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Foraminiferal population dynamics on elevated plastic substrates and in sediments at 4000 m in the Eastern Pacific

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ABSTRACT: Although plastics are becoming more prevalent, even in the far reaches of the deep sea, the influence of these novel attachment surfaces has yet to be systematically studied regarding the ecology and distribution patterns of attached fauna. Herein, we report the abundances and vertical distribution patterns of epibenthic foraminifera living on plastics after 2 yr on the seafloor at 4000 m water depth and compare these populations with those of nearby naturally occurring substrates and their surrounding sediments. After 2 yr, 239 foraminifera were found attached to 4 Seafloor Epibenthic Attachment Cubes (SEA^{3}s). Dominant taxa included *Cibicidoides wuellerstorfi* var. *lobatulus*, *Pyrgoella* sp., and arborescent foraminifera. Variations in colonization height and abundance between plastic types were observed, but no clear drivers of these patterns can be ascertained from this study. Foraminiferal populations from elevated substrates and the nearby sediment cores showed no significant overlap in populations, suggesting that foraminifera colonizing SEA^{3}s did not originate from surrounding sediments and likely recruited from other elevated substrates common in the area (e.g. glass sponges). This study demonstrates that plastics serve as hard substrates which deep-sea foraminifera inhabit and that plastics may persist for extended periods of time, potentially altering ecosystem compositions in environments dominated by soft sediments. There is a significant difference between colonizing epifaunal and sediment populations, which raises interesting questions about colonization and distribution processes in deep bathyal and abyssal environments. Epibenthic foraminifera attached to elevated substrates may be underrepresented in the sedimentary record through preservation and sampling biases.

KEY WORDS: Epibenthic foraminifera · Marine ecology · Colonization experiment · Ocean plastic · Plastic pollution

1. INTRODUCTION

As much as three-fourths of the world's ocean consists of the permanently dark deep sea, representing the largest habitat on Earth (e.g. Norse 1994). Although the abyssal zone accounts for a significant portion of the world's oceans, it remains mostly unexplored, with over 99% of its seafloor having never been observed directly (e.g. Webb et al. 2010). This is especially true for the vast majority of deep-seafloor environments which are covered in soft sediments. These extensive habitats play a significant role in carbon cycling and are susceptible to anthropogenic influences, including climate change, mineral extrac-

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tion, and commercial fishing (Smith et al. 2009, Sweetman et al. 2017). Deep-seafloor ecosystems are influenced in many different ways by anthropogenic activities, including those that impact sea surface export productivity (Smith et al. 2009), habitat heterogeneity (Vanreusel et al. 2010, Venturelli et al. 2018), seawater pH (Chen et al. 2017), and the amount and types of plastics reaching the seafloor (Woodall et al. 2014, Krause et al. 2020). Environmental impacts on ecosystem functioning can operate at decadal to millennial time scales (Yasuhara et al. 2016), but in many cases, we have a very poor understanding of how predicted future environmental changes, of any scale, will affect deep-seafloor ecosystems (Sweetman et al. 2017) or how deep-sea creatures may adapt to anthropogenic pollution (Heaney 2000, Baker et al. 2010, Costello & Chaudhary 2017, Hamdan et al. 2021).

Ubiquitous in the world's oceans, benthic foraminifera (protists) comprise as much as half of the eukaryotic biomass in the deep sea and play a significant role in carbon cycling and trophic networks (Gooday 2003, Gooday & Jorissen 2012). The microfossil record of benthic foraminifera in seafloor sediments also serves as an archive that records paleoceanographic changes based on geochemical, morphological, and ecological proxies (Gooday 2003, Jorissen et al. 2007, Katz et al. 2010, Gooday & Jorissen 2012). Much of the work documenting global distribution patterns and inferred ecological constraints of deep-sea species of benthic foraminifera comes from analyses of living plus dead specimens from core-top sediments (Jorissen et al. 2007). Most studies of deep-sea foraminifera continue to focus on those living on or within seafloor sediments, while less attention has been paid to taxa associated with elevated hard substrates (Venturelli et al. 2018). Although soft sediments characterize much of the deep-sea, hard structures that protrude above the seafloor can be common. Benthic foraminifera are known to colonize these hard structures, called elevated substrates, including manganese nodules (e.g. Mullineaux 1987, 1989), carbonate rocks (Lutze & Thiel 1989), areas where currents winnow away fine sediments leaving behind sand and gravel (e.g. Schönfeld 2002a,b), and biogenic structures, such as worm tubes at methane seeps (Sen Gupta et al. 2007, Burkett et al. 2015) and sponge spicules (Beaulieu 2001a,b), cold-water corals (e.g. Fentimen et al. 2020 and references therein), and sponges (Lintner et al. 2022). While any material rising above the sediment–water interface can serve as a potential attachment surface, substrates may act as habitat islands, generating advantages in feeding (Linke & Lutze 1993), and/or may serve as a refuge from inhospitable seafloor conditions (Sen Gupta et al. 2007). Foraminiferal species commonly attached to elevated substrates at the depths of 4000 m include: *Cibicidoides wuellerstorfi* var. *lobatulus* (Schwager, 1866), *Pyrgoella* sp. (Cushman & White, 1936), and attached arborescent foraminifera (pictured and discussed in Burkett et al. 2020, to be named and described in a future manuscript).

1.1. Island Theory of Biogeography

Benthic populations in the deep sea are highly influenced by the heterogeneity of the ocean seafloor environment, commonly driven by changes in timing and the amount of phytodetrital inputs, bottom-water circulation and composition (e.g. oxygenation), and physical parameters (e.g. temperature, depth, and salinity). Unique areas, such as vents, seeps, whale falls, and shipwrecks, have been documented to be epicenters of vastly different communities on these elevated substrates as well as in the surrounding sediments which tend to radiate outward from the source (e.g. Hamdan et al. 2021). Elevated substrates vary in their composition, which can be biogenic, authigenic, or built materials. Built materials include structures created or modified by humans (Hamdan et al. 2021) and can include structures as large as shipwrecks or as small as a plastic straw. In fact, ships have even been purposely sunk to create additional habitat on the seafloor (e.g. Goeting et al. 2022 and references therein). Plastic materials greater than a few centimeters are a new type of colonizable material in the deep-sea environment. Plastics are becoming ever more prevalent in the deep sea, and macroscopic pieces can serve as elevated substrates in benthic habitats dominated by soft sediments (e.g. Rizzo et al. 2022). Elevated substrates on the seafloor may function as isolated environments as described by the Island Theory of Biogeography, which states that in island-like, or isolated, systems that are disconnected from similar environments, species richness and diversity are dictated by the size and connectivity to the population source (Wilson & MacArthur 2016). Given the extent of soft-sediment-covered surfaces on the seafloor, most hard-bottomed seafloor environments protruding from the seafloor sediments could be considered isolated, especially if attached populations are recruited from the water column as opposed to surrounding sediments (e.g. Meyer et al. 2016).

1.2. Objectives

The objectives of this study were to examine the colonization of plastic after 2 yr of exposure on the seafloor to assess any preferences of deep-sea foraminifera; to determine how elevated plastic structures impact the ecology and distribution patterns of deep-sea benthic foraminifera; and to document the extent to which plastics are colonized after a set period of time. In order to achieve our objectives, we (1) compared vertical distribution patterns of foraminifera on plastics with studies of foraminifera found on other elevated substrates (e.g. Schönfeld 1997, 2002a,b, Beaulieu 2001a,b) to yield insights into why foraminifera live on elevated microhabitats; (2) conducted a comprehensive assessment of the results of Seafloor Epibenthic Attachment Cube (SEA^3) colonization studies from Station M in the NE Pacific Ocean to provide a better understanding of the ecological influence of hard plastic substrates in the deep sea, facilitate the assessment of potentially advantageous features (e.g. height above the seafloor, current direction, etc.), and characterize colonization patterns of hard substrates in the deep sea; and (3) compared elevated populations with infaunal foraminiferal populations in nearby sediments that provide clues about how elevated habitat islands influence deep-sea communities.

