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Microplastic Ingestion by Zooplankton

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ABSTRACT: Small plastic detritus, termed "microplastics", are a widespread and ubiquitous contaminant of marine ecosystems across the globe. Ingestion of microplastics by marine biota, including mussels, worms, fish, and seabirds, has been widely reported, but despite their vital ecological role in marine food-webs, the impact of microplastics on zooplankton remains under-researched. Here, we show that microplastics are ingested by, and may impact upon, zooplankton. We used bioimaging techniques to document ingestion, egestion, and adherence of microplastics in a range of zooplankton common to the northeast Atlantic, and employed feeding rate studies to determine the impact of plastic detritus on algal ingestion rates in copepods. Using fluorescence and coherent anti-Stokes Raman scattering (CARS) microscopy we identified that thirteen zooplankton taxa had the capacity to ingest 1.7−30.6 μm polystyrene beads, with uptake varying by taxa, life-stage and bead-size. Post-ingestion, copepods egested faecal pellets laden with microplastics. We further observed microplastics adhered to the external carapace and appendages of exposed zooplankton. Exposure of the copepod *Centropages typicus* to natural assemblages of algae with and without microplastics showed that 7.3 μm microplastics (>4000 mL^{-1}) significantly decreased algal feeding. Our findings imply that marine microplastic debris can negatively impact upon zooplankton function and health.

1. INTRODUCTION

It has been estimated that up to 10% of plastics produced globally enters our oceans, so it is of little surprise that plastic debris is now a pervasive and resilient pollutant of the marine environment.[1,2] Larger plastic debris, such as monofilament line, plastic strapping, and plastic bags, can entangle, garrotte, drown, or be eaten by an array of marine wildlife.[3] There is compelling evidence that microplastics—small plastic <5 mm in diameter—also negatively impact upon marine biota.[4] Microplastics consist of synthetic polymer products manufactured to be of a small size, such as exfoliates in cosmetics,[5] and those items derived from the fragmentation of larger plastic debris, for example polyester fibers from fabrics,[6] polyethylene fragments from plastic bags[7] and polystyrene particles from buoys and floats.[8] Typically, high-density plastics (e.g., polyvinyl chlorides, polyester) settle out of the water column, whereas low-density plastics (e.g., polyethylene, polystyrene) remain buoyant, although freshwater inputs, storms, and biofilm formation may result in vertical mixing.[9,10] Floating plastic debris is susceptible to local and ocean currents resulting in higher-than-average waterborne microplastic concentrations in areas of confluence.[11]

Microplastics are of environmental concern as their small size makes them available to a wide range of marine biota.[12] Microplastic ingestion has been demonstrated in marine organisms, including amphipods, lugworms, and barnacles,[4] mussels,[13] decapod crustaceans,[14] seabirds,[15] and fish.[16,17] Ingested microplastics might obstruct feeding appendages, aggregate, and block the alimentary canal, limit the food intake of an organism or be translocated into the circulatory system.[13,14] Further, microplastics may introduce toxicants to the organism: first, additives incorporated into a plastic during manufacture to improve its properties (e.g., phthalates for malleability and polybrominated diphenyl ethers (PDE) for heat resistance) might leach out of weathered plastic debris;[18,19] second, the large surface area to volume ratio and hydrophobic properties of microplastics leave them susceptible to the accumulation of hydrophobic organic contaminants (HOCs) which could dissociate post-ingestion.[20]

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The extent to which microplastics are ingested and can impact upon zooplankton is uncertain. Zooplankton have a vital ecological role in marine ecosystems, both as primary consumers in the marine food web, and in the case of meroplankton, consisting of the juvenile life stage of numerous commercially important species. The widespread presence of small plastic debris in the water column makes interactions between zooplankton and microplastics highly likely; indeed, both small plastic debris and zooplankton >333 μm in diameter have been recurrently sampled together in sea surface trawls and by continuous plankton recorders.[4,11,21,22] Zooplankton display a range of feeding modes, which vary by life-stage, species and prey availability.[23] Zooplankton can use a combination of chemo- and mechano-receptors to select prey, and their ability to preferentially feed on one species of algae over other algae, plastic beads or detritus has been demonstrated.[24−26] Laboratory experiments, in which latex beads were used to model algal ingestion, have shown that zooplankton have the potential to ingest small plastics.[26−28] Uptake of these small plastics likely results from indiscriminate feeding modes (e.g., filter-feeding), by which prey with equivalent spherical diameters (ESD) <100 μm are non-selectively fed upon.[23,29]

Due to the complexities of sampling and extracting microplastics from the marine environment, existing studies have largely focused on detritus >333 μm.[1,30] However, there is evidence of very small microplastics (<100 μm) both in the benthos and water column. Sampling of shoreline, estuarine and harbor sediments has shown the presence of ∼20 μm diameter fibrous polymers,[4,6,31] and microplastic fibers, granules, films, and polystyrene spheres ranging in size from 38 μm to 1 mm.[32] In the water column, sampling with a 80 μm mesh in Swedish coastal waters captured 100 000 times greater concentrations of microplastics than when using a 450 μm mesh, with a maximal concentration of 102 000 microplastics per m^3 sampled near a polyethylene production facility.[33] Sampling of microplastics in this size range is exceptional, as such there is currently insufficient data to determine realistic environmental concentrations of these particles.

Here, we investigate the ingestion of minute microplastics, ≤31 μm diameter, by a range of zooplankton species, and examine their impact on zooplankton function and feeding. To explore the hypothesis that zooplankton are capable of ingesting microplastics, 15 zooplankton taxa—representative of abundant mesozooplankton in northeast Atlantic coastal systems—were exposed to polystyrene spheres in the size range 7.3−30.6 μm suspended in natural seawater, then analyzed using fluorescence microscopy. Using the copepod *Temora longicornis*, we explored where 0.4−3.8 μm microplastics accumulate, both internally and externally, using a novel bioimaging technique: coherent anti-Stokes Raman scattering (CARS) microscopy. Finally, to test the hypothesis that microplastics negatively impact upon zooplankton feeding, we exposed the copepod *Centropages typicus* to natural assemblages of algae and polystyrene beads, using fluorometry and flow cytometry to quantify algal ingestion.

