Agreement on the Conservation of Albatrosses and Petrels

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Guidelines for sampling tissues from by-caught dead birds (with applicability for fresh beached carcasses)

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This paper has an attachment: Guidelines For Sampling Tissues From By-caught Dead Birds (With Applicability For Fresh Beached Carcasses) – Slides/Visual Aids

***NOTE**: These guidelines were prepared for sampling freshly dead by-caught birds on-board fishing vessels. This implies that protocols are purposely over-simplified, and in no way intend to replace proper post-mortem examination of birds dying under any other circumstance. Full necropsies are the recommended gold standard when trained personnel are available and/or when investigating mortality events (with the aim of determining cause of death), and should not be replaced by these guidelines. In any case, these protocols provide options for sample collection beyond identifying cause of death*.

JUSTIFICATION

Commercial fishing operations are considered the greatest threat to the survival of many albatross species, and efforts to monitor the impact of fisheries through on-board observers (OBO) is common practice. However, the opportunity to better measure impacts and gain meaningful knowledge by utilizing seabird carcasses recovered from fisheries bycatch is currently under-utilized. With proper sample collection protocols and minimum training, carcasses from by-catch events could not only provide valuable information on the overall health condition, pollution loads, and disease exposure for many species, but also on population-level demographics, distribution patterns, genetics, and feeding habits, among others. 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*".

In this context, the main objective of this guideline is to maximize scientific sampling from albatross and petrels incidentally caught in fisheries, by providing comprehensive, yet simplified, sample collection protocols. Adaptation of protocols to the specific needs and capabilities of each country and fishery type might be required. Improvement to guidelines is expected over time as feedback is received from field users.

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

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. 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.

A thorough list of potential analysis and information that can be obtained from freshly dead (by-caught, stranded, dead at colonies) birds for health (and other) studies is provided in the table below.

Table 1. Diagnostic analysis and information potentially obtainable from samples recovered from dead (by-caught, stranded, dead at colonies) birds.

Sample	Target analysis and outcome information
Whole carcass	Complete necropsy, multiple analysis. May include cause of death determination.
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 or other 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 (must collect within minutes of death)
Skin and skin lesions	-pathogen screening (i.e. poxvirus) -pathology (histology) -genetics (sexing, species identification, others)
Ecto and 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 (visual, stable isotopes) -toxicology (biotoxins, others) -marine debris ingestion -parasites
Stomach content (oil)	-fatty acids (indirect marker of diet during long foraging trips) -toxicology (biotoxins, microplastics) -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, microplastics) -pathogens (viruses, bacteria, fungi, parasites) -genetics (sexing, species identification, geographical origin/migration, phenotypic variation)
Preen gland oil	-microplastics (and/or plastic derived chemicals, ie. phthalates)
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, microplastics)
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.)

Protocols for sample collection and storage

The protocols provided in this guideline (Table 2) were 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. 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. Researchers must define the objective of sample collection, and therefore prioritize types of samples to be collected and stored, adapting the data sheets accordingly.

Of note, and particularly for on-board sample collection by OBO, the reality of OBOP suggests that they struggle as is. Therefore, assigning OBO additional tasks, such as collecting samples from drowned birds, might be challenging. 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. Pilot testing of protocols suggested that 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 personal role in this process, and did not foresee difficulties in implementation should the time and mechanisms be allotted by their programs. All protocols were considered to be simple enough for completion on board; notwithstanding, most OBO preferred the option of collecting full carcasses on board to be later processed by specialized teams on land.

The protocols presented offer three options: a) basic protocol: bird processing at sea (with or without access to cold chain) and b) advanced protocol: bird processing on land. The approach chosen shall respond to the specific characteristics of target fisheries, duration of fishing trips, on board conditions, on-board observer capacity (time, training, engagement), and storage capacity (i.e. access to freezer) (Figure 1). These protocols can also be implemented to obtain samples from birds found dead at colonies or beach strandings, with the caveats expressed above.

