Marine Pollution Bulletin 142 (2019) 470–474

Contents lists available at ScienceDirect

Marine Pollution Bulletin

journal homepage: www.elsevier.com/locate/marpolbul

Detection of ultrafine plastics ingested by seabirds using tissue digestion

Jennifer L. Lavers[a,*], Georgina Stivaktakis[b], Ian Hutton[c], Alexander L. Bond[a,d]

[a] Institute for Marine and Antarctic Studies, University of Tasmania, 20 Castray Esplanade, Battery Point, Tasmania 7004, Australia
[b] Institute for Marine and Antarctic Studies, University of Tasmania, School Road, Newnham, Tasmania 7250, Australia
[c] Lord Howe Island Museum, P.O. Box 157, Lord Howe Island, NSW 2898, Australia
[d] Bird Group, Department of Life Sciences, The Natural History Museum, Akeman Street, Tring, Hertfordshire HP23 6AP, United Kingdom

ARTICLE INFO

Keywords:
Extraction methods
Marine debris
Microplastic
Plastic pollution

ABSTRACT

Plastic debris is a major global threat to marine ecosystems and species. However, our knowledge of this issue may be incomplete due to a lack of a standardized method for quantifying ingested ultrafine particles (1 μm – 1 mm) in wildlife. This study provides the first quantification of ultrafine plastic in seabirds using chemical and biological digestion treatments to extract plastic items from seabird gizzards. The alkaline agent, potassium hydroxide, outperformed the enzyme corolase, based on cost and efficiency (e.g., digestion time). Ultrafine plastics were observed in 7.0% of Flesh-footed Shearwater (*Ardenna carneipes*) gizzards collected from Lord Howe Island, Australia and accounted for 3.6% of all plastic items recovered (13 out of 359 items). Existing methods for extracting ingested plastic from seabirds do not account for ultrafine particles, therefore our results indicate current seabird plastic loads, and the associated physical and biological impacts, are underestimated.

1. Introduction

Plastics have become a revolutionary alternative for industry and society, transforming the delivery of health care and packaging, storage, and transportation of goods (Hammer et al., 2012; PlasticsEurope, 2018). Over the past century, global plastic production has grown exponentially to over 345 million tonnes per annum, the majority of which is designed to be thrown away after single use (PlasticsEurope, 2018; Thompson et al., 2009). A combination of high disposability and gaps in waste management systems has led to plastic debris becoming recognized as a major environmental issue, causing widespread contamination of aquatic and terrestrial environments, and serious economic and ecological harm (Cole et al., 2011; Rocha-Santos and Duarte, 2015; Worm et al., 2017).

Vast areas of the ocean now contain up to 890,000 plastic items km^{-2} with at least 1.1–2.4 million tonnes of new plastic entering the ocean from riverine systems every year (Lebreton et al., 2017). This growing mass of floating debris creates a 'plastic soup' of large and small items distributed throughout the water column (Reisser et al., 2014), entangling or being ingested by > 2240 marine species (Tekman et al., 2019). Once ingested, plastic debris exposes wildlife to toxic substances and associated adverse effects, including endocrine disruption and reduced body condition (Tanaka et al., 2013; Teuten et al., 2009).

Our understanding of this complex issue is increasing rapidly, but information on the quantity of very small particles (e.g., ultrafine plastics 1 μm–1 mm) ingested by marine wildlife has been a conspicuous gap (Mattsson et al., 2018). While such particles may appear harmless due to their small size, they pose a significant threat since tiny particles can penetrate tissues and accumulate in organs, leading to changes in behaviour and metabolism (Cedervall et al., 2012; Mattsson et al., 2017; Wegner et al., 2012). Particle size also plays a pivotal role in the exposure of wildlife to chemicals, as smaller particles are generally more toxic than the corresponding bulk material at the same mass concentration (Santo et al., 2014).

