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Low levels of microplastics (MP) in wild mussels indicate that MP ingestion by humans is minimal compared to exposure via household fibres fallout during a meal☆

Ana I. Catarino [a, *], Valeria Macchia [b], William G. Sanderson [a, c], Richard C. Thompson [d], Theodore B. Henry [a, e]

[a] Center for Marine Biodiversity & Biotechnology, Institute of Life and Earth Sciences, EGIS, Heriot-Watt University, Edinburgh EH14 4AS, UK
[b] School of Applied Science, Edinburgh Napier University, Sighthill Campus, Sighthill Court, Edinburgh EH11 4BN, UK
[c] St Abbs Marine Station, St Abbs, Scottish Borders, TD14 5PW, UK
[d] Marine Biology and Ecology Research Centre, University of Plymouth, Devon PL4 8AA, UK
[e] Department of Forestry, Wildlife and Fisheries, and Center for Environmental Biotechnology, The University of Tennessee, Knoxville, TN, USA

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ABSTRACT

Microplastics (MPs) are the most numerous debris reported in marine environments and assessment of the amounts of MPs that accumulate in wild organisms is necessary for risk assessment. Our objective was to assess MP contamination in mussels collected around the coast of Scotland (UK) to identify characteristics of MPs and to evaluate risk of human exposure to MPs via ingestion of mussels. We deployed caged mussels (*Mytilus edulis*) in an urbanised estuary (Edinburgh, UK) to assess seasonal changes in plastic pollution, and collected mussels (*Mytilus* spp and subtidal *Modiolus modiolus*) from eight sampling stations around Scotland to enumerate MP types at different locations. We determined the potential exposure of humans to household dust fibres during a meal to compare with amounts of MPs present in edible mussels. The mean number of MPs in *M. modiolus* was 0.086 ± 0.031 (SE, $n = 6$)/g ww (3.5 ± 1.29 (SE) per mussel). In *Mytilus* spp, the mean number of MPs/g ww was 3.0 ± 0.9 (SE, $n = 36$) (3.2 ± 0.52 (SE) per mussel), but weight dependent. The visual accuracy of plastic fibres identification was estimated to be between 48 and 50%, using Nile Red staining and FT-IR methodologies, respectively, halving the observed amounts of MPs in wild mussels. We observed an allometric relationship between the number of MPs and the mussels wet weight. Our predictions of MPs ingestion by humans via consumption of mussels is 123 MP particles/y/capita in the UK and can go up to 4620 particles/y/capita in countries with a higher shellfish consumption. By comparison, the risk of plastic ingestion via mussel consumption is minimal when compared to fibre exposure during a meal via dust fallout in a household (13,731–68,415 particles/Y/capita).

1. Introduction

Increasing levels of plastic debris are among the most prominent environmental issues faced by government agencies worldwide (e.g. House of Commons, 2016). Small pieces of plastic [1 μm–5 mm, microplastics (MPs) (Arthur et al., 2009; Browne et al., 2007)] are the most numerous debris reported in marine environments (Eriksen et al., 2013), and contamination by these particulates can present a hazard for aquatic organisms (Cole et al., 2015; Wright et al., 2013). Ingestion of MPs by organisms can facilitate MP exposure across trophic levels (Farrell and Nelson, 2013), including a potential for human exposure via consumption of shellfish (Galloway, 2015). The transfer of small-sized MPs from the lumen of the gastrointestinal tract across epithelial membranes and into internal tissues appears to be minimal (Batel et al., 2016); however, further investigation is necessary, particularly to resolve potential absorption/accumulation of smaller plastic particles (<1 μm, nanoplastics) in tissues.

Despite the numerous concerns regarding the potential negative effects of plastic particles, the establishment of baseline

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* Corresponding author.
E-mail addresses: a.catarino@hw.ac.uk, catarino.anai@gamil.com (A.I. Catarino).

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observations and long-term monitoring programmes are still in their early days, especially relating to the use of marine biota, and are highly regional [e.g. San Francisco Bay, USA (Sutton and Sedlak, 2017)]. The determination of the levels of MP contamination in targeted organisms is crucial as it will allow establishment of a temporal and spatial comparison, and enable assessment of real environmental and human health risks.

Mussels, already well established biomonitors for environmental contaminants (Andral et al., 2011; Beyer et al., 2017; Kimbrough et al., 2008), are good candidates for assessment of MP exposure in the environment (Beyer et al., 2017). Mussels have the ability to filter large volumes of water [e.g. 30 ml min^{-1} for *Mytilus edulis* (Clausen and Riisgard, 1996),] and actively filter and trap suspended particulates such as algae and sediments (Bertolini et al., 2017; Engel et al., 2017). In laboratory experiments, the ingestion and retention of MPs within their gut has been observed [72 h (Ward and Kach, 2009) to up to 96 h (von Moos et al., 2012)]. The enumeration of particles can thus reflect an integrated exposure over time due to MPs retention either within the lumen of their digestive tract, within internal tissues, or even adherent to tissue surfaces. Because of their wide geographical and spatial distribution, that includes intertidal (e.g. *Mytilus* spp) and subtidal (e.g. *Modiolus modiolus*) environments, mussels can provide information on the MP contamination throughout various locations. However, to our knowledge, there are no long-term monitoring programmes specific for MPs contamination of mussels in place, comparable to other contamination assessment programmes such as the Mussel Watch Program led by The U.S. National Oceanic and Atmospheric Administration (NOAA) and the Mediterranean Science Commission (CIESM) Mussel Watch.

