumulation in delphinids from Rio de Janeiro state, south-eastern Brazilian coast. Sci. Total Environ. 433, 123-131.

Lapointe, J.M., Duignan, P.J., Marsh, A.E., Gulland, F.M., Barr, B.C., Naydan, D.k., King, D.P., Farman, C.A., Burek-Huntington, K.A., Lowenstine, L.J., 1998. Meningoencephalitis due to a *Sarcocystis neurona*-like protozoan in Pacific harbor seals (*phoca vitulina richardsi*). J. Parasitol 84, 1184-1189.

Lavery, T.J., Butterfield, N., Kemper, C.M., Reid, R.J., Sanderson, K., 2008. Metals and selenium in the liver and bone of three dolphin species from South Australia, 1988–2004. Sci. Total Environ. 390, 77-85.

Lehnert, K., Seibel, H., Hasselmeier, I.W.,P., Iversen, M., Nielsen, N.H., Heide-Jorgensen, M.P., Prenger-Berninghoff, E., Siebert, U., Change in parasite burden and associated pathology in harbour porpoises (*Phocoena phocoena*) in west Greenland. *In Press*.

Lindsay, D.S., Collins, M.V., Mitchell, S.M., Wetch, C.N., Rosypal, A.C., Flick, G.J., Zajac, A.M., Lindquist, A., Dubey, J.P., 2004. Survival of *Toxoplasma gondii* oocysts in eastern oysters (*Crassostrea virginica*). J. Parasitol. 90, 1054-1057.

Lindsay, D.S., Phelps, K.K., Smith, S.A., Flick, G., Sumner, S.S., Dubey, J.P., 2001. Removal of *Toxoplasma gondii* from sea water by eastern oysers (*Crassostrea virginica*). J. Eukaryot. Microbiol. 48 (Suppl.), 197S-198S.

Lindsay, D.S., Thomas, N.J., Dubey, J.P., 2000. Biological characterisation of *Sarcocystis neurona* isolated from a southern sea otter (*Enhydra lutris nereis*). Int. J. Parasitol. 30, 617-624.

Lipscomb, T.P., Kennedy, S., Moffett, D., Ford, B.K., 1994. Morbilliviral disease in an Atlantic bottlenose dolphin (*Tursiops truncatus*) from the Gulf of Mexico. J. Wildlife Dis. 30, 572-576.

Lipscomb, T.P., Kennedy, S., Moffett, D., Krafft, A., Klaunberg, B.A., Lichy, J.H., Regan, G.T., Worthy, G.A.J., Taubenberger, J.K., 1996. Morbilliviral epizootic in bottlenose dolphins of the Gulf of Mexico. J. Vet. Diagn. Invest. 8, 283-290.

Lipscomb, T.P., Schulman, F.Y., Moffett, D., Kennedy, S., 1994. Morbilliviral disease in Atlantic bottlenose dolphins (*Tursiops truncatus*) from the 1987-1988 epizootic. J. Wildlife Dis. 30, 567-571.

Mardon, D., Stretch, D., 2004. Comparative assessment of water quality at Durban beaches according to local and international guidelines. Water SA 30, 317-323.

McCullough, S.j., McNeilly, F., Allan, G.M., Kennedy, S., Smyth, J.a., Cosby, S.L., McQuaid, S., Rima, B.K., 1991. Isolation and characterisation of a porpoise morbillivirus. Arch. Virol. 118, 247-252.

McFee, W.E., Lipscomb, T.P., 2009. Major pathologic findings and probable causes of mortality in bottlenose dolphins stranded in South Carolina from 1993 to 2006. J. Wildlife Dis. 45, 575-593.

McFee, W.E., Osborne, C.A., 2004. Struvite calculus in the vagina of a bottlenose dolphin (*Tursiops truncatus*). J. Wildlife Dis. 40, 125-128.

McHuron, E.A., Greig, D.J., Colegrove, K.M., Fleetwood, M., Spraker, T.R., Gulland, F.M.D., Harvey, J.T., Lefebvre, K.A., Frame, E.R., 2013. Domoic acid exposure and associated clinical signs and histopathology in pacific harbor seals (*Phoca vitulina richardsi*). Harmful Algae 23, 28-33.

Migaki, G., Gunnels, R.D., Casey, H.W., 1978. Pulmonary cryptococcosis in an Atlantic bottlenose dolphin (*Tursiops truncatus*). Lab. Anim. Sci. 28, 603-606.

Migaki, G., Sawa, T.R., Dubey, J.P., 1990. Fatal disseminated toxoplasmosis in a spinner dolphin (*Stenella longirostris*). Vet. Pathol. 27, 463-464.

Miller, M.A., Gardner, I.A., Kreuder, C., Paradies, D.M., Worcester, K.R., Jessup, D.A., Dodd, E., Harris, M.D., Ames, J.A., Packham, A.E., Conrad, P.A., 2002. Coastal freshwater runoff is a risk factor for *Toxoplasma gondii* infection of southern sea otters (*Enhydra lutris nereis*). Int. J. Parasitol. 32, 997-1006.

Miller, W.G., Adams, L.G., Ficht, T.A., Cheville, N.F., Payeur, J.P., Harley, D.R., House, C., Ridgeway, S.H., 1999. *Brucella*-induced abortions and infections in bottlenose dolphins (*Tursiops truncatus*). J. Zoo Wildlife Med. 30, 100-110.

Miller, W.G., Padhye, A.A., Van Bonn, W., Jensen, E., Brandt, M.E., Ridgway, S.H., 2002. Cryptococcosis in a bottlenose dolphin (*Tursiops truncatus*) caused by *Cryptococcus neoformans* var. *gattii*.. J. Clin. Microbiol. 40, 721-724.

Moore, M.J., Bogomolni, A.L., Dennison, S.E., Early, G., Garner, M.M., Hayward, B.A., Lentell, B.J., Rotstein, D.S., 2009. Gas bubbles in seals, dolphins, and porpoises entangled and drowned at depth in gillnets. Vet. Pathol. 46, 536-547.

Mori, C., Morsey, B., Levin, M., Gorton, T.S., De Guise, S., 2008. Effects of organochlorines, individually and in mixtures, on B-cell proliferation in marine mammals and mice. J. Toxicol. Environ. Health A 71, 266-275.

Moss, B., 2007. Poxviridae: The Viruses and their Replication. In: Knipe, D.M. (Ed.), Fields Virology. Lippincott Williams and Wilkins, Philadelphia. pp. 2906-2947.

Munday, B.L., Mason, R.W., Hartley, W.J., Presidente, P.J., Obendorf, D., 1978. Sarcocystis and related organisms in Australian wildlife: 1. survey findings in mammals. J. Wildlife Dis. 14, 417-433.

Munoz, P.M., Garcia-Castrillo, G., Lopez-Garcia, P., Gonzalez-Cueli, J.C., De Miguel, M.J., Marin, C.M., Barberán, M., Blasco, J.M., 2006. Isolation of *brucella* species from a live striped dolphin *(Stenella coeruleoalba*) in Spain. Vet. Rec. 158, 450-451.

Murdoch, M.E., Reif, J.S., Mazzoil, M., McCulloch, S.D., Fair, P.A., Bossart, G.D., 2008. Lobomycosis in bottlenose dolphins (*Tursiops truncatus*) from the Indian River Lagoon, Florida: Estimation of prevalence, temporal trends, and spatial distribution. Ecohealth 5, 289-297.

Natoli, A., Peddemors, V.M., Hoelzel, A.R., 2008. Population structure of bottlenose dolphins (*Tursiops aduncus*) impacted by by-catch along the east coast of South Africa. Conserv. Genet. 9, 627-636.

Newman, S.J., Smith, S.A., 2006. Marine mammal neoplasia: A review. Vet. Pathol. 43, 865-880.

Nollens, H.H., Rivera, R., Palacios, G., Wellehan, J.F.X., Saliki, J.T., Caseltine, S.L., Smith, C.R., Jensen, E.D., Hui, J., Lipkin, W.I., Yochem, P.K., Wells, R.S., St. Leger, J., Venn-Watson, S., 2009. New recognition of enterovirus infections in bottlenose dolphins (*Tursiops truncatus*). Vet. Microbiol. 139, 170-175.

Nollens, H.H., Wellehan, J.F.X., Saliki, J.T., Caseltine, S.L., Jensen, E.D., Bonn, W., Venn-Watson, S., 2008. Characterization of a parainfluenzavirus isolated from a bottlenose dolphin (*Tursiops truncatus*). Vet. Microbiol. 128, 231-242.

Norris, K.S., 1961. Standardized methods for measuring and recording data on the smaller cetaceans. J. Mammal. 42, 471-476.

O'Hara, T.M., O'Shea, T.J., 2001. Toxicology. In: Dierauf, L.A., Gulland, F.M.D. (Eds.), CRC Handbook of Marine Mammal Medicine. CRC Press Inc, Boca Raton, pp. 471-520.

O'Shea, T.J., Bossart, G.D., Fournier, M., Vos, J.G., 2003. Conclusions and Perspectives for the Future. In: Vos, J.G., Bossart, G.D., Fournier, M., O'Shea, T.J. (Eds.), Toxicology of Marine Mammals. Taylor & Francis, New York, pp. 595-613.

Pallansch, M., Roos, R., 2007. Enteroviruses. In: Knipe, D.M. (Ed.), Fields Virology. Lippincott Williams and Wilkins, Philadelphia, pp. 839-893.

Paniz-Mondolfi, A.E., Reyes, J.O., Dávila, J.L., 2007. Lobomycosis in Venezuela. Int. J. Dermatol. 46, 180-185.

Peddemors, V.M., 1995. The aetiology of bottlenose dolphin capture in shark nets off Natal, South Africa. PhD thesis. University of Port Elizabeth. pp 1-83.

Peddemors, V.M., 1999. Delphinids of Southern Africa: A review of their distribution, status and life history. J. Cetacean. Res. Manage. 1, 157-165.

Peddemors, V.M., Atkins, S., Oosthuizen, W.H., 2004. *Sousa Chinensis* - Indian Humpback Dolphin. In: Friedmann, Y., Daly, B. (Eds.), Red Data Book of the Mammals of South Africa: A Conservation Assessment. Conservation Breeding Specialist Group (SSC/IUCN), Endangered Wildlife Trust, South Africa, pp. 722.

Peddemors, V.M., Oosthuizen, W.H., 2004. *Tursiops Aduncus* - Indian Ocean Bottlenose Dolphin. In: Friedmann, Y., Daly, B. (Eds.), Red Data Book of the Mammals of South Africa: A Conservation Assessment. Conservation Breeding Specialist Group (SSC/IUCN), Endangered Wildlife Trust, South Africa, pp. 722.

Pellett, P.E., Roizman, B., 2007. The Family *Herpesviridae*: A Brief Introduction. In: Knipe, D.M. (Ed.), Fields Virology. Lippincott Williams and Wilkins, Philadelphia, pp. 2479-2499.

Pier, A.C., Takayama, A.K., Miyahara, A.Y., 1970. Cetacean nocardiosis. J. Wildlife Dis. 6, 112-118.

Pierce, G.J., Santos, M.B., Murphy, S., Learmonth, J.A., Zuur, A.F., Rogan, E., Bustamante, P., Caurant, F., Lahaye, V., Ridoux, V., Zegers, B.N., Mets, A., Addink, M., Smeenk, C., Jauniaux, T., Law, R.J., Dabin, W., López, A., Alonso Farré, J.M., González, A.F., Guerra, A., García-Hartmann, M., Reid, R.J., Moffat, C.F., Lockyer, C., 2008. Bioaccumulation of persistent organic pollutants in female common dolphins (*Delphinus delphis*) and harbour porpoises (*Phocoena phocoena*) from western European seas: Geographical trends, causal factors and effects on reproduction and mortality. Environ. Pollut. 153, 401-415.

Powell, J.W.B., Archibald, R.T., Cross, C.A., Rotstein, D.S., Soop, V.M., McFee, W.E., 2009. Multiple congenital cardiac abnormalities in an Atlantic bottlenose dolphin (*Tursiops truncatus*). J. Wildlife Dis. 45, 839-842.

Pugliares, K.R., Bogomolni, A., Touhey, K.M., Herzig, S.M., Harry, C.T., Moore, M.J., 2007. Marine mammal necropsy: An introductory guide for stranding responders and field biologists. Woods Hole Oceanographic Institute Technical Report WHOI-2007-06, 1-132.

Raga, J.A., Banyard, A., Domingo, M., Corteyn, M., Van Bressem, M.F., Fernández, A., Aznar, F.J., Barrett, T., 2008. Dolphin morbillivirus epizootic resurgence, Mediterranean Sea. Emerg. Infect. Dis. 14, 471-473.

Raga, J.A., Fernández, M., Balbuena, J.A., Aznar, F.J., 2009. Parasites. In: Perrin, W.F., Würsig, B., Thewissen, J.G.M. (Eds.), Encyclopedia of Marine Mammals (Second Edition). Academic Press, London, pp. 821-830.

Raverty, S.A., Gaydos, J.K., 2004. Killer whale necropsy and disease testing protocol. Animal Health Center, Ministry of Agriculture, Food and Fisheries, Abbottsford, British Columbia, Canada. pp. 1-63..

Rawson, A.J., Patton, G.W., Hofmann, S., Pietra, G.G., Johns, L., 1993. Liver abnormalities associated with chronic mercury accumulation in stranded Atlantic bottlenose dolphins. Ecotox. Environ. Safe. 25, 41-47.

Reddy, M.L., Dierauf, L.A., Culland, F.M.D., 2001. Marine Mammals as Sentinels of Ocean Health. In: Dierauf, L.A., Gulland, F.M.D. (Eds.), CRC Handbook of Marine Mammal Medicine. CRC Press Inc, Boca Raton, pp. 3-13.

Rehtanz, M., Ghim, S.J., McFee, W., Doescher, B., Lacave, G., Fair, P.A., Reif, J.S., Bossart, G.D., Jenson, A.B., 2010. Papillomavirus antibody prevalence in free-ranging and captive bottlenose dolphins (*Tursiops truncatus*). J. Wildlife Dis. 46, 136-145.

Reid, I.M., 1972. Corpora amylacea of the bovine mammary gland: Histochemical and electron microscopic evidence for their amyloid nature. J. Comp. Pathol. 82, 409-413.

Reidarson, T.H., Griner, L.A., Pappagianis, D., McBain, J., 1998a. Coccidioidomycosis in a bottlenose dolphin. J. Wildlife Dis. 34, 629-631.

Reidarson, T.H., McBain, J., House, C., King, D.P., Stott, J.L., Krafft, A., Taubenberger, J.K., Heyning, J., Lipscomb, T.P., 1998b. Morbillivirus infection in stranded common dolphins from the Pacific Ocean. J. Wildlife Dis. 34, 771-776.

Reidarson, T.H., McBain, J.F., Dalton, L.,M., Rinaldi, M.G., 2001. Mycotic Diseases. In: Dierauf, L.A., Gulland, F.M.D. (Eds.), CRC Handbook of Marine Mammal Medicine. CRC Press Inc, Boca Raton, pp. 337-355.

Reif, J.S., Mazzoil, M.S., McCulloch, S.D., Varela, R.A., Goldstein, J.D., Fair, P.A., Bossart, G.D., 2006. Lobomycosis in Atlantic bottlenose dolphins from the Indian River Lagoon, Florida. J. Am. Vet. Med. Assoc. 228, 104-108.