2. MATERIALS AND METHODS

2.1. Geologic setting

In the NE Pacific, an abyssal plain site known as Station M has been monitored through autonomous vehicles, instrumentation, and experiments deployed over the course of 30 yr by researchers at the Monterey Bay Aquarium Research Institute (MBARI) (e.g. Smith et al. 2020). Located about 220 km west of Point Conception, California, USA (34° 50′ N, 123° 00′ W), Station M lies at a water depth of 4000 m (Fig. 1), where seafloor experimentation suggest substrates are exposed to gentle currents (~2.75 to 1.34 cm s^{-1}, Beaulieu & Baldwin 1998, Beaulieu 2001a). Elevated hard sub-

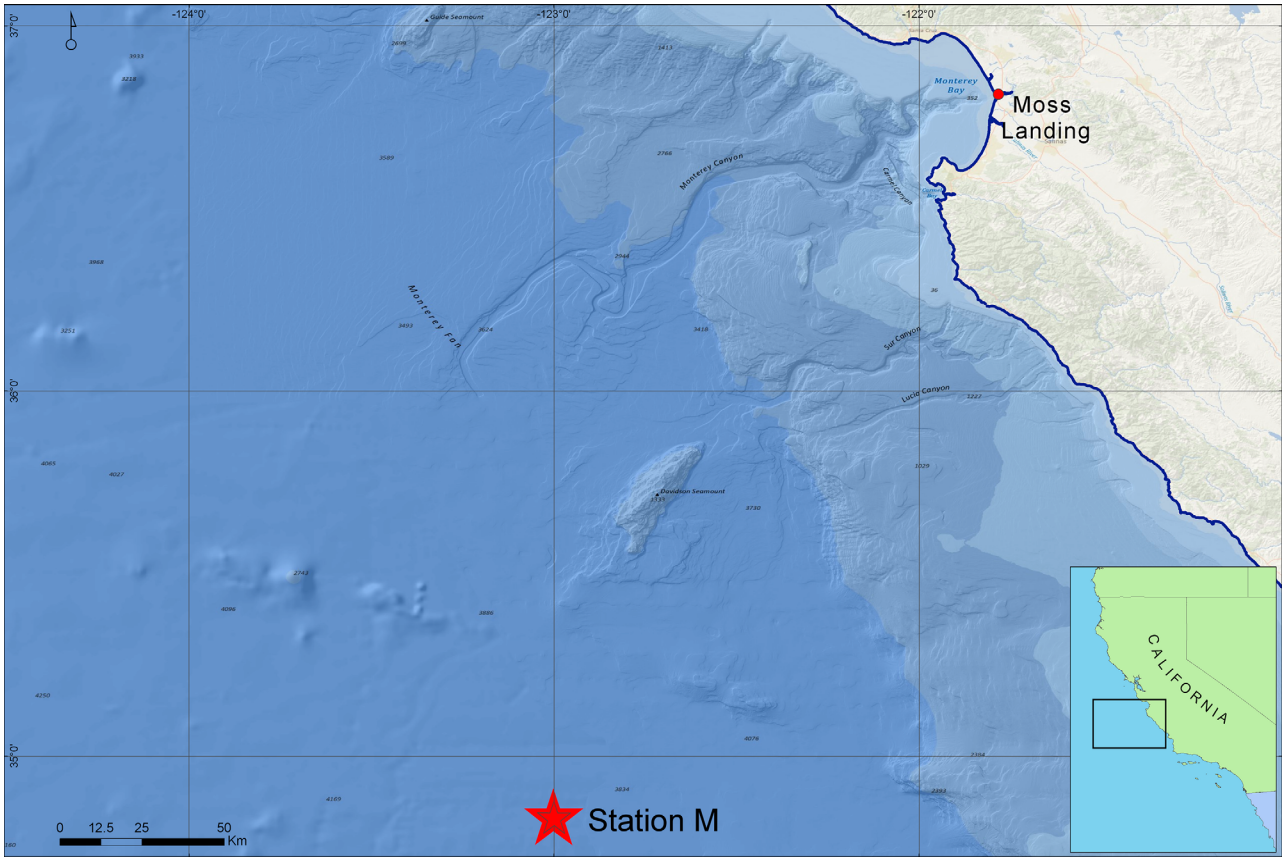

Fig. 1. Station M, designated by the star, is located at 34° 50′ N, 123° 00′ W, about 220 km west of Point Conception, California, USA, at a water depth of about 4000 m. Moss Landing, the location of the Monterey Bay Aquarium Research Institute (MBARI), is identified on the map. Image credit: Linda Kuhntz (MBARI)

strates in the region include a few consolidated outcrops and scattered manganese nodules (Beaulieu 2001b). Biogenic structures, mostly hexactinellid sponges, tend to be the most prolific hard substrates at Station M, extend as high as 1 m above the sediment water interface, and have been documented to occur at a density of ~1118 stalks ha^{-1} from centimeters to meters apart from each other (Beaulieu 2001b). On both natural and artificial materials, benthic foraminifera are among the dominant colonizing organisms (Beaulieu 2001a,b, Burkett et al. 2020). Although total assemblages were not reported, within the sediments at Station M, an *in situ* feeding experiment study concluded that benthic foraminifera assemblages contained predominantly agglutinated (such as *Cyclammina*) and infaunal calcareous species (such as *Globobulimina*, Drazen et al. 1998, Jeffreys et al. 2013).

Biogenic production of carbonate and the accumulation of calcareous materials on the seafloor are influenced by increased carbonate solubility in the deep ocean. The water depth at which the carbonate dissolution rate increases dramatically is known as the 'lysocline.' The water depth at which the rate of calcareous materials accumulating on the seafloor, including calcareous tests of foraminifera, is equal to the rate of dissolution is known as the carbonate compensation depth (CCD). The CCD and lysocline in the region are expected to occur within 4500–5000 m, and at 3500 m, respectively (Broecker & Peng 1982, Chen et al. 1988, Hales 2003). At 4000 m, Station M is near the average ocean CCD and below the average lysocline. The saturation state of bottom waters at abyssal depths is difficult to measure, and it is unclear if bottom waters at Station M are continually undersaturated with respect to carbonate. While *in situ* measurements of carbonate ion corrosion have not been made in this region, sediments and calcite spars were deployed as part of this project to assess carbonate dissolution to evaluate the potential for calcareous foraminiferal test dissolution (Table S2 in the Supplement at www.int-res.com/articles/suppl/m723p001_supp.pdf).

2.2. Elevated materials

SEA^{3}s are experimental units designed to be deployed on the seafloor for plastic substrate experiments (Burkett et al. 2018, 2020). SEA^{3}s are composed of a metal frame that has been coated in Plasti-Dip®, squares of attached plastic mesh completely surrounding the metal cube, and several types of fiberglass and plastic rods attached to the back corner (Fig. 2). A 3D schematic of the SEA^{3}s has been

