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Uptake and Retention of Microplastics by the Shore Crab *Carcinus maenas*

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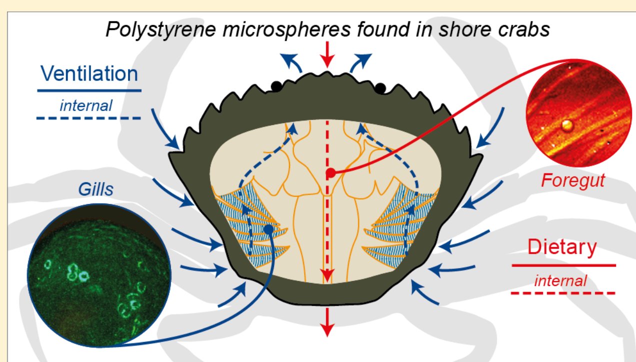
ABSTRACT: Microplastics, plastics particles <5 mm in length, are a widespread pollutant of the marine environment. Oral ingestion of microplastics has been reported for a wide range of marine biota, but uptake into the body by other routes has received less attention. Here, we test the hypothesis that the shore crab (*Carcinus maenas*) can take up microplastics through inspiration across the gills as well as ingestion of pre-exposed food (common mussel *Mytilus edulis*). We used fluorescently labeled polystyrene microspheres (8−10 μm) to show that ingested microspheres were retained within the body tissues of the crabs for up to 14 days following ingestion and up to 21 days following inspiration across the gill, with uptake significantly higher into the posterior versus anterior gills. Multiphoton imaging suggested that most microspheres were retained in the foregut after dietary exposure due to adherence to the hairlike setae and were found on the external surface of gills following aqueous exposure. Results were used to construct a simple conceptual model of particle flow for the gills and the gut. These results identify ventilation as a route of uptake of microplastics into a common marine nonfilter feeding species.



INTRODUCTION

Microplastics, particles of plastics less than 5 mm in length, have been identified as a major marine pollutant. Due to their widespread use and persistence in the environment, microplastics can now be found in marine waters and sediments throughout the world.[1] Particle abundances of 1×10^5 particles/(m^3 of seawater) have been recorded for coastal waters of Sweden[2] and $>1.0 \times 10^3$ particles/(m^2 of sediment) on a beach in Malta.[3] Microplastics can be classified into two groups: primary microplastics that are created at the micro scale for use in cosmetics,[4] as drug vectors,[5] and as virgin preproduction pellets[4] and secondary microplastics that are derived from larger items such as packaging, clothing, or ropes, through photodegredation or physical degradation.[6]

There is increasing evidence that these microplastics can be taken up via ingestion into marine organisms. Several hundred species have now been reported to ingest plastics from the wild,[7] with much of the evidence coming from examination of gut contents postmortem. Species affected include turtles and sea birds,[8] with microplastic found in fish,[9,10] zooplankton,[11,12] and benthic invertebrates.[13−15]

The common mussel *Mytilus edulis* has been identified as a species susceptible to microplastic ingestion.[13] As a filter feeder it will "ventilate" large volumes of water, with ventilation rates of up to 300 mL·min^{-1} at 100% O_2 saturation and 15 °C,[16] increasing its susceptibility to water-borne substances. Browne et al.[13] showed under laboratory conditions that *M. edulus* will accumulate 3.0 and 9.6 μm polystyrene microspheres within the gut, and these plastics had a residence time of over 48 days within its circulation system and fecal pellets. This species forms an important prey species for higher trophic organisms including the shore crab *Carcinus maenas*. Transfer of microplastics can occur from *M. edulus* to the shore crab *C. maenas* under laboratory conditions.[17] While this transfer of microplastic from *M. edulus* to *C. maenas* has been reported, the detailed mechanisms by which these microplastics enter the crab's body are not known.

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In a similar manner to *M. edulus*, *C. maenas* also ventilates water from its surroundings, mainly for respiratory function and at a rate of around 45 $mL \cdot min^{-1}$ when held under similar conditions (100% O_2 saturation, 15 °C) to *M. edulus*.[18] Farrell and Nelson[17] identified microspheres on individual crab gill lamelli, raising the possibility that they had entered the crab gill through ventilation. The ventilation mechanism of *C. maenas* is shown in Supporting Information section 1. The movement of water through the base of the limbs and over the gills facilitates the uptake of small particulate material into the gill chamber and onto the gills.[19] Bacteria have also been found to enter and form colonies within the gill itself.[20] The presence of microspheres on the gills could therefore be due to this ventilatory process, representing an uptake route of microplastics additional to that established via food or oral ingestion.

