Agreement on the Conservation of Albatrosses and Petrels

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Sampling guidelines to assess plastic ingestion in ACAP species

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

SUMMARY

During AC9, the PaCSWG 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 the 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*". In PaCSWG4 we presented draft guidelines to assess the incidence of plastics ingestion in ACAP species (Doc 09) for consideration of the working group. The AC10 Final Report recommended "*it would be useful to separate the guidelines into macro- and microplastic sample acquisition, reflecting the differing complexities in the tasks"*. Thus, this paper provides sampling protocols to asses plastic ingestion (macro, micro and plastic-derived chemicals) with an array of sample type choices that should facilitate collection in diverse settings (i.e. freshly-dead beached or by-caught specimens, live and dead animals at nesting sites, fresh scat sampling from nests, etc.).

RECOMMENDATIONS

Collect samples to assess plastic ingestion whenever an opportunity presents.

1. Macroplastics (>5mm): Collect stomachs from dead birds and voluntary regurgitates from live birds to quantify ingestion.
2. Microplastics (<5mm): Collect stomach content and assess plastic excretion in faecal precursor samples from dead birds. Collect live-bird voluntary regurgitates.
3. Plastic-derived chemicals (additives): Collect tissues from dead animals (i.e. liver, adipose) and/or body fluids from live and/or dead animals (i.e. preen gland oil) to assess absorption and body tissue deposit of plastic-derived chemicals. Link to components of plastic found in stomach. Avoid contamination.
4. Plastic-adsorbed organic contaminants: Collect tissues from dead animals (i.e. liver, adipose) and/or body fluids from live and/or dead animals (i.e. preen gland oil). Link to contaminants adsorbed to plastics in stomach content. Avoid contamination.
5. Non-invasive assessment of macro- and microplastics: collect guano and/or regurgitated boluses. For leached plastic chemicals collect hatched and/or unviable eggs.

Directrices para obtener pruebas a fin de evaluar la ingestión de plásticos por parte de las especies amparadas por el ACAP

RESUMEN

Durante el AC9, el PaCSWG observó la intrusión generalizada de macro y microplásticos en la dieta y el medio ambiente de las aves marinas y expresó su preocupación por los pronósticos de que esto aumentará. Esto se reflejó más tarde en el Informe Final del AC9, que afirmaba "la necesidad de fomentar la investigación para evaluar la exposición, la incidencia y los impactos de los plásticos y microplásticos en el medio marino sobre las especies de ACAP". En el PaCSWG4 presentamos protocolos para evaluar la incidencia de la ingestión de plásticos en las especies ACAP (Doc 09) para su consideración por el grupo de trabajo. El Informe Final del AC10 recomendaba "sería útil separar los protocolos en la adquisición de muestras macro y microplásticos, reflejando las diferentes complejidades de las tareas". Por lo tanto, este documento proporciona protocolos de muestreo para evaluar la ingestión de plásticos (macro, micro y productos químicos derivados de plásticos) con una serie de opciones de tipos de muestras que deberían facilitar la recolección en diversos entornos (por ejemplo, animales recién muertos varados o capturados incidentalmente, animales vivos y muertos en los sitios de reproducción, muestreo de fecas frescas de los nidos, etc.).

RECOMENDACIONES

Recolectar muestras para evaluar la ingestión de plástico cuando se presente la oportunidad.

1. Macroplásticos (>5mm): Recolectar estómagos de aves muertas y regurgitados voluntarios de aves vivas para cuantificar ingestión.
2. Microplásticos (<5mm): Recolectar el contenido estomacal y evaluar la excreción plástica en precursores fecales de aves muertas. Recolectar regurgitados voluntarios de aves vivas.
3. Productos químicos derivados (aditivos): Recoger tejidos de animales muertos (hígado, grasa) y/o fluidos corporales de animales vivos y/o muertos (aceite de la glándula uropígea) para evaluar la absorción y el depósito de productos químicos derivados del plástico en tejidos corporales. Asocie a los componentes plásticos hallados en estómago. Evite contaminación.
4. Contaminantes orgánicos absorbidos: Recolectar tejidos de animales muertos (hígado, grasa) y/o fluidos corporales de animales vivos y/o muertos (aceite de la glándula uropígea). Asocie a los componentes plásticos hallados en estómago. Evite contaminación.
5. Evaluación no invasiva de macro y microplásticos: recoger guano y/o bolos regurgitados. Para productos químicos lixiviados, recoja los huevos incubados y/o inviables.

Lignes directrices relatives à l'échantillonnage en vue d'évaluer l'ingestion de plastique par les espèces inscrites à l'ACAP

RÉSUMÉ

Lors du CC9, le GTSPC a noté l'invasion étendue de macro et microplastiques dans le régime et l'environnement des oiseaux de mer, et a exprimé son inquiétude quant aux prévisions d'augmentation de ces intrusions. Cette préoccupation a été relayée dans le Rapport final du CC9 qui établissait la nécessité d'« encourag[er] la recherche visant à évaluer l'exposition aux déchets plastiques et microplastiques dans l'environnement marin, leur incidence et leurs impacts sur les espèces de l'ACAP ». Lors du GTSPC4, nous avons présenté un projet de lignes directrices destinées à quantifier l'incidence de l'ingestion de plastiques sur les espèces inscrites à l'ACAP (Doc 09) afin que celui-ci soit examiné par le groupe de travail. Le Rapport final du CC10 recommandait qu'il serait utile de distinguer, dans différentes lignes directrices, l'acquisition d'échantillons de macroplastiques et de microplastiques, afin de refléter les complexités dans les tâches à mener. Le présent document fournit donc des protocoles en matière d'échantillonnage destinés à quantifier l'ingestion de plastiques (macro, micro et produits chimiques dérivés du plastique) ainsi qu'une série de choix de types d'échantillons qui devrait permettre de faciliter la collecte d'échantillons dans des contextes divers (à savoir des spécimens fraîchement échoués ou issus de la capture accessoire, des animaux morts ou vifs sur les sites de nidification, des échantillons d'excréments frais provenant des nids, par exemple).

RECOMMANDATIONS

Recueillir des échantillons afin de quantifier l'ingestion de plastiques dès que l'occasion se présente.