Reif, J.S., Peden-Adams, M., Romano, T.A., Rice, C.D., Fair, P.A., Bossart, G.D., 2009. Immune dysfunction in Atlantic bottlenose dolphins (*Tursiops truncatus*) with lobomycosis. Med. Mycol. 47, 125-135.

Resendes, A.R., Juan-Salls, C., Almeria, S., Maj, N., Domingo, M., Dubey, J.P., 2002. Hepatic sarcocystosis in a striped dolphin (*Stenella coeruleoalba*) from the Spanish Mediterranean coast. J. Parasitol. 88, 206-209.

Ridgeway, S.H., 1979. Reported causes of death of captive killer whales (*Orcinus orca*). J Wildlife Dis 15, 99-104.

Riva, G.T., Johnson, C.K., Gulland, F.M.D., Langlois, G.W., Heyning, J.E., Rowles, T.K., Mazet, J.A.K., 2009. Association of an unusual marine mammal mortality event with *Pseudo-nitzschia* spp. blooms along the southern California coastline. J. Wildlife Dis. 45, 109-121.

Roe, W.D., Gartrell, B.D., Gartrell, B.D., Hunter, S.A., 2012a. Freezing and thawing of pinniped carcasses results in artefacts that resemble traumatic lesions. Vet. J. 194, 326-331.

Ross, H.M., Jahans, K.L., MacMillan, A.P., Reid, R.J., Thompson, P.M., Foster, G., 1996. *Brucella* species infection in North Sea and cetacean populations. Vet. Rec. 138, 647-648.

Ross, P.S., 2002. The role of immunotoxic environmental contaminants in facilitating the emergence of infectious diseases in marine mammals. HERA 8, 277-292.

Rotstein, D.S., West, K., Levine, G., Lockhart, S.R., Raverty, S., Morshed, M.G., Rowles, T., 2010. *Cryptococcus gattii* VGI in a spinner dolphin (*Stenella longirostris*) from Hawaii. J. Zoo Wildlife Med. 41, 181-183.

Rowles, T.K., Van Dolah, F.M., Hohn, A.A., 2001. Gross Necropsy and Specimen Collection Protocols. In: Dierauf, L.A., Gulland, F.M.D. (Eds.), CRC Handbook of Marine Mammal Medicine. CRC Press Inc, Boca Raton, pp. 449-470.

Schroeder, J., Wallace, J., Cates, M., Greco, S., Moore, P., 1985. An infection by *Vibrio alginolyticus* in an Atlantic bottlenose dolphin housed in an open ocean pen. J. Wildlife Dis. 21, 437-438.

Seehusen, F., Baumgärtner, W., 2010. Axonal pathology and loss precede demyelination and accompany chronic lesions in a spontaneously occurring animal model of multiple sclerosis. Brain Pathol 20, 551-559.

Šeol, B., Gomerčic, M.Đ., Naglic, T., Gomerčic, T., Galov, A., Gomerčic, H., 2006. Isolation of *Clostridium tertium* from a striped dolphin (*Stenella coeruleoalba*) in the Adriatic Sea.; J. Wildlife Dis. 42, 709-711.

Shlosberg, A., Bellaiche, M., Regev, S., Gal, R., Brizzi, M., Hanji, V., Zaidel, L., Nyska, A., 1997. Lead toxicosis in a captive bottlenose dolphin (*Tursiops truncatus*) consequent to ingestion of air gun pellets. J. Wildlife Dis. 33, 135-139.

Siebert, U., Joiris, C., Holsbeek, L., Benke, H., Failing, K., Frese, K., Petzinger, E., 1999. Potential relation between mercury concentrations and necropsy findings in cetaceans from German waters of the North and Baltic Seas. Mar. Pollut. Bull. 38, 285-295.

Siebert, U., Tolley, K., Víkingsson, G.A., Ólafsdottir, D., Lehnert, K., Weiss, R., Baumgärtner, W., 2006. Pathological findings in harbour porpoises (*Phocoena phocoena*) from Norwegian and Icelandic waters. J. Comp. Pathol. 134, 134-142.

Siebert, U., Wünschmann, A., Weiss, R., Frank, H., Benke, H., Frese, K., 2001. Post-mortem findings in harbour porpoises (*Phocoena phocoena*) from the German north and Baltic seas. J. Comp. Pathol. 124, 102-114.

St. Leger, J., Wu, G., Anderson, M., Dalton, L., Nilson, E., Wang, D., 2011. West Nile virus infection in killer whale, Texas, USA, 2007. Emerg. Infect. Dis. 17, 1531-1533.

St. Leger, J.A., Begeman, L., Fleetwood, M., Frasca, S., Garner, M.M., Lair, S., Trembley, S., Linn, M.J., Terio, K.A., 2009. Comparative pathology of nocardiosis in marine mammals. Vet. Pathol. 46, 299-308.

Stimmer, L., Siebert, U., Wohlsein, P., Fontaine, J.J., Baumgärtner, W., Beineke, A., 2010. Viral protein expression and phenotyping of inflammatory responses in the central nervous system of phocine distemper virus-infected harbour seals (*Phoca vitulina*). Vet. Microbiol. 145, 23-33.

Sweeney, J.C., Miyaki, G., Vainik, P.M., Conklin, R.H., 1976. Systemic mycoses in marine mammals. J. Am. Vet. Med. Assoc. 169, 946-948.

Sweeney, J.C., Ridgway, S.H., 1975. Common diseases of small cetaceans. J. Am. Vet. Med. Assoc. 167, 533.

Taborda, P.R., Taborda, V.A., McGinnis, M.P., 1999. *Lacazia loboi*, the etiologic agent of lobomycosis. J. Clin. Virol. 37, 2031-2033.

Tanabe, S., 2002. Contamination and toxic effects of persistent endocrine disrupters in marine mammals and birds. Mar. Pollut. Bull. 45, 69-77.

Taugenberger, J.K., Atkin, T.J., Atkin, T.J., Fanning, T.G., Krafft, A.E., Moeller, R.B., Kodsi, S.E., Mense, M.G., Lipscomb, T.P., Lipscomb, T.P., 2000. Molecular genetic evidence of a novel morbillivirus in a long-finned pilot whale (*Globicephalus melas*). Emerg. Infect. Dis. 6, 42-45.

Tell, L.A., 2005. Aspergillosis in mammals and birds: Impact on veterinary medicine. Med. Mycol. 43, S71-S73.

Thomas, N.J., Dubey, J.P., Lindsay, D.S., Cole, R.A., Meteyer, C.U., 2007. Protozoal meningoencephalitis in sea otters (*Enhydra lutris)*: A histopathological and immunohistochemical study of naturally occurring cases. J. Comp. Pathol. 137, 102-121.

Torchin, M.E., Lafferty, K.D., Kuris, A.M., 2002. Parasites and marine invasions. Parasitology 124, S137-S151.

Tsai, M., You, H., Tang, Y., Liu, J., 2008. *Shewanella* soft tissue infection: Case report and literature review. Int. J. Infect. Dis. 12, e119-e124.

Turnbull, B.S., Cowan, D.F., 1998. Myocardial contraction band necrosis in stranded cetaceans. J. Comp. Pathol. 118, 317-327.

Turnbull, B.S., Cowan, D.F., 1999a. Angiomatosis, a newly recognized disease in Atlantic bottlenose dolphins (*Tursiops truncatus*) from the Gulf of Mexico. Vet. Pathol. 36, 28-34.

Turnbull, B.S., Cowan, D.F., 1999b. Synovial joint disease in wild cetaceans. J. Wildlife Dis. 35, 511-518.

UNEP-WCMC, 2013. UNEP-WCMC species database: CITES-listed species. 2013, .

Valli, V.E.O., 2007. Lymph Nodes. In: Maxie, M.G. (Ed.), Jubb, Kennedy & Palmer's Pathology of Domestic Animals (Fifth Edition). Elsevier, Edinburgh, pp. 273-283.

Van Bressem, M.F., Jepson, P.D., Barrett, T., 1998. Further insight on the epidemiology of cetacean morbillivirus in the north-eastern Atlantic. Mar. Mammmal Sci. 14, 605-613.

Van Bressem, M.F., Raga, J.A., Guardo, G., Jepson, P.D., Duignan, P.J., Siebert, U., Barrett, T., Santos, M.C., Moreno, I.B., Siciliano, S., Aguilar, A., Waerebeek, K., 2009a. Emerging infectious diseases in cetaceans worldwide and the possible role of environmental stressors. Dis. Aquat. Org. 86, 143-157.

Van Bressem, M.F., Santos, M.C., Oshima, J.E., 2009b. Skin diseases in Guiana dolphins (*Sotalia guianensis*) from the Paranaguá estuary, Brazil: A possible indicator of a compromised marine environment. Mar. Environ. Res. 67, 63-68.

Van Bressem, M.-., Van Waerebeek, K., Garcia-Godos, A., Dekegel, D., Pastoret, P.P., 1994. Herpes-like virus in dusky dolphins, *Lagenorhynchus obscurus*, from coastal Peru. Mar. Mammal Sci. 10, 354-359.

Van Bressem, M.F., Van Waerebeek, K., Pirard, G.E., Desaintes, C., 1996. Genital and lingual warts in small cetaceans from coastal Peru. Dis. Aquat. Org. 26, 1-10.

Van Bressem, M.F., Visser, I.K.G., Bildt, M.W.G., 1991. Morbillivirus infection in Mediterranean striped dolphins (*Stenella coeruleoalba*). Vet. Rec. 29, 471-472.

Van Bressem, M.F., Waerebeek, K., Javier Aznar, F., Raga, J.A., Jepson, P.D., Duignan, P., Deaville, R., Flach, L., Viddi, F., Baker, J.R., Beneditto, A.P., Echegaray, M., Genov, T., Reyes, J., Felix, F., Gaspar, R., Ramos, R., Peddemors, V., Sanino, G.P., Siebert, U., 2009. Epidemiological pattern of tattoo skin disease: A potential general health indicator for cetaceans. Dis. Aquat. Org. 85, 225-237.

Van Bressem, M.F., Waerebeek, K., Jepson, P.D., Raga, J.A., Duignan, P.J., Nielsen, O., Beneditto, A.P., Siciliano, S., Ramos, R., Kant, W., Peddemors, V., Kinoshita, R., Ross, P.S., López-Fernandez, A., Evans, K., Crespo, E., Barrett, T., 2001. An insight into the epidemiology of dolphin morbillivirus worldwide. Vet. Microbiol. 81, 287-304.

Van Bressem, M.F., Waerebeek, K., Reyes, J.C., Dekegel, D., Pastoret, P.P., 1993. Evidence of poxvirus in dusky dolphin (*Lagenorhynchus obscurus*) and Burmeister's porpoise (*Phocoena spinipinnis*) from coastal Peru. J. Wildlife Dis. 29, 109-113.

Van Dijk, J.E., Gruys, E., Mauwen, J.M.V.M. (Eds.), 2007. Colour Atlas of Veterinary Pathology. Sauderns Elsevier, Spain.

Venter, K., 2009. Diet of humpback dolphins (*Sousa plumbea*) along the south-eastern coast of South Africa. Unpublished BSc Honours thesis. Rhodes University, Grahamstown, South Africa. pp. 1-34.

Veraldo, P.E., Kilpper-Balz, R., Biavasco, F., Satta, G., Schleifer, K.H., 1988. *Staphylococcus delphini*, a coagulase-positive species isolated from dolphins. Int. J. Syst. Bacteriol. 38, 436-439.

Waltzek, T.B., Cortés-Hinojosa, G., Wellehan, J.F.X., Gray, G.C., 2012. Marine mammal zoonoses: A review of disease manifestations. Zoonoses Public Hlth. 59, 521-535.

Wang, Q., Chang, B.J., Riley, T.V., 2010. *Erysipelothrix rhusiopathiae.* Vet. Microbiol. 140, 405-417.

Ward, J.R., Lafferty, K.D., 2004. The elusive baseline of marine disease: Are diseases in ocean ecosystems increasing? PLoSBiol 2, e120 2, 542-546.

Wells, R.S., Rhinehart, H.L., Hansen, L.J., Sweeney, J.C., Townsend, F.I., Stone, R., Casper, D., Scott, M.D., Hohn, A.A., Rowles, T.K., 2004. Bottlenose dolphins as marine ecosystem sentinels: Developing a health monitoring system. EcoHealth 1, 246-254.

Woodard, J.C., Zam, S.G., Caldwell, D.K., Caldwell, M.C., 1969. Some parasitic diseases of dolphins. Path. Vet. 6, 257-272.

Woshner, V., Knott, K., Wells, R., Willetto, C., Swor, R., O'Hara, T., 2008. Mercury and selenium in blood and epidermis of bottlenose dolphins (*Tursiops truncatus*) from Sarasota Bay, FL: Interaction and relevance to life history and hematologic parameters. EcoHealth 5, 360-370.

Young, D.D., 1998. Aspects of condition in captive and free-ranging dolphins. Unpublished PhD thesis. Rhodes University, Grahamstown, South Africa. pp 1-436.

Zabka, T.S., Goldstein, T., Cross, C., Mueller, R.W., Kreuder-Johnson, C., Gill, S., Gulland, F.M.D., 2009. Characterization of a degenerative cardiomyopathy associated with domoic acid toxicity in California sea lions (*Zalophus californianus*). Vet Pathol 46, 105-119.

Zappulli, V., Mazzariol, S., Cavicchioli, L., Petterino, C., Bargelloni, L., Castagnaro, M., 2005. Fatal necrotizing fasciitis and myositis in a captive common bottlenose dolphin (*Tursiops truncatus*) associated with *Streptococcus agalactiae*. J. Vet. Diagn. Invest. 17, 617-622.

Appendix A. Glossary

Caudal:	Referring to the direction towards the tail, opposite of cranial (Figure 2).
Cranial:	Referring to the direction towards the head, opposite of caudal (Figure 2).
Dorsal:	Related to or towards the back of the upper side of an animal, opposite of ventral (Figure 2).
Endocardium:	Inner layer of the heart.
Endocardium:	Outside layer of the heart.
Lateral:	Referring to the side on the animal, usually denoted by right or left.
Midline:	Along the median (middle) plane of the body of an animal or organ.
Myocardium:	Bulk of the muscle layer of the heart.
Rostral:	Referring to the direction towards the tip of the nose and mouth, only used when describing direction on the head (Figure 2).
Ventral:	Related to or towards the belly or bottom of an animal, opposite of dorsal (Figure 2).
Zoonosis:	Disease that can be transmitted from animals to man or *vice versa*.

Appendix B. Standard Measurements

Standardized measurements for full morphometric studies that should be taken before every necropsy, as suggested by Norris (1961) with explanatory diagram under 1.5.2.

No.	Measurement	Centimetres (cm)
1	Total length (tip of upper jaw to tail notch	
2	Tip of upper jaw to centre of eye	
3	Tip of upper jaw to apex of melon	
4	Tip of upper jaw to angle of gape	
5	Tip of upper jaw to external auditory meatus	
6	Centre of eye to external auditory meatus (direct)	
7	Centre of eye to angle of gape (direct)	
8	Centre of eye to centre of blowhole (direct)	
9	Tip of upper jaw to blowhole along midline	
10	Tip of upper jaw to anterior insertion of flipper	
11	Tip of upper jaw to tip of dorsal fin	
12	Tip of upper jaw to midpoint of umbilicus	
13	Tip of upper jaw to midpoint of genital aperture	
14	Tip of upper jaw to centre of anus	
15	Projection of lower jaw beyond upper jaw	
16	Tip of upper jaw to posterior extremity of throat creases	
20	Length of throat creases	
21	Girth on a transverse plane intersecting axilla	
22	Girth at maximum	
23	Girth on a transverse plane intersecting the anus	
24	Height and length of eye	
25	Length of mammary slits (right;left)	
26	Length of genital slit : Length of anal opening	
27	Dimensions of blowhole (width:length)	
28	Diameter of external auditory meatus (right:left)	
29	Length of flipper, anterior insertion to tip	
30	Length of flipper, axilla to tip	
31	Maximum width of flipper	
32	Height of dorsal fin, fin tip to base	
33	Dorsal fin base	
34	Width of flukes, tip to tip	
35	Distance from nearest point on anterior border of flukes to notch	
36	Depth of notch between flukes	

Appendix C. Individual Animal Data

Table C.1. Individual animal details including KwaZulu-Natal Sharks Board identification number (KZNSB no.), Port Elizabeth Museum number (PEM no.), species, sex and age category.

