

Disease surveillance in southern African seabird colonies

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1. Introduction

A surveillance programme is necessary to manage diseases and pathogens to minimise the risk of diseases further reducing seabird populations. The programme is envisaged as the systematic on-going collection, collation and analysis of information related to animal health, with the timely dissemination of information to key stakeholders, such as seabird colony managers, rehabilitation centres, state veterinarians, management authorities and government departments, so that remedial action can be taken. This would support effective decision-making including management policy and priority setting and response to disease outbreaks. With regards to wildlife diseases, surveillance programmes can be employed to attain a variety of goals, such as identifying emerging and re-emerging pathogens, which would allow for time-sensitive responses to disease outbreaks by authorities and improve the understanding of causes of morbidity and mortality in wildlife populations.

A disease surveillance programme investigates the pathogens present, species affected, geographic area involved and prevalence of diseases within a population. The integral activities of a surveillance programme are:

- an organised system of sample collection and storage,
- standardised pathogen detection and identification,
- information management,
- data analysis and information dissemination to key stakeholders.

A disease surveillance programme should be:

- representative of the health status of the entire population, throughout their range,
- sensitive in terms of detecting significant mortality or illness events,
- accurate through employing sensitive, specific and appropriate methods of diagnosis,
- timely to allow for prompt intervention if necessary, flexible and readily adaptable to modifications as necessary,
- simple enough to ensure easy and consistent implementation,
- cost-effective; maximizing outputs and minimizing costs whenever possible,
- valuable in terms of providing outputs that are meaningful for stakeholders, and
- sustainable to ensure long-term stakeholder participation.

There is a need to adopt a multidisciplinary and integrative approach to wildlife health assessments, including both research and surveillance. It is vital to give feedback to the stakeholders to ensure their motivation to continue the work. Consequently, communication is a vital part of surveillance. Education is also critical on different levels; there needs to be veterinary training in wildlife health and there needs to be training of people involved in participatory disease surveillance within the local communities.

2. Objectives

On a national level, disease surveillance is important to reduce socio-economic costs to society, particularly those related to the food industry, tourism and biodiversity conservation, and to meet international obligations to detect and report important pathogens that are present in wild animals. More specifically, the following objectives are needed for a disease surveillance programme of southern African seabird colonies:

- I. Establish prevalence of specific pathogens
- II. Detect the presence of new or previously unreported pathogens
- III. Demonstrate the absence of specific pathogens
- IV. Monitor specific disease trends within and among different populations
- V. Facilitate the control of disease and contribute to a disease risk assessment
- VI. Organize biological specimen banks for future epidemiological studies

These objectives will be achieved through both active and passive surveillance. Active surveillance corresponds to the systematic collection and testing of samples from seabird colonies, as well as systematic monitoring of seabird colonies to obtain information and samples from individuals with signs of disease and/or mass mortality events. Passive surveillance, on the other hand, consists of the opportunistic gathering of information on disease and mortality events from field observations, and routine collection and testing of samples from seabirds admitted to rehabilitation centres. Additionally, these objectives can also be reached (and expanded) through the retrospective testing of samples that were systematically collected and stored both from seabird colonies and rehabilitation centres, once routine protocols of collecting are in place.

With regards to Objective III, controlled avian diseases in South Africa will be prioritised for specific testing: Newcastle disease (Newcastle Disease Virus), avian influenza (Influenza A Virus), psittacosis (*Chlamydia psittaci*), tuberculosis (*Mycobacterium avium*), salmonellosis (*Salmonella enteritidis*, *S. gallinarum*, *S. pullorum*) and any disease unknown to South Africa or Namibia (according to the Department of agriculture, forestry and fisheries, <http://www.daff.gov.za/vetweb/Disease%20Control/List%20of%20controlled%20%20notifiable%20Animal%20Diseases%202007.pdf>). The World Health Organisation for Animal Health (OIE) also publishes a list of notifiable diseases, and in addition to those listed above, includes: avian infectious bronchitis, avian infectious laryngotracheitis, avian mycoplasmosis (*Mycoplasma gallisepticum*, *M. synoviae*), duck virus hepatitis, infectious bursal disease and turkey rhinotracheitis. (<http://www.oie.int/animal-health-in-the-world/oie-listed-diseases-2015/>).

