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## Chemoreception drives plastic consumption in a hard coral

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## ABSTRACT

The drivers behind microplastic (up to 5 mm in diameter) consumption by animals are uncertain and impacts on foundational species are poorly understood. We investigated consumption of weathered, unfouled, biofouled, pre-production and microbe-free National Institute of Standards plastic by a scleractinian coral that relies on chemosensory cues for feeding. Experiment one found that corals ingested many plastic types while mostly ignoring organic-free sand, suggesting that plastic contains phagostimulents. Experiment two found that corals ingested more plastic that wasn't covered in a microbial biofilm than plastics that were biofilmed. Additionally, corals retained ~8% of ingested plastic for 24 h or more and retained particles appeared stuck in corals, with consequences for energetics, pollutant toxicity and trophic transfer. The potential for chemoreception to drive plastic consumption in marine taxa has implications for conservation.

## 1. Introduction

Plastics are ubiquitous across almost all facets of modern economies. In 2012, global plastic production was 288 million metric tons (MT), a 620% increase from 1975 (Jambeck et al., 2015). Large amounts of plastic become marine debris entering the ocean through land-based runoff, the fishing industry, and other sources including aquaculture (Andrady, 2011; Thompson, 2015). Microplastics, pieces of plastic up to 5 mm in diameter, are generated through photolytic, thermal-oxidative, hydrolytic, abrasive and biotic weathering of larger pieces (Andrady, 2011). Microplastics began accumulating in the oceans more than four decades ago (Thompson et al., 2004; Thompson et al., 2005) and are now ubiquitous in marine environments, reported in surface water (Barnes et al., 2009), mid-water (Lattin et al., 2004), benthic sediments (Van Cauwenberghe et al., 2015) and deep-sea habitats (Fischer et al., 2015).

Micro and macro plastic debris are consumed through foraging (Moore, 2008; Graham and Thompson, 2009) by a wide variety of animals, including birds, turtles, mammals, fish, and invertebrates (Lusher, 2015). Animals are thought to ingest plastic because it looks like prey (Ryan, 1987; Fukuoka et al., 2016; Boerger et al., 2010), is the correct size (Moore, 2008), or because it is covered by additional organic compounds (Brilliant and MacDonald, 2002; Kastelein and Lavaleije, 1992). Plastic is so widespread that some animals undoubtedly ingest it incidentally while pursuing other prey (Lusher et al., 2013; Bravo Rebollo et al., 2013; Walker and Coe, 1989), or because they are starving and desperate (Kastelein and Lavaleije, 1992; De

Pierrepoint et al., 2005). Consumed plastics are largely indigestible and do not break down in the gut, making them dangerous. Ingestion can result in gut blockage (Stamper et al., 2006), false satiation (Watts et al., 2015; Murray and Cowie, 2011) and reduced energy reserves (Wright et al., 2013a, 2013b; Watts et al., 2015). Apart from physical impacts on marine organisms, commercial plastics leach hundreds of compounds; most have unknown biological effects but plasticizers like phthalates are confirmed environmental estrogens and androgens (Li et al., 2015; Pothitou and Voutsas, 2008; Tharp et al., 2012). In addition to toxicity from chemicals added during manufacturing (Li et al., 2015), plastics adsorb substances that are persistent, bioaccumulative, and toxic (PBTs) from the environment (Engler, 2012; Lee et al., 2013; Besseling et al., 2012; Rochman et al., 2013), creating a vector for PBTs and environmental steroids into marine food webs (Setälä et al., 2014; Wright et al., 2013a, 2013b).

The majority of microplastic ingestion studies focus on marine fish and seabirds. Research on microplastic ingestion by marine invertebrates is still nascent (Lusher, 2015). Invertebrates like corals offer important insights because they rely on chemoreception to capture prey, initiating feeding responses stimulated by compounds found on and in prey items (Lenhoff and Heagy, 1977). Thus, examining ingestion of plastics by corals has implications for how other taxa use chemical senses to interact with plastics.

Coral ecosystems are vital to climate resiliency, maintenance of biodiversity and provision of natural resources for humans (Worm et al., 2006), so it is important to understand the impacts microplastics have on corals. Impacts of plastics can be direct, through ingestion, or

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indirect due to the new substrate generated by plastic surfaces available for colonization by invasive organisms (Barnes, 2002) and exposure to plastic leachates in the environment (Li et al., 2015). Ingestion is the most direct and experimentally tractable route.

Hall et al. (2015) pioneered study of plastic ingestion by corals. Based on concentrations of microplastics remaining in water, corals were estimated to consume microplastic polypropylene (PP) at rates between 1.2 and 55  $\mu\text{g h}^{-1}$  per square centimeter of coral fragment. Our study builds on Hall et al. (2015) by addressing two questions: 1) Do corals ingest pre-production plastic devoid of microbes and contaminants? 2) What are ingestion, egestion and retention rates using environmentally weathered plastic with and without biofilms? We hypothesized that corals would not ingest and retain pre-production or weathered microplastics without a microbial film. Conversely, we expected corals would ingest weathered microplastics with a biofilm because microbes provide surface phagostimulants and nutritional value. We predicted that corals would egest the majority of ingested plastic.