Figure 1. 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)

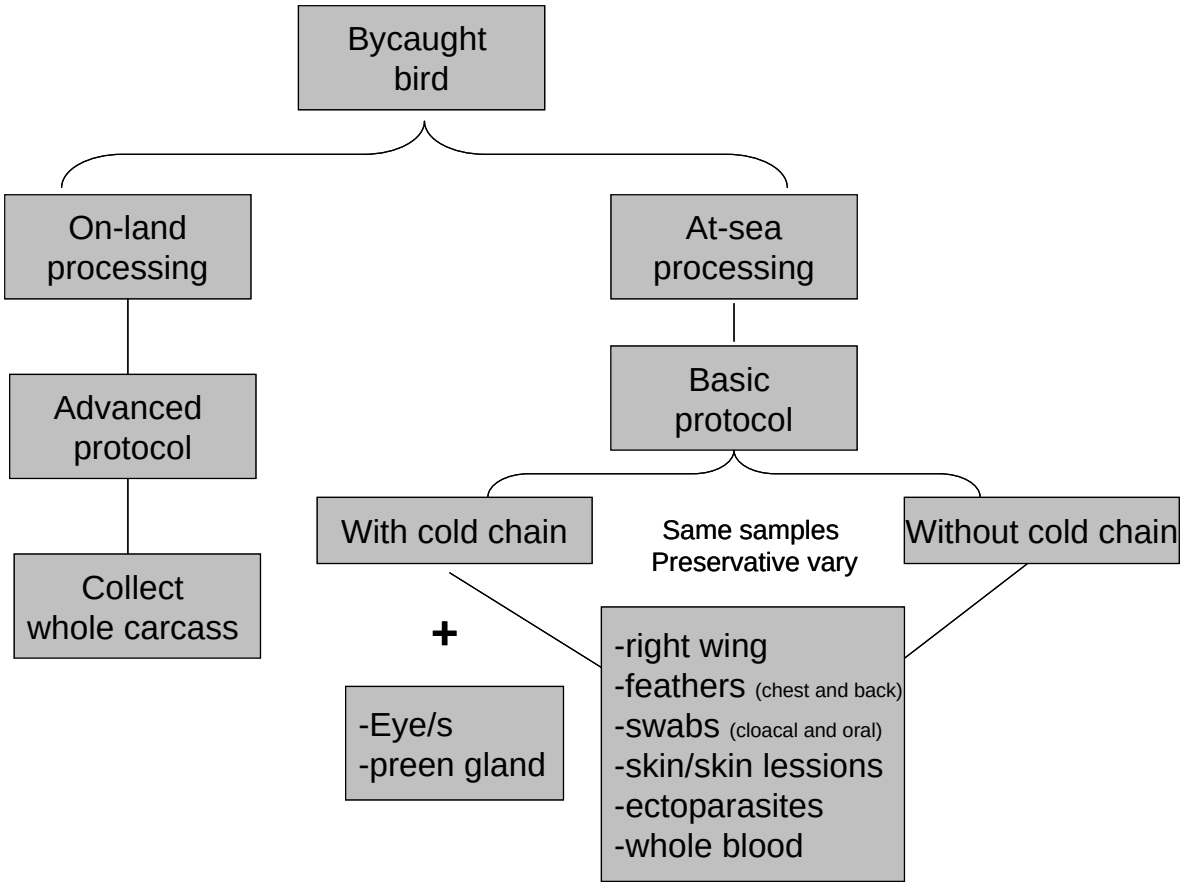


Table 2. Sample collection protocols

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). A glossary on storage temperatures and preservatives is provided below this table.

