Agreement on the Conservation of Albatrosses and Petrels

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Guidelines to assess potential impacts of plastic and microplastic in ACAP species

Marcela Uhart, Patricia Pereira Serafini, Luciana Gallo, Britta Denise Hardesty and Barbara Wienecke

SUMMARY

During AC9, the PCSWG noted the widespread intrusion of both macro and microplastic in the diet and environment of seabirds, and expressed concern about forecasts that this will increase. This was later reflected in AC9 Final Report, which stated "the need to encourage research assessing the exposure to, and incidence and impacts of plastics and microplastics in the marine environment on ACAP species". Considering that several marine plastic and microplastic initiatives are underway, the PCSWG agreed that ACAP could contribute to this topic through various actions. One such action is the production of guidelines to assess the incidence of plastics exposure in ACAP species. Thus, this paper presents draft protocols for surveying live and dead ACAP species, with an array of sample type choices that should facilitate collection in diverse settings (i.e. freshly dead stranded and by-caught specimens, live and dead animals at nesting sites, fresh scats and regurgitated boluses from nests).

RECOMMENDATIONS

To the extent possible, we recommend collecting samples to assess plastic and microplastic exposure in ACAP species, whenever an opportunity presents.

1. Collect stomach contents (dead birds or from regurgitates) to visually classify plastic fragments and assess ingestion.
2. Collect tissues from dead animals (i.e. liver, adipose) and/or body fluids from live and/or dead animals (i.e. preen oil) to assess what is absorbed by the bird and then deposited in body tissues.
3. Collect feces and/or regurgitated boluses for a non-invasive assessment of exposure.

RESUMEN

Durante AC9, el PCSWG destacó la amplia presencia de macro y microplásticos en la dieta y el ambiente de las aves marinas, y expresó su preocupación ante los pronósticos de que ello empeorará en el futuro. Esto fue luego reflejado en el Reporte Final AC9, que resaltó “la necesidad de incentivar investigación para evaluar la exposición, incidencia e impactos de plásticos y microplásticos en el ambiente marino para especies ACAP”. Considerando que existen varias iniciativas sobre plásticos marinos en marcha, el PCSWG acordó que ACAP podría contribuir a este tema mediante varias acciones. Una de ellas es la producción de lineamientos para evaluar la incidencia de exposición a plásticos en especies ACAP. De este modo, aquí se presentan protocolos para el monitoreo de plásticos en especímenes vivos y muertos de especies ACAP, con una diversidad de opciones de tipos de muestras posibles para facilitar la colecta en diversas situaciones (por ejemplo, aves recién muertas por by-catch o varamientos frescos, aves vivas y muertas en colonias reproductivas, heces y regurgitados frescos en nidos).

RECOMENDACIONES

En la medida de lo posible, recomendamos colectar muestras para evaluar exposición a plásticos y microplásticos en especies ACAP, siempre que dicha oportunidad se presente.

1. Colectar contenido estomacal (aves muertas o regurgitados) para clasificación visual de fragmentos plásticos y evaluación de ingesta.
2. Colectar tejidos de animales muertos (ej. hígado, tejido adiposo) y/o fluidos corporales de aves vivas o muertas (ej. aceite de glándula uropígea) para evaluar lo que el ave absorbe y luego deposita en sus tejidos.
3. Colectar heces y/o bolos regurgitados para evaluación de exposición a plásticos de manera no invasiva.

1. INTRODUCTION

Our planet is undergoing increasingly disruptive changes as a result of accelerated human activities. Pressures on natural systems are immense: climate change, pollution and emerging infectious diseases are three of the multitude of threats that are dramatically affecting species and ecosystems around the world (Heard et al. 2013). Understanding the impact of concurrent threats on natural populations is crucial for predicting their evolutionary trajectory and extinction risk.

Among the most threatened vertebrates in the world are the albatrosses and petrels in the order Procellariiformes, which includes many species breeding on isolated oceanic islands (Birdlife International 2012). Because they are top predators, seabirds reflect the set of processes that affect their prey at lower trophic levels, and can therefore be considered sentinels of ocean health (Furness 2003, Cardoso et al. 2014). Hence, they can be very useful indicators of altered ecological processes and environmental conditions (Weimerskirch et al. 2003, Parsons et al. 2008, Grimaldi et al. 2014, Phillips et al. 2016).