Globally, seabirds are considered reliable sentinels of ocean health, with long-term monitoring programs providing valuable information on trends in plastic and chemical contamination in the world's oceans (Burger and Gochfeld, 2004; Lavers and Bond, 2016). Exposure of seabirds to small plastics (e.g., ultrafine particles) is poorly documented (Provencher et al., 2017), but likely occurs through direct ingestion, secondary ingestion via prey, or through the breakup of larger items during the digestion process. Many birds (especially seabirds) have a stomach consisting of two well-defined chambers: the proventriculus and muscular gizzard (Denbow, 2000). Like pumice and some prey hard parts (e.g., squid beaks), the movement of ingested plastic through seabird gastrointestinal tracts appears to be influenced by particle size, with fragments that are too large to pass into the gizzard being retained

* Corresponding author.
E-mail address: Jennifer.Lavers@utas.edu.au (J.L. Lavers).

https://doi.org/10.1016/j.marpolbul.2019.04.001
Received 27 January 2019; Received in revised form 31 March 2019; Accepted 1 April 2019

Table 1
Review of digestive treatments for the extraction of marine plastics from biological tissue samples (adapted from Miller et al. (2017).

Species	Tissue	Treatment (including agent)	Digestion time	Polymer type	Agent accessibility	Cost per sample (AU$)[a]	Source
Copepod (*Temora longicornis*)	Whole animal	500 μg ml^{-1} proteinase-K, 15 ml of sample, incubated for 2 h at 50 °C	2 h	Polyester & nylon fibers, PET, PVC	Uncommon	$22.40	Cole et al. (2014)
Blue mussel *(Mytilus edulis)*	Whole animal	25 ml trypsin 0.3125%, heated magnetic stirrer @ ~38 °C for 30 min	30 min	PET, PA, HDPE, PVC, PP	Uncommon	$3.29	Courtene-Jones et al. (2017a)
Blue mussel *(Mytilus edulis)*	Whole animal	1.5 ml corolase 7089 (activity 966/UHb/ml) in 100 ml MilliQ. Heated magnetic stirrer 1 h @ 60 °C	12 h	Nylon fiber	Uncommon	$1.86	Catarino et al. (2017)
Blue mussel (*Mytilus edulis*)	Whole animal	20 ml 22.5 M 69% HNO_3, 3 mussels per sample. Room temp. overnight	12 h	Nylon fiber, PS	Common	$28.40	Claessens et al. (2013)
12 × terrestrial bird sp.	Oesophagus, intestine	10% KOH (volume not reported), digest at room temp.	2–3 wk	Not reported	Common	$0.29	Zhao et al. (2016)
Mussel/crab/fish	Whole animal	20 ml 10% KOH, incubated @ 60 °C	24 h	LDPE, HDPE, PP, PA, PS	Common	$0.29	Dehaut et al. (2016)
North Sea fish sp.	Stomach, oesophagus, intestine	3× volume of biological sample of 10% KOH solution added, stored @ room temp.	2–3 wk	Not reported	Common	$0.89	Foekema et al. (2013)
Beaked whale (*Mesoplodon mirus*)	Oesophagus, stomach, intestine	3× volume of biological sample of 10% KOH solution added, stored @ room temp.	3 wk	PP and rayon fibers	Common	$0.89	Lusher et al. (2015)
Sprat (*Sprattus sprattus*)	Whole animal	20 ml 1 M KOH, digest at room temp.	2 days	Various plastics[b]	Common	$0.16	Kühn et al. (2017)
Flesh-footed Shearwater (*Ardenna carneipes*)	Gizzard	20 ml 1 M KOH. Digest in water bath @ 60 °C	12 h	Not recorded	Common	$0.16	This study
Flesh-footed Shearwater (*Ardenna carneipes*)	Gizzard	2.5 ml corolase 7089 (activity 840/UHb/ml) in 25 ml filtered water, replaced every 3 h. Digest in water bath @ 60 °C	9 h	Not recorded	Uncommon	$9.30	This study

Cellulose acetate (CA), high-density polyethylene (HDPE), low-density polyethylene (LDPE), polylactic acid (PLA), polyamide (PA), polypropylene (PP), polystyrene (PS), polyvinyl chloride (PVC), polyethylene terephthalate (PET).

[a] Based on prices available from Sigma Aldrich Australia and AB Enzymes in 2017.

[b] Various plastic types broadly categorized into synthetics, rubbers and biodegradables.

in the proventriculus. Importantly, items that are too large to pass directly into the intestine are temporarily retained in the gizzard until they are sufficiently broken up, presumably into ultrafine particles, before they are excreted (Provencher et al., 2018; Terepocki et al., 2017).