3. Specific considerations for disease surveillance of southern African seabird colonies

Past studies have indicated that the prevalence of some pathogens in wild adult seabirds can be remarkably low, often below 1-2%. Such low prevalences imply the need for large sample sizes to identify infected individuals, which may not be compatible with the logistical and financial constraints of sample collection in seabird colonies.

Because colonial seabirds are often under pressure from predators, it may generally be difficult to identify seabirds with external signs of disease, as this would make them vulnerable to predation. Similarly, the presence of carcase-eating animals at colonies also renders it difficult to collect intact carcasses of seabirds. The opportunistic collection of information and samples from sick or deceased seabirds in colonies is therefore difficult, requiring intensive monitoring and possibly biasing samples towards species that are most frequently monitored for other purposes. Rehabilitation centres, on the other hand, may provide valuable opportunities to sample individuals with external signs of disease; the prevalence of pathogens and diseases in these circumstances, however, might not faithfully represent their occurrence in the whole

population. Nevertheless, long term records may provide an indication of health of colonies from which they originate.

Disease surveillance of seabirds is currently constrained by the poor sensitivity and specificity of diagnostic tests that primarily target domestic poultry and that are not yet validated for use in other species. This is particularly relevant for serological tests that rely on secondary antibody reactions (for example: indirect enzyme-linked immunosorbent assay (ELISA) or indirect immunofluorescence assay (IFA)), as commercial kits with secondary antibodies targeting chicken immunoglobulins may not adequately recognize seabird immunoglobulins. Furthermore, because some cross-reactivity or morphological overlap can occur between different pathogens and parasites, even direct diagnostic methods may perform sub-optimally and produce misleading results if applied to seabird species that have been poorly studied. For instance, even polymerase chain reaction (PCR) testing, which is generally considered highly sensitive and specific, may co-amplify genes of non-targeted pathogens or may fail to detect variants and strains of pathogens that are novel or exclusive to particular seabird species.

It is therefore recommended that diagnostic tests for disease surveillance should be validated in the seabird species concerned before testing is conducted more widely. This may not be currently feasible through local commercial laboratories, and thus will need to be developed and conducted in research laboratories. For this, it would be ideal to work in conjunction with the National Zoological Gardens (NZG) that have a Disease Surveillance and Monitoring programme as part of their Research Department. The NZG also houses the Biobank for wildlife samples.

Long-term banking of biological samples from seabirds is a valuable strategy that will allow future validation and/or development of more reliable diagnostic techniques. Biobanking will also be valuable to historically contextualize the emergence of diseases, and to obtain additional information on the epidemiology and pathology of pathogens that were not known or not perceived to be significant at the time of sample collection.

4. Pathogens and parasites causing disease in southern African seabirds

The following list of pathogens and parasites has been compiled from published and ongoing research, but should not be considered complete as it is likely that more pathogens will be detected with further sampling and testing.

- **Avian cholera** caused by *Pasteurella multocida* is possibly the most important infectious disease occurring in southern African seabirds, particularly for Cape cormorant colonies in the Western Cape. The pathogen has been recorded in other seabird species but with lower mortality. The presence of carcasses within a colony is a contributing factor to the occurrence of outbreaks, and the prevention and control of this disease relies primarily on the removal and incineration of infected carcasses.
- **Aspergillosis** is caused by the fungus *Aspergillus* spp., which is ubiquitous in the environment and causes disease predominantly in otherwise immunocompromised seabirds. This is predominantly a slow and pervasive respiratory disease, but may also cause systemic infection and acute mortality.
- **Avian malaria** is caused by the protozoan *Plasmodium* spp. and spread by mosquitoes (Culicidae). This systemic infection can lead to high morbidity and mortality, especially in captivity (including rehabilitation) if adequate precautions are not taken. African penguins are highly susceptible to the

infection, with the disease less common in other seabirds but occasional mortality may also occur in those species. Factors such as standing water (allowing for the breeding of mosquitoes) and the proximity to other bird species in the vicinity (both domestic and wild) can increase the likelihood of disease.

