Agreement on the Conservation of Albatrosses and Petrels

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Protocols for sample collection from bycaught birds for health (and other) studies.

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SUMMARY

Commercial fishing operations are considered the greatest threat to the survival of many albatross species, and fisheries monitoring through on-board observers (OBO) is common practice. However, carcasses recovered from fisheries are currently under-utilized. With proper protocols and training, evaluation of such carcasses could not only provide valuable information on population-level demographics, distribution patterns, genetics, and food habits, but also on overall health condition, pollution loads, and disease exposure, allowing for the establishment of baseline health data for many species, and the early identification of environmental change and pathological processes. Within the context of an ACAP funded project that aims at maximizing scientific sampling from by-caught albatrosses, we have developed comprehensive, yet simplified, sample collection protocols and visual training-aids, which we have delivered through hands-on wetlabs for on-board observers in Argentina, Brazil, Chile, and Peru. Improvement and adaptation of protocols to country and fisheries-specific needs and capabilities is ongoing. OBOs considered protocols simple enough for completion on board and were enthusiastic about collaborating should the time and mechanisms be allotted by their institutions. As we move towards field-testing the protocols we must: a) define mechanisms (i.e. permission to store samples on board and disembark at ports) and sustainability; b) develop or reinforce in-country and/or regional networks to compile, store and utilize collected samples; c) identify countries and financial support for implementation of pilots and proof of concept.

Our goal is that these protocols and methods will be improved by ground-testing and then presented to ACAP to be made available worldwide.

RECOMMENDATIONS

1. That interested ACAP countries proceed to define implementation and sustainability mechanisms (i.e. time assignments, permission to store samples on board and disembark at ports) that would enable on-board observers to complete the sample collection protocols.
2. That ACAP acknowledges current limited seabird health research capacity in S. America and encourages the establishment of a regional “health network” to better deliver on ACAP priorities for proper curation of tissue samples from by caught seabirds.
3. That ACAP recognizes the value of field-testing the developed protocols and endorses pilot implementation phase in selected countries where necessary permits have been secured.

Protocolos para recolectar muestras de aves capturadas a fin de realizar estudios en materia de salud (y otras áreas)

RESUMEN

Se considera que las operaciones de pesca comercial constituyen la mayor amenaza para la supervivencia de muchas especies de albatros; por tal motivo, el monitoreo de pesquerías a través de observadores a bordo de los barcos es una práctica común. Sin embargo, actualmente no se están aprovechando al máximo los cuerpos sin vida que se encuentran en las pesquerías. Con la capacitación y los protocolos adecuados, el estudio de dichos cuerpos aportaría información valiosa no solo sobre los datos demográficos, los patrones de distribución, la genética y los hábitos alimenticios de las poblaciones de aves, sino también sobre las condiciones de salud, las cargas contaminantes y la exposición a enfermedades en términos generales. Esa información permitiría que se establecieran datos de referencia relativos a la salud para muchas especies y que se identificaran cambios ambientales y procesos patológicos de forma temprana. En el marco de un proyecto financiado por el ACAP tendiente a maximizar el muestreo científico tomado de albatros que hayan sido capturados de forma incidental, hemos elaborado material gráfico de capacitación y protocolos de recolección de muestras abarcadores pero simples que fueron entregados a los observadores durante capacitaciones prácticas realizadas en laboratorios húmedos en Argentina, Brasil, Chile y Perú. En este momento, se están mejorando los protocolos y se los está adaptando a las necesidades y capacidades de cada país y pesquería. Los observadores consideraron que los protocolos eran lo suficientemente simples para ser aplicados a bordo de un barco, y expresaron que colaborarán de buen agrado con el proyecto si sus respectivas instituciones asignan el tiempo y los mecanismos necesarios para tal fin. Dado que se acerca el momento de llevar a cabo las pruebas de campo de los protocolos, debemos a) definir los mecanismos (por ejemplo, las autorizaciones para almacenar muestras a bordo y desembarcar en los puertos) y la sostenibilidad; b) crear o reforzar redes a nivel nacional y/o regional para recopilar, almacenar y utilizar las muestras obtenidas; e c) identificar países y sustentos económicos para implementar las pruebas piloto y de concepto.

Nuestro objetivo consiste en que estos protocolos y métodos se perfeccionen mediante las pruebas de campo y luego se presenten ante el ACAP para que estén disponibles en todo el mundo.

RECOMENDACIONES

1. Que los países interesados que forman parte del ACAP procedan a definir los mecanismos de implementación y sostenibilidad (por ejemplo, el reparto del tiempo y las autorizaciones para almacenar muestras a bordo y desembarcar en los puertos) que les permitirían a los observadores completar los protocolos de recolección de muestras en los barcos.
2. Que el ACAP reconozca las limitaciones existentes en Sudamérica en materia de investigación sobre la salud de las aves marinas y promueva la formación de una "red de salud" regional para optimizar la implementación de las medidas prioritarias del ACAP tendientes a lograr la curación adecuada de muestras de tejido de aves marinas capturadas en forma secundaria.
3. Que el ACAP reconozca la importancia de que los protocolos elaborados pasen por pruebas de campo y avale la fase de implementación piloto en determinados países en los que los permisos están asegurados.