A.1. BASIC PROTOCOL AT-SEA WITHOUT ACCESS TO COLD CHAIN				
Sample	**Analysis**	**Supplies needed**	**On-board Storage**	**Laboratory & long-term storage**
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 (optional, helps prevent rotting of tissue at 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)	-if dry, 5 paper envelopes or ziploc bags with 10 feathers from each location (chest/back) -if wet, plastic vial + ethanol	- air-dried and stored at room temperature - if wet, store in ethanol at room temperature	-room temperature (dry feathers in envelopes or 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, 2 of each: -2ml cryovial + RNAlater -polyester tipped swabs	-room temperature *(ideally no longer than 1 week, then freeze)*	-frozen, ideally ultra-freezer

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 forceps	-room temperature *(ideally no longer 1 week for RNAlater)* -samples in ethanol always at room temperature	-RNAlater frozen, ideally ultra-freezer -ethanol at room temperature
Ectoparasites	-parasitology, vector-borne diseases (eg. Rickettsia)	-plastic vial (can be cryovial) + ethanol -forceps	-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 ziploc bags or paper envelopes*	-room temperature *(ideally no longer than 1 week, then freeze)* -samples in ethanol always at room temperature	-frozen, ideally ultra-freezer *(except samples in ethanol always at room temperature)*

A.2. BASIC PROTOCOL AT-SEA WITH ACCESS TO COLD CHAIN (ON-BOARD FREEZER)

Sample	Analysis	Supplies needed	On-board Storage	Laboratory & long-term storage
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	-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)	-if dry, 5 paper envelopes or ziploc bags with 10 feathers from each location (chest/back) -if wet, plastic vial + ethanol	- air-dried and stored 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 cryovials no preservative -polyester tipped swabs	-frozen	-frozen, ideally ultra-freezer
Skin and skin lesions (i.e. poxvirus)	-pathology, pathogen screening by PCR -genetics (sexing, others)	-2 ml cryovial + RNA later -scissors and forceps	-frozen	-frozen, ideally ultra-freezer
Ectoparasites	-parasitology, vector-borne diseases (eg. Rickettsial)	-plastic vial + ethanol -forceps	-room temperature	-room temperature
Whole blood ("touch" blood in cavity or organ with filter paper, or collect from heart or other location with syringe and needle)	-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 ziploc bags or paper envelopes*	-frozen -samples in ethanol always at room temperature	-frozen, ideally ultra-freezer (*except samples in ethanol, always room temperature*)
Eye/s	-biochemistry (plasma biochemistries, nutritional condition, pathology, time of death) -serology (antibodies)	-whirlpack or ziploc bag, one or both eyes, separately -scissors and forceps	-frozen	-frozen, ideally ultra-freezer

	-pathogens (molecular) -toxicology (biotoxins, POPs, etc.)			
Preen gland	-microplastics exposure	-clean sterilized new scalpel blade and forceps -wrap in clean double aluminum foil *Avoid contact with plastics (gloves, etc). Sterilize utensils*	-frozen	-frozen

B. ADVANCED PROTOCOL ON-LAND

On board, collect whole carcass in a double large garbage bag and keep frozen until arrival at the laboratory

Sample	**Analysis**	**Supplies needed**	**Laboratory & long-term storage**
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.)	-air-dried and then collect in a bag	-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)	- air dry, then place in 5 paper envelopes or ziploc bags with 10 feathers from each location (chest/back)	-air-dried and stored at room temperature. If humid location, add silica gel beads to bags. -if long-term storage, freeze

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	-ultra-freezer (UTM, no preservative) -RNAlater can be frozen
Skin lesions	-pathology -pathogen screening by PCR	-2ml or lager vials + 10% formalin -2ml cryovials + UTM -2ml cryovial + RNAlater -2ml cryovials no preservative -scissors and forceps	-room temperature (formalin) -ultra-freezer (UTM, no preservative) -RNAlater can be frozen
Ectoparasites	-parasitology, vector-borne diseases (eg. Rickettsial)	-plastic vial + ethanol -forceps	-room temperature
Whole blood ("touch" blood in cavity or organ with filter paper, or collect from heart or other location with syringe and needle)	-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) + UTM -cryovial (2ml) + ethanol -FTA or 903 cards -Nobuto filter paper -Whatman filter paper *Store filter papers individually in ziploc bags or paper envelopes*	-frozen, ideally ultra-freezer -filter paper can be room temp if short-term storage -samples in ethanol always room temperature
Eye/s	-biochemistry (plasma biochemistries, nutritional condition, pathology, time of death) -serology (antibodies) -pathogens (molecular) -toxicology (biotoxins, POPs, etc.)	-whirlpack or ziploc bag, one or both eyes, separately -scissors and forceps	-frozen, ideally ultra-freezer