- **Other blood parasites** such as the protozoa *Babesia* spp., *Haemoproteus* spp. and *Leucocytozoon* spp. and the bacteria *Borrelia* sp of the relapsing fever group have been described in southern African seabirds. These parasites are considered enzootic and may contribute to morbidity with concurrent infections, especially in immune-compromised individuals.
- **Bacterial infections of respiratory and gastro-intestinal systems** can be caused by a broad variety of bacteria, often opportunist species that take advantage of an immunosuppressive event. It is unlikely to cause high mortality of seabirds in the wild unless another predisposing condition is present (for example, malnutrition, environmental pollution), but highly pathogenic species/strains are also possible and could lead to significant outbreaks.
- **Avian pox** is caused by *Avipoxvirus*, spread by direct contact and biting arthropods and generally causes eye, beak, flipper and feet lesions. These lesions are usually self-limiting and not lethal, but secondary bacterial infections or impairment in the ability to feed can be relevant and result in mortality. A predisposing factor is the presence of biting arthropods, such as mosquitoes and flies, which may act as vectors.
- **Coccidiosis** is caused by the protozoa *Eimeria* spp. and can infect the gastro-intestinal systems or the kidneys. Most infections are asymptomatic and more likely to cause gradual debilitation than high mortality.
- **Cryptosporidiosis** is caused by the protozoa *Cryptosporidium* spp. affecting the respiratory, gastro-intestinal or renal systems. There is likely to be widespread exposure to the pathogen, but clinical disease is generally limited to immune-compromised individuals, and can be particularly relevant for African penguin chicks.
- **Respiratory herpesvirus infection** has been diagnosed in African penguin chicks. It is unclear whether this was primary or secondary cause of death but the virus could possibly cause a mortality event although may also cause decreased long-term breeding productivity.
- **Avian influenza** is known to occur in migratory aquatic birds wintering in South Africa. Most strains present in aquatic birds are low pathogenic avian influenza (LPAI), but occasionally highly pathogenic avian influenza (HPAI) may also occur.
- **Chlamydiosis** is caused by *Chlamydiophila psittaci* and affects a wide range of bird species. Even though it has not yet been reported in African penguins, it is known to cause severe outbreaks in other penguin species. The infection in birds may not always be apparent, but chronic disease or acute mortality may occur. *C. psittaci* is also a zoonotic pathogen, causing respiratory infection in humans.
- **Gastrointestinal helminthiases** by *Contracaecum* spp., *Cardiocephaloides physalis* and *Tetrabothrius* spp. are relatively common in African seabirds. The impact of these parasites is unclear in many cases, but they are likely to contribute to morbidity and mortality of individuals. In particular, *C. physalis* has been implicated in the deaths of African penguin chicks in the Eastern Cape.
- **Visceral helminthiasis** by *Cyathoma phenisci* (respiratory tract) and *Renicola sloanei* (kidneys) have occasionally been identified in southern African seabirds, but their clinical significance is unclear.
- **Ectoparasite infestation** by ticks (*Ornithodoros capensis*, *Ixodes uriae*), fleas (*Parapsyllus* spp.), flies (*Pseudolynchia canariensis*, *Lynchia schoutedeni*), unidentified hypoderatid mites and many different

louse species (particularly *Austrogoniodes* spp.) are relatively common in southern African seabirds, particularly in colonial species. The infection may contribute to the mortality of individuals whose health is otherwise compromised, particularly chicks. Furthermore, these parasites serve as hosts or mechanical vectors for pathogens (for example, *Babesia* spp. and *Borrelia* spp. in ticks, Avipoxvirus in fleas and lice, etc.).