Protocoles pour la collecte d'échantillons sur des oiseaux capturés accidentellement à des fins d'études sanitaires (et autres)

RÉSUMÉ

Les activités de pêche commerciale constituent l'une des plus graves menaces à la survie de nombreuses espèces d'albatros, et la surveillance des pêcheries par des observateurs à bord des navires (OBN) est une pratique courante. Pour autant, les carcasses collectées dans des pêcheries sont à ce jour trop peu utilisées. Grâce à des protocoles et à une formation adéquats, l'examen de telles carcasses pourrait non seulement fournir de précieuses informations en matière de données démographiques des populations, de modèles de répartition, de génétique et d'habitudes alimentaires, mais aussi sur les conditions sanitaires globales, les charges polluantes et l'exposition à des maladies. Cela permettrait d'établir des données de référence sur la santé de nombreuses espèces ainsi que d'identifier à temps les évolutions environnementales et les processus pathologiques. Dans le cadre d'un projet financé par l'ACAP visant à tirer le meilleur profit possible des collectes scientifiques d'albatros victimes de capture accessoire, nous avons élaboré des protocoles exhaustifs, mais simplifiés, pour la collecte d'échantillons, ainsi que des modules didactiques visuels, et les avons transmis sous forme d'aqualaboratoires pratiques aux observateurs à bord des navires en Argentine, au Brésil, au Chili et au Pérou. Le perfectionnement de ces protocoles et leur adaptation aux pays ainsi qu'aux besoins et aux capacités propres à chaque pêcherie est en cours. Les OBN ont jugé les protocoles suffisamment simples pour être remplis à bord, et ont fait preuve d'enthousiasme à l'idée de collaborer, sous réserve que du temps et des mécanismes leur soient octroyés par leurs institutions. La prochaine étape étant d'essayer les protocoles sur le terrain, nous devons : a) définir les mécanismes (p.ex. la permission d'entreposer des échantillons à bord et de

les descendre aux ports) et les moyens d'assurer une durabilité ; b) développer ou renforcer les réseaux à l'échelle des pays et/ou des régions afin de compiler, d'entreposer et d'utiliser les échantillons collectés ; c) identifier des pays et des soutiens financiers pour la mise en œuvre des projets pilotes et de la validation de principe.

Notre objectif est que ces protocoles et méthodes soient améliorés par des essais sur le terrain, puis présentés à l'ACAP afin de pouvoir les diffuser à travers le monde.

RECOMMANDATIONS

1. Que les États membres de l'ACAP intéressés s'efforcent de définir les modalités de la mise en œuvre ainsi que les mécanismes de durabilité (p. ex. octroi de temps à cette fin, permission d'entreposer des échantillons à bord et de les décharger aux ports) afin que les observateurs à bord des navires puissent appliquer les protocoles de collecte des échantillons.
2. Que l'ACAP reconnaisse le caractère limité des capacités actuelles de recherche en matière de santé des oiseaux de mer en Amérique du Sud et encourage la mise en place d'un « réseau sanitaire » régional ayant pour vocation une meilleure réalisation des priorités de l'ACAP relatives à la conservation appropriée des échantillons de tissus prélevés sur des oiseaux capturés accidentellement.
3. Que l'ACAP reconnaisse l'intérêt de tester sur le terrain les protocoles élaborés et prenne en charge la phase pilote de mise en œuvre dans les pays sélectionnés où les autorisations requises ont été obtenues.

1. INTRODUCTION

1.1. Justification and framework

Commercial fishing operations are considered the greatest threat to the survival of many albatross species (Tuck et al. 2001, Baker et al., 2002, Lewison et al. 2004, Rolland et al. 2009, Jimenez et al. 2015), and efforts to monitor the impact of fisheries through on-board observers is common practice. However, the opportunity to better measure impacts and gain meaningful knowledge utilizing seabird bycatch carcasses recovered from fisheries is currently under-utilized. With proper protocols and training, evaluation of carcasses from by-catch events could not only provide valuable information on population-level demographics, distribution patterns, genetics, and food habits, but also on overall health condition, pollution loads, and disease exposure, allowing for the establishment of baseline health data for many species, and the early identification of pathological processes (Mörner et al. 2002, Mallory et al. 2010).