1. Macroplastiques (> 5 mm) : Recueillir les estomacs des oiseaux morts et les contenus stomacaux des oiseaux vivants qui régurgitent volontairement en vue de quantifier l'ingestion de plastiques.
2. Microplastiques (< 5 mm) : Recueillir les bols alimentaires et évaluer l'excrétion de plastiques dans des échantillons fécaux antérieurs provenant d'oiseaux morts. Recueillir les contenus stomacaux des oiseaux vivants qui régurgitent volontairement.
3. Produits chimiques dérivés de plastiques (additifs) : Collecter des tissus sur des animaux morts (foie, tissus adipeux) et/ou des fluides corporels sur des animaux vivants et/ou morts (glande uropygienne) afin de déterminer le taux d'absorption et les dépôts de produits chimiques dérivés du plastique dans les tissus corporels. Liens avec composants de plastique retrouvés dans l'estomac. Éviter toute contamination.
4. Contaminants organiques adsorbés sur le plastique : Collecter des tissus sur des animaux morts (foie, tissus adipeux) et/ou des fluides corporels sur des animaux vivants et/ou morts (glande uropygienne). Liens avec des contaminants adsorbés sur les plastiques se trouvant dans le contenu stomacal. Éviter toute contamination.
5. Évaluation non invasive des macro et microplastiques : recueillir le guano et/ou des bols régurgités. Pour les produits chimiques lixiviels issus du plastique, collecter les œufs éclos et/ou non viables.

1. INTRODUCTION

Among the most threatened vertebrates in the world are the albatrosses and petrels (order Procellariiformes), which include many species breeding on isolated oceanic islands (Birdlife International 2018). 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 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; plastic ingestion has been reported in several species (Acampora et al. 2014, Wilcox et al. 2015, Roman et al. 2016, Roman et al. 2019a). Ingested plastics can be classified by size (NOAA, Barnes et al., 2009, GESAMP). Four our purposes macro plastics means >5mm and micro plastics means <5mm. Macroplastics are most frequently associated with direct health effects when ingested by marine animals due to their potential to cause injuries, suffocation or obstruct the gastrointestinal tract (Pierce et al. 2004; Phillips et al. 2010; Ryan 2016; Roman et al. 2019a). The health effects of microplastics ingested by marine fauna, however, remain poorly understood.

On the other hand, indirect effects of plastic ingestion related to the accumulation of chemicals derived from plastic degradation (e.g. additives such as plasticizers and flame retardants) have also been documented in marine animals (Tanaka et al. 2013, 2015; Fossi et al. 2012, 2014; Hardesty et al. 2015). In addition, PCBs (polychlorinated biphenyls) and POCs (organochlorine pesticides) that have an affinity for organic and plastic particles, on which they tend to be adsorbed (Mato et al., 2002, Endo et al., 2005, Ríos et al., 2007) have also been reported (Colabuono et al. 2010, 2012, Yamashita et al. 2007, 2011, 2018). Most of 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, Fossi et al. 2018). Their accumulation over long periods of time (e.g. chronic leaching from plastic particles retained in the stomach) may affect the life cycle and reproductive success of species, potentially leading to long term harm at the population level (Finkelstein et al. 2007; Hardesty et al. 2015).

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. Considering that marine plastic 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 plastic ingestion in ACAP species. Thus, during PaCSWG4 we provided a draft set of guidelines for consideration of the working group. Comments and recommendations have been incorporated in the current revised sampling protocols to assess plastic ingestion (macro, microplastics and derived chemical compounds) with an array of sample type choices from both live and dead birds and non-invasively from their immediate environment, that should facilitate collection in diverse settings. Due to their particular sampling and diagnostic methodologies, smaller plastics (<1mm) are beyond the scope of these guidelines.

2. SAMPLE ACQUISITION AND STORAGE TO ASSESS PLASTIC INGESTION IN ACAP SPECIES

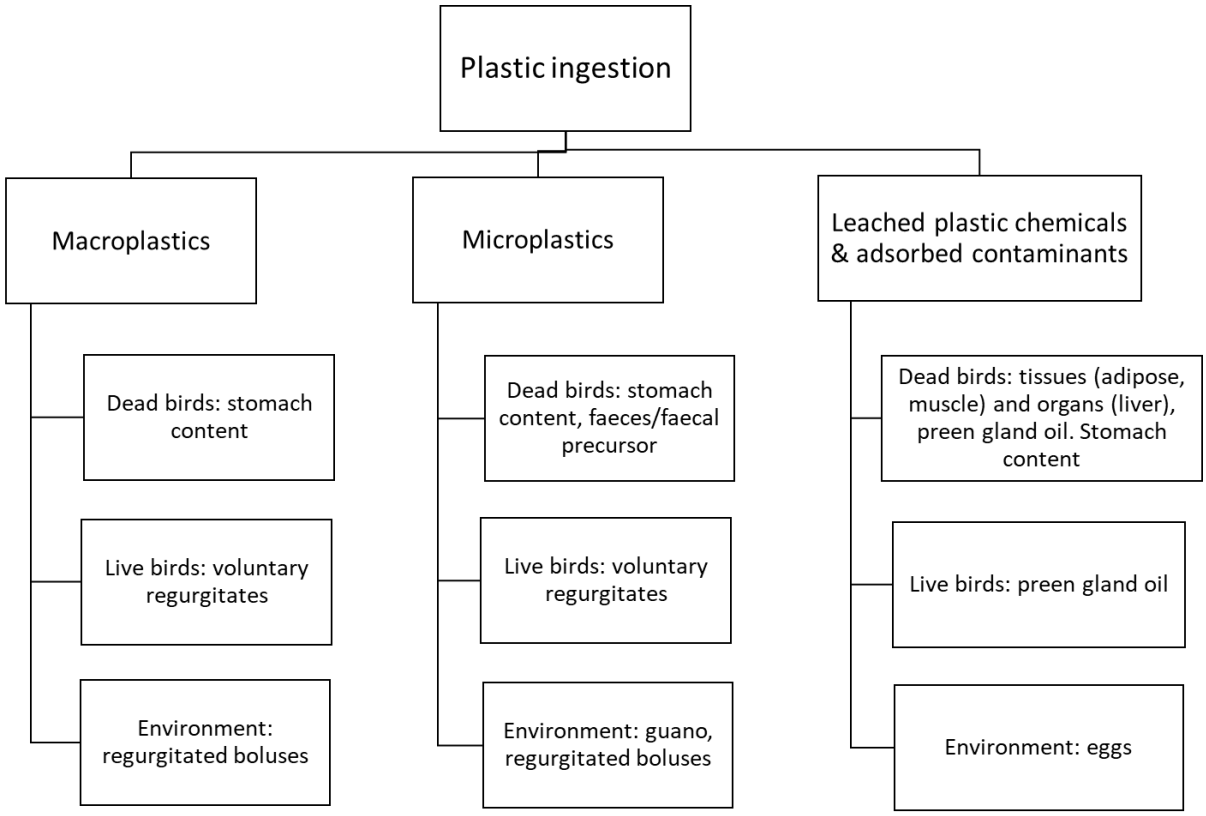
Sampling from dead birds can be performed on recently deceased animals or on frozen carcasses. Therefore, by-caught birds that died recently could be sampled on-board fishing vessels or kept frozen until arrival on land, and then sampled in a lab or other facility. This also applies to beached carcasses and recently deceased birds at nesting sites (though access to cold-chain or freezer might be restricted in these situations, favouring in-situ sampling).

