A standardized necropsy protocol for health investigations of small cetaceans in southern Africa

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Globally, the increasing need to conduct both research and surveillance of the health of wild animal populations has been recognized as an important tool in conservation and management. While such studies on terrestrial wildlife are frequent in the southern African sub-region, their counterparts in the marine environment seem to be largely lacking. Here we report on our experience in establishing and testing a standardized necropsy protocol for small cetaceans adapted for the local context, with the specific aim of sampling for health investigations and monitoring. The necessity, challenge and value of regional standardization in data collection specifically aimed at health investigations, inter-disciplinary collaboration, long-term data banking, and sample storage are discussed in addition to practical and safety considerations. The developed protocol, focusing on the necropsy technique and tissue sample collection, as well as a list of required equipment are available as online supplementary material.

Key words: cetaceans, disease investigation, health, necropsy, surveillance.

INTRODUCTION

The need for effective wildlife health investigations, including both surveillance and research, has become more evident in recent years, with ever-increasing reports of the effects of climate change, biodiversity loss, and emerging animal and human diseases reported in wildlife (Aguirre, Ostfeld, Tabor, House & Pearl, 2002; Harvell *et al.*, 2002; Norman, Digiacomo, Gulland, Meschke & Lowry, 2008; Ryser-Degiorgis, 2013). While this has been recognized and implemented in both terrestrial (Keusch, Pappaioanou, González, Scott & Tsai, 2009; Kock, 1996; Munson & Cook, 1993; Spalding & Forrester,1993) and freshwater (Ferreira & Pienaar, 2011; Huchzermeyer, 2012) wildlife health investigations on the African continent and in the southern African sub-region, similar systematic health examinations of larger vertebrates remain scarce in the marine environment. One hurdle to such investigations may be the necessity for a more integrated, interdisciplinary research approach, as often tools and knowledge from various research fields need to be combined to identify the factors contributing to the emergence of a disease (Ryser-Degiorgis, 2013). In addition, considerations of other factors that may have contributed to death (*e.g.* entanglements, boat strikes, etc.) will also benefit from such an approach (Bester, 2014)

The rise in reports of the number and severity of diseases affecting marine mammals and the perception of a global ecological crisis in the last decade has raised concern over deteriorating ocean health (Gulland & Hall, 2007; Lafferty, Porter & Ford, 2004). Coastal cetacean species, such as *Tursiops aduncus* (Indo-Pacific bottlenose dolphin) and *Sousa plumbea* (Indian Ocean humpback dolphin), are particularly vulnerable to the effects of human activity due to their role as apex predators (long-lived, feed at high trophic levels and thus exhibit bioaccumulation of pollutants) in an inshore habitat (Lane *et al.*, 2014; Reddy, Dierauf & Gulland, 2001; Wells *et al.*, 2004). Consequently,

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coastal dolphins function as good sentinel species to detect early warning signs of disturbances in ocean ecosystem health (Bester, 2014). This information, in turn, allows better characterization and management of potential negative impacts on human and animal health (Bossart, 2006).

Increasing reports of diseases affecting marine mammals are, in part, the result of more dedicated research in this field and the advancement of diagnostic techniques (Gulland & Hall, 2007). However, an increase in the frequency of marine mammal mortalities over the last four decades, particularly due to exposure to harmful algal blooms and morbillivirus outbreaks in the North Atlantic and Mediterranean Sea, have been reported (Gulland & Hall, 2007; Raga *et al.*, 2008; Van Bressem *et al.*, 2009). Changes in frequency of other disease outbreaks are more difficult to assess, owing to a lack of baseline data for most marine communities (Gulland & Hall, 2007; Harvell *et al.*, 1999; Ward & Lafferty, 2004). This, combined with predictions of future increases in disease due to climate change and an increase in stress factors, such as pollutants, commercial use of marine and submarine resources (such as seismic surveys, oil and gas exploration, offshore wind farms, and submarine mining ventures), inter- and intra-specific competition, and habitat destruction, lends new urgency to understanding the causes of marine mammal disease outbreaks (Bester, 2014; Epstein, Sherman, Spanger-Siegfried, Langston & Prasad, 1998; Harvell *et al.*, 1999, 2002; Ward & Lafferty, 2004). However, impediments to wildlife health investigations are largely related to zoological, behavioural and ecological characteristics of wildlife populations and limited access to investigation materials (Ryser-Degiorgis, 2013).