Preen gland	-microplastics exposure	-clean sterilized new scalpel blade and forceps -wrap in clean double aluminum foil *Avoid contact with plastics (gloves, etc). Sterilize utensils*	-frozen
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) -toxicology (heavy metals, POPs, biotoxins, microplastics) -pathogens (viruses, bacteria, fungi, parasites) -genetics (sexing, others)	*Histopathlogy*: -plastic jar + 10% formalin (formalin:tissue 10:1). *All samples in same jar.* Individual samples in: -2 or 5ml cryovial + RNAlater -2 or 5ml cryovial + UTM -2 or 5ml cryovial + ethanol *Pathogens*: -whirlpack bags no preservative *Toxicology*: -sterilized new scalpel blade, clean forceps, wrap in cleaned double aluminum foil*. For microplastics avoid all contact with plastics (gloves, etc). Sterilize utensils* -complete necropsy equipment	-room temperature (formalin and ethanol) -all others frozen, ideally ultra-freezer

Subcutaneous adipose tissue and body fat (heart, kidney)	-fatty acids (indirect marker of diet during long foraging trips, feeding area) -toxicology (heavy metals, POPs, microplastics)	-wrap in clean double aluminum foil (x2) -sterilized new scalpel blade, clean forceps, wrap in clean double aluminum foil. A*void all contact with plastics (gloves, etc). Sterilize utensils*	-frozen or ultra-freezer
Bone	-toxicology (heavy metals, POPs) -minerals (Ca, P, etc.)	-wrap in cleaned double aluminum foil	-frozen or ultra-freezer
Stomach content (solids)	-main prey, recent diet -toxicology (biotoxins, microplastics) -marine debris ingestion -parasites	*Diet and marine debris ingestion:* -whirlpack or ziploc bags *Parasites and diet (isotopes):* -large plastic vial + ethanol *Toxicology*: -microplastics: sterilized glass vial, place aluminum foil under cap -biotoxins: 2 or 5ml cryovial *For microplastics sterilize utensils and avoid all contact with plastics (gloves, etc)*	-frozen, ideally ultra-freezer -room temperature (ethanol)

Stomach content (oil)	-fatty acids (indirect marker of diet during long foraging trips) -toxicology (biotoxins, microplastics)	*Toxicology*: -microplastics and fatty acids: sterilized glass vial, place aluminum foil under cap (x2) -biotoxins: 2 or 5ml cryovial *For microplastics avoid all contact with plastics (gloves, etc). Sterilize utensils*	-frozen or ultra-freezer
Feces	-microplastics exposure -endoparasites -pathogens (molecular: viruses, bacteria, fungi, parasites)	*Microplastics:* -sterilized glass vial, place aluminum foil under cap *Pathogens*: -2ml cryovial + RNAlater -2ml cryovial + UTM *Endoparasites:* -plastic vial + 5% formalin	-RNAlater can be frozen, UTM ultra-freezer -microplastics frozen -endoparasites: room temperature
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 ultra-freezer
Internal parasites	-parasite identification	-plastic vial + 5% formalin	-room temperature

GLOSSARY

Storage:

Room temperature: no refrigeration. Normally between 10 and 20°C

Frozen: domestic freezer, -20°C approx.

