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Ingestion of Nanoplastics and Microplastics by Pacific Oyster Larvae

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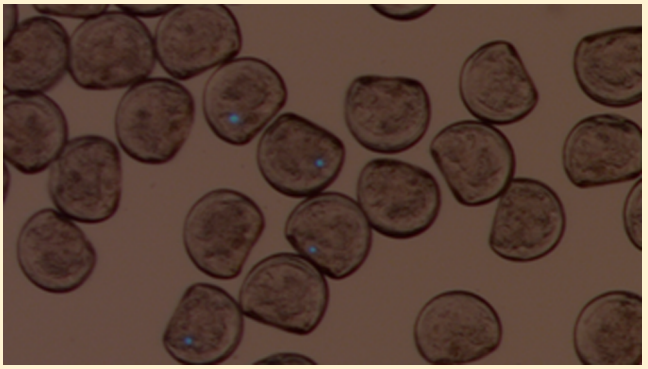
Supporting Information

ABSTRACT: Plastic debris is a prolific contaminant effecting freshwater and marine ecosystems across the globe. Of growing environmental concern are "microplastics"and "nanoplastics" encompassing tiny particles of plastic derived from manufacturing and macroplastic fragmentation. Pelagic zooplankton are susceptible to consuming microplastics, however the threat posed to larvae of commercially important bivalves is currently unknown. We exposed Pacific oyster (*Crassostrea gigas*) larvae (3−24 d.p.f.) to polystyrene particles spanning 70 nm-20 μm in size, including plastics with differing surface properties, and tested the impact of microplastics on larval feeding and growth. The frequency and magnitude of plastic ingestion over 24 h varied by larval age and size of polystyrene particle (ANOVA, $P < 0.01$), and surface properties of the plastic, with aminated particles ingested and retained more frequently (ANOVA, $P < 0.01$). A strong, significant correlation between propensity for plastic consumption and plastic load per organism was identified (Spearmans, $r = 0.95$, $P < 0.01$). Exposure to 1 and 10 μm PS for up to 8 days had no significant effect on *C. gigas* feeding or growth at <100 microplastics mL^{-1}. In conclusion, whil micro- and nanoplastics were readily ingested by oyster larvae, exposure to plastic concentrations exceeding those observed in the marine environment resulted in no measurable effects on the development or feeding capacity of the larvae over the duration of the study.



INTRODUCTION

Plastic is a versatile, durable, and widely used material, with countless applications including in packaging, construction, textiles, and electronics.[1,2] Globally, plastic production has risen rapidly over the past 60 years and we currently produce over 299 million tonnes per annum.[3] Increasingly plastics are being used to manufacture single-use items (e.g., food and beverage packaging), which is resulting in vast amounts of plastic waste being produced every year. While the majority of this waste is discarded via landfill, incinerated, or recycled, immense quantities of improperly disposed plastic waste are entering the aquatic environment via littering, sewage, runoff, landfill leachates, and illegal dumping.[4,5] Recently, Eriksen et al.[6] estimated over 5 trillion pieces of plastic are floating on the surface of the oceans; innumerable amounts of plastic have been further documented on the seafloor.[7−11] The vast majority of this marine plastic litter is categorized as "microplastic", microscopic plastic (1 mm to 1 μm diameter) which can be directly manufactured, with applications in personal care products and media blasting,[12] or derived from the biological, photochemical, and mechanical breakdown of macroplastics.[13] An emerging contaminant of concern are "nanoplastics" (here defined as <1 μm diameter), manufactured for use in powdered coatings, paints, and medicine, generated as a byproduct of plastic manufacture and 3D printing, or derived from the prolonged "nanofragmentation" of larger plastics.[13−15] In nanoparticle (NP) research, a size classification of <100 nm is more commonly applied for NPs, as particles below this size threshold tend to demonstrate colloidal behaviors and characteristics that differentiate them from their larger counterparts.[16]

Plastic debris has impinged on freshwater and marine ecosystems across the globe, including lakes,[17] shelf-seas,[18] midoceanic atolls,[19] deep-sea sediments,[20] and polar ice.[21] Over 630 species, including fish, turtles, cetaceans, seabirds, bivalves, and crustaceans, have been recorded interacting with plastic debris.[22] Ingestion of microscopic plastic fragments and fibers may cause physical harm, such as gut blockages or intestinal perforation,[23,24] or facilitate the transfer of persistent organic pollutants or toxic additives to the organism.[25] Marine zooplankton, including copepods, decapod larvae, bivalve larvae (species unknown), and gelatinous plankton, can ingest nanoplastics and microplastics via suspension feeding and trophic transfer (i.e., consumption of prey containing microplastic).[26−30] Zooplankton are vitally important to marine ecosystems: underpinning marine food webs, playing key roles in marine biogeochemical cycling, and encompassing the

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juvenile life stages of commercially important fish and invertebrate species. In copepods and freshwater zooplankton nanoplastics and microplastics have been associated with reduced feeding and sublethal health impacts.[26,29,31] However, the risk plastic particulates pose to bivalve larvae are currently unknown.

Pacific oysters (*Crassostrea gigas*) are considered a model invertebrate organism and a keystone species within marine ecosystems, with a high economic and ecological importance. Adult *C. gigas* typically grow in shallow subtidal or intertidal waters, where they can form reefs which can provide stability to coastlines, increase biodiversity and improve water quality.[32] Owing to their popularity as seafood and high tolerance to salinity and temperature, *C. gigas* are widely used in aquaculture, which has led to their worldwide distribution. Pacific oysters are fecund, broadcast spawners. Postfertilization, embryos rapidly develop (typically within 48 h) into planktonic veliger larvae that feed upon microalgae to support growth prior to settlement. Owing to their proximity to urban coastlines and large filtration volumes, oysters are considered at risk to anthropogenic pollutants, including tributyltin, organochloride pesticides and heavy metals which can cause toxicity, reduce immune defense and increase mortality.[33,34] Oysters are also susceptible to ingesting microplastics: recently adult *C. gigas* being sold for human consumption, sourced from the northeast Atlantic, were found to contain 0.47 ± 0.16 microplastics g^{-1} ww.[35] Consumption of microplastics by adult oysters is reported to affect feeding and gamete quality.[36] However, the uptake and impacts of nanoplastics and microplastics upon the health of oyster larvae are yet to be explored.