The Agreement on the Conservation of Albatrosses and Petrels (ACAP) has repeatedly recognized the need to establish capacity to collect health and disease exposure information from by-caught carcasses during routine operations as a priority: **ACAP AC7, 2013 Report**, item 9.1.3.28 "…*specifically encourages the development of guidelines for the collection and curation of tissues samples obtained from by caught seabirds*". New information from by-caught animals, albeit not unbiased, encompassing a wide range of species and age categories over broad geographic regions, would greatly increase knowledge on current health threats for albatross and petrels.

In this context, the main objective of this ACAP funded project is to maximize scientific sampling from albatross and petrels incidentally caught in fisheries, by building capacity for standardized sample and health data collection and storage in South American countries. Hitherto, we have developed comprehensive, yet simplified, sample collection protocols and visual training-aids, which we have delivered through hands-on training and wetlabs. Initially, and in close coordination with OBO program leads, we have targeted on-board observers (OBO) in Argentina and Brazil in the Atlantic, and Chile, and Peru in the Pacific. Improvement and adaptation of protocols to the specific needs and capabilities of each country and fishery type is ongoing. In this document we present a thorough compilation of information that can be obtained from by-caught birds for health (and other) studies, and share standardized/simplified protocols for sample collection and storage we have developed over the past year. Finally we provide specific recommendations (and/or a list of pending goals) for field-testing the protocols and delivering improved and complete versions to ACAP for worldwide distribution.

2. COLLECTION AND POTENTIAL USE OF BYCAUGHT BIRD SAMPLES

2.1. Scope of information obtainable from by-caught birds (health -and other- studies)

The simple collection of feathers and small tissue samples from bycaught birds can provide crucial information to determine their susceptibility to disease and damage associated with pollutants, amongst other significant health-related factors. Furthermore, with the same effort involved in collecting samples for health assessments, information on demographics, distribution patterns and migration, identification of individuals and genetic characterization of little-known species, feeding habits in non-breeding times and overlap between species, information on food chains and dependence on fishing discards, inter alia, can be easily obtained. The scope/extent of possible studies will depend largely on on-board conditions and storage capacity, as well as the "enthusiasm" of on-board observers and prioritization by OBO program leads (see below). A comprehensive compilation of potential analysis, with

emphasis on health studies, from samples obtainable from bycaught birds is presented in ANNEX I.

2.2. Protocols for sample collection and storage

We developed detailed yet simplified sample collection protocols and visual training aids for biological sample collection and storage (see ANNEX II-IV). For this, we reviewed the literature for publications using samples/specimens which could be easily obtained from bycaught/dead birds (over 100 articles), and compiled existing OBO information collection protocols in target countries. We initially defined a tiered three step approach for protocol complexity (basic, intermediate and advanced), which later evolved into a two-pronged scheme: a) basic: at sea (with or without access to cold chain) and b) advanced: on land. This approach responded to the specific characteristics of fisheries in the target countries, duration of fishing trips, on board conditions, and storage capacity (i.e. access to freezer) and was further narrowed with feedback from OBO during wetlabs (see ANNEX II).

2.3. Training workshops and wetlabs

Sample collection and storage protocols from by-caught animals were shared with select on-board observer teams and programs in Argentina, Chile, Brazil (following consent and interest of the parties involved) (May 2015), and Peru (June 2015). Workshops were delivered in partnership with local stakeholders and Birdlife International's South American Seabird Program. During workshops we transferred capacity to local teams via theoretical classes and group discussions (i.e. feasibility of proposed approaches) and practiced manual dexterity for sample collection through hands-on wetlabs. Feedback and discussions also fed a list of road-blocks (potential and currently existent) and issues requiring resolution prior to protocol implementation (i.e. authorization for sample or carcass storage on board). We also distributed sample collection and storage kits purchased with ACAP support, as well as data collection sheets and other necessary materials.

Improvement and adaptation of protocols is ongoing. In Argentina we have obtained permits from the National Veterinary Service (SENASA) to collect and store samples on board and disembark them at selected ports, allowing for proximate launch of protocol pilot testing.

3. MAIN CONCLUSIONS AND SPECIFIC RECOMMENDATIONS

There is clearly not a “one size fits all” alternative for sample collection protocols from bycaught birds. Individualities of each OBO program as well as differences between fisheries in relation to duration of trips, types of vessels, on-board conditions and storage capacity (i.e. cold chain), have required additional simplification of protocols following workshops. It is also likely that more tailored adaptations will have to be made to fit each country’s needs and/or interests. In any case, the protocols we designed are deliberately conceived to focus on the collection of as few as three or four samples per bird, but yield a myriad of potential diagnostic options (see Annex I). Furthermore, the diversity of uses and information from samples can be increased dramatically by collecting replicate samples and storing each in a different preservative. Thus, narrowing or expanding the scope of protocols to respond to specific research needs is viable and relatively straightforward.