The lack of data on ultrafine particles in seabirds is due, in part, to the difficulty in quantifying these tiny particles and, until recently, a lack of standardized methodology which provided a framework and motivation to collect such data (Provencher et al., 2017). To date, the focus of seabird plastic studies has been on lavage (stomach flushing) of live animals which underestimates ingested debris (Hutton et al., 2008), or necropsy of deceased animals using a 1 mm mesh size to separate debris items, thus providing no data on the presence of very small items (e.g., van Franeker et al., 2011). Ultrafine plastics can also be overlooked due to concealment by biological tissue, and visual separation is both difficult and time consuming. However, without data on ultrafine particles, plastic loads in individual birds are underestimated, and information on this emerging threat is therefore lacking.

Recently, chemical digestion techniques have been used to enhance the quantification of plastic by removing biological tissues. While the majority of research has used tissues from fish, two studies obtained rapid, complete digestion of soft tissues from avian intestines using chemicals such as potassium hydroxide (KOH) over a period of 3–14 days (Kühn et al., 2017; Zhao et al., 2016). However, results of some studies suggest digestive treatments, including KOH, hydrogen peroxide (H_2O_2), and hydrochloric acid (HCl) are inconsistent or can cause alterations to plastic polymers (Avio et al., 2015; Dehaut et al., 2016). Enzymatic digestion has been proposed as a safer alternative to chemical digestion and has been recently used to extract ingested plastics from shellfish with no evidence of damage to the plastics (Catarino et al., 2017; Courtene-Jones et al., 2017b).

In eastern Australia, the ingestion of macro- (> 5 mm) and microplastic particles (1–5 mm) by Flesh-footed Shearwaters (*Ardenna carneipes*) has been linked to perforations of the digestive tract, and a range of sublethal effects (Lavers et al., 2014) which likely contribute to the decline of this species (Lavers, 2015; Lavers et al., 2019; Reid et al., 2013). No data exist on the ingestion of ultrafine particles in Flesh-footed Shearwaters, or seabirds more broadly, and few methods are available for obtaining this type of data, especially from muscular/fibrous tissues, such as the gizzard where such items are likely to accumulate. Therefore, the aims of this study were to 1) quantify ingested ultrafine plastics in Flesh-footed Shearwaters, and 2) identify digestive treatments for the extraction and quantification of marine plastics from seabird gizzards.

2. Materials and methods

2.1. Sample collection and preparation

During 4–9 May 2017 and 1–9 May 2018, freshly deceased Flesh-footed Shearwater fledglings (80–90 days old; e.g., road kill) were collected on Lord Howe Island, New South Wales (31.5191°S, 159.0649°E). The gizzard from each bird was extracted intact, placed in a numbered bag, and stored at −20 °C. Samples were then transported to the University of Tasmania where they were thawed for 1–2 h, weighed using an electronic balance to 0.00001 g, and the length and width recorded with digital Vernier calipers to 0.01 mm. Care was taken to prevent contamination of samples with ultrafine plastic particles from outside sources: all samples were processed in the presence of a fume hood, and work benches and tools were wiped clean with sterile paper and 70% ethanol between samples, all lab consumables were made of glass, and researchers wore lab coats and clothing made of non-synthetic materials whenever possible.

2.2. Digestion treatments

Two digestion agents, 1 M KOH (Kühn et al., 2017) and 2.5 ml corolase 7089 (Catarino et al., 2017), were selected for this study, based on cost, accessibility, treatment time, known or predicted effect on plastic polymers, and ease of transport (Table 1).

When digesting samples using KOH, we followed the procedures similar to those outlined by Kühn et al. (2017). However, as this protocol was developed for fish tissue (sprat *Sprattus sprattus*), we completed initial tests to determine an optimal digestion protocol for seabird gizzards. In brief, a sub-set of gizzards was randomly assigned to two test groups: room temperature (18–21 °C) and heat bath (60 °C). This temperature (60 °C) was chosen as it is below the melting point of most polymers. The room temperature samples achieved near complete digestion (i.e., only oily fatty deposits remained) after 15 days of exposure, while small remnants of the gizzards remained after only 10 h of exposure to the heat bath. To ensure complete digestion, for our final protocol each gizzard was placed in a glass flask containing 20 ml of 1 M KOH solution, then in a 60 °C water bath for 12 h. The residual solution was then passed through nested mesh sieves in descending size order (4.75, 1.00, and 0.33 mm) to capture the liberated plastics. KOH was chosen because it is inexpensive (AU$0.16 per gizzard), readily available, can be safely transported, and the treatment time was relatively short.