In this study we investigate the threat plastic particulates pose to *C. gigas* larvae. First we test the capacity for oyster larvae to ingest nanoplastic and microplastic polystyrene (PS) varying in size and surface properties. Second we investigate the relationship between the frequency of plastic consumption with the average number of particles ingested per larvae. Lastly, we explore whether microplastics can impact the feeding capacity and growth of *C. gigas* during larval development.

MATERIALS AND METHODS

Oyster Larvae. Pacific oyster (*C. gigas*) larvae were obtained through strip spawning of conditioned adults (Guernsey Sea Farms, Channel Islands). Larvae were transported on filters (held on ice), and then transferred to multiple flasks containing 4 L of artificial seawater (ASW; 1 μm filtered) and algae for a minimum 2 h recovery period, at a stocking density of 5 larvae mL^{-1}. Prior to experimental use, subsamples were taken to verify larval viability (i.e., motility, shape) and conduct counts. All experiments were conducted within a controlled temperature laboratory (20 °C; 16:8 light:dark).

Seawater. Artificial seawater was prepared on site using purified water and marine salts (Tropic Marin).[37] Seawater had a salinity of 33 ± 0.3 ppt and pH 8.22−8.24.

Algae. Two algal strains were cultured on site: the haptophyte *Isochrysis galbana* (Centre for Sustainable Aquatic Research, Swansea) and the diatom *Phaeodactylum tricornutum* (Marine Biological Association, Plymouth). Algae were maintained on Guillard's F/2 (SigmaG0154), enhanced with sodium metasilicate for *P. tricornutum*, in an algal incubator (22 °C, 45 rpm, 16:8 light:dark, Infors HTMultitron Pro). For feeding and growth experiments we used a mix of *Isochrysis* spp. (*T. iso* strain) and *Chaetoceros calcitrans* (5:1 ratio; Guernsey Sea Farms, Channel Islands). Algal size, biovolume and population density was verified using coulter counter (Beckman Multisizer 3).

Nanoplastic and Microplastic Preparation. Standard and fluorescently labeled PS beads were purchased from Spherotech (70 nm−20 μm diameter, 470 nm excitation). To remove any preservatives (i.e., 0.01% NP40 detergent, 0.02% sodium azide), stock solutions were centrifuged (10 000 rpm at 20 °C for 10 min; Biofuge) and the supernatant pipetted off. Pelleted beads were washed by repeatedly suspending the particles in Milli-Q, centrifuging (per previous parameters) and removing the supernatant. Prior to experimental use, beads were suspended in ASW (0.02 μm filtered), and stored in the dark at 2 °C to limit microbial growth. Microplastic stocks were sonicated (20 kHz, Cole Palmer 130 W ultrasonic processor) for 10 s prior to quantification and experimental use. Stock concentrations were ascertained using coulter counter (Beckman Multisizer 3) for >4 μm beads, and flow cytometry (BD Accuri C6) for <4 μm particulates, and serial dilutions performed for microscopy counts. The mass equivalent dose of plastic used in each experiment was calculated using the density, size and concentration of PS (Supporting Information (SI) Table S.1, Table S.2).

Nano- and microplastic sizes were confirmed using transmission electron microscopy (TEM): in brief, 2% stock solutions were pipetted onto Pioloform coated hexagonal 100 mesh copper grids and dried at 60 °C for 5 min. Samples were viewed using TEM (Jeol JEM-1400 TEM operated at 120 kV) and micrographs taken at nominal magnifications using a systematic uniform random sampling pattern (SI Figure S1). Particles (n > 50) were systematically selected using sizing and circularity parameters, and Feret diameter calculated using ImageJ (SI Table S.3). To ascertain whether the plastics aggregated in seawater, PS beads were added to seawater at a concentration of 1000 microplastics mL^{-1}, sonicated and maintained for 24 h. Postexposure, subsamples were viewed under a microscope (Olympus SZX16, ×10 objective) and aggregation behavior observed.

Nanoplastic and Microplastic Consumption. To determine the size range of plastic that can be ingested by juvenile *C. gigas*, we exposed larvae (3, 10, and 24 d.p.f.) to fluorescent PS beads: 0.07, 0.16, 0.87, 1.84, 4.1, 7.3, 10.2, and 20.3 μm diameter. Larvae were incubated for 24 h in 200 mL of lightly aerated ASW (1 μm filtered), containing 1000 microplastics mL^{-1} (excluding control), and 25 000 cells mL^{-1} of both *I. galbana* and *P. tricornutum* (n = 5 per treatment). We further assessed whether oyster larvae differentially consume microplastics with varying surface properties. Using the protocols described above, we exposed 8 d.p.f. larvae to either: 0.87 μm fluorescent PS, 0.99 μm aminated PS (PS-NH_2), or 0.94 μm carboxylated PS (PS-COOH). Postexposure, larvae were fixed (4% buffered formaldehyde), and subsequently visualized under a microscope with GFC fluorescence (≥1.84 μm PS beads: Olympus SZX16, ×6−×10 objective, 100 larvae $replicate^{-1}$; 0.87−0.99 μm PS beads: Leica DMI 4000, ×20 objective, 25 larvae $replicate^{-1}$; ≤ 0.16 μm PS beads: Zeiss Observer.Z1, ×40 oil objective, 10 larvae $replicate^{-1}$). Larvae were systematically assessed for the presence or absence of nano- and microplastics, and where present, the number of PS particles ingested per larvae was recorded.

Ingestion Rate. Assessment of oyster larvae feeding was conducted by adapting the protocols of Cole et al.[26] In brief: 74 mL glass bottles were filled with AFSW (1 μm filtered), 50 000

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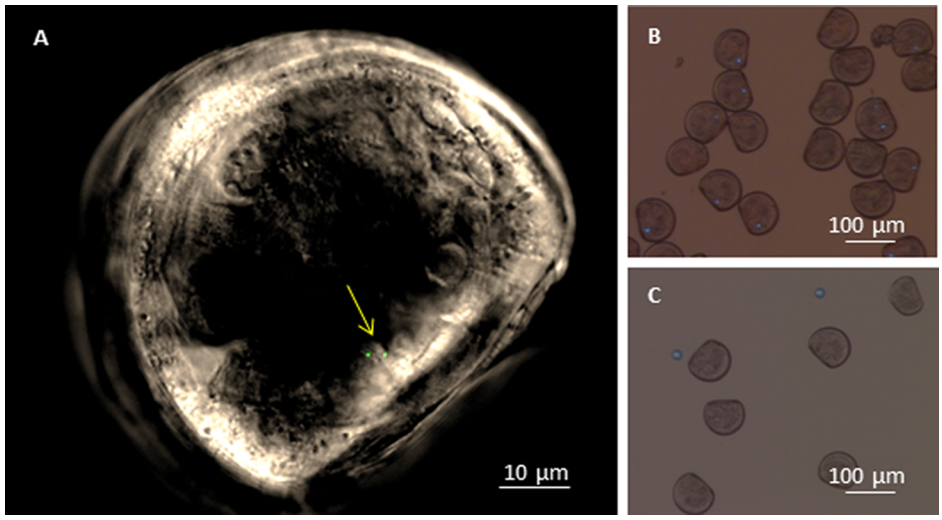