- **Assymptomatic infections** by *Mycoplasma* sp, *Salmonella* sp and Newcastle Disease Virus have been identified in great white pelicans. The high prevalence of *Mycoplasma* sp and *Salmonella* sp in this species suggests that the pelican may act as a reservoir of infection for other seabirds and humans.
- **Antibodies** against pathogens such as *Mycoplasma* sp, Newcastle Disease Virus, Infectious Bursal Disease Virus, Infectious Bronchitis Virus, Avian Encephalomyelitis Virus and Avian Reovirus have been identified in southern African seabirds but their significance for these populations is unclear.

5. Targeted surveillance

5.1. Sample collection

5.1.1. Sample size

Sample sizes are a compromise between statistical power for representation of the study population, disturbance to the colony, diagnostic test performance, expected pathogen prevalence, and logistical and financial constraints. A large sample size is often necessary, especially when the expected prevalence is very low or unknown.

The sample sizes estimated in Table 1 were calculated considering a finite population. For these estimates, the prevalence of unknown pathogen was estimated as 10% for African penguins, Cape gannets, Cape cormorants and Bank cormorants (based on serological health survey results conducted in 2010-2013) and 20% for Great white pelicans and Kelp gulls (based on Assunção et al 2007). Sensitivity and specificity were both set at 95%; these parameters assume that only validated methods are employed, and that non-validated methods may not perform to the same level. Precision was set at 10%; this is lower than the usual standard (5%) but is considered a more realistic approach considering limitations of resources and logistics involved in sampling seabird colonies. The sample sizes proposed in Table 1, with a total of approximately 2800 seabirds sampled yearly, are therefore likely to be at the low end of those needed for statistical significance but are thought to be sufficient for an initial assessment of pathogen prevalence in these populations. A more detailed analysis of statistical power and sample sizes can be conducted at later stages of this surveillance program, incorporating the preliminary findings from the first samples tested and the input from an epidemiologist.

Species were selected based on conservation status (African penguins, Cape gannets, Cape cormorants and Bank cormorants) or possibility of being carriers of disease (Great white pelican and Kelp gull), ease of collecting samples from birds that are routinely monitored, and those occurring on the same colony. Colonies were selected based on numbers of breeding seabirds, routine monitoring already being conducted, colonies with different species breeding together and ease of collection of samples. It should therefore be emphasized that this is by no means a definitive sampling scheme, but merely a starting point for a targeted surveillance programme.

These samples should be evenly distributed across age-classes (chicks, juveniles, adults) in order to ensure detection of pathogens that are restricted to particular age-classes. Sampling effort should be consistent

each year in terms of age-class composition, species and time of year (this is dependent on the breeding cycle of each species); if this is not possible, sampling adult individuals across seasons for species that are present year-round is also recommended to identify seasonal trends of pathogens. The ease and feasibility of capturing birds and the potential disturbance to the colony will be determining factors in this approach and the specific sampling protocol needs to be determined for each colony, based on field assessment during the surveillance programme.

5.1.2. Who will collect the samples

Ideally, the sample collection would be made by people that are already working with these species. These may be conservationists that routinely conduct monitoring, or field biologists that are already handling the birds for monitoring or other research purposes. This will help to limit the disturbance caused by a disease surveillance programme, as well as to minimise additional handling of birds. However, on many colonies there is minimal handling and therefore sampling would cause disturbance and it is important to limit this as much as possible.

A participatory approach allows many stakeholders (with “in the field” personnel) to be involved in the programme and increases the number of samples collected as well as providing insights that might be critical to understand the epidemiology of any disease. Field personnel would need to receive basic training to participate in this disease surveillance programme, and a core field team could be selected to undergo further training to ensure the good quality and consistency of sample collection. Sample collection will need to be overseen and co-ordinated by a central person to ensure that the correct permits are obtained and that collection and storage of samples is done correctly and sent to the appropriate laboratories or organisations.