Cetaceans are generally difficult to study due to their entirely aquatic lifestyle, resulting in a paucity of information regarding disease presence and prevalence. Therefore, carcasses of either stranded or incidentally caught animals provide a unique opportunity to collect valuable data that would not be obtainable in any other way. These data may provide information on the life history and ecology of a species, as well as help assess individual and population health (Geraci & Lounsbury, 1993; Lane *et al.*, 2014; Norris, 1961; Rowles, van Dolah & Hohn, 2001; Pugliares *et al.*, 2007). Here we report on the experience and lessons learned from developing a protocol for cetacean health investigations in the southern African sub-region (Lane *et al.*, 2014), highlight the need and advantage of standardizing sampling between research groups, and provide our developed protocol and associated information as extensive online material. The objective of developing a standardized necropsy protocol was to facilitate disease testing and health assessment of small cetaceans in southern Africa. Our approach was to integrate histopathological sampling and sampling for health investigations into a standard museum sample and data collection protocol, focusing on step-by-step dissection technique and tissue sample collection. We also included information on pathological descriptions, as well as the development of guidelines on defrosting carcasses to ensure good quality samples for health investigations and monitoring.

In countries with long, often remote coastlines, such as South Africa, various individuals, often associated with different research groups, may be responsible for collecting data from carcasses of stranded or incidentally caught animals. While some data collection, largely on morphological measurements, has been standardized internationally (Norris, 1961), to date, there have been no published protocols for southern Africa. Health investigation results, in particular, are seldom directly comparable if they have not been standardized (Gulland & Hall, 2007); in addition, all aspects of data collection can benefit from standardized collection protocols (Rowles *et al.*, 2001). Some protocols have previously been developed internationally to standardize data collection from dead marine mammals (Geraci & Lounsbury, 1993; Kuiken & Hartmann, 1993; Rowles *et al.*, 2001; Raverty & Gaydos, 2004; Pugliares *et al.*, 2007), but were found to be too technical to guide lay personnel in the dissection and evaluation of dolphin carcasses. In addition, these protocols did not take into consideration the personnel, training, technical infrastructure, and capabilities of support institutions, such as laboratories, available in the sub-region. Limited resources, lack of trained personnel and remoteness of locations may limit the capacity to conduct detailed cetacean necropsies and sampling in southern Africa, which may lead to important diseases and trends remaining unobserved. In addition, stranding networks and general information on cetaceans outside of South Africa may be limited, resulting in a lower chance of detecting diseases that may be spreading through marine mammal populations. Southern African coastal weather conditions are generally hot and humid, causing rapid tissue decomposition, which may further hamper disease investigations. Finally, South Africa lies on a major shipping route and thus the potential to import vectors of disease from anywhere in the world is high.

MATERIALS AND METHODS

The protocol for health investigations on small cetaceans (see online supplement) was developed for dolphins incidentally caught in shark nets off the KwaZulu-Natal coast, South Africa (Lane *et al.*, 2014). Gill nets are deployed off the South African east coast by the KwaZulu-Natal Sharks Board (KZNSB) to reduce the risk of shark–human interactions (Cockcroft, Ross & Peddemors, 1990; Cockcroft, 1994). Approximately 20 dolphins are incidentally caught (by-caught) annually in the shark nets (KwaZulu-Natal Sharks Board 2009).

Dolphins caught in the shark nets present an opportunity to study average individuals in relation to health, and therefore can be used as representatives of the overall health status of the population (Jauniaux *et al.*, 2002). In contrast, dolphins found stranded and dead along the coast, have an unkown cause of death and need be carefully considered when making inferences regarding overall dolphin population health (Jauniaux *et al.*, 2002). The majority of the dolphins incidentally caught in the shark nets are the coastal species *T. aduncus* and *S. plumbea*, although pelagic *Delphinus capensis* (long-beaked common dolphins) and *Stenella* spp. (spotted and spinner dolphins) are also caught (Cockcroft *et al.*, 1990).

A long-standing agreement exists between the 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 is accessioned to the Graham Ross Marine Mammal Collection.

Evaluation of dolphins incidentally caught in the shark nets was performed under research permits issued to the PEM/Bayworld by the South African Departments of Environmental Affairs and Agriculture, Forestry and Fisheries (RES2012/40 and RES2013/19).