Figure 1. Ingestion of nanoplastics and microplastics by 3 d.p.f. oyster larvae varied with particulate size. (A) Oyster larva following ingestion of 160 nm fluorescent PS beads (arrow). (B) 1.84 μm PS beads were ingested by ~20% of *C. gigas* larvae. (C) Larvae were unable to ingest 20.3 μm PS beads. Images acquired using AxioVision LE software (Zeiss) and inverted microscope (Zeiss Observer.Z1), and cellSens software (Olympus) and stereomicroscope (Olympus SZX16).

cells mL^{-1} of algae (*T. iso* and *C. calcitrans* mix; 5:1 ratio) and PS microplastic beads (1 or 10 μm) to give total plastic concentrations of: 0, 1, 10, 100, or 1000 microplastics mL^{-1} (n = 4). Oyster larvae (9 d.p.f.) were added to each bottle at a concentration of 5 larvae mL^{-1}, with the exception of controls to determine algal growth without predation. Bottles were rotated on a plankton wheel (<5 rpm) for 24 h. Samples were preserved (4% buffered formaldehyde) and subsequently analyzed using a coulter counter (Beckman Multisizer3) to determine algal concentrations and biovolume. Algal ingestion rate was calculated by carbon biomass (ng C larvae^{-1} day^{-1}), using the equation of Frost.[38]

Growth. We conducted an eight-day exposure to gauge the effect of microplastics on larval size. Oyster larvae (5 larvae mL^{-1}) were incubated in 400 mL glass beakers, containing 300 mL AFSW (1 μm filtered) and 50 000 cells mL^{-1} of algae (*T. iso* and *C. calcitrans* mix; 5:1 ratio), supplied with light aeration and a cover to limit airborne contamination; full water changes were conducted every other day. We used three treatments: plastic free control (n = 6), 1 μm PS microplastics (100 microplastics mL^{-1}; n = 6) and 10 μm PS microplastics (100 microplastics mL^{-1}; n = 6). Following the exposure larvae were visualized (Zeiss Observer.Z1; ×10 objective), photographed and the images digitally analyzed to determine larval size (ImageJ: larvae selected by circularity and size; size calculated as pixels).

Statistics. Statistical analysis was conducted using R, and graphs prepared using Microsoft Excel. Analysis of variance (ANOVA) with Tukey's posthoc test was used to compare the various treatments. Spearman's rank test was used to assess correlation between percentage of larvae ingesting plastic, and average number of beads consumed. Data is reported as mean ± standard error. P values <0.05 considered significant.

RESULTS

Nanoplastic and Microplastic Size and Aggregation. The diameter of nano- and microplastic beads largely conformed to the sizes provided by the supplier, although differences were observed (SI Table S3). Throughout this paper we continue to use the manufacturer's size classes for consistency. All beads, with the exception of the 70 nm particles which were irregular in shape and size, appeared spherical (SI Figure S1). Owing to their small size, we were unable to visualize 70 nm beads internalized by larvae using fluorescent-coupled microscopy. We therefore excluded 70 nm data from these results. Visual inspection of fluorescent plastic particulates (>0.16 μm) incubated in seawater for 24 and 48 h showed no evidence of aggregation.

Plastic Consumption. Uptake experiments revealed that planktonic oyster larvae have the capacity to ingest both nanoplastics and microplastics. Oyster larvae of all ages (3, 10, and 24 d.p.f.) demonstrated capacity for consuming nano-PS (0.16 and 0.87 μm diameter; Figure 1A). Further, larvae of all ages tested showed the ability to ingest 1.84−7.3 μm diameter polystyrene particulates (Figure 1B), however 20.3 μm PS was only bioavailable to larger 24 d.p.f. *C. gigas* larvae (Figure 1C).

Larval age and the size of plastic had a significant impact on plastic consumption (percentage uptake; ANOVA, P < 0.001; Figure 2A−C) and the average numbers of beads consumed per larvae (mean plastic per larvae; ANOVA, P < 0.01; Figure 2E−G). With 3 and 10 d.p.f. oysters, the proportion of individuals ingesting plastic, and average number of plastics present per individual, decreased with increasing plastic size. For example, all 3 d.p.f. larvae consumed 160 nm beads, with an average load of 10.5 particles per larva, whereas 1.8 μm PS microplastics were only visualized in 23% of individuals, with an average microplastic load of 0.26 beads larva^{-1}. Older 24 d.p.f. larvae showed the capacity to consume the full range of microplastics proffered, with 0.87, 7.3, and 10.4 μm diameter beads consumed by approximately 80% of individuals. Aminated-PS was consumed by approximately half the 8 d.p.f. larvae, whereas carboxylated and nonfunctionalized PS was ingested by 10−20% of larvae (ANOVA, P < 0.01; Figure 2D). The mean number of beads per larva was also significantly greater for aminated PS (ANOVA, P < 0.05; Figure 2H).

We identified a strong, significant correlation between the mean number of beads (including nanoplastic, microplastics and particles with differing surface properties) consumed per larvae and the percentage of individuals showing uptake (Spearmans, r = 0.95, P < 0.01; Figure 3).