2. MATERIALS AND METHODS

2.1. Zooplankton Sampling. Zooplankton sampling was conducted between November 2011 and October 2012 at Station L4 (50° 15′N, 4° 13′W), a coastal site located in the western English Channel 12 km south of Plymouth, UK.[34,35] A 200 μm mesh was used to collect zooplankton via horizontal surface tows and vertical hauls. Collected zooplankton were held in 2 L of seawater within a coolbox, and transported to controlled-temperature facilities at Plymouth Marine Laboratory (Plymouth, UK). For all experimental procedures, we maintained the zooplankton at ambient sea-surface temperatures (ranging 10−17 °C depending on sampling date). Specimens were hand-selected under a dissecting microscope within two hours of sampling, and then collectively held in 2 L of filtered seawater (0.22 μm Millipore filter) for 24 h to allow full gut depuration. In all, fourteen mesozooplankton taxa (size: 0.2−20 mm), representative of the most commonly occurring zooplankton in the western English Channel and covering a range of life-stages and life-strategies, in addition to cultured *Oxyrrhis marina*, a heterotrophic dinoflagellate (size: 15−30 μm), were selected for microplastic ingestion studies (Table 1).

2.2. Natural Seawater Preparation. For the algal ingestion studies, natural seawater (5 L) was collected from the sea surface at station L4, passed through a 200 μm mesh into a polycarbonate carboy and returned to the laboratory within 2 h. The seawater was further screened with a 100 μm mesh to ensure the removal of any grazing micrometazoans then stored in the dark for 24 h at ambient sea-surface temperature to maintain the natural communities of algae at normal concentrations. Prior to experimental work, the seawater was mixed thoroughly by gentle inversion of the water in the carboy.

2.3. Microplastics. Exposures used commercial polystyrene spheres (SPHERO Spherotech). With global production rates of 10.6 million tons in 2001, polystyrene is the fourth most commonly produced polymer in the world and its presence as a constituent of marine debris is commonly reported.[30,36] The bead sizes used in each experiment (0.4−30.6 μm) were selected to be comparable with the prey size range of the zooplankton exposed.[23,37]

2.4. Microplastic Ingestion by Zooplankton. To ascertain whether zooplankton ingest microplastics we conducted exposures using fluorescent polystyrene beads, and used microscopy to assess uptake. Microplastic suspensions were made up by pipetting 20 μL of 7.3, 20.6, or 30.6 μm diameter fluorescently labeled (yellow fluorescence: 400−500 nm excitation, 450−550 nm emission) polystyrene spheres into glass vials containing 20 mL of filtered seawater (0.1% v/v: 3000 beads mL^{-1} (7.3 μm); 2240 beads mL^{-1} (20.6 μm); 635 beads mL^{-1} (30.6 μm)), then mixed through repeated inversion. With larger zooplankton (e.g., copepods, decapod larvae, chaetognaths), individual specimens were added directly to the vial (n = ≥6 per exposure), and fitted to a rotating plankton wheel (<5 rpm) for 24 h. For smaller zooplankton or those with low survivability in the laboratory (e.g., bivalve larvae, gelatinous holoplankton, *O. marina*), individual specimens were exposed to microplastic suspensions in Petridishes (n = ≥6 per exposure) at ambient sea temperature for 1 h (with the exception of bivalve larvae which were exposed for 24 h using this method). Post-exposure, zooplankton were washed with filtered seawater and transferred to Eppendorf tubes containing 1 mL of 4% formalin . Ingestion was ascertained by viewing specimens at ×40−400 magnification with an Olympus IMT2 inverted light microscope with fluorescence to determine the presence of polystyrene beads (fluorescing yellow-green) within the alimentary canal or body cavity of the zooplankton. To better understand the interactions between zooplankton and microplastics, both live and preserved copepods and select zooplankton specimens were viewed under the microscope for

Table 1. Capacity for a Range of Zooplankton to Ingest Microplastics, Demonstrated Using Fluorescent Microscopy[a]

organism	taxonomy	microplastic ESD (μm)	exposure duration (h)	ingestion (Y/P/N?)
Holoplankton (Copepods)				
Acartia clausi	Copepoda (Calanoida)	7.3	24	yes
Acartia clausi	Copepoda (Calanoida)	20.6	24	no
Acartia clausi	Copepoda (Calanoida)	30.6	24	partial
Calanus helgolandicus	Copepoda (Calanoida)	7.3	24	yes
Calanus helgolandicus	Copepoda (Calanoida)	20.6	24	yes
Calanus helgolandicus (juv.)	Copepoda (Calanoida)	20.6	24	yes
Calanus helgolandicus	Copepoda (Calanoida)	30.6	24	partial
Centropages typicus	Copepoda (Calanoida)	7.3	24	yes
Centropages typicus	Copepoda (Calanoida)	20.6	24	yes
Centropages typicus	Copepoda (Calanoida)	30.6	24	yes
Temora longicornis	Copepoda (Calanoida)	7.3	24	yes
Temora longicornis	Copepoda (Calanoida)	20.6	24	yes
Temora longicornis	Copepoda (Calanoida)	30.6	24	yes
Holoplankton (Other)				
Doliolidae	Tunicata	7.3	1	yes
Euphausiidae	Euphausiacea	20.6	24	yes
Parasagitta sp.	Chaetognatha	20.6	1	no
Parasagitta sp.	Chaetognatha	30.6	24	no
Obelia sp.	Cnidaria (Hydrozoa)	20.6	1	partial
Siphonophorae	Cnidaria (Hydrozoa)	20.6	1	no
Meroplankton				
Bivalvia (larvae)	Mollusca	7.3	24	yes
Brachyura (megalopa)	Decapoda	20.6	24	yes
Brachyura (zoea)	Decapoda	20.6	24	no
Caridea (larvae)	Decapoda	20.6	24	yes
Paguridae (larvae)	Decapoda	20.6	24	partial
Porcellanidae (zoea)	Decapoda	30.6	24	partial
Microzooplankton				
Oxyrrhis marina	Dinoflagellata	7.3	1	yes