In addition, the reality of OBOP suggests that they struggle as is. Therefore, assigning OBO additional tasks might be challenging at best. However, it may be possible to gradually implement the protocols starting with the more advanced or fine-tuned programs, and/or only assign them to a few more willing or skilled individuals identified during training. On this note, most OBO were enthusiastic about the data that could be generated through the use of the protocols, understood the value of such information and of their critical role in this process, and did not foresee difficulties in implementation should the time and mechanisms be allotted by their institutions. All protocols were considered to be simple enough for completion on board, which was one of the primary challenges we faced.

During the following year we intend to: a) beta-test protocols, collect feedback and re-test improved protocols (i.e. Argentina and potentially Brazil); b) compile feedback from field tests, and prepare final, ground-tested versions of protocols and guidelines; c) submission of final protocols and guidelines to ACAP for potential implementation in other parts of the world.

3.2. Recommendations

A final definition of tailored protocols requires field-testing in at least a few scenarios. This pilot implementation of protocols will require:

3.2.1. Defining implementation and sustainability mechanisms

Given some of the road-blocks identified, we recommend that interested ACAP countries proceed to define implementation and sustainability mechanisms (i.e. time assignments, permission to store samples on board and disembark at ports) that would enable on-board observers to complete the sample collection protocols.

3.2.2. Establishing in-country and/or regional "health network"

Given current limited seabird health research capacity in S. America, we recommend that ACAP acknowledges this gap and encourages the establishment of in-country and/or regional "health networks" to better deliver on ACAP priorities for proper curation of tissue samples from by caught seabirds.

3.2.3. Endorsing pilot implementation phase

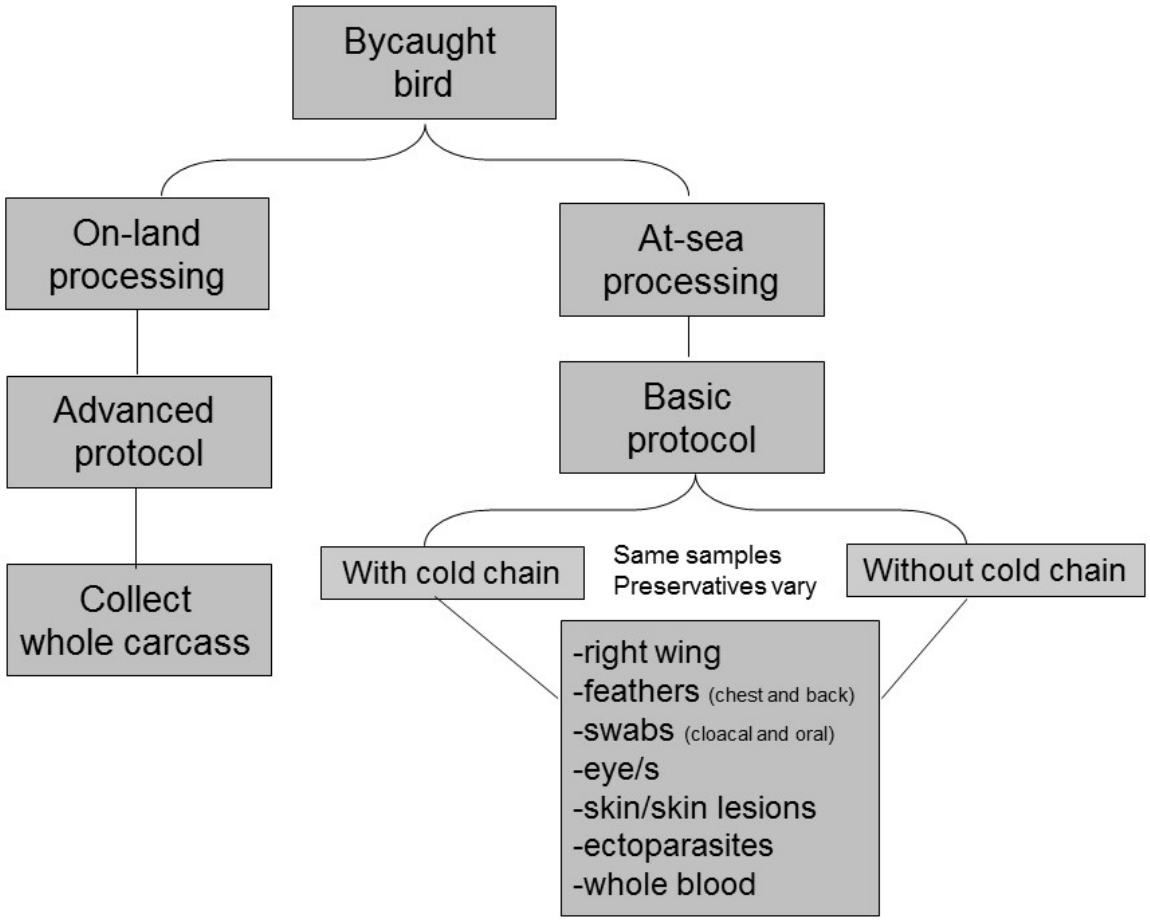
There is currently potential to deploy pilot trials of protocols in at least one or two countries (i.e. Brazil and Argentina). In Brazil, the link between government, NGOs and Universities already in place via Plan ACAP would allow for relatively smooth implementation and proof of concept in the short term. In Argentina, INIDEP OBOP have expressed interest in initiating sample collection, several scientists are aligned for sample reception, and permits from the National Veterinary Service (SENASA) to collect and store samples on board and disembark them at selected ports have been obtained. We therefore recommend that ACAP recognizes the value of field-testing the developed protocols and endorses pilot implementation phase in selected countries where necessary permits have been or might soon be secured.

