Agreement on the Conservation of Albatrosses and Petrels

Eleventh Meeting of the Advisory Committee

Florianópolis, Brazil, 13 – 17 May 2019

Project ACAP 2018-02

Prevalence and magnitude of plastic exposure (macro and microplastics and select chemical compounds) in albatrosses and petrels off the shores of Argentina and Brazil

Secretariat

SMALL GRANTS PROJECT PROGRESS REPORT TO THE ADVISORY COMMITTEE

Project No: ACAP 2018-02
Project Title: Prevalence and magnitude of plastic exposure (macro and microplastics and select chemical compounds) in albatrosses and petrels off the shores of Argentina and Brazil

Project initiated by: *Brazil*

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Summary of project activities (max 300 words)

Objective 1: Evaluate incidence and magnitude of plastic ingestion in dead birds

June-July 2018: PIs coordinated collection, submission and sampling of bird carcasses with partners.

July-November 2018: Updated and refined sampling protocols and datasheets for sample collection. These followed a revised version of ACAP protocol PaCSWG4 Doc 09 and were discussed and agreed upon by all project partners.

August 2018-present: Birds were necropsied and samples including gastrointestinal tract, preen gland, liver and breast feathers were collected and stored appropriately, both in Brazil and Argentina. Morphometric measurements and photos were also taken. Environmental control samples were collected due to the ubiquitous nature of plasticizers in the environment.
A full suite of supplementary tissues (ie. spleen, brain, eye, whole blood, kidney, bone, tracheal and cloacal swabs) were collected and adequately stored for complementary and future studies (toxicology, infectious diseases, etc), both in Brazil and Argentina.

Objective 2: Macroscopically estimate the prevalence of plastic ingestion and characterize items present in the gut

August-November 2018: Prepared protocols for gastrointestinal macroplastics recovery and classification, specifically for this study.

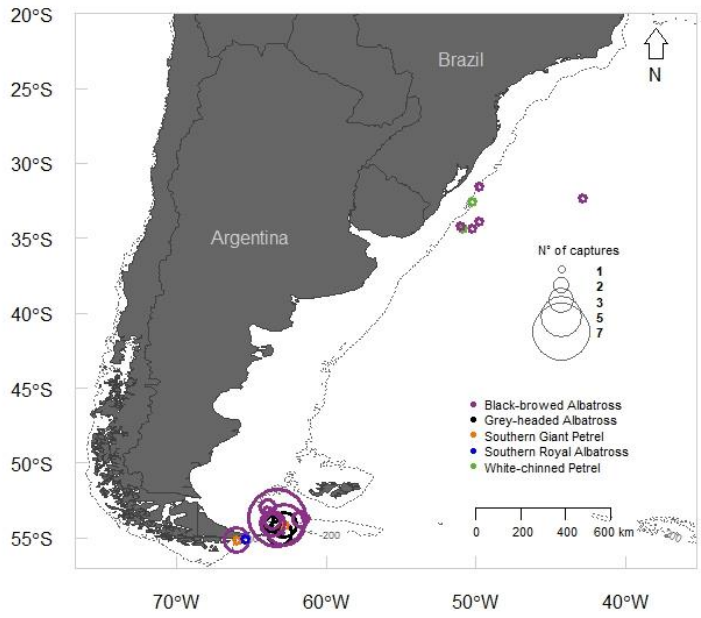
Objective 3: Establish diagnostic capacity for select chemical compounds derived from plastic degradation (phthalates) in Argentina and Brazil. Objective 4) Perform chemical analysis to identify and quantify phthalates in select tissues from dead birds.

July 2018-present: In Argentina, we are validating laboratory methods developed by Hardesty et al. (2015) for identifying and quantifying plasticizers in samples from dead birds, capacity that will be transferred to a selected lab in Brazil later this year. All necessary supplies, including chemical standards for processing samples in both countries, were purchased.

Project outcomes (if any accomplished to date, detailed by objective) (max 300 words)

Objective 1: We developed detailed and simplified protocols for sample collection (see Appendix II and III).

