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Short communication

Trophic level transfer of microplastic: *Mytilus edulis* (L.) to *Carcinus maenas* (L.)

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ABSTRACT

This study investigated the trophic transfer of microplastic from mussels to crabs. Mussels (*Mytilus edulis*) were exposed to 0.5 μm fluorescent polystyrene microspheres, then fed to crabs (*Carcinus maenas*). Tissue samples were then taken at intervals up to 21 days. The number of microspheres in the haemolymph of the crabs was highest at 24 h (15 033 ml^{-1} ± SE 3146), and was almost gone after 21 days (267 ml^{-1} ± SE 120). The maximum amount of microspheres in the haemolymph was 0.04% of the amount to which the mussels were exposed. Microspheres were also found in the stomach, hepatopancreas, ovary and gills of the crabs, in decreasing numbers over the trial period. This study is the first to show 'natural' trophic transfer of microplastic, and its translocation to haemolymph and tissues of a crab. This has implications for the health of marine organisms, the wider food web and humans.

1. Introduction

Pollution of the marine environment by microplastic (particles <5 mm (Arthur et al., 2008)) is a global problem of growing concern (Sutherland et al., 2011). Many of these effects have yet to be studied and the long-term consequences remain largely unknown (Moore, 2008). Moreover, the amount of plastic in the sea is probably underestimated by a considerable amount; Kukulka et al. (2012) predicted there may be 2.5 times the measured surface volume of plastic in the oceans, due to mixing in the water column.

Microplastic is ingested by many marine invertebrates as the particles are in the size range of plankton (Browne et al., 2008). Particles can also accumulate in sediment (Thompson et al., 2004), suggesting that these would be available to many benthic species. Invertebrates with a range of feeding methods have been shown to ingest microplastic including; filter feeders (mussels, barnacles), deposit feeders (lugworms) and detritivores (amphipods, sea cucumbers) (Browne et al., 2008; Thompson et al., 2004; Graham and Thompson, 2009). 10 μm polystyrene microspheres were ingested by polychaetes, bivalves, echinoderms and bryozoans (Ward and Shumway, 2004). Microspheres ingested by mussels were translocated from the gut into the circulatory system and persisted for over 48 days (Browne et al., 2008).

Plastic contains organic contaminants, either added during manufacture or adsorbed from the seawater (Teuten et al., 2009). Plastic can concentrate contaminants, up to the order of 10^6 (Mato et al., 2001), potentially acting as both source and vector for the contaminants. Contaminated plastic has been found on beaches all around the world (Ogata et al., 2009). PCBs can transfer from plastic to streaked shearwater chicks (Teuten et al., 2009) and hydrophobic contaminants have been found to be transported to sediment-dwelling organisms via plastic (Teuten et al., 2007).

Even without the complication of pollutants desorbing from the plastic, the microplastic itself can have deleterious effects. Many marine organisms, including 26 species of cetaceans and 44% of seabirds, have been observed to ingest large and small plastic debris. Effects include blockage of the digestive tract and false satiation (Moore, 2008). In terrestrial mammals, ingested plastic microparticles were taken up by the gastrointestinal epithelium of rodents into their lymphatic system (Hussain et al., 2001), showing cellular damage (Lam et al., 1993) and thrombosis (Nemmar et al., 2003).

There is potential for microplastic to enter the food chain, but there is little evidence. Plastic particles found in the scat of fur seals (*Arctocephalus* spp.) were believed to have been ingested by lantern fish (*Electrona subaspera*), which were in turn eaten by the seals (Eriksson and Burton, 2003). Pieces of fish seeded with strands of polypropylene were fed to Norway lobsters (*Nephrops norvegicus*) and the plastic was present in their stomachs 24 h later (Murray and Cowie, 2011). If it could be proven that microplastic can be transferred from one trophic level to the next, there could be detrimental implications for bioaccumulation and biomagnification.

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The blue mussel (*Mytilus edulis*) is a common species with worldwide distribution and is an important food source for many animals including crabs (Bayne, 1976). The shore crab (*Carcinus maenas*) is a common species which has a varied diet, including mussels, and is predated by many other species (Crothers, 1968). If trophic level transfer of microplastic does occur from *M. edulis* to *C. maenas* then there could be implications for the rest of the food web.

The aim of this study was to investigate the trophic level transfer of microplastic from mussels to crabs; to give an indication of the amount of microplastic transferred to the crabs and an indication of the length of time the microplastic persisted.

2. Materials and methods

The twenty-four crabs (*Carcinus maenas)* were all females of the red colour morph to minimise gender and type bias. They had a mean carapace width of 51.13 mm ± 4.46 and a mean wet weight of 32.54 g ± 10.83 ($n = 24$). They had been starved for 3 days prior to the start of the experiment, then placed in individual identical 2.5 L buckets with loosely-fitted lids and 1 L of seawater (16 °C). They were left to acclimatise for 1 h.