ANNEX I. Diagnostic analysis and information potentially obtainable from samples recovered from bycaught birds.

Sample	**Target analysis and outcome information**
Whole carcass	Complete necropsy, multiple analysis.
Primary feathers from right wing	-stable isotopes (diet during known molting period, geographical origin/migration) -corticosterone (stress) -contaminants (heavy metals, persistent organic pollutants (POPs), trace elements)
Chest and back feathers	-stable isotopes (diet during feather growth, feeding area, trophic relationships) -contaminants (heavy metals, persistent organic pollutants (POPs), trace elements) -genetics (sexing, species identification, , phenotypic variation; viral genome if pathogens present) -viral pathogens -corticosterone (stress)
Cloacal and oral swabs	-pathogens (viruses, bacteria, fungi, parasites) -genetics (sexing, others)
Eye	-biochemistry (plasma biochemistries, nutritional condition, pathology, time of death) -serology (antibodies to specific pathogens) -pathogens -toxicology (biotoxins, POPs, etc.) -vision function
Skin and skin lesions	-pathogen screening by PCR (i.e. poxvirus) -pathology (histology) -genetics (sexing, species identification, others)
Ecto & endoparasites	-parasitology, vector-borne diseases (eg. rickettsial)
Whole blood from heart (or other location)	-serology (antibodies) -genetics (sexing, species identification/confirmation, geographical origin/migration) -stable isotopes (recent diet) -pathogens (viruses, bacteria, fungi, hemoparasites) -contaminants (heavy metals, POPs) -biotoxins -reproductive status (hormones)

Stomach content (solids)	-main prey, recent diet -toxicology (biotoxins, others) -marine debri ingestion -parasites
Stomach content (oil)	-fatty acids (indirect marker of diet during long foraging trips) -toxicology (biotoxins) -parasites
Gonads	-past and present reproductive activity
Tissues (liver, kidney, spleen, lung, heart, thyroid, brain)	-histopathology (damage caused by diseases, nutritional status, general health state) -toxicology (heavy metals, POPs, biotoxins) -pathogens (viruses, bacteria, fungi, parasites) -genetics (sexing, species identification, geographical origin/migration, phenotypic variation)
Subcutaneous adipose tissue and body fat (heart and kidneys)	-fatty acids (indirect marker of diet during long foraging trips, feeding area) -toxicology (heavy metals, POPs)
Bone	-toxicology (heavy metals, POPs) -minerals (Ca, P, etc.)
Cerebrospinal fluid	-biochemistry (plasma biochemistries, nutritional condition, pathology, time of death) -serology (antibodies) -pathogens -toxicology (biotoxins, POPs, etc.)

ANNEX II. Tiered approach for protocol complexity (basic/advanced & on-land/at-sea), based on characteristics of fisheries, duration of fishing trips, on board conditions, and storage capacity (access to cold chain).



ANNEX III. Data collection protocols.

This table provides a detailed guide for the collection and preservation of samples obtained from bycaught birds and the type of diagnostic tests which can be performed, with emphasis on health studies (pathogens, nutritional status, and general health status).

Sample	**Analysis**	**Supplies needed**	**On-board Storage**	**Laboratory & long-term storage**
A.1. BASIC PROTOCOL AT-SEA WITHOUT ACCESS TO COLD CHAIN				
Whole right wing (primary feathers) (cut at joint)	a) feathers: -stable isotopes (diet known molting period, geographical origin) -corticosterone (stress) -toxicology (heavy metals) b) bone: -toxicology (heavy metals, POPs) -minerals (Ca, P, etc.)	-bag -salt (for joint)	-room temperature (wing must be dry)	-room temperature
Chest and back feathers (40-50 of each). *Pluck feathers, do not cut.*	-stable isotopes (diet during feather growth, feeding area, trophic relationships) -Contaminants (heavy metals, POPs, trace elements) -genetics (sexing, geographical origin/migration, viral genome, phenotypic variation) -viral pathogens -corticosterone (stress)	-5 ziplock o whirlpack bags with 10 feathers from each location (chest/back) -if dry, add silica gel to each bag -if wet, plastic vial + ethanol	- air-dried and stored in bags at room temperature. - if wet, store in ethanol at room temperature	-room temperature (dry feathers in bags, wet feathers in ethanol)
Cloacal (C) and oral (O) swabs	-pathogens (molecular) (viruses, bacteria, fungi, parasites) -genetics (sexing, others)	In all cases C and O separately, 1 of each: -2ml cryovial + RNAlater -2ml cryovial + UTM -polyester tipped swabs	-room temperature *(ideally no longer than 1 week)*	-frozen, ideally ultra-freezer

