



# Assessment tools for microplastics and natural fibres ingested by fish in an urbanised estuary<sup>☆</sup>

Jennifer E. Halstead<sup>a, \*</sup>, James A. Smith<sup>a</sup>, Elizabeth A. Carter<sup>b</sup>, Peter A. Lay<sup>b</sup>,  
Emma L. Johnston<sup>a</sup>

<sup>a</sup> Evolution & Ecology Research Centre, School of Biological, Earth & Environmental Sciences, University of New South Wales, Sydney, NSW 2052, Australia

<sup>b</sup> Vibrational Spectroscopy Core Facility, The School of Chemistry, The University of Sydney, NSW 2006, Australia

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## ABSTRACT

Microplastics and fibres occur in high concentrations along urban coastlines, but the occurrence of microplastic ingestion by fishes in these areas requires further investigation. Herein, the ingestion of debris (i.e., synthetic and natural fibres and synthetic fragments of various polymer types) by three benthic-foraging fish species *Acanthopagrus australis* (yellowfin bream), *Mugil cephalus* (sea mullet) and *Gerres subfasciatus* (silverbidy) in Sydney Harbour, Australia has been quantified and chemically speciated by vibrational spectroscopy to identify the polymer type. Ingested debris were quantified using gut content analysis, and identified using attenuated total reflectance Fourier transform infrared (ATR-FTIR) and Raman microspectroscopies in combination with principal component analysis (PCA). The occurrence of debris ingestion at the time of sampling ranged from 21 to 64% for the three species, and the debris number ranged from 0.2 to 4.6 items per fish for the different species, with ~53% of debris being microplastic. There was a significant difference in the amount of debris ingested among species; however, there was no difference among species when debris counts were standardised to fish weight or gut content weight, indicating that these species ingest a similar concentration of debris relative to their ingestion rate of other material. ATR-FTIR microspectroscopy successfully identified 72% of debris. Raman spectroscopy contributed an additional 1% of successful identification. In addition, PCA was used to non-subjectively classify the ATR-FTIR spectra resulting in the identification of an additional 9% of the debris. The most common microplastics found were polyester (PET), acrylic-polyester blend, and rayon (semi-synthetic) fibres. The potential of using Raman microspectroscopy for debris identification was investigated and provided additional information about the nature of the debris as well as the presence of specific dyes (and hence potential toxicity).

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## 1. Introduction

Society's consumption of plastic products has increased exponentially in recent years. This consumption combined with inadequate waste management has positioned plastic as one of the most prevalent forms of anthropogenic pollution in the marine environment (Jambeck et al., 2015; Ivar do Sul and Costa, 2014). This form of pollution now has a global distribution (Bergmann et al., 2000; GESAMP, 2016; Helm, 2017) and is consumed by numerous species from a range of habitats with diverse feeding strategies; e.g.

pelagic and benthic fishes, (Davison and Asch, 2011; Choy and Drazen, 2013; Romeo et al., 2015; Mizraji et al., 2017; McGoran et al., 2017) filter feeders (De Witte et al., 2014) and benthic infauna (Van Cauwenberghe et al., 2015).

This study applies the definition of microplastic as artificial polymers and plastics smaller than 5 mm (Arthur et al., 2009; Wright et al., 2013). They can be manufactured in this size class (e.g. beads or pre-production resin pellets), or can result from the fragmentation of larger plastic items. Fibrous synthetic particles (fibres) are a particularly prominent type of microplastic in the marine environment (Claessens et al., 2011). One major source of fibres is clothing, and 70 million tonnes of fibres are used in the global clothing industry yearly. Fibres from clothing, as well as other sources, can enter the marine environment through sewage, or stormwater outputs (Carr et al., 2016; Mintenig et al., 2017). This

<sup>☆</sup> This paper has been recommended for acceptance by Eddy Y. Zeng.

\* Corresponding author.

E-mail address: [jenniferehalstead@gmail.com](mailto:jenniferehalstead@gmail.com) (J.E. Halstead).

fibre contamination typically outnumbers other microplastic types with fibres over three times more prevalent in areas that are downstream of wastewater outfalls (Browne et al., 2010) and close to larger urban centres (Dris et al., 2015). Microplastic particles, including fibres, are problematic because they are a form of contamination that is small enough to be ingested by marine animals (GESAMP, 2016; Bergmann et al. 2000; Helm, 2017). This is a concern because ingested polymer fragments of this size can transfer from prey to predators (Farrell and Nelson, 2013), and potentially act as a vector for delivery of toxic chemicals to organisms to cause adverse biological impacts (Chua et al., 2014; Browne et al., 2013, 2015; Rochman et al., 2013, 2015). It is important to note that the potential role of microplastics as vectors for toxic chemical loads is not conclusive, and requires further studies of long-term effects to determine the environmental relevance (Koelmans et al., 2016).

Fish are known to ingest microplastic (Foekema et al., 2013; Lusher et al., 2013; Collard et al., 2015; Naidoo et al., 2016; Rummel et al., 2016; Mizraji et al., 2017), making them a potential vector of toxic chemicals through food chains and into human diets (Rochman et al., 2015). Due to typically high concentrations of microplastic (including both natural and synthetic fibres) along urbanised coasts (Mathalon and Hill, 2014), there is a need for more research in these areas to quantify and assess the extent of microplastic ingestion by marine biota, and the ecological significance of the phenomenon (Lönnstedt and Eklöv, 2016).