Sampling from live birds can be performed opportunistically when handling birds for other purposes, or specifically for plastics investigation. Sampling live animals, however, requires specific training and skills as well as appropriate permits, and should therefore be restricted to personnel with the necessary expertise and approvals. Also, biases inherent to each sampling approach should be considered during study design.

Studies aiming to assess plastics leached chemicals and adsorbed contaminants, must consider sampling within a controlled setting (i.e. lab or similar facility) to 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 2.7. Under field conditions, and to the extent possible, materials must be single-use until they can be re-sterilized in order to avoid contamination. When sampling, contact with plastics, latex, etc. (gloves, bags, vials, syringes, others), should be avoided. Nitrile gloves are recommended.

Whenever possible, we encourage collection of samples for immediate use as well as archival storage. If immediate interest is only a quick macroscopic assessment of plastic ingestion, it would still be ideal if the collection and storage of samples was done in such a way as to allow more dedicated complementary studies in the future (i.e. assessment of microplastics and/or leached chemicals). For this, however, collection, handling and storage need to follow strict procedures to avoid contamination and invalidation of often irreplaceable samples. In the sections dedicated to each plastic type, we offer guidance for the simplest approach possible. Yet if samples are intended for immediate as well as future use, the more complex sterility processes will have to be followed (“clean” in text and tables implies heat-treated). When in doubt, handle all specimens with extra care and place in heat-treated aluminium foil prior to storage in other containers (i.e. sealable plastic bags).

IN ALL CASES: Wash hands thoroughly with soap and water prior to and after sample collection for personal protection.



Summary of sample types and sources for assessment of macro- and microplastics, leached plastic chemicals and adsorbed contaminants.

2.1. Macroplastics:

Sample type	***Sample source***	***Analytical method and references***	***Sample collection and storage***
Solid stomach content	Live (voluntary regurgitant)	Visual classification of plastic items: *Carey et al. 2011, Lavers et al. 2014*, *Provencher et al. 2014*	* Put whole stomach or recovered stomach contents in sealable plastic bag* and store in freezer for later transport/analysis. * metal or plastic tray, forceps/tweezers, water to separate/ID plastic items * optional 1 and/or 5 mm sieve
	Dead (proventriculus and gizzard)	Visual classification of plastic items from the proventriculus and gizzard: *Colabuono et al. 2009, Jimenez et al. 2015, Ryan et al. 2016, Roman et al. 2016, 2019, Hyrenbach et al. 2017, Provencher et al. 2014, 2018, Van Franeker et al. 2011*	
Regurgitated feed boluses	Environmental	Visual classification of plastic items in chick boluses: *Hyrenbach et al. 2017, Copello and Quintana 2003*	* forceps/tweezers * boluses preserved in 70% alcohol at room temperature or frozen in sealable plastic bag * metal or plastic tray, forceps/tweezers, water to separate/ID plastic items * 1 and/or 5 mm sieve

***** if chemical analysis will be performed, wrap in clean aluminium foil prior to storing in plastic bag.

2.1.1. Gastrointestinal tract sampling: In dead birds, the gastrointestinal tract or stomachs (proventriculus and gizzard) can be recovered through dissection or during necropsy (for details, see Van Franeker 2004). Stomach contents from live birds can be collected in many species from adults and large chicks by spontaneous, voluntary regurgitation (Carey et al. 2011, Lavers et al. 2014, Provencher et al. 2004). Stomach lavage is highly invasive and not recommended for this purpose only. Note that the gizzard of Procellariiforms (except albatross) is separated from the proventriculus by an isthmus juncture where hard items can become lodged and are not easily regurgitated (Furness et al. 1985). This can cause injury and bias the sample obtained in live birds.

Once retrieved, place gastrointestinal tract/regurgitate samples in sealable plastic bag (previously, wrap in clean aluminium foil if chemical analysis will also be performed) and store frozen for later transport/analysis. For macroplastics analysis, stomach content can be washed and sieved through a 1 to 5 mm mesh to facilitate separation of large items. Recovered solids can be stored until visual analysis for fragment classification by colour, size, type (Provencher et al. 2017).

2.1.2. Environmental sampling

Regurgitated feed boluses: regurgitated pellets of indigestible material (boluses) containing both debris and natural food items can be found in nesting colonies. Use forceps to place fresh intact feed-boluses individually in sealable plastic bags and store frozen until visual analysis (Colabuono et al. 2009, Jimenez et al. 2015, Ryan et al. 2016, Hyrenbach et al. 2017, Provencher et al 2014, 2018, Van Franeker et al. 2011). Alternatively, boluses can be preserved in screw top vials with 70% ethanol at room temperature (Copello and Quintana 2003). Boluses should be dissolved with water and sieved through a 1 to 5 mm mesh. Recovered solids can be stored until visual analysis for fragment classification by colour, size, type (Provencher et al. 2017).