5.1.3. What samples to collect

This list is recommended based on experience gained while conducting a seabird health survey in 2010-2013. Samples need to be timeously sent to the laboratory and stored in specific conditions otherwise haemolysis and cellular metabolism cause changes that influence the results. The following data and samples should be obtained from each bird:

- Date of sampling
- Location
- Species
- Age
- Breeding status
- Body measurements: species-specific protocol (in most cases comprises head length, bill length, bill depth and body mass)
- 2 capillary tubes of blood (from foot or leg vein), which will be used to prepare:
 - 2 blood smears fixed in methanol
 - drops of blood onto FTA paper and air-dried
 - 1 capillary tube into transport medium
- 1 tracheal swab in transport medium
- 1 cloacal swab in transport medium
- Faecal sample in buffered formalin
- 6 feathers in ziplock bag (short back feathers with the skin tag intact)
- Photographs for Biobank identification, including distinguishing characteristics of the bird

- Photographs of chest spot pattern (penguins)

Veterinary procedures, such as taking a blood sample and collecting swabs, need to be conducted by people authorised by the South African Veterinary Council. This authorisation is recommended for people commonly working in the field. The collection of samples will be limited if personnel do not have veterinary authorisation.

Sampling kits will be provided by the National Zoological Gardens of South Africa (NZG) Biobank. Two kits will be available, one for sampling live birds within a colony, and one for opportunistic sampling of dead birds. The live bird kits will include RNAlater (transport medium for blood and swabs), as well as FTA paper. Kits for dead birds will include tubes containing buffered formalin, ziplock bags for storage of organs / lesions at -20°C (freezer), tubes with ethanol for parasites (ecto and endo), as well as a blood tube for blood clots. All samples received will be processed and banked according to international Best Practice guidelines, and managed according to established standard operating procedures. Full, detailed protocols will be supplied once the project is operational.

5.2. Detection of pathogens

For the targeted surveillance, samples will be banked with the NZG Biobank to be available for disease research. Bio-banking of Namibian samples needs to be investigated in more detail as additional permits will be required; the NZG is currently working with the Namibian government on this issue. It is foreseen that the research resulting from this surveillance programme will be similar to those published by previous health evaluation studies conducted in other seabird species. In particular, the approach used by Assunção et al (2007) will be favoured, i.e. PCR testing of a wide range of pathogens.

6. Scanning surveillance

6.1. Sample collection

6.1.1. Sample size

Scanning surveillance means sampling opportunistically, hence sample size will be dependent on number of seabirds sent to rehabilitation centres or the number of carcasses found in the field. These samples are an important resource of a surveillance programme and it is worthwhile to put effort into their collection at the different colonies and in organising transport of sick and dead birds to rehabilitation centres.

6.1.2. Who will collect the samples

Field personnel involved in the targeted surveillance effort, as well as other persons working at seabird colonies and the general public will be able to contribute in the sample collection. In particular, training of field teams is invaluable in observing a seabird that may be sick, identifying a freshly deceased carcass and the importance of collecting different species. Rehabilitation centres that concentrate on the rehabilitation of seabirds in South Africa serve as a network of people that receive sick and dead birds and who can be trained to take samples for testing; this can also be carried out by veterinarians associated with the centres.

6.1.3. What samples to collect

Similarly to the targeted surveillance, it is recommended that samples include blood smear, faeces in buffered formalin and tracheal and cloacal swabs on live birds and also tissues and lesions from dead birds. It is not possible to collect from every bird admitted, so priority should be made on freshly dead carcasses

from the colonies and birds that die shortly after admission to the centre. It is also possible to collect samples from abandoned eggs (antibody, toxicology and heavy metal levels), so although this needs to be tested in southern African seabirds, it is reasonable to recommend their collection for future research.