KZNSB no.	PEM no.	Species	Sex	Age
RB 10001	N 3573	*Tursiops*	female	<2 y
UMD 09089	N 3574	*Tursiops*	male	>12 y
UMD 09088	N 3575	*Tursiops*	female	>12 y
KAR 09074	N 3576	*Tursiops*	female	<2 y
TON 10002	N 3578	*Tursiops*	female	>12 y
SUN 09055	N 3581	*Tursiops*	female	2-12 y
BAL 10004	N 3583	*Tursiops*	female	2-12 y
RB 10003	N 3584	*Sousa*	male	>12 y
ZIN 10008	N 3590	*Tursiops*	male	>12 y
TB 10004	N 4339	*Tursiops*	female	>12 y
RB 104	N 4341	*Sousa*	male	>12 y
DUR 2050	N 4342	*Tursiops*	female	<2 y
RAM 10005	N 4343	*Tursiops*	female	<2 y
UMZ 0941	N 4344	*Tursiops*	female	<2 y
DUR 09951	N 4345	*Tursiops*	male	<2 y
UMH 10003	N 4346	*Tursiops*	female	<2 y
TON 10001	N 4347	*Tursiops*	female	<2 y
DUR 10025	N 4348	*Tursiops*	male	<2 y
TB 10003	N 4349	*Tursiops*	male	<2 y
RB 10006	N 4351	*Sousa*	female	2-12 y
SAL 10003	N 4352	*Tursiops*	male	2-12 y
PAR 10026	N 4355	*Tursiops*	female	2-12 y
RB 10014	N 4529	*Tursiops*	female	2-12 y
ZIN 10015	N 4530	*Sousa*	female	<2 y
UMD 10007	N 4531	*Tursiops*	female	<2 y
DUR 10079	N 4532	*Tursiops*	female	<2 y
DUR 10089	N 4533	*Tursiops*	male	2-12 y
SAN 10011	N 4534	*Tursiops*	female	2-12 y
GLN 10005	N 4535	*Tursiops*	female	2-12 y
SAL 11003	N 4536	*Tursiops*	female	<2 y
SAL 11004	N 4537	*Tursiops*	female	2-12 y
MG 11005	N 4539	*Tursiops*	female	<2 y
TO 11001	N 4541	*Tursiops*	male	2-12 y
ZIN 11010	N 4542	*Sousa*	male	2-12 y
DUR 12020	N 4639	*Tursiops*	female	<2 y
WIN 12002	N 4640	*Tursiops*	male	<2 y
DUR 12016	N 4641	*Tursiops*	male	<2 y
ST 11004	N 4642	*Tursiops*	female	<2 y
GLN 11005	N 4643	*Tursiops*	female	>12 y
RAM 11003	N 4645	*Tursiops*	male	<2 y

Appendix D. Pathology Pictures

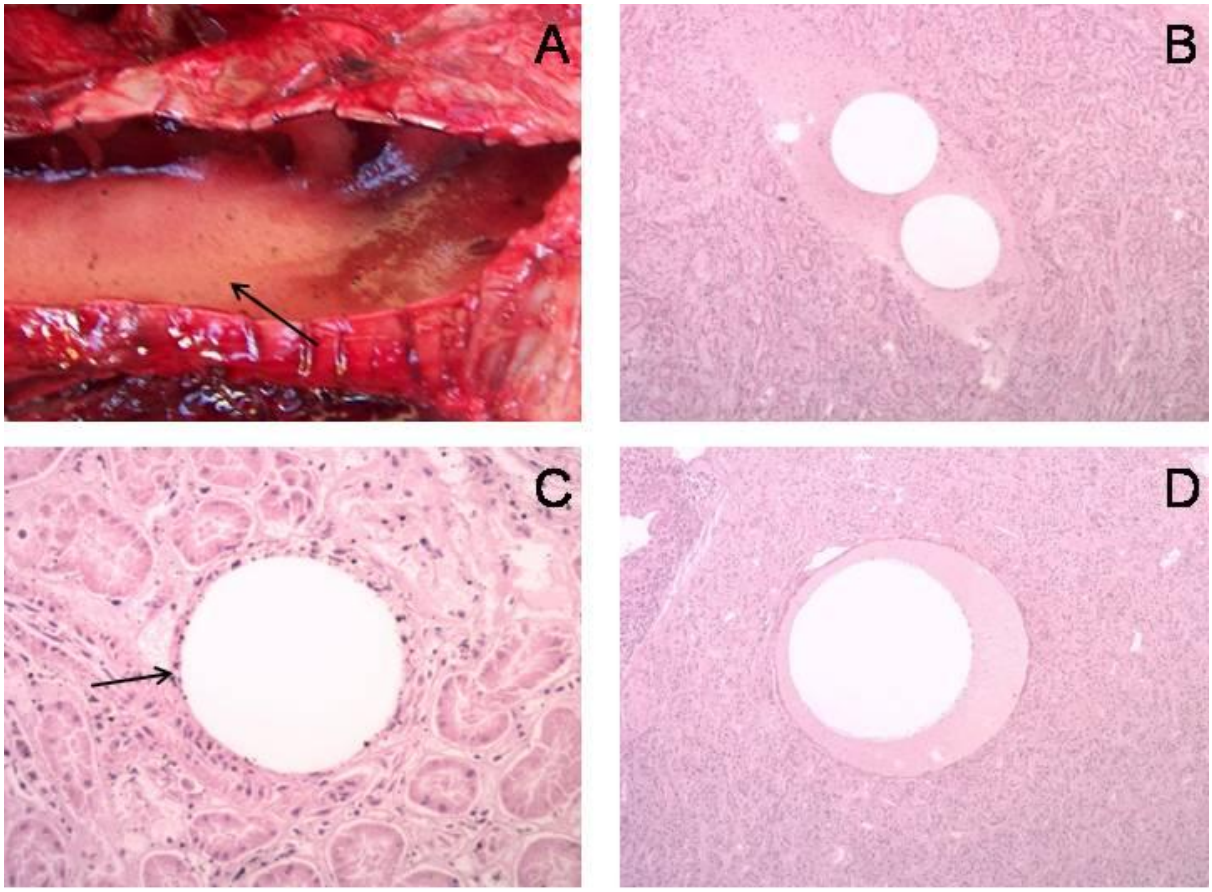

Figure D.1. Incidental findings. A: Macrograph froth in the trachea (black arrow) indicative of agonal pulmonary oedema. B: Kidney with blood vessel containing serum with discrete gas emboli (Haematoxylin and Eosin, x40). C: Kidney with gas bubble compressing surrounding tissue (black arrow) (Haematoxylin and Eosin, x200). D: Testis with distended blood vessel containing gas bubble (Haematoxylin and Eosin, x40).

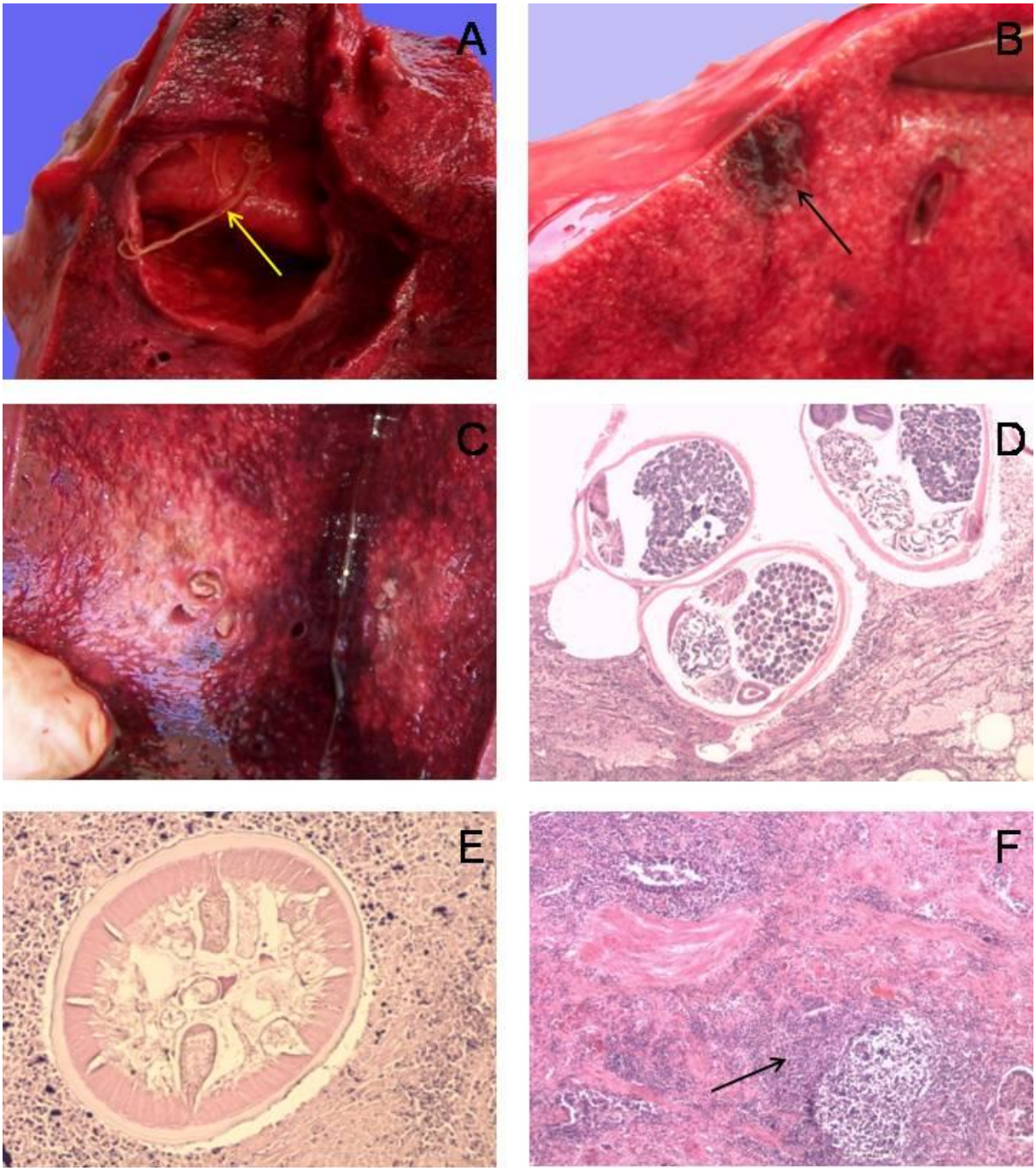

Figure D.2. Pulmonary lesions. A: Ectatic bronchus containing thin, long, white helminths (arrow) identified as *Halocercus* sp. B: Moderate focal pneumonia. C: Locally extensive chronic exudative pneumonia. D: Pulmonary helminths surrounded by inflammatory infiltrates (Haematoxylin and Eosin, x40). E: Cross section of helminth with surrounding pneumonia (Haematoxylin and Eosin, x100). F: Severe multifocal pneumonia (arrow) consisting of interstitial infiltration of lymphocytes, plasma cells and eosinophils (Haematoxylin and Eosin, x40).

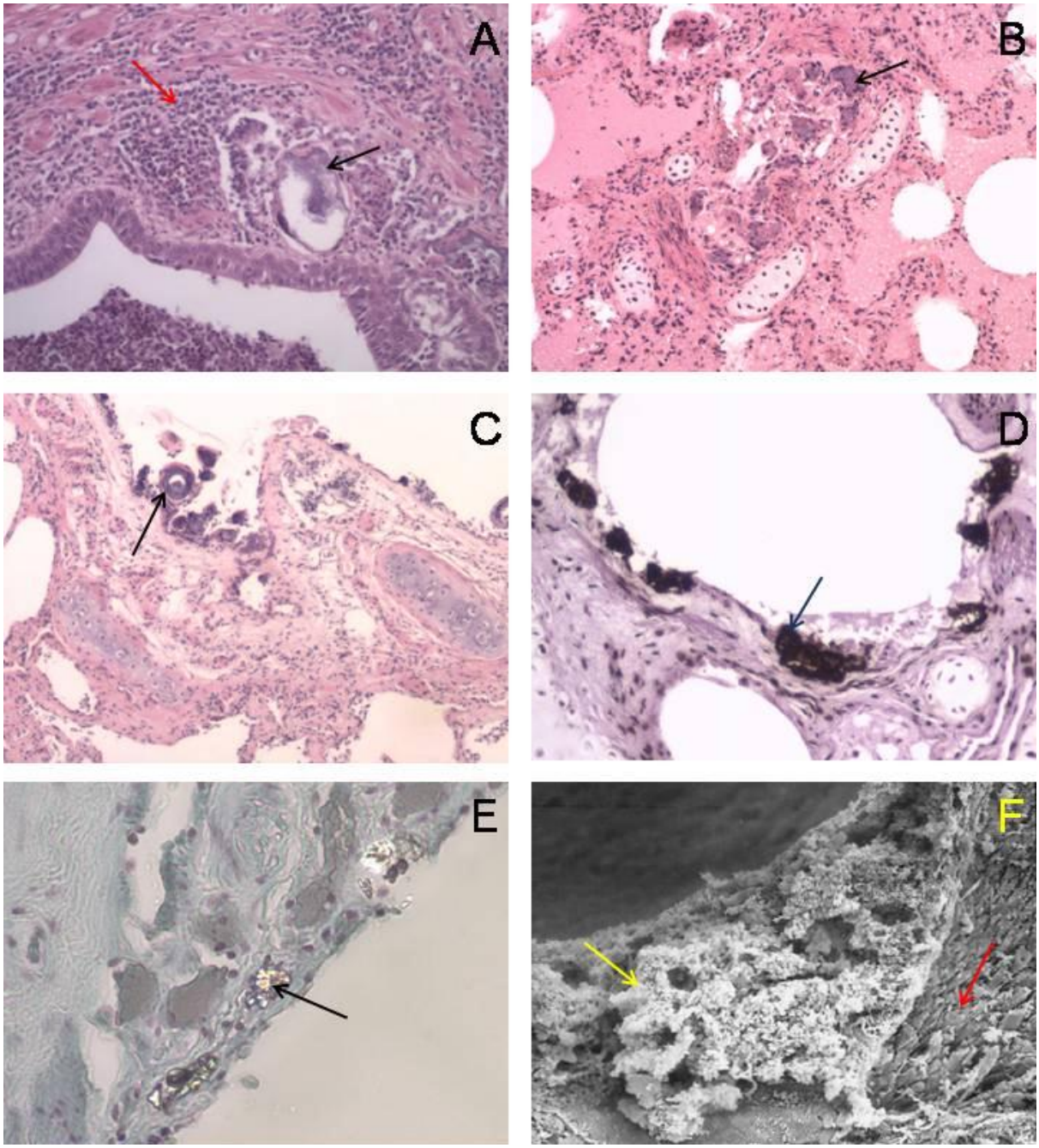

Figure D.3. Bronchiolar calcification. A: Inflammation (red arrow) associated with possible early mucosal lesions (black arrow) (Haematoxylin and Eosin, x200). B –C: Foci of calcification within the bronchiolar and bronchial mucosa respectively (Haematoxylin and Eosin, x100). D: Von Kossa staining confirming mineralisation (x200). E: Gram stain where mineralized foci (arrow) are birefringent (x200). F: Scanning electro-micrograph of foci of calcification (yellow arrow) next to normal epithelium with cilia (red arrow).