The internal anatomy of the shore crab has evolved to fit its omnivorous lifestyle. The gut is divided into fore-, mid-, and hindgut. The foregut contains a complex gastric mill that functions to grind and mix plant and animal tissue, including hard organic materials such as animal shells. A ventral filter in the cardiac stomach then separates gut contents by size; particles >100 nm pass to the hindgut and are eliminated in the feces, while particles <100 nm pass to the hepatopancreas for digestion.[21−23] Typical gut residence time for food is around 12 h, with an evacuation time through the fecal pellets of 12−48 h.[22]

The gills serve different functions depending on their anatomical location. Gills located to the posterior of the gill chamber (gills 7−9) have higher Na^+/K^+-ATPase activity compared with those to the anterior (gills 1−6).[24] Thus, a major function of the posterior gills is ion exchange, while the anterior gills serve as the major site for respiration and oxygen uptake. The many lamellae within the gills provide a large potential surface area for exogenous material adherence.

The aim of this study was to investigate the uptake, location, residence time, and clearance of microplastics (polystyrene microspheres, 8−10 μm in diameter) in the shore crab *C. maenas* via two alternative exposure routes: (1) direct (ventilatory) exposure via water and (2) exposure via diet by use of microplastics-dosed mussels, *Mytilus edulis*.

■ MATERIALS AND METHODS

Experimental Rationale. We first assessed the ability of *Carcinus maenas* to uptake and retain 8−10 μm polystyrene microspheres through its ventilatory mechanism. To do this, crabs were fitted with a mask designed to enable measurement of ventilation and then exposed to 10 μm fluorescently labeled microspheres. Subsequent experiments explored the presence of microspheres on individual gills and the extent to which microspheres were retained on the gills. We then assessed the ability of *C. maenas* to take up and retain microspheres through ingestion of mussels, *Mytilus edulis,* which had been exposed to microspheres. The location and abundance of the microspheres was determined by microscopy, and results used to construct a conceptual model of flow and retention.

Study Organisms. Crabs and mussels were collected from the River Exe estuary, Devon (U.K.), 50°35.2′ N, 3°23.59′ W, in batches throughout 2013. Animals were held in groups of 20 in 20 L tanks [artificial seawater (ASW): salinity 33 practical salinity units (PSU), temperature 12−13 °C] for at least 4 days prior to the experiment to depurate. A further six *C. maenas* were collected from the Troon Harbour, Clyde Sea Area, Scotland (U.K.) (55°32.49′ N 4°39.50′ W) in August 2013 and held in natural seawater (NSW) collected from the same location. Only male *C. maenas* were used, to avoid any potential bias relating to sex.

Ventilation. Exposures were conducted for periods of 24 h and 21 days and to concentrations between 9.4×10^5 and 4.0×10^4 $microspheres \cdot L^{-1}$.

Ventilation Mask. To test the hypothesis that microplastics are taken up into the gill chamber via the ventilation route, crabs were exposed to fluorescently labeled 10 μm polystyrene microspheres (9.4×10^5 $microspheres \cdot L^{-1}$ in 17 L of NSW). Exposures were for 16 h through the water column and used a modified tank system, which ensures the only entry point of the tank water into the crab was through the ventilation mechanism. The tank design is described in more detail by Taylor:[18] A mask was fitted around the mouth of the crab and attached to the external carapace and the animals were then allowed to recover for 2 h, confirmed by a steady-state respiration rate in accordance with Taylor.[18] The end of the mask was then connected to the exit tube on the side of the tank, taking away the exhalent water. As the mouth parts of the crab are covered, the only way the water (and any particles in the tank) can enter the crab is through the ventilatory route. Six male *C. maenas* were used in this study. Once attached to the tank (one for each crab), crabs were then left to settle for a further 30 min prior to addition of microspheres to the tank. After 16 h, 1 mL of hemolymph was taken via the base of the third walking leg, and gills were dissected as one sample and kept at −20 °C for further analysis.

Microsphere Location on Gills. To determine the location of microsphere settlement on the gills, gills (1−9) were dissected from an additional six crabs that were exposed to 4.0×10^4 $microspheres \cdot L^{-1}$ (1 L of ASW) in individual 3 L tanks for 16 h. Gills were frozen at −20 °C until further analysis, as described above.

Residence Time on Gills. To estimate the residence time of microplastics within the gills of *C. maenas,* 22 crabs were exposed to 4.0×10^4 $microspheres \cdot L^{-1}$ (1 L of ASW) in individual tanks as described above for 16 h. After 16 h, 12 crabs were removed and dispatched, and the gills were analyzed as described above.

To test if crabs were able to retain microspheres between water changes, the water from tanks holding the remaining 12 crabs was changed and the crabs were left for 24 h. Microsphere abundance in the water at each water change (every 2 days for 22 days) was determined by vacuum-filtering the whole 1 L of wastewater from the crab tank (plus 300 mL used to rinse the crab) through Whatman No. 2 filter paper (pore size 8 μm). Microspheres were counted on the filter paper under a florescent microscope (Leica DMI 4000B). To do this, filter papers were divided into 95 cm squares, and the average abundance of microspheres in five squares were determined and multiplied by 95 to estimate the total microsphere abundance in the filtered water. After 22 days, the remaining 11 crabs were dispatched, and the gills were analyzed as described above.