6.2. Detection of pathogens

In the case of permitted seabird rehabilitation facilities in South Africa, it is recommended that these organisations undertake disease testing as part of their diagnostic work for the animals in their care. These facilities may also serve as hubs to receive carcasses and collect samples from recently deceased seabirds. Pathogen detection should be carried out by a veterinarian or trained personnel under the supervision of a veterinarian to help determine cause of death and to collect samples to send to laboratories for pathogen identification. In outstanding circumstances, sample collection from carcasses may also be conducted by field teams; support and training will be necessary to ensure these teams are capable of conducting a superficial post-mortem examination and sample collection.

Examples of routine tests to be done for disease surveillance are listed in Table 2 along with laboratories used in the Western Cape, samples needed and approximate costs involved. Duplicate samples for biobanking should be taken at the same time so these can be stored adequately for future research (see above for kits provided by the NZG Biobank). It is important to bear in mind that commercial laboratories can be costly, especially the PCR testing, so the rehabilitation centres need to bear this in mind. A veterinary laboratory exists at Onderstepoort, University of Pretoria but this poses problems in terms of transport and transport permit conditions so it is best to use local commercial laboratories instead.

When a pathogen is identified, it is important to do as thorough a diagnostic work-up as possible to ensure the most specific diagnosis. This is often difficult when dealing with opportunistic sample collection (for example autolysis of carcasses), the tests used may not be validated for the specific species and often the pathogen may only be detected after the disease event has resolved. Therefore doing as wide a range of tests is recommended, and collecting samples for biobanking will ensure that further research can be done on these samples.

7. Information management

As previously indicated, a co-ordinator will be critical to assist the supervision of the programme in general in terms of the application of permits, training of fieldwork personnel, arrangement of sample kit delivery to fieldworkers, supervision of collection of samples, correct storage of samples, and to ensure that samples are sent to the correct laboratories or organisations. It is also critical that this co-ordinator supervises the standardised recording of data and collation of that data in a centralised database, which will be done in conjunction with the data required for the Biobank.

The rehabilitation centres will need to enter their relevant data into a database set up by the project co-ordinator, who will also ensure that this is regularly updated. Implications of data-sharing will be discussed with each centre that is submitting data and appropriate protocols introduced. SAEON (South African Environmental Observation Network) has protocols that can be incorporated. The project coordinator will need to incorporate all data from targeted and scanning surveillance programmes, set up a database so that all data can be reliably searched, retrieved and analysed as well as be securely archived and preserved over time.

The project co-ordinator needs to ensure the appropriated epidemiological statistical analysis of the data is undertaken, as well as obtaining expert opinion on the results and the relevance of the results (both pathogen and statistical). This is collaborative work and all parties must be recognised for their input. The coordinator will be responsible to follow up with those conducting research and collate all results, and will play a central role in mediating the intellectual property and information sharing of the findings of the programme, to ensure that all contributors have the option of co-authorship in the resulting scientific publications.

The National Zoological Gardens of South Africa is a declared national research facility of the National Research Foundation, and therefore, very clear structures and mandates are in place to promote, coordinate and facilitate research activities. The NZG Biobank as a research platform of the NZG will be responsible for the sample curation, as well as the meta-data linked to the individual and population. These biomaterials and data are accessible for future research projects. Access and benefit sharing of samples will be managed by the NZG Research Ethics and Scientific Committee; a committee comprising external members representing higher education institutions and welfare organisations following strict evaluation and review processes.

8. Communication of surveillance results

Reports will be issued on a yearly basis to Department of Environmental Affairs (Oceans and Coasts), CapeNature and SANParks as the primary governmental organisations involved in the conservation of southern African seabirds. If the Namibian samples are also submitted as part of the project, then reports will also be issued to the Namibian Ministry of Fisheries and Marine Resources. Any reportable diseases recorded will be reported to the state veterinary authorities immediately upon confirmation of the diagnosis.