The protocol for this study was approved by the Research Committee of the Faculty of Veterinary Science, the Animal Use and Care Committee of the University of Pretoria (Protocol V011/12) and the Ethics and Scientific Committee of the National Zoological Gardens of South Africa (P10/23).

A locally adapted necropsy protocol was developed by combining methods described in existing protocols and adapting them to local conditions (see Geraci & Lounsbury, 1993; Kuiken & Hartmann, 1991; Raverty & Gaydos, 2004; Rowles *et al.*, 2001; Pugliares *et al.*, 2007). The developed protocol was then tested during serial dissections on a total of 46 *T. aduncus* and five *S. plumbea* between April 2010 and April 2012, the results of which are described elsewhere (Lane *et al.*, 2014). Further changes were made to the protocol based on the results of the study to ensure that it was adapted to local conditions. Sampling protocols for parasites and microbiology were developed in consultation and in accordance with available local laboratory expertise. In addition, a data capture report and checklist were developed to be used with the necropsy protocol. All tissue samples were lodged with the Graham Ross Marine Mammal Collection at the PEM and histological slides were banked at the National Zoological Gardens, Pretoria.

RESULTS

The necropsy protocol is given in Appendix I in the online supplement. The data capture report form and checklist are given in Appendix II in the online supplement. Results should be reported in as much detail as possible, irrespective of the experience of the person conducting the necropsy. Observation and information contributed by laypeople (*i.e.* not formulated in the correct technical terms) can contribute important information on macroscopic pathological changes observed during necropsy. In addition, digital photography presents a powerful tool in documenting such changes, thus aiding the identification of underlying etiology. If information is not available, or an organ is not evaluated, a reason should be given to aid in the further development and modification of this protocol. A suggested equipment list is given in Appendix III in the online supplement and a list of standard external measurements in Appendix IV in the online supplement.

Legislation

The dissection, handling, and research on marine mammals in South Africa is controlled by the Marine Living Resources Act (Act 18 of 1998), which states that permits are required for these actions to be performed 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/).

Therefore, if a live stranded cetacean is found on

Table 1. Organizations to be contacted when a live stranded cetacean is found.

Province	Organization	Contact details
KwaZulu-Natal	KwaZulu-Natal Sharks Board	031 566 0400
	Ezemvelo KZN Wildlife	033 845 1002/ 083 380 6298
Eastern Cape	Port Elizabeth Museum/Bayworld	071 724 2122/ 041 584 0650
Western Cape	Department of Environmental Affairs: Oceans and Coasts	021 402 3173
	Mammal Research Institute, Whale Unit	082 570 8212

the beach, a regulatory body should be contacted as soon as possible. As stranded animals may be carrying diseases easily transmittable to humans, it is also important not to touch or interact with the animal. As one of the first steps, the locally responsible organization should be contacted (Table 1). It needs to be stressed that even in the event of a dead cetacean, any assistance from lay people not affiliated with the listed institutions in Table 1 needs to be covered under a permit from the Department of Environmental Affairs.

In terms of the Animal Health Act (Act 7 of 2002), any controlled or notifiable disease as well as any disease that has not previously occurred in the Republic of South Africa (www.info.gov.za/documents) should be reported as soon as possible to a provincial or national state veterinary department. A detailed list of these may be found at http://www.daff.gov.za/.

