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Ingestion of microplastic debris by green sea turtles (*Chelonia mydas*) in the Great Barrier Reef: Validation of a sequential extraction protocol

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ABSTRACT

Ocean contamination by plastics is a global issue. Although ingestion of plastic debris by sea turtles has been widely documented, contamination by microplastics (< 5 mm) is poorly known and likely to be under-reported. We developed a microplastic extraction protocol for examining green turtle (*Chelonia mydas*) chyme, which is multifarious in nature, by modifying and combining pre-established methods used to separate microplastics from organic matter and sediments. This protocol consists of visual inspection, nitric acid digestion, emulsification of residual fat, density separation, and chemical identification by Fourier transform infrared spectroscopy. This protocol enables the extraction of polyethylene, high-density polyethylene, (aminoethyl) polystyrene, polypropylene, and polyvinyl chloride microplastics > 100 µm. Two macroplastics and seven microplastics (two plastic paint chips and five synthetic fabric particles) were isolated from subsamples of two green turtles. Our results highlight the need for more research towards understanding the impact of microplastics on these threatened marine reptiles.

1. Introduction

Plastics are one of the most common and persistent pollutants in coastal and marine environments worldwide (Gall and Thompson, 2015; Moore, 2008). Anthropogenic marine debris was first identified as an issue in the Great Barrier Reef two decades ago (Haynes, 1997). Recent estimates suggest that > 5 trillion pieces of plastic debris, weighing > 250,000 tons, may be floating in the world's oceans (Eriksen et al., 2014). These estimates of plastic pollution are higher if particles in beach sand and those deposited onto seafloors are also included (Galgani et al., 2015).

Plastic pollutants are broadly divided into two categories; macroplastics (> 5 mm) and microplastics (< 5 mm, Barnes et al., 2009; Moore, 2008). Both macro- and microplastics are ubiquitous and widespread in the marine environment; polluting the ocean surface, water column, and benthos (Cole et al., 2011; Galgani et al., 2015; Woodall et al., 2014). Microplastic pollutants are broadly classified as either primary or secondary microplastics (Cole et al., 2011). Primary microplastics are deliberately manufactured in the sub-visible size range, such as pelletised raw materials for manufacture of plastic products (Ashton et al., 2010) and plastic beads destined for use in processes and applications such as air-blasting, medicinal vectors and cosmetic exfoliants (Cole et al., 2011; Fendall and Sewell, 2009). Secondary microplastics are created by the physical, chemical, and biological degradation of plastic debris in the environment (Cole et al., 2011; Duis and Coors, 2016; Moore, 2008).

Marine life is mainly impacted by plastic debris through the processes of entanglement and ingestion (Derraik, 2002). Ingested macroplastics can either pass through the intestinal tract, or accumulate there for several months, effectively blocking the tract and/or reducing the feeding stimulus with lethal or sub-lethal effects (Laist, 1987; Lutz, 1990; Nelms et al., 2016; Santos et al., 2015). Ingestion of macroplastics has been implicated in the mortality of a wide range of organisms including sea birds (Provencher et al., 2014), cetaceans (Jacobsen et al., 2010; Laist, 1987), sirenians (Beck and Barros, 1991; Ceccarelli, 2009; Laist, 1987) and sea turtles (Santos et al., 2015).

Similarly, ingestion of microplastics has also been reported for a wide range of marine wildlife including fishes (Foekema et al., 2013),

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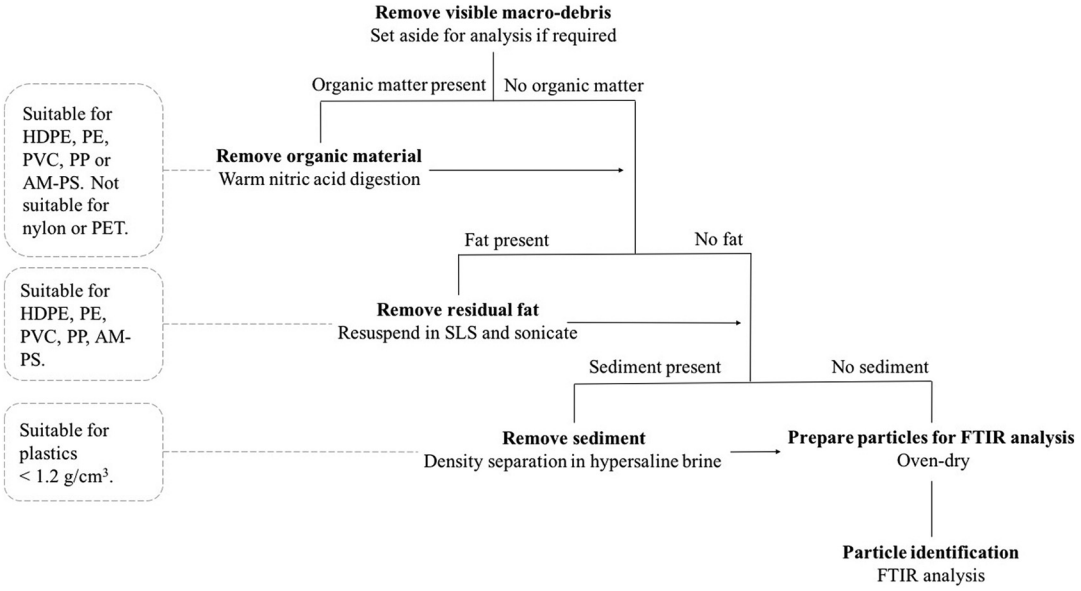