All parties involved in the collection of data will also receive yearly reports. Reports will include all data collected, source of data, test results, expert opinion and preliminary statistical analysis. It is important that all aspects of the surveillance data are addressed in the analysis of the data, i.e. public health (zoonotic diseases), domestic animal health (notifiable diseases), wildlife conservation and management (wildlife health) and environmental health (stability of ecosystems). The parties involved in collection of data will have preference in the publication of the data they provided, and collaboration between all parties for multi-institutional research will be encouraged.

National and local management authorities will be responsible for updating any disease risk assessments for individual colonies based on these results and to keep updated disease contingency plans based on the latest available research and information.

9. Critical assessment and Governance of the programme

It is necessary to determine critical success factors (those factors needed to be in place to achieve the overall mission), specific objectives and goals, resource and capacity needs and organisational responsibility in order for the programme to succeed. These in turn must be monitored, re-evaluated and managed in order to continue with a sustainable long-term programme that offers valuable data. Table 3 summarises the critical success factors; currently Dr. Nola Parsons is funded through the Bayworld Centre for Research and

Education (BCRE) for 2015 to write this guideline document and will continue in the role of co-ordinator in 2016, if this funding continues.

Currently SANCCOB is the responsible party for developing guidelines for an African penguin disease surveillance and diagnosis programme (BMP action point 4.5.4.1) and to conduct a disease risk assessment for seabird breeding colonies (BMP action point 4.5.4.3). The management authorities are responsible to implement this programme (BMP action point 4.5.4.2) as well as to draft disease contingency plans for African penguin colonies (BMP action point 4.5.4.4).

The project co-ordinator will need to co-ordinate and organise the different organisations involved from government (Department of Environmental Affairs and Department of Agriculture), management authorities (CapeNature and SANParks), rehabilitation centres, biobank facilities and collaborative partnerships. This will likely be done by a non-government organisation.

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Table 1. Estimated sample sizes needed for a targeted disease surveillance programme in southern African seabird colonies. Data of number of breeding pairs were obtained from Department of Environmental Affairs (2014, unpublished data), CapeNature (2014, unpublished data), Simmons et al (2015) and Kemper et al (2007). Numbers of individual adults were calculated by multiplying the number of breeding pairs by 3.2 (Crawford & Boonstra 1994) for African penguins and multiplying by 2 for all other species.

	Bird Island	St Croix Island	Dyer Island	Stony Point	Boulders	Robben Island	Dassen Island	Vondeling Island	Malgas Island	Lambert's Bay	Possession Island	Halifax Island	Ichaboe Island	Mercury Island	Total estimated population and total samples
African penguin															
No. breeding pairs	2837	8685	1148	2045	942	964	2112	116	30		594	852	661	3171	24157
Estimated no. individuals	9078	27792	3674	6544	3014	3085	6758	371	96		1901	2726	2115	10147	77302
Sample size	58	58	58	58	57	57	58	51	37		57	57	57	58	721
Great white pelican															
No. breeding pairs							416								416
Estimated no. individuals							832								832
Sample size							105								105
Cape gannet															
No. breeding pairs	79403								18200	4037	380		10500	2200	114720
Estimated no. individuals	158806								36400	8074	760		21000	4400	229440
Sample size	58								58	58	54		58	58	344
Cape cormorant															
No. breeding pairs		31	12210	900		918	154	103	616	115	2440		10655	1590	29732
Estimated no. individuals		62	24420	1800		1836	308	206	1232	230	4880		21310	3180	59464
Sample size		30	58	57		57	49	46	56	47	58		58	57	573
Bank cormorant															
No. breeding pairs				51		94	55	5	11				217	2505	2938
Estimated no. individuals				102		188	110	10	22				434	5010	5876
Sample size				37		45	38						52	58	230
Kelp gull															
No. breeding pairs			213			1251	1999	61	118	92	1494	272	72		10229
Estimated no. individuals			426			2502	3998	122	236	184	2988	544	144		20458
Sample size			94			114	116	61	80	73	115	98	66		817
Total each colony	116	88	210	152	57	273	366	158	231	178	284	155	291	231	2790
Total province	EC	204							WC	1625			Namibia	961	