We accessed 50 birds caught by fisheries operating off southern Brazil and Argentina (Figure below) between 2015 and 2017. The birds were provided by the on-board observer programs of the Albatross Task Force in Argentina, and Projeto Albatroz in Brazil. Bycaught birds included 32 Black-browed albatrosses (*Thalassarche melanophris*), two Atlantic Yellow-nosed albatrosses (*T. chlororhynchos*), five White-chinned petrels (*Procellaria aequinoctialis*), two Southern Giant Petrels (*Macronectes giganteus*), one Southern royal albatross (*Diomedea epomophora*). Sex proportion was similar (46% males and 44% females, 10% to be determined), and 82% were adults.



In addition, samples were collected from beach cast bird carcasses recovered in the states of São Paulo, Paraná and Santa Catarina in Brazil and Chubut Province in Argentina. In Brazil, carcasses were provided by Projeto de Monitoramento de Praias da Bacia de Santos and processed by R3 Animal and CEMAVE / ICMBio team (data is being collated), whereas in Argentina 3 carcasses (all juveniles, 2 males 1 female) were recovered opportunistically.

Objective 2: We developed detailed protocols for gastrointestinal macroplastics recovery and classification (see Appendix IV) with advice from J.A. Van Franeker (Wageningen University & Research, Netherlands).

Objectives 3 and 4: For phthalate analysis in Argentina we selected the Laboratorio Centralizado de Química Orgánica y Cromatografía (CCT CENPAT) in Puerto Madryn. We

are in the process of validating methods by Hardesty et al. (2015).

Pending outcomes:

a) Examine the collected digestive tracts according to the protocols previously described.
b) Run chemical analysis for phthalates in preen gland and livers in Argentina
c) Transfer techniques for chemical analysis to selected laboratory in Brazil (Universidade Federal de Santa Catarina in Florianópolis, SC, Brazil).

Were the funds spent in accordance with the original budget? Please provide an acquittal of the funds spent to date (max 100 words)

Total grant budget: AUD 20,000 Year 1: AUD 5,025. Total spent to date: AUD 1,820

Detailed expenditures are provided as total spent per category/total budgeted
Equipment, supplies, and materials for field and sample collection and storage AUD 400/400
Technical services for initial sample processing and laboratory test setup AUD 750/1475
Courier for sample shipment AUD 120/550
Travel for necropsies, sample collection and training within Argentina AUD 500/2400
Communications AUD 50/200

Were there any unforeseen difficulties with the project? (max 300 words)

Logistics for accessing by-caught birds in Argentina is challenging since they need to be transported either by boat or air out of Tierra del Fuego (terrestrial transport is excluded because of passage through Chile). Through collaborations we managed to get the recovered specimens shipped to a central location for sample collection, from where they need to be again transferred to the laboratory. This implies extensive paperwork and logistics coordination, which is possible but not simple. For future banking of specimens and/or samples a more simplified mechanism needs to be developed to expedite the process.

The process for banking bycaught birds is more streamlined in Brazil. Yet in Brazil beach cast carcasses are shared with other pre-established projects, which requires careful coordination for accessing specific samples for this study.

Have you identified any questions or issues that need to be addressed further? (max 300 words)

Plasticizers (phthalates esthers) are a diverse group of chemicals (25+ compounds) that are used by the plastic industry. When absorbed, these compounds are potentially toxic and are known to induce a broad variety of chronic and sub-lethal toxic effects, including endocrine dysfunction, immune response disruption, mutagenesis and carcinogenesis (Finkelstein et al. 2007; Teuten et al. 2009; Hirai et al. 2011). Studies on leaching chemical compounds and chronic and sub-lethal effects from ingestion of microplastics in seabirds, and particularly long-lived species like albatrosses and petrels, are lacking. Continued access to beach cast and bycaught birds, as well as those entering rehabilitation, could greatly contribute to advance pending research.

Appendix I. List of references.

Cadogan, D.F., Howick, C.J., 2012. Plasticizers, in: Ullmann's Encyclopedia of Industrial Chemistry. Springer, Weinheim, pp. 599–618.