Eye	-vision function	-10 ml vial + eye preservative(*) -scissors and tweezers	-room temperature	-room temperature
Skin and skin lesions (i.e. poxvirus)	-pathology, pathogen screening by PCR -genetics (sexing, others)	-2 ml cryovial + RNA later -2ml cryovial + ethanol -scissors and tweezers	-room temperature *(ideally no longer 1 week for RNAlater)*	-RNAlater frozen, ideally ultra-freezer -ethanol room temperature
Ectoparasites	-parasitological, vector-borne diseases (eg. Rickettsial)	-plastic vial (can be cryovial) + ethanol - tweezers	-room temperature	-room temperature
Whole blood ("touch" blood in cavity or organ with filter paper, or collect from heart with syringe and needle or any location)	-serology (antibodies) -genetics (sexing, geographical origin/migration, species) -stable isotopes -pathogens (viruses, bacteria, fungi, hemoparasites) -contaminants (heavy metals, POPs) -biotoxins	-syringe and needle -cryovial (2ml) + RNAlater -cryovial (2ml) + ethanol -FTA or 903 cards -Nobuto filter paper -Whatman filter paper -store filter papers individually in ziplocks with silica gel	-room temperature *(samples in ethanol always at room temperature; samples in RNAlater if longer than 1 week, freeze)*	-frozen, ideally deep freeze ideally *(except samples in ethanol always at room temperature)*
A.2. BASIC PROTOCOL AT-SEA WITH ACCESS TO COLD CHAIN (ON-BOARD FREEZER)				
Whole right wing (primary feathers)	a) feathers: -stable isotopes (diet known molting period, geographical origin) -corticosterone (stress) -toxicology (heavy metals) b) bone: -toxicology (heavy metals, POPs) -minerals (Ca, P, etc.)	-bag	-frozen	-frozen

Chest and back feathers (40-50 of each). *Pluck feathers, do not cut.*	-stable isotopes (diet during feather growth, feeding area, geographical origin, trophic relationships) -Contaminants (heavy metals, POPs, trace elements) -genetics (sexing, geographical origin/migration, viral genome, phenotypic variation) -viral pathogens -corticosterone (stress)	-5 ziplock o whirlpack bags with 10 feathers from each location (chest/back) -silica gel in each bag for dry feathers	- air-dried and stored in bags at room temperature. *- if wet, store in bags and freeze or place in ethanol and store at room temperature*	-same condition of arrival (room temperature or frozen)
Cloacal (C) and oral (O) swabs	-pathogens (molecular) (viruses, bacteria, fungi, parasites) -genetics (sexing, others)	In all cases C and O separately, 1 of each: -2ml cryovial + RNAlater -2ml cryovial + UTM -2ml cryovials no preservative -polyester tipped swabs	-frozen	-frozen, ideally ultra-freezer
Eye/s	-biochemistry (plasma biochemistries, nutritional condition, pathology, time of death) -serology (antibodies) -pathogens (molecular) -toxicology (biotoxins, POPs, etc.) -vision function	-whirlpack or Ziploc bag + no preservative (one eye) - 10 ml vial + eye preservative (one eye) -scissors and tweezers	-frozen bag no preservative -room temperature eye preservative	-frozen, ideally ultra-freezer -room temperature eye preservative
Skin and skin lesions (i.e. poxvirus)	-pathology, pathogen screening by PCR -genetics (sexing, others)	-2 ml cryovial + RNA later -scissors and tweezers	-frozen	-frozen, ideally ultra-freezer
Ectoparasites	-parasitological, vector-borne diseases (eg. Rickettsial)	-plastic vial + ethanol - tweezers	-room temperature	-room temperature