For the enzymatic digestion, we used 2.5 ml of corolase (activity 840 UHb) in 25 ml of filtered H_2O following methods similar to Catarino et al. (2017). As this protocol was developed for mollusk tissue (blue mussel *Mytilus edulis*), we again completed tests to determine an appropriate strategy for seabird gizzards. A sub-set was randomly assigned to three test groups: 0.5 ml of corolase in 50 ml filtered H_2O, 1.5 ml of corolase in 50 ml filtered H_2O, and 2.5 ml corolase in 25 ml filtered H_2O with the gizzard placed in a fresh solution every 3 h over a 9 h period. Samples in the first two groups did not exhibit any evidence of digestion after 20 h, while the gizzards treated with higher enzymatic concentration were completely digested within 9 h. Based on these results, we used this combination as our final protocol.

Once the digestions were complete, the residual solution was passed through nested mesh sieves as described above. When required (i.e., for very small particles), plastic items were confirmed through visual identification using a dissecting microscope and floatation in a weak salt solution. Results are reported as frequency of occurrence (FO), number, and mass of plastics, with summary data as the mean ± standard deviation with range.

3. Results

A total of 57 Flesh-footed Shearwaters gizzards were examined. Mean mass, length, and width was 2.05 ± 0.66 g, 22.48 ± 4.27 mm, and 17.52 ± 2.60 mm, respectively. A total of 359 pieces of plastic were recovered, with 91.5% of gizzards containing plastic with an average of 0.05 ± 0.06 g and 6.02 ± 5.50 pieces of plastic (Table S1). The majority (60.4%; $n = 217$) of recovered plastic items were in the micro-plastic (1.00–4.74 mm) size range, with mean length 3.02 ± 1.00 mm and width 2.11 ± 0.86 mm. Macro-plastics (≥ 4.75 mm) were also abundant, accounting for 35.9% ($n = 129$) of items recovered (length 8.38 ± 7.03 mm, width 3.24 ± 1.95 mm). Ultrafine debris (i.e., items extracted from the 0.99–0.33 mm sieve) accounted for 3.6% of all items (13/359 items). These 13 particles were recovered from six of the 57 gizzards (FO: 7.01%). Mean length, width, and mass of the 13 ultrafine particles were 0.95 ± 0.21 mm, 0.58 ± 0.22 mm, and 0.0005 ± 0.0009 g, respectively. All the ultrafine particles were classified as hard fragments (Fig. 1).

The cost per gizzard using 20 ml of 1 M KOH was $0.16/sample (2017 AUD). Corolase was inherently more expensive and needed to be replenished at 3-hourly intervals due to the activity potency, increasing the cost per gizzard to $9.30 (2017 AUD). Digestion using the KOH

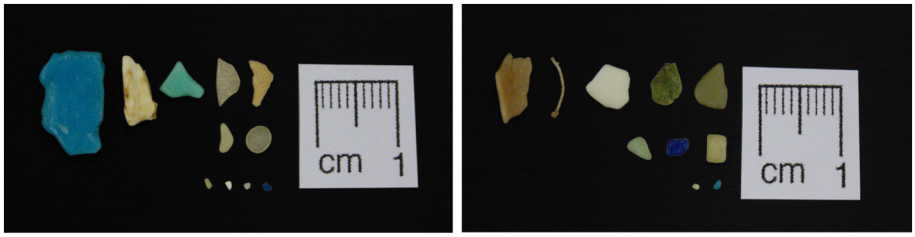

Fig. 1. Plastic items extracted from two Flesh-footed Shearwater gizzards after chemical and enzymatic digestion. In each panel, macro-plastics (> 4.75 mm) are shown in the top row, micro-plastics (> 1.00–4.74 mm) in the middle row, and ultrafine particles (1 μm–1 mm) along the bottom row.

solution was straightforward, requiring little or no ongoing adjustment during the digestion process while the corolase digestion required replacing the corolase with a fresh batch every 3 h to maintain the subculture *Bacillus subtilis* activity potency (total volume used over 10 h is 7.5 ml corolase in 75 ml filtered H_2O). KOH is also readily available from a variety of commercial suppliers whereas corolase 7089 could only be purchased in bulk (~25 kg) from a limited number of overseas suppliers (Table 1).