Finkelstein, M.E., Grasman, K.A., Croll, D.A., Tershy, B.R., Keitt, B.S., Jarman, W.M., Smith, D.R., 2007. Contaminant-associated alteration of immune function in black-footed albatross (*Phoebastria nigripes*), a North Pacific predator. Environmental Toxicology and Chemistry: An International Journal 26, 1896–1903.

Hardesty, B.D., Holdsworth, D., Revill, A.T., Wilcox, C., 2015. A biochemical approach for identifying plastics exposure in live wildlife. Methods in Ecology and Evolution 6, 92–98.

Hirai, H., Takada, H., Ogata, Y., Yamashita, R., Mizukawa, K., Saha, M., Kwan, C., Moore, C., Gray, H., Laursen, D., 2011. Organic micropollutants in marine plastics debris from the open ocean and remote and urban beaches. Marine Pollution Bulletin 62, 1683–1692.

Teuten, E.L., Saquing, J.M., Knappe, D.R., Barlaz, M.A., Jonsson, S., Björn, A., Rowland, S.J., Thompson, R.C., Galloway, T.S., Yamashita, R., 2009. Transport and release of chemicals from plastics to the environment and to wildlife. Philosophical Transactions of the Royal Society of London B: Biological Sciences 364, 2027–2045.

Appendix II. Protocols for sampling collection and storage to assess plastic exposure (macro and microplastics and select chemical compounds) in ACAP species.

SAMPLE COLLECTION AND STORAGE

When sampling, avoid contact with plastics, latex, etc. (gloves, bags, vials, syringes, others). To avoid contamination, wear nitrile gloves for sample collection.

Sampling can be performed on recently dead animals or on carcasses stored frozen and then thawed.

Data recording:

Use the datasheet provided to record information about animals sampled and origin of carcass.

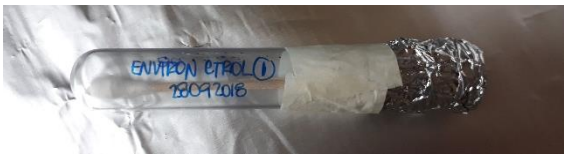
***Bird sampling*:**

During the necropsy collect:

1) **gastrointestinal (GI) tract** from the esophagus to the first portion of the duodenum. Tie a knot with cotton string at each end to avoid leaks.
2) Whole **preen gland (PG).** Avoid squeezing the gland while handling.
3) at least half the **liver (LIVER).**
4) **breast feathers** (2-5). Pluck feathers, do not cut.

Environmental control:
Wave a swab (cotton tip, wooden handle) in the air without it touching anything (do this for about a minute). Place the swab in a glass vial, cover with a lid of aluminum foil, seal it with paper tape, and label appropriately (see picture A below). Do this for 1-3 tubes, depending on the duration of the sampling session.

Picture A. Environmental control.



Storage:

- ✓ Place gastrointestinal tract in a ziploc bag, label and store at -20°C.
- ✓ Wrap preen gland and liver, separately, in a double layer of aluminum foil, label and store at -20°C
- ✓ Place feathers (air-dried) in paper envelope, label and store at room temperature.
- ✓ Place cotton-tip swab in a glass vial with a lid of aluminum foil, seal it with paper tape, label and store at -20ºC.

Cleaning sample collection:
Before necropsies:

- glass vials and reusable utensils (eg. tweezers, scissors, scalpel) should be washed thoroughly with distilled water and a brush. Then wash with solvents (3 times each):

1st methanol or acetone, 2nd dichloromethane (DCM), 3rd hexane. Alternatively, replace washes with solvents by heating the material to 450 °C for 6 hs.

- Aluminum foil should be heated to 450 °C for 6 hs.

During necropsies

- To avoid contamination between individuals use new scalpel blade, and reusable utensils should be washed thoroughly with running water and detergent. Then distilled water and a brush. Rinse several times.