[a]Microplastic uptake is based upon the number of individuals in a treatment ($n = \geq 6$) that contained beads in their alimentary canals or body cavity following 1 or 24 h exposures to either 7.3, 20.6, or 30.6 μm fluorescent polystyrene beads. ESD = equivalent spherical diameter. Scoring system: yes (>50%); partial (<50%); no (0%).

varying lengths of time to observe the feeding process, ingestion, gut passage, and egestion of polystyrene beads.

2.5. Interactions between Microplastics and Copepods. To explore the internal distribution and external adherence of microplastics in zooplankton, we first exposed the copepod *Temora longicornis* to polystyrene beads and then employed CARS microscopy (see below) to visualize their uptake. Microplastic suspensions were formulated by adding 12 μL of 0.4, 1.7, or 3.8 μm diameter non-labeled polystyrene spheres to 24 mL of filtered seawater (0.05% v/v: 1×10^6 beads mL^{-1} (0.4 μm), 380×10^3 beads mL^{-1} (1.7 μm), and 40×10^3 beads mL^{-1} (3.8 μm)), which were mixed through inversion and sonication. Individual *T. longicornis* ($n = \geq 6$ per exposure) were added to each vial, rotated at <5 rpm at ambient sea temperature for 24 h. Post-exposure, specimens were poured onto a 200 μm mesh suspended in filtered seawater (to prevent damage to the copepods), washed gently, preserved in 4% formalin and then transferred to the bioimaging suite at the University of Exeter (Exeter, UK).

2.6. Coherent Anti-Stokes Raman Scattering (CARS) Microscopy. CARS microscopy is a novel microscopy technique that provides label-free contrast, based on vibrational spectroscopy[38] which has exceptional capability for locating polymer particles within biological tissues with subcellular precision.[39,40] CARS imaging was performed using a custom-built microscopy system based on a commercial confocal laser-scanning microscope and a synchronized dual-wavelength picosecond laser source. Laser excitation was provided by an optical parametric oscillator (OPO) (Levante Emerald, APE, Berlin) pumped with a frequency doubled Nd:vandium picosecond oscillator (High-Q Laser Production GmbH). The pump laser generated a 6 ps, 76 MHz pulse train at 532 nm with adjustable output power up to 10 W. The OPO produced collinear signal and idler beams with perfect temporal overlap and provided continuous tuning over a range of wavelengths. The signal beam was used as the pump, ranging from 670 to 980 nm and fundamental of Nd:vandium (1064 nm) used as the Stokes beam. The maximum combined output power of the pump and Stokes was approximately 1 W, which was attenuated to reduce the power at the sample to between 15 and 30 mW. To improve the transmission of the near-IR excitation through the commercial microscope (IX71 and FV300, Olympus UK) the galvanometer mirrors were replaced with silver mirrors and the tube lens was replaced with a MgF2 coated lens. The collinear pump and Stokes beams were directed onto the scanning confocal dichroic which was replaced by a silver mirror with high reflectivity throughout the visible and NIR (21010, Chroma Technologies, Bellows Falls, VT). The forward-CARS signal was collected by the air condenser, transmitted by the dichroic mirror and directed onto a red-sensitive photomultiplier tube (R3896, Hamamatsu Photonic UK). The epi-CARS signal was collected using the objective lens and separated from the pump and Stokes beams by a long-wave pass dichroic mirror (z850rdc-xr, Chroma Technologies) and directed onto a second R3896 photomultiplier tube at the rear microscope port. The CARS signal was isolated at each photodetector using a single band-pass filters centered at the anti-Stokes wavelengths. Imaging was performed using either a 60× water immersion, or 20× air objective (UPlanS Apo, Olympus UK).

2.7. Impact of Microplastics on Copepod Feeding. To determine whether microplastics negatively impact upon a copepod's ability to ingest natural prey, we exposed the copepod *Centropages typicus* to natural assemblages of algae with and without microplastics, and compared algal ingestion