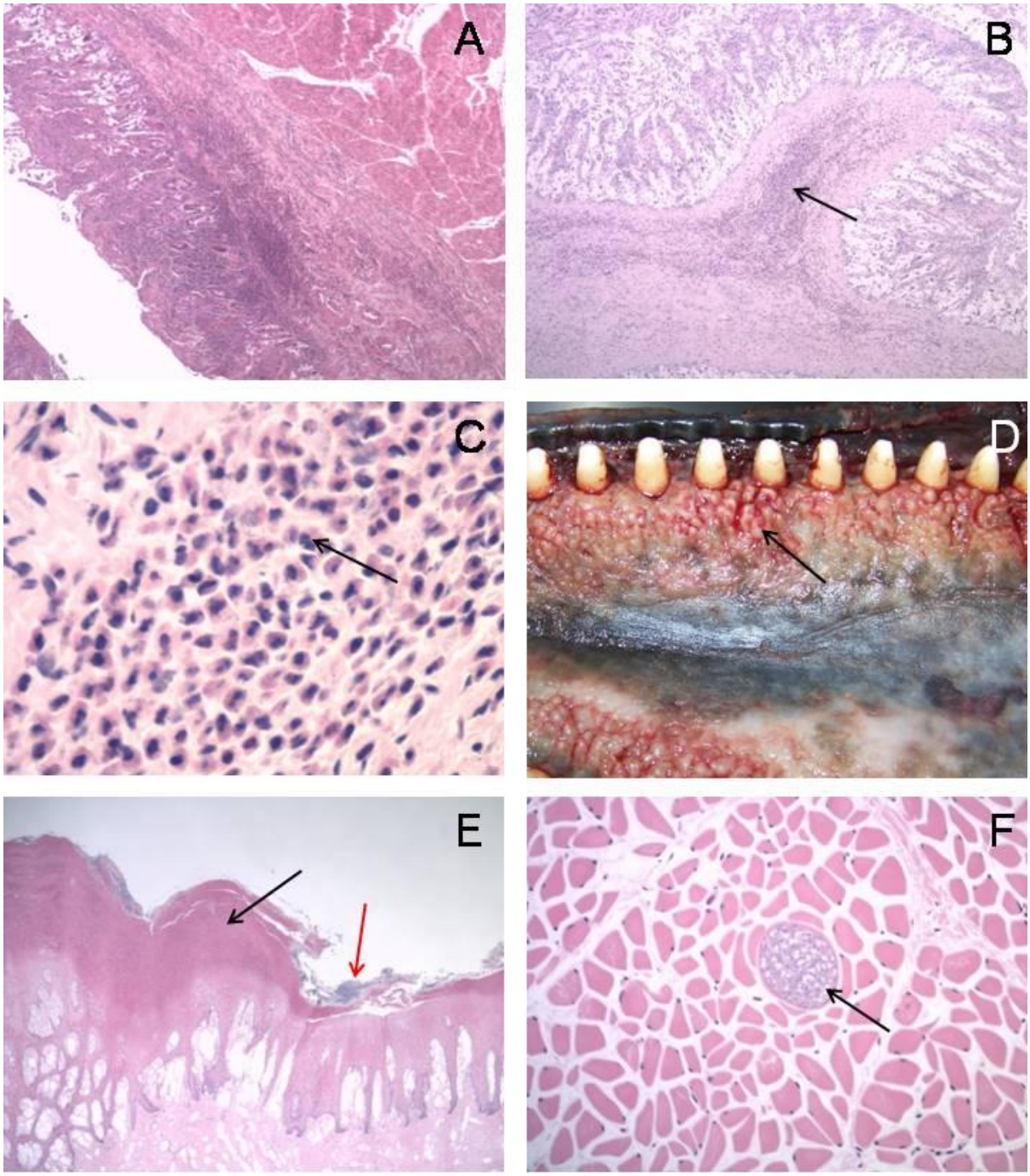

Figure D.4. Gastrointestinal tract lesions. A: Severe locally extensive acute eosinophilic enteritis (Haematoxylin and Eosin, x40). B: Moderate diffuse subacute eosinophilic and lymphoplasmacytic enteritis (Haematoxylin and Eosin, x40). C: Inflammation in the intestine was mainly eosinophilic (black arrow) in nature (Haematoxylin and Eosin, x400). D: Macroscopic irregular firm protrusions on the gingival mucosa of the hard palate. E: Severe locally extensive hyperplasia of gingival mucosa (black arrow) with mixed colonies of bacteria (Haematoxylin and Eosin, x20). F: Protozoal cyst (suspected *Sarcocystis* sp.) in the lingual muscle (black arrow) (Haematoxylin and Eosin, x400).

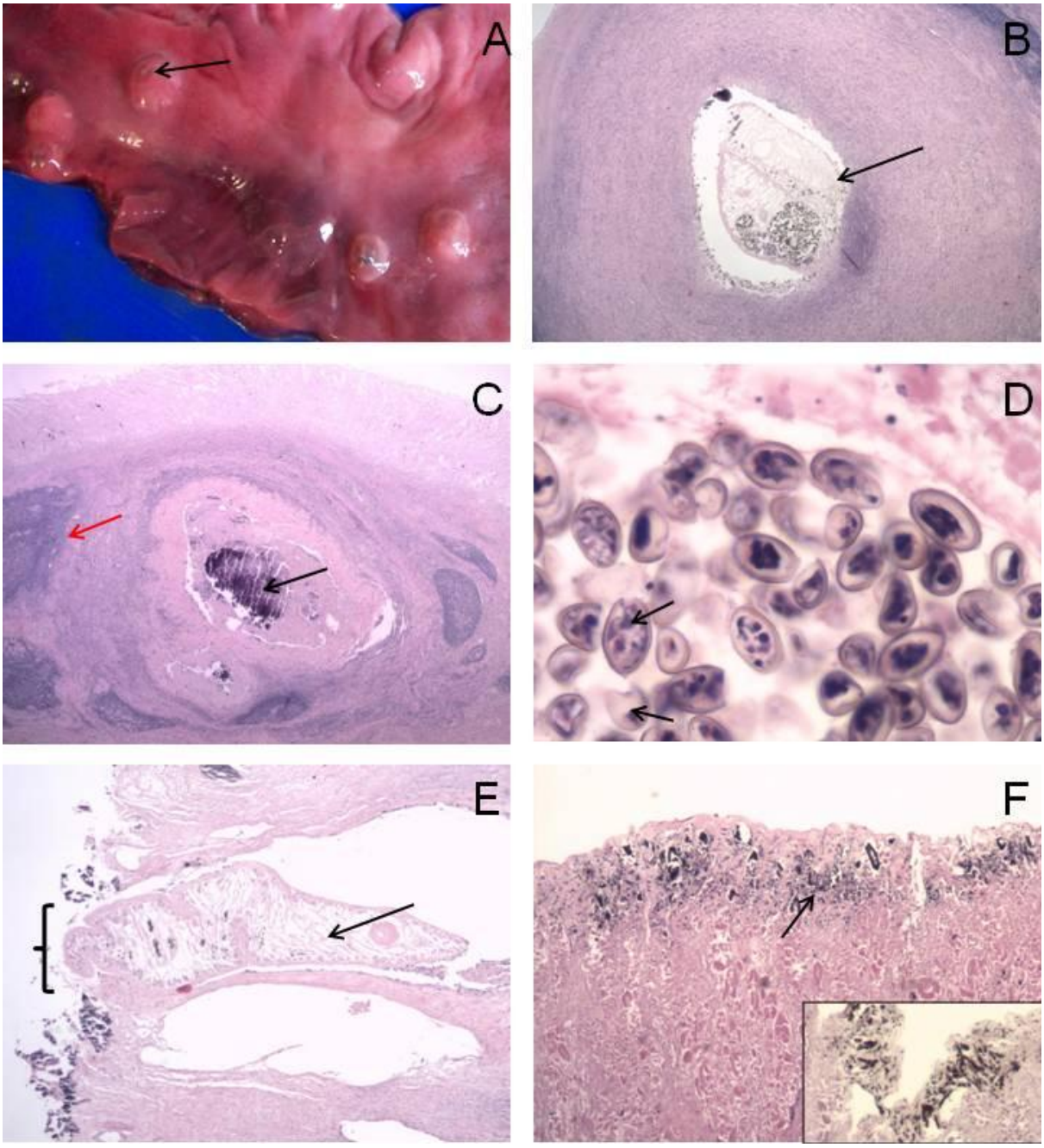

Figure D.5. Trematode and associated lesions. A: Macroscopic firm, round, parasitic nodules, up to 1 cm in diameter, occurring either singularly or in clusters of up to six nodules, mainly in the pyloric compartment, with small pore opening to the gastric lumen (arrow). B: Severe focal chronic parasitic gastritis with central adult trematode from the family Brachycladiinae (black arrow) (Haematoxylin and Eosin, x20). C: Parasitic nodule with dystrophic calcification of cellular debris (black arrow), and marked follicular lymphoid hyperplasia (Haematoxylin and Eosin, x20). D: Trematode eggs were embryonated, with one operculum and one polar spine (Haematoxylin and Eosin, x400). E: Parasitic nodule with adult trematode (black arrow) with pore visible (bracket) (Haematoxylin and Eosin, x40). F: Moderate multifocal dystrophic mineralisation of the gastric mucosa (Haematoxylin and Eosin, x40) confirmed on Von Kossa stain (insert) (x40).

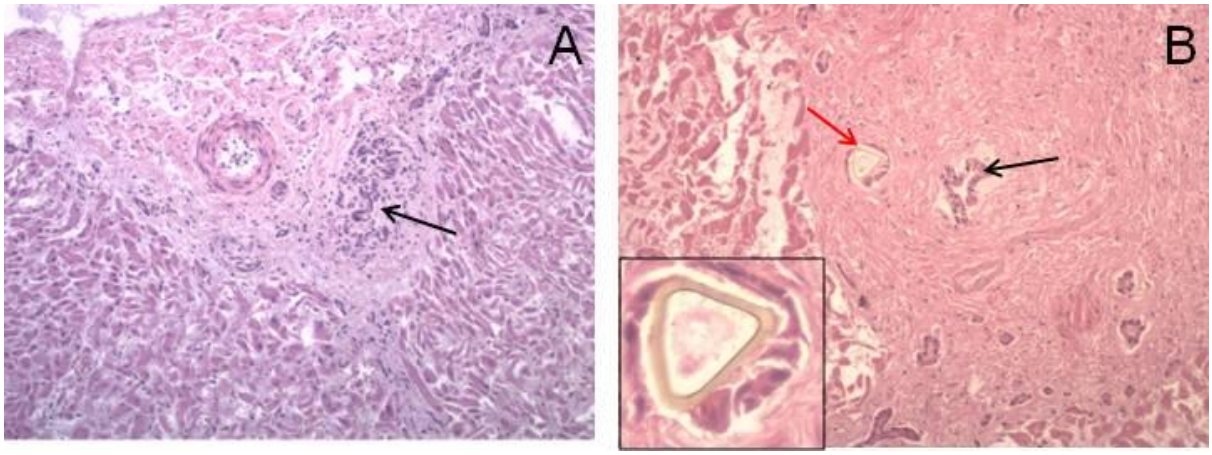

Figure D.6. Hepatic lesions. A: Mild multifocal bile ductular hyperplasia (black arrow) within the portal triad (Haematoxylin and Eosin, x40): B: Severe hepatic periportal fibrosis associated with a free trematode egg (red arrow) (Haematoxylin and Eosin, x100) also occurring within the bile ductules (insert (Haematoxylin and Eosin, x400)).

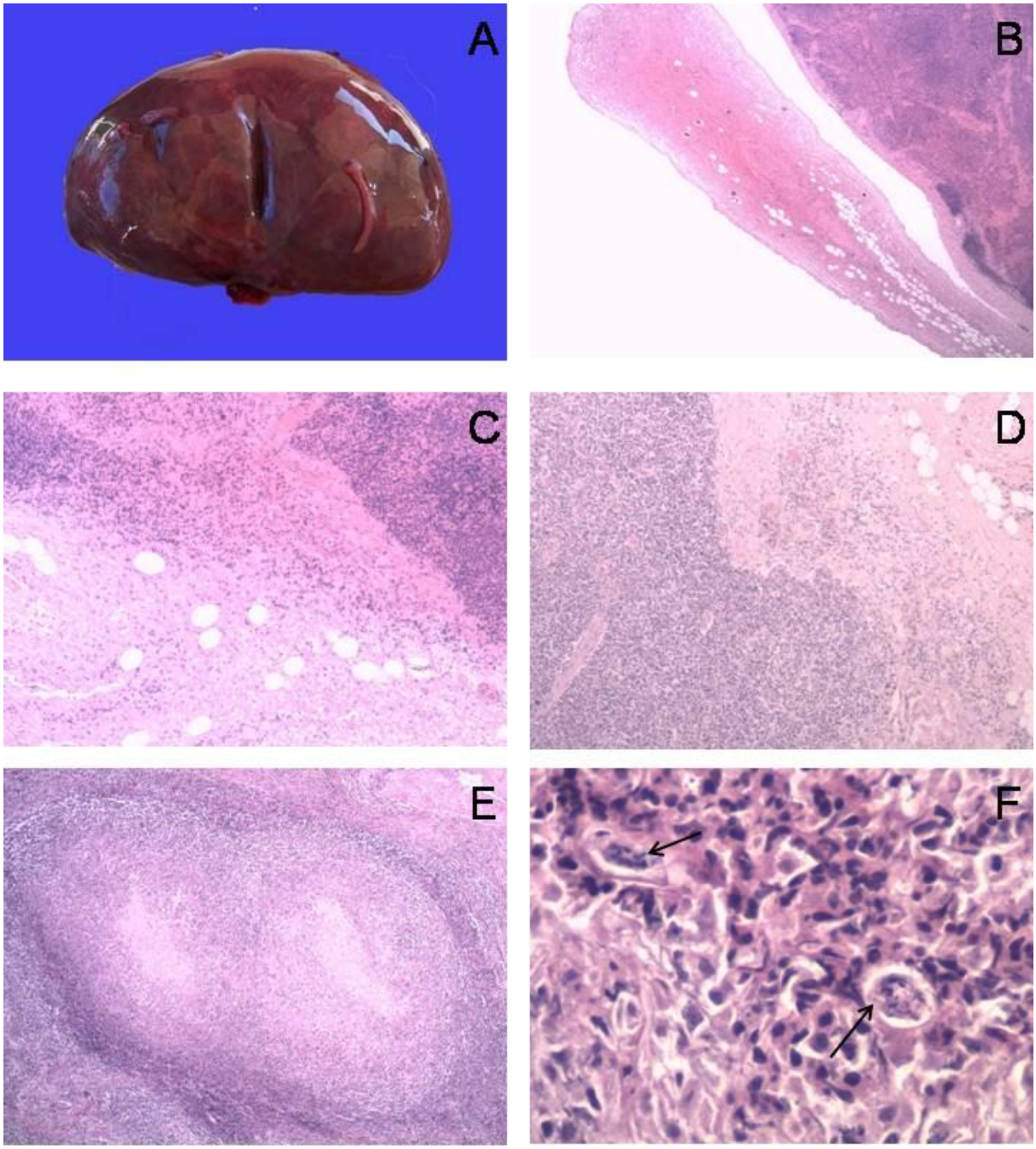

Figure D.7. A: Macroscopic splenic tags. B: Splenic tag that consisted of mature fibrovascular connective tissue (Haematoxylin and Eosin, x20). C: Mild multifocal subacute lymphoplasmacytic and eosinophilic capsular splenitis often accompanied the fibrovascular tags (Haematoxylin and Eosin, x100). D: Mesenteric lymph node with moderate multifocal eosinophilic capsular lymphadenitis (Haematoxylin and Eosin, x100) E: Severe focal necro-granulomatous mesenteric lymphadenitis (Haematoxylin and Eosin, x40). F: Nematode larvae (black arrows) associated with mesenteric lymphadenitis (Haematoxylin and Eosin, x200).