Feeding. Two methods were used to feed crabs with food that contained polystyrene microspheres. First, crabs were fed with fresh mussels that had themselves been exposed to microspheres through the water. Second, crabs were fed with jellified mussels (described below); this was done to standardize the microsphere content in the mussel tissue offered to the crabs.

Pre-exposed Mussel. Fresh mussels were dosed with 10 μm polystyrene microspheres following protocols from Browne et al.[13] Fifty mussels (57.2 ± 2.4 cm shell length) were glued onto wooden sticks and individually suspended into a 500 mL beaker with 300 mL of aerated ASW, and the suspension was stirred by use of magnetic stir bars. Mussels were allowed to settle for 30 min prior to feeding with algae (Isochrysis 1800, Reed Mariculture, 5−6 μm cell diameter) at a concentration of 4.0×10^6 cells·L^{-1}, and after polystyrene microspheres were added in a concentration of 1.0×10^6 microspheres·L^{-1} (300 mL of ASW), the mussel was left to feed for 4 h.

Processed Mussel Feed. Jellified mussel homogenate was made by adding 105 g of mussel homogenate (spiked with 10 μm polystyrene microspheres) to 13 g of gelatin (Sigma, G2500) dissolved in 70 mL of distilled water (70 °C for 30 min); this mixture was vortexed and allowed to set overnight at 5 °C. Cubes were cut of approximately 2 cm^3 (1.95 ± 0.07 g). The final concentration of polystyrene microspheres was $4.0 \times 10^3 \pm 154$ microspheres·g^{-1} (based on analysis of six replicate cubes).

Feeding Assay. Two trials (24 h and 21 days) were carried out with one feed containing the microspheres taking place on day 0 (days labeled as "days since last feed"). Crabs were fed in tanks of 1 L ASW for 0.5 h to allow direct feeding on the prey item. Each crab was then moved to a clean tank of 1 L ASW. At each sampling point, 1 mL of hemolymph was taken via the base of the third walking leg and foreguts were removed from each crab and frozen at −20 °C.

First, 42 male *C. maenas* were fed with fresh mussels [1.5×10^5 microspheres/(g of mussel)] and left to consume for 0.5 h. Crabs were sampled at set time points between day 1 and day 21 postfeed. Water changes occurred every 2 days where fecal pellets were collected and frozen at −20 °C until further analysis for microplastic abundance determination.

Second, a 24 h experiment was conducted with jellified mussels [4.0×10^3 microspheres/(g of food)]. Crabs were sampled between 0.5 and 24 h.

Tissue Analysis. Each tissue was weighed and homogenized in 5 mL of deionized (DI) water. Fecal pellet and hemolymph samples were homogenized with 1 mL of DI water.

Six 20 μL aliquots of each tissue sample were pipetted into individual wells of a black UV 96-well plate, and microspheres were counted under a florescent microscope (Leica DMI 4000B, I3 filter excitation range 450−490 nm). An average of the six aliquots was determined, and the number of microspheres was expressed as total number within each tissue.

Fluorescent Microscopy. Images of fecal pellets were taken via bright-field microscope image (×5). Fluorescent microspheres contained within the fecal pellet were then imaged with a superimposed fluorescence I3 filter (see above) image of the same pellet.

Coherent Raman Scattering Microscopy. Coherent Raman scattering microscopy (CRS) is a multiphoton microscopy technique that provides label-free contrast of both the target sample and surrounding biological matrix, based on vibrational spectroscopy. In order to investigate microplastic uptake and biodistribution within the crabs, exposures were set up as previously described[11] and key tissues were excised to be imaged by CRS spectroscopy.

Three-dimensional imaging was performed to confirm the location of particles within the biological tissue. A series of two-dimensional images (in the $x-y$ plane) acquired over the depth range of interest (i.e., the z-direction) were acquired by incremental adjustment of the focus between a frame to create a "z-stack" of images that was subsequently rendered for three-dimensional visualization. It was found that z-steps of 1.5 μm were sufficient to provide adequate resolution of the 8 μm particles and surrounding biological structures of interest.

To investigate any association of microplastic on or within the gills, two crabs were exposed to nonlabeled 8 μm polystyrene microspheres (4.0×10^3 microspheres·L^{-1} ASW) for 24 h. The gills were dissected fresh and analyzed with CRS microscopy. To investigate any association of microplastic on or within the foregut, two crabs were fed via jellified mussel homogenate dosed with nonlabeled polystyrene microspheres (4.0×10^3 microspheres·g^{-1}).

Statistics. To test if the factor "day" (i.e., resident time of microspheres) described the variation in the number of polystyrene microspheres in both the gills and the foregut, independent measures (each crab) were tested with analysis of variance (ANOVA; Minitab) and a Tukey post hoc test. Normality of residuals and homogeneity of variances were assessed visually; when assumptions were not met, then a Kruskall−Wallis test was used to compare means.