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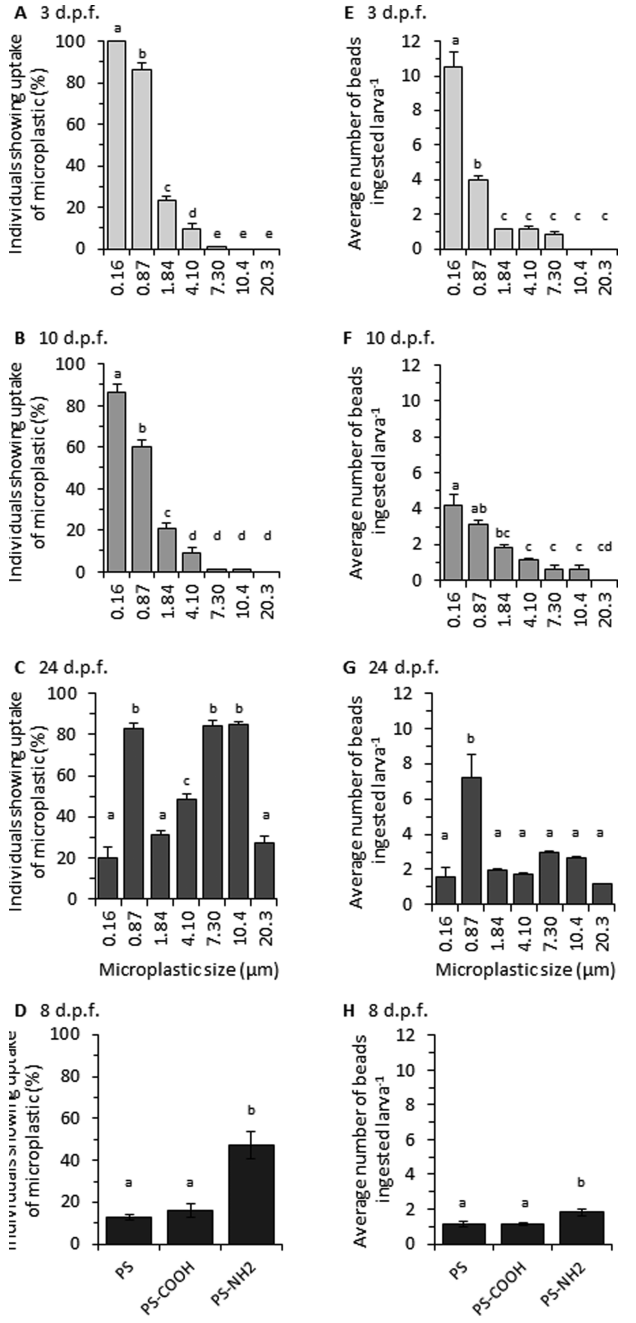

Figure 2. Ingestion of nanoplastics and microplastics by planktonic oyster larvae varied with particulate size, surface properties and the age of larvae. Left column: Proportion of oyster larvae (3, 10, 24, and 8 d.p.f.) containing PS following a 24 h exposure period (n = 5 per treatment). Right column: Mean number of microplastics beads present within these oyster larvae (3, 10, 24, and 8 d.p.f.) following a 24 h exposure period (n = 5 per treatment). Bars show mean values, error bars show standard error; different letters denote statistical significance (P < 0.05, ANOVA) between treatments.

Ingestion Rate. In the absence of plastic, larvae consumed an average of 0.7 and 0.8 ng C larvae^{-1} day^{-1} across the two experimental setups. The presence of 1 μm microplastics had a subtle, albeit significant effect on the algal ingestion rates (ANOVA, P < 0.05; Figure 4A), owing to reduced algal ingestion rates of larvae exposed to 1000 microplastics mL^{-1}, which were significantly lower than larvae exposed to 1 microplastic mL^{-1} (ANOVA Tukey PostHoc test, P = 0.049).

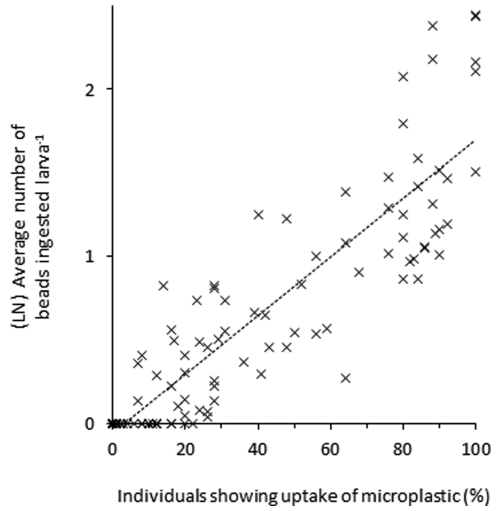

Figure 3. Relationship between the number of oyster larvae demonstrating uptake of nanoplastics and microplastics (natural log transformation) and the mean number of PS particles ingested per larvae (3, 8, 10, and 24 d.p.f.) following a 24 h exposure (R_s = 0.99, n = 35, P < 0.01).

However, 10 μm microplastics had no significant impact on algal ingestion rates (ANOVA, P = 0.60; Figure 4B).

Growth. Following 8 d exposure, there was no significant difference in the size of the oyster larvae (ANOVA, P = 0.53; Figure 4C) irrespective of treatment.

DISCUSSION

Our study demonstrates that planktonic *C. gigas* larvae can readily ingest waterborne nanoplastic and microplastic PS particles. We identified a strong, significant relationship between the number of individuals ingesting plastic and the number of microplastics consumed per individual. In this first study to assess the risk microplastics pose to bivalve larvae, we found 1 and 10 μm PS had limited impact on algal feeding, with no observed consequence for larval growth at 100 microplastics mL^{-1}.

We identified that nano-PS (<1 μm) could be internalized by oyster larvae (3–24 d.p.f.). Although 70 nm PS was too small to be visualized, we observed 160 nm PS beads in 100% of 3 d.p.f. larvae and >80% of 10 d.p.f. larvae, and 870 nm PS in >80% of 3 and 24 d.p.f. larvae. Nanoparticles <100 nm in size have unique properties that may enhance their toxicity to organisms; these particles can pass through cellular membranes inducing cytotoxic damage,[39] and their large surface area to volume ratio and surface properties dramatically increase sorption of hydrophobic compounds such as planar PCBs.[40] We were unable to determine whether nano-PS (>160 nm) had translocated across the gut epithelia but deem this an important consideration for future research efforts. In vitro experiments have identified that carboxylated nano-PS beads (20–60 nm) can penetrate cells, interfere with the endoplasmic reticulum and inhibit cytochrome P450 isoenzymes, presumably by inducing conformational changes.[41] In vitro, nano-PS (24 nm) can bind to apolipoprotein A-I in fish cells, potentially limiting lipid acquisition from fat reserves.[42] Uptake of nano-PS has been demonstrated in zooplankton, including the copepod *Tigriopus japonicus* (50 and 500 nm),[29] freshwater *Daphnia magna* (24 nm)[31,42] and sea urchin larvae.[43] In *T. japonicus*, exposure to 50 nm PS (12.5 μgmL^{-1}; two-generation chronic