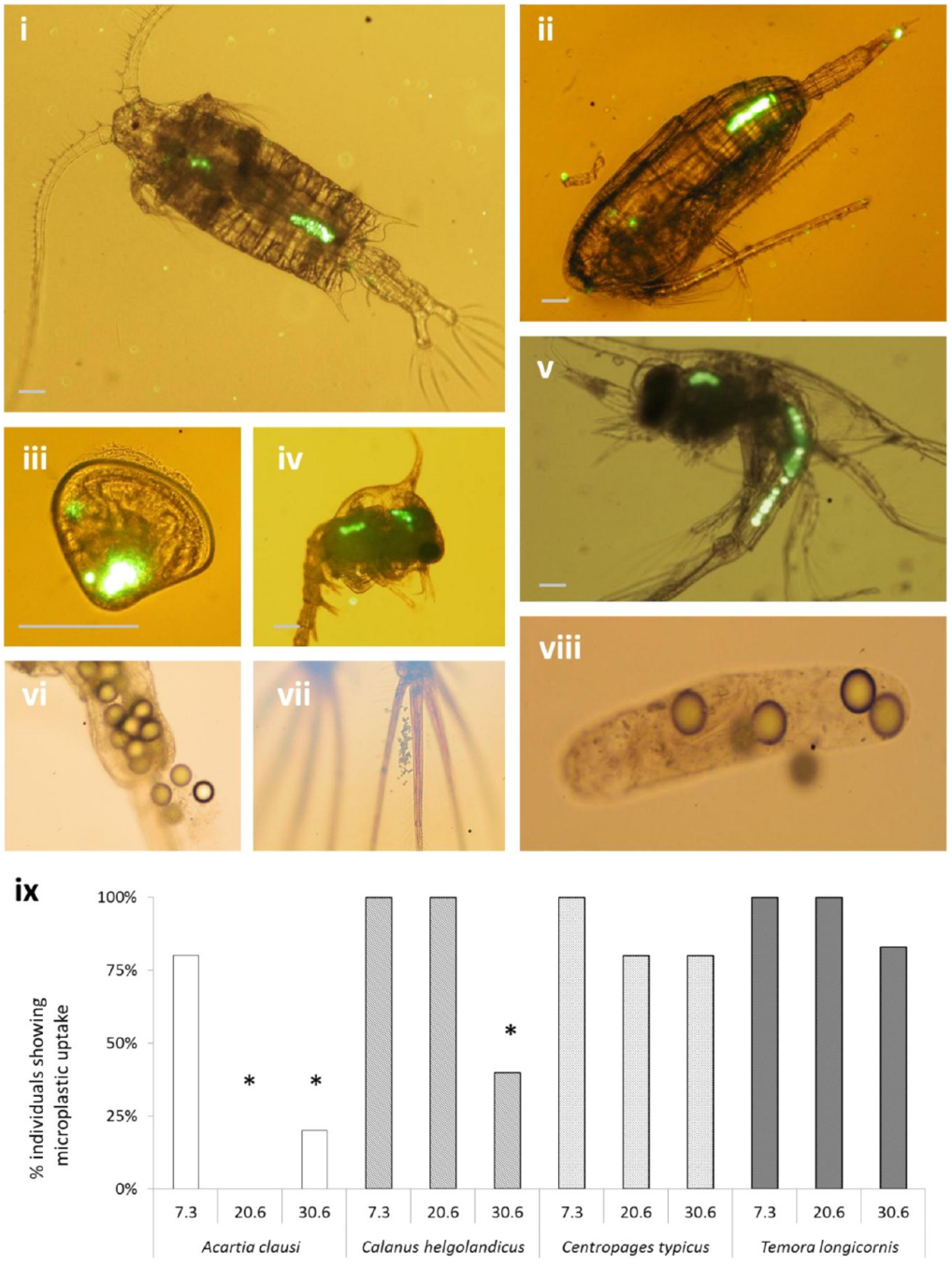

Figure 1. Microplastics of different sizes can be ingested, egested and adhere to a range of zooplankton, as visualized using fluorescence microscopy: (i) the copepod *Centropages typicus* containing 7.3 μm polystyrene (PS) beads (dorsal view); (ii) the copepod *Calanus helgolandicus* containing 20.6 μm PS beads (lateral view); (iii) a D-stage bivalve larvae containing 7.3 μm PS beads (dorsal view); (iv) a Brachyuran (decapod) larvae (zoea stage) containing 20.6 μm PS beads (lateral view); (v) a Porcellanid (decapod) larvae, containing 30.6 μm PS beads (lateral view); (vi) 30.6 μm PS beads in the posterior-gut of the copepod *Temora longicornis* during egestion, (vii) 1.4 μm PS beads trapped between the filamental hairs of the furca of *C. typicus*; (viii) a *T. longicornis* faecal pellet containing 30.6 μm PS beads; (ix) proportion of copepods (*Acartia clausi*, *Calanus helgolandicus*, *Centropages typicus*, and *Temora longicornis*) with microplastics in their guts following 24 h of exposure to 7.4, 20.6, and 30.6 μm polystyrene beads. * denotes statistically significant ($P \leq 0.05$) lower consumption of larger beads compared with that of 7.3 μm beads. Scale bar (gray line): 100 μm.

rates between treatments. In our initial experiment, designed to identify the size of microplastic that would have the greatest impact on *C. typicus* feeding, we exposed individual *C. typicus* specimens (n = ≥6 per exposure) to 23 mL of natural seawater containing 0 or 23 μL of 7.3 or 20.6 μm fluorescent polystyrene beads (0.1% v/v), rotated at <5 rpm for 24 h. To quantify algal concentrations within the natural seawater pre- and post-exposure, we vacuum filtered the exposure media through a glass fiber filter, and then transferred the filter to 7 mL of acetone, held at 4 °C in the dark for 24 h. The chlorophyll levels within the acetone solution were measured using a Turner fluorometer. Since 7.3 μm microplastics had the most notable impact on *C. typicus* feeding, we conducted a further experiment to establish a dose−response relationship between microplastic concentration and food uptake. Microplastic suspensions consisted of 0, 2.5, 5, 10, or 20 μL additions of