To test if the number of polystyrene microspheres varied on each gill, a repeated measures ANOVA (SPSS) was used on the gill contained within the gill chamber with the most microspheres present (with a Greenhouse−Geisser correction to meet the assumption of sphericity of the data). A Bonferroni pairwise test was then used to test which gills had the greatest abundance. $p < 0.05$ was treated as significant in all tests.

Model of Microplastic Retention. A simple box model was constructed to investigate how organ physiology may interact with potential for retaining plastic microspheres. The model (described in further detail in Supporting Information sections 5 and 6) is

$$\mathrm{d}P_\mathrm{F} = \omega\left(\frac{P_\mathrm{S}}{V_\mathrm{S}} - \frac{P_\mathrm{F}}{V_\mathrm{F}}\right) - aG + \mathrm{d}P_\mathrm{R}$$

$$\frac{\mathrm{d}P_\mathrm{R}}{\mathrm{d}t} = aG - \mathrm{d}P_\mathrm{R}$$

$$\frac{\mathrm{d}P_\mathrm{S}}{\mathrm{d}t} = -\omega\frac{P_\mathrm{S}}{V_\mathrm{S}} \quad \text{or} \quad \frac{\mathrm{d}P_\mathrm{S}}{\mathrm{d}t} = -\omega\left(\frac{P_\mathrm{S}}{V_\mathrm{S}} - \frac{P_\mathrm{F}}{V_\mathrm{F}}\right) \tag{1}$$

where P_S is the number of microspheres from the source (either water or food), P_F is the number of microspheres inside the organ held in "free" suspension (either in the water or food), and P_R is the number of retained microspheres trapped or entangled within hairs or folds on the organ's surface V_S is the volume (in liters) of the source and V_F is the volume of the organ in question. For the purposes of our model, we assumed the organ functions as a well-mixed environment, with a flow applied through the organ at rate ω, where the flow of microspheres is controlled by the gradient between the concentration of microspheres inside the organ and the concentration of microspheres at the organ source. We assumed that free microspheres can become entangled at rate a and that retained microplastics can re-enter free suspension at rate d, where the maximum number of free plastics that can become entangled is G, given by the step function:

$$G = \begin{cases} M - P_\mathrm{R} & \text{when } P_\mathrm{F} > M - P_\mathrm{R} \\ P_\mathrm{F} & \text{when } P_\mathrm{F} \leq M - P_\mathrm{R} \end{cases} \tag{2}$$