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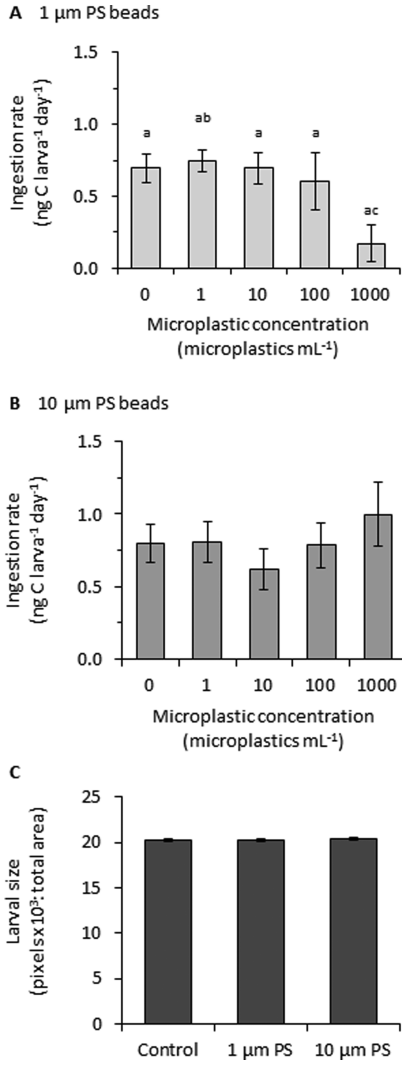

Figure 4. Microplastics had limited impact on oyster larvae. Mean ingestion rate (ng C larvae^{-1} mL^{-1}) of algal prey by 8 d.p.f. planktonic oyster larvae exposed to (A) 1 μm PS microplastics (n = 5 per treatment; P = 0.706, ANOVA); (B) 10 μm microplastics (n = 5 per treatment; P = 0.102, ANOVA). (C) The mean size, measured as pixels (ImageJ), of larvae following 8 d exposure: plastic free control, 1 μm [100 microplastics mL^{-1}] and 10 μm PS [100 microplastics mL^{-1}].

toxicity test) increased development times and mortality of nauplii and copepodites in the F0 generation, and exposure to 500 nm plastics was associated with a reduction in egg production. Similarly, in *D. magna,* exposure to nano-PS (220 μg/mL; pre-exposed to algae) resulted in reduced growth, survival and neonate clutch size, and increased neonate abnormalities (68% occurrence) at 32 mg L^{-1}. Nanoplastics (<100 nm) can aggregate in water,[44] although we saw no evidence of this in these studies with plastics >160 nm in size, increasing their bioavailability to adult bivalves.[45] Exposure to nano-PS aggregates can result in adult mussels (*Mytilus edulis*) closing their shells and increasing faeces and pseudofaeces production to limit uptake.[44]

Our observations that microplastics can be ingested by oyster larvae adds to growing evidence that zooplankton are highly susceptible to consuming plastic particulates. Marine zooplankton, including pelagic copepods,[26,27,30,46] benthic copepods,[29] sea urchin larvae,[28] euphausiids,[46] salps,[47] decapod larvae[27] and unidentified bivalve larvae,[27] have been shown to consume microplastic. Microplastic consumption in zooplankton is largely attributed to suspension feeding, a relatively indiscriminate feeding mode by which large volumes of water are filtered by the organism to remove particles of nutritional value. Oyster larvae feed by beating their cilia, drawing water through their oral groove and velum, where cilia filter particulates out.[48] Gerdes[49] notes filtering activity of oyster larvae can range from 0.5 to 100 μL larva^{-1} h^{-1} depending on larval age and food availability. We identified that the maximal size of plastic consumed by larvae increased with larval age: 7.3 μm in 3 d.p.f.; 10.4 μm in 10 d.p.f.; 20.3 μm (the maximum bead size used in this experiment) in 24 d.p.f. larvae. As *C. gigas* larvae develop and grow, expansion of the oral grooves will allow for larger particles to be consumed. In the Portugese oyster *Crassostrea angulata*, the oral groove of larvae is approximately 20−35% of velum diameter and expands in size as larvae develop.[48] We found there was no lower size limit of plastics that could be ingested, however, the frequency of 0.16 μm PS consumption decreased in aging larvae: 100% uptake in 3 d.p.f.; 86% in 8 d.p.f.; 20% in 20 d.p.f. larvae. In copepods, setal spacing on the maxillae limit efficient uptake of smaller particles,[50] and we speculate that reduced uptake of nanoparticles in older larvae may result from larger cilia spacing in the velum. The limitations of these physical constraints would suggest particulate uptake is limited mainly by the size and abundance of the particle; therefore, in an environmental setting, microplastic uptake by oyster larvae would be proportional to the localized concentrations of plastic.

We observed that aminated PS beads were present in a greater number of larvae than carboxylated and standard PS beads. TEM imaging showed all these beads were of equal size (0.80−0.81 μm diameter), therefore any differences likely relate to the surface properties of the plastic. Our results suggest that aminated PS were either: (1) consumed more readily, and/or (2) retained in the intestinal tract for longer, than standard and carboxylated PS. At face value, plastics with amine and carboxyl groups on their surface will have positive and negative surface charges, respectively. Determining the net surface charge of particles would typically be assessed using zeta-potential or their electrophoretic ability,[51] but these methods are not appropriate with high-ionic strength media such as seawater.[52] Our expectation is that in seawater the surface charge of these particles will be rendered obsolete by the overwhelming number of charged ions (H^+ and OH^-) that are electrostatically attracted to the plastics' surface (Richard Handy, personal communications). The surface charge of the plastics within the intestinal tracts of oyster larvae is unknown. Torre et al.[43] observed that carboxylated PS (40 nm) formed microaggregates which accumulated in the digestive tracts of developing sea urchin (*Paracentrotus lividus*) embryos. Retention of aminated PS is a concern for developing oyster larvae as in vitro studies have demonstrated these cationic particles can cause cellular apoptosis and embryonic malformations.[43]

Our finding that propensity for microplastic uptake correlates strongly with the number of plastics ingested by an individual larvae is of particular interest. Analysis of environmental data indicates this correlation is evident elsewhere, including ocean foraging fulmars beached on the coastline of western Europe (Spearmans, r = 0.91, P < 0.01)[53] and fish sampled from the western English Channel (Spearmans, r = 0.84, P < 0.01).[54] This trend indicates that where a high proportion of a

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population is shown to ingest plastic, the amount of plastic those individuals consume and retain is expected to be high too, and vice versa. This trend may be useful to modellers looking to predict the impacts of plastic to aquatic biota.