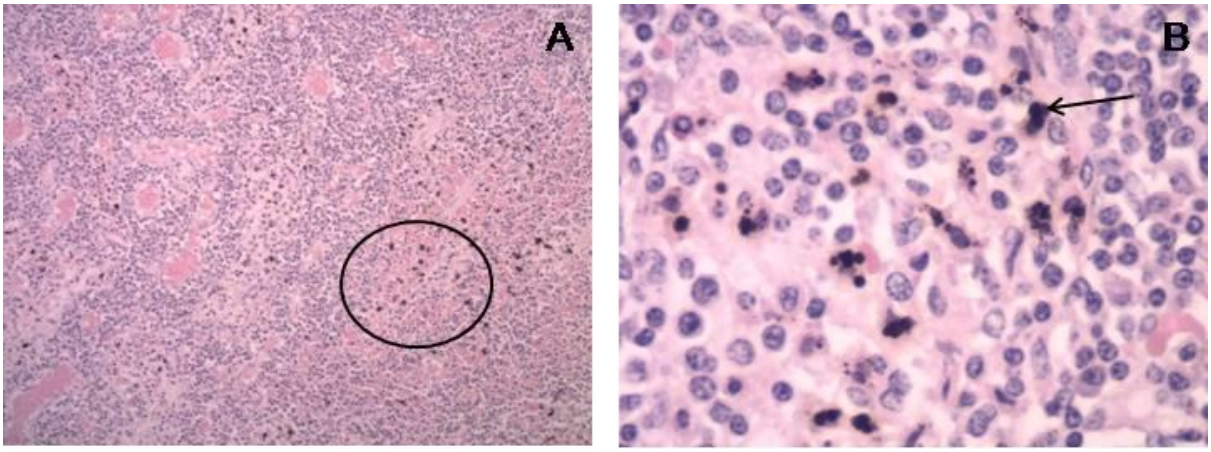

Figure D.8. A: Foreign material accumulation in the marginal lymph node of the lung (Haematoxylin and Eosin, x100). B: Particulate matter (possibly carbon) accumulation in macrophages in the marginal lymph node of the lung (Haematoxylin and Eosin, x400)

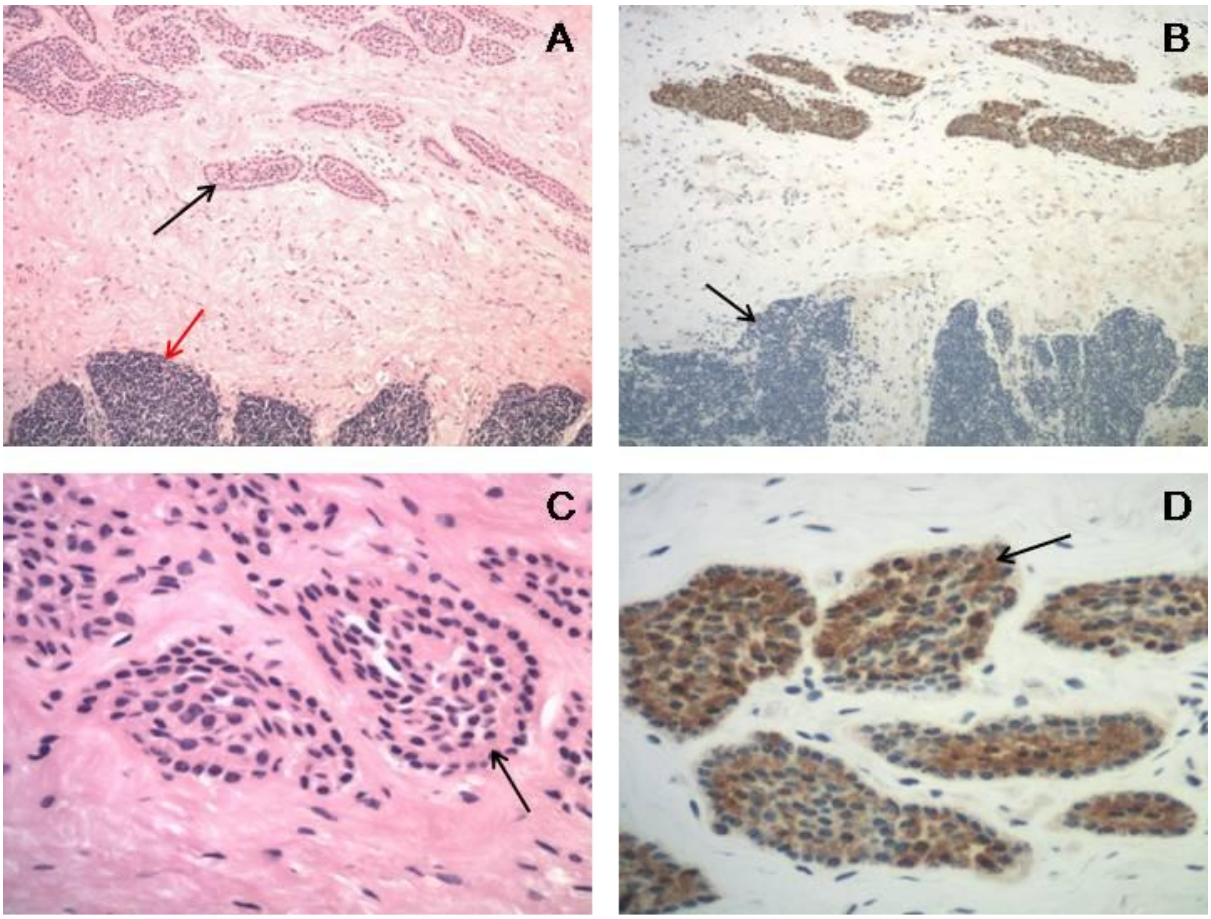

Figure D.9. Neuroendocrine glands. A: Acini of low cells (black arrow) found next to the thymus (Haematoxylin and Eosin, x100). B: Chromogranin A stain of A. C: Acini were composed of low cuboidal cells (black arrow) (Haematoxylin and Eosin, x400). D: Immunolabelling of chromogranin A in neuroendocrine cells, haematoxylin counterstain (x400).

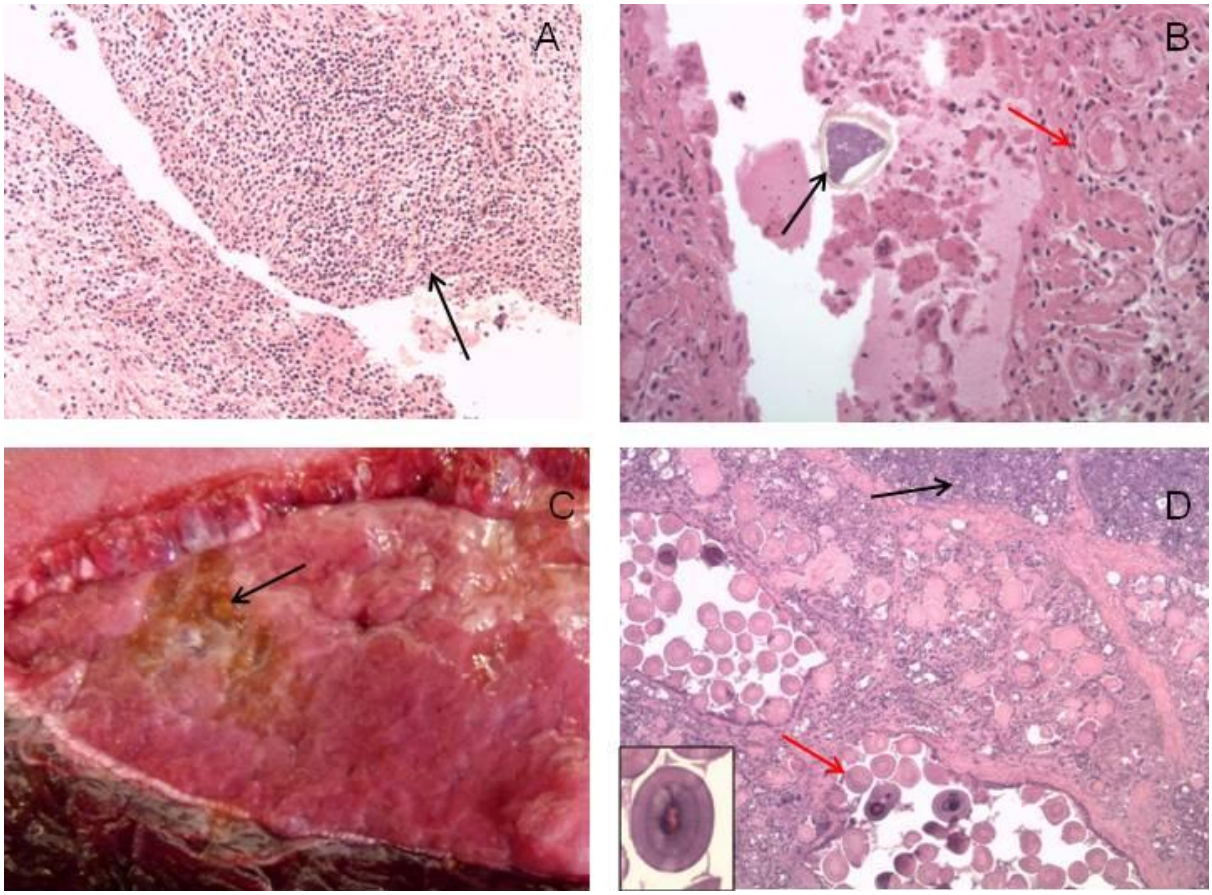

Figure D.10. Female reproductive tract lesions. A: Severe lymphoplasmacytic endometritis (black arrow) (Haematoxylin and Eosin, x100). B: Mild multifocal eosinophilic endometritis (red arrow) associated with a trematode egg (black arrow) (Haematoxylin and Eosin, x200). C: Macrograph of mammary gland showing focal mastitis (black arrow). D: Large numbers of *corpora amylacea* (red arrow and insert) within dilated ducts adjacent to moderate multifocal subacute lymphoplasmacytic mastitis (black arrow) (Haematoxylin and Eosin, x40).

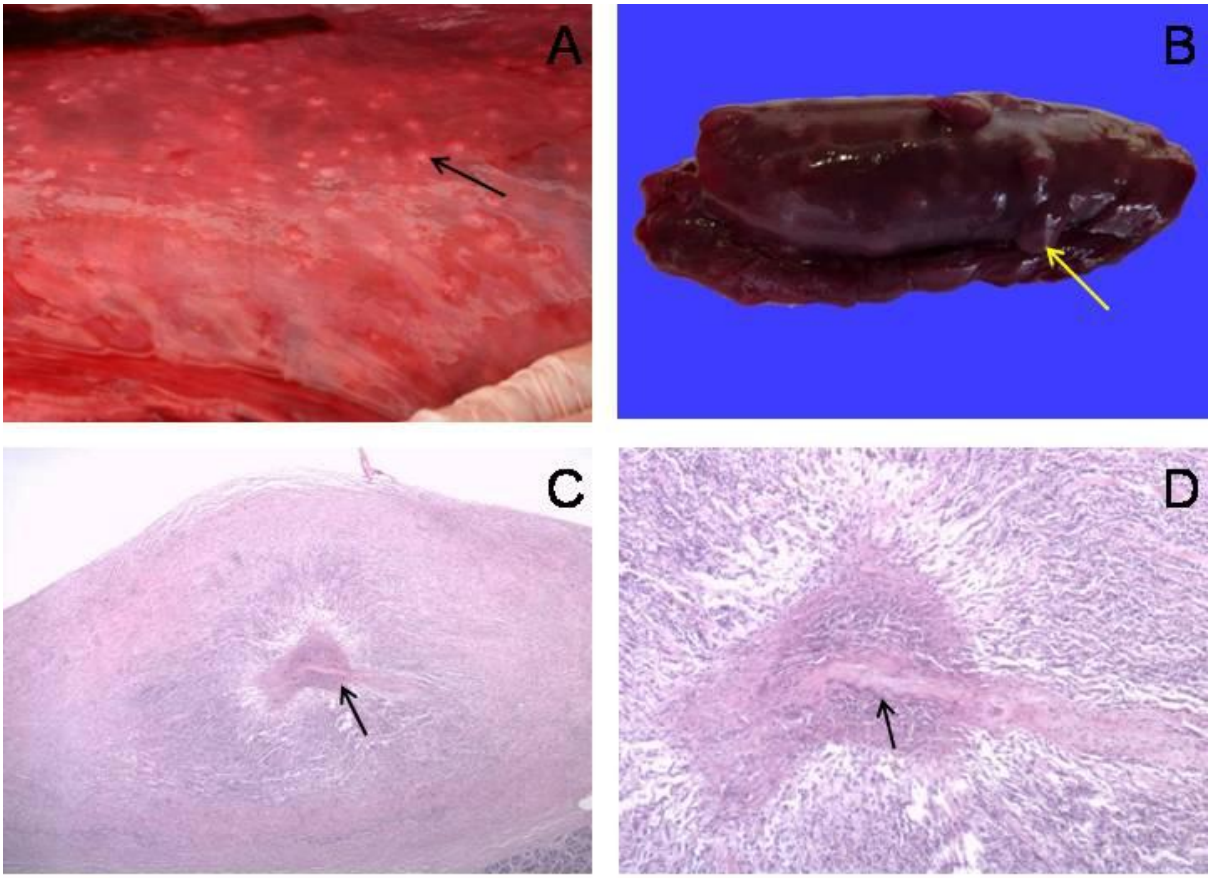

Figure D.11. Male reproductive tract and peritoneal lesions. A: Peritoneum with multiple, slightly raised, firm white nodules, also found on the testes, liver, kidneys and mesenteric lymph nodes. B: Multiple firm nodules found on the testis. C: Severe focal granulomatous orchitis (Haematoxylin and Eosin, x20). D: Central area of necrosis (black arrow), resembling a migration tract (Haematoxylin and Eosin, x40).