4. Discussion

While our understanding of plastic pollution is increasing rapidly, there are key areas where a lack of information limits our ability to accurately describe the pressures plastic imposes on species and ecosystems. Quantitative data on the presence of ultrafine particles is one such area. Recent findings from a handful of studies of fish and invertebrates suggest that particles in this size range are abundant and have the potential to cause significant harm (Chen et al., 2017; Lu et al., 2016; Shen et al., 2019; Wegner et al., 2012). To date, there is no information on the hazard ultrafine particles may pose for birds. This is due, in part, to the difficulty of identifying and working with tiny particles, particularly in the field (e.g., remote areas where lab facilities are limited or unavailable). However, the recent development of a standardized method for describing ingested plastic in birds enables reliable data to be collected on small particles under most scenarios (Provencher et al., 2017).

Plastic items ingested by seabirds have been traditionally collected in one of two ways: necropsy of dead birds, or lavage (stomach flushing) of live birds. The latter is a non-lethal method of collecting samples from a random sub-set of the population (Barrett et al., 2007). However, lavage suffers from imperfect detection as only the proventriculus is emptied, and a small proportion of plastic items are missed due to tissue obstruction (Hutton et al., 2008). Successful digestion of soft tissues and recovery of plastics in marine invertebrates (e.g., Courtene-Jones et al., 2017b) suggested chemical or enzymatic digestion may provide an opportunity to separate ingested plastics from seabird tissues in a rapid and reliable manner. We found that digestion of seabird gizzards using KOH was both time and cost effective, with all soft tissue digested, leaving only plastic debris and hard biological fragments (e.g., squid beaks). The addition of a 60 °C water bath reduced previous published treatment times by ~75% (from 2 days to 12 h; Table 1) with no apparent consequences. In contrast, digestion of seabird gizzards using corolase was more challenging due to the high cost and limited accessibility of this reagent.

Current protocols for examining ingested plastic in seabirds typically use a 1 mm mesh sieve to capture and sort liberated plastics into size classes after necropsy or lavage (Provencher et al., 2017). Sieves with smaller dimensions are uncommon as they can become blocked with biological material, and items < 1 mm have been considered rare (OSPAR, 2015) or of low interest in studies of seabirds as they are assumed to pass through the digestive tract (Provencher et al., 2017). Our results demonstrate that discounting ingested plastics < 1 mm underestimates plastic load in 7% of Flesh-footed Shearwaters. While the mass of ultrafine particles in each of these birds was low (0.0005 ± 0.0009 g), additional plastic particles passing through seabirds' digestive tracts increase the risk of harm from chemical pollutants and infectious agents present on the plastics' surface (Kirstein et al., 2016; Lavers et al., 2014). It is possible some, or all, of the ultrafine particles arose through the breakup of larger items in either the proventriculus or through the mechanical grinding of the gizzard. As noted by Terepocki et al. (2017), plastic fragments in two seabird species decreased in size from the proventriculus and gizzard, with the smallest items being detected in the intestines.

While our findings have improved our understanding of the occurrence of ultrafine particles in seabirds, there remains no data on nano-sized plastics for any seabird species. Both size classes play an important role in facilitating the translocation of toxins and microbes, with nano-plastics able to pass through the gut epithelial membrane and circulatory system of organisms (Mattsson et al., 2018; Shen et al., 2019; von Moos et al., 2012). Further development of extraction and quantification methods for ultrafine and nano-sized particles will enable research on a wider range of species, and for larger numbers of samples to be processed quickly in both laboratory and field settings.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.marpolbul.2019.04.001.