Recommended sample label: standard bird identifier (species initials, can use common or scientific name) – number of sample (sequential for the same species). Date of sampling (ddmmyyyy) - type of sample (i.e. GI tract, liver, preen gland –use initials). Examples: BBA01 - 02042019 PG. Which stands for: Black browed albatross, sample no 1 for this species, from 2nd April 2019, Preen gland. BBA01 - 02042019 LIVER. Which stands for: Black browed albatross, sample no 1 for this species, from 2nd April 2019, Liver.

Appendix III. Datasheet for plastic exposure (macro and microplastics and select chemical compounds) in ACAP species

Collection data

Person who performed necropsy | **Date** month / day / year | **Site** Lat / Long | **Colony / fishing vessel**

Animal Identification code= Species initials (common or scientific name) – sample # (sequential for same sp) | Ring | **Sex:** male ☐ female ☐ UNK ☐ | **Age class:** adult ☐ juvenile ☐ chick ☐

BY-CAUGHT ☐ **DEAD AT COLONY** ☐ **BEACH STRANDING** ☐

Condition of carcass:
- ☐ Fresh
- ☐ Moderate descomposition
- ☐ Advanced descomposition
- ☐ Mummified

Oil:
- ☐ None
- ☐ Slightly (2-33% of body)
- ☐ Moderate (34-66% of body)
- ☐ Heavily (67-100% of body)

Photos: (taken with ID code)
- ☐ Head
- ☐ Back
- ☐ Chest
- Body fat*: ☐ photo#1 ☐ photo#2

Evidence of

	Yes	No
	☐	☐

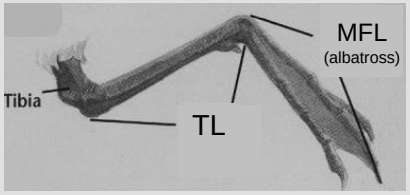
Morphometrics

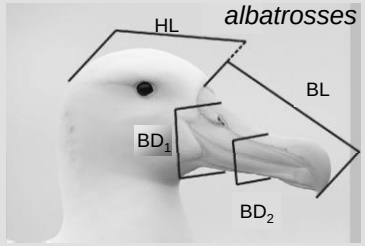
WL= BD=

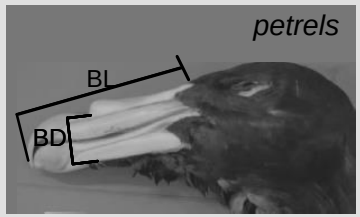
TL= $BD_{1,2(albatross)}$=

BL= $HL_{(albatross)}$=

Weight= $MFL_{(albatross)}$=







Keel angle **(draw)=

* **photo #1** after opening the skin, showing the chest muscles and subcutaneous tissue, and **photo #2** after opening the body cavity, showing the presence of abdominal visceral fat and cardiac fat.

**Keel angle: 1° Use 25 cm flexible wire, 2° Press over chest (before necropsy), 3° Use wire to draw chest silhouette (below wire) on data sheet. Use back of datasheet if necessary.

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

Animal identification code:

Sample	FROZEN -20°C			ROOM TEMPERATURE	Sample label
	Double aluminum foil	Ziploc	Glass vial	Paper envelope	Example: BBA01 - 02042019 PG Which stands for: Black browed albatross, sample no 1 for this species, from 2nd April 2019, Preen gland.
Preen gland	☐				
Liver	☐				
Gastrointestinal tract		☐			
Breast feathers (2-5 air-dried)				☐	
Environmental control (1-3 per session)			☐		

Note: Before necropsies, glass vials and reusable utensils (eg. tweezers, scissors, scalpel) should be washed thoroughly with distilled water and a brush. Then, wash with solvents (3 times each): 1st methanol or acetone, 2nd dichloromethane, 3rd hexane. Alternatively, replace washes with solvents by heating the material to 450 °C for 6hs . Aluminum foil should be heated to 450 °C for 6hs. To avoid contamination between individuals during necropsies use new scalpel blade, and reusable utensils should be washed thoroughly with running water and detergent. Then distilled water ad a brush. Rinse several times.