Whole blood ("touch" blood in cavity or organ with filter paper, or collect from heart with syringe and needle or any location)	-serology (antibodies) -genetics (sexing, geographical origin/migration, species) -stable isotopes -pathogens (viruses, bacteria, fungi, hemoparasites) -contaminants (heavy metals, POPs) -biotoxins	-syringe and needle -cryovial (2ml) + RNAlater -FTA or 903 cards -Nobuto filter paper -Whatman filter paper -store filter papers individually in ziplocks with silica gel	-frozen	-frozen, ideally deep freeze
B. ADVANCED PROTOCOL ON-LAND **(the following in addition to all samples in basic protocols above)** *collect whole carcass in a double large garbage bag and keep frozen until arrival at the laboratory*				
Cloacal (C) and oral (O) swabs	-pathogens (viruses, bacteria, fungi, parasites)	In all cases C and O separately, 1 of each: -2ml cryovial + RNAlater -2ml cryovial + UTM -2ml cryovials no preservative -polyester tipped swabs	-deep freeze	
Skin lesions	-pathology -pathogen screening by PCR	-2ml cryovials + 10% formalin -2ml cryovials + UTM -2ml cryovials + Trizol -2ml cryovials no preservative -scissors and tweezers	-room temperature (formalin) -deep freeze (UTM,Trizol, no preservative)	
Gonads	-reproductive activity	-plastic vial + 10% formalin (formalin:tissue 10:1).	-room temperature	

Tissues (liver, kidney, spleen, lung, heart, thyroid, brain)	-histopathology (damage caused by diseases, nutritional status, general health state) -toxicology (heavy metals, POPs, biotoxins) -pathogens (viruses, bacteria, fungi, parasites) -genetics (sexing, others)	-plastic vial + 10% formalin (formalin:tissue 10:1). *All samples in same vial.* -2 or 5ml cryovial + RNAlater -2 or 5ml cryovial + UTM -2 or 5ml cryovial + Trizol -2ml cryovial + ethanol - whirlpack bags no preservative -complete necropsy equipment	-room temperature (formalin and ethanol) -deep freeze (RNAlater, UTM, Trizol and whirlpack)
Subcutaneous adipose tissue and body fat (heart, kidney)	-fatty acids (indirect marker of diet during long foraging trips, feeding area) -toxicology (heavy metals, POPs)	-glass tube with Teflon cap, chloroform with 0.01% antioxidant BHT -whirlpack or ziplock bags -scalpel, scissors, tweezers	-frozen or deep freeze
Bone	-toxicology (heavy metals, POPs) -minerals (Ca, P, etc.)	-whirlpack or ziplock bags -scalpel, scissors, tweezers	-frozen or deep freeze
Stomach content (solids)	-main prey, recent diet -toxicology (biotoxins) -marine debris	-whirlpack or ziplock bags (x2), -large plastic vial + ethanol	-frozen, ideally deep freeze (bags) -room temperature (ethanol)
Stomach content (oil)	-fatty acids (indirect marker of diet during long foraging trips) -toxicology (biotoxins)	-fatty acids: glass tube with Teflon cap, chloroform with 0.01% antioxidant BHT -biotoxins: 2 or 5ml cryovial (x2)	-frozen or deep freeze (oil in glass tube and cryovials)
Cerebrospinal fluid	-biochemistry (plasma biochemistries, nutritional condition, pathology, time of death) -serology (antibodies) -pathogens -toxicology (biotoxins, POPs, etc.)	-2ml cryovial -syringe and needle	-frozen or deep freeze
Internal parasites	- parasite identification	-parasites: plastic vial + 5% formalin	-room temperature

Storage glossary:
Room temperature: no refrigeration. Normally between 10 and 20°C
Frozen: domestic freezer, -20°C approx.
Deep freeze: ultra-freezer, -70°C approx. Note: dry ice yields similar temperature, ideal for sample transport.
Liquid nitrogen: -160°C. Requires special dewar and handling caution.

Preservatives:
Ethanol: off the shelf ethanol 96°. Must be stored at room temperature before and after use.
RNAlater: RNA*later*® solution is a nontoxic tissue storage reagent that rapidly permeates tissues to stabilize and protect cellular RNA. RNA*later*® solution minimizes the need to immediately process tissue samples or to freeze samples in liquid nitrogen for later processing. Can be stored at room temperature before and after use. https://www.thermofisher.com/ar/es/home/brands/product-brand/rnalater.html
Eye preservative: For vision analysis. 30ml 95% ethanol + 20 ml formaldehyde + 10 ml glacial acetic acid + 30 ml distilled water. Must be stored at room temperature before and after use.
Formalin*: 5% (50ml commercial formaldehyde + 950ml distilled water), 10% (100ml commercial formaldehyde + 900ml distilled water + 4gr (1 tsp) table salt). Must be stored at room temperature before and after use.
Trizol*: *TRIzol* Reagent is a complete, ready-to-use reagent for the isolation of high-quality total RNA or the simultaneous isolation of RNA, DNA, and protein from a variety of biological samples. Can be stored at room temperature before use, but requires ultra-freezing once sample is collected
https://www.thermofisher.com/order/catalog/product/15596026
***Caution**: Formalin and Trizol are toxic and should not be handled or used without proper training and personal protective equipment.*
UTM/UVT: Universal Viral Transport Media (UTM™, Universal Transport Medium or UVT, BD™ Universal Viral Transport System) is a room temperature stable viral transport medium for collection, transport, maintenance and long term freeze storage of Viruses, Chlamydia, Mycoplasma and Ureaplasma specimens. Can be stored at room temperature before and after use.
http://www.bd.com/ds/productCenter/CT-ViralTransport.asp
http://www.copanusa.com/products/collection-transport/utm-viral-transport/
Filter papers (903 Protein Saver/FTA card/Whatman): Filter papers are widely used for blood preservation to detect DNA or RNA by PCR. They can also be used for antibody, protein and biotoxin detection. Can be stored at room temperature before and after use.
http://www.gelifesciences.com/webapp/wcs/stores/servlet/catalog/en/GELifeSciences-ar/products/AlternativeProductStructure_21577/
http://www.gelifesciences.com/webapp/wcs/stores/servlet/catalog/en/GELifeSciences-ar/products/AlternativeProductStructure_17096/
http://www.gelifesciences.com/webapp/wcs/stores/servlet/productById/en/GELifeSciences-ar/28419265

