Potential microplastic sizes of 6 common Caribbean spong species collected June 21, 2019 in Bocas del Toro, Panama

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Project
» Collaborative Research: Investigations into microbially mediated ecological diversification in sponges (Ecological Diversification in Sponges)

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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, even though they are widely distributed across benthic habitats, can process large volumes of seawater, and can retain small particles within their water filtration systems. This study examines the presence of potential MP (PMP) in wild marine sponges and seawater collected in Bocas del Toro, Panamá. Subsurface seawater and tissue from six common Caribbean sponge species was collected in Saigon Bay, a heavily impacted, shallow-water coral reef. Seawater samples were filtered onto glass fiber filters to retain any PMP present and sponge tissue was digested with bleach, heated and filtered. Filters were examined using fluorescence microscopy to quantify PMP. An average of 107 ± 25 PMP L–1 was detected in seawater from Saigon Bay with particles ranging in size between 10 µm and ~3,000 µm. The number of PMP found in sponge tissue ranged between 6 ± 4 and 169 ± 71 PMP g–1 of dry tissue. Most particles found in sponge samples were very small (10–20 µm), but fibers greater than 5,000 µm were detected. Our results indicate that PMP exists within the tissues of the sponges we studied, but future studies should confirm the presence of MP in sponges using chemical analysis. Most importantly, the discrepancy between low levels of PMP in our sponge samples and high levels in the surrounding seawater highlights the potential for sponges to resist and/or egest MP. Finally, we provide a critical evaluation of our methods to improve their use in future MP work with benthic marine organisms.

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Coverage

Location: Bocas del Toro Panama, Saigon Bay. This site is adjacent to the Smithsonian Tropical Research Institute.
Spatial Extent: **Lat**:9.345775 **Lon**:-82.256381
Temporal Extent: 2019-06-21

Methods & Sampling

Sponge and seawater samples were collected from Saigon Bay near Isla Colón, Bocas del Toro, Panamá. Sponge samples were collected on 21 June 2019 from ~6 to 8 m below the surface during an outbound tide so that any pollutants concentrated near the developed area were likely pulled through the bay. 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*, *Amphimedon compressa*, *Callyspongia aculeata*, *Ircinia campana*, *Mycale laevis,* and *Niphates erecta*. 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. Each sponge section was wrapped in aluminum foil underwater and placed in a mesh bag for transport.

Four liters of seawater were collected at the same time and depth as the sponge samples. Four clean (washed with soap and water and rinsed three times with MilliQ®) 1 L 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 seawater 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 seawater filtered for the seawater samples was between 3.6 and 4.0 L. Counts were normalized to seawater sample volume for quantification of PMP concentrations.

Seawater 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 4 h) 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 (MilliQ®) and this excess water (~100 mL) was also filtered to maximize sample transfer. Seawater 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 seawater sample filter. A procedural blank was run in parallel to each of the three seawater samples, generating three MilliQ blanks (i.e., ~1 L of MilliQ was added to a clean beaker, filtered, and the filter was stored at –20 °C).

Each of the 18 individual sponge samples (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 MilliQ (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 dry) with a steel utility blade. Subsamples were used to minimize tissue digestion time and the dry mass of each subsample varied because sponge tissue density varied across species even though each original sponge sample was approximately equal in length (~2–4 cm). Final PMP counts were normalized to subsample dry mass during data analysis. Each subsample was cut into pieces with a utility blade and added to a clean 20 mL glass scintillation vial and covered with foil. In total, 54 subsamples were generated (6 species × 3 replicates × 3 subsamples). 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 causes minimal physical degradation of plastic particles (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, but we recommend filtering bleach before use for future studies (see “Evaluation of methods and considerations”). Still, we used procedural blanks (see following paragraph) to evaluate the degree of pre-existing experimental contamination in our samples. Vials with sponge tissue and bleach were heated to 60 °C for 2 h to expedite digestion (Conley et al., 2019; Payton, Beckingham & Dustan, 2020). 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 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; approximately 10 mL of bleach was added to six

clean scintillation vials, heated, and filtered.

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. Each filter was mounted onto a microscope slide with a few drops of MilliQ to secure the filter onto the slide. The filter was then surveyed at 100× total magnification in a sweeping motion vertically and laterally until the entire filter was visually surveyed, and the number of detected PMP was recorded. Because the seawater sample filters were so concentrated with PMP, each filter and its corresponding MilliQ blank was surveyed independently three times. The first survey resulted in counts that were consistently much greater than those resulting from the second and third surveys, while the latter two surveys resulted in consistently similar counts. Therefore, we concluded that the elevated counts from survey 1 were a result of human error, and the mean number of PMP for each seawater and MilliQ blank filter was determined using only counts from the second and third surveys. A 10 × 10 mm reticle net grid on one eyepiece in the microscope was used to measure the size of detected PMP. The size of nearly every PMP found in the sponge subsamples and corresponding blanks and the sizes of at least 15% of PMP found in the seawater samples and corresponding MilliQ 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. These size categories were chosen post hoc based on the sizes of the particles detected on the filters. They serve to demonstrate the frequency of different particle sizes in all samples and blanks, and to identify any differences in particle uptake or retention by sponges based on particle size.

Data Processing Description

Potential microplastic (PMP) concentrations are reported as number of PMP per liter (PMP L–1) for seawater samples and number of PMP per gram of dry tissue (PMP g–1) for sponge samples. The blank-corrected number of PMP g–1 was determined for each of the 54 subsamples representing the six species. These 54 values were then grouped by replicate to produce a mean number of PMP g–1 for each replicate. These true replicate values (*N*= 3) were then grouped by species to produce a mean number of PMP g–1for each species.

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Data Files

File
955389_v1_pmpsize.csv (Comma Separated Values (.csv), 61.69 KB) MD5:db0be4673d37adc73a054fa7e11fbac4 Primary data file for dataset ID 955389, version 1

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Related Publications

Collard, F., Gilbert, B., Eppe, G., Parmentier, E., & Das, K. (2015). Detection of Anthropogenic Particles in Fish Stomachs: An Isolation Method Adapted to Identification by Raman Spectroscopy. Archives of Environmental Contamination and Toxicology, 69(3), 331–339. https://doi.org/10.1007/s00244-015-0221-0
Methods

Conley, K., Clum, A., Deepe, J., Lane, H., & Beckingham, B. (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. https://doi.org/10.1016/j.wroa.2019.100030
Methods

Fallon, B. R., & Freeman, C. J. (2021). Plastics in Porifera: The occurrence of potential microplastics in marine sponges and seawater from Bocas del Toro, Panamá. PeerJ, 9, e11638. Portico.

https://doi.org/10.7717/peerj.11638
Results

Loh, T.-L., & Pawlik, J. R. (2014). Chemical defenses and resource trade-offs structure sponge communities on Caribbean coral reefs. Proceedings of the National Academy of Sciences, 111(11), 4151–4156. doi:10.1073/pnas.1321626111
Methods

Payton, T. G., Beckingham, B. A., & Dustan, P. (2020). Microplastic exposure to zooplankton at tidal fronts in Charleston Harbor, SC USA. Estuarine, Coastal and Shelf Science, 232, 106510. https://doi.org/10.1016/j.ecss.2019.106510
Methods

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Parameters

Parameter	Description	Units
Sample_type	Samples are either seawater samples collected from Bocas del Toro, Panama, MilliQ water blank, sponge tissue, or bleach blank.	unitless
Sample_replicate	Sample replicate (1-3 for seawater samples; B1-B3 for MilliQ water blank; sponge tissue [sponge name: A.cau: Aplysina cauliformis, A.com: Amphimedon compressa, C.acu: Callyspongia aculeata, I.cam: Ircinia campana, M.lae: Mycale laevis, N.ere: Niphates erecta], sponge species replicate number [1-3], sponge species replicate subsample number [1-3]; bleach blank number [1-3] and bleach blank subsample number [1-2].	unitless
Length_um	Length of microplastic	micrometers (um)
Width_um	Width of microplastic	micrometers (um)
sampling_lat	Sampling latitude	decimal degrees
sampling_lon	Sampling longitude	decimal degrees
sampling_date	Sampling date	unitless