2.2. Microplastics:

Sample type	***Sample source***	***Analytical method and references***	***Sample collection and storage***
Faeces	Environmental	*V*isual examination of plastic items from bird faeces: *Gil-Delgado 2017*	*use disposable wooden spatulas to collect faeces in screw top vials or sealable plastic bags* and store in freezer for later transport/analysis. * metal or plastic tray, forceps/tweezers, water to separate/ID plastic items * 1 mm sieve
Regurgitated feed boluses		Visual classification of plastic items in chick boluses: *Hyrenbach et al. 2017*	* forceps/tweezers * boluses preserved in 70% alcohol at room temperature or frozen in sealable plastic bag * metal or plastic tray, forceps/tweezers, water to separate/ID plastic items * 1 mm sieve

Stomach content	Dead (gizzard, proventriculus, intestine and cloaca)	Visual analysis and Fourier-transform infrared (FTIR) spectroscopy of GI tract content in marine mammals and birds: *Lusher et al 2015, Avery-Gomm 2016, 2018* Visual analysis bird GI tract: *Franeker et al. 2011, Provencher et al. 2018* Visual analysis from last 10 cm of bird intestine and cloaca: *Provencher et al. 2018*	* Put GI tract in sealable plastic bag*, and store in freezer for later transport/analysis * metal or plastic tray, forceps/tweezers, water to separate/ID plastic items * 1 mm sieve
	Live (voluntary regurgitate)	Visual analysis from regurgitates: *Provencher et al. 2014*	* Put stomach content in sealable plastic bag*, and store in freezer for later transport/analysis * metal or plastic tray, forceps/tweezers, water to separate/ID plastic items * 1 mm sieve

***** if chemical analysis will be performed, wrap in clean aluminium foil prior to storing in plastic bag.

2.2.1.a. Gastrointestinal tract sampling: In dead birds, the gastrointestinal tract (proventriculus, gizzard, intestine, cloaca) can be recovered through dissection or necropsy (for details, see Van Franeker (2004). Stomach contents from live birds can be collected from adults and large chicks by spontaneous regurgitation in many species (Provencher et al. 2004). Stomach lavage is highly invasive and not recommended for this purpose. Note that the gizzard of Procellariiforms (except albatross) is separated from the proventriculus by an isthmus juncture where hard items can become lodged and are not easily regurgitated (Furness et al. 1985). This can cause injury and bias the sample obtained in live birds.

Once retrieved, place gastrointestinal tract/regurgitate in sealable plastic bag (previously wrap in clean aluminum foil if chemical analysis will be performed), and store in freezer for later transport/analysis.

2.2.1.b. **Gastrointestinal tract content analysis for microplastics:**

Step 1: enzyme digestion or solvent extraction (Cole et al. 2014, Lusher et al. 2017, Provencher et al. 2018) will help to remove all organic material.

Step 2: filtration with a sieve. The mesh size selected determines the minimum size that is targeted for sampling. One-millimetre sieves are commonly used and recommended for microplastics diagnosis.

Step 3: visual, chemical or physical characterization of particles. For particles 1–5 mm, visual evaluation by stereo-microscope is usually used for material recovered through sieving (Lusher et al. 2015, Avery-Gomm et al. 2016, 2018, Gil-Delgado et al. 2017, Provencher et al. 2014, 2018, Franeker et al. 2011). If smaller particles (< 1mm) are targeted, chemical and physical characterization of recovered materials by spectroscopic techniques, such as Fourier-transform infrared (FTIR) and Raman spectroscopy, are particularly useful for identifying microplastics in the 500–50µm and 50–1µm size, respectively (Kappler et al. 2016).

2.2.2. Environmental sampling: Fresh scats and regurgitated feed-boluses can be collected to assess plastic ingestion 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.

a) Scat sampling: Scoop faeces with disposable wooden spatulas. Place in sealable plastic bags or vials and freeze. Alternatively, collect faeces, dry at room temperature, weigh and then freeze for later visual analysis (Gil-Delgado et al. 2017). A rapid-screening method to detect and quantify microplastics based on fluorescent tagging with Nile Red stain in combination with density separation can also be used (Maes et al. 2017).

b) Regurgitated feed boluses: regurgitated pellets of indigestible material (boluses) containing both debris and natural food items can be found in nesting colonies. Use forceps to place fresh intact feed-boluses individually in plastic sealable bags and store frozen until visual analysis (Hyrenbach et al. 2017).

2.3. Plastic-derived chemicals (additives):

Sample type	***Sample source***	***Analytical method and references***	***Sample collection and storage***
Preen gland oil	live	-phtalates in preen gland oil (recent 3–6 months exposure): *Hardesty et al. 2015*	* clean sterilized metal spatula * glass vial with aluminium foil under the cap (or use lids with PTFE liners). *alternatively, wipe the gland with a glass microfiber filter and save it in a cleaned aluminium foil envelope
	dead	-phtalates in preen gland oil (recent 3–6 months exposure): *Hardesty et al. 2015*	* clean sterilized new scalpel blade and tweezers/forceps * cleaned double aluminium foil to collect dissected gland
Stomach content (oil and plastic items)	live (voluntary regurgitate)	-leaching of flame retardants (PBDEs) in stomach oil and other solutions (e.g. fish oil): *Tanaka et al. 2015*	* sterilized glass vial, place aluminium foil under cap (or use lids with PTFE liners).
	dead	-flame retardants (PBDEs) in ingested plastics*: Tanaka et al 2013, 2015* -leaching of PBDEs in stomach oil and other solutions (e.g. fish oil): *Tanaka et al. 2015*	* metal tray, clean sterilized new scalpel blade and clean forceps/tweezers * wrap stomach in cleaned double aluminium foil, and store in freezer for later transport/analysis.
Abdominal adipose tissue and pectoral muscle	dead	-phtalates and other compounds in whale blubber and muscle: *Fossi et al. 2012, 2014* -flame retardants (PBDEs) in bird adipose tissue: *Tanaka et al. 2013, 2015*	* clean sterilized new scalpel blade and clean forceps/tweezers * cleaned double aluminium foil
Organs (ie. liver)	dead	-flame retardants (PBDEs) in bird liver: *Tanaka et al. 2015*	* clean sterilized new scalpel blade and clean forceps/tweezers * cleaned double aluminium foil
Eggs	environmental	- flame retardants (PBDEs): *Jaspers et al. 2005, Polder et al. 2008*	* cleaned double aluminium foil

If possible, preen gland, solid stomach contents, adipose tissue and liver should be collected from the same animal to link detection of chemical compounds from plastics in the gastrointestinal system with their presence in preen-gland oil and body tissues.