In experiment one, we handed fragments of pre-production plastic, microbe-free National Institute of Standards and Technology (NIST) plastic, weathered plastics, and organic-free sand to *Astrangia poculata* polyps, recording the firing of nematocysts and/or ingestion of the fragment. In experiment two, we used a feeding chamber to expose *A. poculata* to either unfouled or biofouled microplastics for 30 min. At the end of the feeding trial the corals were moved to a container where they egested plastic for 24 h. At the end of the 24 h the amount of egested plastic was measured. Coral skeletons and flesh were dissolved and the amount of plastic retained after 24 h determined. Experimentally weathered plastics were used in experiment two because they represent plastics encountered by corals in the ocean; weathering may affect retention rates due to differences in particle shape or surface area (Watts et al., 2015; Wright et al., 2013a, 2013b). We used a mixture of polystyrene (PS), low-density polyethylene (LDPE) and high-density polyethylene (HDPE) due to their prevalence in oceanic surface waters (Hidalgo-Ruz et al., 2012). A 24 h egestion period was included immediately after the feeding trial because the ability to egest plastic reduces the likelihood of physical blockage, decreases the time for desorption of contaminants, and reduces trophic transfer of microplastics (Wright et al., 2013a, 2013b). Consumption and retention were directly measured by weighing plastic from the egestion tank and recovered from corals after their skeletons and flesh were dissolved.

## 2. Methods & materials

### 2.1. Coral collection & husbandry

Our study coral, *A. poculata* (formerly *A. danae*), is a small, euryhaline, facultatively symbiotic, solitary stony coral found in temperate to tropical coastal waters (Jacques and Pilson, 1980; Peters et al., 1988). We chose *A. poculata* because it is well studied, primarily heterotrophic, hardy in laboratory settings, and applicable to other scleractinian corals (Jacques et al., 1983). We collected *A. poculata* on shell hash from sites in Back Sound and Bogue Sound, North Carolina (lat/lon: ~34.708, -76.710 on 2/22/2016 and 34.702, -76.621 11/16/2015) using a scallop dredge.

Coral was kept in ambient seawater and transferred to rectangular 105 L single pass water tables with an inlet flow of 72–90 L/h and circulating flow provided by a 1117 L/h pump. Electric heaters maintained temperature at 18–22°C. Salinity for the time of year the experiment occurred is typically 32.9 psu (SD = 1.45 psu) and mean pH is 7.9 (SD = 0.027) (Johnson et al., 2013). Supplemental fluorescent light was provided 3 m overhead of the water tables, with a L:D cycle of 14:10. Corals were fed by delivering ~0.33 g newly hatched *Artemia* nauplii, ~0.4 g freeze dried zooplankton and ~1.25 g pulverized cichlid pellets to the tanks 4–5 times per week. To prepare coral colonies, the extraneous shell hash substrate was cut away from colonies by using diagonal cutters. This occurred at least two days before a given colony

was used in an experiment.

### 2.2. Experiment 1: plastic and sand preparation

Microbe-free NIST standard HDPE and LDPE pellets, as well as pre-production pellets of polypropylene (PP), polyethylene terephthalate (PET), polycarbonate (PC), polyvinyl chloride (PVC) and polystyrene (PS) were broken into pieces suitable for *A. poculata* to ingest. NIST pellets were assumed to be microbe-free due to the manufacturing and packaging protocols followed during their production. In order to minimize risk of microbial contamination and avoid chemical changes induced by heat, we fragmented NIST and clean plastic pellets into small pieces using liquid nitrogen and a hammer. Approximately 250 mg of each variety of plastic was contained in a clean, double-layered aluminum foil pouch, sandwiched between two steel plates and dipped in a bath of liquid nitrogen for 1 min. The plates were then immediately struck with a hammer several times, shattering plastic into fragments of varying sizes. Pouches were opened and particles of 500–1000  $\mu\text{m}$  diameter were collected with sterilized forceps. Sand samples were collected from a NOAA beach (lat/lon: 34.718, -76.671) next to the Duke University Marine Lab and organics were removed by baking in a furnace at 500 °C for 4 h.

### 2.3. Experiment 1: measuring responses

Coral colonies were put in 4 cm deep glass specimen dishes with 800 ml seawater and placed under a dissection scope. Each colony was fed a single type of plastic or sand by delivering particles to the tentacles or outer oral disc of polyps, with individual polyps being fed no more than one particle and individual colonies being fed between 1 and 5 particles. Sand and plastic were not introduced on different days or at different times of the day. For positively buoyant plastic, plastic shards were adhered to ridges of sterilized forceps without directly gripping the shards. Then, shards were gently put into contact with a coral tentacle for no more than 1–2 s, after which forceps were withdrawn. Plastic would either be fired upon by cnidocytes and drawn away from the forceps (recorded as “firing cnidocyte”), or ignored and removed from the tentacle along with the forceps (recorded as “no response”). If particles were ingested after cnidocytes fired, we recorded this as “ingesting particle”. For neutral or negatively buoyant plastic and sand, shards or grains were pushed under the water surface and allowed to fall or drift into contact with tentacles or the outer oral disc. Care was taken not to introduce particles around corals' mouth, agitate the coral or allow forceps to make direct contact with tentacles or the oral discs.