A recurrent issue associated with microplastic exposure in marine biota is an impact on their feeding capacity. For example, microplastics can significantly reduce algal feeding in the pelagic copepods *Centropages typicus* (20 μm PS; > 4000 microplastics mL^{-1}) and *Calanus helgolandicus* (20 μm PS; 65 microplastics mL^{-1}).[26,27] In the benthic polychaete *Arenicola marina* unplasticized polyvinyl chloride (uPVC) microplastics (<5% dw sediment) and PS microplastics (7.5% dw sediment) significantly reduced feeding activity.[55,56] In *C. helgolandicus* and *A. marina,* this reduction in feeding has been associated with a decrease in energetic reserves, egg size and hatching success and survival.[26,56,57] We observed that 100 microplastics mL^{-1} did not significantly impact algal biomass ingestion rates or larval growth in *C. gigas*. We speculate that microplastics are readily ingested and passed by oyster larvae owing to the limited selectivity and relative simplicity of their feeding apparatus; conversely, with copepods, the complex mouthparts allow for intermittent rejection of non-nutritious plastic particles, reducing time allocated to algal feeding.[26,58]

Pelagic sampling practices typically have lower size thresholds of 200−333 μm, precluding the sampling of microplastics below this size range.[59,60] However, by using 50 μm hand nets, Kang et al.[61] found that waterborne concentration of plastics in Geoje Bay, South Korea exceeded 15 500 microplastics m^{-3} (<0.02 microplastics mL^{-1}). Photodegradation and fragmentation of plastic is expected to result in plastics becoming smaller and more numerous over time.[14,15] If we assume a liberal 1000-fold higher plastic concentration for nano- and microplastics of the size bioavailable to oyster larvae, based on the maximal reported filtration rate (100 μL h^{-1}) for oyster larvae, we calculate oyster larvae could filter <36 plastics day^{-1}. According to our results, this would be insufficient to affect feeding or growth. However, we cannot rule out that over time, chronic exposure to nanoplastics and microplastics, including plastics of different shapes, size and polymer plastics with adhered contaminants (e.g., PCBs, PBDEs), might have cytotoxic effects which could affect the development of *C. gigas* larvae. Furthermore, the uptake of plastics by oyster larvae may represent a route by which plastics can enter the food web at large. With their small size, large cohorts and a limited escape response, oyster larvae are bioavailable to a range of marine planktivores. The trophic transfer of nanoplastics and microplastics is widely documented, including: algae to zooplankton to fish;[42] copepods to mysid shrimp;[30] fish to Norwegian lobster;[62] and mussels to crabs.[63] The potential for biomagnification of plastic particulates up the food chain is of particular concern for organisms at higher trophic levels, seafood biosecurity, and ultimately human health.[39,64]

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.5b04099.

Additional information as noted in the text (PDF)

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) Cole, M.; Lindeque, P.; Halsband, C.; Galloway, T. S. Microplastics as contaminants in the marine environment: A review. *Mar. Pollut. Bull.* **2011**, *62*, 2588−2597.

(2) Thompson, R. C.; Swan, S. H.; Moore, C. J.; vom Saal, F. S. Our plastic age. *Philos. Trans. R. Soc., B* **2009**, *364* (1526), 1973−1976.

(3) PlasticsEurope *Plastics - the facts 2014/2015*, 2015. http://www.plasticseurope.org/Document/plastics-the-facts-20142015.aspx?FolID=2.

(4) Derraik, J. G. B. The pollution of the marine environment by plastic debris: a review. *Mar. Pollut. Bull.* **2002**, *44* (9), 842−852.

(5) Jambeck, J. R.; Geyer, R.; Wilcox, C.; Siegler, T. R.; Perryman, M.; Andrady, A.; Narayan, R.; Law, K. L. Plastic waste inputs from land into the ocean. *Science* **2015**, *347* (6223), 768−771.

(6) Eriksen, M.; Lebreton, L. C.; Carson, H. S.; Thiel, M.; Moore, C. J.; Borerro, J. C.; Galgani, F.; Ryan, P. G.; Reisser, J. Plastic Pollution in the World's Oceans: More than 5 Trillion Plastic Pieces Weighing over 250,000 Tons Afloat at Sea. *PLoS One* **2014**, *9* (12), e111913.

(7) Claessens, M.; Meester, S. D.; Landuyt, L. V.; Clerck, K. D.; Janssen, C. R. Occurrence and distribution of microplastics in marine sediments along the Belgian coast. *Mar. Pollut. Bull.* **2011**, *62* (10), 2199−2204.

(8) Ivar do Sul, J. A.; Spengler, A.; Costa, M. F. Here, there and everywhere. Small plastic fragments and pellets on beaches of Fernando de Noronha (Equatorial Western Atlantic). *Mar. Pollut. Bull.* **2009**, *58* (8), 1236−1238.

(9) Lattin, G. L.; Moore, C. J.; Zellers, A. F.; Moore, S. L.; Weisberg, S. B. A comparison of neustonic plastic and zooplankton at different depths near the southern California shore. *Mar. Pollut. Bull.* **2004**, *49* (4), 291−294.

(10) Watters, D. L.; Yoklavich, M. M.; Love, M. S.; Schroeder, D. M. Assessing marine debris in deep seafloor habitats off California. *Mar. Pollut. Bull.* **2010**, *60* (1), 131−138.

(11) Schulz, M.; Krone, R.; Dederer, G.; Wätjen, K.; Matthies, M. Comparative analysis of time series of marine litter surveyed on beaches and the seafloor in the southeastern North Sea. *Mar. Environ. Res.* **2015**, *106*, 61−67.

(12) Zitko, V.; Hanlon, M. Another Source of Pollution by Plastics: Skin Cleansers with Plastic Scrubbers. *Mar. Pollut. Bull.* **1991**, *22* (1), 41−42.

(13) Andrady, A. L. Microplastics in the marine environment. *Mar. Pollut. Bull.* **2011**, *62* (8), 1596−1605.

(14) Koelmans, A. A.; Besseling, E.; Shim, W. J., Nanoplastics in the aquatic environment. In *Marine Anthropogene Litter*; Bergmann, M., Gutow, L., Klages, M., Eds.; Springer: Berlin, 2015; pp 325−340.

(15) Andrady, A. L., Persistence of plastic litter in the oceans. In *Marine Anthropogenic Litter*; Springer, 2015; pp 57−72.