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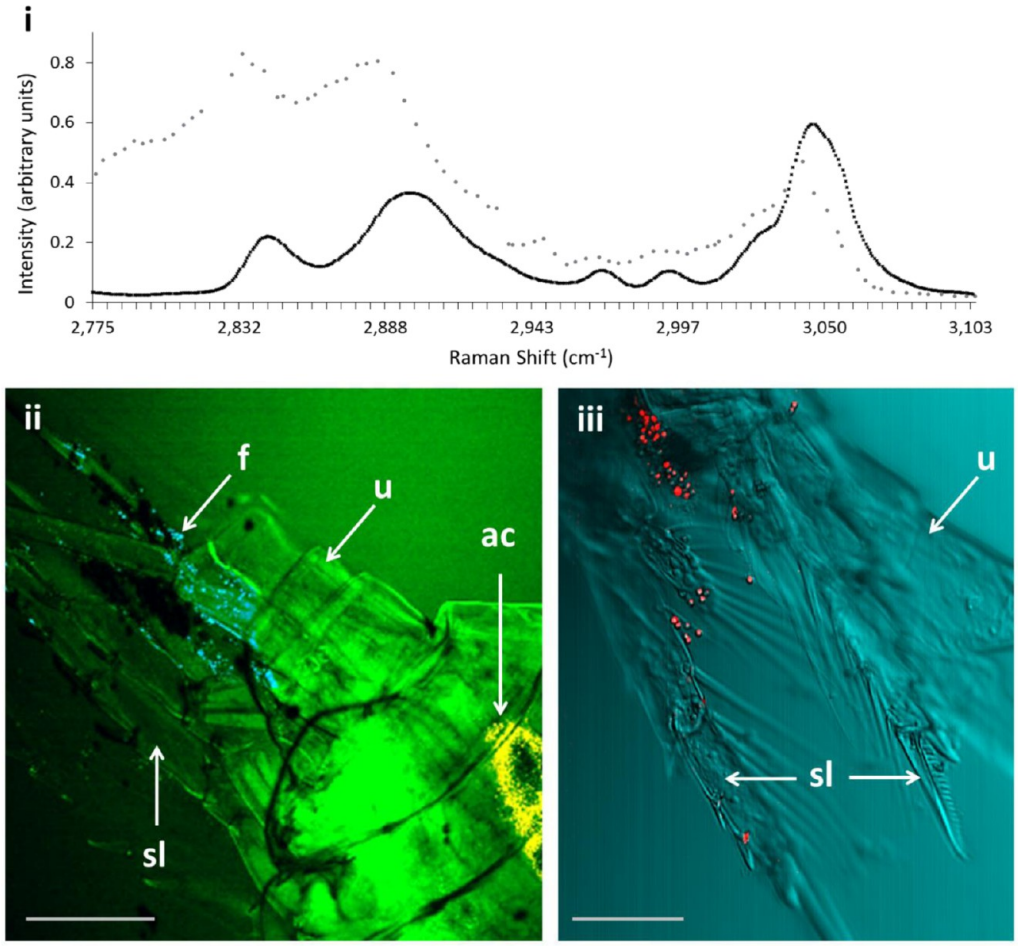

Figure 2. Coherent anti-Stokes Raman scattering (CARS) microscopy: (i) Spontaneous [black] and stimulated [grey] peaks for polystyrene beads, Raman shifts of 2845 cm^{-1} (C−H) and 3050 cm^{-1} (aromatic C−H) were used to visualize the polystyrene; (ii) 3.4 μm microplastics accumulated in the alimentary canal [ac] of the copepod *Temora longicornis* (yellow dots); beads further adhered to the exterior of the copepod's urosome [u], furca [f] and posterior swimming legs [sl] (blue dots); (ii) 3.4 μm microplastics (red dots) adhered to the external surface of the posterior swimming legs of *T. longicornis*. Scale bar [gray line]: 50 μm.

7.3 μm fluorescent polystyrene beads in 23 mL of natural seawater. A 1.8 mL aliquot of natural seawater was taken from all vials at T_0 and fixed with 40 μL of 50% glutaraldehyde (4% final concentration), inverted for 2 min, refrigerated at 4 °C for 30 min and subsequently snap-frozen in liquid nitrogen and stored in a −80 °C freezer prior to analysis using analytical flow cytometry. Individual *C. typicus* (n = ≥6 per exposure) were added to experimental vials, while controls (with no copepod) were set up to determine natural growth or decline of algae over the exposure period. The vials were incubated on a rotating plankton wheel (5 rpm) for 24 h in the dark. Post-exposure (T_{24}), a further 1.8 mL aliquot was fixed (as with T_0). Flow cytometric analysis was carried out on thawed natural seawater samples using a BD Accuri C6 flow cytometer.[41] Particle abundance data was subsequently used to calculate the ingestion rates of algae by *C. typicus*.[42]

2.8. Statistical Analysis. Data was analyzed using Microsoft Excel. Student's t tests were used to compare experimental data with controls, with significant difference attributed where $P \leq 0.05$. Regression analysis was used to analyze the correlation between algal ingestion rates and microplastic concentration.

3. RESULTS

3.1. Microplastic Ingestion by Zooplankton. The majority of zooplankton (13 of 15) exposed to polystyrene beads (7.3−30.6 μm) demonstrated the capacity to ingest microplastics (Table 1). Organisms exhibiting uptake included copepods (Figure 1i, ii), bivalve larvae (Figure 1iii) and decapod larvae (Figure 1iv, v). Only two specimens—chaetognaths (*Parasagitta* sp.) and siphonophorae (Cnidaria)—showed no evidence of ingestion. All four species of copepods examined demonstrated some affinity for ingesting microplastics, with *Centropages typicus* and *Temora longicornis* able to consume 7.3, 20.6, and 30.6 μm polystyrene beads (Figure 1ix). The other copepods showed evidence of size-based selectivity: *Acartia clausi* ingested 7.3 μm beads but ingested significantly fewer 20.6 and 30.6 μm beads, and *Calanus helgolandicus* showed significantly less affinity for 30.6 μm beads than for 7.3 μm beads. The decapod Brachyurans demonstrated variability in microplastic ingestion depending upon life-stage: brachyuran zoea showed no affinity for 20.6 μm beads, while the more developed brachyuran megalopa readily ingested such beads. *Obelia* sp., Paguridae larvae and Porcellinidae (zoea) exhibited individual variability in their ability to ingest polystyrene beads, with less than half the