Fig. 1. Sequential extraction protocol for the extraction of microplastics from green turtle chyme showing polymer suitability.

cetaceans (Lusher et al., 2015), zooplankton (Sun et al., 2017) and sea turtles (Santos et al., 2015). Like macroplastics, ingested microplastics can impact organisms physically (Wright et al., 2013) and increasing concern has been expressed regarding their capacity to act as a vector for toxic chemicals (Besseling et al., 2013; Derraik, 2002; Moore, 2008; Von Moos et al., 2012). Once ingested, chemical effects can occur via three processes: 1) leaching: plasticisers, UV stabilisers, and other chemicals added to polymers during production leach into the organism post-ingestion; 2) sorption: pollutants such as polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs), metals, and pesticides adsorbed onto microplastics from the surrounding environment are released internally post-ingestion; and 3) trophic flow: accumulated toxins are bioaccumulated through the food chain (Bejgarn et al., 2015; Hamlin et al., 2015; Koelmans et al., 2014).

One iconic animal impacted by marine debris is the sea turtle. All seven turtle species are known to be affected by plastic debris globally (Clukey et al., 2017; Gall and Thompson, 2015; Nelms et al., 2016). Two factors that likely increase the risk of plastic ingestion by sea turtles relative to other marine species are: 1) visual feeding strategies which select for structures analogous to jellyfish and soft floating plastics, and 2) backward-facing oesophageal papillae which inhibit regurgitation and facilitate particle accumulation in the gut (Schuyler et al., 2014; Vegter et al., 2014; Wyneken, 2001).

Of all sea turtle species, the green turtle (*Chelonia mydas*, Linnaeus 1758) and leatherback turtle (*Dermochelys coriacea*, Vandelli 1761) are the most susceptible to marine debris because of their respective herbivorous and gelatinous diets (Di Beneditto and Awabdi, 2014; Schuyler et al., 2013). Plastic debris can also become entangled among green turtle food sources such as seagrass leaves and macroalgae (Awabdi et al., 2013). Microplastics have been found in sea turtle stomach content in Brazil and the North Atlantic (Mascarenhas et al., 2004; Pham et al., 2017), raising concerns about potential cumulative impacts of microplastics on these slow-growing animals, including dietary dilution and malnutrition (Nelms et al., 2016).

Methods for extracting microplastics have been developed for a range of sample matrices. Visual assessment using microscopy is routinely used to extract microplastics from waste water, sea water, sediments, ice, plant matter, biological tissues, and whole organisms. Density separation is commonly used to extract microplastics from water or sediments (Claessens et al., 2013; Hidalgo-Ruz et al., 2012; Reisser et al., 2013; Thompson et al., 2004). Chemical digestion is used to extract microplastics from whole organisms (Claessens et al., 2013) and from ingested material, for example, from pelagic fish or cetacean chyme (i.e. ingested material and digestive tract fluid) (Foekema et al., 2013; Lusher et al., 2015). Many of these methods are suitable and efficient for either homogenously organic or homogenously inorganic sample matrices; however, each of them alone is unlikely to be suitable for microplastic extraction from green turtle chyme. Green turtle chyme can have a diverse organic composition and can also contain sediments. In fact, when green turtles shift from their pelagic stage to coastal benthic habitats, their diet broadens from mainly animal matter such as jellyfish and sponges to include herbivorous components, particularly seagrass, algae, and associated sediments and epibionts (Bjorndal, 1997). Due to the diverse diet of coastal turtle populations, chyme from non-pelagic green turtles is expected to be relatively complex, comprising a range of organic (plant and animal material) and inorganic (mineral and sediment) matrices. Therefore, a protocol capable of efficiently extracting microplastics from all matrices is required in order to accurately establish contamination levels. The objective of this study is to develop and validate a microplastic extraction protocol suitable for investigating green turtle chyme samples, thereby improving method harmonisation in marine debris research (Tate et al., 2012).

2. Materials and methods

2.1. Sample collection and preparation

Two unsuccessfully rescued green turtles (Turtle A: StrandNet #55364 and Turtle B: StrandNet #53584), collected near Cairns (central Great Barrier Reef, Australia) were used for this study. Turtle A was a juvenile with a curved carapace length of 45.4 cm. Turtle B was an adult female with a curved carapace length of 103 cm. Foreguts (including oesophagus, stomach, and small intestine) of both turtles were necropsied, the rest of the digestive tract being required for a different study. The foregut content was visually inspected and any visible macroplastics were removed for subsequent analysis. For each turtle, chyme was transferred to a metal bucket and homogenised by manual stirring using a metal spoon.

2.2. Sequential extraction protocol

A preliminary pilot test using chemical digestion (HNO_3, 69.5%) of green turtle chyme was unsuccessful, as some fat and sediments remained. A sequential extraction protocol (Fig. 1) was therefore