### 2.4. Experiment 2: plastic preparation

Weathered pre-production pellets of polystyrene (PS), low-density polyethylene (LDPE) and high-density polyethylene (HDPE) were provided by collaborators at Scripps Institute of Oceanography. Pellets were approximately 3 by 4.8 mm. At Scripps, pellets were held in open pyrex containers and exposed to sunlight, an arid climate, and occasional rain for five years (December 2010–January 2016). After each rain event, Scripps personnel emptied the glass containers of water as quickly as possible.

We shattered the pellets in a new Magic Bullet food processor and used geological sieves to create a plastic mixture with particle sizes of 125–1000  $\mu\text{m}$ , the size range of *A. poculata* prey (Boschma, 1925). The resulting plastic mixture consisted of similar proportions of PP, LDPE and HDPE. After sorting, particles were rinsed in DI water and dried overnight in a fume hood. After shattering, the majority of the plastic surface area was assumed to be free of microbes because of new surface area generated by shattering.

We saved half of the weathered plastic particles in a dry, sealed container (“unfouled plastic”) and the other half in a 75  $\mu\text{m}$  mesh container (“bio-fouled plastic”). The mesh container was submerged in

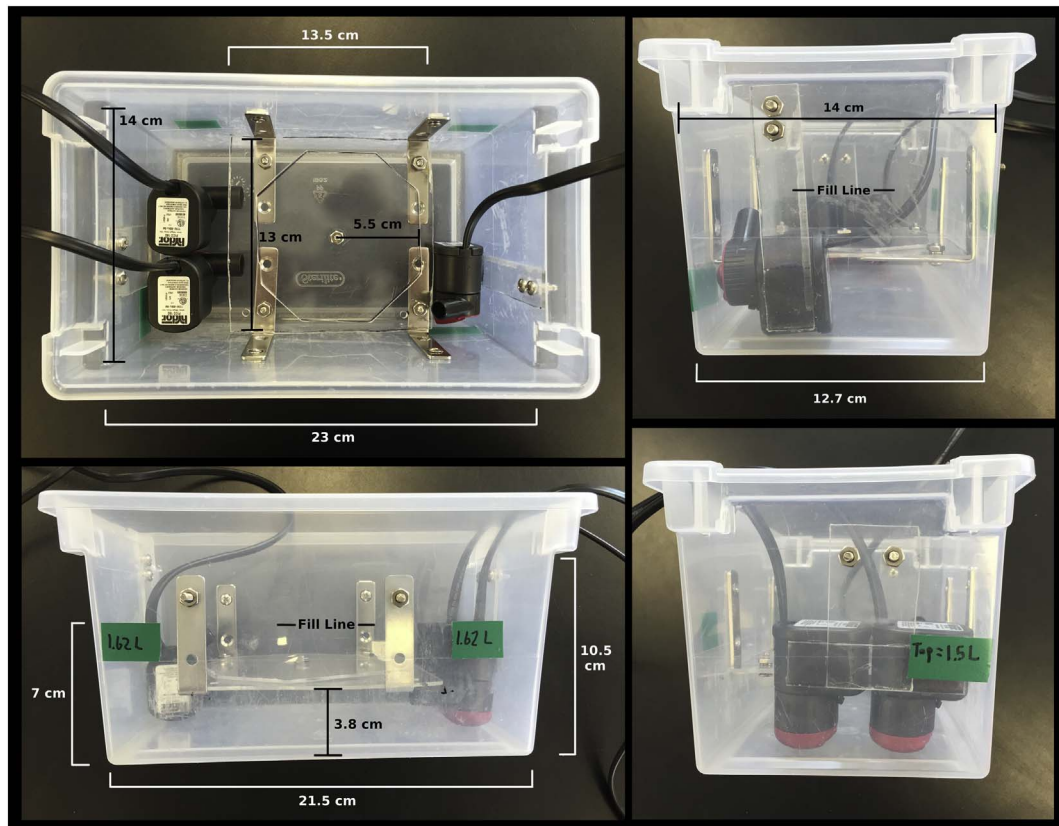


Fig. 1. Diagram and dimensions of the feeding chamber used to circulate plastic and feed corals. (A) Top-down view of the feeding chamber, showing the hexagonal rotating platform where coral was placed. (B) End view showing a pump directed upward to break surface tension at the fill line and push plastic particles underwater. (C) Side view of the feeding chamber. (D) End view showing the two main circulation pumps.

a single pass seawater table with sand filtered seawater from the Beaufort Inlet for 1 week, which is long enough for a bacterial biofilm thickness to form (Lobelle and Cunliffe, 2011; Dang et al., 2008; Jones et al., 2007). The biofouled plastics were stored in a lidded glass container of aged seawater prepared following Rittschof et al. (1992) and stored at 6 °C. All aged seawater was aged for at least 2 weeks, with a salinity of 35–37 psu and a pH of 8.15–8.2.

## 2.5. Experiment 2: feeding chamber

Feeding chamber flow was generated by 2 adjustable-output Hydor Pico 160 pumps set to 450 L/h and 1 adjustable flow Hydor Pico 70 pump set to 87 L/h (Fig. 1). A circulation pump was directed to break surface tension and force buoyant plastic into the water column. Settings were selected during preliminary trials where flow rates were adjusted until polyps in the chamber were extended comparatively to polyps remaining in the water table.

For each trial, we added 13–17 colonies of *A. poculata* (360–380 living polyps in total) to a feeding chamber filled with 1.62 L of aged seawater. All trials took place in 34–35 psu salinity water at 20–21 °C, with the exception of one trial, which occurred at 17 °C. The feeding chamber was partially immersed in a single pass water table that maintained a constant temperature. After adding colonies to the chamber, specimens were exposed to chamber flow conditions for 45 min prior to addition of plastic.

We then introduced 500 mg of either unfouled (8 trials) or bio-fouled (8 trials) plastic into the feeding chamber. Corals were allowed to feed on plastic for 30 min. Particles were delivered to polyps evenly by rotating the platform holding the coral colonies 180° half way through the trial.

After 30 min, corals were rinsed with aged seawater to dislodge any plastic attached to the polyp or substrate and transferred to an egestion

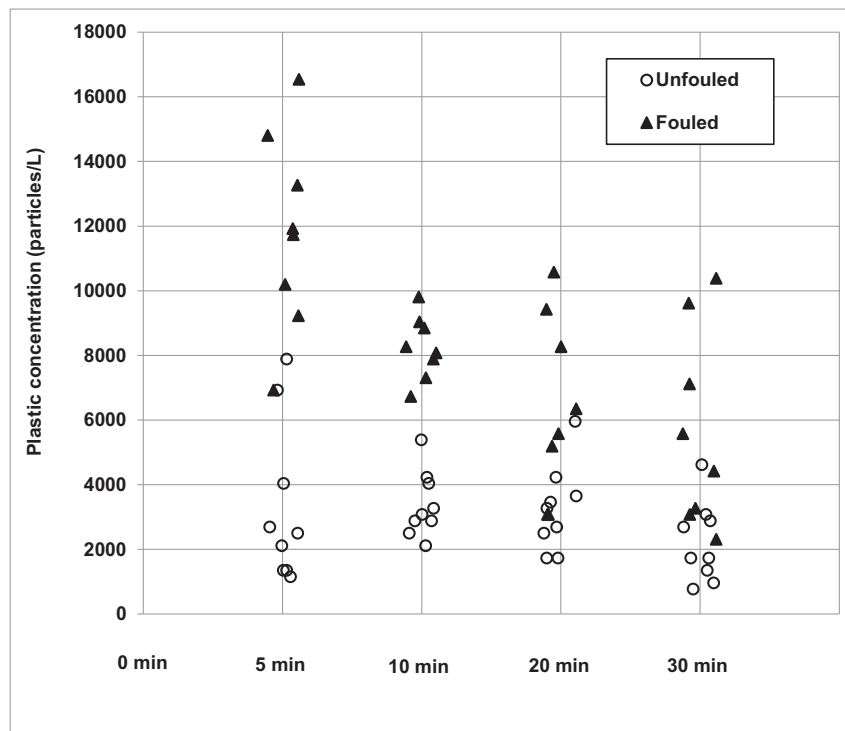
tank filled with 1.42 L of aged seawater (~143 cm<sup>2</sup> surface area). Just before adding the corals, the aged seawater was aerated for 30 min. The egestion tank was also temperature controlled by partial immersion in the same water table. Colonies were left undisturbed for 24 h, allowing them to egest plastic they had consumed. A standing water environment was maintained to reduce the likelihood of polyps re-ingesting plastic. After 24 h, colonies were rinsed with aged seawater and placed in a dry beaker.

To estimate the amount of added plastic that remained in the water column throughout the feeding trials, we ran 17 trials (9 with unfouled plastic and 8 with bio-fouled plastic) without any corals. 5.2 ml water samples were taken from the water column 5 min, 10 min, 20 min and 30 min into each trial while avoiding buoyant plastic on the surface of the water. The samples were emptied onto a vacuum filter, rinsed with DI water and dried before particles counts were taken using a stereoscope.

Bio-fouled plastic was more than twice as concentrated as unfouled plastic in the feeding chamber water column (Wilcoxon Rank Sum,  $w = 101$ ,  $p < 0.001$ ). The greatest concentration of both unfouled and bio-fouled plastic occurred during the first 10 min of the experiment and then dropped off as particles adhered to submerged bubbles (Fig. 2).

## 2.6. Experiment 2: coral dissolution

To recover plastic retained in the polyps after 24 h, we dissolved the coral polyp, skeleton, and shell hash substrate and then filtered the particles from solution. We soaked colonies in 8.25% sodium hypochlorite for 24 h, then dissolved the skeletons in 37% HCl for 30 min. A second 24-h soak in 8.25% sodium hypochlorite dissolved tissues liberated from skeletons. Particulate material was trapped on a 125 µm sieve between each acid/bleach wash, removing dissolved organic or



**Fig. 2.** Plastic particle concentrations in the feeding chamber from four time points. Concentrations were extrapolated from 5.2 ml water samples, and each point represents a sample.

inorganic matter. The resulting sample was transferred to a 50 ml centrifuge vial and consisted of plastic particles and indigestible mineral detritus, along with ~20–40 ml of DI water. We used a saturated sodium chloride solution (~35.88 g NaCl per 100 ml H<sub>2</sub>O at 20 °C) to float plastic particles and spun the sample in a centrifuge (581 × g for 10 min). The centrifuge forced mineral detritus to the base of the vial where it was then removed with a pipet. The plastic particles floated at the top of the saturated solution. Vial contents were emptied onto a vacuum filter and rinsed with DI water, leaving only plastic fragments on the filter. In summary, the particles were sorted by size, chemistry, and density. The plastic left over had a different buoyancy than sand, did not dissolve in acid or bleach, and was hard and transparent, distinguishing it from organic detritus or mineral particles. After drying the filter (50 °C for 3 h), fragments were transferred to a petri dish and weighed on a microbalance.

To measure the amount of plastic egested by corals during the 24 h egestion period, the same methods were applied to the contents of the egestion tank. Contents were emptied into a 125 µm sieve and transferred to a beaker. The samples were chemically treated and weighed using the same methods described above.

## 2.7. Experiment 2: analysis

For feeding chamber trials, weights of ingestion, egestion and plastic retention were first normalized by the number of polyps (360–380) in a given trial. Because data were not normally distributed, we then compared ingestion, egestion and retention weights between unfouled and bio-fouled treatments using Wilcoxon sum rank tests. Wilcoxon tests were also used to compare the percent of total ingested unfouled and bio-fouled plastic retained in corals after 24 h.

## 3. Results

### 3.1. Experiment one: hand feeding trials

Coral polyps reacted very differently to plastic and organic-free sand (Table 1). Organic-free sand elicited very weak cnidocyte response (1/10 particles) and corals only consumed one sand particle. Rejection of

**Table 1**

Number of particles offered to *A. poculata* polyps, the number of particles that elicited cnidocyte responses, and the number of particles ingested.

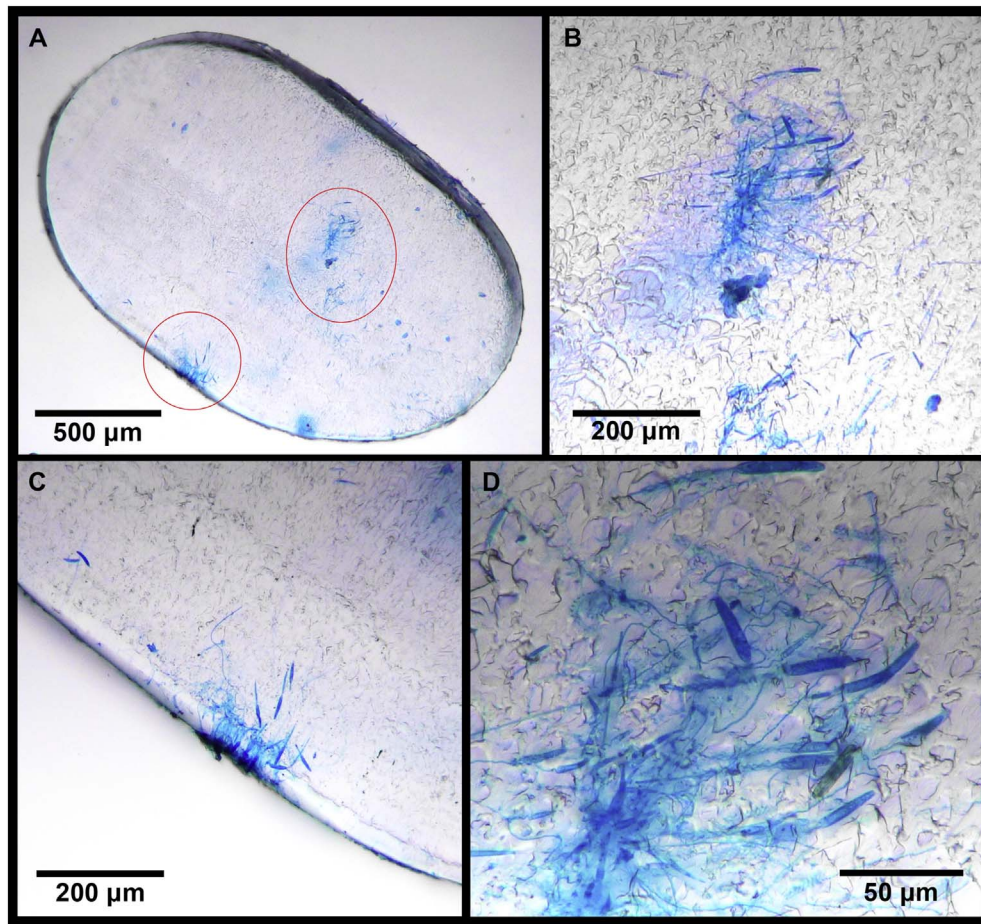
Particle type	Offered	Cnidocytes Fired	Ingested
Organic-free sand	10	1	1
NIST LDPE	10	10	9
NIST HDPE	10	9	8
Polyethylene terephthalate	10	10	8
Polyvinyl chloride	10	10	10
Polycarbonate	10	9	8
Polystyrene	10	9	8
Polypropylene	10	10	10
Weathered, unfouled plastic	10	10	10

sand particles was typified by grains slowly being rolled off of a tentacle base or oral disc over the course of several minutes, presumably by ciliary action as no responses by tentacles were observed. In contrast, more than 90% of polyps fired cnidocytes with all eight plastic types offered and more than 80% of plastic particles offered were ingested, including microbe-free NIST varieties. When plastic particles were ingested, corals moved particles from the tentacles to the mouth over the course of 30 s to several minutes, eventually swallowing the plastic. When polyps did not ingest NIST particles, 66% of the time this appeared due to positively buoyant forces freeing particles from attached cnidocytes. We confirmed that cnidocytes were firing on plastic by offering particles to polyps and then staining the particles with a protein stain and imaging under a microscope (Fig. 3).

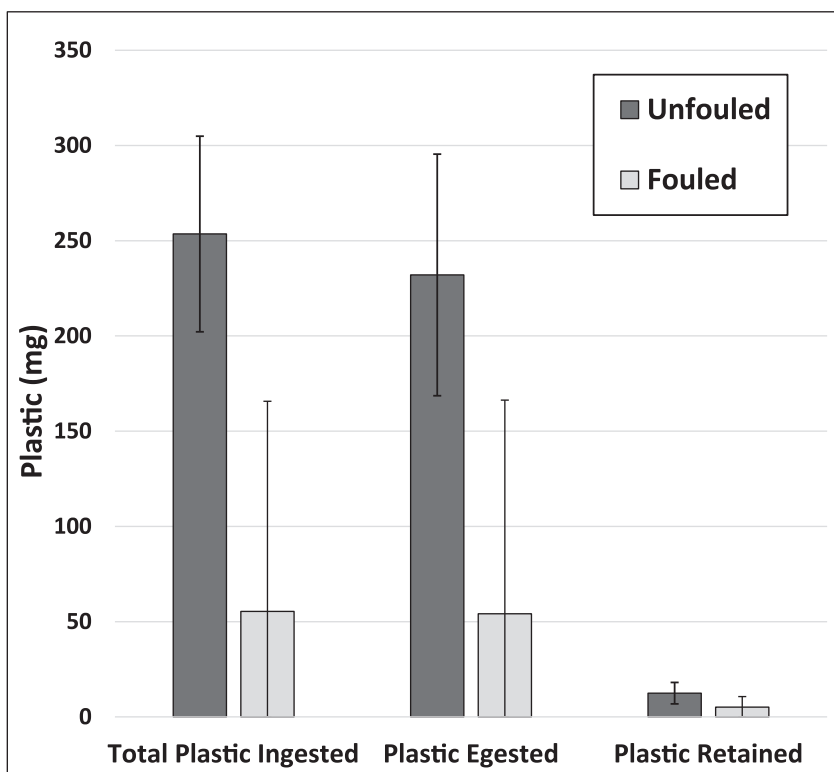
### 3.2. Experiment two: effects of microbial fouling

Corals consumed significantly more unfouled than bio-fouled plastics (Wilcoxon Rank Sum,  $w = 69$ ,  $p < 0.001$ ), Fig. 4. A median of 254 (51 IQR) mg unfouled weathered plastic per trial was ingested, compared to a median of 55 (110 IQR) mg weathered bio-fouled plastic (Wilcoxon Rank Sum,  $w = 69$ ,  $p < 0.001$ ). There was a 3 to 5-fold difference between unfouled and bio-fouled plastics for ingestion, egestion and retention, with median weights for bio-fouled plastic being about 1/3 to 1/5 of the unfouled treatment (Wilcoxon Rank Sum Test





**Fig. 3.** A thin polycarbonate slice was offered to a coral polyp and removed before it could be ingested. Slice was stained with Coomassie Blue and imaged at 10 × (A), 20 × (B and C) and 40 × (D).



**Fig. 4.** Comparison of the median total plastic consumed, plastic egested and plastic retained. Blue bars are the unfouled plastic treatment, orange bars are the bio-fouled treatment. Error bars are the inter-quartile range.

[egestion],  $w = 69$ ,  $p < 0.001$  [retention],  $w = 78$ ,  $p < 0.001$ ).

During the 24 h egestion period, corals egested a median of 94.3% (4.1 IQR) of the plastic they ingested. The percent of plastic egested was not significantly different between unfouled and bio-fouled treatments (Wilcoxon Rank Sum,  $w = 34$ ,  $p = 0.89$ ). Between 81.4% and 91.1% of this egestion took place within the first 6 h. Corals retained a median of 5.7% (4.1 IQR) of total ingested plastic after 24 h, with plastic retained in all trials. Retention rate was not significantly different between unfouled and bio-fouled treatments (Wilcoxon Rank Sum,  $w = 38$ ,  $p = 0.89$ ).

#### 4. Discussion

This study aimed to determine whether corals will ingest a variety of plastic types, and if a microbial biofilm affects ingestion or retention rates of plastics, compared to plastics without a biofilm. The study used a combination of ex-situ methods to present coral colonies with individual particles of plastic, as well as subject a number of colonies to 30 min in a “feeding chamber” with concentrations of biofouled and unfouled plastic. In experiment one, polyps actively captured and ingested all tested plastics, including weathered, pre-production and microbe-free NIST varieties, and exhibited a very weak response to organic-free sand (Table 1). Contrary to our hypothesis, experiment two demonstrated that microbial fouling greatly reduced the amount of plastic ingested, though significant amounts of biofouled plastic were still consumed (Fig. 4). Microbial fouling did not influence the proportion of plastic retained.

Prey capture and feeding by *A. poculata* and other anthozoans is under chemosensory control (Boschma, 1925; Lindstedt, 1971a; Thorington and Hessinger, 1988; Thorington and Hessinger, 1998; Muir Giebel et al., 1988) and consist of 3 separate chemically-mediated behaviors: 1) Batteries of cnidocytes are triggered to fire by compounds such as *N*-acetylated sugars, found associated with cell membranes, mucin and chitin of prey surfaces (Thorington and Hessinger, 1998). 2) A feeding incitant such as apurine then triggers tentacles to move captured prey to the mouth (Lindstedt, 1971b). 3) A feeding stimulant such as reduced glutathione (GSH, found in internal fluids of prey) triggers reversed ciliary beating on the oral disc, mouth and pharynx, resulting in ingestion (Lindstedt, 1971b). Proline and GSH are widespread feeding stimulants for cnidarians (Lindstedt, 1971a) but a variety of common constituents of internal fluids of prey can fulfill this role (Lenhoff and Heagy, 1977; Lehman and Porter, 1973; Mariscal and Lenhoff, 1968). Thus, from the results of experiment one, it appears many plastic particles release phagostimulants and are treated similarly to prey. It is unlikely that any tested anthozoan phagostimulants are in unfouled plastics.

Across experiments one and two, prey capture and feeding behaviors were observed with NIST categories of plastic as well as weathered plastic, showing that this phenomenon is not a product of the weathering process, and likewise that weathering does not eliminate the response. In experiment one, NIST particles typically spent  $< 5$  s in the water column before making contact with tentacles and eliciting feeding responses, suggesting ingestion is not a result of early colonization by microbes. The organic-free sand particles spent a similar time in the water column and elicited a very weak response from corals (Table 1). There are two likely explanations for these results: 1) Microbial films obscure phagostimulants on plastic or provide feeding deterrents. 2) Soaking plastic in sea water (as occurred with the bio-fouled treatment) leaches and removes phagostimulants or changes surface properties, causing plastic to chemoreception less attractive.

In all our trials, corals retained a small proportion of ingested plastic through the end of the experiment, at least 24 h. This is surprising because anthozoans digest food rapidly (Boschma, 1925; Nicol, 1959; Yonge, 1931). In a previous study, *A. poculata* fully digested copepods in 3 h and retained ingested non-food particles for an average of 50 min (Boschma, 1925). These data, combined with our observation that most

particles were egested in  $< 6$  h suggest that retained plastic was stuck in the coral polyp and support reports of plastic particles found wrapped in mesenterial tissues (Hall et al., 2015).

Zooplankton (Desforges et al., 2015), insects (Yang et al., 2015; Gaylor et al., 2012), fish (Hoss and Settle, 1990), birds (Wilcox et al., 2015), and cetaceans (Baulch and Perry, 2014) are reported to ingest plastics. Because plastics are not composed of known phagostimulants, plastic consumption has been assumed to be a result of visual (Kastelein and Lavaleije, 1992; De Pierrepoint et al., 2005; Schuyler et al., 2014; Mrosovsky et al., 2009) or tactile (Moore, 2008) misidentification or from flavoring by organic compounds on plastic surfaces (Brilliant and MacDonald, 2002; Kastelein and Lavaleije, 1992). Anthozoans have no visual senses and their chemical receptors are analogous to gustatory receptors in higher phyla in that they discriminate food. Consumption of unbiofilmed plastic by cnidarians indicates that plastic, though indigestible and leaching toxic compounds (Li et al., 2015) have chemistries that cause consumption.

