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Plastic ingestion by fish in the Southern Hemisphere: A baseline study and review of methods

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ABSTRACT

Plastic ingestion is well documented among marine birds and sea turtles but fewer studies have investigated ingestion in fish, particularly in the Southern Hemisphere. We investigated the frequency of plastic ingestion in 21 species of fish and one species of cephalopod. The overall occurrence of plastic ingestion was 0.3%. Two micro-plastic items were recovered from the gastrointestinal tract of a single Antarctic toothfish (*Dissostichus mawsoni*). Ingestion rates were similar to other studies of fish conducted in both the Northern and Southern Hemispheres, however comparisons across species and locations are challenging due to the lack of consistency in the identification and classification of plastic debris. In response, we propose a standardised sampling protocol based on the available literature to provide a stronger basis for comparisons among existing and future studies of plastic ingestion in fish.

1. Introduction

Plastic pollution is widespread throughout the world's marine environments (Eriksen et al., 2014; Thompson et al., 2004). Current production, use, and disposal of plastic materials is not sustainable and presents significant concerns in terms of its introduction and subsequent accumulation in the global oceans (Thompson et al., 2009). Marine plastic debris originates from land and sea, entering the ocean as a result of both deliberate and accidental actions. Research suggests there are five trillion plastic items, weighing more than 243,978 million metric tonnes (MT), currently floating at the ocean's surface (Eriksen et al., 2014; Jambeck et al., 2015). Once present in the marine environment, plastic items are dispersed via oceanic currents and wind patterns, resulting in their global manifestation which extends throughout the water column (Barnes et al., 2009; Lebreton et al., 2012). In addition to this ubiquitous distribution, there are regions where debris is known to accumulate in substantial concentrations, most notable are the five oceanic gyres located in each of the major ocean basins (Eriksen et al., 2014). Of great concern is that these same regions often exhibit increased abundance of wildlife due to associated upwelling processes and biological productivity (Jantz et al., 2013).

Plastic debris presents a significant threat to marine biota (Gall and Thompson, 2015; Vegter et al., 2014). Negative encounters between wildlife and marine plastic pollution have increased from 267 species in 1997 (Laist, 1997) to 693 species in 2015 (Gall and Thompson, 2015), demonstrating an increase of nearly 75% in less than two decades. Major threats to marine life are from entanglement, or the direct and sub-lethal effects of ingestion, exposing wildlife to pollutants absorbed to the surface of plastic particles (Chua et al., 2014; Lavers and Bond, in press; Lavers et al., 2014; Tanaka et al., 2013). Throughout the water column, plastic objects exist in a variety of colours, shapes, sizes and densities (Reisser et al., 2014). These items degrade slowly in the marine environment, persisting for long periods of time, and are subsequently available for entry into the marine food web via ingestion by zooplankton (Cole et al., 2013), invertebrates (Graham and Thompson, 2009), fish (Davison and Asch, 2011), sea turtles (Di Beneditto and Awabdi, 2014), birds (Lavers et al., 2014) and marine mammals (Gall and Thompson, 2015).

Once ingested, plastic debris can contribute to a wide range of impacts including internal blockages and disrupted digestion (Hjelmeland et al., 1988; Jackson et al., 2000), biomagnification of harmful chemicals associated with plastics up the food web (Farrell and Nelson, 2013; Teuten et al., 2009), and a growing list of sub-lethal effects including morbidity (Lavers et al., 2014), liver toxicity (Rochman et al., 2013), endocrine disruption (Rochman et al., 2014) and neurotoxic effects (Oliveira et al., 2013). There is conflicting evidence in the literature regarding the retention times of plastic in the stomachs and intestines of marine wildlife (Hoss and Settle, 1990; Ryan, 2015) and the ability of fish to pass plastic items through their digestive tract (Hoss and Settle, 1990; Van Noord et al., 2013).

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Ingestion of marine plastic by fish was first reported by Carpenter et al. (1972). The majority of studies since then have been undertaken during the past five years focusing almost exclusively on Northern Hemisphere species (Anastasopoulou et al., 2013; Boerger et al., 2010; Davison and Asch, 2011; Foekema et al., 2013; Jantz et al., 2013; Lusher et al., 2013; Romeo et al., 2015). Far less research has been conducted on Southern Hemisphere species (Cliff et al., 2002; Di Beneditto and Awabdi, 2014; Ramos et al., 2012) as well as in freshwater environments (Faure et al., 2012; Sanchez et al., 2014). Of the handful of plastic ingestion studies conducted in the Southern Hemisphere (Appendix 1), none have investigated fish in Australian waters.

Recent estimates suggest plastic pollution is present in substantial quantities in Southern Hemisphere marine environments (Eriksen et al., 2014) and is therefore available for ingestion by fish and other species. Exceptionally high rates of plastic ingestion by seabirds foraging in the Tasman Sea off eastern Australia (Lavers et al., 2014), along with sporadic reports from southern opah (*Lampris immaculatus*) in the Southern Ocean (Jackson et al., 2000), and tiger sharks (*Galeocerdo cuvier*) in northern Australia (Stevens and McLoughlin, 1991) provide further evidence of plastic in Australian waters. However, observations of ingestion were incidental to the primary aims of most of these studies and as a result, the frequency of plastic ingestion and its associated impacts on Australian fish remains largely unknown.