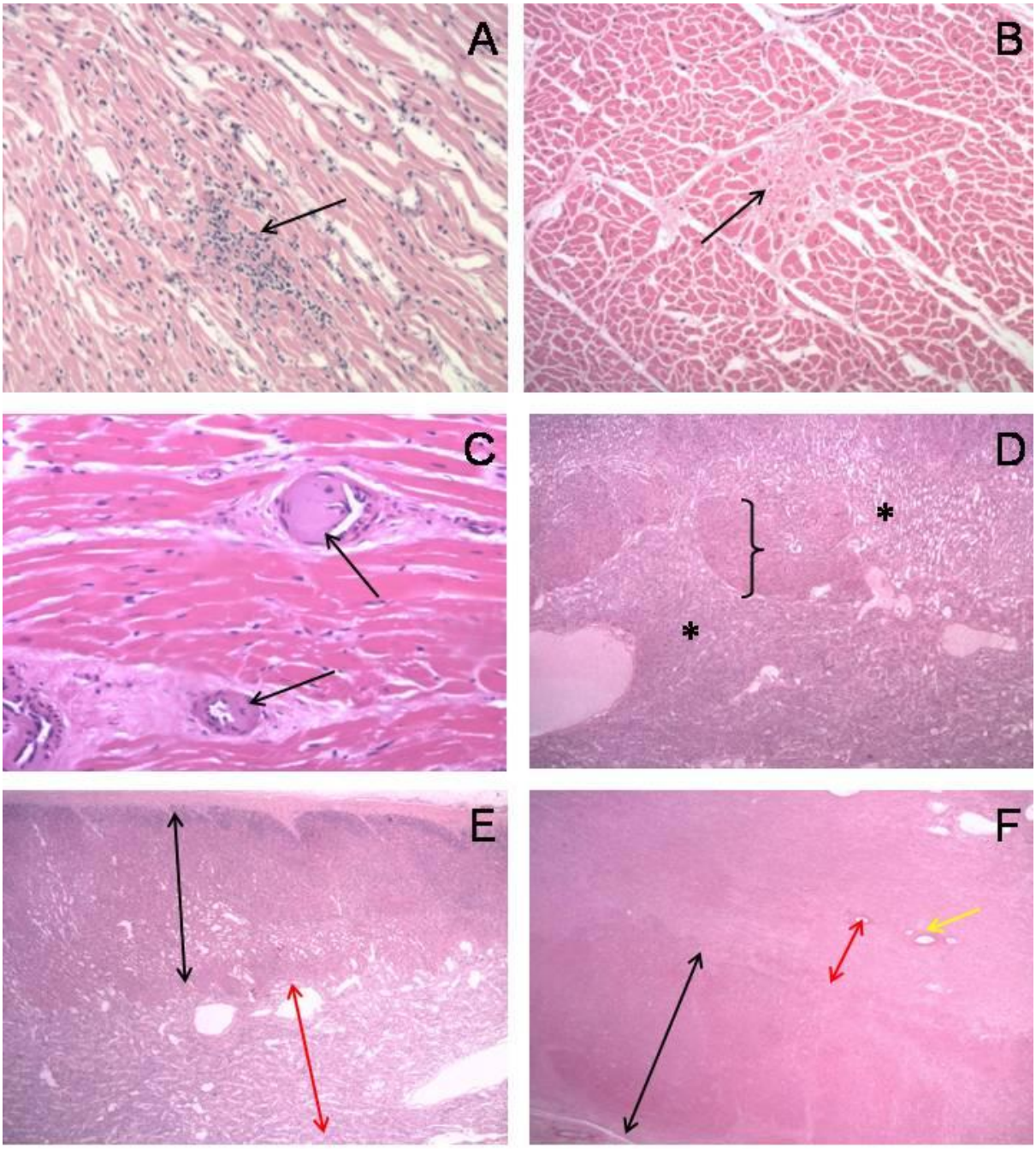

Figure D.12. Cardiovascular and adrenal lesions. A: Mild focal subacute lymphoplasmacytic myocarditis (arrow) (Haematoxylin and Eosin, x200). B: Mild focal myocardial fibrosis (arrow) (Haematoxylin and Eosin, x100). C: Arteriosclerosis of two blood vessels (arrows) in the myocardium (Haematoxylin and Eosin, x200). D: Mild multifocal nodular adrenal cortical hyperplasia as evident by cortical nodules (brackets) surrounded by adrenal medulla (*), with normal appearing cortex (red arrow) (Haematoxylin and Eosin, x20). E: Normal adrenal gland with cortex (black arrow) and medulla (red arrow) of approximately the same width (Haematoxylin and Eosin, x20). F: Moderate diffuse adrenal cortical hyperplasia as seen by an increase in the ratio of cortex (black arrow) to medulla (red arrow) (Haematoxylin and Eosin, x20).

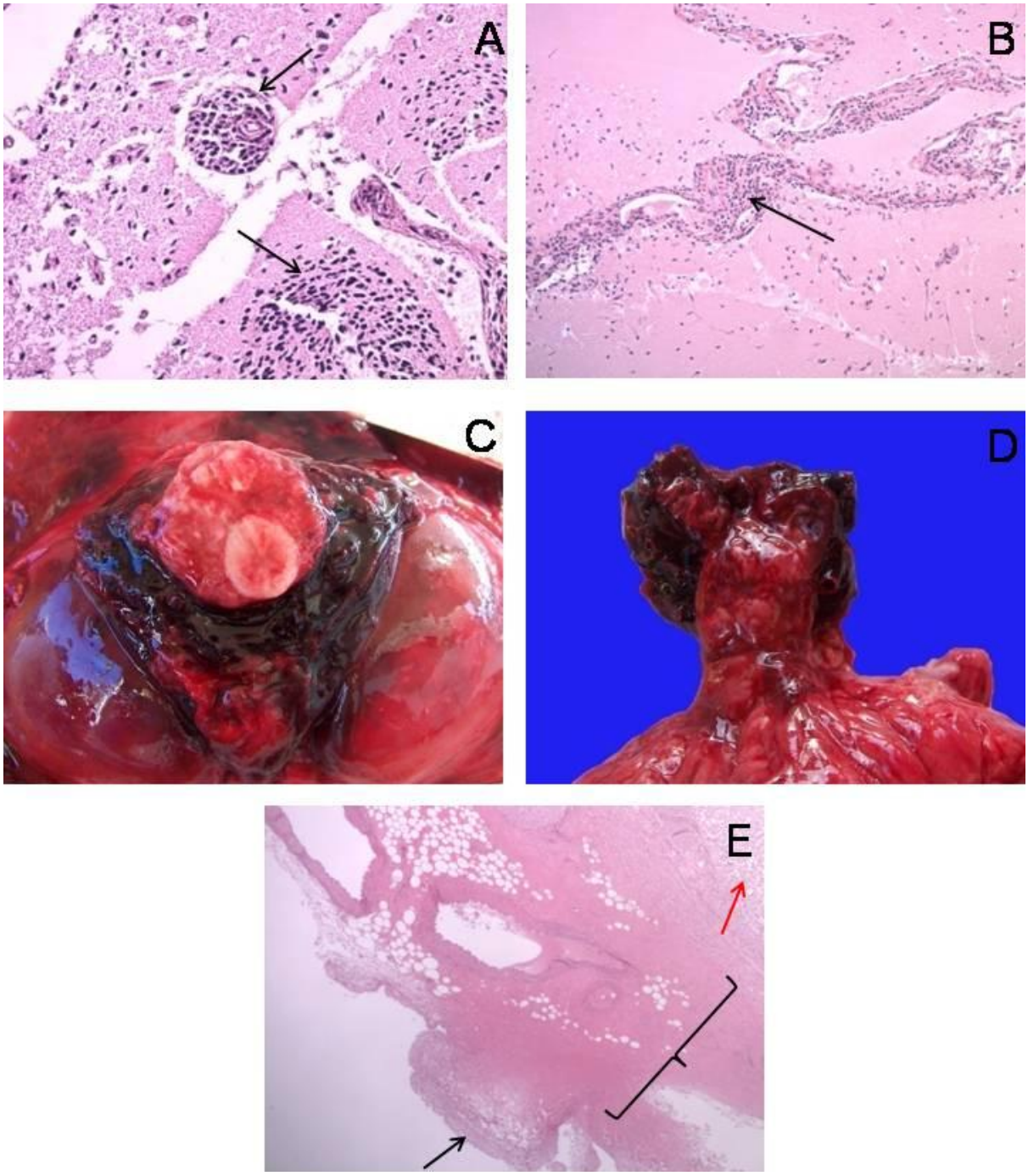

Figure D.13. Central nervous system lesions A: Moderate multifocal subacute lymphoplasmacytic perivascular encephalitis (Haematoxylin and Eosin, x200). B: Moderate multifocal subacute lymphoplasmacytic meningitis (Haematoxylin and Eosin, x100). C – D: Haemorrhage around the spinal cord as it exits the *foramen magnum*. E: Histology of previous with the haemorrhage visible (bracket) between the spinal cord (red arrow) and synovium of the occipital joint (black arrow) (Haematoxylin and Eosin, x20).

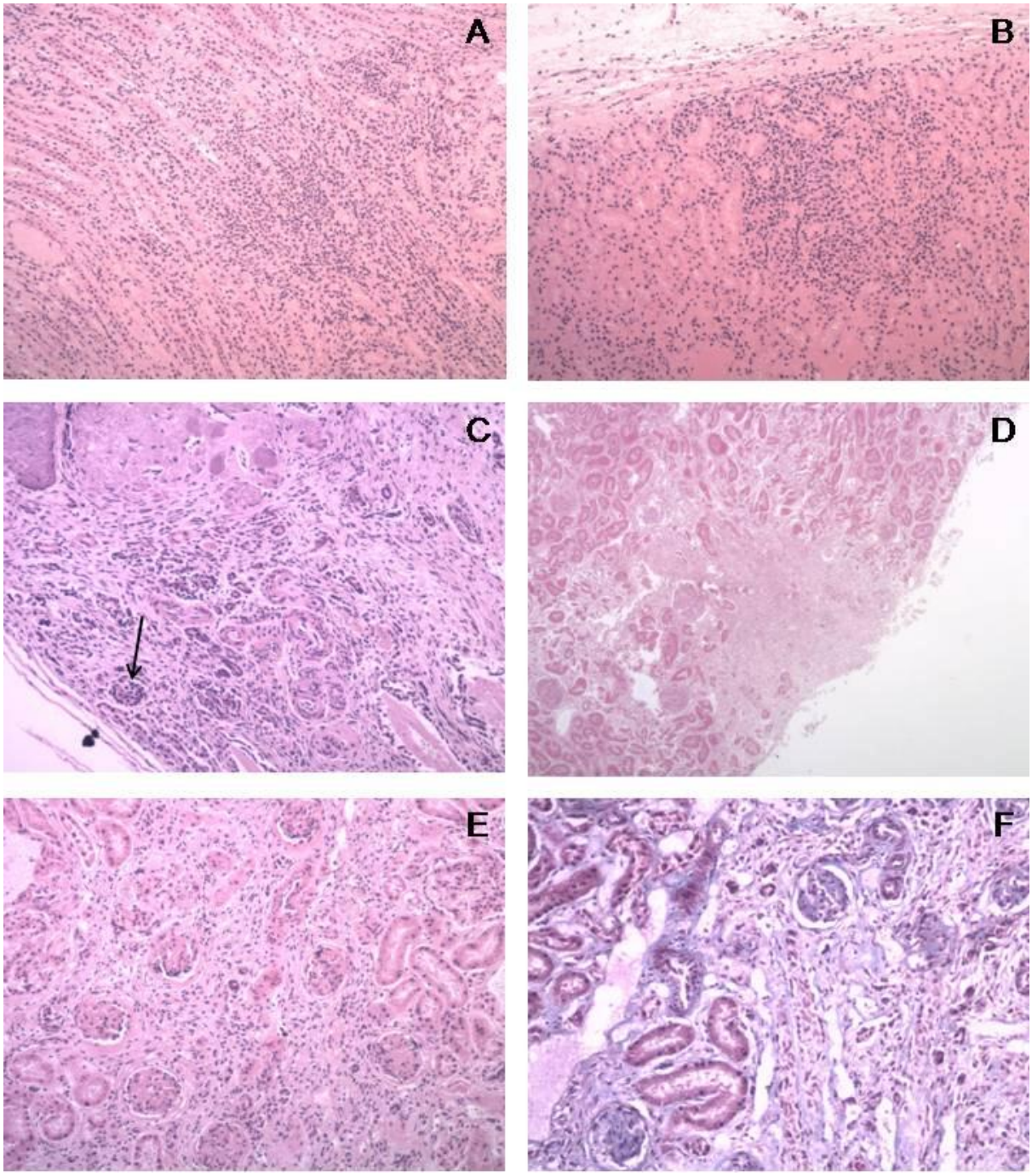

Figure D.14. Renal lesions. A: Mild multifocal subacute lymphoplasmacytic interstitial nephritis (Haematoxylin and Eosin, x20). B: Mild multifocal subacute lymphoplasmacytic subcapsular interstitial nephritis (Haematoxylin and Eosin, x100). C: Mild focal renal hypoplasia with immature glomeruli (black arrow) (Haematoxylin and Eosin, x20). D: Mild multifocal renal cortical fibrosis (Haematoxylin and Eosin, x40). E: Mild multifocal chronic ascending pyelonephritis (Haematoxylin and Eosin, x200) F: Masson's trichrome confirming fibrosis associated with the pyelonephritis (x100).

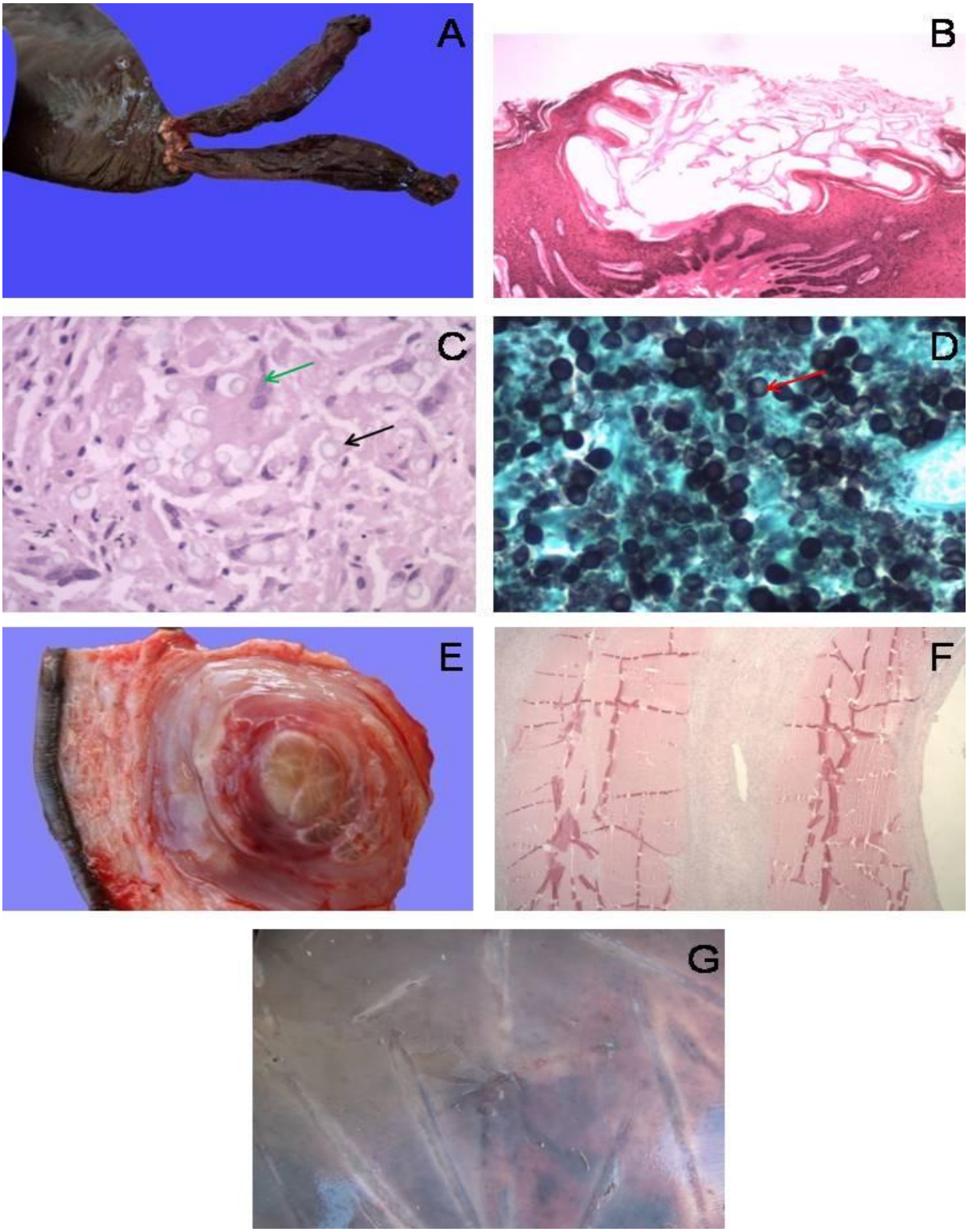

Figure D.15. Skin and subcutis. A: Flipper with *Xenobalanus globicipitis* attached. B: Attachment site of *X. globicipitis* with arborizing epidermal hyperplasia with no inflammatory reaction (Haematoxylin and Eosin, x40). C: Lobomycosis with fungal cells (black arrows) and multinucleate giant cells (green arrow) (Haematoxylin and Eosin, x400). D: GMS of previous lesion highlighting fungal cells (x400). E: Macroscopic appearance of nodular dermatitis. F: Histological appearance of nodular severe chronic deep dermatitis with granulation tissue, and compartmentalised hyaline material (Haematoxylin and Eosin, x20). G: Net marks found on all animals.