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
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.
***Caution**: Formalin is 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 viral specimens. Can be stored at room temperature before 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/Nobuto): Filter papers are widely used for blood preservation to detect pathogen or host 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, yet have a longer life if frozen.
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
https://www.coleparmer.com/i/advantec-800700-nobuto-blood-filter-strip-100-pk/0664440

Recommendations on how to clean and sterilize utensils and materials for plastic exposure surveys
Properly cleaning glass vials, aluminum foil and re-usable utensils such as spatulas and forceps prior to sample collection and storage is essential. Because cleaning procedures require use of solvents and heating to high temperatures, consider contacting a local lab for help or resort to collaborators who may provide you with pre-cleaned materials and kits for the field. All glassware and re-usable utensils should be washed thoroughly with distilled water and a brush. Rinse several times. Then, wash with an organic solvent (such as dichloromethane, Merck Suprasolv) three times and heat to 450 °C overnight to remove any traces of organic material. Aluminum foil should be heated to 450 °C overnight (adapted from Hardesty et al 2015*).
*Hardesty, B. D., Holdsworth, D., Revill, A. T., & Wilcox, C. 2015a. A biochemical approach for identifying plastics exposure in live wildlife. *Methods in Ecology and Evolution*, *6*(1), 92-98.

Datasheets for sample collection from bycaught birds

This general data sheet must **always be completed**, regardless of the site where the samples are ultimately collected. The ID assigned to the bird in this sheet will accompany all samples collected from this individual.

Bycatch data

Fishing vessel

Vessel position Lat Long

Date month day year

Describe fishing gear: (# 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

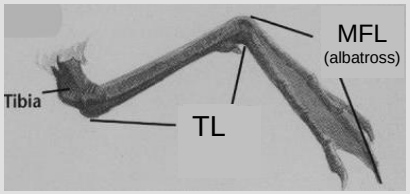
Morphometrics

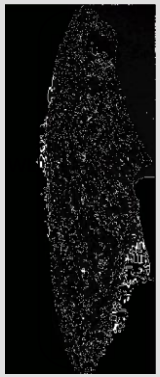
WL= BD=

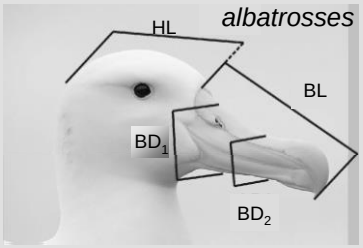
TK= $BD_{1,2(albatross)}$=

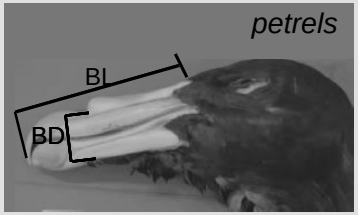
BL= $HL_{(albatross)}$=

Weight= $MFL_{(albatross)}$=









Keel angle (draw)=

Glossary: **WL**= wing length, **TL**= tarsus length, **BL**= bill length, **BD**= bill depth, **HL**= head length, **MLF**= middle finger length (with nail)

*Keel angle: 1° Use 25 cm flexible wire. 2° Press over chest. 3° Use wire to draw chest silhouette on data sheet.

This datasheet should be used as a guideline for **samples collected on board** a fishing vessel **WITH freezer capacity**. If the whole carcass is collected, fill in the first line only. If the bird is sampled, then follow the sheet and proceed with sample collection.

On-board sample collection

WITH COLD CHAIN (freezer)

Animal identification code:

Sample	Store frozen			Store at room temperature
	No preservative	**RNAlater (vial)**	**Filter paper**	**Ethanol (vial)**
Whole carcass *(if collected don't fill rest of sheet)*	Double or triple bag ☐			
Right wing	bag ☐			
Chest feathers	Ziploc or envelope ☐			
Back feathers	Ziploc or envelope ☐			
Cloacal swab	**2°** ☐	**1°** ☐		
Oral swab	**2°** ☐	**1°** ☐		
Ectoparasites				☐
Eye/s (one or both eyes separately)	Ziploc R ☐ L ☐			
Preen gland *	Double aluminum foil ☐			
Skin/skin lesions	**3°** ☐	**1°** ☐		**2°** ☐
Whole blood (circle type of filter paper used)		**1°** ☐	**3º** 903 / FTA /whatman/Nobuto Number ☐	**2°** ☐

PRIORITY: recommended priority order: 1°, 2°, 3°. In boxes, either "tick-off" as collected, or write in number of samples collected. (ie. 2 vials). *use clean sterilized new scalpel blade and forceps, and AVOID all contact with plastics during sampling.