Twenty-four live mussels (*Mytilus edulis*)(8 × 3 replicates) were scrubbed to remove any organisms from their shells, placed in individual identical 600 ml glass beakers with 400 ml of seawater (16 °C) and left to acclimatise for 15 min. They had a mean shell length of 50.71 mm ± 4.83 and a mean wet weight of 4.52 g ± 1.67 ($n = 24$). 50 μl of 0.5 μm green fluorescent polystyrene microspheres (estimated 411 million) (Duke Scientific Corporation) was added to each beaker. All mussels were observed to open their shells and appeared to be feeding (siphons or mantle extended) within 5 min of the microspheres being added to the beaker and for >90% of the exposure time. There was assumed to be some natural variation in the retention and uptake of microspheres (see discussion of Kach and Ward, 2008), and as 54% of them produced yellow/green-tinted pseudofaeces within 1 h. One hour was therefore set as exposure period to avoid excess removal of particles from mussels. After 1 h, the mussels were removed from the beakers, cut open and the soft tissue removed from the shell. Thirteen of them had microspheres in high enough concentrations to be visible to the naked eye when cut open. The tissue from one randomly selected mussel was placed in a bucket with each crab. All the crabs had eaten >75% of their mussel within 30 min.

At each sample period, three crabs were sampled for replication, three replicates being the minimum needed for basic statistical analyses, thereby reducing non-essential use of experimental animals. For sampling, the crabs were placed in a bucket of ice for 45 min to anaesthetise them, and a sample of haemolymph removed from the arthrodial membrane at the base of the first pereopod, using a 1 ml syringe and 0.6 × 25 mm needle (Terumo). The sample was placed in a 2 ml eppendorf with 2.5 times the volume of 20% formalin as a preservative and anti-coagulant. The crabs were then using 70% ethanol.

The first 9 crabs were sampled at 1, 2 and 4 h after the crabs were given the mussels. The remaining crabs were transferred to a tank with through-flow seawater (16 °C) and sampled at 24 h, 4, 7, 14 and 21 days. They were fed with plastic-free fish every three days.

Three control crabs (mean carapace width: 50.33 mm ± 9.02, mean weight: 35.92 g ± 15.72) were each fed one mussel (mean shell length: 49.33 mm ± 4.62, mean wet weight: 4.40 g ± 0.55) that had not been exposed to microspheres. Samples of haemolymph were taken after 24 h.

A 25 μl sub-sample of each of the haemolymph samples was examined under a fluorescence microscope (Nikon eclipse E800). Each crab was dissected by cutting round the edge of the carapace and removing it.5 mm diameter samples of stomach, hepatopancreas, ovary and gill were examined under the fluorescence microscope. The microspheres were counted under ×100 and ×200 magnification.

3. Results and discussion

Microspheres were found in tissue samples from the stomach (Fig. 1), hepatopancreas, ovary and gills (Fig. 2). The microspheres were found in the highest concentrations in the 5 mm diameter samples of stomach at 1 h (1025 ± SE 556), 2 h (883 ± SE 589) and 4 h (1007 ± SE 572), but none at later time samples. Even after just 1 h, microspheres were present in the 5 mm diameter samples of hepatopancreas (65 ± SE 55), as well as ovary (68 ± SE 66) and gills (75 ± SE 63). The samples of stomach, hepatopancreas and ovary had the most number of microspheres at 1 h, the gill at 2 h (167 ± SE 44). There were no microspheres seen in any of the samples by 21 days. There was a large variation in the number of microspheres in the tissue samples due to uneven distribution of microspheres throughout the tissues and inaccuracies inherent in the method used. This was highlighted as an area of future study.

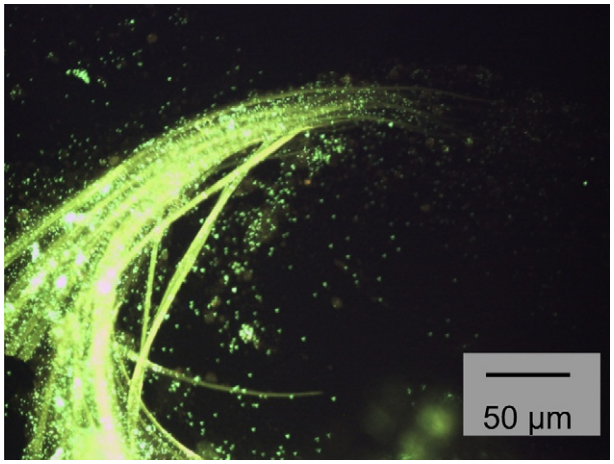

Fig. 1. Micrograph showing 0.5 μm fluorescent microspheres in the filtering hairs inside the cardiac stomach of a crab sampled at 1 h×200 magnification.