Acknowledgements

We are grateful for the support of the Lord Howe Island community, especially T. Adams, L. Bryce, and J. & C. Schick. Financial and logistical support was provided by Detached Foundation, L. Bryce, C. Noone, and Living Ocean Foundation (especially B. & I. Fulton). The University of Tasmania Animal Ethics (A16357), New South Wales Office of Environment and Heritage (SL100169), and Lord Howe Island Board (LHIB 06/16) granted permission for this work, with support from the Australian Bird and Bat Banding Service (ABBBS). J. Benjamin kindly provided the photos for Fig. 1 and two anonymous reviewers provided feedback on earlier versions of this manuscript.

References

Avio, C.G., Gorbi, S., Regoli, F., 2015. Experimental development of a new protocol for extraction and characterization of microplastics in fish tissues: first observations in commercial species from Adriatic Sea. Mar. Environ. Res. 111, 18–26.
Barrett, R.T., Camphuysen, C.J., Anker-Nilssen, T., Chardine, J.W., Furness, R.W., Garthe, S., Huppop, O., Leopold, M.F., Montevecchi, W.A., Veit, R.R., 2007. Diet studies of seabirds: a review and recommendations. ICES J. Mar. Sci. 64, 1675–1691.
Burger, J., Gochfeld, M., 2004. Marine birds as sentinels of environmental pollution. EcoHealth 1, 263–274.
Catarino, A.I., Thompson, R., Sanderson, W., Henry, T.B., 2017. Development and optimization of a standard method for extraction of microplastics in mussels by enzyme digestion of soft tissues. Environ. Toxicol. Chem. 36, 947–951.
Cedervall, T., Hansson, L.-A., Lard, M., Frohm, B., Linse, S., 2012. Food chain transport of nanoparticles affects behaviour and fat metabolism in fish. PLoS One 7, e32254.
Chen, Q., Gundlach, M., Yang, S., Jiang, J., Velki, M., Yin, D., Hollert, H., 2017. Quantitative investigation of the mechanisms of microplastics and nanoplastics toward zebrafish larvae locomotor activity. Sci. Total Environ. 584-585, 1022–1031.
Claessens, M., Van Cauwenberghe, L., Vandegehuchte, M.B., Janssen, C.R., 2013. New techniques for the detection of microplastics in sediments and field collected organisms. Mar. Pollut. Bull. 70, 227–233.
Cole, M., Lindeque, P., Halsband, C., Galloway, T.S., 2011. Microplastics as contaminants