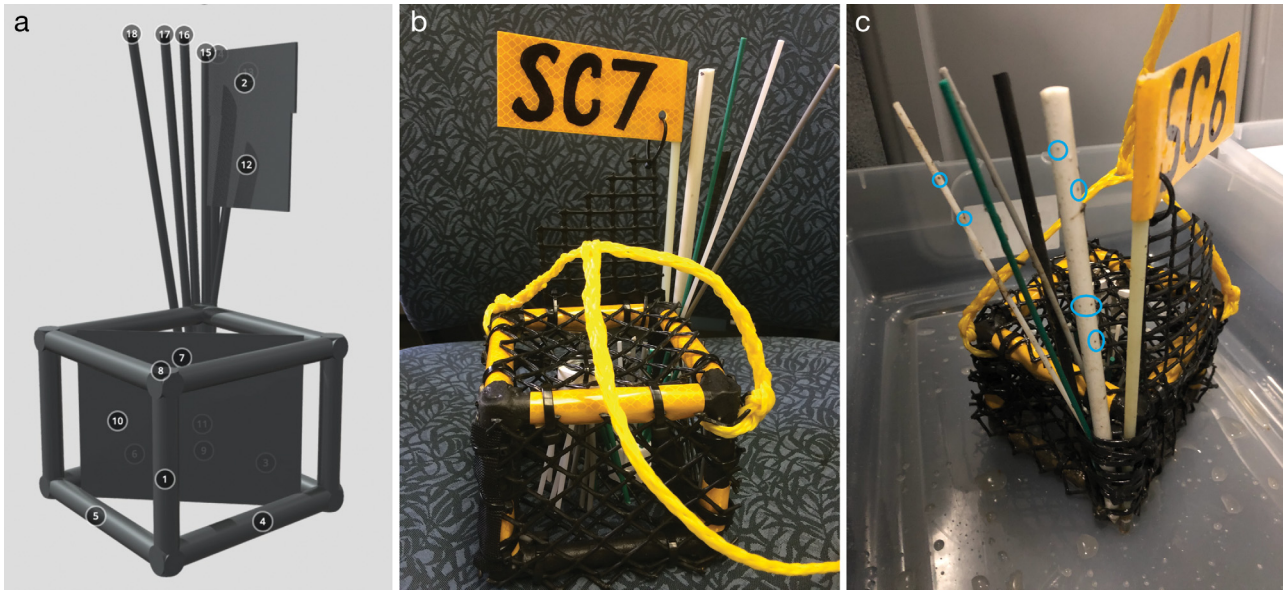

Fig. 2. (a) A 3D model of the Seafloor Epibenthic Attachment Cube (SEA^3) — which can be found on Sketchfab — design including metal frame covered in PlastiDip® (1), identification flag (2), Side 2 Mesh (4), Side 3 Mesh (5), Middle Mesh (7), Top Mesh (8), Flag Mesh (12), Black Flagpole (13), Fiberglass Flagpole (14), Main flagpole (15), White ABS Flagpole (16), Grey PVC Flagpole (17), Green PP Flagpole (18). Additional identification numbers (3, 6, 9, 10, 11) illustrate labeled features which can be seen on the opposite side of the digital model. Please see SketchFab for details (https://skfb.ly/6YWpY). (b) Photo of SEA^{3}7 prior to deployment. (c) Photo of SEA^{3}6 after recovery and prior to disassembly and picking. Attached foraminifera are visible, with the naked eye, especially on the white fiberglass and plastic rods (blue circles)

created and published on SketchFab (Fig. 2a, https://skfb.ly/6YWpY). Several plastic and fiberglass rods, referred to as flagpoles, were attached to assess the effect of added height and composition on colonization (Fig. 2a; see points 13–18 in the SketchFab schematic). Additionally, a square of mesh was added to the middle of the frame (Fig. 2a; point 7) and a small triangle or square of mesh was connected to the identification flag and the top of the frame, called the Flag Mesh (Fig. 2a; point 12). All materials were attached to the frame with plastic zip ties.

2.3. Deployment and recovery

On 16 November 2017, we deployed 4 SEA^{3}s (SEA^{3}6–SEA^{3}9) at Station M using the RV 'Western Flyer' and the ROV 'Doc Ricketts.' Each cube was transported to and from the seafloor in a covered biobox. Using the ROV's manipulator arm, SEA^{3}s were set on the seafloor in the desired location in soft substrates near glass sponges (proximity to elevated substrates provided in Fig. 3) and pushed slightly into the sediment to secure the SEA^3 on the seafloor (Fig. 3). Approximately 2 yr later, in November of 2019, the ROV grasped the polypropylene handles to retrieve the SEA^{3}s (Fig. 3) that were then placed in separate containers within sealed bioboxes on the ROV's basket and stored in a 2°C cooler onboard. Once onshore, the 2 bioboxes, each containing 2 separate SEA^{3}s, were transferred into a van, packed in ice, and transported to California State University, Bakersfield. The bioboxes with SEA^3 containers were stored in a walk-in refrigerator during processing. SEA^{3}s were disassembled, labeled, and placed in seawater extracted from their individual containers, and foraminifera were removed with a sterile scalpel and fine-tipped paint brush. Each flagpole was examined for foraminifera in a 1 cm lined dish to determine the height of foraminiferal colonization. Because some flagpoles were not completely straight, the base of the flagpole was placed on the 0 cm line and the curvature of the flagpole was maintained to help alleviate any discrepancies between actual height above

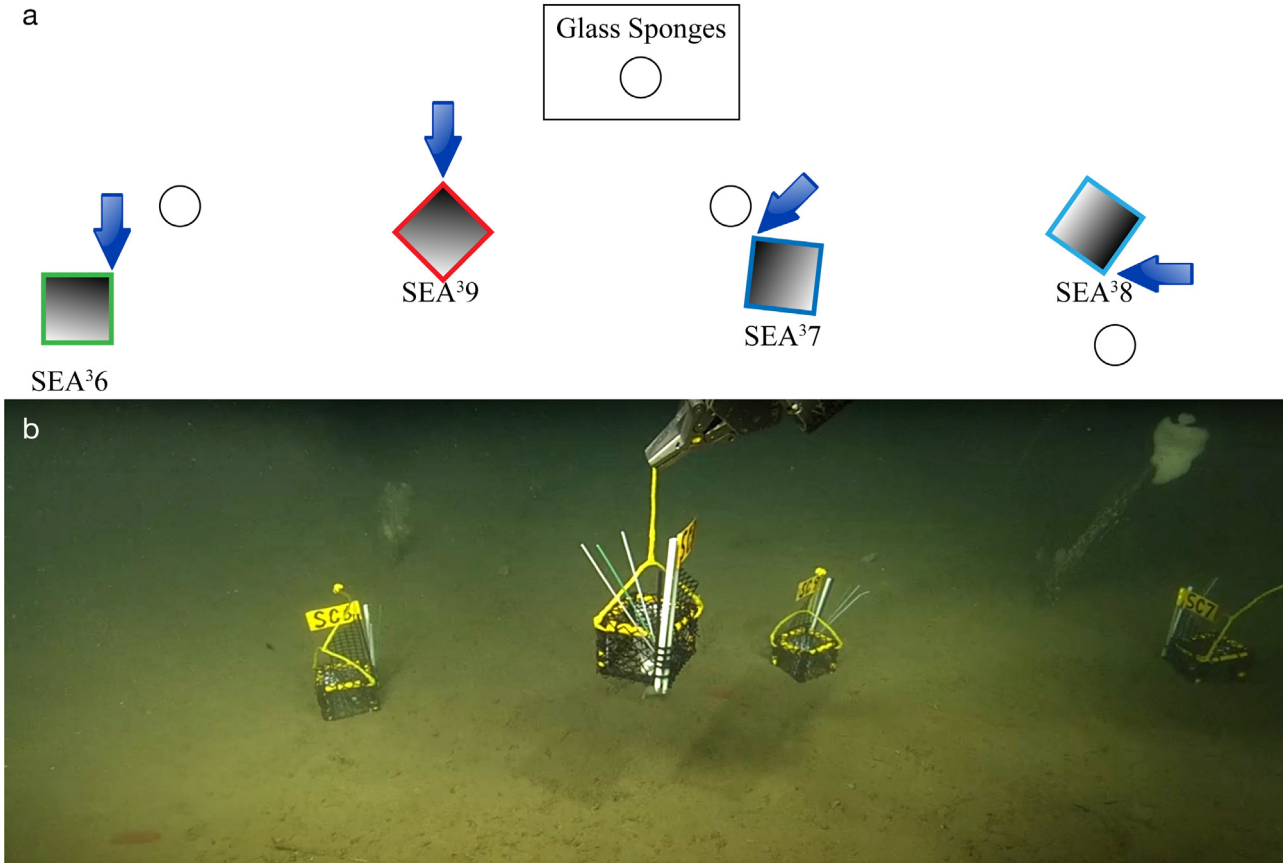

Fig. 3. Orientation of SEA^{3}s and nearby glass sponges. (a) Schematic of the locations of materials. White circles represent glass sponges; colored squares represent SEA^{3}s. The corner shaded the darkest with a blue arrow marks the corner to which the flagpoles were attached. (b) Deployment of the SEA^{3}s

the seafloor and bend of the plastic material. While this arrangement of flagpoles may create a margin of error in actual colonization height above the seafloor, results provide information about the relative vertical distributions of these elevated foraminifera. Additionally, because the SEA^{3}s were pushed into the sediment slightly, the base of the plastic does not represent the sediment–water interface (SWI). Observations of the lack of colonization of elevated foraminifera as well as sediment line, and comparisons with photographs were used to identify the SWI for each set of plastic rods. A new SWI position for each flagpole was defined, and all results presented represent this height measurement. As a result of the time required to disassemble SEA^{3}s and examine all 36 elevated flagpoles and mesh, not all cage mesh material was examined in a timely fashion. As a result, this study reports only the SEA^3 plastic and fiberglass flagpoles which were completely picked. A single cube, SEA^{3}9, was completely picked (including the surrounding mesh and top squares), and a detailed comparison of colonization differences between species abundances on various aspects of the SEA^3 materials is reported here (Figs. 4 & 6; Table S1).

Flagpoles composed of varied materials were incorporated to assess the potential for substrate preference. While some plastic materials were apparent from manufacturing details, others required Fourier transform infrared spectroscopy (FTIR) measurements to confirm the compositions (SEA^3 mesh and black flagpole). FTIR measurements made at California State University indicate the mesh of the SEA^{3}s is composed of polyethylene while the black flagpoles are a silicon polymer and polyethylene terephthalate (PET). The results from FTIR are limited in that the compositions were interpreted with the use of a free database (limiting the search capabilities), and while the black flagpole may be a blend of polymers, or a copolymer, it may have a coating which cannot be penetrated by the 2 µm resolution of the instrument; thus, these should be considered preliminary analyses. Flagpoles with clear compositions from the manufactures included fiberglass (main and thick white), acrylonitrile-butadiene-styrene (ABS) (white), polyvinyl chloride (PVC) (grey), and polypropylene (PP) (green). All plastic materials were smooth to microscopically pitted and were examined under a microscope. Foraminifera locations, from flagpole top to the line created by the materials pressing into the sediment were documented to determine the role of height on foraminiferal abundance on different colonizable materials. Flagpoles were attached to the

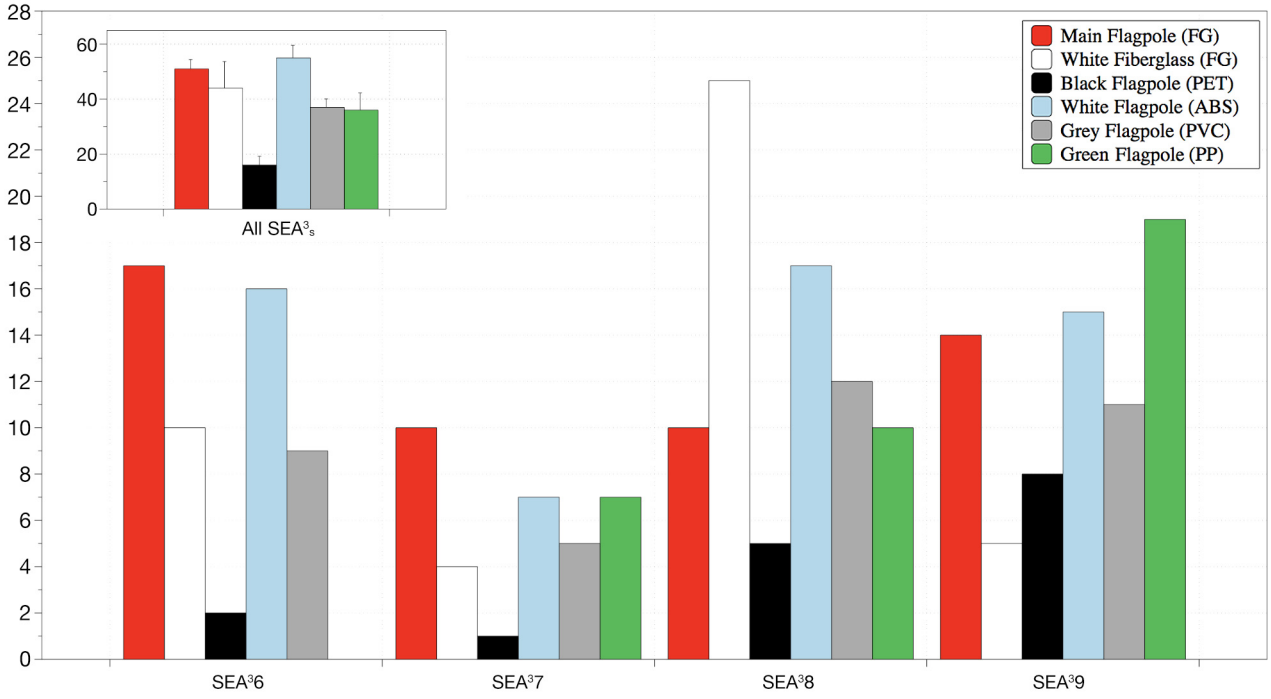

Fig. 4. Number of foraminifera attached to each SEA^3 flagpole. Each flagpole is denoted as a distinct color. The Main Flagpole is composed of fiberglass and has the reflective tape identification flag attached to the top. The White Fiberglass Flagpole is slightly larger in diameter than the Main Flagpole (also made from fiberglass). The Black Flagpole is composed of a silicon polymer and/or polyethylene terephthalate (PET) plastic, while the White Flagpole is made from acrylonitrile-butadiene-styrene (ABS), the Grey Flagpole is made from polyvinyl chloride (PVC), and the Green Flagpole is made from polypropylene (PP)

struts of the cubes from 0 to ~8 cm to facilitate a stable attachment and retention of all flagpoles. These areas were not likely exposed or intact after the disassembly of materials and were not examined.

To ascertain the degree of carbonate saturation at Station M, calcite spars were placed inside the SEA^{3}s in small, porous PVC containers that were suspended a few centimeters above the SWI inside each cube. These containers allowed adequate water flow while also being configured so the calcite spar would not fall out as the SEA^3 was deployed and later picked up by the ROV. The dry weight of these spars was recorded using a 4 decimal place scale before and after the deployment of the experimental substrates. These measurements are archived in Table S2 in the Supplement.

2.4. Sediment cores

Two sediment cores were collected during the recovery of the SEA^{3}s and upon recovery from the ROV and stored in a walk-in refrigerator on board the ship until they were sliced and preserved on shore. Slices were taken at 0.5 and 1 cm intervals, sieved, and wet picked, based on the methods of Corliss & Emerson (1990). Samples were preserved in a 4% formalin solution buffered with Borax and stained with 65 ml of Rose Bengal (1 g l^{-1}) at California State University, Bakersfield. Samples were then transported to Oklahoma State University and washed over 150 and 63 µm sieves until each sample was separated, and foraminifera were wet picked and identified (Figs. 5, 7 & 8; Table S3). After foraminifera counts were established from the >150 µm fraction and were standardized to abundances per 50 cm^3.

2.5. Calculation of foraminiferan distribution

The average living depth (ALD) and average vertical maximum (AVM) were calculated for samples within the sediment and those on elevated substrates, respectively. The larger size fraction facilitated our comparison with attached fauna which almost never fell below the >150 µm size fraction. Juvenile *Cibicidoides wuellerstorfi* attached to an adult from Hydrate Ridge were observed to have a test diameter of at least 150 µm even when they were only composed of 3 chambers (Burkett et al. 2018).