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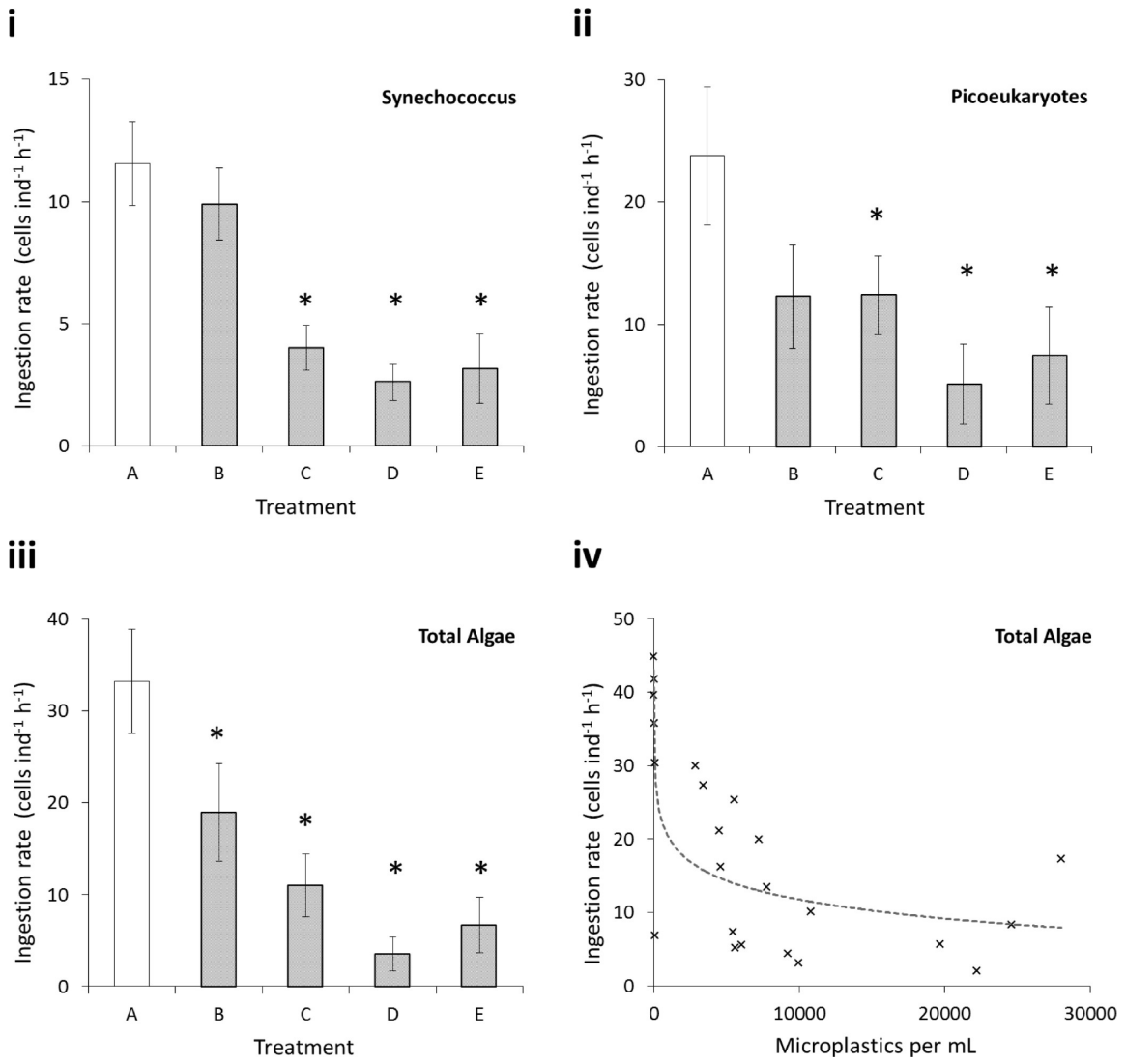

Figure 3. Exposure to increasing concentrations of microplastics in the copepod *Centropages typicus* (n = ≥5). Treatments comprise seawater containing natural assemblages of algae [A] with 4000 [B], 7000 [C], 11 000 [D], and 25 000 [E] 7.3 μm polystyrene beads per mL. * denotes statistically significant ($P \leq 0.05$) lower ingestion rates (cells individual^{-1} hour^{-1}) than in controls. Graphs show ingestion rates of (i) *Synechococcus* sp.; (ii) Picoeukaroytes; (iii) all algae present; (iv) plot comparing positive *C. typicus* algal ingestion rates at differing microplastics concentrations—logarithmic regression: $R^2 = 0.70$ ($P \leq 0.05$).

exposed specimens in a cohort showing evidence of microplastic uptake.

Live observations of copepods, euphausids, and doliolids found microplastics were ingested via filter-feeding. In copepods and euphausids, this process relied upon the rapid movement of the swimming legs and external appendages, which generated a feeding current that indiscriminately drew surrounding beads toward the organism. With doliolids, we observed the microplastics being drawn through the anterior siphon into their body cavity, where the polystyrene beads were entrapped and drawn toward the gut. *Oxyrrhis marina*, a single celled heterotrophic dinoflagellate, demonstrated a more direct method of ingestion, locating particles with their flagella and then engulfing the polystyrene beads. Post-ingestion, copepods typically aggregated beads within the anterior midgut, shifted them to the posterior midgut via peristaltic action (Figure 1i, ii) and egested them within densely packed faecal pellets (Figure 1vi, viii). Typically, microplastic-laden faecal pellets were egested within hours. In the absence of food, individual microplastic beads could remain in the intestinal tract of *C. helgolandicus* for up to 7 days (data not shown). During observations of both live and preserved zooplankton specimens, including copepods, decapod larvae and euphausids, microplastics often adhered to the specimens' external surfaces. In copepods that died during the exposure period, polystyrene beads would coat the carapace in vast numbers; similarly, beads were observed to cling to the shed carapace of a molting *C. helgolandicus* copepodite. In live specimens, microplastics were found to concentrate between the external appendages of copepods, including the swimming legs, feeding apparatus, antennae, and furca (Figure 1vii).