(16) Petersen, E. J.; Diamond, S. A.; Kennedy, A. J.; Goss, G. G.; Ho, K.; Lead, J.; Hanna, S. K.; Hartmann, N. B.; Hund-Rinke, K.; Mader, B. Adapting OECD aquatic toxicity tests for use with manufactured nanomaterials: key issues and consensus recommendations. *Environ. Sci. Technol.* **2015**, *49* (16), 9532−9547.

(17) Eerkes-Medrano, D.; Thompson, R. C.; Aldridge, D. C. Microplastics in freshwater systems: A review of the emerging threats, identification of knowledge gaps and prioritisation of research needs. *Water Res.* **2015**, *75*, 63−82.

DOI: 10.1021/acs.est.5b04099
Environ. Sci. Technol. 2015, 49, 14625−14632

(18) Lusher, A. L.; Burke, A.; O'Connor, I.; Officer, R. Microplastic pollution in the Northeast Atlantic Ocean: Validated and opportunistic sampling. *Mar. Pollut. Bull.* **2014**, *88* (1), 325−333.
(19) Costa, M.; Ivar do Sul, J.; Silva-Cavalcanti, J.; Araújo, M.; Spengler, Â.; Tourinho, P. On the importance of size of plastic fragments and pellets on the strandline: a snapshot of a Brazilian beach. *Environ. Monit. Assess.* **2010**, *168* (1), 299−304.
(20) Woodall, L. C.; Sanchez-Vidal, A.; Canals, M.; Paterson, G. L.; Coppock, R.; Sleight, V.; Calafat, A.; Rogers, A. D.; Narayanaswamy, B. E.; Thompson, R. C. The deep sea is a major sink for microplastic debris. *R. Soc. Open Sci.* **2014**, *1* (4), 140317.
(21) Obbard, R. W.; Sadri, S.; Wong, Y. Q.; Khitun, A. A.; Baker, I.; Thompson, R. C. Global warming releases microplastic legacy frozen in Arctic Sea ice. *Earth's Future* **2014**, *2* (6), 315−320.
(22) Gall, S.; Thompson, R. The impact of debris on marine life. *Mar. Pollut. Bull.* **2015**, *92* (1), 170−179.
(23) van Franeker, J. A., *Fulmar Litter EcoQO Monitoring in the Netherlands 1979−2008 in relation to EU Directive 2000/59/EC on Port Reception Facilities*; IMARES: Wageningen UR, 2010; Vol. IMARES Report C027/10.
(24) Wilcox, C.; Van Sebille, E.; Hardesty, B. D., Threat of plastic pollution to seabirds is global, pervasive, and increasing. In *Proceedings of the National Academy of Sciences*, 2015; 201502108.
(25) Bakir, A.; Rowland, S. J.; Thompson, R. C. Enhanced desorption of persistent organic pollutants from microplastics under simulated physiological conditions. *Environ. Pollut.* **2014**, *185*, 16−23.
(26) Cole, M.; Lindeque, P.; Fileman, E.; Halsband, C.; Galloway, T. The impact of polystyrene microplastics on feeding, function and fecundity in the marine copepod *Calanus helgolandicus*. *Environ. Sci. Technol.* **2015**, *49* (2), 1130−1137.
(27) Cole, M.; Lindeque, P.; Fileman, E.; Halsband, C.; Goodhead, R.; Moger, J.; Galloway, T. S. Microplastic ingestion by zooplankton. *Environ. Sci. Technol.* **2013**, *12*, 6646−6655.
(28) Kaposi, K. L.; Mos, B.; Kelaher, B.; Dworjanyn, S. A. Ingestion of microplastic has limited impact on a marine larva. *Environ. Sci. Technol.* **2013**, *48* (3), 1638−1645.
(29) Lee, K.-W.; Shim, W. J.; Kwon, O. Y.; Kang, J.-H. Size-Dependent Effects of Micro Polystyrene Particles in the Marine Copepod *Tigriopus japonicus*. *Environ. Sci. Technol.* **2013**, *47* (19), 11278−11283.
(30) Setälä, O.; Fleming-Lehtinen, V.; Lehtiniemi, M. Ingestion and transfer of microplastics in the planktonic food web. *Environ. Pollut.* **2014**, *185*, 77−83.
(31) Besseling, E.; Wang, B.; Lürling, M.; Koelmans, A. A. Nanoplastic affects growth of S. obliquus and reproduction of D. magna. *Environ. Sci. Technol.* **2014**, *48* (20), 12336−12343.
(32) Grabowski, J. H.; Brumbaugh, R. D.; Conrad, R. F.; Keeler, A. G.; Opaluch, J. J.; Peterson, C. H.; Piehler, M. F.; Powers, S. P.; Smyth, A. R. Economic valuation of ecosystem services provided by oyster reefs. *BioScience* **2012**, *62* (10), 900−909.
(33) Luna-Acosta, A.; Renault, T.; Thomas-Guyon, H.; Faury, N.; Saulnier, D.; Budzinski, H.; Le Menach, K.; Pardon, P.; Fruitier-Arnaudin, I.; Bustamante, P. Detection of early effects of a single herbicide (diuron) and a mix of herbicides and pharmaceuticals (diuron, isoproturon, ibuprofen) on immunological parameters of Pacific oyster (Crassostrea gigas) spat. *Chemosphere* **2012**, *87* (11), 1335−1340.
(34) Chu, F.-L.; Hale, R. Relationship between pollution and susceptibility to infectious disease in the eastern oyster, Crassostrea virginica. *Mar. Environ. Res.* **1994**, *38* (4), 243−256.
(35) Van Cauwenberghe, L.; Janssen, C. R. Microplastics in bivalves cultured for human consumption. *Environ. Pollut.* **2014**, *193*, 65−70.
(36) Sussarellu, R.; Soudant, P.; Lambert, C.; Fabioux, C.; Corporeau, C.; Laot, C., Microplastics: effects on oyster physiology and reproduction. In *International workshop on fate and impact of microplastics in marine ecosystems (MICRO2014)*; Plouzane (France), 2014.
(37) Atkinson, M. J.; Bingman, C. Elemental composition of commercial seasalts. *Journal of Aquariculture and Aquatic Sciences* **1998**, *8* (2), 39−43.
(38) Frost, B. W. Effect of size and concentration of food particles on the feeding behaviour of the marine planktoinic copepod *Calanus pacificus*. *Limnol. Oceanogr.* **1972**, *17*, 805−815.
(39) Bouwmeester, H.; Hollman, P. C.; Peters, R. J. Potential health impact of environmentally released micro-and nanoplastics in the human food production chain: experiences from nanotoxicology. *Environ. Sci. Technol.* **2015**, *49* (15), 8932−47.
(40) Velzeboer, I.; Kwadijk, C.; Koelmans, A. Strong sorption of PCBs to nanoplastics, microplastics, carbon nanotubes, and fullerenes. *Environ. Sci. Technol.* **2014**, *48* (9), 4869−4876.
(41) Fröhlich, E.; Kueznik, T.; Samberger, C.; Roblegg, E.; Wrighton, C.; Pieber, T. R. Size-dependent effects of nanoparticles on the activity of cytochrome P450 isoenzymes. *Toxicol. Appl. Pharmacol.* **2010**, *242* (3), 326−332.
(42) Cedervall, T.; Hansson, L.-A.; Lard, M.; Frohm, B.; Linse, S. Food chain transport of nanoparticles affects behaviour and fat metabolism in fish. *PLoS One* **2012**, *7* (2), e32254.
(43) Della Torre, C.; Bergami, E.; Salvati, A.; Faleri, C.; Cirino, P.; Dawson, K. A.; Corsi, I. Accumulation and Embryotoxicity of Polystyrene Nanoparticles at Early Stage of Development of Sea Urchin Embryos Paracentrotus lividus. *Environ. Sci. Technol.* **2014**, *48* (20), 12302−12311.
(44) Wegner, A.; Besseling, E.; Foekema, E.; Kamermans, P.; Koelmans, A. Effects of nanopolystyrene on the feeding behavior of the blue mussel (Mytilus edulis L.). *Environ. Toxicol. Chem.* **2012**, *31* (11), 2490−2497.
(45) Ward, J. E.; Kach, D. J. Marine aggregates facilitate ingestion of nanoparticles by suspension-feeding bivalves. *Mar. Environ. Res.* **2009**, *68* (3), 137−142.
(46) Desforges, J.-P. W.; Galbraith, M.; Ross, P. S. Ingestion of Microplastics by Zooplankton in the Northeast Pacific Ocean. *Arch. Environ. Contam. Toxicol.* **2015**, *69* (4), 320−330.
(47) Chan, W. Y.; The impact of microplastics on salp feeding in the tropical Pacific. *ANU Undergrad. Res. J.* **2012**, *4*.
(48) Qiu, T.; Liu, Y.; Zheng, J.; Zhang, T.; Qi, J. A feeding model of oyster larvae (Crassostrea angulata). *Physiol. Behav.* **2015**, *147*, 169−174.
(49) Gerdes, D. The Pacific oyster Crassostrea gigas. *Aquaculture* **1983**, *31* (2), 195−219.
(50) Frost, B. W. Feeding Behavior of *Calanus pacificus* in Mixtures of Food Particles. *Limnol. Oceanogr.* **1977**, *22* (3), 472−491.
(51) Hassellöv, M.; Readman, J. W.; Ranville, J. F.; Tiede, K. Nanoparticle analysis and characterization methodologies in environmental risk assessment of engineered nanoparticles. *Ecotoxicology* **2008**, *17* (5), 344−361.
(52) Neihof, R. A.; Loeb, G. I. The surface charge of particulate matter in seawater. *Limnol. Oceanogr.* **1972**, *17* (1), 7−16.
(53) van Franeker, J. A.; Blaize, C.; Danielsen, J.; Fairclough, K.; Gollan, J.; Guse, N.; Hansen, P.-L.; Heubeck, M.; Jensen, J.-K.; Le Guillou, G.; Olsen, B.; Olsen, K.-O.; Pedersen, J.; Stienen, E. W. M.; Turner, D. M. Monitoring plastic ingestion by the northern fulmar Fulmarus glacialis in the North Sea. *Environ. Pollut.* **2011**, *159* (10), 2609−2615.
(54) Lusher, A.; McHugh, M.; Thompson, R. Occurrence of microplastics in the gastrointestinal tract of pelagic and demersal fish from the English Channel. *Mar. Pollut. Bull.* **2012**, *67* (1−2), 94−99.
(55) Wright, S. L.; Rowe, D.; Thompson, R. C.; Galloway, T. S. Microplastic ingestion decreases energy reserves in marine worms. *Curr. Biol.* **2013**, *23* (23), 1031−1033.
(56) Besseling, E.; Wegner, A.; Foekema, E. M.; van den Heuvel-Greve, M. J.; Koelmans, A. A. Effects of Microplastic on Fitness and PCB Bioaccumulation by the Lugworm Arenicola marina (L.). *Environ. Sci. Technol.* **2013**, *47* (1), 593−600.
(57) Wright, S.; Rowe, D.; Thompson, R. C.; Galloway, T. S. Microplastic ingestion decreases energy reserves in marine worms. *Curr. Biol.* **2013**, *23* (23), 1031−1033.