in the marine environment: a review. Mar. Pollut. Bull. 62, 2588–2597.
Cole, M., Webb, H., Lindeque, P.K., Fileman, E.S., Halsband, C., Galloway, T.S., 2014. Isolation of microplastics in biota-rich seawater samples and marine organisms. Sci. Rep. 4, 4528.
Courtene-Jones, W., Quinn, B., Gary, S.F., Mogg, A.O.M., Narayanaswamy, B.E., 2017a. Microplastic pollution identified in deep-sea water and ingested by benthic invertebrates in the Rockall trough, North Atlantic Ocean. Environ. Pollut. 231, 271–280.
Courtene-Jones, W., Quinn, B., Murphy, F., Gary, S.F., Narayanaswamy, B.E., 2017b. Optimisation of enzymatic digestion and validation of specimen preservation methods for the analysis of ingested microplastics. Anal. Methods 9, 1437–1445.
Dehaut, A., Cassone, A., Frère, L., Hermabessiere, L., Himber, C., Rinnert, E., Rivière, G., Lambert, C., Soudant, P., Huvet, A., Duflos, G., Paul-Pont, I., 2016. Microplastics in seafood: benchmark protocol for their extraction and characterization. Environ. Pollut. 215, 223–233.
Denbow, D.M., 2000. In: Whittow, C.G. (Ed.), Gastrointestinal anatomy and physiology. Academic Press, London, pp. 299–326.
Foekema, E.M., De Gruijter, C., Mergia, M.T., van Franeker, J.A., Murk, A.J., Koelmans, A.A., 2013. Plastic in North Sea fish. Environ. Sci. Technol. 47, 8818–8824.
van Franeker, J.A., Blaize, C., Danielsen, J., Fairclough, K., Gollan, J., Guse, N., Hansen, P.L., Heubeck, M., Jensen, J.K., Le Guillou, G., Olsen, B., Olsen, K.O., Pedersen, J., Stienen, E.W., Turner, D.M., 2011. Monitoring plastic ingestion by the Northern Fulmar *Fulmarus glacialis* in the North Sea. Environ. Pollut. 159, 2609–2615.
Hammer, J., Kraak, M.H.S., Parsons, J.R., 2012. Plastics in the marine environment: The dark side of a modern gift. In: Whitacre, D.M. (Ed.), Reviews of Environmental Contamination and Toxicology. Springer Science, pp. 1–44.
Hutton, I., Carlile, N., Priddel, D., 2008. Plastic ingestion by flesh-footed shearwaters, *Puffinus carneipes*, and wedge-tailed shearwaters, *Puffinus pacificus*. Pap. Proc. R. Soc. Tas. 142, 67–72.
Kirstein, I.V., Kirmizi, S., Wichels, A., Garin-Fernandez, A., Erler, R., Löder, M., Gerdts, G., 2016. Dangerous hitchhikers? Evidence for potentially pathogenic *Vibrio* spp. on microplastic particles. Mar. Environ. Res. 120, 1–8.
Kühn, S., van Werven, B., van Oyen, A., Meijboom, A., Bravo Rebolledo, E.L., van Franeker, J.A., 2017. The use of potassium hydroxide (KOH) solution as a suitable approach to isolate plastics ingested by marine organisms. Mar. Pollut. Bull. 115, 86–90.
Lavers, J.L., 2015. Population status and threats to flesh-footed shearwaters (*Puffinus carneipes)* in south and Western Australia. ICES J. Mar. Sci. 72, 316–327.
Lavers, J.L., Bond, A.L., 2016. Ingested plastic as a route for trace metals in Laysan Albatross (*Phoebastria immutabilis*) and Bonin Petrel (*Pterodroma hypoleuca*) from Midway Atoll. Mar. Pollut. Bull. 110, 493–500.
Lavers, J.L., Bond, A.L., Hutton, I., 2014. Plastic ingestion by flesh-footed shearwaters (*Puffinus carneipes*): implications for chick body condition and the accumulation of plastic-derived chemicals. Environ. Pollut. 187, 124–129.
Lavers, J.L., Hutton, I., Bond, A.L., 2019. Changes in technology and imperfect detection of nest contents impedes reliable estimates of population trends in burrowing seabirds. Glob. Ecol. Conserv. 17, e00579.
Lebreton, L.C.M., van der Zwet, J., Damsteeg, J.-W., Slat, B., Andrady, A., Reisser, J., 2017. River plastic emissions to the world's oceans. Nat. Commun. 8, 15611.
Lu, Y., Zhang, Y., Deng, Y., Jiang, W., Zhao, Y., Geng, J., Ding, L., Ren, H., 2016. Uptake and accumulation of polystyrene microplastics in zebrafish (*Danio rerio*) and toxic effects in liver. Environ. Sci. Technol 50, 4054–4060.
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*. Environ. Pollut. 199, 185–191.
Mattsson, K., Johnson, E.V., Malmendal, A., Linse, S., Hansson, L.-A., Cedervall, T., 2017. Brain damage and behavioural disorders in fish induced by plastic nanoparticles delivered through the food chain. Sci. Rep. 7, 11452.