3.2. Interactions between Microplastics and Copepods. CARS microscopy used a blend of transmitted light to capture the structure of the copepod, and Raman shifts of 2845 cm^{-1} (C−H) and 3050 cm^{-1} (aromatic C−H) to visualize the polystyrene (Figure 2i). *Temora longicornis* ingested both 1.7 and 3.8 μm polystyrene beads; use of Z-stacking—in which 2D images at incremental focal plains are layered together to form a

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3D image—confirmed that microplastics clumping in the posterior midgut were, indeed, internalized (Figure 2ii; yellow dots), but sufficient resolution to identify microplastic translocation was not possible. CARS imaging confirmed that microplastics adhere to the external appendages of the zooplankton: polystyrene beads (0.4−3.8 μm) accumulated between the filamental hairs on appendages, including the furca (Figure 2iii; blue dots), rear swimming legs (Figure 2iii; red dots) and antennules, and between the segments of the carapace, particularly around the urosome and swimming legs.

3.3. Impact of Microplastics on Copepod Feeding. Using chlorophyll concentration as a proxy for algal abundance, we identified that 7.3 μm microplastics had a significant impact on algal ingestion by the copepod *Centropages typicus* (data not shown) and identified a significant dose−response relationship between ingestion rates and the concentration of 7.3 μm polystyrene beads. Exposed to seawater—containing natural assemblages of algae—*C. typicus* ingested ∼12 *Synechococcus* sp. ind^{-1} h^{-1} (Figure 3i) and ∼24 picoeukaryotes ind^{-1} h^{-1} (Figure 3ii). These ingestion rates decreased when additionally exposed to ∼4000 microplastics mL^{-1}; this decrease was statistically significant at concentrations of ≥7000 microplastics mL^{-1} (t test: $P \leq 0.05$). When considering all of the <20 μm ESD algal groups identified using flow cytometry − *Synechococcus* sp., picoeukaryotes, nanoeukaryotes, and cryptophytes—, in combination (hereafter referred to as "total algae"), *C. typicus* presented total algal ingestion rates of ∼34 algae ind^{-1}h^{-1} in the absence of microplastics. Total algal ingestion rates for *C. typicus* were significantly reduced with the addition of ≥4000 microplastics mL^{-1} (t test: $P \leq 0.05$; Figure 3iii). Furthermore, we identified a strong, logarithmic relationship ($R^2 = 0.70$, $P \leq 0.05$) between the ingestion rate of total algae and microplastic concentration (Figure 3iv).

4. DISCUSSION

Our results show that a range of zooplankton common to the northeast Atlantic can ingest microplastics (1.4−30.6 μm diameter), with capacity for uptake varying between species, life-stage, and microplastic size. Microplastics were indiscriminately ingested via filter-feeding and later egested in faecal pellets, typically within a matter of hours. Microplastics accumulated on the external surface of dead zooplankton, and were found trapped between the external appendages of live copepods. We visualized 1.7 and 3.8 μm polystyrene beads clustered within the alimentary canal and aggregated between the setae and joints of external appendages. Lastly, we demonstrated that the presence of 7.3 μm polystyrene beads could significantly reduce the algal ingestion rate of the copepod *Centropages typicus*, in a dose−response relationship.

We demonstrated that 13 zooplankton taxa—including holoplankton, meroplankton, and microzooplankton—have the capacity to ingest polystyrene beads in the absence of natural food. All four copepod species showed uptake of microplastics, with varying degrees of selectivity: *T. longicornis* and *C. typicus* ingested 7.3, 20.6, and 30.6 μm beads, whereas *A. clausi* and *C. helgolandicus* fed on 7.3 μm beads but less frequently ingested larger beads. Using CARS microscopy, we further identified that *T. longicornis* could ingest 1.7 and 3.8 μm microplastics; however, we found no evidence of 0.4 μm beads being ingested. Brachyuran larvae only ingested 20.6 μm polystyrene beads as megalopa (postzoea larvae), with no uptake observed when in the earlier zoea stage. Microplastics were also ingested by the filter-feeding euphausids and doliolids, and *Oxyrrhis marina*, a heterotrophic dinoflagellete that ingests motile or immotile prey through engulfment via a non-permanent cytosome.[43] These findings corroborate the results of several previous studies, which documented the uptake of <100 μm microplastics by *Acartia tonsa*,[28] *Calanus pacificus* adults, copepodites and nauplii,[26,44,45] *Oxyrrhis marina*,[46] ciliates,[47,48] echinoderm larvae[27] and salps.[49]

We did not observe microplastic uptake in *Parasagitta* sp. (chaetognaths) following 1 or 24 hour exposures to 30.6 μm beads, or siphonophorae (Cnidaria) exposed to 20.6 μm plastics, possibly as a result of handling stress, or more likely because these zooplankton are raptorial predators and feed actively, so were not enticed to capture the immotile microplastics.[37] Furthermore, only 10−50% of *Obelia* sp., Paguridae larvae and Porcellinidae (zoea) specimens presented with polystyrene beads in their intestinal tracts post-exposure. As we also observed size-selective ingestion in *A. clausi* and *C. helgolandicus*, it is important to consider how microplastics may impact on different zooplankton feeding strategies. Zooplankton use both mechanoreception (i.e., detection of pressure disturbances within the water) and chemoreception (i.e., detection of infochemicals emitted by algal cells) to sense prey.[29,37] As such, the clean immotile beads used in our algal-free experiments are less likely to be detected by exposed zooplankton, although it is possible that aged microplastics, that have developed biofilms during their residence within the marine environment,[10] may generate a chemosensory response; this effect was observed in the copepod *Eurytemora affinis* which more readily ingested beads spiked with bacteria than when offered beads alone.[50] While some copepods will continuously filter-feed regardless of prey availability, others (e.g., *C. pacificus*, *A. tonsa*) can limit their movement and filter-feed at reduced rates to conserve energy when faced with low food concentrations.[51,52] The presence of algae promotes greater uptake of microplastics in the filter-feeding copepods *Calanus pacificus*[26] and *Eucalanus pileatus* CV copepodites;[53] notably, *A. clausi* only ingests 16 μm polystyrene beads in the presence of algae.[24] Some zooplankton can ingest or reject prey upon capture, depending on surface characteristics and charge of the particle, both echinoderm larvae and the copepods *A. clausi* and *E. pileatus* can reject plastic beads that coalesced within their mouthparts.[27,53,54] The presence of microplastics may also alter the behavior of zooplankton, limiting their capacity to feed; in *Acartia tonsa* copepodites, contact with 45 μm plastic beads caused the organisms to "jump", limiting time dedicated to feeding bouts and reducing their clearance rates by 60%.[55]