Contamination is a very important concern for plastic-derived chemical compound sampling. Wear nitrile, not latex, gloves when collecting samples.

Note that the use of properly cleaned utensils and supplies for sample collection and storage is essential for assessment of plastic-derived chemicals. Should you lack capacity to have properly prepared materials at hand, you should disregard collecting samples for plastic chemical compound analysis.

2.3.1. Seabird preen-gland oil sampling:

a) 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 clean double aluminium foil, label and store in freezer. Open the preen gland in the lab using a clean scalpel blade and transfer gland oil (frozen or cold typically at this point, not warm and liquid) to a pre-cleaned stainless-steel spatula. Return spatula to glass vial. Make sure clean (heat-threatened at 450ºC) aluminium foil is placed over the top of the vial before screwing on the plastic lid (or use lids with PTFE liners). Alternatively, if only a small amount of oil is found in the gland, wipe the open gland with a glass microfiber filter to absorb the preen oil. Store in a cleaned aluminium foil envelope.
b) To collect preen gland oil from live birds, 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. Following Hardesty et al. (2015), 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 aluminium foil (heat-threatened at 450ºC) is placed over the top of the vial before screwing on the plastic lid (or use lids with PTFE liners). Alternatively, follow Yamashita et al. (2018) and wipe preen gland oil using glass microfiber filter. Avoid contact with bird feathers. Store in a cleaned aluminium foil envelope.

2.3.2. Gastrointestinal tract sampling: In dead birds, the gastrointestinal tract can be extracted through dissection or during necropsy (for details, see Van Franeker (2004). Be careful in tying both ends to preserve oil and solid items in content. Stomach contents form live birds can be collected from adults and large chicks by spontaneous regurgitation in many species (Provencher et al. 2004). Stomach lavage is highly invasive and not recommended for this purpose only. Note that the gizzard of Procellariiforms (except albatross) is separated from the proventriculus by an isthmus juncture where hard items can become lodged and are not easily regurgitated (Furness et al. 1985). This can cause injury and bias the sample obtained in live birds.

Once retrieved, wrap gastrointestinal tract/regurgitate in clean double aluminium foil and store frozen for later transport and processing. Open the digestive tract in the lab using a clean scalpel blade and transfer oil (frozen or cold typically at this point, not warm and liquid) to a pre-cleaned and sterilized glass container with aluminium foil under the plastic lid (or use lids with PTFE liners). Freeze for later analysis of leaching compounds (Tanaka et al. 2015). Recovered solids from the gastrointestinal tract (see 2.3.1) should be wrapped in cleaned double aluminium foil and stored frozen until chemical analysis (Tanaka et al. 2013, 2015).

2.3.3. Dead seabird adipose and muscle tissue sampling: After collection with sterilized scalpel, use clean forceps to place tissue in clean, double aluminium foil, and store frozen (Tanaka et al. 2013, 2015, Fossi et al. 2012, 2014).

2.3.4. Dead seabird liver tissue sampling: 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 aluminium foil, and store frozen (Tanaka et al. 2015).

2.3.5. Egg sampling: Plastic contaminants may transfer from the mother to the eggs (Jaspers et al. 2005, Polder et al. 2008, Provencher 2019). Hatched or unviable eggs can be collected from nests, wrapped in foil, and frozen until analysis.

2.3.6. Blanks and control samples: collect at least three ENVIRONMENTAL blanks while sampling at a given site. To collect a blank sample, open the glass vial, wave the spatula in the air without it touching anything. Do this for about the same amount of time it takes to sample the bird. Replace the spatula in the tube, label with date, location, time, etc. and BLANK. 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.

2.4. Adsorbed toxic compounds:

Sample type	***Sample source***	***Analytical method and references***	***Sample collection and storage***
Preen gland oil	live	-POPs i.e. PCBs, DDTs, and HCHs in preen gland oil from live birds: *Yamashita et al. 2018*	* clean sterilized metal spatula * glass vial with clean aluminium foil under the cap (or use lids with PTFE liners). *alternatively, wipe gland with glass microfiber filter and store in cleaned aluminium foil * store in freezer for later transport/analysis
	dead	-POPs in preen gland oil: *Yamashita et al. 2007*	* clean sterilized new scalpel blade and tweezers/forceps * wrap in cleaned double aluminium foil and store in freezer for later transport/analysis
Stomach content (plastic items)	dead	-PCBs and OCPs adsorbed to ingested plastics: *Colabuono et al. 2010, Yamashita et al. 2011*	* metal tray, clean sterilized new scalpel blade and clean forceps/tweezers * wrap stomach in clean aluminium foil and place in sealable plastic bag. *store in freezer for later transport/analysis
Abdominal adipose tissue and pectoral muscle	dead	-PCBs and OCPs in adipose tissue and muscle: *Colabuono et al. 2012, Yamashita et al. 2007, 2011*	* clean sterilized new scalpel blade and clean forceps/tweezers * cleaned double aluminium foil * store in freezer for later transport/analysis
Organs (ie. liver)	dead	-PCBs and OCPs in liver: *Colabuono et al. 2012*	* clean sterilized new scalpel blade and clean forceps/tweezers * cleaned double aluminium foil * store in freezer for later transport/analysis

Note: *Preen gland, solid stomach contents, adipose tissue and liver should be collected from the same animal to link detection of plastic-adsorbed contaminants in the gastrointestinal system with their presence in preen-gland oil and body tissues.*

The use of properly cleaned utensils and supplies for sample collection and storage is essential for assessment of adsorbed contaminants. Should you lack capacity to have properly prepared materials at hand, you should disregard collecting samples for these analyses.

2.4.1. Seabird preen-gland oil sampling:

a) To collect the preen gland in deceased birds, use a clean sterilized, new scalpel blade to excise the gland (help yourself with clean forceps/tweezers if needed). Place the gland in cleaned double aluminium foil, label and store in freezer. Open the preen gland in the lab using a clean scalpel blade and transfer gland oil (frozen or cold typically at this point, not warm and liquid) to a pre-cleaned stainless-steel spatula. Return spatula to glass vial. Alternatively, if only a small amount of oil is found in the gland, wipe the open gland with a glass microfiber filter to absorb the oil. Store in a cleaned aluminium foil envelope (Yamashita et al. 2007).
b) To collect preen gland oil from live birds, 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. Following Yamashita et al. (2018), wipe preen gland oil using glass microfiber filter. Store in a cleaned aluminium foil envelope.

2.4.2. Gastrointestinal tract sampling: In dead birds, the gastrointestinal tract can be extracted through dissection or during necropsy (for details, see Van Franeker (2004). Be careful in tying both ends to preserve oil and solid items in content. Stomach contents form live birds can be collected from adults and large chicks by spontaneous regurgitation in many species (Provencher et al. 2004). Stomach lavage is highly invasive and not recommended for this purpose only. Note that the gizzard of Procellariiforms (except albatross) is separated from the proventriculus by an isthmus juncture where hard items can become lodged and are not easily regurgitated (Furness et al. 1985). This can cause injury and bias the sample obtained in live birds.

Once retrieved, wrap the gastrointestinal tract/regurgitate in cleaned aluminium foil and place in sealable plastic bag. Store frozen for later transport and post-processing. Open the digestive tract in the lab using a clean scalpel blade. Recovered solids (see 2.3.1. Gastrointestinal tract sampling) can be wrapped in cleaned, double aluminium foil and stored frozen until chemical analysis (Yamashita et al. 2011, Colabuono et al. 2010).

2.4.3. Dead seabird adipose tissue sampling: Collect with sterilized scalpel and use clean forceps to place tissue in clean, double aluminium foil, and store frozen (Colabuono et al. 2012, Yamashita et al. 2007, 2011).

2.4.4. Dead seabird liver tissue sampling: To collect liver tissue, dissect the whole organ or a large section with a new sterilized scalpel blade. Use clean forceps to place tissue in clean, double aluminium foil, and store frozen (Colabuono et al. 2012).

2.5. Sample labelling 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_ Xx 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 labelling vials. If vial has no label, or when labelling aluminium foil, use paper or masking tape to create a label. When transferring samples always make sure 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 aluminium foil sheets to wrap samples from the same individual (for plastic-derived chemical analysis), or ziploc bag (other analysis).

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.

2.6. Supplies needed

1. Glass vials for preen gland oil, stomach oil, etc.: any clean vial can be used. A recommendation is using Corning “single use” centrifuge tubes which can be ordered from most lab suppliers. An example is Corning, product no 99502-10: 10ml (16x114mm) disposable glass screw cap centrifuge tubes with lids with PTFE liners. VWR catalogue no 33502-140. To reduce costs of PTFE lids, place aluminium foil (heat-threatened at 450ºC) under common plastic lids.

2. 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

3. Disposable wooden spatulas: also found as flat wooden tongue depressors.

3. Aluminium foil: commercial cooking aluminium foil.

4. Microfiber filter wipe: Whatman GFF, 47 mm diameter can be used to wipe the preen gland to collect oil for chemical analysis. https://www.sigmaaldrich.com/catalog/product/aldrich/wha1825047?lang=en&region=US

5. Sealable plastic bags: commercial sealable plastic bags (e.g. Ziploc)

6. Sieves: 5 and/or 1mm mesh

7. metal trays

2.7. Recommendations for cleaning and sterilizing utensils and materials for plastic-derived chemicals

Properly cleaning glass vials, aluminium foil and re-usable utensils (e.g. tweezers, scissors, scalpel) 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.

Prior to sample collection, glass vials and reusable utensils should be washed thoroughly with distilled water and a brush. Rinse several times. 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 hours. Aluminium foil should be heated to 450°C for 6 hours.

To avoid contamination between individuals during sampling, use new scalpel blade for each animal; reusable utensils should be washed thoroughly with running water and detergent and a brush and then rinsed with distilled water several times. To avoid contamination between samples taken from the same individual, 1-wash utensils with water, 2-dry with paper towel, and 3-rinse with alcohol.

3. CONCLUDING REMARKS

The aim of these guidelines is to present general and simple sampling options to assess plastic ingestion (macro- and microplastics, leached chemicals and adsorbed compounds) in ACAP species. These protocols can be applied broadly both in the field by non-expert personnel (i.e. environmental and dead bird sampling), as well as by specialized personnel in the case of live birds or teams performing full necropsies in controlled settings.

At least four levels of analysis can be performed on the sample types suggested in the protocols above. With an increasing level of complexity (and generally increasing costs), it is possible to perform:

1) visual analysis to classify plastic items (macro- and microplastics >1mm) from stomach contents (e.g. Provencher et al. 2014, 2018, Colabuono 2009, Van Franeker et al. 2011, Roman et al. 2016, 2019), boluses (Hyrenbach et al. 2017, Copello and Quintana 2003) and faeces (Gil-Delgado et al. 2017);
2) rapid-screening (detection and quantification) of microplastics (size limit of detection is defined by magnification and optical resolution) based on selective fluorescent staining using Nile Red, followed by density-based extraction, filtration and visual analysis (Maes et al. 2017);
3) chemical and physical characterization of nano-scale microplastics by spectroscopic techniques such as FTIR (500–50 µm) and Raman (50–1 um) (Kappler et al. 2016, Lusher et al. 2015, Avery-Gomm et al. 2016, 2018);
4) chemical analysis to identify and quantify specific plastic-derived (additives) compounds from preen (Hardesty et al. 2015a), and stomach oils (Tanaka et al. 2015), plastic items (Tanaka et al. 2013, 2015), organs and tissues (Fossi et al. 2012, 2014, Tanaka et al. 2013, 2015).
5) chemical analysis to identify and quantify specific plastic-adsorbed compounds from preen (Yamashita et al. 2007, 2018), plastic items (Colabuono et al. 2010, Yamashita et al. 2011), organs and tissues (Colabuono et al. 2012, Yamashita et al. 2007, 2011).

6) chemical analysis of plastic resin types (i.e. polyethylene, polystyrene, polyvinyl, polypropylene. etc.) from chick regurgitated feed-boluses (Nilsen et al. 2015 -not included in tables).

Methods development for sampling and analysis of plastics is an important, emerging area of research and development in marine litter science (e.g. Galgani et al. 2011). There is consensus that assessing the pervasiveness of plastic exposure in seabirds requires adoption of standardized methods to facilitate cross-species comparisons (Moser and Lee, 1992; van Franeker et al. 2011; Avery-Gomm et al. 2016; Provencher et al. 2014). These guidelines aim to provide selected methods and options based on the authors experience and should be re-visited frequently to incorporate newer, simpler and cheaper technologies as they become available.

4. REFERENCES

Acampora, H., Schuyler, Q. A., Townsend, K. A., & Hardesty, B. D. 2014. Comparing plastic ingestion in juvenile and adult stranded short-tailed shearwaters (*Puffinus tenuirostris*) in eastern Australia. *Marine Pollution Bulletin*, *78*, 63–68.

Avery-Gomm, S., Valliant, M., Schacter, C. R., Robbins, K. R., Liboiron, M., Daoust, P. Y., Rios, L. M., Jones, I. L., 2016. A study of wrecked dovekies (*Alle alle*) in the western North Atlantic highlights the importance of using standardized methods to quantify plastic ingestion. *Marine Pollution Bulletin,* 113, 75–80.

Avery-Gomm, S., Provencher, J. F., Liboiron, M., Poon, F. E., & Smith, P. A. 2018. Plastic pollution in the Labrador Sea: an assessment using the seabird northern fulmar *Fulmarus glacialis* as a biological monitoring species. *Marine Pollution Bulletin*, *127*, 817–822.

Barnes, D. K. A., Galgani, F., Thompson, R. C., Barlaz, M. 2009. Accumulation and fragmentation of plastic debris in global environments. *Philosophical Transactions of the Royal Society B*, 364, 1985−1998.

BirdLife International. 2018. The IUCN Red List of Threatened Species 2018. http://www.birdlife.org.

Carey, M. J. 2011. Intergenerational transfer of plastic debris by Short-tailed shearwaters (*Ardenna tenuirostris*). *Emu,* 111: 229–234.

Cardoso, M. D., de Moura, J. F., Tavares, D. C., Gonçalves, R. A., Colabuono, F. I., Roges, E. M., & Siciliano, S. 2014. The Manx shearwater (*Puffinus puffinus*) as a candidate sentinel of Atlantic Ocean health. *Aquatic Biosystems*, *10*, 6.

Colabuono, F. I., Barquete, V., Domingues, B. S., & Montone, R. C. 2009. Plastic ingestion by Procellariiformes in southern Brazil. *Marine Pollution Bulletin*, *58*, 93–96.

Colabuono, F. I., Taniguchi, S., & Montone, R. C. 2010. Polychlorinated biphenyls and organochlorine pesticides in plastics ingested by seabirds. *Marine Pollution Bulletin*, *60*, 630–634.

Colabuono, F. I., Taniguchi, S., & Montone, R. C. 2012. Organochlorine contaminants in albatrosses and petrels during migration in South Atlantic Ocean. *Chemosphere*, *86*, 701–708.

Cole, M. *et al.* 2014. Isolation of microplastics in biota-rich seawater samples and marine organisms. *Nature Scientific Reports,* **4,** 4528.

Dauwe, T., Jaspers, V., Covaci, A., Schepens, P., & Eens, M. 2005. Feathers as a nondestructive biomonitor for persistent organic pollutants. *Environmental Toxicology and Chemistry*, *24*(2), 442–449.

Eriksson, C., & Burton, H. 2003. Origins and biological accumulation of small plastic particles in fur seals from Macquarie Island. *AMBIO 32*, 380–384.

Fossi, M. C., Panti, C., Baini, M., Lavers, J. L. 2018. A review of plastic-associated pressures: cetaceans of the Mediterranean Sea and Eastern Australian shearwaters as case studies. *Frontiers in Marine Science*, *5,* 173.

Furness, R. W. 2003. Impacts of fisheries on seabird communities. *Scientia Marina,* 67 (Suppl.2), 33–45.

Goldsworthy, S. D., M. A. Hindell, & H. M. Crowley. 1997. Diet and diving behaviour of sympatric fur seals *Arctocephalus gazella* and *A. tropicalis* at Macquarie Island. In: *Marine Mammal Research in the Southern Hemisphere, Status Ecology and Medicine Vol 1*. Hindell, M. and Kemper, C. (eds). Surrey, Beatty & Sons, Chipping Norton, NSW 2170. 151–163.

Grimaldi, W. W., Seddon, P. J., Lyver, P. O. B., Nakagawa, S., & Tompkins, D. M. 2015. Infectious diseases of Antarctic penguins: current status and future threats. *Polar Biology*, *38*, 591–606.

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*, 92–98.

Hardesty, B. D., Good, T. P., & Wilcox, C. 2015b. Novel methods, new results and science-based solutions to tackle marine debris impacts on wildlife. *Ocean & Coastal Management*, *115*, 4–9.

Heard, M. J., Smith, K. F., Ripp, K. J., Berger, M., Chen, J., Dittmeier, J., Goter, M., McGarvey, S. T., & Ryan, E. 2013. The threat of disease increases as species move toward extinction. *Conservation Biology*, *27*, 1378–1388.

Hyrenbach, K. D., et al. 2017. Plastic ingestion by Black-footed Albatross *Phoebastria nigripes* from Kure Atoll, Hawai'i: Linking chick diet remains and parental at-sea foraging distributions. *Marine Ornithology*, *45*, 225–236.

Jaspers, V., Covaci, A., Maervoet, J., Dauwe, T., Voorspoels, S., Schepens, P., & Eens, M. 2005. Brominated flame retardants and organochlorine pollutants in eggs of Little owls (*Athene noctua*) from Belgium. *Environmental Pollution*, *136*, 81–88.

Jiménez, S., Domingo, A., Brazeiro, A., Defeo, O., Phillips, R.A. 2015. Marine debris ingestion by albatrosses in the southwest Atlantic Ocean. *Marine Pollution Bulletin* 96, 149–154.

Käppler, A., Fischer, D., Oberbeckmann, S., Schernewski, G., Labrenz, M., Eichhorn, K. J., & Voit, B. 2016. Analysis of environmental microplastics by vibrational microspectroscopy: FTIR, Raman or both? *Analytical and Bioanalytical Chemistry*, *408*, 8377–8391.

Lavers J. L., Bond, A. L., & Hutton, I. 2014. Plastic ingestion by Flesh-footed Shearwaters (*Puffinus carneipes*): Implications for fledgling body condition and the accumulation of plastic-derived chemicals. *Environmental Pollution, 187*,124-129.

Lusher, A. L., Hernandez-Milian, G., O'Brien, J., Berrow, S., O'Connor, I., & Officer, R. 2015. Microplastic and macroplastic ingestion by a deep diving, oceanic cetacean: the True's beaked whale *Mesoplodon mirus*. *Environmental Pollution*, *199*, 185–191.

Lusher, A. L., Welden, N. A., Sobral, P., & Cole, M. 2017. Sampling, isolating and identifying microplastics ingested by fish and invertebrates. *Analytical Methods*, *9*, 1346–1360.

Maes, T., Jessop, R., Wellner, N., Haupt, K., & Mayes, A. G. 2017. A rapid-screening approach to detect and quantify microplastics based on fluorescent tagging with Nile Red. *Nature Scientific Reports*, *7,* 44501.

Nilsen, F., Hyrenbach, K. D., Fang, J., & Jensen, B. 2014. Use of indicator chemicals to characterize the plastic fragments ingested by Laysan albatross. *Marine Pollution Bulletin,* 87: 230–236.

Parsons, M., Mitchell, I., Butler, A., Ratcliffe, N., Frederiksen, M., Foster, S., & Reid, J. B. 2008. Seabirds as indicators of the marine environment. ICES *Journal of Marine Science, 65*,1520–1526.

Pazos, R. S., Maiztegui, T., Colautti, D. C., Paracampo, A. H., & Gómez, N. 2017. Microplastics in gut contents of coastal freshwater fish from Río de la Plata estuary. *Marine Pollution Bulletin*, *122*, 85–90.

Phillips, R. A., *et al.* 2016. The conservation status and priorities for albatrosses and large petrels. *Biological Conservation*, *201*, 169–183.

Polder, A., Venter, B., Skaare, J.U., & Bouwman, H. 2008. Polybrominated diphenyl ethers and HBCD in bird eggs of South Africa. *Chemosphere*, *73*, 148–154.

Provencher, J. F., *et al.* 2014. Prevalence of marine debris in marine birds from the North Atlantic. *Marine Pollution Bulletin*, *84*,411–417.

Provencher, J. F., Vermaire, J. C., Avery-Gomm, S., Braune, B. M., & Mallory, M. L. 2018. Garbage in guano? Microplastic debris found in faecal precursors of seabirds known to ingest plastics. *Science of the Total Environment*, *644*, 1477–1484.

Provencher J. F. 2019. Seabirds and Plastics Pollution: Birds as Monitoring Tools and Vectors. Oral session AAAS Annual Meeting Washington DC February 14–17. https://aaas.confex.com/aaas/2019/meetingapp.cgi/Paper/24179

Ryan, P. G., De Bruyn, P. N., & Bester, M. N. 2016. Regional differences in plastic ingestion among Southern Ocean fur seals and albatrosses. *Marine Pollution Bulletin*, *104*, 207–210.

Roman, L, QA Schuyler, BD Hardesty and KA Townsend. 2016. Prevalence and selectivity of anthropogenic debris ingestion in eastern Australian avifauna. PLoS One. http://dx.doi.org/10.1371/journal.pone.0158343.

Roman, L., Hardesty, B. D., Hindell, M., & Wilcox, C. 2019a. A quantitative analysis linking seabird mortality and marine debris ingestion. *Nature Scientific Reports*. https://doi.org/10.1038/s41598-018-36585-9

Roman, L., Lowenstine, L., Parsley, L. M., Wilcox, C., Hardesty, B. D., Gilardi, K., & Hindell, M. 2019b. Is plastic ingestion in birds as toxic as we think? Insights from a plastic feeding experiment. *Science of the Total Environment, 665,* 660–667.

Tanaka, K., Takada, H., Yamashita, R., Mizukawa, K., Fukuwaka, M., & Watanuki, Y. 2013. Accumulation of plastic derived chemicals in tissues of seabirds ingesting marine plastics. *Marine Pollution Bulletin, 69*,219-222.

Tanaka, K., Takada, H., Yamashita, R., Mizukawa, K., Fukuwaka, M., & Watanuki, Y. 2015. Facilitated leaching of additive-derived PBDES from plastic by seabirds´ stomach oil and accumulation tissues. *Environmental Science & Technology, 49*,11799-11807.

Van Franeker, J. 2004. *Save the North Sea Fulmar-Litter-EcoQO Manual Part 1: Collection and dissection procedures*. Alterra Report 672, Wageningen, the Netherlands.

Van Franeker, J. A., Blaize, C., Danielsen, J., Fairclough, K., Gollan, J., Guse, N., & Turner, D. M. 2011. Monitoring plastic ingestion by the northern fulmar *Fulmarus glacialis* in the North Sea. *Environmental Pollution, 159*, 2609e2615.

Weimerskirch, H., P. Inchausti, C. Guinet, & Barbraud, C. 2003. Trends in birds and seals population as indicators of a system shift in the Southern Ocean. *Antarctic Science, 15*,249–256.

Wilcox, C., Van Sebille, E., & Hardesty, B. D. 2015. Threat of plastic pollution to seabirds is global, pervasive, and increasing. *Proceedings of the National Academy of Sciences*, *112*, 11899–11904.

Wilcox, C., Mallos, N. J., Leonard, G. H., Rodriguez, A., & Hardesty, B. D. 2016. Using expert elicitation to estimate the impacts of plastic pollution on marine wildlife. *Marine Policy, 65*, 107–114.

Yamashita, R., Takada, H., Murakami, M., Fukuwaka, M. A., & Watanuki, Y. 2007. Evaluation of noninvasive approach for monitoring PCB pollution of seabirds using preen gland oil. *Environmental Science & Technology, 41*, 4901–4906.

Yamashita, R., Takada, H., Fukuwaka, M. A., & Watanuki, Y. 2011. Physical and chemical effects of ingested plastic debris on short-tailed shearwaters, *Puffinus tenuirostris*, in the North Pacific Ocean. *Marine Pollution Bulletin*, *62*, 2845–2849.

Yamashita, R., *et al.* t 2018. Global monitoring of persistent organic pollutants (POPs) using seabird preen gland oil. *Archives of Environmental Contamination and Toxicology*, *75*, 545–556.