Mattsson, K., Jocic, S., Doverbratt, I., Hansson, L.-A., 2018. Chapter 13 - Nanoplastics in the aquatic environment. In: Zeng, E.Y. (Ed.), Microplastic Contamination in Aquatic Environments. Elsevier, pp. 379–399.
Miller, M.E., Kroon, F.J., Motti, C.A., 2017. Recovering microplastics from marine samples: a review of current practices. Mar. Pollut. Bull. 123, 6–18.
von Moos, N., Burkhardt-Holm, P., Kohler, A., 2012. Uptake and effects of microplastics on cells and tissue of the blue mussel *Mytilus edulis* L. after an experimental exposure. Environ. Sci. Technol. 46, 11327–11335.
OSPAR, 2015. Guidelines for Monitoring of Plastic Particles in Stomachs of Fulmars in the North Sea Area, OSPAR Commission Agreement 2015-03e. (26pp).
PlasticsEurope, 2018. Plastics - the Facts 2018: An Analysis of European Plastics Production, Demand and Waste Data. PlasticsEurope Market Research Group, Brussels, Belgium.
Provencher, J.F., Bond, A.L., Avery-Gomm, S., Borrelle, S.B., Bravo Rebolledo, E.L., Hammer, S., Kühn, S., Lavers, J.L., Mallory, M.L., Trevail, A.M., van Franeker, J.A., 2017. Quantifying ingested debris in marine megafauna: a review and recommendations for standardization. Anal. Methods 9, 1454–1469.
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. Sci. Total Environ. 644, 1477–1484.
Reid, T., Hindell, M., Lavers, J.L., Wilcox, C., 2013. Re-examining mortality sources and population trends in a declining seabird: using Bayesian methods to incorporate existing information and new data. PLoS One 8, e58230.
Reisser, J., Slat, B., Noble, K., du Plessis, K., Epp, M., Proietti, M., de Sonneville, J., Becker, T., Pattiaratchi, C., 2014. The vertical distribution of buoyant plastics at sea. Biogeosci. Discuss. 11, 16207–16226.
Rocha-Santos, T., Duarte, A.C., 2015. A critical overview of the analytical approaches to the occurrence, the fate and the behavior of microplastics in the environment. Trends Anal. Chem. 65, 47–53.
Santo, N., Fascio, Torres, F., Guazzoni, N., Tremolada, P., Bettinetti, R., Mantecca, P., Bacchetta, R., 2014. Toxic effects and ultrastructural damages to *Daphnia magna* of two differently sized ZnO nanoparticles: does size matter? Water Res. 53, 339–350.
Shen, M., Zhu, Y., Zhang, Y., Zeng, G., Wen, X., Yi, H., Ye, S., Ren, X., Song, B., 2019. Micro(nano)plastics: unignorable vectors for organisms. Mar. Pollut. Bull. 139, 328–331.
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. Mar. Pollut. Bull. 69, 219–222.
Tekman, M.B., Gutow, L., Macario, A., Haas, A., Walter, A., Bergmann, M., 2019. LITTERBASE.
Terepocki, A.K., Brush, A.T., Kleine, L.U., Shugart, G.W., Hodum, P., 2017. Size and dynamics of microplastic in gastrointestinal tracts of Northern Fulmars (*Fulmarus glacialis*) and Sooty Shearwaters (*Ardenna grisea)*. Mar. Pollut. Bull. 116, 143–150.
Teuten, E.L., Saquing, J.M., Knappe, D.R.U., Barlaz, M.A., Jonsson, S., Björn, A., Rowland, S.J., Thompson, R.C., Galloway, T.S., Yamashita, R., Ochi, D., Watanuki, Y., Moore, C., Viet, P.H., Tana, T.S., Prudente, M., Boonyatumanond, R., Zakaria, M.P., Akkhavong, K., Ogata, Y., Hirai, H., Iwasa, S., Mizukawa, K., Hagino, Y., Imamura, A., Saha, M., Takada, H., 2009. Transport and release of chemicals from plastics to the environment and to wildlife. Philos. Trans. Royal Soc. B. 364, 2027–2045.
Thompson, R.C., Swan, S.H., Moore, C.J., vom Saal, F.S., 2009. Our plastic age. Philos. Trans. Royal Soc. B. 364, 1973–1976.
Wegner, A., Besseling, E., Foekema, E.M., Kamermans, P., Koelmans, A.A., 2012. Effects of nanopolystyrene on the feeding behavior of the blue mussel (*Mytilus edulis* L.). Environ. Toxicol. Chem. 31, 2490–2497.
Worm, B., Lotze, H.K., Jubinville, I., Wilcox, C., Jambeck, J.R., 2017. Plastic as a persistent marine pollutant. Annu. Rev. Environ. Resour. 42, 1–26.
Zhao, S., Zhu, L., Li, D., 2016. Microscopic anthropogenic litter in terrestrial birds from Shanghai, China: not only plastics but also natural fibers. Sci. Total Environ. 550, 1110–1115.