A significant threat for seabirds is ocean pollution by marine debris; impacts from both macro and microplastics have been reported in several species (Vince & Hardesty 2017, Wilcox et al. 2015). There is a need, however, to understand the consequences of plastics entering the food chain, and of the sub-lethal impacts of ingestion, including endocrine disruption. The Final Report of the Ninth Meeting of the Advisory Committee of the Agreement on the Conservation of Albatrosses and Petrels stated, in item 9.1.3 on *Threats and prioritisation,* "the need to encourage research assessing the exposure to, and incidence and impacts of plastics and microplastics in the marine environment on ACAP species" (AC9 Final Report).

During AC9, the Population and Conservation Status Working Group (PCSWG) noted the widespread intrusion of both macro and microplastic in the diet and environment of seabirds, and expressed concern about forecasts that this will increase. The PCSWG acknowledged that designing research that can conclusively pinpoint impacts of plastics on seabirds remains a major challenge. Considering that marine plastic and microplastic initiatives are underway by others including the Convention on Migratory Species (CMS), the Commission for the Conservation of Antarctic Marine Living Resources (CCAMLR) and the International Maritime Organization (IMO), the PCSWG agreed that ACAP could contribute to this topic through various actions. One such action is the production of guidelines to assess the incidence of plastics exposure in ACAP species. Thus, this paper presents draft protocols for surveying live and dead ACAP species, with an array of sample type choices that should facilitate collection in diverse settings.

2. SAMPLING PROTOCOLS FOR PLASTIC AND MICROPLASTIC EXPOSURE ASSESSMENT IN ACAP SPECIES

Within the intersessional period of May 2016 and July 2017 we explored options for plastics and microplastics assessment protocols in ACAP species, in an effort to identify technically-robust yet field and non-expert friendly alternatives. Because contamination is a very important concern for plastics sampling, we have found simplifying protocols somewhat challenging. This is particularly due to the need to use specialized containers (ie. sterilized glass vials, sterilized metal spatulas) as well as keeping any plastic or latex (from gloves for example) away from the bird sampling area, which may be hard to achieve under field conditions. Notwithstanding, a list of potential options for sampling are described in the following table, including both live and dead birds, as well as non-invasive samples collected from the environment. **Note that the use of properly cleaned utensils and supplies for sample collection and storage is essential for plastics assessment. Should you lack capacity to have properly prepared materials at hand, you should disregard collecting samples for plastics analysis. This applies to all sample types and collection options presented below.**

sample origin	***sample type/specimen***	***type of analysis***	***reference***	***supplies needed***
live bird	preen gland oil content	chemical	*Hardesty et al 2015a*: phtalates in preen gland oil (recent 3-6 months exposure)	*clean sterilized metal spatula *glass vial with aluminum foil under the cap
	Solid stomach content (voluntary regurgitate)	visual	*Carey 2011; Bond and Lavers 2013; Provencher et al 2014*: visual classification of plastic items	*metal tray, forceps/tweezers, rinse bottle with water to separate/ID as plastic, aluminum foil
	Oil stomach content (voluntary regurgitate)	chemical	*Tanaka et al 2015*: leached compounds in stomach oil	*sterilized glass vial, place aluminum foil under cap
dead bird	preen gland	chemical	*Hardesty et al 2015a*: phtalates in preen gland oil (recent 3-6 months exposure)	*clean sterilized new scalpel blade and tweezers/forceps *cleaned double aluminum foil
	stomach content (solid and oil)	visual and chemical	*Tanaka et al 2015*: leached compounds in stomach oil and liver tissue; *Van Franeker et al 2011*: solid stomach contents.	*solids: metal tray, forceps/tweezers, rinse bottle with water to separate/ID as plastic, aluminum foil *oil: sterilized glass vial, place aluminum foil under cap
	feces	chemical (Nile Red stain)	*Maes et al 2017*: rapid screening (detection and quantification) by fluorescent tagging with Nile Red stain	*clean sterilized new scalpel blade and forceps/tweezers *clean sterilized metal spatula *glass vial with aluminum foil under cap
	adipose tissue	chemical	*Fossi et al 2012 and 2014*: phtalates and other derivatives in whale blubber; *Tanaka et al 2013*: compounds in bird abdominal adipose tissue	*clean sterilized new scalpel blade and clean forceps/tweezers *cleaned double aluminum foil
	organs (ie. liver)	chemical	*Tanaka et al 2015*: leached compounds in stomach oil and bird liver tissue	*clean sterilized new scalpel blade and clean forceps/tweezers *cleaned double aluminum foil
environment	regurgitated feed boluses	chemical	*Nilsen et al 2015*: classify types of plastic resin in fragments from chick regurgitated feed-boluses	*clean sterilized forceps/tweezers *cleaned double aluminum foil
	feces	chemical (Nile Red stain)	*Maes et al 2017*: rapid screening (detection and quantification) by fluorescent tagging with Nile Red stain	*clean sterilized metal spatula *glass vial with aluminum foil under the cap