Observations:

This datasheet should be used as a guideline for **samples collected on board** a fishing vessel **WITHOUT** freezer capacity.

On-board sample collection

NO COLD CHAIN – ALL SAMPLES STORED AT ROOM TEMPERATURE

Animal identification code:

Sample	No preservative	Vial RNAlater*	Vial Ethanol	Filter paper
Right wing (should be dry)	bag: ☐			
Chest feathers	air dry** then envelope ☐		if wet ☐	
Back feathers	air dry** then envelope ☐		If wet ☐	
Cloacal swab (x 2)		☐		
Oral swab (x 2)		☐		
Ectoparasites			☐	
Skin/skin lesions		**1°** ☐	**2°** ☐	
Whole blood (circle type of filter paper used)		**1°** ☐	**2°** ☐	**3°** 903 / FTA / whatman Number ☐

PRIORITY: recommended priority order: 1°, 2°, 3°. In boxes, either "tick-off" as collected, or write in number of samples collected. (ie. 2 vials). *if possible, RNAlater samples should be frozen after 1 week at room temperature. ** air-dry in warm room on ship (ie. kitchen).

Observations:

This datasheet should be used as a guideline for **samples collected on land WITH freezer and/or ultra-freezer capacity**. On board, collect whole carcass in double large garbage bags and keep frozen until arrival at the laboratory

On-land sample collection

Animal identification code:

	Store frozen			At room temperature	
Samples	**No preservative**	**RNAlater**	**UTM (*ultrafreezer*)**	**Formalin 5-10%**	**Ethanol**
Right wing (should be dry)	bag ☐				
Chest feathers	Ziploc or envelope ☐ Number				
Back feathers	Ziploc or envelope ☐ Number				
Cloacal swab	**3º** ☐	**2º** ☐	**1º** ☐		
Oral swab	**3º** ☐	**2º** ☐	**1º** ☐		
Ectoparasites					☐
Skin/skin lesions	**4º** ☐	**2º** ☐	**1º** ☐	**3º** 10% ☐	
Whole blood (circle type of filter paper used)	**4º** 903 / FTA / whatman /Nobuto Number ☐	**3º** ☐	**1º** ☐		**2º** ☐
Eye/s (one or both eyes separately)	Ziploc or Whirlpack R ☐ L ☐				
Preen gland (avoid contact with gloves)	Double aluminum foil ☐				
Gonads				10% ☐	

Continue in Sheet 3

Organ tissues (detail all tissues collected. Each organ separately, except when placed in formalin). *Always collect full set of tissues in formalin (tissue:formalin ration 1:10) and a full set frozen. Priority organs to sample: liver, spleen, kidney.*	**1º** Whirlpack (pathogens) ☐ Aluminum foil (toxicology) ☐	**3º** ☐	**2º** ☐	10% (all samples same jar) ☐	**4º** ☐
Subcutaneous adipose tissue / Body fat (circle sample collected)	Aluminum foil (X2) ☐				
Bone	Aluminum foil ☐				
Stomach content (solids)	**1º** Whirlpack/Ziploc (X2) ☐ Glass vial ☐ Cryovial (X2) ☐				**2º** ☐
Stomach content (oil)	Cryovial (X2) ☐ Glass vial (X2) ☐				
Feces	**2º** Glass vial ☐	**3º** ☐	**1º** ☐	**4º** 5% ☐	
Cerebrospinal fluid	Cryovial ☐				
Internal parasites				5% ☐	

PRIORITY: recommended priority order: 1°, 2°, 3°, 4º. In boxes, either "tick-off" as collected, or write in number of samples collected. (ie. 2 vials). For microplastics use clean sterilized new scalpel blade and forceps, AVOID all contact with plastics during sampling, and store in glass vial with aluminum foil under plastic cap or wrap in double aluminum foil.

Observations: