Plastics in Porifera: the occurrence of microplastics in Caribbean sponges and seawater (#56751)

First submission

Guidance from your Editor

Please submit by **25 Jan 2021** for the benefit of the authors (and your $200 publishing discount) .

Structure and Criteria
Please read the 'Structure and Criteria' page for general guidance.

Custom checks
Make sure you include the custom checks shown below, in your review.

Author notes
Have you read the author notes on the guidance page?

Raw data check
Review the raw data.

Image check
Check that figures and images have not been inappropriately manipulated.

Privacy reminder: If uploading an annotated PDF, remove identifiable information to remain anonymous.

Files

Download and review all files from the materials page.

5 Figure file(s)
2 Table file(s)
2 Raw data file(s)
1 Other file(s)

Custom checks

Field study

- Have you checked the authors field study permits?
- Are the field study permits appropriate?

Structure and Criteria

Structure your review

The review form is divided into 5 sections. Please consider these when composing your review:

1. **BASIC REPORTING**
2. **EXPERIMENTAL DESIGN**
3. **VALIDITY OF THE FINDINGS**
4. General comments
5. Confidential notes to the editor

You can also annotate this PDF and upload it as part of your review

When ready submit online.

Editorial Criteria

Use these criteria points to structure your review. The full detailed editorial criteria is on your guidance page.

BASIC REPORTING

- Clear, unambiguous, professional English language used throughout.
- Intro & background to show context. Literature well referenced & relevant.
- Structure conforms to PeerJ standards, discipline norm, or improved for clarity.
- Figures are relevant, high quality, well labelled & described.
- Raw data supplied (see PeerJ policy).

EXPERIMENTAL DESIGN

- Original primary research within Scope of the journal.
- Research question well defined, relevant & meaningful. It is stated how the research fills an identified knowledge gap.
- Rigorous investigation performed to a high technical & ethical standard.
- Methods described with sufficient detail & information to replicate.

VALIDITY OF THE FINDINGS

- Impact and novelty not assessed. Negative/inconclusive results accepted. *Meaningful* replication encouraged where rationale & benefit to literature is clearly stated.
- All underlying data have been provided; they are robust, statistically sound, & controlled.
- Speculation is welcome, but should be identified as such.
- Conclusions are well stated, linked to original research question & limited to supporting results.

Standout reviewing tips

The best reviewers use these techniques

Tip	*Example*
Support criticisms with evidence from the text or from other sources	*Smith et al (J of Methodology, 2005, V3, pp 123) have shown that the analysis you use in Lines 241-250 is not the most appropriate for this situation. Please explain why you used this method.*
Give specific suggestions on how to improve the manuscript	*Your introduction needs more detail. I suggest that you improve the description at lines 57- 86 to provide more justification for your study (specifically, you should expand upon the knowledge gap being filled).*
Comment on language and grammar issues	*The English language should be improved to ensure that an international audience can clearly understand your text. Some examples where the language could be improved include lines 23, 77, 121, 128 – the current phrasing makes comprehension difficult.*
Organize by importance of the issues, and number your points	*1. Your most important issue* *2. The next most important item* *3. …* *4. The least important points*
Please provide constructive criticism, and avoid personal opinions	*I thank you for providing the raw data, however your supplemental files need more descriptive metadata identifiers to be useful to future readers. Although your results are compelling, the data analysis should be improved in the following ways: AA, BB, CC*
Comment on strengths (as well as weaknesses) of the manuscript	*I commend the authors for their extensive data set, compiled over many years of detailed fieldwork. In addition, the manuscript is clearly written in professional, unambiguous language. If there is a weakness, it is in the statistical analysis (as I have noted above) which should be improved upon before Acceptance.*

Plastics in Porifera: the occurrence of microplastics in Caribbean sponges and seawater

Bailey R Fallon Corresp., 1 , **Christopher J Freeman** 1

1 Department of Biology, College of Charleston, Charleston, SC, United States

Corresponding Author: Bailey R Fallon
Email address: fallonbr@g.cofc.edu

Microplastics (MP) are now considered ubiquitous across global aquatic environments. The ingestion of MP by fish and other marine vertebrates is well studied, but the ingestion of MP by marine invertebrates is not. Sponges (Phylum Porifera) are particularly understudied when it comes to MP ingestion. This is surprising considering that marine sponges are widespread in benthic habitats around the globe, process large volumes of water, and are capable of retaining small particles within their water filtration systems. This study examines the presence of MP in wild Caribbean sponges. Subsurface seawater and tissue from six common Caribbean sponge species was collected in Saigon Bay, a heavily impacted, shallow-water coral reef in Bocas del Toro, Panamá. Water samples were filtered onto glass fiber filters to retain any MP present and sponge tissue was digested with bleach, heated and filtered. Filters were examined using fluorescence microscopy to quantify potential microplastics (PMP). An average of 107±25 PMP per liter was detected in seawater from Saigon Bay with particles ranging in size between 10 µm and ~3000 µm. The number of PMP found in sponge tissue ranged between 6±4 and 169±71 PMP per g of dry tissue. Most particles found in sponge samples were very small (10–20 µm), but fibers greater than 5000 µm were detected. Our results indicate an abundance of MP in Caribbean seawater, and also suggest that sponges may be resistant to chronic MP exposure.

Plastics in Porifera: the occurrence of microplastics in Caribbean sponges and seawater

Bailey R. Fallon[1] and Christopher J. Freeman[1]

[1]Department of Biology, College of Charleston, Charleston, South Carolina, USA

Corresponding Author:
Bailey Fallon[1]

Email address: fallonbr@g.cofc.edu

Abstract

Microplastics (MP) are now considered ubiquitous across global aquatic environments. The ingestion of MP by fish and other marine vertebrates is well studied, but the ingestion of MP by marine invertebrates is not. Sponges (Phylum Porifera) are particularly understudied when it comes to MP ingestion. This is surprising considering that marine sponges are widespread in benthic habitats around the globe, process large volumes of water, and are capable of retaining small particles within their water filtration systems. This study examines the presence of MP in wild Caribbean sponges. Subsurface seawater and tissue from six common Caribbean sponge species was collected in Saigon Bay, a heavily impacted, shallow-water coral reef in Bocas del Toro, Panamá. Water samples were filtered onto glass fiber filters to retain any MP present and sponge tissue was digested with bleach, heated and filtered. Filters were examined using fluorescence microscopy to quantify potential microplastics (PMP). An average of 107±25 PMP per liter was detected in seawater from Saigon Bay with particles ranging in size between 10 µm and ~3000 µm. The number of PMP found in sponge tissue ranged between 6±4 and 169±71 PMP per g of dry tissue. Most particles found in sponge samples were very small (10–20 µm), but fibers greater than 5000 µm were detected. Our results indicate an abundance of MP in Caribbean seawater, and also suggest that sponges may be resistant to chronic MP exposure.

Introduction

As humans continue to expand across the globe, our collective impact on the environment is amplified (Crutzen, 2002; Zalasiewicz et al., 2010; Lewis & Maslin, 2015). The detrimental effects of anthropogenic pollutants such as nutrients, chemicals, and sediment on the environment are well known, but the release of microplastics (MP) has been of increasing concern (Browne, Galloway & Thompson, 2007; Thompson et al., 2015; Waller et al., 2017). Microplastics are defined as any plastic particle that is between 100 nm and 5 mm in size and include spheres, pellets, fibers and other small plastics commonly used in cosmetics, clothing, pharmaceuticals and industrial products (Zitko & Hanlon, 1991; Thompson et al., 2004; Betts, 2008; Arthur, Baker & Bamford, 2009; Koelmans et al., 2015). They can be introduced to the environment via sewage, wastewater treatment effluents, industrial spills and runoff, and via the ~~degradation~~ of larger plastics (Browne et al., 2011; Cole et al., 2011; Conley et al., 2019). The progressive fragmentation of MP and their dynamic position in the water column due to wave action may impact planktonic, nektonic, and benthic organisms directly (Browne, Galloway & Thompson, 2007; Browne et al., 2008; Thompson et al., 2009; Wright, Thompson & Galloway, 2013). In addition, organisms encountering or consuming MP may be exposed to organic pollutants, heavy metals and pathogenic microbes bound to their surfaces (Mato et al., 2001; Hirai et al., 2011; Zettler, Mincer & Amaral-Zettler, 2013; Lamb et al., 2018; Rotjan et al., 2019; Dudek et al., 2020).

Much of the existing research on MP ingestion has revolved around vertebrates, with fish being the most studied group of aquatic organisms (de Sá et al., 2018). Studies that investigate MP ingestion by marine invertebrates are of mounting importance if we are to better understand the overall ~~role~~ of MP in the marine world (Wright, Thompson & Galloway, 2013; de Sá et al., 2018). Marine ciliates, calanoid copepods, amphipods, lugworms, blue mussels, Pacific oysters, sea cucumbers, sea anemones, corals, lobsters and the larvae of several invertebrate phyla have been known to ingest MP in laboratory settings, and many ingest MP *in situ* (Wilson, 1973; Ward & Targett, 1989; Hart, 1991; Christaki et al., 1998; Ward, Levinton & Shumway, 2003; Thompson et al., 2004; Browne et al., 2008; Graham & Thompson, 2009; Ward & Kach, 2009; Murray & Cowie, 2011; Hall et al., 2015; Sussarellu et al., 2015; Allen, Seymour & Rittschof, 2017; Rotjan et al., 2019). Several of these taxa exhibit some ability to select particles based on size or type, and some can defecate, regurgitate or otherwise egest the particles (Zebe & Schiedek, 1996; Wilson, 1973; Powell & Berry, 1990; Thompson et al., 2004; Graham & Thompson, 2009; Sussarellu et al., 2015; Hankins, Duffy & Drisco, 2018; Rotjan et al., 2019). Detrimental effects of MP ingestion by these animals include tissue inflammation, neurotoxicity, energy depletion, reduced skeletal growth rates, increased stress, and reduced immune function, feeding and reproduction (Besseling et al., 2013; von Moos, Burkhardt-Holm & Köhler, 2012; Avio et al., 2015; Cole et al., 2015; Sussarellu et al., 2015; Chapron et al., 2018; Hankins, Duffy & Drisco, 2018; Reichert et al., 2018; Tang et al., 2018; Rotjan et al., 2019). However, these impacts are highly variable across species, suggesting that some invertebrates may be more vulnerable to MP ingestion than others.

Sponges (Phylum Porifera) are particularly understudied in MP research, despite the fact that they are globally distributed across benthic ecosystems (Van Soest et al., 2012; de Sá et al., 2018). As prolific filter feeders, sponges often exhibit high pumping rates (0.005–0.6 liters of water per second per liter of sponge tissue) ~~and~~ can therefore process large volumes of water through their canals and greater aquiferous systems (Reiswig, 1974; McMurray, Pawlik & Finelli, 2014; Pawlik, Loh & McMurray, 2018). In fact, sponge communities may overturn the water column (up to 30 m deep) every 1–56 days (Pile, Patterson & Witman, 1996; Savarese et al., 1997; McMurray, Pawlik & Finelli, 2014; Pawlik, Loh & McMurray, 2018). As sponges draw water through their system of internal canals and chambers, they retain food particles including diatoms, cyanobacteria, viruses, flagellates, ciliates and yeast cells (Reiswig, 1971/1974/1975/1990; Frost, 1978; Imsiecke, 1993; Pile, Patterson & Witman, 1996; Pile et al., 1997; Ribes, Coma & Gili, 1999; Kowalke, 2000; Hadas et al., 2006; Maldonado et al., 2010). These food particles are typically smaller than 70 µm in diameter (Ribes, Coma & Gili, 1999) because sponge ostia (exterior, incurrent openings) rarely exceed 60 µm and typically prohibit particles greater than 50 µm from entering the sponge (Reiswig, 1971; Simpson, 1984). The removal of these food types by sponges plays an essential role in nutrient cycling on coral reefs (Lesser, 2006; Van Soest et al., 2012; de Goeij et al., 2013; Pawlik, Burkepile & Thurber, 2016; de Goeij et al., 2017). Importantly, as sponges increase their dominance on many coral reefs, their influence on overall reef function may become amplified (Zea, 1993; McMurray, Henkel & Pawlik, 2010; Colvard & Edmunds, 2011; Villamizar et al., 2013). Their widespread distribution, ability to retain small particles, and their prolific water filtering make sponges ideal candidates for evaluating MP abundance in marine systems.

Few studies have examined MP ingestion by sponges. One laboratory study exposed the temperate sponges *Tethya bergquistae* Hooper & Wiedenmayer (1994) and *Crella incrustans* Carter (1885) to 1 µm and 6 µm plastic beads and found no significant impact of the beads on sponge respiration or food particle retention (Baird, 2016). The study concluded that sponges may be resistant to MP exposure. Other laboratory studies have used plastic beads (0.1, 0.2, 0.5, 1.0, 4.0 and 5.7 µm in diameter) to study sponge physiology and have demonstrated the uptake of the beads in sponge tissues (Willenz & Van de Vyver, 1982; Turon, Galera & Uriz, 1997;

Leys & Eerkes-Medrano, 2006). Recently, Girard et al. (2020) examined the presence, abundance and diversity of microparticulate pollutants in tropical sponges from North Sulawesi, Indonesia. They found that sponges do take up foreign particles, including MP such as polystyrene, and incorporate them into their skeletons and other internal tissues (Girard et al., 2020). The authors reported a maximum concentration of 612 foreign particles per g of dry sponge tissue, and concluded that sponges may act as bioindicators of marine microparticulate pollutants (Girard et., 2020). Modica, Lanuza & García-Castrillo (2020) also recently found microfibers embedded on the surfaces of preserved museum sponge specimens representing 31 families. The authors predicted that the sponges, originally collected off the northern coast of Spain, were actively collecting fibers from the surrounding water and had been doing so for over 20 years (Modica, Lanuza & García-Castrillo, 2020).

Sponges are particularly abundant on Caribbean reefs with a high biomass, species diversity, and a percent cover that exceeds that of reef-building corals (Loh & Pawlik, 2014; Easson et al., 2015; de Bakker et al., 2017; Pawlik, Loh & McMurray, 2018). Many Caribbean sponges feed heterotrophically on dissolved and particulate organic matter (DOM and POM), but some also rely on cyanobacterial symbionts for nutrition (Erwin & Thacker, 2008; Freeman et al., 2015; McMurray et al., 2016; Rix et al., 2016). Like sponges, MP is also likely common in the Caribbean. Bosker, Guaita & Behrens (2018) found an average of 261 MP/kg of sediment on four Lesser Antilles beaches while Acosta-Coley et al. (2019) found over 100 particles/m^2 on some Colombian beaches. Garcés-Ordóñez et al. (2019) found up to 2,863 MP/kg of dry soil in polluted mangrove forests in Colombia while Rose & Webber (2019) found up to 0.00573 MP/L in surface water in the heavily polluted Kingston Harbor of Jamaica. However, surface measurements may seriously underestimate MP abundance (Gallo et al., 2018). For example, it is estimated that about one twelfth of the total number of MP present in the ocean ends up on the surface, with about the same fraction occurring in subsurface waters and the rest occurring on the seafloor and on beaches (Andrady et al., 2011). Wright, Thompson & Galloway (2013) also noted that benthic suspension and deposit feeders may be exposed to biofouled and other high-density MP that sink to the benthos. Together, these studies suggest that Caribbean sponge communities are likely exposed to MP pollution close to the benthos.

This study is the first to investigate the presence of MP in Caribbean sponges and to report a subsurface MP concentration in Caribbean seawater. We predicted that Saigon Bay, a heavily-impacted area in the Bocas del Toro archipelago of Panamá, would be polluted with MP. We further predicted that marine sponges in the bay would be collecting these particles via filter feeding because sponges select food that is very small (<70 µm) and that is within the size range for particles considered to be MP (100 nm–5000 µm). We used fluorescence microscopy to identify and quantify suspected MP and refer to detected particles as potential MP (PMP) per Covernton et al. (2019). We report the occurrence of PMP in six tropical sponge species and in seawater from Panamá and address the ecological implications of our findings.

Materials & Methods

Study site and sample collection

Sponge and seawater samples were collected from Saigon Bay near Isla Colón, Bocas del Toro, Panamá (Fig. 1). Saigon Bay sits immediately adjacent to houses, hotels and docks and is susceptible to anthropogenic pollution (Collin, 2005; Gochfeld, Schloder & Thacker, 2007; Easson et al., 2015; Fig. 1). The bay also experiences a large degree of boat traffic, which may bring pollutants from other parts of the archipelago into the area. Bocas del Toro also has an underdeveloped waste disposal infrastructure (Aronson et al., 2004; Carruthers et al., 2005; Gochfeld, Schloder & Thacker, 2007; Easson et al., 2015). With heavy and frequent rains (3–5 m per year), much of this waste enters the surrounding waterways via runoff and some is often observed floating in the area (Caruthers et al., 2005; Collin et al., 2005; Kaufmann & Thompson, 2005; Gochfeld, Schloder & Thacker, 2007; Easson et al., 2015; B. Fallon, 2019, pers. obs.).

Sponge samples were collected on 21 June 2019 from ~6–8 ~~meters~~ below the surface during an outbound tide so that any pollutants concentrated near the developed area were likely pulled through the bay (Fig. 1). A small (~5–8 cm; 0.08–1.0 g dry mass) section of sponge was removed by hand or by steel blade from three individuals (N=3 replicates) of each of the six study species: *Aplysina cauliformis* Carter (1882), *Amphimedon compressa* Duchassaing de Fonbressin & Michelotti (1864), *Callyspongia vaginalis* Lamarck (1814), *Ircinia campana* Lamarck (1814), *Mycale laevis* Carter (1882) and *Niphates erecta* Duchassaing de Fonbressin & Michelotti (1864). These species were chosen as they represent some of the most dominant sponge species in the Caribbean (Loh & Pawlik, 2014) and include a diversity of growth forms and physiologies. Each sponge section was wrapped in aluminum foil underwater and placed in a mesh bag for transport.

Four liters of water were collected at the same time and depth as the sponge samples. Four, clean ~~one-liter~~ glass jars were covered in foil and sealed with metal lids before descent. The jars were opened and filled at depth, and then re-covered with foil and sealed. This was replicated twice more on 1 July and 6 July 2019 at the same site during outbound tides. A total of three water samples (~4 L each) were obtained for this study. It should be noted that ~100–200 ml of seawater occasionally leaked from one of the glass jars. Thus, the total volume of water filtered for the water samples was between 3.6 and 4.0 L. Counts were normalized to water sample volume for quantification of PMP concentrations.

Sample processing

Water samples (N=3) were processed separately on or close to their respective collection days (21 June, 1 July and 6 July 2019). Seawater (~4 L) was vacuum filtered onto a pre-combusted (450°C for ~~four hours~~) 0.7 µm pore size (Whatman™ 1825-047 GF/F) glass microfiber filter. The four glass jars and sides of the filtration funnel were rinsed with analytical grade water and this excess water (~100 ml) was also filtered to maximize sample transfer. Water sample filters were then covered with another pre-combusted filter, wrapped in foil and stored at -20°C until further analysis. Any PMP later found on the cover filters were added to the total number of PMP recorded for its corresponding water sample filter. One procedural blank was run ~~along~~ with each of the three water samples (i.e., ~1 L of analytical grade water was added to a clean beaker, filtered, and the filter was stored at -20°C).

Each of the 18 individual sponge sections (three per species) was divided in approximately half using a steel utility blade: one half for preliminary analysis and methods development and the other half for final analysis. Each half was rinsed thoroughly with analytical grade water (as we were only interested in PMP retained within the sponge body), weighed on a clean piece of foil, wrapped in foil and frozen at -20°C until further analysis. The halves used for final PMP analysis were lyophilized and each sample was partitioned into three subsamples (~0.05–0.3 g) with a steel utility blade. Subsamples were used to minimize tissue digestion time. Each subsample was cut into pieces with a utility blade and added to a clean 20 ml glass scintillation vial and covered with foil, producing 54 subsamples. The dry weight of each subsample was recorded and approximately 5–10 ml of household bleach (Clorox®, 6% sodium hypochlorite) was added to each scintillation vial to digest the organic tissue. Bleach was used because it rapidly digests sponge tissue and because it ~~shows~~ minimal degradation of plastic particles (Hooper, 2003; Collard et al., 2015). The bleach we used was not pre-filtered to remove potential plastic contaminants before use because the high viscosity of bleach slows filtering time considerably. However, we used procedural blanks (see below) to evaluate the degree of contamination ~~in our samples~~. Vials with sponge tissue were heated ~~(up~~ to 60°C) ~~on a hot plate for two hours~~ to expedite digestion. If necessary, additional bleach was added to the vials to digest any remaining tissue.

After bleach digestion, each subsample (N=54) was filtered onto a pre-combusted 0.7 µm pore size (Whatman™ 1825-047 GF/F) glass microfiber filter. Approximately 5–10 ml of pure analytical grade water (MilliQ®) was added to the glass filtration funnel prior to the digested sponge subsample in order to minimize filtering time. After the sample was fully filtered, the sides of the funnel were rinsed with excess MilliQ to ensure maximum sample retention onto the filter. The filter was then removed and kept in a covered aluminum foil dish until further analysis. A total of six procedural blanks were run alongside the subsamples (i.e., ~10 ml of bleach was added to six clean scintillation vials, heated, and filtered).

Positive controls

Positive controls with known MP types were used to demonstrate plastic fluorescence behavior as well as the minimal effect of bleach and heat on that behavior. Control MP were generated by cleaning common laboratory and consumer plastics (such as spray bottles, dish ware, monochromatic clothing, etc.) with 100% isopropyl alcohol and shaving particles (<5 mm) into

20 ml glass scintillation vials with a steel utility blade. Plastic type was identified by the recycling label or clothing tag on each unit of plastic. Ten plastic types were used including high density polyethylene (HDPE), low density polyethylene (LDPE), polyethylene (PE), polyethylene terephthalate (PETE), polypropylene (PP), polystyrene (PS), polyvinyl chloride (PVC), “Other” and the clothing fibers polyolefin and polyester (only clothes made with 100% polyolefin or polyester were sampled). Different colors of the same plastic type were collected when possible. Two sets of scintillation vials were prepared for the positive controls: one set for bleach digestion with heat and another control set to be processed only with MilliQ and without heat~~, producing 20 vials~~. The bleach set was processed according to the sponge sample procedure, and 5–10 ml of MilliQ was added to the control set vials that were not heated. All positive controls were filtered according to the sponge filtering procedure.

PMP visualization

All filters were analyzed for PMP presence using an E600 Nikon Eclipse fluorescence microscope fitted with a UV-1A fluorescence filter block (EX 360–370, DM 400, BA 400). Potential MP was distinguished from fluorescing background material (inorganic sand grains, proteinaceous spongin, invertebrate cuticle fragments, etc.) based on the brightness and color of fluorescence (Figs. 2, S1). Plastic fluoresced stronger and with an electric blue color when compared to these other materials, which had a dulled, blue-green fluorescence (Figs. 2, S1). The entire filter of each sample and blank was visually surveyed for PMP presence and the number and sizes of detected PMP were recorded. The size of nearly every PMP found in the sponge subsamples and corresponding blanks and at least 15% of PMP found in the water samples and corresponding blanks ~~were~~ recorded. The number and sizes of PMP in the positive controls were not recorded as they served only to demonstrate plastic fluorescence behavior and the effect of bleach and heat on that behavior. Only particles greater than or equal to 10 μm in maximum length were recorded for any filter. Particle sizes were categorized into nine groups based on maximum length: 10–20 μm, 21–50 μm, 51–100 μm, 101–300 μm, 301–500 μm, 501–1000 μm, 1001–3000 μm, 3001–5000 μm and >5000 μm. Particle sizes are reported in a stacked bar chart (Fig. 3) and do not reflect blank-corrected values (i.e., the proportion of particles within each size category represents the percent ~~out~~ of total particles surveyed and may reflect the sizes of potential contaminants). The number of PMP on sample filters was corrected based on the average number of PMP found on the corresponding blank filters. These corrections were not done on the basis of size (i.e., 10–20 μm particles in the blank were not subtracted from 10–20 μm particles in the sample) as we aimed only to evaluate general background contamination. Occasionally, this correction led to a negative value, and in these cases, the PMP value for the sponge subsample was adjusted to zero.

Mitigating contamination

Since plastic is abundant in field and laboratory settings, several steps were taken to minimize sample contamination. Nitrile gloves and 100% cotton cloths and lab coats were used at all times during sample processing. However, it is possible that cotton fibers from these materials were counted in blanks and samples because cotton (cellulose) may autofluoresce under UV light (Malinowska et al., 2015). The particular fluorescence behavior of cotton cellulose was not tested in this study. Glassware was used in place of plasticware and all glassware and samples were covered with foil when not in use. Glassware and metal utensils were cleaned with soap and water and rinsed three times with MilliQ or analytical grade water before use. Lastly, dry sponge samples were cut into scintillation vials under a laminar flow hood to reduce airborne contamination. Though these steps were taken to minimize contamination, we also recognize that false positives are still possible.

Data analysis

Potential microplastic (PMP) concentrations are reported as number of PMP per liter (PMP/L) for seawater samples and number of PMP per g of dry tissue (PMP/g) for sponge samples. ~~Recall that six sponge species were chosen for this study, that three individual sponges (N=3 replicates) were sampled for each species, and that three subsamples were taken from each sponge replicate (6 species × 3 replicates × 3 subsamples = 54 subsamples).~~ The blank-corrected number of PMP/g was determined for each of the 54 subsamples. These 54 values were then grouped by replicate to produce a mean number of PMP/g for each replicate. These true replicate values were then grouped by species to produce a mean number of PMP/g for each species. A one-way ANOVA test followed by a Tukey's HSD pairwise multiple comparisons test was used in R to determine any significant differences in mean PMP concentrations between the six sponge species.

Results

Plastic fluorescence behavior and positive controls

Plastic particles fluoresced electric blue when exposed to UV light (except for red PP, which fluoresced pink) and often fluoresced much brighter when compared to other materials (sand grains, spongin, chitin, etc.), which had a blue-green and dulled fluorescence (Figs. 2, S1). Some plastic types (e.g., HDPE, PVC) showed weak to no fluorescence, while others (e.g., Other, PETE, Polyester, PP) showed intermediate to strong fluorescence (Fig. S1). Lightly colored plastics (e.g., clear, white, yellow, light blue, red) fluoresced more often and stronger than darkly colored plastics (e.g., black, brown, gray, green, dark blue), though some light plastics did not fluoresce at all (Fig. S1). Some plastics (e.g., "Other") in the control MilliQ set showed small flecks of florescent material even if the plastic itself did not fluoresce (Fig. S1G, H). Exposure to bleach and heat showed little to no effect on plastic fluorescence behavior (Fig. S1).

Seawater

An average of 107±25 particles per liter of seawater was found in water samples collected from Saigon Bay. The PMP detected in seawater varied in size, though very small (10–20 μm) particles ~~made up about one fourth (~~ ~25% ~~)~~ of the total number of particles (Fig. 3). The corresponding blanks had proportionately fewer very small particles (~16%), with ~~about a fourth (~~ ~27% ~~)~~ of all particles in the blanks being 101–300 μm in maximum length (Fig. 3). Although one large fiber (3001–5000 μm) was found in the water samples, no large fibers were found in the blanks, and no very large fibers (>5000 μm; technically outside the range of MP) were found in the water samples or water blanks. The number of particles detected in the water blanks never exceeded 10% of those found in the samples, indicating that there was minimal contamination during sample processing (Gago et al., 2016).

Sponge

The number of PMP per g of dry tissue varied across the six sponge species (one-way ANOVA, df=5, F=5.358, p=0.0081; Fig. 4, Table 2). *Callyspongia vaginalis*, *A. cauliformis*, *N. erecta* and *I. campana* showed the highest concentrations of particles (mean±SE 169±71, 113±23, 75±38 and 71±20 PMP/g, respectively; Table 1), while *A. compressa* and *M. laevis* showed lower concentrations (14±2 and 6±4 PMP/g, respectively; Fig. 4, Table 1). However, there were few significant pairwise differences in mean PMP concentration between species (Fig. 4, Table 2). The number of particles in the procedural blanks was sometimes greater than that in the sponge subsamples themselves (17 ~~out~~ of the 54 subsamples had a negative net number of particles). As such, PMP counts in the sponge blanks as a percentage of counts in sponge subsamples sometimes exceeded 100%. This finding is concerning because a blank percent of 10% has previously been used as a threshold to signify that sample counts are significantly greater than blank counts (Gago et al., 2016). A relatively high level of background PMP in our subsamples may have been the result of using non-filtered bleach and/or the use of very small amounts of tissue (~0.05–0.3 g) for each subsample (see below for further discussion). However, our blank-corrected values still offer some insight into the presence of PMP in wild sponge tissue.

Most PMP found in all sponge samples and blanks was very small (10–20 µm). Very small PMP made up about half of the total number of PMP found in *A. cauliformis*, *A. compressa*, *C. vaginalis*, *M. laevis* and the blanks, while they comprised about 32% and 25% of all particles found in *I. campana* and *N. erecta*, respectively (Fig. 3). Very large particles or small fibers (501–1000 µm) and medium fibers (1001–3000 µm) together also comprised a large percent (~25–31%) of the total number of particles found in some sponge species (*I. campana, M. laevis, N. erecta*), but not in the blanks (Fig. 3). Large (3001–5000 µm) and very large fibers (>5000 µm; technically outside the range of MP) were ~~also~~ found only in ~~the~~ sponge samples but not in the blanks, making up about 7%, 4%, and 4% of the particles found for *N. erecta*, *C. vaginalis* and *M. laevis*, respectively (Fig. 3).

Discussion

Microplastic in seawater

An average concentration of 107±25 PMP/L of seawater in Saigon Bay is striking. Few studies have investigated MP concentrations in Caribbean seawater but reports of surface concentrations have not exceeded 0.00573 MP/L (Law et al., 2010; Rose & Webber, 2019). Previous studies targeted larger particles (>335 µm, collected via plankton tows), while we targeted smaller particles (>10 µm, collected as bulk samples close to the benthos). Surface MP concentrations in the world's coastal waters and oceans are also reported as lower than ours, though these studies again targeted larger size fractions. Colton, Knapp & Burns (1974) reported a concentration of 0.000067 MP/L (>947 µm, plankton tows) in the open northwest Atlantic Ocean while Doyle et al. (2011) reported a maximum of 0.00019 MP/L (>505 µm, plankton tows) in the coastal Northeast Pacific Ocean. Aliabad, Nassiri & Kor (2019) reported a maximum of 0.00114 MP/L (>333 µm, plankton tows) in the Gulf of Oman while Payton, Beckingham & Dustan (2020) reported a maximum of 0.6 MP/L (>43 µm, grab samples) in the estuarine Cooper River of South Carolina.

Recent findings suggest that previous studies significantly underestimate MP concentrations in seawater because plankton tow nets (300–1000 µm mesh) are commonly used when sampling the upper 1 m of the water column (Covernton et al., 2019). Kang et al. (2015) and Barrows et al. (2017) concluded that these tows allow smaller MP (<300 µm) and fibers (due to their small width) to pass through holes in the nets, and that these studies may be underestimating seawater MP concentrations by orders of magnitude. Covernton et al. (2019) compared the suitability of *in situ* sieve versus bulk sample methods to measure MP abundance in seawater. They found that bulk seawater samples collected in one-liter glass jars and filtered directly onto 8-µm pore size filters resulted in PMP concentrations that were on average 8.5 times higher than samples that were collected in 10-L buckets, sieved using a 63 µm mesh in the field, and then filtered (Covernton et al., 2019). They concluded that studies using plankton nets may underestimate MP concentrations by up to four orders of magnitude compared to studies that target smaller (<100 µm) plastics (Covernton et al., 2019). The authors highlighted the necessity of using bulk seawater samples and sensitive filtration methods (ability to detect plastics down to 10 µm) when assessing the exposure of marine organisms to MP pollution (Covernton et al., 2019).

An average seawater concentration of 107 PMP/L in our study compares better with studies that used bulk samples and sensitive filtration methods. Covernton et al. (2019) filtered grab surface seawater samples onto an 8 µm filter and reported 5.28 MP/L in coastal British Columbia, Canada. Jiang et al. (2020) pumped surface seawater through 50 µm net and reported 6.5 MP/L in the South Yellow Sea. Norén & Naustvoll (2010) pumped surface seawater through a 10 µm filter and reported 102 MP/L in Swedish coastal waters. Though our finding (107 PMP/L) is very similar to that of the Swedish study (Norén & Naustvoll, 2010), our subsurface value is still elevated compared to the other studies. Because we sampled subsurface seawater and used different methods (grab samples filtered directly onto a 0.7 µm filter) compared to other surface water studies, it is uncertain whether our seawater value is relatively high compared to reported values. Still, a concentration of 107 PMP/L is concerning and warrants further investigation of subsurface seawater at additional sites and over time in the Caribbean using bulk samples and sensitive filtration.

Microplastic in sponges

This is the first study to evaluate the presence of MP in wild Caribbean sponges and our results indicate that ~~the sponges~~ do ingest MP. The concentration of PMP in sponge tissue was generally low (6–169 PMP/g) for all species. Girard et al. (2020) examined microparticulate pollutants (minerals, shell fragments, cotton, polystyrene, etc.) in tropical sponges from Indonesia. Like us, the authors used bleach-digested dried sponge subsamples (0.0022–0.011 g dry) and vacuum-filtered them onto 1 μm pore size membranes (Girard et al., 2020). Using Raman spectroscopy, they detected 91–612 foreign particles (5–200 μm in size) per g of dry tissue (Girard et al., 2020). These values included all targeted microparticulates, but the authors also reported that one sample of *Ircinia* had a polystyrene concentration of 159 particles/g of dry tissue (Girard et al., 2020). As such, our results (6–169 PMP/g) align well with those of Girard et al. (2020), especially considering that PMP would only be a fraction of the total particles they detected.

Most PMP (up to 65%) found in our sponge samples were very small (10–20 μm), while PMP within the same size range made up only about one quarter of those found in seawater. This suggests that sponges may demonstrate some selectivity in MP ingestion, preferring very small particles. This is not surprising considering that sponges typically feed on microorganisms smaller than 70 μm (Ribes, Coma & Gili, 1999). Moreover, laboratory studies have demonstrated the retention of microbeads (<5.7 μm) in sponge tissues, which supports the idea that sponges prefer very small particles (Schmidt, 1970; Willenz & Van de Vyver, 1982; Imsiecke, 1993).

A total of 20 large fibers (3001 μm to >5000) were detected in the sponge samples but they were absent in blanks and present only once in the seawater samples. This finding suggests that sponges may concentrate synthetic fibers from seawater as they filter feed. Although we rinsed the outside of our samples prior to analysis in an attempt to isolate MP retained within the sponge aquiferous system, it is possible that the fibers we detected were embedded on the surface of the sponges (Modica, Lanuza & García-Castrillo, 2020). It is also plausible that the fibers were stuck within the sponges' internal canals after having passed through the ostia because fiber width, regardless of maximum length, never exceeded 10 µm.

The location of MP within the bodies of ~~our~~ sponge species is unknown, but recent studies have highlighted the presence of microparticulate pollutants in the ectosome (outer layer of the sponge body), inner mesohyl, and around the choanocyte chambers of northern Atlantic and western Pacific sponges (Modica, Lanuza & García-Castrillo, 2020; Girard et al., 2020). The latter study predicted that some particles were captured on the sponge surface by exopinacocytes and were subsequently drawn into the body, while other particles were drawn passively into the aquiferous system via ostia and were later phagocytized by choanocytes (Girard et al., 2020). The authors also suggested that non-spiculate sponges tended to incorporate larger (>50 µm) particles into their skeletons whereas spiculate sponges tended to incorporate smaller (<50 µm) particles into their ectosome (Girard et al., 2020). We did not perform histological experiments in our study and so cannot report the location of PMP within Caribbean sponge tissues. However, because we examined both non-spiculate (*A. cauliformis* and *I. campana*) and spiculate (*A. compressa*, *C. vaginalis*, *M. laevis* and *N. erecta*) sponges, it is possible that these Caribbean species may be incorporating MP into their tissues in ways suggested by Girard et al. (2020). Furthermore, the calcareous sponge *Sycon coactum* Urban (1906) has been shown to egest microbeads (up to 1.0 µm) by action of choanocytes, which can engulf the beads and carry them into excurrent chambers (Leys & Eerkes-Medrano, 2006). The ability of other sponge species to egest MP is unknown, but future work should use histological methods to better understand how MP enter the sponge body, where they are being retained, and whether more species can egest MP.

Variation in PMP concentration across sponge species may relate to differences in sponge morphology and/or physiology. Tissue density, pumping rate, aquiferous system complexity, and/or microbial abundance may impact PMP abundance and retention because these traits impact the volume and residence time of water processed by sponges (Reiswig, 1974; Weisz, Lindquist & Martens, 2008; Easson et al., 2015). Interestingly, Girard et al. (2020) observed that particle incorporation by sponges was independent of particle material. In other words, the authors suggested that the sponges would take up particles based on what was available in the surrounding water, and that any differences in the composition of incorporated particles between species depended only on particle spatial variation (Girard et al., 2020). Additionally, Modica, Lanuza & García-Castrillo (2020) found that fiber abundance in sponge ectosomes was independent of sponge species, habitat type and depth, and that fibers were likely ubiquitous in the surrounding seawater. Similarly, we also cannot yet conclude that varying sponge characteristics influence particle uptake and retention because there were few significant differences in mean PMP concentration between our species (Fig. 4, Table 2). Future studies should aim to identify any such relationships across additional species.

Ecological implications

A relatively high concentration of PMP in seawater from Bocas del Toro represents an elevated exposure of marine and human life to MP. The archipelago is home to numerous species of sponges, corals, polychaetes, tunicates, nemerteans, echinoderms, molluscs, crustaceans, hydroids, bryozoans, sipunculans, flatworms and anemones, and diverse species of commercial and non-commercial fishes such as snapper, grouper, grunts, butterflyfish, parrotfish and sharks (Collin, 2005; Seemann et al., 2014). High PMP concentrations in the archipelago's coastal waters means that these local species are susceptible to MP ingestion. Most of the seafood sold in Bocas del Toro restaurants such as lobster, octopus and commercial fishes is locally sourced (Dorsett & Rubio-Cisneros, 2019). Thus, the people that visit or live on the islands may be at risk for the consumption of contaminated seafood. This risk, as well as the flow of MP into local waterways, is only expected to increase as tourism and residency continue to increase (Easson et al., 2015; World Bank, 2018; Dorsett & Rubio-Cisneros, 2019).

Scaling our data to appreciable values helps to illuminate the story of MP in Caribbean sponges. An average concentration of 87 PMP/g across all sponge species in this study equates to >8,000 PMP particles in a sponge that weighs 100 g (dry), or a sponge that is approximately 1.5 L (McMurray, Blum & Pawlik, 2008; Girard et al., 2020). This number agrees well with that reported by Girard et al. (2020) who predicted that at least 10,000 microparticulates (sum of MP, minerals, etc.) per sponge may exist in some demosponges (100 g dry) from Indonesia. Furthermore, using known pumping rates (~0.09–0.48 L sec^{-1} L^{-1}) and tissue densities (~89–155 g/L) for sponges that are congeneric with our Caribbean species (Weisz, Lindquist & Martens, 2008; Fiore, Freeman & Kujawinski, 2017; Pawlik, Loh & McMurray, 2018), and an ambient seawater concentration of 107 PMP/L, we would predict that a 100 g sponge could be passing between 25,000 and 174,000 particles through its body every hour. These values are far greater than that (8,000 PMP) which we would predict to be present in a sponge at any moment in time as our samples would indicate. This finding supports the hypothesis that Caribbean sponges have some capacity to resist MP ingestion and/or that they have some ability to egest the particles.

Interestingly, despite the presence of PMP in every species, the sponges from which samples were taken appeared to be healthy and functional (open ostia, no evidence of necrosis, large individuals). Based on this gross examination, we did not detect an effect of MP ingestion on sponges in Saigon Bay. From laboratory experiments, Baird (2016) also reported an absence of effect ~~as~~ MP exposure ~~showed~~ little impact on temperate sponge respiration. In addition, relatively low concentrations of PMP in sponge tissue despite there being ~107 PMP/L of seawater in Saigon Bay support the idea that tropical sponges have some capacity to resist MP ingestion. As selective filter feeders, perhaps sponges can adjust their pumping rates in response to pulses of MP, as sometimes occurs with increased sediment load (Gerrodette & Flechsig, 1979; Maldonado et al., 2010; McMurray et al., 2016). Girard et al. (2020) suggested that sponges may act as bioindicators of general microparticulate pollutants, but our results indicate that marine sponges may be resistant to specifically MP exposure and therefore may not be the best indicators of MP pollution. Therefore, increased spatial and temporal sampling is needed to test the potential for sponges to act as bioindicators of MP in aquatic environments.

Evaluation of methods and considerations

We acknowledge that the methods used in this study have some limitations. Only fluorescence microscopy was used to identify and quantify suspected MP. The lack of secondary verification, such as by Raman or FT-IR spectroscopy, requires us to refer to the particles detected as potential microplastics (PMP) per Covernton et al. (2019). This method raises several concerns. Firstly, the lack of additional verification methods means that the number of particles detected in our samples may be positively skewed owing to false positives. However, Payton, Beckingham & Dustan (2020) noted that fewer MP in water samples were detected using fluorescence microscopy than when using brightfield microscopy alone, indicating the potential also for some negative bias. In our positive controls, we confirmed that not all plastic types fluoresce under our microscopy conditions, and that there is variation in fluorescence strength and color between plastic types. Since we only counted particles that fluoresced strongly with an electric blue color (i.e., the fluorescence behavior of white and clear fragments of PETE and PP), our results may reflect the presence of only particular plastic types and therefore underestimate the true number of MP present in the samples.

We also recognize that an appreciable number of particles were found in the blanks for the sponge study, sometimes amounting to more than were found in the sponge subsamples themselves. While the counts in the water blanks as a percentage of counts in water samples was low (<10%), water blanks were not digested with bleach. This suggests that MP present in commercial bleach products may have created a higher PMP background level in our sponge subsamples. This background contamination might be reduced by pre-filtering the bleach solution, and furthermore it's contribution to sample counts would be diminished if larger dry tissue samples (>0.3 g dry) were analyzed. Still, even if some of the PMP in the sponge samples are artifacts of PMP in bleach, it is striking how few PMP were found in the sponge samples when compared to their concentration in the surrounding seawater.

The methods used in this study offer an efficient and cost-effective way to evaluate the presence of PMP in marine sponges. The use of bleach to digest organic material showed little to no effect on the physical integrity and fluorescence behavior of plastic particles. This method of evaluation agrees with Collard et al. (2015) and other recent studies (J. Lynch, 2019, pers. comm.). We recommend the use of bleach in future MP studies owing to its capacity to digest soft tissues, its limited effect on MP, and to its cost efficiency, but recommend filtering it before use to reduce potential background contamination.

Conclusions

This study surveys the occurrence of PMP in wild sponges and in subsurface seawater from Bocas del Toro, Panamá. Digestion of dry tissue using household bleach is a time- and cost-effective method for evaluating MP presence because it has little to no effect on plastic integrity or fluorescence behavior. We recommend this technique with some additional solution preparation for future MP work. A PMP concentration of ~107 PMP/L in subsurface seawater from Saigon Bay compares well with or is greater than previous surface reports that used bulk samples and sensitive filtration techniques (down to 10 µm). As a result, we recommend the continued use of such techniques along with the use of subsurface samples when evaluating the exposure of benthic filter-feeding organisms to MP. Our results further indicate that Caribbean sponges do ingest MP, and that sponges may preferentially collect fibers and very small (10–20 µm) particles. The relatively low occurrence of PMP (6–169 PMP/g) in seemingly healthy sponges, however, suggests that sponges may be somewhat resistant to MP ingestion or retention. Lastly, the presence of PMP in sponges and seawater from Saigon Bay indicates that humans and marine animals are exposed to MP in Bocas del Toro. This exposure is expected to increase with a growth in population and tourism. This study highlights the lack of MP research in the Caribbean, and future work should be aimed at evaluating the presence and impact of MP in this beloved and highly-frequented region.

Acknowledgements

We would like to thank the Smithsonian Tropical Research Institute for lab space, field supplies and boat access. We also thank the Hollings Marine Laboratory, College of Charleston, Grice Marine Laboratory, M. Janech, A. Bland, P. Lee and N. Schanke for technical support, lab space and supplies. Thanks to C. Easson, C. Fiore, D. Gonzalez, S. Czwalina, A. Stephens and J.

Thurnham for their assistance with sample collection. We also thank S. Czwalina for assistance with sample processing and A. Parry for help with data analysis. Finally, this research would not have been possible without the advice and guidance of B. Beckingham, J. Lynch, L. Jonas, K. Dudek, C. Fiore, C. Easson, G. Lôbo-Hajdu, M. Janech, R. Thacker and P. Dustan.

References

Acosta-Coley, I., M. Duran-Izquierdo, E. Rodriguez-Cavallo, J. Mercado-Camargo, D. Mendez-Cuadro, and J. Olivero-Verbel. 2019. Quantification of microplastics along the Caribbean Coastline of Colombia: Pollution profile and biological effects on Caenorhabditis elegans. *Marine Pollution Bulletin* 146:574–583.

Aliabad, M.K., M. Nassiri, K. Kor. 2019. Microplastics in the surface seawaters of Chabahar Bay, Gulf of Oman (Makran Coasts). Marine Pollution Bulletin 143:125–133.

Allen, A.S., A.C. Seymour, and D. Rittschof. 2017 Chemoreception drives plastic consumption in a hard coral. *Marine Pollution Bulletin* 124:198–205.

Andrady, A.L. 2011. Microplastics in the marine environment. *Marine Pollution Bulletin* 62(8):1596–1605.

Aronson, R., I. Macintyre, C. Wapnick, and M. O'Neill. 2004. Phase shifts, alternative states and the unprecedented convergence of two reef systems. *Ecology* 85:1876–1891.

Arthur, C., J. Baker, and H. Bamford (eds.). 2009. *Proceedings of the International Research Workshop on the Occurrence, Effects and Fate of Microplastic Marine Debris. Sep. 9–11, 2008.* NOAA Technical Memorandum NOS-OR&R-30.

Avio, C.G., S. Gorbi, M. Milan, M. Benedetti, D. Fattorini, G. d'Errico, M. Pauletto, L. Bargelloni, and F. Regoli. 2015. Pollutants bioavailability and toxicological risk from microplastics to marine mussels. *Environmental Pollution* 198:211C222.

Baird, C.A. 2016. Measuring the effects of microplastics on sponges. M.S. Thesis, Victoria University of Wellington.

Barrows, A.P.W., C.A. Neumann, M.L. Berger, and S.D. Shaw. 2017. Grab vs. neuston tow net: a microplastic sampling performance comparison and possible advances in the field. *Analytical Methods* 9:1446–1453.

Besseling, B., A. Wegner, E.M. Foekema, M.J. Heuvel-Greve, and A.A. Koelmans. 2013. Effects of microplastic on fitness and PCB bioaccumulation by the lugworm *Arenicola marina* (L.) *Environmental Science and Technology* 47:593–600.

Betts, K. 2008. Why small plastic particles may pose a big problem in the oceans. *Environmental Science and Technology* 42(24):8995–8995.

Bosker, T., L. Guaita, and P. Behrens. 2018. Microplastic pollution on Caribbean beaches in the Lesser Antilles. *Marine Pollution Bulletin* 133:442–447.

Browne, M.A., A. Dissanayake, T.S. Galloway, D.M. Lowe, and R.C. Thompson. 2008. Ingested microscopic plastic translocates to the circulatory system of the mussel, *Mytilus edulis* (L.). *Environmental Science and Technology* 42(13):5026–5031.

Browne, M.A., P. Crump, S.J. Nivens, E. Teuten, A. Tonkin, T. Galloway, and R. Thompson. 2011. Accumulation of microplastics on shorelines worldwide: sources and sinks. *Environmental Science and Technology* 45(21):9175–9179.

Browne, M.A., T.S. Galloway, and R. Thompson. 2007. Microplastic – an emerging contaminant of potential concern? *Integrated Environmental Assessment and Management* 3:559–561.

Carruthers, T.J.B., P.A.G. Barnes, G.E. Jacome, and J.W. Fourqurean. 2005. Lagoon scale processes in a coastally influenced Caribbean system: implications for the seagrass *Thalassia testudinum*. *Caribbean Journal of Science* 41:441–455.

Carter, H.J. 1882. Some sponges from the West Indies and Acapulco in the Liverpool Free Museum described, with general and classificatory remarks. *Annals and Magazine of Natural History* 9(52):266–368.

Carter, H.J. 1885. Descriptions of sponges from the neighbourhood of Port Phillip Heads, South Australia. *Annals and Magazine of Natural History* 16(94):277–368.

Chapron, L., E. Peru, A. Engler, J.F. Ghiglione, A.L. Meistertzheim, A.M. Pruski, A. Purser, G. Vétion, P.E. Galand, and F. Lartaud. 2018. Macro- and microplastics affect cold-water corals growth, feeding and behaviour. *Scientific Reports* 8:1–8.

Christaki, U., J.R. Dolan, S. Pelegri, and F. Rassoulzadegan. 1998. Consumption of picoplankton-size particles by marine ciliates: effects of physiological state of the ciliate and particle quality. *Limnology and Oceanography* 43:458–464.

Cole, M., P. Lindeque, C. Halsband, and T.S. Galloway. 2011. Microplastics as contaminants in the marine environment: A review. *Marine Pollution Bulletin* 62:2588–2597.

Cole, M., P. Lindeque, C. Halsband, and T.S. Galloway. 2015. The impact of polystyrene microplastics on feeding, function and fecundity in the marine copepod *Calanus helgolandicus*. *Environmental Science and Technology* 49:1130–1137.

Collard, F., B. Gilbert, G. Eppe, E. Parmentier, and K. Das. 2015. Detection of anthropogenic particles in fish stomachs: An isolation method adapted to identification by Raman spectroscopy. *Archives of Environmental Contamination and Toxicology* 69:331–339.

Collin, R. 2005. Ecological monitoring and biodiversity surveys at the Smithsonian Tropical Research Institute's Bocas del Toro Research Station. *Caribbean Journal of Science* 41(3):367–373.

Colton, J.B., F.D. Knapp, B.R. Burns. 1974. Plastic particles in surface waters of the Northwestern Atlantic. *Science* 185(4150):491–497.

Colvard, N.B., and P.J. Edmunds. 2011. Decadal-scale changes in abundance of non-scleractinian invertebrates on a Caribbean coral reef. *Journal of Experimental Marine Biology and Ecology* 397(2):153–160.

Conley, K., A. Clum, J. Deepe, H. Lane, and B. Beckingham. 2019. Wastewater treatment plants as a source of microplastics to an urban estuary: Removal efficiencies and loading per capita over one year. *Water Research X* 3:100030.

Covernton, G.A., C.M. Pearce, H.J. Gurney-Smith, S.G. Chastain, P.S. Ross, J.F. Dower, and S.E. Dudas. 2019. Size and shape matter: A preliminary analysis of microplastic sampling technique in seawater studies with implications for ecological risk assessment. *Science of the Total Environment* 667:124–132.

Crutzen, P.J. 2002. Geology of mankind. *Nature* 415:23.

de Bakker, D.M., F.C. van Duyl, R.P.M. Bak, M.M. Nugues, G. Nieuwland, and E.H. Meesters. 2017. 40 years of benthic community change on the Caribbean reefs of Curaçao and Bonaire: the rise of slimy cyanobacterial mats. *Coral Reefs* 36(2):355–367.

de Goeij et al. 2017. Chapter 8: Nutrient fluxes and ecological functions of coral reef sponges in a changing ocean. In: Carballo, J.L., and J.J. Bell, eds. *Climate Change, Ocean Acidification and Sponges*. Cham: Springer Nature, 373–410.

de Goeij, J. M., D. Van Oevelen, M.J. Vermeij, R. Osinga, and J.J. Middelburg. 2013. Surviving in a marine desert: The sponge loop retains resources within coral reefs. *Science* 342:108–110.

de Sá, L.C., M. Oliveira, F. Ribeiro, T.L. Rocha, and M.N. Futter. 2018. Studies of the effects of microplastics on aquatic organisms: What do we know and where should we focus our efforts in the future? *Science of the Total Environment* 65:1029–1039.

Dorsett, N., and T. Rubio-Cisneros. 2019. Many tourists, few fishes: Using tourists' and locals' knowledge to assess seafood consumption on vulnerable waters of the archipelago of Bocas del Toro, Panamá. *Tourism Management* 74:290–296.

Doyle, M.J., W. Watson, N.M. Bowlin, S.B. Sheavly. 2011. Plastic particles in coastal pelagic ecosystems of the Northeast Pacific Ocean. *Marine Environmental Research* 71:41–52.

Duchassaing de Fonbressin, P., and G. Michelotti. 1864. Spongiaires de la mer Caraibe. *Natuurkundige verhandelingen van de Hollandsche maatschappij der wetenschappen te Haarlem* 21(2):1–124.

Dudek, K.L., B.N. Cruz, B. Polidoro, and S. Neuer. 2020. Microbial colonization of microplastics in the Caribbean Sea. *Limnology and Oceanography Letters* 5:5–17.

Easson, C.G., K.O. Matterson, C.J. Freeman, S.K. Archer, and R.W. Thacker. 2015. Variation in species diversity and functional traits of sponge communities near human populations in Bocas del Toro, Panamá. *PeerJ* 3:e1385.

Erwin, P.M., and R.W. Thacker. 2008. Phototrophic nutrition and symbiont diversity of two Caribbean sponge-cyanobacteria symbioses. *Marine Ecology Progress Series* 362:139–147.

Fiore, C.L., C.J. Freeman, and E.B. Kujawinski. 2017. Sponge exhalent seawater contains a unique chemical profile of dissolved organic matter. *PeerJ* 5:e2870.

Freeman, C.J., D.M. Baker, C.G. Easson, and R.W. Thacker. 2015. Shifts in sponge-microbe mutualisms across an experimental irradiance gradient. *Marine Ecology Progress Series* 526:41–53.

Frost, T.M. 1978. In situ measurement of clearance rates for the freshwater sponge *Spongilla lacustris*. *Limnology and Oceanography* 23:1034–1039.

Gago, J., F. Galgani, T. Maes, and R.C. Thompson. 2016. Microplastics in seawater: Recommendations from the Marine Strategy Framework Directive implementation process. *Frontiers in Marine Science* 3:219.

Gallo, F., C. Fossi, R. Weber, D. Santillo, J. Sousa, I. Ingram, A. Nadal, and D. Romano. 2018. Marine litter plastics and microplastics and their toxic chemicals components: the need for urgent preventive measures. *Environmental Sciences Europe* 30:13.

Garcés-Ordóñez, O., V.A. Castillo-Olaya, A.F. Granados-Briceñoc, L.M.B. García, L.F.E. Díaz. 2019. Marine litter and microplastic pollution on mangrove soils of the Ciénaga Grande de Santa Marta, Colombian Caribbean. *Marine Pollution Bulletin* 145:455–462.

Gerrodette, T., and Flechsig, A.O. 1979. Sediment-induced reduction in the pumping rate of the tropical sponge *Verongia lacunosa*. *Marine Biology* 55:103–110.

Girard, E.B., A. Fuchs, M. Kaliwoda, M. Lasut, E. Ploetz, W.W. Schmahl, and G. Wörheide. 2020. Sponges as bioindicators for microparticulate pollutants?. *Ecology, Environment & Conservation*.

Gochfeld, D.J., C. Schloder, R.W. Thacker. 2007. Sponge community structure and disease prevalence on coral reefs in Bocas del Toro, Panamá. In: Custodio, M.R., G. Lobo-Hajdu,

E. Hajdu & G. Muricy, eds. *Porifera Research: Biodiversity, Innovation and Sustainability, Serie Livros 28*. Rio De Janeiro: Museu Nacional, 335–343.

Graham, E.R., and J.T. Thompson. 2009. Deposit- and suspension-feeding sea cucumbers (Echinodermata) ingest plastic fragments. *Journal of Experimental Marine Biology and Ecology* 368(1):22–29.

Hadas, E., D. Marie, M. Shpigel, M. Ilan. 2006. Virus predation by sponges is a new nutrient-flow pathway in coral reef food webs. *Limnology and Oceanography* 51:1458–1550.

Hall, N.M., K.L.E. Berry, L. Rintoul, M.O. Hoogenboom. 2015. Microplastic ingestion by scleractinian corals. *Marine Biology* 162:725–732.

Hankins, C., A. Duffy, and K. Drisco. 2018. Scleractinian coral microplastic ingestion: potential calcification effects, size limits, and retention. *Marine Pollution Bulletin* 135:587–593.

Hart, M.W. 1991. Particle capture and the method of suspension feeding by echinoderm larvae. *Biology Bulletin* 180(1):12–27.

Hirai, H., H. Takada, Y. Ogata, R. Yamashita, K. Mizukawa, M. Saha, C. Kwan, C. Moore, H. Gray, D. Laursen, E.R. Zettler, J.W. Farrington, C.M. Reddy, E.E. Peacock, and M.W. Ward. 2011. Organic micropollutants in marine plastics debris from the open ocean and remote and urban beaches. *Marine Pollution Bulletin* 62(8):1683–1692.

Hooper, J.N.A. 2003. 'Sponguide'. Guide to Sponge Collection and Identification, pp. 4–6. Queensland Museum, Queensland.

Hooper, J.N.A., and F. Wiedenmayer. 1994. Porifera. In: Wells, A. ed. *Zoological Catalogue of Australia. Volume 12*. Melbourne: CSIRO, 442.

Imsiecke, G. 1993. Ingestion, digestion, and egestion in *Spongilla lacustris* (Porifera, Spongillidae) after pulse feeding with *Chlamydomonas reinhardtii* (Volvocales). *Zoomorphology* 113:233–244.

Jiang, Y., Y. Zhao, X. Wang, F. Yang, M. Chen, and J. Wang. 2020. Characterization of microplastics in the surface seawater of the South Yellow Sea as affected by season. *Science of the Total Environment* 724:138375.

Kang, J.-K., O.Y. Kwon, K.-W. Lee, Y.K. Song, and W.J. Shim. 2015. Marine neustonic microplastics around the southeastern coast of Korea. *Marine Pollution Bulletin* 96(1–2): 304–312.

Kaufmann, K.W., and R.C. Thompson. 2005. Water temperature variation and the meteorological and hydrographic environment of Bocas del Toro, Panamá. *Caribbean Journal of Science* 41:392–413.

Koelmans, A.A., E. Besseling, W.J. Shim. 2015. Nanoplastics in the aquatic environment. In: Bergmann, M., L. Gutow, and M. Klages, eds. *Marine Anthropogenic Litter*. Cham: Springer, 329–344.

Kowalke, J. 2000. Ecology and energetics of two Antarctic sponges. *Journal of Experimental Marine Biology and Ecology* 247:85–97.

Lamarck, J.-B. 1814. Sur les polypiers empâtés. *Annales du Museum national d'Histoire naturelle* 20:294–458.

Lamb, J.B., B.L. Willis, E.A. Fiorenza, C.S. Couch, R. Howard, D.N. Rader, J.D. True, L.A. Kelly, A. Ahmad, J. Jompa, C.D. Harvell. 2018. Plastic waste associated with disease on coral reefs. *Science* 359:460–462.

Law, K.L., S. Morét-Ferguson, N.A. Maximenko, G. Proskurowski, E.E. Peacock, J. Hafner, and C.M. Reddy. 2010. Plastic accumulation in the North Atlantic Subtropical Gyre. *Science* 1192321.

Lesser, M. P. 2006. Benthic-pelagic coupling on coral reefs: feeding and growth of Caribbean sponges. *Journal of Experimental Marine Biology and Ecology* 328:277–288.

Lewis, S., and M. Maslin. 2015. Defining the Anthropocene. *Nature* 519:171–180.

Leys, S.P., and D.I. Eerkes-Medrano. 2006. Feeding in a calcareous sponge: particle uptake by pseudopodia. *Biological Bulletin* 211:157–171.

Loh, T.-L., and J.R. Pawlik. 2014. Chemical defenses and resource trade-offs structure sponge communities on Caribbean coral reefs. *PNAS* 111(11):4151–4156.

Maldonado, M., X. Zhang, X. Cao, L. Xue, H. Cao, and W. Zhang. 2010 Selective feeding by sponges on pathogenic microbes: a reassessment of potential for abatement of microbial pollution. *Marine Ecology Progress Series* 403:75–89.

Malinowska, K.H., T. Rind, T. Verdorfer, H.E. Gaub, and M.A. Nash. 2015. Quantifying synergy, thermostability, and targeting of cellulolytic. *Analytical Chemistry* 87: 7133−7140.

Mato, Y., T. Isobe, H. Takada, H. Kanehiro, C. Ohtake, and T. Kaminuma. 2001. Plastic resin pellets as a transport medium for toxic chemicals in the marine environment. *Environmental Science and Technology* 35(2):318–324.

McMurray, S.E., J.E. Blum, and J.R. Pawlik. 2008. Redwood of the reef: growth and age of the giant barrel sponge *Xestospongia muta* in the Florida Keys. *Marine Biology* 155:159–171.

McMurray, S.E., J.R. Pawlik, and C.M. Finelli. 2014. Trait-mediated ecosystem impacts: how morphology and size affect pumping rates of the Caribbean giant barrel sponge. *Aquatic Biology* 23:1–13.

McMurray, S.E., T.P. Henkel, and J.R. Pawlik. 2010. Demographics of increasing populations of the giant barrel sponge *Xestospongia muta* in the Florida Keys. *Ecology* 91(2):560–570.

McMurray, S.E., Z.I. Johnson, D.E. Hunt, J.R. Pawlik, and C.M. Finelli. 2016. Selective feeding by the giant barrel sponge enhances foraging efficiency. *Limnology and Oceanography* 61(4):1271–1286.

Modica, L., P. Lanuza, and G. García-Castrillo. 2020. Surrounded by microplastic, since when? Testing the feasibility of exploring past levels of plastic microfibre pollution using natural history museum collections. *Marine Pollution Bulletin* 151:110846.

Murray, F., and P.R. Cowie. 2011. Plastic contamination in the decapod crustacean *Nephrops norvegicus* (Linnaeus, 1758). *Marine Pollution Bulletin* 62(6):1207–1217.

Norén, F., and L. Naustvoll. 2010. Survey of microscopic anthropogenic particles in Skagerrak. *TA* 2779:1–20.

Pawlik, J.R., D.E. Burkepile, and R.V. Thurber. 2016. A vicious circle? Altered carbon and nutrient cycling may explain the low resilience of Caribbean coral reefs. *Bioscience* 66:470–476.

Pawlik, J.R., T.-L. Loh, and S.E. McMurray. 2018. A review of bottom-up vs. top-down control of sponges on Caribbean fore-reefs: what's old, what's new, and future directions. *PeerJ* 6:e4343.

Payton, T.G., B.A. Beckingham, and P. Dustan. 2020. Microplastic exposure to zooplankton at tidal fronts in Charleston Harbor, SC USA. *Estuarine, Coastal and Shelf Science* 232:106510.

Pile, A.J., M.R. Patterson, and J.D. Witman. 1996. *In situ* grazing on plankton <10μm by the boreal sponge *Mycale lingua*. *Marine Ecology Progress Series* 141:95–102.

Pile, A.J., M.R. Patterson, M. Savarese, V.I. Chernykh, and V.A. Fialkov. 1997. Trophic effects of sponge feeding within Lake Baikal's Littoral zone. 2. Sponge abundance, diet, feeding efficiency, and carbon flux. *Limnology and Oceanography* 42:178–184.

Powell, M.D., and A.J. Berry. 1990. Ingestion and regurgitation of living and inert materials by the estuarine copepod *Eurytemora affinis* (Poppe) and the influence of salinity. Es*tuarine, Coastal and Shelf Science* 31(6):763–773.

Reichert, J., J. Schellenberg, P. Schubert, and T. Wilke. 2018. Responses of reef building corals to microplastic exposure. *Environmental Pollution* 237:955–960.

Reiswig, H.M. 1971. *In situ* pumping activities of tropical demospongiae. *Marine Biology* 9:38–50.

Reiswig, H.M. 1974. Water transport, respiration and energetics of three tropical marine sponges. *Journal of Experimental Marine Biology and Ecology* 14:231–249.

Reiswig, H.M. 1975. Bacteria as food for temperate-water marine sponges. *Canadian Journal of Zoology* 53:582–589.

Reiswig, H.M. 1990. In situ feeding in 2 shallow-water hexactinellid sponges. In: Rützler, K., ed. *New Perspectives in Sponge Biology*. Washington: Smithsonian Institution Press, 504–510.

Ribes, M., R. Coma, and J.-M. Gili. 1999. Natural diet and grazing rate of the temperate sponge *Dysidea avara* (Demospongiae, Dendroceratida) throughout an annual cycle. *Marine Ecology Progress Series* 176:179–190.

Rix, L., J.M. de Goeij, C.E. Mueller, U. Struck, J.J. Middelburg, F.C. van Duyl, F.A. Al-Horani, C. Wild, M.S. Naumann, D. van Oevelen. 2016. Coral mucus fuels the sponge loop in warm- and cold-water coral reef ecosystems. *Scientific Reports* 6(1):18715.

Rose, D., and M. Webber. 2019. Characterization of microplastics in the surface waters of Kingston Harbour. *Science of the Total Environment* 664:753–760.

Rotjan, R.D., K.H. Sharp, A.E. Gauthier, R. Yelton, E.M.B. Lopez, J. Carilli, J.C. Kagan, and J. Urban-Rich. 2019. Patterns, dynamics and consequences of microplastic ingestion by the temperate coral, *Astrangia poculata*. *Proceedings of the Royal Society B* 286:20190726.

Savarese, M., M.R. Patterson, V.I. Chernykh, V.A. Fialkov. 1997. Trophic effects of sponge feeding within Lake Baikal's littoral zone. 1. In situ pumping rates. *Limnology and Oceanography* 42:171−178.

Simpson, T.L. 1984. *The Cell Biology of Sponges*: Springer.

Schmidt, I. 1970. Phagocytose et pinocytose chez les Spongillidae. *Zeitschrift für vergleichende Physiologie* 66:398–420.

Seemann, J., C.T. González, R. Carballo-Bolaños, K. Berry, G.A. Heiss, U. Struck, and R.R. Leinfelder. 2014. Assessing the ecological effects of human impacts on coral reefs in Bocas del Toro, Panamá. *Environmental Monitoring and Assessment* 186:1747–1763.

Sussarellu, R., M. Suquet, Y. Thomas, C. Lambert, C. Fabioux, M.E.J. Pernet, N.L. Goïc, V. Quillien, C. Mingant, Y. Epelboin, C. Corporeau, J. Guyomarch, J. Robbens, I. Paul-

Pont, P. Soudant, and A. Huvet. 2015. Oyster reproduction is affected by exposure to polystyrene microplastics. *PNAS* 113(9):2430–2435.

Tang, J., X. Ni, Z. Zhou, L. Wang, and S. Lin. 2018. Acute microplastic exposure raises stress response and suppresses detoxification and immune capacities in the scleractinian coral *Pocillopora damicornis*. *Environmental Pollution* 243:66–74.

The World Bank. (2018). Online database. https://data.worldbank.org/indicator/ST.INT.ARVL?locations=PA, Accessed date: 18 May 2020.

Thompson, R.C. 2015. Microplastics in the marine environment: Sources, consequences and solutions. In: Bergmann, M., L. Gutow, and M. Klages, eds. *Marine Anthropogenic Litter*. Cham: Springer, 185.

Thompson, R.C., C.J. Moore, F.S. vom Saal, S.H. Swan. 2009. Plastics, the environment and human health: current consensus and future trends. *Philosophical Transactions of the Royal Society of London B: Biological Science* 364(1526):2153–2166.

Thompson, R.C., Y. Olsen, R.P. Mitchell, A. Davis, S.J. Rowland, A.W.G. John, D. McGonigle, and A.E. Russell. 2004. Lost at sea: where is all the plastic? *Science* 304:838.

Turon, X., J. Galera, and M.J. Uriz. 1997. Clearance rates and aquiferous systems in two sponges with contrasting life-history strategies. *Journal of Experimental Zoology* 278:22–36.

Urban, F. 1906. Kalifornische Kalkschwämme. *Archiv für Naturgeschichte* 72(1):33–76.

Van Soest, R.W.M., N. Boury-Esnault, J. Vacelet, M. Dohrmann, D. Erpenbeck, and N.J. De Voogd. 2012. Global diversity of sponges (Porifera). *PLoS ONE* 7:e35105. doi: 10.1371/journal.pone.0035105.

Villamizar, E., M.C. Diaz, K. Rützler, and R. de Nobrega. 2013. Biodiversity, ecological structure, and change in the sponge community of different geomorphological zones of the barrier fore reef at Carrie Bow Cay, Belize. *Marine Ecology* (Berlin):10.1111/maec.12099.

von Moos, N., P. Burkhardt-Holm, and A. Köhler. 2012. Uptake and effects of microplastics on cells and tissue of the blue mussel *Mytilus edulis* L. after an experimental exposure. *Environmental Science and Technology* 46:11327–11335.

Waller, C.L., H.J. Griffiths, C.M. Waluda, S.E. Thorpe, I. Loaiza, B. Moreno, C.O. Pacherres, and K.A. Hughes. 2017. Microplastics in the Antarctic marine system: An emerging area of research. *Science of the Total Environment* 598:220–227.

Ward, J.E., and D.J. Kach. 2009. Marine aggregates facilitate ingestion of nanoparticles by suspension-feeding bivalves. *Marine Environmental Research* 68(3):137–142.

Ward, J.E., and N.M. Targett. 1989. Influence of marine microalgal metabolites on the feeding behavior of the blue mussel *Mytilus edulis*. *Marine Biology* 101:313–321.

Ward, J.E., J.S. Levinton, and S.E. Shumway. 2003. Influence of diet on pre-ingestive particle processing in bivalves: I: transport velocities on the ctenidium. *Journal of Experimental Marine Biology and Ecology* 293(2):129–149.

Weisz, J.B., N. Lindquist, and C.S. Martens. 2008. Do associated microbial abundances impact marine demosponge pumping rates and tissue densities? *Oecologia* 155:367–376.

Willenz, P., and G. Van de Vyver. 1982. Endocytosis of latex beads by the exopinacoderm in the fresh water sponge *Ephydatia fluviatilis*: an in vitro and in situ study in SEM and TEM. *Journal of Ultrastructure Research* 79:294 –306.

Wilson, D.S. 1973. Food size selection among copepods. *Ecology* 54(4):909–914.

Wright, S.L., R.C. Thompson, and T.S. Galloway. 2013. The physical impacts of microplastics on marine organisms: a review. *Environmental Pollution* 178:483–492.

Zalasiewicz, J., M. Williams, W. Steffen, P. Crutzen. 2010. The new world of the Anthropocene. *Environmental Science & Technology* 44:2228–2231.

Zea, S. 1993. Cover of sponges and other sessile organisms in rocky and coral reef habitats of Santa Marta, Colombian Caribbean Sea. *Caribbean Journal of Science* 29:75–88.

Zebe, E., and D. Schiedek. 1996. The lugworm *Arenicola marina*: a model of physiological adaptation to life in the intertidal sediments. *Helgoland Marine Research* 50(1):37–68.

Zettler, E.R., T.J. Mincer, and L.A. Amaral-Zettler. 2013 Life in the ‘plastisphere’: microbial communities on plastic marine debris. *Environmental Science and Technology* 47:7137–7146.

Zitko, V., and M. Hanlon. 1991. Another source of pollution by plastics: Skin cleaners with plastic scrubbers. *Marine Pollution Bulletin* 22:41–42.

Table 1(on next page)

Abundance of potential microplastics (PMP) in sponge and water samples.

Table 1. Abundance of potential microplastics (PMP) in sponge and water samples.

Sample type	Mean PMP/g for sponges and PMP/L for water (+/- standard error)
A. cauliformis	113 (+/- 23)
A. compressa	14 (+/- 2)
C. vaginalis	169 (+/- 71)
I. campana	71 (+/- 20)
M. laevis	6 (+/- 4)
N. erecta	75 (+/- 38)
Subsurface seawater	107 (+/- 25)

Table 2(on next page)

Results from a Tukey's HSD pairwise multiple comparisons test of mean potential microplastic (PMP) abundance across sponge species in R. Significant differences ($p<0.05$) are boldfaced.

Table 2. Results from a Tukey's HSD pairwise multiple comparisons test of mean potential microplastic (PMP) abundance across sponge species in R. Significant differences ($p<0.05$) are boldfaced.

Pairwise comparison	Adjusted p-value
A. cauliformis - A. compressa	0.1269
A. cauliformis - C. vaginalis	0.9701
A. cauliformis - I. campana	0.9291
A. cauliformis - M. laevis	**0.0360**
A. cauliformis - N. erecta	0.8863
A. compressa - C. vaginalis	**0.0365**
A. compressa - I. campana	0.4709
A. compressa - M. laevis	0.9687
A. compressa - N. erecta	0.5416
C. vaginalis - I. campana	0.5608
C. vaginalis - M. laevis	**0.0101**
C. vaginalis - N. erecta	0.4893
I. campana - M. laevis	0.1661
I. campana - N. erecta	0.9999
M. laevis - N. erecta	0.2016

Figure 1

Map of Saigon Bay located off the coast of Isla Colón, the main island of the Bocas del Toro archipelago of Panamá.

The star indicates sample collection site. Note the high level of development on the northeastern border of Saigon Bay. Image © 2020 CNES/Airbus © 2020 Europa Technologies © 2020 Google.

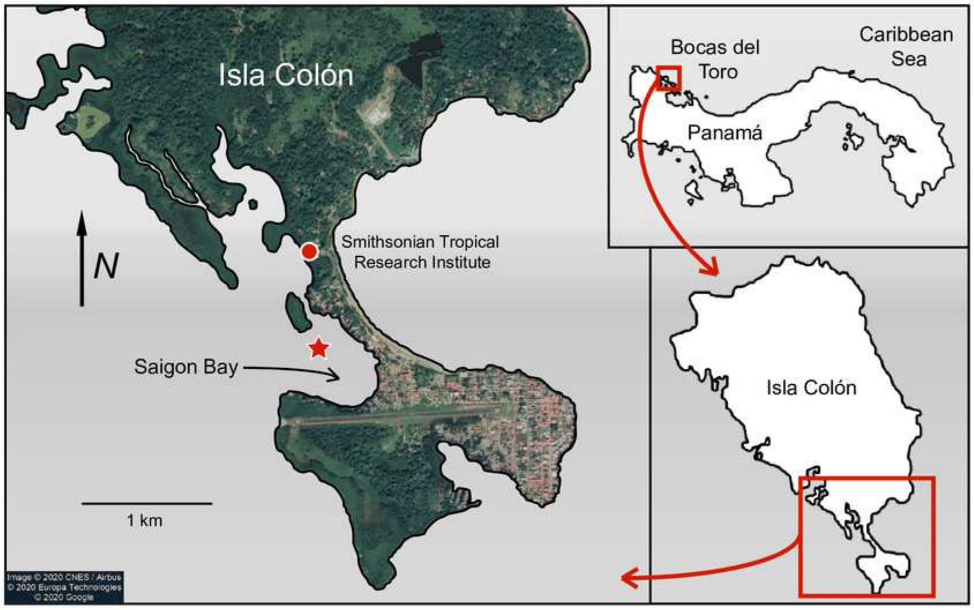

Isla Colón
N
Smithsonian Tropical
Research Institute
Saigon Bay
1 km
Bocas del
Toro
Caribbean
Sea
Panamá
Isla Colón

Figure 2

Potential microplastics (PMP) on the filters of sponge samples, water samples and blanks.

The top two rows include samples from the six sponge species: (A) *A. cauliformis*. (B) *A. compressa*. (C) *C. vaginalis*. (D) *I. campana*. (E) *M. laevis*. (F) *N. erecta*. The bottom two rows include seawater samples (G–J) as well as one blank from the seawater study (K) and one blank from the sponge study (L). Note the dulled, blue-green autofluorescence (indicated by white arrows) of spongin fragments, sand grains and two copepods in images A, D, G and I, respectively, as it compares with the bright, electric blue autofluorescence (indicated by red arrows) of PMP. Images were taken at 100× total magnification .

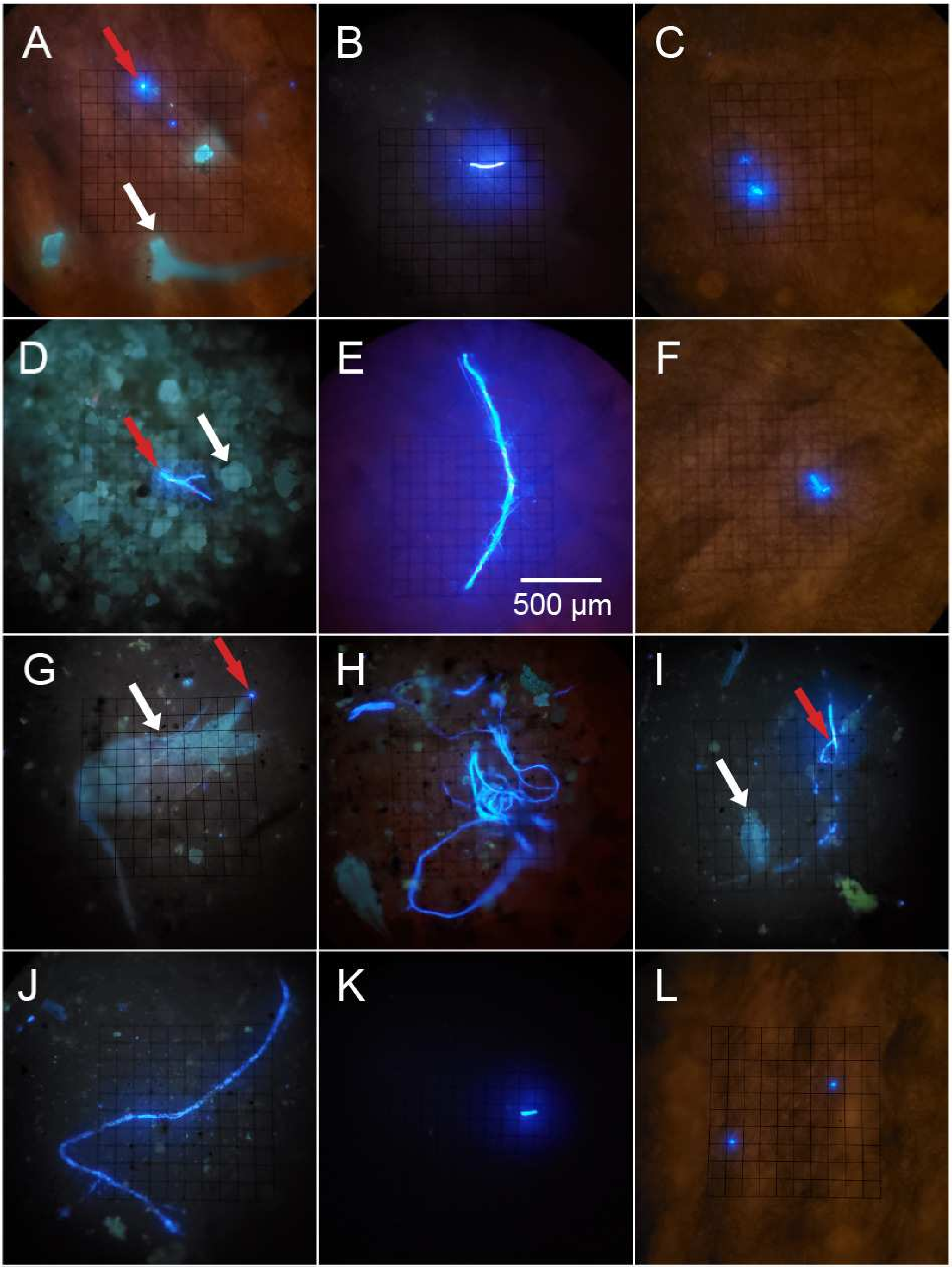

A
B
C
D
E
500 µm
F
G
H
I
J
K
L

Figure 3

The relative abundance (percent out of total) of potential microplastic (PMP) sizes detected in sponge and seawater samples and blanks.

Colors within the bars indicate the size of particles in micrometers (µm). Note the presence of large (3001–5000 µm) and very large (>5000 µm) fibers only in some sponge samples.

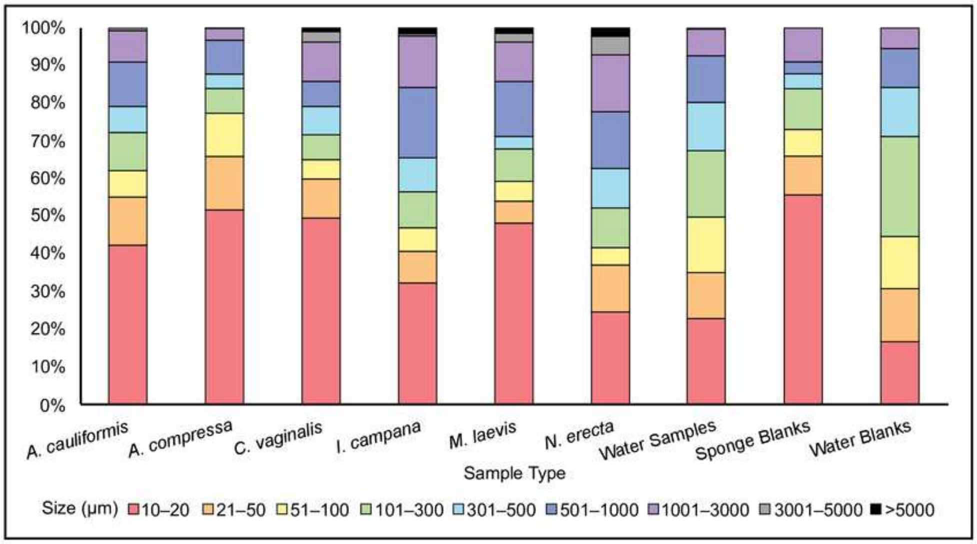
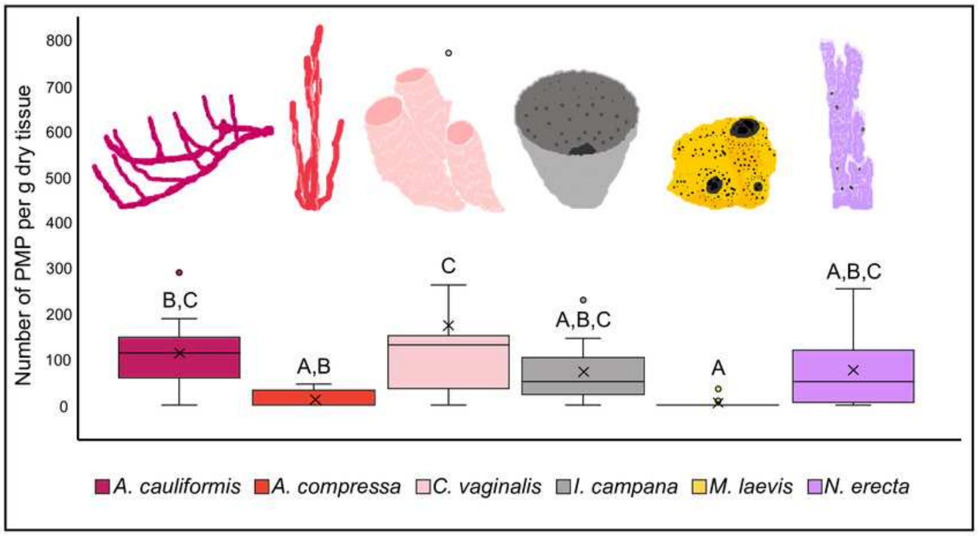

100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
A. cauliformis
A. compressa
C. vaginalis
I. campana
M. laevis
N. erecta
Water Samples
Sponge Blanks
Water Blanks
Sample Type
Size (µm) 10–20 21–50 51–100 101–300 301–500 501–1000 1001–3000 3001–5000 >5000

Figure 4

Number of potential microplastics (PMP) per gram of dry sponge tissue.

Box plots are median inclusive and the dots indicate statistical outliers while the “x” in each plot represents the mean. Letters above each plot indicate significant pairwise difference (Tukey’s test, $p<0.05$).


Number of PMP per g dry tissue
800
700
600
500
400
300
200
100
0
B,C
A,B
C
A,B,C
A
A,B,C
A. cauliformis
A. compressa
C. vaginalis
I. campana
M. laevis
N. erecta