Trophic and direct transfer of microplastics into the common shore crab *Carcinus maenas*

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Summary
Microscopic plastic debris is emerging as a global conservation issue. Ingestion of microplastics has been demonstrated in a range of marine animals both in the laboratory and in the oceans, but we know little of how microplastics may be transferred across trophic levels. Here, we used mussels (*Mytilus edulis*) and crabs (*Carcinus maenas)* to investigate the bioaccumulation and trophic transfer of 10μm fluorescently labelled polystyrene microspheres. Organisms were exposed to microplastics through trophic transfer (oral ingestion) or direct exposure (via the ventilation process) and internal distribution, gut transit and depuration were studied using dissection and fluorescence microscopy. Our results show that microplastics were rapidly distributed across body tissues and provide gut transit times in our trophic model and through uptake across the gill in our ventilation model. We will present an update of our results and discuss their wider ecological implications.

Introduction

Microplastics- particles of plastics less than 5mm in length - have been identified as a widespread and potential major pollutant in the marine environment, yet our knowledge of their bioavailability and biological effects is scant (Wright *et al.*, 2013). In particular, we know little of the potential routes of uptake of microplastics into animals and the potential for them to be transferred across trophic levels.

The common mussel *Mytilus edulis* (*M. edulis*) is a sessile filter feeding bivalve that is susceptible to plastic microsphere contamination (Browne *et al.*, 2008). This species forms an important prey species for higher trophic organisms including the common shore crab *Carcinus maenas (C. Maenas)* Trophic transfer of microplastic from *M edulis* to *C maenas* has been reported (Farrell and Nelson, 2013), but the detailed mechanisms by which these microplsatics enter the body or are distributed internally are not known. Questions for further study include the residence time of microplastics in different tissues and depuration rates from the gut and other internal organs. Additionally, dietary uptake of microplastics may not be the only mechanism of entry; other physiological processes i.e. gill ventilation using inhaled water from the environment may be involved. Ecologically these microspheres could also be transferred to further trophic levels resulting in a biomagnifying effect, potentially transferring to our marine food resources.

The aim of this study was to investigate the uptake, translocation and clearance of microplastics into *Carcinus maenas* by two alternative routes: trophic transfer from the mussel *Mytilus edulis* and through direct exposure in the water.

Materials and Methods

Carcinus maenas and *Mytlis edulis* were collected from the Exe estuary, Devon (UK). For the study of trophic transfer, three trials were carried out with only one feed taking place at the start of the experiment. Firstly, male *C. maenas* (n=42) were fed with *M. edulis* which had previously been exposed to 10μm fluorescent polystyrene microspheres (1000 spheres/ml filtered artificial seawater) for 4 hours. Crabs were sampled at seven time points between 1 day and 3 weeks post feed. The second (crab*s,* n=40) and third (crabs, n=36) experiments were carried out using jellified mussels (homogenised mussel: distilled water: gelatine. 105:70:13) which were spiked with the same 10μm fluorescent polystyrene microspheres at known concentrations. Crabs were sampled at seven time points between ½ hour and 24hrs.

For the study of direct transfer, 12 male *C. maenas* were attached (via a latex mask) to a modified tank system designed by Taylor (1976) which collects exhalant water, and thus monitors the ventilation process. Ventilation rate and oxygen consumption were recorded prior to a recovery period of 2hrs. Microspheres (same as above) were then added to the main tank at a concentration of 1,000 per ml and ventilation rate and oxygen consumption were re-recorded over the following 2hrs.

For all experiments, haemolymph, stomach, gills and hepatopancreas were excised from each crab and homogenised. Six 20µl aliquots of each sample were pipetted in to a black UV 96 well plate and the number of microspheres counted under a florescent microscope. Numbers of microspheres were expressed as total within tissue.

Results and Discussion

The uptake and distribution of these polystyrene microspheres over the first 24hr period and an extended 3 week period was recorded. Results indicate that the dosing of microplastics into each tissue occurs over the first 30 minutes of the feed. The abundance of microspheres then remained constant in each tissue over the first 24hrs. After this, the quantity of microspheres decreased in internal tissues, with most being voided by the crab by day 7. However, some particles remained in tissues for up to 22 days. Unlike other studies (Farrell and Nelson, 2013) the microspheres in this study were very rarely seen in the heamolymph in either the short or long term. This could be due to our use of larger microspheres of 10 µm diameter compared with the 5µm microspheres used by Farrell and Nelson (2013). We found microspheres on the gills at many time points thought the study, although in low numbers.

Future work will explore the effects of repeated feeding events, longer depuration rates and different polymer types and shapes, to determine what influence these variables have on microplastics ingestion. Finally, many studies of decapods in the wild (Murray & Cowie, 2011) have only looked at the amount of plastics within the stomach; if indeed plastics are also being taken up in to the gill surface (albeit in lower numbers compared to the stomach) this could be ecologically important and worthy of further investigation in the field.

References

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