Table 2. An example of diagnostic testing, samples needed and costs for scanning surveillance at a rehabilitation centre in the Western Cape. Petrol costs have been calculated using the round trip distance at a rate of R3.40/km. In the Eastern Cape, samples would need to be sent to the Provincial Veterinary Laboratory in Grahamstown (approximately R1591 return from Cape St Francis), IDEXX collects from Port Elizabeth but Vetdiagnostix only operates from Durban and Cape Town. In Namibia, all samples would need to be sent to the Central Veterinary Laboratory in Windhoek (approximately R100 postage Lüderitz to Windhoek).

Test	Laboratory	Samples needed	Collection and costs	Cost of sampling kit	Cost of test (incl vat)
Bacteriology - Aerobic culture	IDEXX	Swab transport medium	supplied by lab	R 5.00	R 469.00
Bacteriology - Aerobic and anaerobic culture	IDEXX	Swab transport medium	supplied by lab	R 5.00	R 662.00
Virus culture (per virus)	Provincial Veterinary Laboratory: Stellenbosch	Swab transport medium or tissue sample	transport needed - R340 petrol	R 5.00	R 452.58
Histopathology	Vetdiagnostix	Tissue samples in formalin	supplied by lab	supplied by lab	R 267.00
Avian DNA sexing	Molecular Diagnostic Services	Drop of blood on filter paper, in eppendorf tube	postage/courier - R30	R 1.00	R 90.00
Avian pathogen tests (per test)	Molecular Diagnostic Services	Tracheal swab	postage/courier - R30	R 5.00	R 200.00
Serology - haemagglutination tests - Newcastle Disease and Avian Influenza H5 and H6 (per test)	Provincial Veterinary Laboratory: Stellenbosch	0.5 - 1ml serum sample frozen -20°C	transport needed - R340 petrol	R 6.00	R 15.00
PCR - surveillance screening - Newcastle Disease and Avian Influenza H5 and H6 (per test)	Provincial Veterinary Laboratory: Stellenbosch	Tracheal swab	transport needed - R340 petrol	R 5.00	R 148.00

Table 3. Critical success factors needed to ensure the set-up of a long-term disease surveillance programme for southern African seabird colonies.

Critical success factors	Resources needed	Responsible party	Time frame
Funding of a programme co-ordinator	Salary	To be determined	As soon as possible
Project proposal and approval of funding	Funding	Co-ordinator	As soon as possible
Permits in place	Project approval	Co-ordinator	Beginning 2016
Sample kits	NZG Biobank will fund and supply all sample kits	NZG Biobank	As soon as project is approved
Training of people to collect samples	Funding	Co-ordinator	Beginning 2016
Training of people to identify sick and freshly dead carcasses	Funding	Co-ordinator	Beginning 2016
Distribution of sample kits to relevant people	NZG Biobank will fund and supply all sample kits	NZG Biobank and Co-ordinator	As soon as project is approved
Resources to collect samples (capacity, transport, time) - colony	Capacity, transport, time (shared other work)	Conservation authorities	Beginning 2016
Collection of samples	Permits, SAVC authorisation, training, sample kits	Researchers, colony managers, co-ordinator	Middle 2016
Storage of samples	NZG Biobank in place	NZG Biobank	As soon as project is approved
Data and sample collation	Salary in place	Co-ordinator	Ongoing
Training of people to conduct post mortems and collect samples	Funding	Co-ordinator	Beginning 2016
Resources to collect samples (capacity, transport, diagnostic tests) - rehabilitation centres	Funding, permits, training, sample kits	Rehabilitation centres	Middle 2016
Set up disease surveillance database	Salary in place	NZG Biobank and co-ordinator	End 2016
Epidemiological analysis of data collected	Set up network of expert collaborators	Co-ordinator	End 2017
Annual reports to permitting organisations	Salary in place	Co-ordinator	December each year
Annual reports to all parties involved in collection of samples	Salary in place	Co-ordinator	December each year