2.1. Dead Birds (By-caught, stranded, dead at colonies)

Sampling can be performed on recently dead animals or on frozen carcasses. Therefore, freshly-dead by-caught birds could be sampled on-board fishing vessels or kept frozen until arrival on land, and then sampled in a lab or other facility. This also applies to beached carcasses and recently dead birds at nesting sites (though access to freezer might be restricted in these situations, favoring in-situ sampling). Sample collection within a controlled setting would reduce contamination risk and enable having all proper sampling utensils and supplies easily at hand. Details on cleaning and sterilizing utensils are provided below in 2.8. IN ALL CASES: When sampling, avoid contact with plastics, latex, etc. (gloves, bags, vials, syringes, others). *Wash hands thoroughly with soap and water prior to after sample collection.*

1. **Dead seabird preen gland sampling:** Hardesty et al (2015) have identified plasticizers in preen-gland oil. Following Hardesty seabird preen gland sampling protocol, to collect the preen gland in deceased birds, simply use a clean sterilized, new scalpel blade to excise the gland (help yourself with clean forceps/tweezers if needed), avoiding all contact with plastics, gloves, etc. Place the gland in double aluminum foil, label and store in freezer. If you use forceps, these should be clean and sterile as well. Under field conditions, note that all materials will be single-use until they can be re-sterilized in order to avoid contamination (see section 2.8 below).
2. **Dead seabird stomach content sampling:** In this case, stomach and/or gastrointestinal contents (oil and solids) can be recovered during necropsy.
    a. Oil should be placed in pre-cleaned and sterilized glass containers with aluminum foil under the plastic lid and then frozen.
    b. Solids should be washed and sieved through a 1 mm mesh following methods in Van Franeker et al. (2011). Recovered solids can be wrapped in cleaned, double aluminum foil and stored frozen until visual (macro and microscopic) analysis.
3. **Dead seabird scat sampling:** Maes et al. (2017) developed a rapid-screening approach to detect and quantify microplastics based on fluorescent tagging with Nile Red stain. In this case, use clean sterilized new scalpel blade and forceps/tweezers to cut the intestine near the cloaca, and then scoop feces with a clean sterilized stainless steel spatula. Place in a glass vial with aluminum foil under the cap and freeze.
4. **Dead seabird adipose tissue sampling:** The abdominal adipose tissue has been used to assess polybrominated diphenyl ethers (PBDEs) exposure in seabirds (Tanaka et al. 2013). After collection with sterilized scalpel, use clean forceps to place tissue in clean, double aluminum foil, and store frozen.
5. **Dead seabird liver tissue sampling:** Tanaka et al (2015) found leached plastics compounds in stomach oil and liver tissue. To collect liver tissue, place the whole organ or dissect a large section with a new sterilized scalpel blade. Use clean forceps to place tissue in clean, double aluminum foil, and store frozen.