ALD was calculated based on the equation of Jorissen et al. (1995):

$$\mathrm{ALD}x = \sum(n_i \times D_i)/\mathrm{N} \quad (1)$$

where Σ is from all sedimentary intervals examined and is expressed as $i = 0,x$, where x is the lower boundary of the deepest sample, n_i is the number of specimens in interval i, D_i is the midpoint of the interval i, and N is the sum of individuals in all intervals. Therefore, to calculate AVM of foraminifera colonizing elevated substrates, the ALD equation was modified as follows:

$$\mathrm{AVM}x = \sum(n_i \times D_i)/\mathrm{N} \quad (2)$$

where Σ is from all intervals of the elevated substrate examined and is expressed as $i = 0,x$, where x is the highest point of the elevated substrate, n_i is the number of specimens in interval i, D_i is the midpoint of the interval i, and N is the sum of individuals in all intervals.

3. RESULTS

3.1. Elevated materials: abundances vs. composition

Of the 246 foraminifera on flagpoles, *Cibicidoides wuellerstorfi* var. *lobatulus*, *Pyrgoella* sp. (Fig. 5), and an arborescent foraminiferan dominated (Fig. 4; Table S1). No significant differences existed between foraminiferal colonization densities of flagpoles of different compositions with the exception of the black flagpole (Fig. 4). The black flagpole containing silicone polymer and PET material had consistently low colonization numbers (total of 16), while all other flagpoles had higher but similar numbers. One of the white fiberglass flagpoles had the highest total foraminifera count found on any flagpole (25 individuals on SEA^{3}8, Table 1).

3.2. Elevated materials: average vertical maximum

Foraminiferal AVM was determined for all flagpoles and showed no clear patterns when graphed (Fig. 6). AVM was calculated by combining the heights and total foraminifera per plastic type (Eq. 2) and showed consistent maxima between 16 and 19 cm despite a max height of foraminifera on the flagpoles being between 25 and 27 cm above the seafloor. No clear patterns exist for specific foraminifera groups or types of flagpoles being colonized.

3.3. Elevated materials: recruitment and dispersal

Of the 4 SEA^{3}s, SEA^{3}7 had the lowest foraminiferal abundance (37 individuals), while SEA^{3}s 9 and 8 had more than double that abundance (74 and 80 individu-

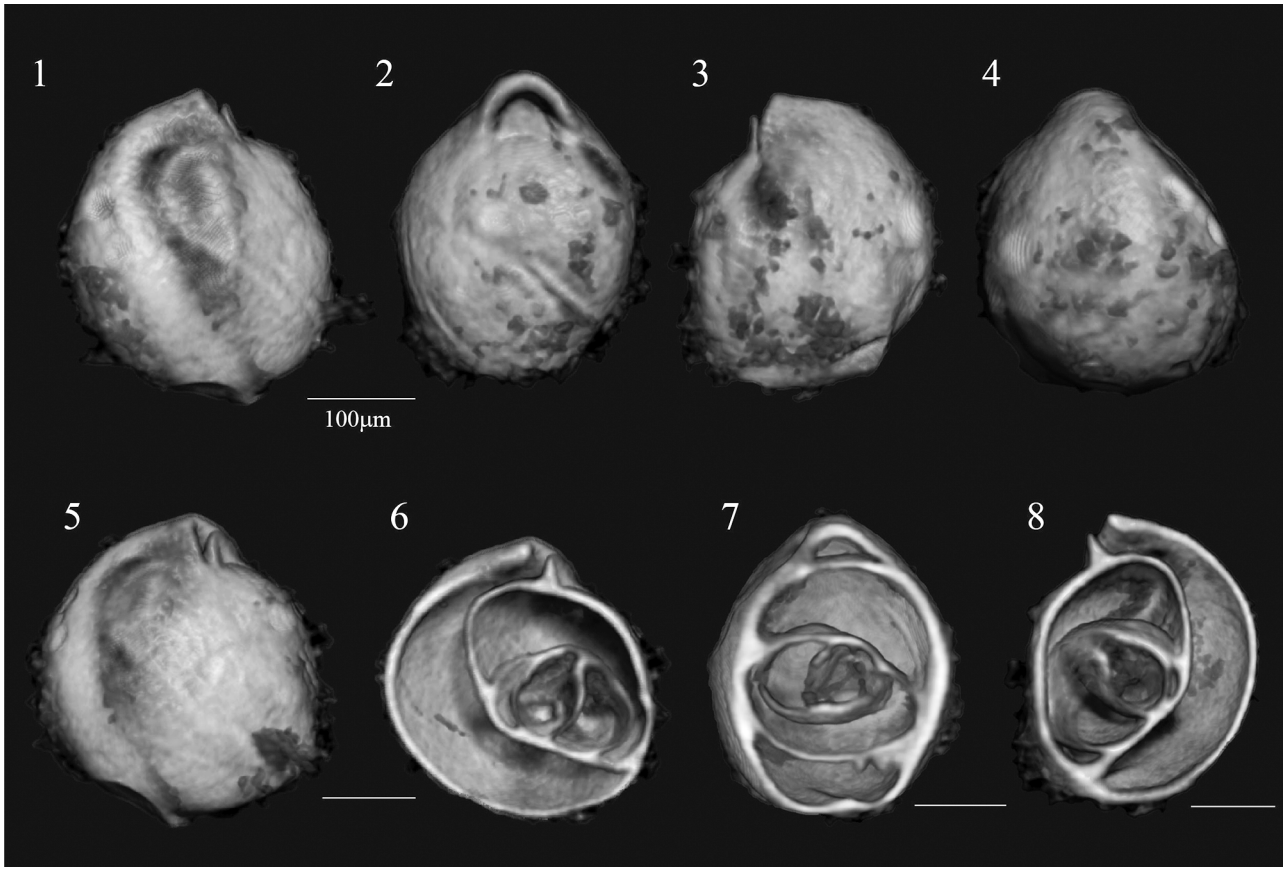

Fig. 5. MicroCT images of a *Pyrgoella* sp. found attached to SEA^{3}s after the 2 yr MBARI deployment. Pictured is 1 of 2 specimens removed from a large cyst attached to the elevated plastic experiment. All scale bars are 100 µm (although not all bars are the same length). (1) Side view, (2) apertural view, (3) side view, (4) back view, (5) side view, before digital sectioning, (6) side view from image 5 showing digital sectioning revealing internal structure, (7) apertural view from image 2 showing digital sectioning revealing internal structure, (8) side view from image 3 showing digital sectioning revealing internal structure

als, respectively). SEA^{3}6 yielded 55 individual foraminifera (Fig. 4, Table 1) SEA^{3}6 had 4 individual foraminifera which were atttached to the very top of flagpoles. These were given a height of 27 cm and are noted in Table S1.

Visual observations and video footage during deployment and recovery clearly show several glass sponges within a few meters of the SEA^{3}s (Fig. 3). The corner of the SEA^3 where the flagpoles were attached is indicated in Fig. 3 as the darkest portion of the inside of the square. With a close proximity to a glass sponge, SEA^{3}7 had the overall lowest foraminiferal abundances. Conversely, SEA^{3}9, which was located furthest from glass sponges, had the second highest foraminiferal abundances of all the SEA^{3}s.