The three species investigated in this present study were yellowfin bream (*Acanthopagrus australis*; Owen, 1853), sea mullet (*Mugil cephalus*; Linnaeus, 1758) and silverbiddy (*Gerres subfasciatus*; Cuvier, 1830). Each of these species is widely distributed along eastern Australia in coastal areas and estuaries (Kailola et al., 1993), and each is commercially harvested, with sea mullet comprising the largest catch by weight of all fished species in NSW (Stewart et al., 2015). Yellowfin bream is also heavily fished recreationally (Stewart et al., 2015). In NSW, sea mullet and yellowfin bream are classified as 'fully fished' and silverbiddy as 'uncertain' (Stewart et al., 2015). Each of these three species is consumed by humans, in particular yellowfin bream, which is generally considered a table fish.

These species share a general 'benthic' feeding strategy, with yellowfin bream eating mostly benthic invertebrates as well as some plant material and fish (Kailola et al., 1993; Hadwen et al., 2007; Truong et al., 2017), sea mullet consuming detritus and benthic microalgae as well as infauna largely indiscriminately (Kailola et al., 1993; Hadwen et al., 2007; Whitfield et al., 2012), and silverbiddy likely consuming benthic invertebrates as well some algae and sediment (based on a closely related species; Platell et al., 1999). This would likely expose them to microplastics and natural fibres on the substrate, and potentially accumulating within the sediment (Barnes et al., 2009; McGoran et al., 2017). Measuring rates of microplastic ingestion enables a better understanding of the threat of microplastics to marine food webs and possibly human health. Sydney is the most populated city in Australia with more than 4.6 million inhabitants (Johnston et al., 2015), and its harbour has been chronically contaminated with metals, organochlorines, hydrocarbons and dioxins (Birch, 2000; Birch and Taylor, 2002). To the authors knowledge there are no published studies to date that have investigated microplastic pollution in this Harbour (Mayer-Pinto, 2015). This lack of research poses a potential issue for properly evaluating the health of the ecosystem. It may also be of concern from a human health perspective, as more research is carried out on the risks of microplastic present in commonly ingested seafoods. Recreational fishing pressure in Sydney Harbour is almost double that in nearby estuaries, despite public health warnings over fish tissue contamination (Hedge et al., 2013).

One limitation to our understanding of the extent of microplastic and natural fibre pollution and ingestion has been the difficulty in accurately identifying the constituent polymers and chemical additives of microplastics and fibres. Previous microplastic research has commonly used visual examination for microplastic debris identification, which subsequently lacks information about the specific polymer type or chemical composition of the microplastic (Choy and Drazen, 2013; Romeo et al., 2015; Desforges et al., 2014). Over 200 different chemicals have been detected in marine microplastic sampling (Rani et al., 2015), and without a more detailed understanding of the polymer type, it is difficult to understand how harmful specific types of microplastic can be to biota (Lusher et al., 2013). Additionally, the subjective nature of visual identification means researchers may inaccurately estimate quantities of microplastics when assessing the stomach contents of commonly studied animals, such as fish (Collard et al., 2015), for example by not distinguishing between natural and synthetic fibres (Lenz et al., 2015; Remy et al., 2015), both of which have potential toxicity.

In the past five years 50 published studies have used vibrational spectroscopy (Raman and infrared (IR)) to characterise and identify non-destructively the chemical composition of microplastics. Vibrational spectroscopy measures the energy that it takes for the bonds between atoms within molecules to vibrate. Raman and IR spectroscopy provide complementary information about the functional groups (e.g. C-H, C=C, C=O) present in a sample.

FTIR (Fourier transform infrared) spectroscopy is recognised as a very effective technique for microplastic identification (Mecozzi et al., 2016), although many researchers have used infrared spectrometers and sampling accessories that are not entirely well-suited to the collection of microscopic samples. Typical infrared sampling accessories used to analyse microplastics include; compression of the sample in a diamond anvil cell (DAC), production of a cast film, or the use of a macroscopic attenuated total reflectance (ATR) accessory (Obbard et al., 2014; Acosta-Coley and Olivero-Verbel, 2015; Nor and Obbard, 2014). More recent studies have employed FTIR microspectroscopy, which collects data using either transmission, reflectance or micro-ATR sampling modes (Song et al., 2014; Käppler et al., 2016). FTIR microspectroscopy enables investigation of suitable samples as small as 10 to 20 microns, however this can be limited by the sample thickness. An ATR crystal mounted on a microscope objective (micro-ATR) allows samples of any thickness to be measured including dark (highly absorbing) samples as well as providing information about the sample surface (Kazarian and Chan, 2010).

Raman microspectroscopy offers non-contact measurements of samples with a spatial resolution in the order of ~1 µm. A range of different laser sources (excitation lines) from the high-energy ultraviolet (UV) through to visible and near-IR (NIR) light can be used to generate a spectrum. It is well known that using visible light for Raman spectroscopic analysis of plastics can generate both a Raman signal as well as a strong fluorescence which is generally attributable to the pigments, additives or impurities within the sample (Siesler, 2011). To bleach or quench the fluorescence, which can mask Raman spectral features, a sample is often exposed to the laser for a longer time period. The use of a 785 nm wavelength excitation line is less likely to excite fluorescence and has a better scattering efficiency c.f. 1064 nm (Siesler, 2011).

Microplastics research could benefit from applying multivariate statistical techniques (chemometrics) to the complex spectroscopic data obtained with current technology (Comnea-Stancu et al., 2017). Principal component analysis (PCA) is one such form of chemometrics that analyses the data variance and identifies the independent principal components (PC) within the data set. PCA generates a PC score and PC loadings plots, which together describe

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