Appendix E. Tables Containing Complete Pathological Findings per Organ System

Table E.1. Complete pathological findings for the respiratory tract, indicating occurrence (lesion/number of organ evaluated) and percentage by species, age group, and region (for both species combined).

Lesion	Total	*T. aduncus*			*S. chinensis*			Region	
		Calf	Juvenile	Adult	Calf	Juvenile	Adult	North	South
Combined pneumonia	37/40	17/19	10/10	5/6	1/1	2/2	2/2	27/30	10/10
	93%	89%	100%	83%	100%	100%	100%	90%	100%
Mild to severe, multifocal subacute eosinophilic and lymphoplasmacytic parasitic pneumonia	8/40	6/19	0/10	1/6	0/1	0/2	1/2	6/30	2/10
	20%	32%	0%	17%	0%	0%	50%	20%	20%
Mild to moderate, multifocal to diffuse, acute to subacute eosinophilic and variably lymphoplasmacytic pneumonia	34/40	15/19	9/10	5/6	1/1	2/2	2/2	24/30	10/10
	85%	79%	90%	83%	100%	100%	100%	80%	100%
Mild to moderate, multifocal, subacute lymphoplasmacytic pneumonia	3/40	2/19	1/10	0/6	0/1	0/2	0/2	3/30	0/10
	8%	11%	10%	0%	0%	0%	0%	10%	0%
Mild to moderate, multifocal to diffuse interstitial fibrosis	12/40	4/19	0/10	5/6	0/1	1/2	2/2	10/30	2/10
	30%	21%	0%	83%	0%	50%	100%	33%	20%
Mild to moderate, multifocal, subacute eosinophilic and lymphoplasmacytic tracheo-bronchitis	12/40	8/19	4/10	0/6	0/1	0/2	0/2	7/30	5/10
	30%	42%	40%	0%	0%	0%	0%	23%	50%
Moderate to severe, multifocal to diffuse acute alveolar oedema	32/40	16/19	8/10	6/6	1/1	1/2	0/2	24/30	8/10
	80%	84%	80%	100%	100%	100%	0%	80%	80%
Moderate to severe, multifocal to diffuse, acute alveolar emphysema	32/40	17/19	9/10	4/6	0/1	1/2	1/2	24/30	8/10
	80%	89%	90%	67%	0%	50%	50%	80%	80%

Table E.1. (cont.)

Mild to severe, multifocal follicular lymphoid hyperplasia of the bronchial associated lymphoid tissue	18/40	9/19	6/10	0/6	1/1	1/2	1/2	14/30	4/10
	45%	47%	60%	0%	100%	50%	50%	47%	40%
Mild to moderate, multifocal, bronchial and bronchiolar mucosal mineralisation	33/40	16/19	8/10	5/6	1/1	2/2	1/2	25/30	8/10
	83%	84%	80%	83%	100%	100%	50%	83%	80%
Mild, multifocal pneumoconiosis	3/40	0/19	0/10	2/6	0/1	1/2	0/2	2/30	1/10
	8%	0%	0%	33%	0%	50%	0%	7%	10%
Mild to moderate, multifocal alveolar macrophage hyperplasia (histiocytosis)	6/40	3/19	0/10	1/6	1/1	1/2	0/2	6/30	0/10
	15%	16%	0%	17%	100%	50%	0%	20%	0%
Small numbers of corpora amylacea free in the alveoli	2/40	0/19	2/10	0/6	0/1	0/2	0/2	2/30	0/10
	5%	0%	20%	0%	0%	0%	0%	7%	0%
Mild to moderate, multifocal to diffuse, subacute eosinophilic to lymphoplasmacytic pleuritis	12/40	6/19	4/10	0/6	0/1	2/2	0/2	9/30	3/10
	30%	32%	40%	0%	0%	100%	0%	30%	30%
Mild multifocal pleural fibrosis	4/40	1/19	1/10	0/6	0/1	1/2	1/2	4/30	0/10
	10%	5%	10%	0%	0%	50%	50%	13%	0%
Pulmonary haemosiderosis	1/40	0/19	0/10	0/6	0/1	0/2	1/2	1/30	0/10
	3%	0%	0%	0%	0%	0%	50%	3%	0%

Table E.2. Complete pathological findings for the gastrointestinal tract, indicating occurrence (lesion/number of organ evaluated) and percentage by species, age group, and region (for both species combined).

Lesion	Total	*T. aduncus*			*S. chinensis*			Region	
		Calf	Juvenile	Adult	Calf	Juvenile	Adult	North	South
Mild, multifocal, subacute lymphoplasmacytic and variably eosinophilic glossitis	10/34	3/19	3/8	4/5	0/1	0/0	0/1	9/25	1/9
	29%	16%	38%	80%	0%	0%	0%	36%	11%
Glossal muscle sarcocystosis	1/34	1/19	0/8	0/5	0/1	0/0	0/1	0/25	1/9
	3%	5%	0%	0%	0%	0%	0%	0%	11%
Mild, multifocal, subacute lymphoplasmacytic pharyngitis	5/32	4/14	0/10	1/5	0/1	0/1	0/1	5/24	0/8
	16%	29%	0%	20%	0%	0%	0%	21%	0%
Mild, multifocal, subacute, lymphoplasmacytic and variably eosinophilic sialoadenitis	8/32	2/14	2/10	4/5	0/1	0/1	0/1	6/24	2/8
	25%	14%	20%	80%	0%	0%	0%	25%	25%
Peyer's patches present in intestine	22/40	11/19	6/10	3/6	1/1	1/2	0/2	19/30	3/10
	55%	58%	60%	50%	100%	50%	0%	63%	30%
Mild, multifocal, subacute variably eosinophilic and lymphoplasmacytic oesophagitis	4/27	2/12	0/8	2/3	0/1	0/1	0/2	3/19	1/8
	15%	17%	0%	67%	0%	0%	0%	16%	13%
Mild, multifocal, subacute lymphoplasmacytic and eosinophilic gastritis	28/38	11/19	8/9	6/6	1/1	1/1	1/2	20/28	8/10
	74%	58%	89%	100%	100%	100%	50%	71%	80%
Moderate to severe, multifocal, chronic parasitic gastritis	12/38	5/19	5/9	2/6	0/1	0/1	0/2	8/28	4/10
	32%	26%	56%	33%	0%	0%	0%	29%	40%
Mild to severe, multifocal to diffuse, subacute, variably lymphoplasmacytic and eosinophilic enteritis	27/40	9/19	10/10	6/6	0/1	2/2	0/2	21/30	6/10
	68%	47%	100%	100%	0%	100%	0%	70%	60%
Tonsillar lymphoid follicles present	22/32	11/14	7/10	3/5	0/1	0/1	1/1	17/24	5/8
	69%	79%	70%	60%	0%	0%	100%	71%	63%

Table E.2. (cont.)

Mild to moderate, subacute, lymphoplasmacytic and variably eosinophilic multifocal hepatitis	5/39	3/19	2/10	0/6	0/1	0/2	0/1	4/29	1/10
	13%	16%	20%	0%	0%	0%	0%	14%	10%
Mild to moderate, subacute, lymphoplasmacytic and eosinophilic periportal hepatitis	20/39	11/19	4/10	5/6	0/1	0/2	0/1	16/29	4/10
	51%	58%	40%	83%	0%	0%	0%	55%	40%
Mild, chronic, lymphoplasmacytic and eosinophilic parasitic portal hepatitis	2/39	0/19	1/10	1/6	0/1	0/2	0/1	1/29	1/10
	5%	0%	10%	17%	0%	0%	0%	3%	10%
Moderate, multifocal, subacute lymphoplasmacytic and eosinophilic cholangitis	4/39	1/19	1/10	0/6	1/1	1/2	0/1	4/29	0/10
	10%	5%	5%	0%	100%	50%	0%	14%	0%
Mild to severe, portal and subcapsular, bile ductular hyperplasia	17/39	7/19	3/10	5/6	1/1	1/2	0/1	12/29	5/10
	44%	37%	30%	83%	100%	50%	0%	41%	50%
Mild to moderate, portal fibrosis	10/39	2/19	3/10	4/6	0/1	1/2	0/1	7/29	3/10
	26%	11%	30%	67%	0%	50%	0%	24%	30%
Mild to moderate, multifocal, hepatic capsular fibrosis	3/39	0/19	1/10	2/6	0/1	0/2	0/1	2/29	1/10
	8%	0%	10%	33%	0%	0%	0%	7%	10%
Mild, multifocal, subacute lymphoplasmacytic and eosinophilic pancreatitis	2/35	2/17	0/9	0/5	0/1	0/2	0/1	2/26	0/9
	6%	12%	0%	0%	0%	0%	0%	8%	0%

Table E.3. Complete pathological findings for the lympho-haemopoeitic system, indicating occurrence (lesion/number of organ evaluated) and percentage by species, age group, and region (for both species combined).

Lesion	Total	*T. aduncus*			*S. chinensis*			Region	
		Calf	Juvenile	Adult	Calf	Juvenile	Adult	North	South
Mild, multifocal, splenic capsular fibrosis	18/39	7/19	6/10	4/5	0/1	0/2	1/2	10/29	8/10
	46%	37%	60%	80%	0%	0%	50%	34%	80%
Mild to moderate, multifocal, subacute, variably lymphoplasmacytic and eosinophilic capsular splenitis	12/39	4/19	4/10	4/5	0/1	0/2	0/2	8/29	4/10
	31%	21%	40%	80%	0%	0%	0%	28%	40%
Mild to moderate, multifocal splenic lymphoid hyperplasia	7/39	2/19	2/10	3/5	0/1	0/2	0/2	6/29	1/10
	18%	11%	20%	60%	0%	0%	0%	21%	10%
Mild to moderate, multifocal, acute to subacute, eosinophilic and variably lymphoplasmacytic cervical capsular lymphadenitis	10/39	4/18	3/10	3/6	0/1	0/2	0/2	6/29	4/10
	26%	22%	30%	50%	0%	0%	0%	21%	40%
Mild, multifocal cervical lymphoid hyperplasia	8/39	3/18	1/10	2/6	1/1	1/2	0/2	4/29	4/10
	21%	17%	10%	33%	100%	50%	0%	14%	40%
Mild, cervical lymph node haemosiderosis	1/39	0/18	0/10	1/6	0/1	0/2	0/2	0/29	1/10
	3%	0%	0%	17%	0%	0%	0%	0%	10%
Mild to severe, multifocal, acute to subacute, eosinophilic and variably lymphoplasmacytic mesenteric capsular lymphadenitis	19/39	5/19	5/10	5/5	0/1	2/2	2/2	15/29	4/10
	49%	26%	50%	100%	0%	100%	100%	52%	40%
Mild, multifocal, mesenteric lymphoid hyperplasia	6/39	3/19	2/10	1/5	0/1	0/2	0/2	6/29	0/10
	15%	16%	20%	20%	0%	0%	0%	31%	0%
Mild, multifocal, mesenteric lymphoid hypoplasia	1/39	0/19	0/10	1/5	0/1	0/2	0/2	0/29	1/10
	3%	0%	0%	20%	0%	0%	0%	0%	10%
Mild, multifocal, mesenteric haemosiderosis	4/39	1/19	1/10	1/5	1/1	0/2	0/2	2/29	2/10
	10%	5%	10%	20%	100%	0%	0%	7%	20%

Table E.3. (cont.)

Mild, multifocal, foreign pigment accumulation in the mesenteric lymph node	1/39	0/19	0/10	0/5	0/1	0/2	1/2	1/29	0/10
	3%	0%	0%	0%	0%	0%	50%	7%	0%
Mild to moderate, multifocal, subacute, eosinophilic and lymphoplasmacytic marginal lymph node of the lung capsular lymphadenitis	14/30	5/13	5/8	2/6	0/1	2/2	0/0	9/22	5/8
	47%	38%	63%	33%	0%	100%	0%	41%	63%
Mild, multifocal, marginal lymph node of the lung lymphoid hyperplasia	3/30	1/13	1/8	0/6	0/1	1/2	0/0	3/22	0/8
	10%	8%	13%	0%	0%	50%	0%	14%	0%
Mild, multifocal, marginal lymph node of the lung lymphoid hypoplasia	1/30	0/13	1/8	0/6	0/1	0/2	0/0	0/22	1/8
	3%	0%	13%	0%	0%	0%	0%	0%	13%
Mild, multifocal, marginal lymph node of the lung haemosiderosis	3/30	0/13	2/8	1/6	0/1	0/2	0/0	2/22	1/8
	10%	0%	25%	17%	0%	0%	0%	9%	13%
Mild, multifocal, foreign material accumulation in the marginal lymph node of the lung	3/30	0/13	2/8	1/6	0/1	0/2	0/0	1/22	2/8
	10%	0%	25%	17%	0%	0%	0%	9%	13%
Mild, multifocal, subacute lymphoplasmacytic thymitis and peri-thymitis	2/21	2/15	0/4	0/1	0/1	0/0	0/0	1/14	1/7
	10%	13%	0%	0%	0%	0%	0%	7%	14%
Small numbers of peri-thymic cuboidal cells	2/21	0/15	1/4	0/1	1/1	0/0	0/0	1/14	1/7
	10%	0%	25%	0%	100%	0%	0%	7%	14%
Mild to moderate, multifocal to diffuse, predominantly eosinophilic myelopoiesis	21/31	9/16	7/9	3/3	1/1	0/1	1/1	16/23	5/8
	68%	56%	78%	100%	100%	0%	100%	70%	63%

Table E.4. Complete pathological findings for the reproductive system, indicating occurrence (lesion/number of organ evaluated) and percentage by species, age group, and region (for both species combined).