One of the challenges to assessing the impacts of plastic in marine environments is the lack of standardised methodologies used across studies, making comparisons among them problematic. For example, there are inconsistencies in the size classes used to describe plastic items. While mega- (>100 mm), macro- (>20 mm), meso- (>5 mm) and micro-plastic (<5 mm) classifications are generally accepted in most studies (Barnes et al., 2009; Romeo et al., 2015; Ryan et al., 2009; Sanchez et al., 2014), others have used varying size categories (Dantas et al., 2012; Eriksen et al., 2014; Romeo et al., 2015).

The primary aims of this study were twofold: to address the paucity of quantitative and qualitative information regarding plastic ingestion by fish in Australian waters by describing the frequency of occurrence, size, and types of plastic ingested; and to develop a standardised sampling protocol from the available literature that will maximise the value of data collected in future studies as well as facilitating direct comparisons among them.

2. Materials and methods

2.1. Specimen collection and analysis

Twenty-one species of fish and one species of cephalopod were collected for this study (Table 1). The majority of fish were wild-caught with a small subset obtained from Australian fish markets. Most marine specimens were sampled from southeast Australian waters, Nichol's lanternfish (*Gymnoscopelus nicholsi*) were sourced from the Southern Ocean (Fig. 1), as were Antarctic toothfish (*Dissostichus mawsoni*), which were recovered from illegal gillnets deployed off the Banzare Banks (Fig. 1). The freshwater species Shannon galaxias (*Paragalaxias dissimilis*) was collected from the Great Lake in the central northern region of Tasmania. Fish were primarily caught during 2010–2015, however *G. nicholsi* specimens were sampled during the 1990 Australian Antarctic Division's KHIPPER cruise. Freshly caught fish were frozen after capture, while samples of *G. nicholsi* and *P. dissimilis* were preserved in 10% formalin and 70% ethanol, respectively.

To ensure a sterile working environment free of plastic contamination, all laboratory surfaces and equipment were cleaned using 100% ethanol and then visually inspected for the presence of plastic fragments. Necroscopies were undertaken in a laminar flow cabinet to prevent airborne contamination. The majority of fish were whole, allowing measurements of total length (TL), a straight line measure (not measured over the curve of the body) from the tip of the snout to the longest lobe of the caudal fin (cm), body weight (g), girth (maximum length between the ventral and dorsal sides; cm), sex (male, female or immature, where determinable) and general body condition, determined by the presence of physical injury and/or parasites. *Platycephalus bassensis* were provided to the project as stomachs only and associated biological data were unavailable. Inspection of the intestinal contents could not be undertaken for species where only stomachs or stomach contents were provided, including *P. bassensis* and *Conger verreauxi*.

Where intact fish were available, the entire gastrointestinal tracts were dissected from the tip of the oesophagus to the vent. Visual inspection was undertaken as per Di Beneditto and Awabdi (2014) to determine if any ulcerations, perforations, or obstructions were caused due to ingested plastic items. Full stomachs were weighed using an electronic balance (precision ± 0.0001 g). Contents of the digestive tract were washed into a clean petri dish and empty stomachs were re-weighed to determine content mass. Plastics were identified via visual inspection and buoyancy tests in deionised water (Hidalgo-Ruz et al., 2012). Contents were passed through a series of Tyler sieves (0.33, 1.00, and 4.75 mm) and carefully examined under a dissecting microscope to determine their likely nature (e.g., prey or plastic).

2.2. Plastic analysis

Potential plastic items, including unidentified and miscellaneous objects, removed from the gastrointestinal tracts of fish were rinsed gently to remove organic materials, dried, and weighed using an electronic balance. Each item was examined under a dissecting microscope and categorised by colour, type, degree of degradation, malleability, and provenance wherever possible. The longest and widest dimensions were recorded using vernier callipers. Items were analysed by Fourier Transform Infrared Spectrometry (FT-IR) at the University of Tasmania's Central Science Laboratory to determine polymer type. FT-IR analyses were performed using a Bruker Vertex 70 Spectrometer with a DLaTGS room temperature detector. The larger sample was run using Zinc Selenide Attenuated Total Reflectance (ZeSe ATR) at 4500–600 wavenumbers (cm^{-1}) with a resolution of four wavenumbers (cm^{-1}). Thirty-two scans were performed for the background and the sample. Microscopic samples were run with a Bruker Hyperion 3000 microscope using a 20× ATR Germanium objective and an MCT detector (liquid nitrogen cooled) at 4000–500 wavenumbers (cm^{-1}) with a four wavenumber (cm^{-1}) resolution. One-hundred and twenty-eight scans were performed for the background and samples. Spectral processing included atmospheric compensation, cutting (e.g., from 4000 to 3500 wavenumbers cm^{-1}), and an extended ATR correction. All output spectra were compared to the FT-IR Raman spectral library to determine the identities of the samples using the first derivative search function.

2.3. Development of standardised approach

A search of the available literature was performed using databases Scopus, Web of Science, and ScienceDirect. The key words used for each database search included combinations of “fish”, “plastic”, “marine debris”, “polyethylene”, “packaging”, “synthetic”, and “litter”. Information on the type of study, sampling procedure, laboratory analyses, plastic identification processes and plastic categorisation systems was extracted from each article. Each report was critically reviewed and data collated to allow for a comparative analysis and general overview of project approaches.

3. Results

3.1. Presence of plastic marine debris

A total of 342 fish from 21 species, representing 17 fish families of class Actinopterygii (ray-finned fishes) were examined (Table 1). Five cephalopod specimens were also analysed (Table 1). Of the 347 samples, plastic was present in one individual (0.3%). Two micro-plastic

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