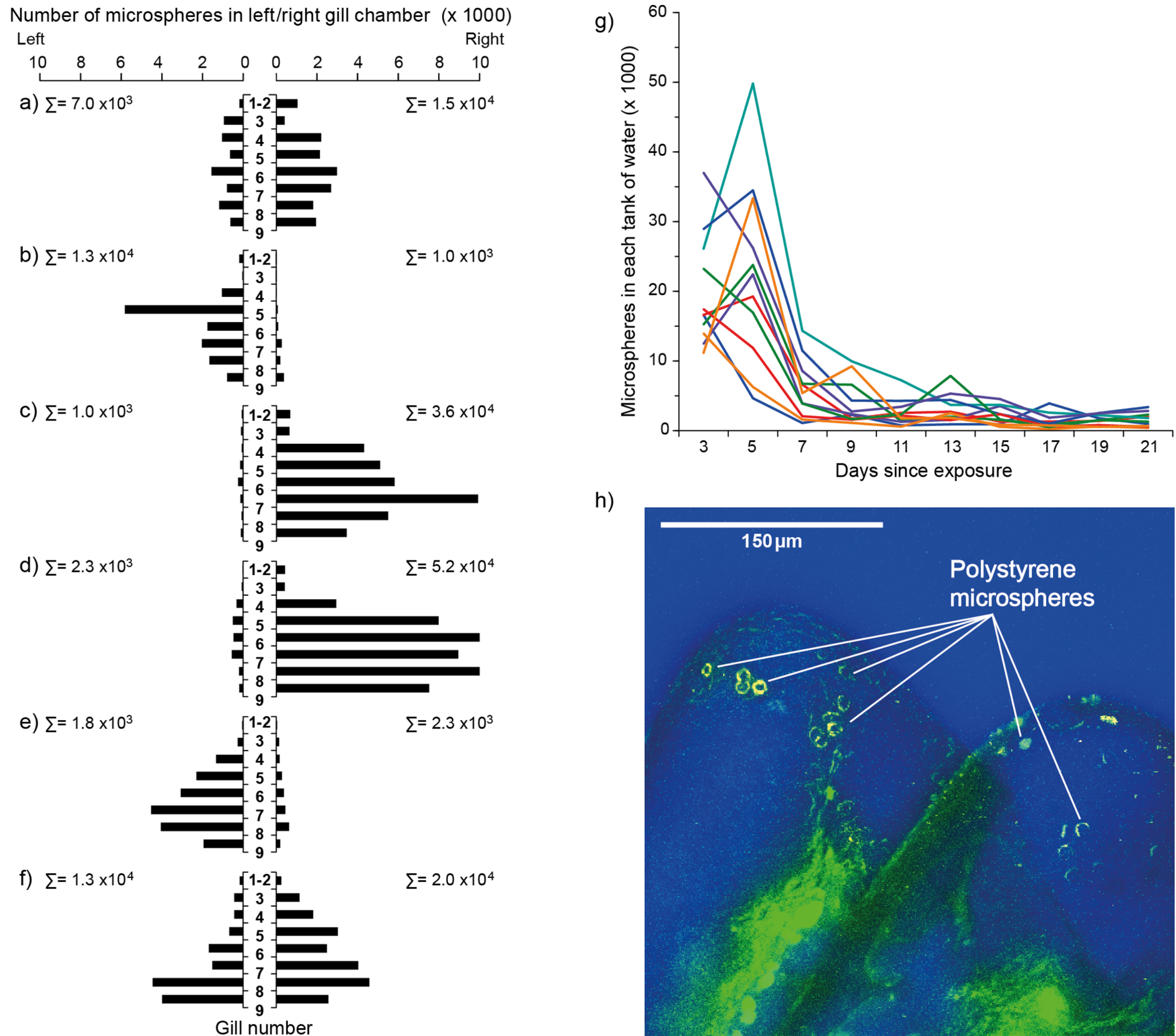

Figure 1. Ventilation exposure of crabs to polystyrene microspheres; crabs were exposed to 4.0×10^4 microspheres·L^{-1} in 1 L of artificial seawater for 16 h. (a−f) Number of microspheres on each gill of six crabs, sum (Σ) of all microspheres on each side. (g) Number of microspheres expelled to tank water (1 L) per day postexposure. (h) Coherent Raman scattering microscopy image of two gill lamellae tips with 8 μm microspheres imaged at 3050 cm^{-1} adhering to the outside of the surface.

Here M is the theoretical maximum number of plastics that could become trapped on the organ's surface.

By solving the model for steady state (by setting $dP_F/dt = 0$ and $dP_R/dt = 0$) under the first condition for G, we determined the equilibrium of our system to be when the concentration of free microplastics within the organ was equal to that of the organ's source and that peak retained microplastics was given by

$$P_R^{\max} = \frac{aM}{a + d} \tag{3}$$

Parameters were estimated from data presented in this paper, based on the allometric relationships of size and volume of foregut and gills in a medium-sized crab, defined in Supporting Information section 6. To calibrate the model, a and d were calculated. Estimates of M and $P_R^{\max}$ were used in the rearranged eq 3:

$$d = a\left[\left(\frac{M}{P_R^{\max}}\right) - 1\right] \tag{4}$$

Therefore, d can be determined as a factor of a, which can be calibrated to the data.

RESULTS

Ventilation. *Microplastic Uptake.* All six masked crabs that were exposed to 9.4×10^5 microspheres·L^{-1} (in 17 L of NSW) had detectable numbers of microspheres on their gills. There was variation in the uptake, with the total number of microspheres on the gills ranging between 3.1×10^4 (0.39% initial exposure concentration) and 6.2×10^5 microspheres (7.7% initial concentration). Microspheres were not detected in the hemolymph of any of the crabs sampled.

Location of Microspheres on Gills. Figure 1a−f shows the total number of microspheres detected, in each of the right and left gill chambers and within each gill, of the six *C. maenas*