DOI: 10.1021/acs.est.5b04099
Environ. Sci. Technol. 2015, 49, 14625−14632

(58) Kiørboe, T. How zooplankton feed: mechanisms, traits and trade-offs. *Biological Reviews* **2011**, *86*, 311−339.
(59) Cole, M.; Webb, H.; Lindeque, P. K.; Fileman, E. S.; Halsband, C.; Galloway, T. S. Isolation of microplastics in biota-rich seawater samples and marine organisms. *Sci. Rep.* **2014**, *4*, 4528.
(60) Hidalgo-Ruz, V.; Gutow, L.; Thompson, R. C.; Thiel, M. Microplastics in the marine environment: a review of the methods used for identification and quantification. *Environ. Sci. Technol.* **2012**, *46* (6), 3060−3075.
(61) Kang, J.-H.; Kwon, O. Y.; Lee, K.-W.; Song, Y. K.; Shim, W. J. Marine neustonic microplastics around the southeastern coast of Korea. *Mar. Pollut. Bull.* **2015**, *96* (1−2), 304−312.
(62) Murray, F.; Cowie, P. R. Plastic contamination in the decapod crustacean Nephrops norvegicus (Linnaeus, 1758). *Mar. Pollut. Bull.* **2011**, *62* (6), 1207−1217.
(63) Watts, A. J.; Lewis, C.; Goodhead, R. M.; Beckett, S. J.; Moger, J.; Tyler, C. R.; Galloway, T. S. Uptake and retention of microplastics by the shore crab Carcinus maenas. *Environ. Sci. Technol.* **2014**, *48* (15), 8823−8830.
(64) Galloway, T. S., Micro-and nano-plastics and human health. In *Marine Anthropogenic Litter*; Springer, 2015; pp 343−366.

DOI: 10.1021/acs.est.5b04099
Environ. Sci. Technol. 2015, 49, 14625−14632