Lesion	Total	*T. aduncus*			*S. chinensis*			Region	
		Calf	Juvenile	Adult	Calf	Juvenile	Adult	North	South
Mild, multifocal, subacute lymphoplasmacytic and variably eosinophilic oophoritis	5/27	1/14	2/7	1/4	0/1	1/1	0/0	4/19	1/8
	19%	7%	29%	25%	0%	100%	0%	21%	13%
Mild, multifocal, ovarian mineralisation	3/27	0/14	0/7	3/4	0/1	0/1	0/0	2/19	1/8
	11%	0%	0%	75%	0%	0%	0%	11%	13%
Mild to moderate, multifocal, subacute eosinophilic and lymphoplasmacytic endometritis	11/27	4/14	2/7	4/4	0/1	1/1	0/0	8/19	3/8
	41%	29%	29%	100%	0%	100%	0%	42%	38%
Moderate, multifocal, acute eosinophilic parasitic endometritis	1/27	0/14	0/7	1/4	0/1	0/1	0/0	1/19	0/8
	4%	0%	0%	25%	0%	0%	0%	5%	0%
Mild to moderate, multifocal, subacute eosinophilic and lymphoplasmacytic metritis	6/27	2/14	0/7	3/4	0/1	1/1	0/0	4/19	2/8
	22%	14%	0%	75%	0%	100%	0%	21%	25%
Mild, multifocal, subacute lymphoplasmacytic mastitis	3/7	2/3	1/2	0/2	0/0	0/0	0/0	3/6	0/1
	43%	66%	50%	0%	0%	0%	0%	50%	0%
Mild to moderate, multifocal, mammary ductular ectasia	5/7	2/3	1/2	2/2	0/0	0/0	0/0	4/6	1/1
	71%	66%	50%	100%	0%	0%	0%	67%	100%
Small to large numbers of mammary corpora amylacea	3/7	0/3	2/2	1/2	0/0	0/0	0/0	2/6	1/1
	43%	0%	100%	50%	0%	0%	0%	33%	100%
Mild, multifocal, mammary dystrophic calcification	2/7	1/3	0/2	1/2	0/0	0/0	0/0	1/6	1/1
	29%	33%	0%	50%	0%	0%	0%	17%	100%
Mild to moderate, multifocal ,acute to subacute eosinophilic and variably lymphoplasmacytic capsular orchitis and epididymitis	6/13	1/5	1/3	1/2	0/0	1/1	2/2	6/11	0/2
	46%	20%	33%	50%	0%	100%	100%	55%	0%
Mild, multifocal, subacute lymphoplasmacytic balanitis	2/5	2/4	0/0	0/0	0/0	0/0	0/1	2/4	0/1
	40%	50%	0%	0%	0%	0%	0%	50%	0%

Table E.5. Complete pathological findings for the cardiovascular system, indicating occurrence (lesion/number of organ evaluated) and percentage by species, age group, and region (for both species combined).

Lesion	**Total**	***T. aduncus***			***S. chinensis***			**Region**	
		Calf	Juvenile	Adult	Calf	Juvenile	Adult	North	South
Mild, multifocal, subacute lymphoplasmacytic myocarditis	15/39	8/19	7/10	0/6	0/1	0/1	0/1	11/29	4/10
	38%	42%	70%	0%	0%	0%	0%	38%	40%
Mild, multifocal, subacute lymphoplasmacytic epicarditis	3/39	2/19	0/10	1/6	0/1	0/1	0/1	3/29	0/10
	8%	11%	0%	17%	0%	0%	0%	10%	0%
Mild, multifocal, subacute lymphoplasmacytic endocarditis	7/39	6/19	1/10	0/6	0/1	0/1	0/1	4/29	3/10
	18%	32%	10%	0%	0%	0%	0%	14%	30%
Mild to moderate, multifocal to diffuse, myocardial fibrosis	10/39	0/19	4/10	5/6	0/1	1/1	0/1	8/29	2/10
	26%	0%	40%	83%	0%	100%	0%	28%	20%
Mild segmental arteriosclerosis	1/39	0/19	0/10	0/6	0/1	1/1	0/1	1/29	0/10
	3%	0%	0%	0%	0%	100%	0%	3%	0%
Moderate, focal, metastatic endocardial calcification	1/39	1/19	0/10	0/6	0/1	0/1	0/1	1/29	0/10
	3%	5%	0%	0%	0%	0%	0%	3%	0%

Table E.6. Complete pathological findings for the endocrine system indicating, occurrence (lesion/number of organ evaluated) and percentage by species, age group, and region (for both species combined).

Lesion	Total	*T. aduncus*			*S. chinensis*			Region	
		Calf	Juvenile	Adult	Calf	Juvenile	Adult	North	South
Mild, multifocal, subacute lymphoplasmacytic adrenal adenitis	7/37	4/18	1/10	1/5	1/1	0/1	0/2	5/28	2/9
	19%	22%	10%	20%	100%	0%	0%	18%	22%
Mild to moderate, nodular to diffuse, adrenal cortical hyperplasia	18/37	8/18	4/10	3/5	0/1	1/1	2/2	15/28	3/9
	49%	44%	40%	60%	0%	100%	100%	54%	33%
Mild, multifocal, acute adrenal capsular haemorrhage	1/37	1/18	0/10	0/5	0/1	0/1	0/2	1/28	0/9
	3%	6%	0%	0%	0%	0%	0%	4%	0%
Mild, multifocal, subacute lymphoplasmacytic thyroiditis	2/27	1/13	0/7	1/4	0/1	0/1	0/1	1/19	1/8
	7%	8%	0%	25%	0%	0%	0%	5%	13%
Small numbers of acute thyroid cyst	1/27	1/13	0/7	0/4	0/1	0/1	0/1	1/19	0/8
	4%	8%	0%	0%	0%	0%	0%	5%	0%
Mild, multifocal, subacute lymphoplasmacytic peri-thyroidal steatitis	2/27	1/13	1/7	0/4	0/1	0/1	0/1	1/19	1/8
	7%	8%	14%	0%	0%	0%	0%	5%	13%
Single peri-thyroidal sarcocysts	1/27	0/13	0/7	0/4	0/1	0/1	0/1	0/19	1/8
	4%	0%	0%	0%	0%	0%	0%	0%	13%
Mild, multifocal, subacute lymphoplasmacytic pituitary adenitis	2/6	2/3	0/2	0/0	0/0	0/1	0/0	2/4	0/2
	33%	66%	0%	0%	0%	0%	0%	50%	0%
Mild, multifocal, subacute lymphoplasmacytic pituitary perineuritis	1/6	1/3	0/2	0/0	0/0	0/1	0/0	1/4	0/2
	17%	33%	0%	0%	0%	0%	0%	25%	0%
Small numbers of small pituitary cysts	1/6	0/3	2/2	0/0	0/0	1/1	0/0	1/4	2/2
	17%	0%	100%	0%	0%	100%	0%	25%	100%

Table E.7. Complete pathological findings for the central nervous system, indicating occurrence (lesion/number of organ evaluated) and percentage by species, age group, and region (for both species combined).

Lesion	Total	*T. aduncus*			*S. chinensis*			Region	
		Calf	Juvenile	Adult	Calf	Juvenile	Adult	North	South
Mild, multifocal, subacute lymphoplasmacytic meningoencephalitis	7/18	3/9	4/6	0/1	0/1	0/1	0/0	5/10	2/8
	39%	33%	67%	0%	0%	0%	0%	50%	25%
Mild, multifocal, acute meningeal haemorrhage	2/18	1/9	0/6	0/1	0/1	1/1	0/0	1/10	1/8
	11%	11%	0%	0%	0%	100%	0%	10%	13%
Severe, focal, acute peri-dural spinal haemorrhage	1/18	0/9	1/6	0/1	0/1	0/1	0/0	1/10	0/8
	6%	0%	17%	0%	0%	0%	0%	0%	0%
Mild ,multifocal satellitosis	2/18	1/9	1/6	0/1	0/1	0/1	0/0	1/10	1/8
	11%	11%	17%	0%	0%	0%	0%	10%	13%
Moderate, locally extensive, acute cerebellar herniation	1/18	1/9	0/6	0/1	0/1	0/1	0/0	1/10	0/8
	6%	11%	0%	0%	0%	0%	0%	10%	0%

Table E.8. Complete pathological findings for the urinary system, indicating occurrence (lesion/number of organ evaluated) and percentage by species, age group, and region (for both species combined).

Lesion	Total	*T. aduncus*			*S. chinensis*			Region	
		Calf	Juvenile	Adult	Calf	Juvenile	Adult	North	South
Mild to moderate, multifocal, subacute lymphoplasmacytic and variably mild interstitial nephritis	13/39	5/19	4/10	3/6	1/1	0/2	0/1	11/29	2/10
	33%	26%	40%	50%	100%	0%	0%	38%	20%
Mild, multifocal, subacute lymphoplasmacytic capsular and subcapsular nephritis	3/39	2/19	0/10	0/6	1/1	0/2	0/1	3/29	0/10
	8%	11%	0%	0%	100%	0%	0%	10%	0%
Mild, multifocal, renal cortical dystrophic calcification	3/39	2/19	0/10	0/6	1/1	0/2	0/1	3/29	0/10
	8%	11%	0%	0%	100%	0%	0%	10%	0%
Mild, multifocal, subacute lymphoplasmacytic peri-renal steatitis	5/39	3/19	2/10	0/6	0/1	0/2	0/1	5/29	0/10
	13%	16%	20%	0%	0%	0%	0%	17%	0%
Mild, focal, renal cortical fibrosis	1/39	0/19	0/10	0/6	0/1	0/2	1/1	1/29	0/10
	3%	0%	0%	0%	0%	0%	100%	3%	0%
Small numbers of mineral deposits in tubular lumen	7/39	3/19	3/10	1/6	0/1	0/2	0/1	5/29	2/10
	18%	16%	30%	17%	0%	0%	0%	17%	20%
Mild, multifocal, follicular lymphoid hyperplasia at the cortico-medullary junction	11/39	5/19	3/10	3/6	0/1	0/2	0/1	9/29	2/10
	28%	26%	30%	50%	0%	0%	0%	31%	20%
Mild, multifocal, subacute lymphoplasmacytic and eosinophilic cystitis	10/36	2/19	5/10	1/3	0/1	2/2	0/1	8/27	2/9
	28%	11%	50%	33%	0%	100%	0%	30%	22%
Mild, multifocal, cystic dystrophic calcification	1/36	0/19	1/10	0/3	0/1	0/2	0/1	0/27	1/9
	3%	0%	10%	0%	0%	0%	0%	0%	11%
Mild to moderate, focal, umbilical dystrophic calcification	3/36	3/19	0/10	0/3	0/1	0/2	0/1	2/27	1/9
	8%	16%	0%	0%	0%	0%	0%	7%	11%
Small amount of haematoidin in umbilical artery	4/36	4/19	0/10	0/3	0/1	0/2	0/1	2/27	2/9
	11%	21%	0%	0%	0%	0%	0%	7%	22%

Table E.9. Complete pathological findings for the musculoskeletal system, indicating occurrence (lesion/number of organ evaluated) and percentage by species, age group, and region (for both species combined).

Lesion	Total	*T. aduncus*			*S. chinensis*			Region	
		Calf	Juvenile	Adult	Calf	Juvenile	Adult	North	South
Mild to moderate, multifocal, subacute lymphoplasmacytic and eosinophilic skeletal myositis	7/37	1/18	2/10	3/4	0/1	1/2	0/2	7/28	0/9
	19%	6%	20%	75%	0%	50%	0%	25%	0%
Mild, focal, acute skeletal haemorrhage	1/37	0/18	1/10	0/4	0/1	0/2	0/2	0/28	1/9
	3%	0%	10%	0%	0%	0%	0%	0%	11%
Mild, multifocal, subacute lymphoplasmacytic and eosinophilic diaphragmatic serositis	4/36	1/19	2/9	1/3	0/1	0/2	0/2	3/27	1/9
	11%	5%	22%	33%	0%	0%	0%	11%	11%
Mild, multifocal, subacute lymphoplasmacytic and eosinophilic diaphragmatic myositis	3/36	0/19	2/9	1/3	0/1	0/2	0/2	3/27	0/9
	8%	0%	22%	33%	0%	0%	0%	11%	0%
Mild, multifocal, subacute diaphragmatic interstitial fibrosis	1/36	0/19	0/9	1/3	0/1	0/2	0/2	1/27	0/9
	3%	0%	0%	33%	0%	0%	0%	4%	0%
Mild, multifocal, diaphragmatic muscle fibre atrophy	1/36	0/19	0/9	1/3	0/1	0/2	0/2	1/27	0/9
	3%	0%	0%	33%	0%	0%	0%	4%	0%

Table E.10. Complete pathological findings for the skin and subcutis, indicating occurrence (lesion/number of organ evaluated) and percentage by species, age group, and region (for both species combined).

Lesion	Total	*T. aduncus*			*S. chinensis*			Region	
		Calf	Juvenile	Adult	Calf	Juvenile	Adult	North	South
Severe, locally extensive, chronic granulomatous fungal dermatitis and cellulitis (consistent with lobomycosis)	1/40	0/19	0/10	0/6	0/1	0/2	1/2	1/30	0/10
	3%	0%	0%	0%	0%	0%	50%	3%	0%
Mild, multifocal, subacute lymphoplasmacytic dermatitis	2/40	0/19	1/10	0/6	0/1	0/2	1/2	2/30	0/10
	5%	0%	10%	0%	0%	0%	50%	7%	0%
Mild, multifocal, subacute lymphoplasmacytic cellulitis	2/40	2/19	0/10	0/6	0/1	0/2	0/2	1/30	1/10
	5%	11%	0%	0%	0%	0%	0%	3%	10%
Moderate, multifocal, pseudoacanthomatous epidermal hyperplasia	3/40	1/19	1/10	1/6	0/1	0/2	0/2	3/30	0/10
	8%	5%	10%	17%	0%	0%	0%	10%	0%
Fibroma	1/40	0/19	0/10	1/6	0/1	0/2	0/2	1/30	0/10
	3%	0%	0%	17%	0%	0%	0%	3%	0%

Table E.11. Complete pathological findings for the organs of special senses (eye and ear), indicating occurrence (lesion/number of organ evaluated) and percentage by species, age group, and region (for both species combined).

Lesion	Total	*T. aduncus*			*S. chinensis*			Region	
		Calf	Juvenile	Adult	Calf	Juvenile	Adult	North	South
Spheroid bodies (artefact)	23/35	9/16	7/9	4/6	1/1	1/1	1/2	17/25	6/10
	66%	56%	78%	67%	100%	100%	50%	68%	60%
Mild, multifocal, optic nerve melanosis	1/35	0/16	0/9	1/6	0/1	0/1	0/2	1/25	0/10
	3%	0%	0%	17%	0%	0%	0%	5%	0%
Mild to moderate, multifocal, peri-neural dystrophic mineralisation	4/35	1/16	1/9	2/6	0/1	0/1	0/2	3/25	1/10
	11%	6%	11%	33%	0%	0%	0%	12%	10%
Mild, multifocal, subacute lymphoplasmacytic peri-neuritis	2/35	0/16	0/9	2/6	0/1	0/1	0/2	1/25	1/10
	6%	0%	0%	33%	0%	0%	0%	4%	10%
Mild, multifocal, subacute lymphoplasmacytic scleritis	4/35	3/16	0/9	1/6	0/1	0/1	0/2	4/25	0/10
	11%	19%	0%	17%	0%	0%	0%	16%	0%