Table 1. Total number of foraminifera per Seafloor Epibenthic Attachment Cube (SEA^3) flagpole by flagpole type, including maximum flagpole height and average vertical maximum (AVM) across all SEA^{3}s. AVM was calculated based on the average living depth (Jorissen et al. 1995). See Eq. (2) in Section 2.5 for details

Total on each	SEA^{3}6	SEA^{3}7	SEA^{3}8	SEA^{3}9	AVM (cm)	Max height (cm)
Main flagpole	18	11	10	14	17	26
White fiberglass	10	6	25	5	16	26
Black flagpole	2	1	5	8	17	25
White flagpole	16	7	18	17	19	27
Grey flagpole	9	5	12	11	18	27
Green flagpole	–	7	10	19	19	27
Total	55	37	80	74	–	–

Table 2. (a) Attachments to all mesh parts of SEA^{3}9. (b) Comparison of the attachment between SEA^3 mesh facing the inside of the SEA^3 vs. the outside

Portion of SEA^{3}9	Average vertical max. (cm)	Max. height (cm)	Total *Cibicidoides wuellerstorfi* var. *lobatulus*	Total *Pyrgoella* sp.	Total arborescent	Total misc. species	Total foraminifera
(a)							
Flag Mesh	5.45	15	24	12	2	0	38
Middle Mesh	3.63	9	8	3	3	1	15
Side 1	5.57	13	13	9	9	0	31
Side 2	5.89	11	21	12	7	1	41
Side 3	5.55	13	14	6	0	0	20
Side 4	5.27	10	19	13	3	0	35
Average all	5.89						Total 180
(b)							
Portion of SEA^{3}9							
Side 2 Inside	6	11	4	2	5	1	12
Side 2 Outside	5.84	11	17	10	2	0	29
Side 4 Inside	2.86	10	12	2	0	0	14
Side 4 Outside	5.55	10	7	11	3	0	21

3.4. Elevated materials: SEA^{3}9 mesh

Mesh sections from SEA^{3}9 were removed and examined for total foraminifera abundances and AVM, and were used to compare abundances between the interior and exterior mesh surfaces of 2 cube sides (Table 2). Total foraminiferal abundances were similar for most mesh areas of SEA^{3}9 with a maximum of 41 (Side 2) and a minimum of 15 (Middle Mesh). The average number of foraminifera per mesh section was 30 individuals, with a total of 180 specimens on all mesh surfaces combined. In comparison, a combined total of 74 specimens were found on the flagpoles of SEA^{3}9, despite the flagpoles having a much smaller surface area than the mesh.

Total abundances of foraminifera collected from SEA^{3}9 are reported in Table 2, and a schematic of the SEA^3 with the labeled parts is provided in Fig. 2a. The Flag Mesh contained a total of 38 foraminifera, consisting of 24 *C. wuellerstorfi* var. *lobatulus* 12 *Pyrgoella* sp., and 2 arborescent foraminifera. The middle mesh inside of the cube had the lowest number of foraminifera with a total of 15 and was made up of 8 *C. wuellerstorfi* var. *lobatulus*, 3 *Pyrgoella* sp., 3 arborescent, and 1 unidentified specimen logged as miscellaneous. Mesh sides from SEA^{3}9 averaged a total of 32 individuals, including an average of 17 *C. wuellerstorfi* var. *lobatulus*, 10 *Pyrgoella* sp., and 5 arborescent foraminifera (Table S3). Mesh side AVM displayed maxima between 5.5 and 7.5 cm. When deployed, SEA^{3}s are pushed slightly into the sediment to ensure stability. Therefore, foraminifera in these AVM would have resided between 3 and 5 cm above the seafloor. Two of the 4 sides of SEA^{3}9 were examined in relation to the orientation of the mesh to the SEA^3 (facing outward vs. inward). Nearly double the number of foraminifera were found on the outside of the mesh versus inside.

3.5. Sediment cores

Agglutinated foraminifera (Figs. 5, 7, & 8) dominated the 2 sediment cores examined, with an average of 158 per 50 cm^3, compared to an average of 77 per 50 cm^3 of calcareous samples, and commonly included large individuals well over 1 mm in length (e.g. Fig. 7, no. 3: *Nodosinum gaussicum*; 12: *Saccorhiza ramosa*; and 13: *Martinottiella variabilis*). Additionally, interesting foraminifera such as *Psammosphaera parva* (Flint, 1899) seem to utilize sponge spicules within their tests (Fig. 7, no. 14) and *Hormosina globulifera* (Brady, 1879) were present (Fig. 7, no. 1). The ALDs of foraminifera varied slightly between cores for both agglutinate (1–1.5 cm in tubecore 1, TC1, and 1.5–2 cm in TC2) and calcareous specimens (2–2.5 in TC1 and 2.5–3 cm in TC2). Total core abundances were relatively low (140–20 per 50 cm^3) but contained *Globobulimina affinis* (Fig. 8, no. 3a,b), which were prevalent deeper within the core and made up the majority of calcareous fauna. In addition to stained foraminifera, these sediment cores contained abundant sponge spicules, pristine planktonic foraminifera, and phytodetrital materials.

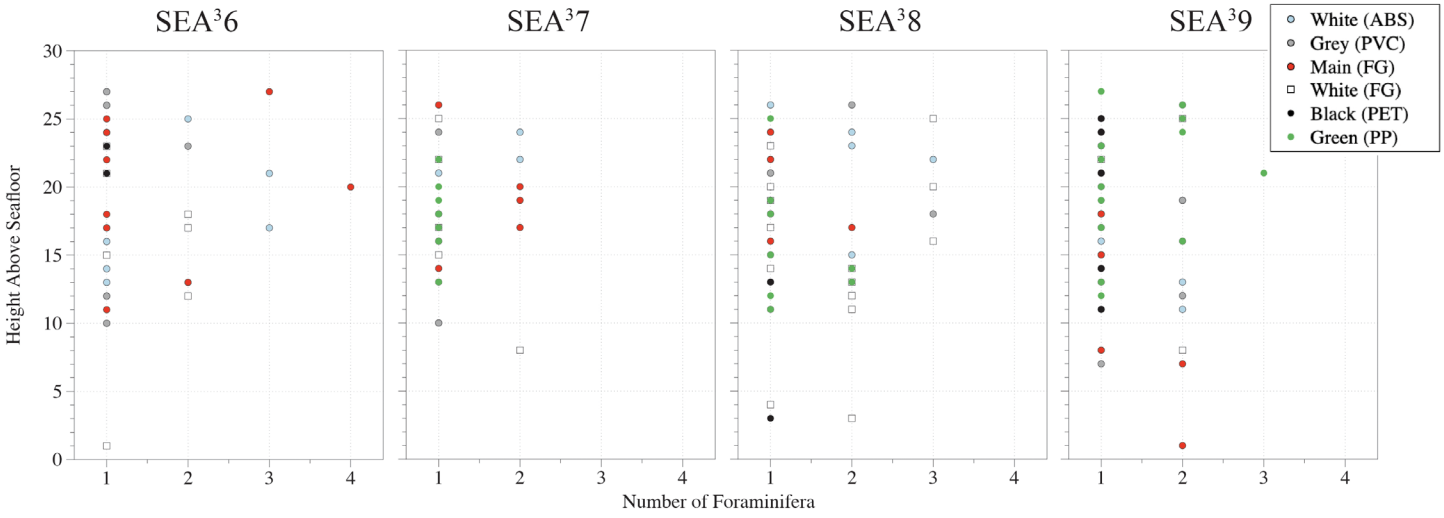

Fig. 6. Vertical attachment height above the sediment–water interface (height above seafloor, cm) was documented for foraminifera on each flagpole as summarized in Table 1. Colonization patterns of the flagpoles do not suggest any preference for colonization at the maximum available height. Whatever the cause, foraminiferal colonization average vertical maximum falls between 16 and 18 cm, while the colonizable material extends up to 27 cm. Flagpoles were attached to the struts of the cubes from 0 cm to about 8 cm

4. DISCUSSION

4.1. Elevation preferences

As was seen in previous 1 yr SEA^3 deployments at Station M (Burkett et al. 2020), there are differences in foraminiferal colonization patterns depending on the composition of the substrate. Results of the 2 yr experimental deployments (this study) are consistent with previous observations in colonization differences with several types of plastics deployed for 1 yr at this site (Burkett et al. 2020). The present study focuses on the distribution pattern of attached epibenthic foraminifera and comparisons with their counterparts in surrounding sediments. Foraminifera seem to avoid the PlastiDip® spray-on plastic covering the steel frame. Flagpoles of different plastics were attached to the struts of the cubes from 0 cm to about 8 cm, and this explains the low colonization of individuals within this range of heights. Some deep-sea foraminiferal studies have focused on differences between colonization and texture of the substrate (e.g. Van Dover et al. 1988). While the present study did not focus on textural differences, the plastics used here would all be described as primarily smooth with occasional micro-pitting or linear features visible under the microscope. Smooth materials (i.e. glass rods) were deployed previously at Station M (Beaulieu 2001a), and these were dominated by attached foraminifera.