Note: *If possible, preen gland, stomach contents, adipose tissue and liver should be collected from the same animal to link detection of plastic in the gastrointestinal system with its presence in preen-gland oil and other tissues.*

2.2. Live Birds

Sampling can be performed opportunistically when handling birds for other purposes, or specifically for plastics and microplastics investigation. Based on field conditions, available time and logistics, a suite of options are available and presented below. Keep in mind that sterilized clean utensils are needed as well as freezer storage capacity, which can be challenging in the field. Details on how to clean and sterilize utensils are provided below in 2.8. IN ALL CASES: when sampling, avoid contact with plastics, latex, etc. (gloves, bags, vials, syringes, others). *Wash hands thoroughly with soap and water prior to after sample collection.*

1. **Live seabird preen gland sampling:** This is a minimally-invasive technique developed by Hardesty et al. (2015), which targets collection of preen-gland oil for biochemical analysis of plasticizers. To collect oil, gently massage the preen gland at the upper base of the tail, giving a gentle squeeze after massaging the gland to express a very modest amount of oil. Carefully remove sterilized stainless steel spatula from glass vial and swab over oil gland to pick up exudate. Return spatula to glass vial without touching any plastic. Make sure clean foil is placed over the top of the vial before screwing on the plastic lid. *Because gloves can't be worn, make sure to wash your hands thoroughly with soap and water prior to after sample collection.*
2. **Live seabird stomach content sampling:** Stomach contents can be collected from adults and large chicks by spontaneous regurgitation for many species. Stomach lavage is highly invasive and not recommended for this purpose only.
    a. Oil should be placed in pre-cleaned and sterilized glass containers with aluminum foil under the plastic lid and then frozen.
    b. Solids should be washed and sieved through a 1 mm mesh following methods in Van Franeker et al. (2011). Recovered solids can be wrapped in cleaned, double aluminum foil and stored frozen until visual (macro and microscopic) analysis.

2.3 Environmental sampling

Fresh scats and regurgitated feed-boluses can be collected to assess plastic and microplastic exposure in seabirds. Collection of these samples from within and near nests is a good option when working at breeding colonies. It is non-invasive and does not require handling birds. Care must be taken, however, to avoid collecting samples from areas with presence of plastic debris and to follow all proper sample collection and storage recommendations to avoid contamination (i.e. sterilize materials, don't touch with gloves, etc.).

1. **Seabird scat environmental sampling**: A fluorescence staining method (such as Nile Red stain used by Maes et al. 2017), in combination with density separation, provides a simple and sensitive approach to highlighting most common polymer fragments in feces of seabirds. Scoop feces with a clean sterilized stainless steel spatula. Place in a pre-cleaned and sterilized glass container with aluminum foil under the cap, and freeze.

2. **Regurgitated feed boluses**: many seabirds regurgitate pellets of indigestible material (boluses) containing both debris and natural food items. Nilsen et al (2014) developed a method to classify plastic debris by their resin components (chemicals characteristic of each type of plastic), that can be applied to small, weathered, and degraded fragments. This complements visual classification of larger, identifiable items. Use clean forceps to place feed-bolus in clean, double aluminum foil, and store frozen.

2.4. Blanks and control samples

Hardesty (seabird preen gland sampling protocol) recommends collecting three environmental blanks in the course of sampling at a site. Their protocol requests that date, location, time, etc. be recorded for these environmental blanks, and labeled BLANK. To run a blank simply open the tube, wave the spatula in the air without it touching anything. Do this for about the same amount of time it takes to sample the preen gland oil from a bird (a minute or so). Replace the spatula in the tube, label appropriately.

In addition, one blank must be kept as a TRANSPORT blank. This will not be opened, but will be run with the other samples to ensure there is no contamination during submission of samples to the laboratory.

2.5. Feathers:

Hardesty (seabird preen gland sampling protocol) recommends collecting 2-5 breast feathers from each animal sampled for plastics exposure for isotope studies. This will allow linking information on plastics exposure with diet/trophic information. Feathers should be stored in a labeled paper envelope with the same ID used for the plastics samples.