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Instruments

Dataset-specific Instrument Name	E600 Nikon Eclipse fluorescence microscope
Generic Instrument Name	Fluorescence Microscope
Dataset-specific Description	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).
Generic Instrument Description	Instruments that generate enlarged images of samples using the phenomena of fluorescence and phosphorescence instead of, or in addition to, reflection and absorption of visible light. Includes conventional and inverted instruments.

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Project Information

Collaborative Research: Investigations into microbially mediated ecological diversification in sponges (Ecological Diversification in Sponges)

Coverage: Caribbean coast of Panama

NSF Award Abstract:
Coral reefs represent a paradox because, despite their immense productivity and biodiversity, they are found in nutrient-poor habitats that are equivalent to "marine deserts." High biodiversity is often associated with a division of resources that allows many types of organisms to coexist with minimal competition. Indeed, unlike many other organisms on coral reefs, sponges are adapted to efficiently remove bacteria, phytoplankton, and dissolved organic matter from seawater by filter-feeding. Sponges are a dominant component of coral reefs worldwide and in the Caribbean, where their biomass exceeds that of reef-building corals. For almost a quarter century, the success of sponges in the Caribbean has been linked to their filter-feeding ability. However, recent work demonstrated that coexisting sponges on Caribbean reefs host unique communities of bacteria that might allow sponges to access multiple pools of nutrients that are not available to other organisms. In this project, the investigators will test the hypothesis that ecologically dominant sponge species in the Caribbean have unique metabolic strategies that are mediated by their associations with microbes that live within the sponge body. This research will combine manipulative field experiments with a novel combination of modern analytical tools to investigate both filter-feeding by sponge hosts and the metabolic pathways of their microbes. This work will advance our understanding of the ecological and evolutionary forces that have helped shape the species present on Caribbean coral reefs. Additionally, this project will support three early-career investigators and provide training opportunities for graduate and undergraduate students at Nova Southeastern University, Appalachian State University, Stony Brook University, and Smithsonian Marine Station. The investigators will also develop innovative outreach programs that expand existing platforms at their institutions to increase public engagement and scientific literacy.

Marine sponges have been widely successful in their expansion across ecological niches in the Caribbean, with biomass often exceeding that of reef-building corals and high species diversity. However, whether this success is linked to efficient heterotrophic filter-feeding on organic carbon in the water column or to their evolutionary investment in microbial symbionts is yet to be fully elucidated. Microbial symbionts expand the metabolic capabilities of host sponges, supplementing heterotrophic feeding with inorganic carbon and nitrogen, mediating the assimilation of dissolved organic matter, and facilitating recycling of host-derived nitrogen. Despite these benefits, microbial symbiont communities are widely divergent across coexisting sponge species and there is substantial variation in host reliance on symbiont-derived carbon and nitrogen among host sponges; therefore, these associations likely mediate the ecological diversification of coexisting sponge species. The goal of this project is to test this transformative hypothesis by adopting an integrative approach to assess the individual components of holobiont metabolism (i.e., microbial symbionts and sponge host) in ten of the most common sponge species in the Caribbean. The investigators will isolate autotrophic and heterotrophic metabolic pathways and explore potential links between microbial symbiont community

composition and the assimilation of particulate and dissolved organic matter (POM and DOM) from seawater. This project will elucidate whether Caribbean sponge species are on similar or divergent evolutionary trajectories, and will provide information that is critical for our understanding of how conditions in the Caribbean basin have shaped the evolution of benthic organisms.

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Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-1929293

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