After 2 yr on the seafloor, foraminiferal abundances were similar between fiberglass, ABS, PVC, and PP materials, with lower abundances on silicon polymer and/or PET plastics. With the exception of the PlastiDip® covering of the cube and the black PET flagpole, which had relatively low colonization rates, foraminiferal distributions indicate that plastic type is not the driving force in colonization of plastics. The black plastic is likely to have low foraminiferal numbers, as FTIR analyses suggest it contains polyethylene, which has been shown to be highly resistant to degradation (Gao & Sun 2021 and references therein). The unique nature of the microbial communities adapting to these degradation-resistant plastics may result in the relatively low foraminiferal abundances observed on these materials.

Vertical colonization maxima occurred between 16 and 18 cm, which was well below the flagpole tops, many of which extended to 27 cm above the sediment. Assuming that individual foraminifera are mobile when initially recruited to the substrate, it is likely they will move to an optimal attachment site. The idea of deep-sea foraminifera moving to find better conditions for attachment is supported by experimental observations by Wollenburg et al. (2018), who documented that *Cibicidoides mundulus* (Brady, Parker & Jones, 1888) placed in a pressurized experimental chamber moved to the point of maximum flow within the first 24 h and remained attached for the 2 wk experiment. Without *in situ* observations at Station M, we speculate that food acquisition is the rea-

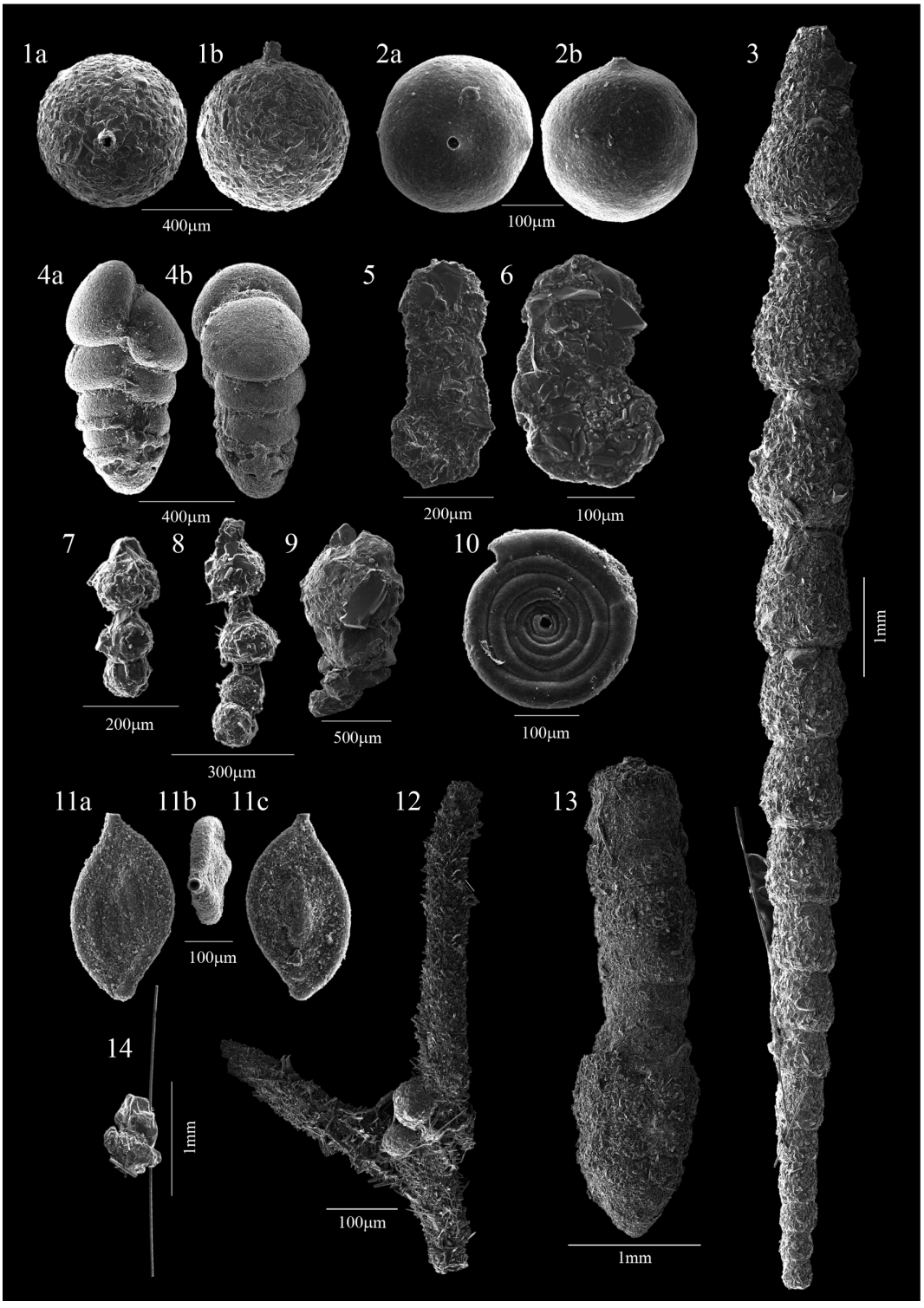

Fig. 7. (1a,b) *Hormosina globulifera* (Brady, 1879) from the sediments of core TC2 in the 0–1 cm interval. (2a,b) *Hormosina* (Brady, 1879) sp. from TC2 at 1–1.5 cm. (3) *Nodosinum gaussicum* (Rhumbler, 1913) from TC1 at 0–1 cm interval. (4a,b) *Karreriella bradyi* (Cushman, 1911) from the TC2 in the 0–1 cm interval. (5) *Eratidus foliaceus* (Brady, 1881) from TC1 in the 1–1.5 cm interval. (6) *Eratidus foliaceus* from TC1 in the 0–1 cm interval. (7) *Hormosinelloides guttifer* (Brady, 1881) from TC2 in the 0–1 cm interval. (8) *H. guttifer* (Brady, 1881) from TC1 in the 0–1 cm interval. (9) *Reophax horridus* (Schwager, 1865) from TC1 in the 1–1.5 cm interval. (10) *Glomospira gordialis* (Jones & Parker, 1860) from TC2 in the 1.5–2 cm interval. (11a–c) *Spirosigmoilina tenuis* (Čžjžek, 1848) from TC1 in the 0–1 cm interval. (12) *Saccorhiza ramosa* (Brady, 1879) from TC1 in the 0–1 cm interval. (13) *Martinottiella variabilis* (Schwager, 1866) from TC1 in the 0–1 cm interval. (14) *Psammosphaera parva* (Flint, 1899) with sponge spicules from TC1 in the 1–1.5 cm interval