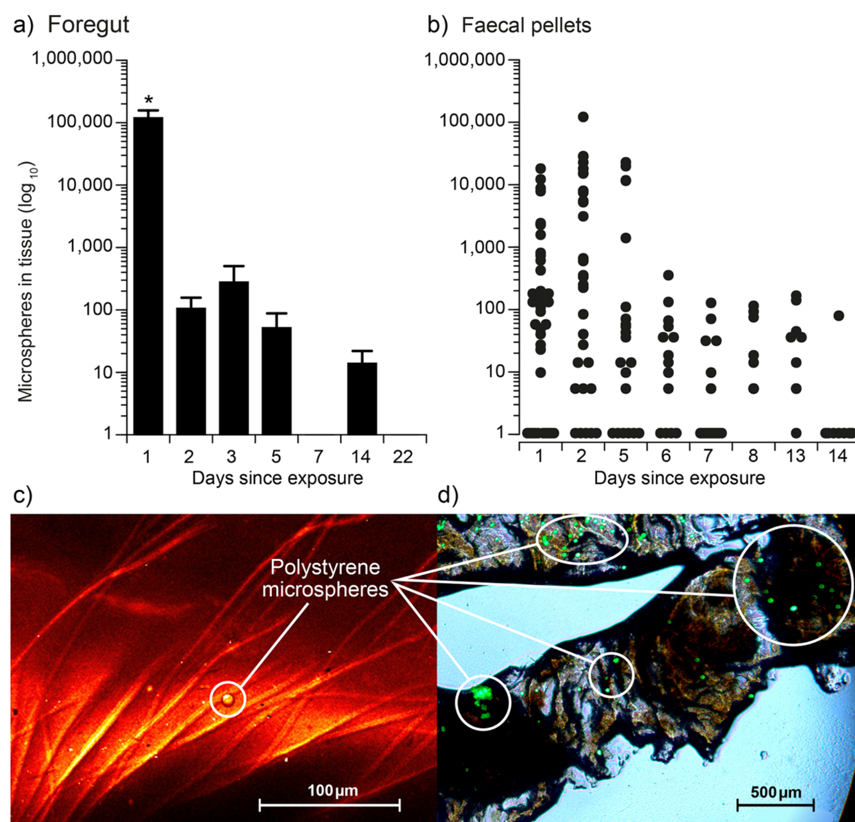

Figure 2. (a, b) Dietary exposure of crabs to polystyrene microspheres; crabs were exposed to 1.5×10^5 microspheres$\cdot$L^{-1} in 1 L of artificial seawater for 16 h. (a) Mean ± SE of the abundance of microspheres within the foregut of six crabs sampled at different time points postfeed. Asterisk indicates a significant difference between the number of microspheres in the foreguts at day 1 and any other day (Tukey, $p < 0.05$). (b) Individual value plots of the abundance of microspheres found in each sample of fecal pellets at different days postfeed. (c, d) Dietary exposure of crabs to 4.0×10^3 polystyrene microspheres$\cdot$L^{-1} in 1 L of artificial seawater for 16 h: (c) coherent Raman scattering image at 2845 cm^{-1}, showing microspheres associating with the internal hairs of the crab foregut, and (d) bright-field microscope image of a fecal pellet from a crab that was fed 3 days previously, with a superimposed fluorescence I3 (excitation range 450−490 nm) image of the same pellet. This shows the fluorescently labeled microspheres located within the fecal pellet.

exposed to microspheres within the surrounding water for 16 h. The number of microspheres contained in the gills differed between the individual gills. Four crabs had more microspheres within the right gill chamber, and two individuals contained more in the left chamber.

There was a significant difference in the number of microspheres relative to the gill number within the chamber [repeated measures ANOVA, $F = 9.517$, $p = 0.005$ with a Greenhouse−Geisser correction]. A Bonferroni pairwise comparison for all individual gills indicated that the gills in the most posterior positions (6−9) had, on average, a significantly higher number of microspheres compared with gills in the anterior positions (1−4) (Bonferroni, $p < 0.05$).

Residence Time on Gills. Figure 1g shows the abundance of microspheres remaining in the water after each water change. On day 3, between 1.1×10^4 and 3.7×10^4 microspheres were detected in the water. This was higher on day 5, at 5.0×10^4, and then reduced to 1.4×10^4 on day 7. On day 21 the number of microspheres detected in the water was between 4.0×10^2 and 3.3×10^3. All 11 crabs were still expiring microspheres after 21 days. These crabs had microspheres associated with the gills with an average of 1000 ± 300 (standard error, SE) microspheres within all gills. This was, however, significantly less than detected for crabs sacrificed after the initial 16 h exposures [$(5.9 \times 10^3) \pm (1.6 \times 10^3)$ SE; Kruskall−Wallis, $H_{1,22} = 10.57$, $p = 0.001$).

Imaging Microspheres on Gills. Coherent Raman scattering microscopy of two gill lamellae tips showed the microspheres associated with the gill epidermis (Figure 1h) This, and a three-dimensional animated reconstruction (Supporting Information section 3), indicates these microspheres had not penetrated into the tissue of the gill tip.

Feeding. *Uptake of Microspheres from the Diet.* In the 24 h postfeed experiment, all crabs sampled had microspheres within the foregut for the first 6 h (with the exception of one crab at 2 h postfeed). At 24 h, three out of six crabs had plastic detected in the foregut. There was a significant decrease in the number of microspheres detected in the foregut of *C. maenas* over the 24 h period (see Supporting Information section 2) (ANOVA, $F_{5,35} = 7.12$, $p < 0.001$). The Tukey post hoc tests indicated crabs sampled at 24 h had significantly less microspheres in the foregut than at other times (apart from at 2 h, where the data were skewed because in one crab no microspheres were detected). No crabs had any microspheres detected in the hemolymph.