Observations:

Appendix IV. GI MACROPLASTICS COLLECTION AND CLASSIFICATION

We will follow Van Franeker et al. (2011) with modifications by Colabuono et al. (2009) and Jimenez et al. (2015).

1) GI tract macroplastics collection.

- ✓ Remove the frozen digestive tract from the freezer. Cut and separate the different sections, namely the esophagus, proventriculus and ventriculus.
- ✓ Put each section in individual metal or plastic trays and leave at room temperature for 2 hours, until thawed.
- ✓ Then, focus on one tray at a time.
- ✓ Place the contents from each GI section in a sieve with a 1 mm mesh. Rinse thoroughly with cold running water to remove mucus from the digestive walls and digested soft food components. If sticky substances hamper further processing of the litter objects, use hot running water and detergents (as needed) to clean them. (Note: if food items are found they can be stored in ziploc bags and frozen at -20C for diet studies).
- ✓ After rinsing, transfer remaining elements of the stomach contents (debris caught in the sieve) to a petri-dish.
- ✓ Allow debris items in petri-dishes to air-dry (24-48hs), cover, and store at room temperature. Next step will be to classify items under a binocular microscope (it can be done another day).

2) Visual analysis and classification of stomach contents:

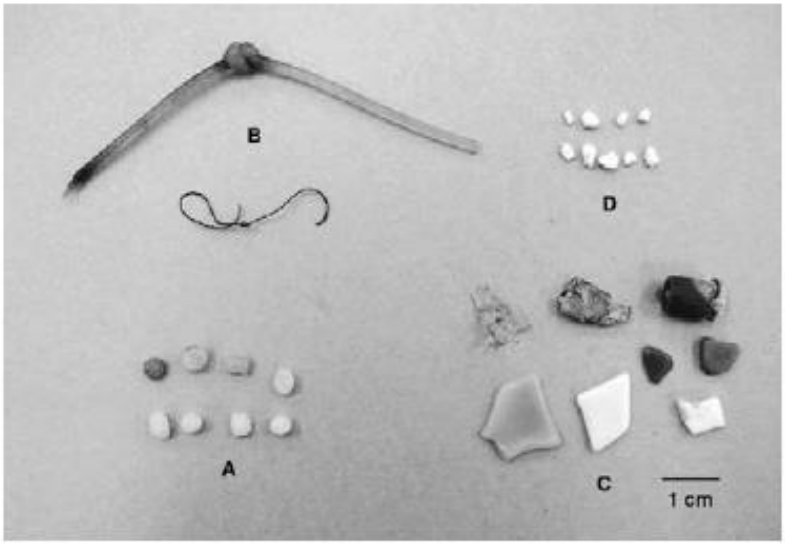
- ✓ Examine all debris items found in each part of the digestive tract and stored in the petri dishes.
- ✓ Categorize and then store items separately (by type, not individually) in paper envelopes:

Categories include (see pictures B and C below):

1) **plastic fragments** (rigid pieces of larger objects or pieces of plastic bags and packaging);
2) **plastic pellets** (which have either polyethylene or polypropylene as the raw material in the form of small spheres or cylinders);
3) **nylon line**;
4) **polystyrene** and/or polyurethane
5) rubbish other than plastic (aluminum foil, paper, wood, pieces of metals, fish hook, and other).

- ✓ After sorting by type of item, record the precise number of items and their combined mass for each category of plastic. Weights should be recorded using electronic weighing scales to an accuracy of 0.0001.

Picture B. From Colabuono et al. 2009. Plastics categories found in the digestive tract of Procellariifomes. The plastic **pellets (A)** were found in the ventriculus of Great shearwater. The plastic line used in longline fisheries, the **nylon (B)** and the hard and flexible **plastic fragments (C)** were found in the digestive tract of beached Great shearwater and Antarctic fulmar specimens. The **polystyrene (D)** were found in the ventriculus of Black-browed albatross.



Picture C. From Van Franeker et al. 2011 Suppl mat (with modifications). An extreme accumulation of plastics in the stomach content of a single bird.