Carcass preparation and defrosting

Owing to a number of factors, such as difficult access to animals, lack of personnel and limited funding, stranded or incidentally caught cetaceans are often frozen before necropsies are performed (Siebert *et al*., 2001, 2006). Freezing commonly causes artefacts, and thus may impair histological interpretation of lesions, but additional further decay (autolysis and putrefaction) may also occur during the thawing process (Roe, Gartrell & Hunter, 2012). Although a common belief appears to be that any sampling for health and pathological investigations is futile once a carcass has been frozen, in our experience, a lot of useful information can be drawn from such samples, even though the material may have some limitations. In fact, an increasing number of samples from both by-caught and stranded animals would aid in setting a much needed baseline for health investigations in the region by adding information on what is ‘normal’, what is not (*i.e.* pathology), and what is due to decay. However, in order to obtain samples useful for health investigations two factors are critical: time between death and freezing and the way a frozen carcass is defrosted. It seems obvious that time between death and freezing should be kept to a minimum, but in our experience this is an often neglected fact, particularly with live strandings, as all efforts understandably go into rescuing and refloating the animal, but little thought goes into the next steps if the animal does not survive. A careful assessment of whether a carcass is frozen or kept in a coolroom for a few days is necessary, depending on the time when a necropsy is possible. Keeping a carcass in a cool environment for a few days is preferable if it can be ensured that the necropsy will take place within 2–3 days. If the possibility for a necropsy cannot be assessed immediately, it is advisable to freeze the carcass as soon as possible. However, care needs to be taken that the carcass is frozen solidly, which may take a few days for large animals. Similarly, the defrosting process as described in the protocol (see the online supplement) is important as slow defrosting in a cool environment will yield better samples than defrosting in direct sunlight.

Zoonoses and personnel safety

Many diseases that can cause disease in humans (zoonoses) have been associated with cetaceans, and not all such organisms have been identified (Raverty & Gaydos, 2004; Waltzek, Cortés-Hinojosa, Wellehan & Gray, 2012). In addition, even normal appearing tissue may carry pathogens and can thus have the potential to infect humans. Persons coming into close contact with live or dead cetaceans 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

Table 2. List of the most important zoonotic diseases associated with dolphins (from Waltzek *et al.*, 2012).

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 toxemia
Calicivirus infection	Calicivirus	Influenza-like symptoms, rarely hepatitis
Blastomycosis	*Ajellomyces dermatitidis*	Cellulitis and lymphadenitis
Lobomycosis	*Lacazia loboi*	Localized skin infection

et al. (2012). The most important diseases known to have been transmitted from dolphins to humans are summarized in Table 2 (Waltzek *et al.*, 2012). To date, no zoonotic diseases have been identified in cetaceans off the South African coast, which may partly be due to the lack of effective disease investigation as well as a general lack of awareness of zoonotic disease by medical doctors examining marine mammal personnel. However, recently established systematic health investigations of by-caught dolphins in South Africa showed the first reported cases of *Sarcocystis* and *Lobomycosis* for the region (Lane *et al.*, 2014), thus highlighting the value and importance of a good protocol to obtain material of diagnostic value. Other bacteria and viruses, such as *Brucella* spp. (Corbel, 1997), *Salmonella* spp. (Ridgway, 1979), *Mycobacterium* spp. (Higgens, 2000), West Nile virus (St. Leger *et al.*, 2011), influenza viruses (Ridgway, 1979), and *Aspergillus* spp. (Higgens, 2000) have been isolated from cetaceans elsewhere and are known zoonoses. Therefore, caution should be exercised whenever handling any organic material, and the apparent absence of a disease should not result in complacency when working with organic material. A detailed list of basic safety precautions is included in the developed protocol (see the online supplement).

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.

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 stranded, rather than being incidentally caught, extra caution should be taken, since such animals may have come ashore as a result of disease. In South Africa, local municipalities have been mandated by the Constitution of the Republic of South Africa (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 transmission to humans is unlikely to occur, carcasses may be buried on the beach or towed out to sea.

DISCUSSION

Combining a protocol for health investigations and monitoring with an existing museum protocol was not as straightforward as one may expect and a number of important points can be made regarding the experience and lessons learned from this exercise. Foremost, careful consideration needs to be taken on how to dissect and sample the carcass to avoid compromising sampling for either task (*i.e.* standard biological sampling and pathological/health sampling). This has an important bearing on the order of necropsy and we found that a step-by-step illustrated guide as developed here (see the online supplement) not only helped personnel to adapt to a new way of sampling, but also ensured standardization and best quality sampling between different carcasses and/or researchers. Special consideration must be given to the collection, preservation and storage of samples. The use of buffered formalin for histopathological sampling is imperative and may not be standard procedure for museum collections at present. Non-buffered formalin rendered many of the samples unusable for histopathological investigations due to the large amount of stain deposit on the samples. Furthermore, the condition of the carcass, storage between death and necropsy, particularly with regards to freezing and defrosting procedures, proved to have important implications for the quality of the samples obtained. The necropsy protocol developed here (see the online supplement) resulted in reasonable quality histopathological samples, useful in conducting health assessment of dolphins. Even though some freezing artefacts remained on histopathological examination, tissue evaluation was possible in the majority of cases (Lane et al., 2014). The developed defrosting method (see the online supplement) improved the quality of the samples significantly.

The results of our systematic health assessment (Lane *et al.*, 2014) indicated a need for continued health monitoring of coastal dolphin populations and further research into disease pathophysiology and anthropogenic factors affecting these populations, particularly as a number of diseases not previously documented in South African dolphins were reported in our study. Although the protocol was developed for work with incidentally caught animals, it has become increasingly evident that its real value lies in giving advice and guidance to lay people and students needing to conduct post-mortem dissections on stranded small cetaceans in remote locations. While detailed necropsies and health investigations are often perceived as only being feasible in a laboratory setting and thus not achievable under often challenging field condition, we experienced that the developed protocol was easily used in the field as all the equipment necessary was basic and standard field equipment which should be present in any stranding response toolkit. Nevertheless, the initial phase of getting used to a new, more detailed protocol, which incorporates sampling for health investigations may take some time and an initial, slow necropsy in a controlled setting (*i.e.* without the risk of the incoming tide washing the carcass away) may be advisable. Increasing experience with necropsies will allow the researcher/lay person to discern what is possible under the often precarious conditions of conducting necropsies on stranded animals, assist in determining what is 'normal' and what may pose a lesion (*i.e.* if in doubt take a sample), and when tissue is too autolysed for any histopathological or other health investigations.

Furthermore, standardizing necropsy methodology between regions and research groups would result in a range of benefits: it would lead to comparable data less affected by sampling bias, enable more productive, coordinated research, which in turn would allow a better overview and more valid conclusions regarding regional patterns and temporal trends. Regional or pooled data would allow a more objective and measurable approach to be made regarding the evaluation of changes in ecosystem health, including statistically more powerful analyses of risk factors and causal factors for disease. In addition, it would highlight new, important areas for future research into animal and ecosystem health/conservation medicine (Norman, 2008). Increased awareness and education of both scientists and lay people should also result in shorter response times to strandings, and thus assist in timely notification of mortality events and could be instrumental in developing and implementing control measures. A systematic approach to necropsies would furthermore assist in the early detection of new infectious agents and diseases, including those that would potentially be catastrophic to marine mammal populations (Groch *et al.*, 2014). A short response time and proper preservation of samples is of the essence to ensure scientifically sound data. This is even more so the case under the warm climatic conditions prevalent in South Africa. At a time when there are increasing concerns regarding the cause of strandings, particularly mass strandings, through anthropogenically generated phenomena, such as harmful algal blooms (Flewelling *et al.*, 2005; Pyenson *et al.*, 2014) or seismic operations (Southall, Rowles, Gulland, Baird & Jepson, 2013), such events may remain undetected if samples are not collected or adequately preserved. Autolysis due to delayed detection or inadequate preservation greatly limits the interpretation of gross necropsy and histological findings and can also influence the sensitivity of diagnostic tests (Ryser-Degiorgis, 2013). In addition, time is a quantitative scale against which all other aspects of disease should be measured (Ryser-Degiorgis, 2013). As patterns of disease emergence may only be recognized over time and new laboratory tools are continuously being developed, long-term data storage and banking of samples with adequately equipped facilities is another important aspect of both research and surveillance of wildlife diseases (Cutler, 2010; Jones *et al.*, 2008; Norman *et al.*, 2012). Samples must be stored in a way to allow subsequent use for retrospective analyses and it may be necessary to accumulate samples until an adequate sample size is achieved (Ryser-Degiorgis, 2013).

It is hoped that this standard necropsy protocol will encourage a more complete health investigation of incidentally caught and stranded cetaceans in the region and assist in expanding the current knowledge of diseases affecting dolphin populations in southern Africa. Identification of subtle gross pathological changes as well as acquisition of some samples (such as the extraction of ears) may require a certain expertise and training. Such skill can be obtained over time by previously untrained staff, but does present a problem in areas/organizations with a lack of training opportunities or high staff turnover. Thus, it is imperative to ensure continuity of at least one trained person per area or research group to ensure consistency in record keeping and sampling as well as data quality and repeatability. In many aspects of the investigation process collaboration with international or regional experts is essential until local capacity and expertise is achieved.

It is expected that requirements for sampling may change over time as new evidence emerges and new tests are developed, and therefore protocols should be adaptable to such changing circumstances (Raverty & Gaydos, 2004). At present, there is a need for the development and validation of certain diagnostic tests within the region (for example, certain toxin and infectious disease assays) and capacity for performing these will need to be developed locally (Butler, 2006; Jones *et al.*, 2008). At present, local pathology testing laboratories have experience with testing for marine bacteria and fungi, but few laboratories are available to test for viruses and protozoans by culture technique and/or immunohistochemistry. In addition, laboratories in general often rely on domestic animal surveillance approaches and on diagnostic tests validated only for domestic species in their investigations into wildlife pathogens (Ryser-Degiorgis, 2013).

Finally, increased communication of both methods (practices and protocols) and results, as well as training of veterinary and wildlife health staff, needs to be implemented to ensure cross disciplinary standardization of data collection, since multidisciplinary and integrative approaches are needed for both research and surveillance (Belant & Deese, 2010; Ryser-Degiorgis, 2013). As the importance of knowledge on the health of wild populations is increasingly recognized, scientific standards need to be of a level that allows comparison of results (Butler, 2006). Perhaps surprisingly, a unique aspect and challenge to wildlife health studies appears to be the integration of the collection of both disease and basic biological data (Ryser-Degiorgis, 2013). Most studies state that the latter is frequently lacking. While there is extensive data collection on biological parameters for marine mammals in the southern African region, synthesis and collation of biological data on a regional level is lacking, and investigations into the health status of animals and populations appear to be absent. In addition, a number of institutions are experiencing changes in staff dealing with by-catch and/or strandings and in view of that standardized protocols will assist in ensuring continued, long-term collection of standardized data/samples.

Continued basic and applied research into both the general biology and ecology of the wildlife populations under investigation remains of high importance to understand the biology and ecology of pathogens, their hosts, and the environment, both in time and space (Belant & Deese, 2010; Ryser-Degiorgis, 2013). Ecological data, such as food availability, and environmental characteristics, like temperature, are important to inform health studies (Ryser-Degiorgis, 2013). To understand disease epidemiology and its effects on the biology of animals and populations, one needs to critically evaluate the effects of ecological parameters on the disease, particularly in view of global change in the marine environment. In marine mammal studies the challenge often lies in obtaining knowledge on prey availability and abundance as well as drawing any deductions on nutritional requirements of the species under investigation. Difficulties inherent in wildlife health investigations may be the absence of data for species-specific systems, such as anatomy, pathology and ecology; this is often challenging in poorly studied species, such as humpback dolphins, for example. But even for better known species, gaps in our knowledge on certain aspects of natural history, behaviour, anatomy and physiology can hinder a complete picture of the factors influencing disease processes.

In our experience, information on feeding habits, basic nutritional requirements, both gross and microscopic appearance of normal tissues, expected parasite fauna and age at first reproduction are all critical data for understanding a disease, but may be unavailable for a number of small cetacean populations in the region. Long-term storage of data as facilitated by many museums may assist in addressing some of these issues, but most population data, such as population size, density, age structure, sex ratio, recruitments, habitat utilization, presence of sympatric species and migration behaviour can only be addressed through field studies and are all critical to the understanding of pathogen transmission and maintenance (Ryser-Degiorgis, 2013). The combination of health surveillance data and wildlife ecological data are required to determine whether emerging diseases are the result of an introduction of infected animals or due to changes in the population dynamics of a host or vector (Ryser-Degiorgis, 2013). Effective wildlife health investigation is a two-step process with a descriptive phase (what, who, where and when) and an analytical and experimental approach (how, why) to study causal relationships. While the foundation of health investigations and monitoring for small cetacean populations in the region has been laid with the establishment of a necropsy protocol that includes systematic sampling for health investigations (Lane *et al.*, 2014; see the online supplement), to date this has only served the purpose of the first, descriptive phase and only for the waters of the southeastern coastline of South Africa. This illustrates the long-term approach needed to arrive at clear causality and effect scenarios for wildlife disease investigations on marine mammals in the region. As such, both investigation and surveillance are ongoing processes and need clearly defined methods and long-term goals to be successful. Use of standardized protocols, such as the one described here, can contribute towards effective wildlife health investigations and fruitful long-term research efforts.

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