The establishment of MPs baseline levels in field mussels is problematic due to the difficulty of inter-studies comparisons. Currently, methods using enzymatic digestion have been developed to assess MP contamination in mussels, enabling a standard quantification of MPs (Catarino et al., 2017; Courtene-Jones et al., 2017). However, early works have used a variety of soft tissue digestions, some of which are aggressive to pH-sensitive polymers resulting in their destruction (Claessens et al., 2013). Recently, less aggressive digestion methods such as with the use of hydrogen peroxide and enzymatic digestion of soft tissue have reported a maximum number of 3 particles/g wet weight (ww) of tissue in farmed (Huahong, 2017, personal communication) *M. galloprovincialis* sampled in a food market in China (Li et al., 2015), and 4.44 particles/g ww of tissue in *M. edulis* from the west coast of Scotland (Courtene-Jones et al., 2017), respectively. However, representative concentration of particles associated with mussels over time and/or over a large geographic area are unknown.

Many species of the *Mytilus* genus (e.g. *M. edulis*, *M. galloprovincialis*, *M. californianus*) are of substantial commercial value as seafood items (Food and Agriculture Organization of the United Nations, 2017), and there are concerns about the potential for MP transfer and exposure in humans via ingestion (Galloway, 2015; Rochman et al., 2015; Van Cauwenberghe and Janssen, 2014). A potential load of 11,000 MPs per year to European shellfish consumers has been hypothesized (Van Cauwenberghe and Janssen, 2014), even if so far there is no evidence of the ingestion of MPs by humans through the food chain (CONTAM, 2016; Galloway, 2015). Furthermore, a recent statement issued by the European Food Safety Authority (EFSA) Panel for Contaminants in the Food Chain concludes that occurrence data in shellfish food items is limited (CONTAM, 2016), which implies that exposure levels are largely unknown.

Scotland offers a privileged space to assess plastic contamination in mussels, due to the large coastline (11,800 km) facing both the North Atlantic and the North Sea and the wide distribution of various mussels species. Blue mussel (*M. edulis*) farming is a significant economic activity with a registered production of 7732 tonnes in 2016 for the table market (Scottish Government, 2017) and the establishment of baseline data on the current status of MPs contamination in Scotland will have a significant impact in conservational policies. Furthermore, other species, such as the horse mussel (*Modiolus modiolus*), have a special conservational status (Kent et al., 2016) and are protected in all of OSPAR regions (OSPAR Commission, 2009). However, there is no information on the relationship of MPs with this species. Preliminary studies have shown that there is potential to use mussels to monitor the presence of MPs in the Scottish coast, and MP contamination has been reported in *M. edulis* specimens from the estuary of the Forth (Edinburgh) (Catarino et al., 2017) and in the west coast of Scotland (Courtene-Jones et al., 2017).

The aim of this project was to provide baseline information on the presence of MPs in mussels collected from intertidal and subtidal locations around Scotland, and to assess temporal variation of the MPs associated with *Mytilus edulis* placed in a caged field experiment. In particular, the objectives were: 1) to quantify the presence of MPs in *Mytilus spp* collected at various locations along the Scottish coast, 2) to assess presence of MPs in a subtidal mussel species (*Modiolus modiolus*) and 3) to assess presence of MPs in caged *Mytilus edulis* placed in Edinburgh (a highly populated area) over time (1 year). This work is the first to report on MPs associated with the protected species *M. modiolus* and to use displaced and caged mussels (*M. edulis*) to assess MP contamination. Finally, to clarify the potential human exposure to MPs via mussel consumption, when compared to other sources, we quantified the amount of airborne fibres that food items contaminated within regular household spaces, during the preparation and consumption of a meal. We compared the amount of MPs present within mussels with the amount of MPs that humans potentially consume via airborne fibre contamination of food items within typical households in Edinburgh UK.

2. Materials and methods

2.1. Port Edgar: caged deployed mussels

Live *Mytilus edulis* obtained from Scottish commercial suppliers, Scottish Shellfish Association, were transferred to Heriot-Watt University (HWU), Edinburgh, UK, and maintained at 10 °C in a temperature controlled chamber on 12-12 h light cycle in a static seawater tank (up to 1/3 seawater renewal every week) and fed a diet of live algae (a mixture of *Tetraselmis suecica* and *Tisochrysis lutea*) alternated with commercial Shellfish Diet 1800® (Reed Mariculture, USA). In 2015, during exposure periods, 16–18 mussels were held in the intertidal zone between Spring tides (two weeks) and evenly distributed in cylindrical stainless-steel cages (10 × 8 cm, height and diameter respectively, Fig. S1) in the estuary of the Forth River, Edinburgh, UK, in Port Edgar (N 55°, 59′42″, W 3°,24′30″). A passive sampler (Fig. S1) was attached to each cage, which consisted of a stainless-steel wired scrubber (i.e. pad pot cleaner) of spheroid shape of 5.5 × 2.5 cm. Following exposure, mussels and scrubs were collected and frozen until processing for enumeration of MPs. The number of processed samples per campaign was nine mussels, three randomly selected mussels per cage, and two passive samplers, i.e. scrubbers. To check for MPs presence and control the number of particles mussels might already have prior to exposure, reference mussels from the main stock were processed following the same procedure.