ANNEX IV

Datasheets for sample collection from bycaught birds

Bycatch data

Fishing vessel

Vessel position Lat Long

Date month day year

Fishing gear characteristics: (# of hooks, spacing of floats and, light-stick, bait type and condition, snood weights,etc.)

Sample collector

Animal Identification code= Species code animal # Ring

Age class: adult juvenile

Bycatch observations(strangled, entangled, drowned):

Picture # (taken with ID code) head back chest

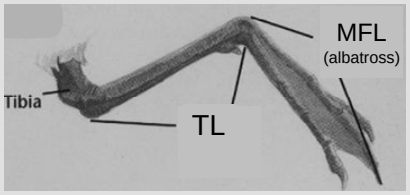
Morphology

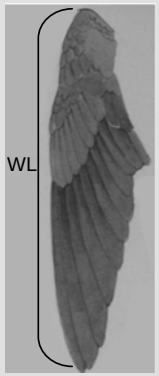
WL= BD=

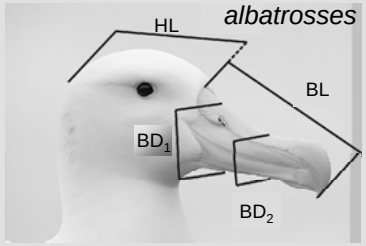
BL= $BD_{1,2(albatross)}$=

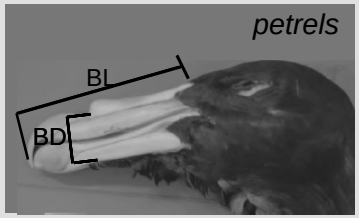
TL= $HL_{(albatross)}$=

Weight= $MFL_{(albatross)}$=









Keel angle (draw)=

On-board samples (on-board freezer)

Sample	Preservation						Storage
	No preservative	RNAlater	Ethanol	UTM	Filter paper	Eye preservative	
Whole carcass *(if collected don't fill rest of sheet)*	garbage bag ☐						Frozen
Right wing	garbage bag ☐						Bags frozen Ethanol room temperature
Chest feathers	Ziplock #: ☐		vial ☐				
Back feathers	Ziplock #: ☐		vial ☐				
Cloacal swab	☐			☐			Frozen
Oral swab	☐	☐		☐			
Ectoparasites			☐				Room temperature
Eye/s	Whirlpack: ☐					vial ☐	Whirlpack frozen Eye preservative room temperature
Skin/skin lesions		☐	☐				Room Temperature
Whole blood (circle type of filter paper used)		☐	☐		**Type** 903 / FTA / whatman **Number** ☐		-filter paper= Room temperature or frozen -ethanol: room temperature -RNAlater= frozen

Observations

On board samples (NO on-board freezer)

Sample	Preservation						Storage
	No preservative	RNAlater*	Ethanol	UTM*	Filter paper	Eye preservative	
Right wing	waste bag: ☐						Samples should be **dry**, add silica gel to each bag. *If wet, do not collect wing.* Place feathers in ethanol if wet or air-dry and store with no preservative
Chest feathers	Ziplock #: ☐		☐				
Back feathers	Ziplock #: ☐		☐				
Cloacal swab		☐		☐			Room temperature *(if possible, freeze after 1 week at room temperature)*
Oral swab		☐		☐			
Eye/s						☐	Room temperature
Ectoparasites			☐				Room temperature
Skin/skin lesions		☐	☐				Room temperature
Whole blood (circle type of filter paper used)		☐	☐		**Type** 903 / FTA / whatman **number** ☐		Room temperature *(if possible, freeze after 1 week at room temperature)*

***if possible, samples in RNAlater and UTM should be frozen after 1 week at room temperature.**

Observations