2.6. Sample labeling and data collection

We recommend including the following information on sample labels:
Three or four letter code - standard bird identifier (species initials, can use common or scientific name)
Date – yyyymmdd
Xxx- type of sample (i.e. liver, preen gland –use initials)
Xx – number of sample (sequential for the same bird)
Example: BBA_20150402_ PG_01
Which stands for: *Black browed albatross, from 2nd April 2015, Preen gland, sample no 1*

When collecting several samples from the same animal, use same identifier but change sample type and number of sample.

Use permanent marker for labeling vials. If vial has no label, or when labeling aluminum foil, use paper or masking tape to create a label. When transferring samples always revise that the labels are in good condition (re-label as necessary). For identification purposes it helps to have all samples from the same animal together. You can use clean, large aluminum foil sheets to wrap samples from the same individual.

In addition to recording types and numbers of samples collected from each individual animal, record location of sample collection as well as the person collecting the sample in your datasheets. This way, each sample will be linked to a site and responsible person.

2.7. Supplies needed

4. **Glass vials** for preen gland oil, stomach oil, etc.: any vial can be used. A recommendation is using Corning “single use” centrifuge tubes which can be ordered from just about any lab supplier. An example are *Corning, product no 99502-10: 10ml (16x114mm) disposable glass screw cap centrifuge tubes with lids with PTFE liners. VWR catalogue no 33502-140.*
5. **Stainless steel spatulas:** To reduce costs, two-headed spatulas can be purchased and then cut in half. An example can be found at: *http://www.sampling.com/stainless_micro_spatulas.html*
6. **Aluminum foil:** commercial cooking aluminum foil.

2.8. Recommendations on how to clean and sterilize utensils and materials:

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.

In a nutshell, 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 2015a).

3. DISCUSSION

The aim of this Working Paper is to present general sampling options that are fairly straightforward and simple, and can therefore be applied broadly both in the field by non-expert personnel, as well as by specialized teams performing full necropsies in controlled settings. The options presented here should allow assessing exposure of plastic, and particularly microplastics, in ACAP species. Towards that goal, collecting samples to assess what is absorbed by the bird and then deposited in tissues (i.e. liver) and/or body fluids (i.e. preen oil) is an important supplement to solely documenting ingestion, which is known to be very prevalent in these species based on published information. At least three levels of analysis can be performed on the sample types suggested in the protocols above. With an increasing level of complexity, it is possible to use stomach contents to visually classify plastic fragments (e.g. Van Franeker et al. 2011), to use scats for a rapid assessment of exposure to plastics via a "quick and simple" Nile Red fluorescent stain (Maes et al. 2017) and, finally, in the case of preen oil and tissues, to perform chemical analysis to identify specific plastic compounds (Hardesty et al. 2015a and b).

Two issues were particularly relevant when developing these sample collection protocols. One was that protocols should be simple and not require special training, so they can be performed by a large number of people with access to birds. The other was that the scope/extent of possible studies would be greatly enhanced if the protocols could be applied

to both live and dead birds, as well as providing options for non-invasive sample collection. In the case of dead birds, in addition to those potentially found at breeding sites, options for sampling are carcasses found on beaches by stranding networks and by-caught birds recovered by on-board observers. At nesting sites, if live birds are not handled, environmental scats and regurgitated boluses can be collected non-invasively, allowing for larger sample sizes and requiring minimum dexterity and supplies. In all cases, adoption of protocols and engagement in sampling efforts will largely depend on field conditions and storage capacity. In the case of on-board observers, it'll be contingent on whether sample collection must occur on board or can be done on frozen carcasses once on shore by a ground-based team. *We emphasize that special care is necessary to avoid contamination, particularly since plastics are everywhere, including most supplies and utensils we normally use.*

Considering that marine litter is a growing environmental concern and that plastic pollution is a global problem, understanding not only the prevalence of plastics exposure in ACAP species but also its potential impact to the bird´s health would represent a social incentive to reducing litter input and impacts on marine ecosystems. These guidelines intend to enable acquisition of more evidence to understand the size of the problem for albatross' health and survival. Plastic pollution remediation will require not only engagement of governments and international collaborations, but also social awareness and mobilization once regional organizations or governments alone cannot resolve this exponentially increasing global environmental problem.

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