In these experiments it is unclear which additives or sorbed molecules triggered chemoreceptors to cause ingestion, especially in the experiment one plastics, which were weathered but never soaked in seawater where they could have adsorbed contaminants. Most pollutants tested reduce feeding rates in most, though not all, marine invertebrates examined: crustaceans, mollusks, polychaetes, and corals (Weis, 2014). Although studies on the effects of pollutants on chemoreceptors in Cnidaria are rare, Ormond and Caldwell (1982) found that crude oil acted as a feeding inducer, and suggested that some component of the oil was stimulating the same chemosensory elements as the food extracts offered. Hydrocarbons found in petroleum derivatives are just one type of pollutant, and in addition to plasticizer leachates, marine plastics have been found to concentrate many types of PBTs, sometimes 1,000,000-fold higher than seawater (Engler, 2012; Mato et al., 2001). Further studies are needed to determine the specific substances released from marine plastics that activate chemosensory pathways in marine invertebrates, including the overlap with added plasticizers and the many chemicals that plastics adsorb in seawater.

Plastic within the digestive tract impacts animals by leaching residual monomers, catalysts and a plethora of additives (Li et al., 2015), and through desorption of PBTs absorbed from the environment (Andrady, 2011; Rochman et al., 2013). Plastic consumption is energetically expensive because plastic takes up space normally filled by food (Watts et al., 2015; Wright et al., 2013a, 2013b; Cole et al., 2015), and might result in a false sense of satiation (Watts et al., 2015; Murray and Cowie, 2011). Plastic can also cause morbidity by occluding the digestive tract (Stamper et al., 2006). The shape of plastic can affect how likely it is to get stuck in the digestive tract, and Watts et al. (2015) found that fibers tended to form tangled balls in the guts of crabs. Environmental weathering, which breaks plastic into irregular shapes, may increase the residence time of plastics within animals.

The plastic retention rates observed in this study are related in part to the shape and form of the shattered test plastic, which can influence residence time (Watts et al., 2015). The concentration of plastic and delivery method we used in our feeding trials may also influence retention. We chose elevated plastic concentrations to better detect differences in ingestion between unfouled and bio-fouled treatments. The concentration of unfouled plastic in our study (mean =  $24 \text{ mg l}^{-1}$ , SEM = 13) was within the range of peak turbidity regimes in some nearshore coral reefs (Anthony, 1999; Browne et al., 2015a, 2015b), while the biofouled plastic concentration (mean =  $70 \text{ mg l}^{-1}$ , SEM = 10) was almost twice these levels. Inshore corals including *A. poculata* have higher tolerances to turbidity and sedimentation; recent studies suggest that inshore corals can tolerate pulsed turbidity events of  $30\text{--}40 \text{ mg l}^{-1}$  with little to no negative effects (Browne et al., 2015a, 2015b). Anthony found inshore corals had higher feeding efficiencies at concentrations of  $32 \text{ mg l}^{-1}$ , compared to mid-reef conspecifics that had a tendency to contract their polyps at high turbidity levels (Anthony, 2000). Inshore corals demonstrated a slight decrease in

feeding efficiency, though rates did not saturate at the levels examined (Anthony, 2000). Thus it is possible that the elevated concentration of biofouled plastics contributed to the observed differences between biofouled and unfouled ingestion rates. It is also possible that phagostimulants on the biofouled plastic surfaces leached out during the biofouling process before the feeding trials. We suggest future coral microplastic ingestion experiments control for the presence of microbial films, levels of phagostimulants, and include an egestion period. These factors greatly influence the amount of plastic ingested and retained by coral polyps, and consequently have important implications for studies of energetics, trophic ecology, and toxicology.

While our study highlights the need for research into the impact of plastic pollution on corals, a critically important group of organisms to human and ocean health, it also raises questions about why animals ingest plastics. As more and more marine organisms at the base of the food web are shown to ingest microplastics, it is important to know whether they are targeting microplastics, and if so, is chemoreceptor activation the reason for ingestion. Our study has implications for many taxa that use chemosensory cues to detect prey. The challenge of marine plastic pollution will only become more intractable in the coming decades as the amount of solid waste generated annually in the world cities continues to rise and is expected to be 2.2 billion tons by the year 2025 (Hoornweg and Bhada-Tata, 2012). Plastic currently makes up ~10% of global municipal waste, and this proportion is expected to increase in the future (Hoornweg and Bhada-Tata, 2012). The propensity for plastic to mimic the taste, smell, appearance and texture of food items will have increasingly dire consequences for environmental and human health. Waste management will continue to be a challenge, especially in developing countries, making novel policy solutions critical. Future studies should identify which varieties of common consumer plastic are consumed most readily and isolate monomers, additives, production residues or combinations of the above that trigger the gustatory receptors in organisms, including vertebrates. If compounds can be identified, the chemical composition of plastics may be modified to either add or remove compounds to make plastic less likely to activate chemoreceptors.

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