Post-ingestion, polystyrene beads were observed to coalesce within the midgut of copepods prior to egestion. While gut-retention times of these microplastics were typically similar to natural food items (i.e., egestion occurred within hours), a follow-up experiment found some *Calanus helgolandicus* individuals retained microplastics for up to 7 days. Microplastics found in the marine environment include fibers, granules, and fragments manufactured from a range of polymers;[30] if such irregularly shaped and fibrous microplastics were ingested, they may become entangled within the intestinal tract, potentially resulting in a nonbiodegradable gut-blockage and greater gut-retention times. Plastic fibers entangle within the intestinal tracts of Nephrops in this manner,[14] wheras fish[16,17] and seabird dissections[15] have demonstrated that marine wildlife can retain a range of plastic detritus within their stomachs near-indefinitely. Prolonged gut-retention times of

plastics and gut-blockages in zooplankton may limit the ability of these organisms to ingest and digest food, and may pose a toxic risk. During manufacture, a suite of additives (e.g., plasticisers, flame-retardants, antimicrobials) are added to plastics, and large surface area to volume ratio and hydrophobic properties of microplastics make them particularly susceptible to the adherence of waterborne contaminants (e.g., PCBs, DDT, and PAHs).[19] The leaching of additives and disassociation of toxic contaminants post-ingestion has been modeled in polychaete worms[56] and demonstrated in streaked shearwaters.[57] In zooplankton, as with other marine biota, these contaminants might be considered endocrine-disruptors, carcinogenic, or toxic, with repercussions for growth, sexual development, fecundity, morbidity, and mortality.[58,59] Of further concern is trophic-transfer: microplastics (and contaminants released from microplastics) within lower-trophic, keystone organisms such as zooplankton may result in the trophic-transfer of these contaminants up the food-chain, with the potential for bioaccumulation and therefore adverse health consequences in higher trophic organisms.

Copepods that died during exposures, and shed molts of copepodites, were coated in microplastics—presumably because of hydrophobic- or static-attractions between the negatively charged polystyrene (average zeta potential: −41.8 mV) and organic material—a process that acts to concentrate microplastics from the surrounding seawater. Our observations of microplastic-laden faecal pellets egested by copepods provided no indication that passage through the alimentary canal had any discernible impact on the microplastics. However, plastics may alter the density and structural integrity of faecal pellets with potential repercussions on vertical carbon flux.[60] During our studies, we also found microplastics were becoming trapped between the external appendages and carapace segments of live copepods. We found that very small microplastics (0.4−3.8 μm) became lodged between the filamental hairs and setae of the antennules, furca, and the swimming legs.[29,61] As these appendages have key roles in copepod function and behavior, this may have repercussions for locomotion, ingestion, mating, and mechanoreception, that may limit their ability to detect prey, feed, reproduce, and evade predators.

We found that the presence of 7.3 μm beads significantly reduced the amount of algae eaten by the copepod *Centropages typicus*, whereas 20.6 μm beads showed no discernible impact on algal consumption. This suggests *C. typicus* can preferentially feed upon algae over 20.6 μm beads (but could not differentiate between the algae and 7.3 μm beads), or, that only the smaller beads impact on copepod feeding (i.e., 7.3 μm beads are small enough to become entrapped between external appendages or be recurrently ingested). A similar finding has been observed with *Acartia clausi* and *Calanus pacificus* nauplii, which selectively fed upon small algae while avoiding larger beads, but could not discriminate between algae and beads of a similar size.[24,45,54] We found that a concentration of 4000 beads mL^{-1} was enough to result in significantly reduced algal ingestion rates. This relationship reached saturation at concentrations of >5000 beads mL^{-1}. Two previous studies have found similar results, where the ingestion rates of the copepod *A. clausi*[24] and *C. pacificus*[45] were significantly reduced by the presence of beads of a similar size to the algae. A reduction in algal feeding may have severe consequences for copepods, as limited energy intake, in particular with species that have minimal lipid reserves (e.g., *Centropages*, *Acartia*), could result in decreased fecundity and growth, or increased mortality.[24,62] We do not yet know whether 5000 particles mL^{-1} can be considered an environmentally relevant concentration for microplastics <10 μm in size. Perpetual fragmentation of plastic litter, coupled with the increasing popularity of household products containing microscopic plastic exfoliates,[5] suggests marine plastic debris is becoming, on average, smaller over time.[63] However, due to the complexities of sampling and extraction, and in the absence of unified sampling methodologies, microplastics are still considered to be an under-researched fraction of marine litter, with no consistent data relating to plastic detritus <333 μm in diameter.[1,30,64] Further, we must consider that microplastics made of polymers other than polystyrene, potentially laden with chemical additives or adhered contaminants, could result in different interactions with zooplankton with variable impacts on function.

Our findings confirm that ingestion of marine microplastic debris by zooplankton in the ocean is feasible. Potential impacts include reduced function and health of the individual, trophic-transfer of contaminants to predators, and the egestion of faecal pellets containing microplastics. Better knowledge of the extent of microplastic contamination of oceans waters is now a research imperative.

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Notes

The authors declare no competing financial interest.

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