Resident Time in Foregut. There was a significant reduction in the number of microspheres within the foreguts of the crabs over the 21 day period of sampling (ANOVA, $F_{6,38} = 18.91$, $p < 0.001$, $\log_{10}$ transformed) (Figure 2a). Crabs sampled from day 2 onward had a significantly lower number of microspheres within the foregut compared with crabs sampled at 24 h (Tukey $P < 0.05$). Polystyrene microspheres were detected in all crabs

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after 24 h (Figure 2a); this declined to around 50–66% of the crabs examined between days 2 and 5. At days 7 and 22 postfeed, there were no detectable microspheres in the foregut; however, they were detectable at day 14.

Microspheres in Fecal Pellets. Figure 2b shows the number of microspheres found in fecal samples from each crab that were held in the experiment for 14 days following feeding. Microspheres were found in 72% of fecal pellets sampled over the duration of the experiment. Seven out of the eight crabs on day 13 had detectable microspheres within their fecal pellets, compared with only one out of the eight crabs sampled on day 14. Fecal pellets from this single crab on day 14 had 76 microspheres, compared with 1.9×10^4 microspheres from the same crab at day 5.

Imaging Microspheres in Foregut and Fecal Pellet. Figure 2c shows a coherent Raman scattering microscope image of the foregut lining of a crab fed with nonlabeled microspheres spiked into jellied mussel. The featured microsphere can be seen associated with the internal setae. The animated reconstruction of the foregut lining shows this association in 3D (Supporting Information, section 2). Figure 2d shows the layered image of bright-field and fluorescent signals of the microspheres within the fecal pellet from a crab 16 h postfeed, showing clusters of microspheres throughout the pellet.

Model. The estimated P_R^{max} for the foregut was 279 (average microspheres at day 3; Figure 2a). M is estimated to be 318, calculated by $S \times D \times H$ (see Supporting Information section 6). Therefore, the relationship between d and a is

$$d = 0.1428a$$

The effects of various values of a are shown in Supporting Information section 7.

The estimated P_R^{max} for the gills was 64 545 (average total amount of microspheres that were recorded being released from the crab over 21 days; Figure 1g). M is estimated to be 51 350, calculated by $S \times H$ (see Supporting Information section 6) Therefore, the relationship between d and a is

$$d = 7.308161a$$

The effects of various values of a are shown in Supporting Information section 8.

In the model (output shown in Supporting Information sections 7 and 8), microspheres are treated as two separate populations, "free" plastic (shown as a red line), which are microspheres caught up in the food or ventilated straight through the gill chamber and not in contact with the trapping hairs or gill lamellae, and retained plastic (shown as a blue line), which are trapped via these structures (as seen in Figures 1h and 2c). The sum of these two populations is displayed as the black line. Microspheres are assumed to be able to change from free to retained and back again. The biological plausibility of this assumption is based on the observation that association of microplastics with biological structures does not involve physical bonding. We assume, therefore, that microplastics will eventually be removed by the mechanical action of the gut and self-cleaning mechanisms of the gills.

DISCUSSION

The data presented show that there is uptake of microplastics, via both ventilation from the water column and dietary transfer from dosed mussels as a food source, in the common shore crab *Carcinus maenas*. Uptake of microspheres was not equal across the different gills within individual animals, with a greater uptake of microplastics observed in the posterior gills (6–9) compared with anterior gills (1–4). Residence time of microspheres within exposed crabs was found to vary depending on the route of microplastic exposure. No microspheres were detected in the foregut or fecal pellets after 14 days in crabs exposed to these plastics through the diet. In contrast, crabs exposed through the ventilation route were still eliminating detectable numbers of microspheres into the surrounding seawater 21 days after completion of exposure. These data have been used to construct a simple box model, in which a mechanistic interpretation of the movement and retention of microplastics in the gut and gills has been postulated.

Crabs tended to exhibit asymmetry in the microplastics accumulated in their gill systems, which we speculate may be due to the pumping mechanism of the scaphognathite being more dominant on one side of the gill chamber. Posterior gills (6–9) were found to contain a significantly greater proportion of microspheres compared with anterior gills (1–4) regardless of which gill chamber was the more dominant in accumulating microspheres. The posterior gills have a larger surface area than anterior gills and thus are more likely to lodge microspheres in their lamellae. The posterior gills function for both ion exchange and respiration.[25] The potential for both of these processes to be affected by these particles remains unclear. Furthermore, it is not known how this finding compares with the behavior of other naturally occurring particles such as colloids, clay minerals, etc., and this warrants further investigation in considering the potential for toxicological effects of these polystyrene microspheres found in association with the gill tissue.