Microspheres were found in all haemolymph samples (Fig. 3). There was a significant (p-value: 0.003, ANOVA) trend of increase in the number of microspheres up to 24 h (15 033 ml^{-1} ± SE 3147), after which the number decreased, though some microspheres were still present at 21 days (267 ml^{-1} ± SE 120). There was no obvious change in the physical or behavioural condition of the crabs after ingestion of the microspheres, up to 21 days. This was a preliminary study, using the minimum number of replicates possible, in order to provide direct evidence of trophic transfer, and highlight areas on which to concentrate further studies.

Norway lobsters (*Nephrops norvegicus*) also ingest microplastic via their food, though this did not show natural trophic level transfer as the *N. norvegicus* were fed pieces of fish seeded with strands of polypropylene (Murray and Cowie, 2011). Microplastic has been found to affect even the primary trophic level, potentially acting as another entry source into the food chain. Positively-charged nano-sized plastic particles adsorbed to the cellulose constituent of algae (*Chlorella* spp. and *Scenedesmus* spp.), which hindered photosynthesis and could affect the sustainability of marine food webs (Bhattacharya et al., 2010).

Kach and Ward (2008), showed that mussels had a 14% retention efficiency for 0.5 μm fluorescent polystyrene microspheres. In

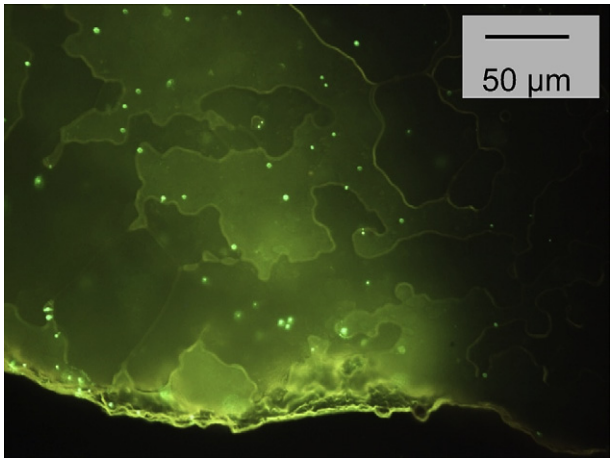

Fig. 2. Micrograph showing 0.5 μm fluorescent microspheres on a gill lamella of a crab sampled at 1 h×200 magnification.

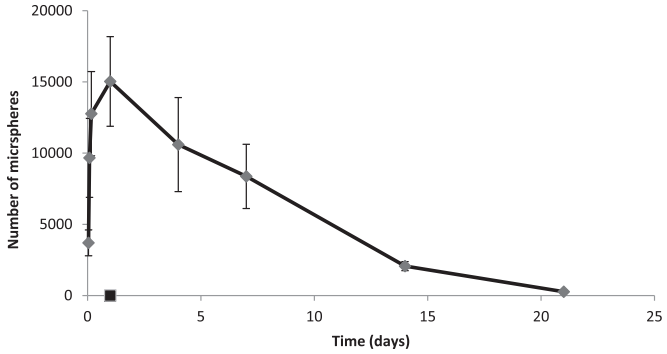

Fig. 3. The mean number of 0.5 μm microspheres ml^{-1} of haemolymph ± SE ($n = 3$) against time (days) since the ingestion of the mussel. Square marker is control sampled at 24 h ($n = 3$).

this study, the mussels were exposed to an estimated 411 million microspheres. At 14% retention efficiency, they would retain 57.54 million microspheres.

It was calculated that the number of microspheres in the crab's entire haemolymph at 24 h was 163 111 ± 34 140 microspheres. This is 0.04% of the number of microspheres to which the mussels were exposed and 0.28% of the estimated number of microspheres retained by the mussels. This estimate was based on the maximum number of microspheres in the haemolymph and does not include an estimate of the number of microspheres in the tissues of the crabs, as a robust estimate of this was not elucidated during this study. This was one mussel eaten by one crab and bioaccumulation and biomagnification could increase the amount of microplastic in both consumers and prey.

This research was conducted under laboratory conditions. In the wild, mussels could be exposed to a range of type and size of microplastic, as well as their natural food and could be exposed to various concentrations over their lifetime. In a similar way, crabs could be exposed directly to microplastic as well as ingesting it via mussels and other prey. Larval and juvenile stages may be particularly vulnerable and require further study.

4. Conclusion

Although the amount of microplastic that transferred from *Mytilus edulis* to *Carcinus maenas* was small, this study has demonstrated that trophic transfer occurs between mussels and crabs, and that microplastic can translocate to the haemolymph and tissues of the crab. This study increases concern for the potential for microplastic to reach higher trophic levels, for the accumulation of environmental pollutants and for the health of animals, including humans.

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