In this study, no microspheres were found in the crab's hemolymph at any of the sampling points for either the ventilatory or food exposure routes. This suggests that no translocation of plastic particles of this particular size occurred from either the foregut or gills into the hemolymph. Distribution from the gills or gut to other organs was not studied here; however, it is unlikely that further distribution from the foregut to digestive organs occurred, because passage between the gut and hepatopancreas is protected by a filter press that allows only nanosized particles to pass through.[21–23]

Farrell and Nelson[17] showed translocation of microspheres into the hemolymph of *C. maenas*, following exposure through food, to a sphere size of 0.5 μm (we used 10 μm spheres). Browne et al.[13] showed that both 3 and 9.6 μm microspheres translocate into the hemolymph of *M. edulis*. In that work, 3 μm spheres were detected in significantly higher amounts than 9.6 μm sized particles. As this study failed to detect 10 μm microspheres within the hemolymph, this suggests a size bias in translocation of microspheres across gill membranes and the foregut, although further work is needed to support this hypothesis in the crab.

According to Hopkin and Nott,[22] the digestion and absorption of food items after feeding takes about 12 h in *C. maenas*, with the excretory phase lasting 12–48 h. This suggests that microspheres, still residing in the foregut after 72 and 120 h (5 days), have a slower transit time compared with normal food items. Our study of the abundance of microspheres within the fecal pellets showed that microspheres take over 6 times longer to leave the body compared to the average excretory phase for food waste.

Carcinus maenas has two mechanisms to remove particles from the gills. First, each gill chamber contains a gill raker or

flabella. This sweeps over the gills with its setae and the particles are then pushed into the water current heading to the exhalent channel.[26] Second, the scaphognathite can reverse the flow of water momentarily to dislodge particles from the gills.[19] It is apparent that the microspheres in this study were not being efficiently removed by this process. The difference between retention times of microplastic in the gills and gut could be due to many factors, both physiological and morphological. One such factor could be the larger surface area of the gills compared to the foregut. Gills have many folds between the gill lamellae, giving a greater likelihood of adherence. The foregut has fewer folds than the gills but features hairlike setae that are thought to prevent large food particles from entering the midgut.[27] These setae will increase the surface area. These structures are shown in Figure 2 to be associated with the ingested microsphere, which may be a way in which microspheres are trapped and retained within the foregut for longer than 74 h. At week 14, two out of the six animals removed had detectable plastic within the foregut, both estimated at around 38 microspheres. As data points were independent of each other, it is entirely possible that some crabs had small amounts left in the foregut and the others at day 7 and 22 did not. Microspheres could also be cycling in and out of the gill chamber through the ventilation route.

We constructed a simple box model in an attempt to provide a conceptual framework for these findings and to provide a future means of testing and evaluating the importance of particle clearing mechanisms in different organisms. A focus for future work could be in determining how factors such as food disaggregation, bioaccumulation, and altered polymer type, particle size, and shape (for example, fibers or films) affect these observations. In addition, the flow of particles has been viewed simplistically here as a linear motion, while in practice this is unlikely to be the case. In addition, it was assumed that microplastics could move freely between retained and freely moving populations, and the concept of permanent retention has not been incorporated into the calculations. Exact representative values for a and d have not been found, but ranges of values that can explain the data have been presented. It is likely that each individual crab could have very different a, d, and M values. More data describing the movement of microspheres between retained and free fractions, for example, from tracking the internal movement of particles within individual animals would help to develop these models in future. These concepts represent an important research gap for future studies.

Both oral and ventilatory uptake routes may be important in natural populations where crabs occur in regions of high plastic pollution; however, it is difficult to say which route is more important on the basis of these laboratory studies. *M. edulis* is known to have high ventilation rates of around 300 $mL \cdot min^{-1}$ at optimal conditions (100% O_2 saturation and 15 °C).[16] This is considerably higher than the ventilation rate of *C. meanas*, which is around 45 $mL \cdot min^{-1}$ for a crab of 60−80 g in the same oxygen and temperature regime.[18] Therefore, on ventilation rates alone, *M. edulis* is likely more susceptible to microplastics ingestion.

Retention time of microplastics within the gills and gut of the crab lasted for 2−3 weeks depending on the uptake route. This means that, following a single exposure, there is a period of up to 3 weeks where these plastics are available for transfer to the next trophic level. *C. maenas* predators includes invertebrates, fish, birds, and mammals.[28] All of these organisms, if they consume the whole crab, could take up microplastic via trophic transfer. Fecal pellets are also a source of nutrition for benthic detritivores, potentially acting as a route for transfer of microplastics to benthic food webs. The dietary uptake of microplastic in marine animals has been a widely reported phenomenon.[8−15] To our knowledge, this is the first study that has shown a major secondary route of uptake into a marine organism via the gills.

■ ASSOCIATED CONTENT

Supporting Information

Diagram of water movement through the gill chamber, number of microspheres in the crab foregut over first 24 h, 3D reconstruction of the gills showing no penetration into the tissue, 3D reconstruction of the foregut lining showing association with the internal setae, details of the model of microplastic retention, a table of model parameters, and calibration of the foregut and gill models. This material is available free of charge via the Internet at http://pubs.acs.org/

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Notes

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■ ABBREVIATIONS

ASW artificial sea water
NSW natural sea water
CRS coherent Raman scattering

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