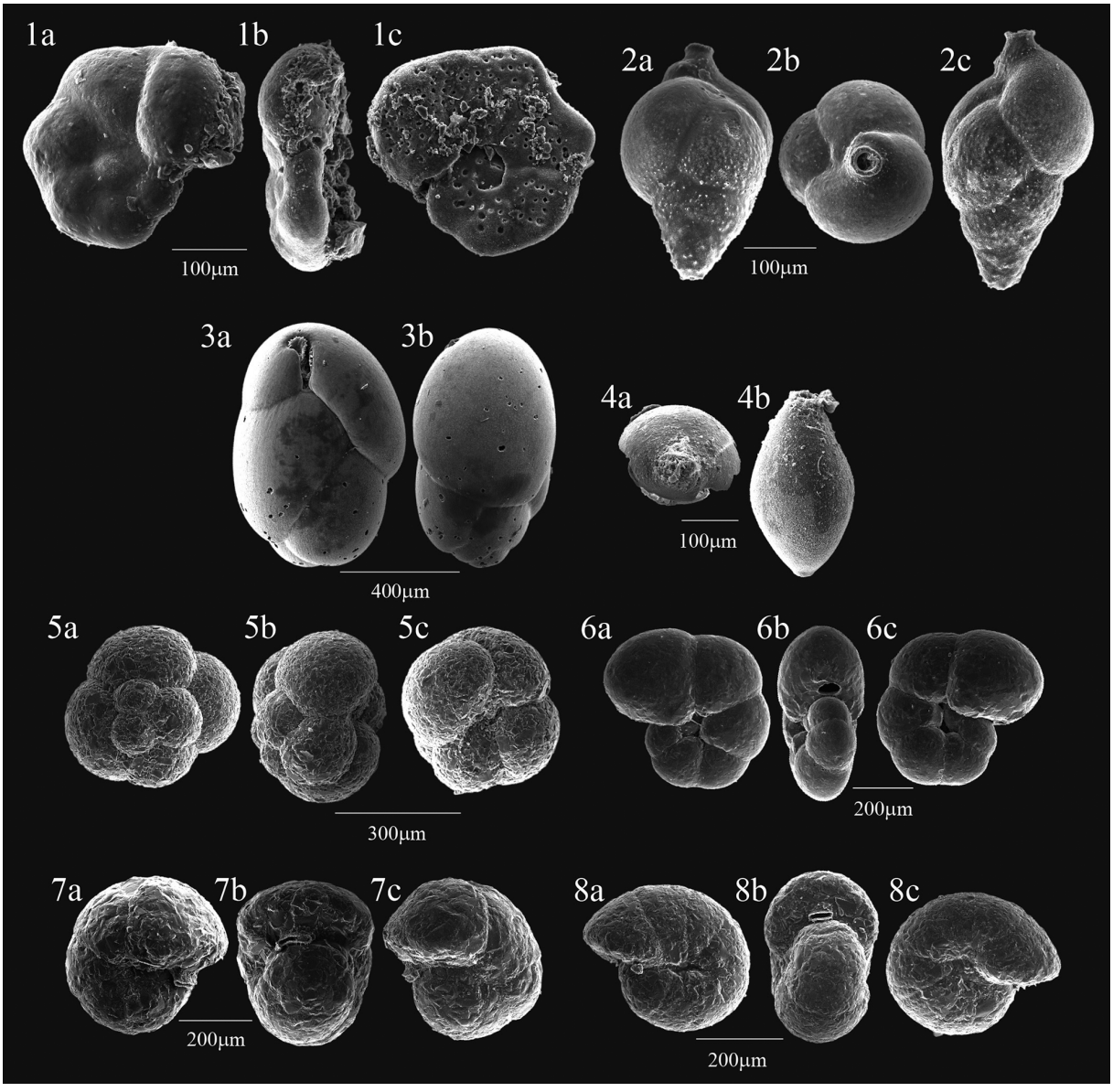

Fig. 8. Scanning electron microscopy images of foraminifera from the sediments at Station M. (1a–c) *Cibicidoides wuellerstorfi* var. *lobatulus* (Schwager, 1866). This is the only specimen collected from the sediment found in TC1 in the 0–1 cm interval. (2a–c) *Uvigerina* (d'Orbigny, 1826) sp. from the sediments of TC1 at 0–1 cm. (3a,b) *Globobulimina affinis* (d'Orbigny, 1839) common in deeper portions of TC1 at 2–2.5 cm. (4a,b) *Lagena* (Walker & Jacob, 1798) spp., broken in apertural view (4a) from TC2 at 0–1 cm. (5a–c) *Paratrochammina challengeri* (Brönnimann & Whittaker, 1988) from TC2 at 0–1 cm. (6a–c) *Haplophragmoides* (Cushman, 1910) sp. from TC1 in the 2.5–3 cm interval. (7a–c) *Cribrostomoides* (Cushman, 1910) sp. from TC1 at 1–1.5 cm. (8a–c) *Cribrostomoides subglobosus* from TC1 at 1–1.5 cm

son 16–18 cm is preferred for attachment. Avoidance of seafloor conditions could be achieved at greater heights on the flagpoles and has been proposed as a potential driver in the colonization of elevated substrates, especially at methane seeps (e.g. Lutze & Thiel 1989, Bernhard 2000, Bernhard et al. 2006, 2010, Sen Gupta et al. 2007). The gentle flow above the SWI at Station M may hint at the height of attachment preferences we see in benthic foraminifera at this location (e.g. 2.19 cm s^{-1} at 2.5 m and 1.34–2.75 cm s^{-1} at ~10 cm above the SWI in Beaulieu & Baldwin 1998, Beaulieu 2001a). Oxygen and temperature conditions of bottom waters in this area are likely similar within the first few centimeters above the SWI, suggesting it is likely that food acquisition is the motivation for foraminifera attaching at the observed range of heights on substrates (as suggested by Lutze & Thiel 1989). The occurrence of *C. wuellerstorfi* on elevated

substrates has been previously documented (e.g. Lutze & Thiel 1989, Burkett et al. 2020), and specimens of *C. wuellerstorfi* have been found with test shapes which conform to the substrate to which they were attached, including SEA^{3}s, tubeworms, and rocks (Burkett et al. 2015, 2018, 2020). Growing in place with morphological conformation to the substrate suggests that these individuals are sacrificing mobility, presumably as a result of having found an acceptable location which may be optimal for the acquisition of sufficient food.

Plastics have become a ubiquitous feature on the ocean floor, reaching even the most remote deep-sea locations and persisting for significant periods of time (e.g. Krause et al. 2020). To date, there is a paucity of studies examining impacts of large plastic pollution on deep-sea populations. Microbial communities associated with seafloor plastics may serve as an important food source for benthic communities, but trophic relationships of foraminifera and microbial communities on plastic debris remain speculative. Recent studies suggest microbes and other deep-sea fauna may have developed specializations for the consumption of plastics (e.g. Agostini et al. 2021). It is likely that much of the colonization and influence of plastics is dependent on the composition and presence of toxic or trace elemental materials (e.g. Henderson et al. 2018, Sarker et al. 2020). Since at least some foraminifera are known to consume bacteria (e.g. Bernhard & Bowser 1992, Goldstein & Corliss 1994), it is possible that the abundance and type of bacteria on plastics and other substrates as well as the acquisition of suspended food particles may influence colonization preferences and distribution patterns. From the results presented in this study, it remains difficult to ascertain the most important variable(s) driving colonization densities, orientations, or preferences in attached benthic foraminifera, and what influence, if any, other attached flora and fauna play in deep-sea foraminiferal ecology.

Consistency in the species found on surrounding biogenic substrates in the past and the repeated findings of these species on previous SEA^3 experimental deployments suggest they are highly adapted to an elevated epibenthic microhabitat. Based on the fact that only 1 *Cibicidoides wuellerstorfi* var. *lobatulus* was observed in sediment cores collected in the region (reported as 3 specimens when extrapolated to numbers per 50 cm^3, Table S3), it seems unlikely that this species spends any part of its life cycle within the sediments at this site. This single specimen may have been living within the sediment or could have fallen from a nearby elevated substrate (Fig. 8, no. 1a–c). Although cores were not collected from directly under the SEA^{3}s, given (1) the existence of source populations on nearby glass sponges (Beaulieu 2001b), (2) the ubiquity of elevated biogenic substrates in the area, and (3) the lack of significant dissolution of the calcite spars, it would seem reasonable to find at least some *C. wuellerstorfi* tests in the sediment. The presence of significant *C. wuellerstorfi* var. *lobatulus* populations dominating elevated materials and not sediments has implications for our understanding of the dispersal and genetic exchange mechanisms of benthic foraminifera populations, taphonomic analyses, and the impact of hard substrates on living and fossil assemblages of foraminifera. Further ecologic and taphonomic study is needed to examine the preservation potential of these elevated epibenthics, as their presence in the sediments of Station M is drastically underrepresented when compared with substrates. The fate of abundant calcareous foraminifera on elevated substrates once they die is the key to understanding the dissimilarity in calcareous foraminiferal populations between sediments and elevated substrates. Calcareous foraminifera examined on SEA^{3}s adhered their tests to the substrate using organic and/or mineral outgrowths in the same manner as reported by Dubicka et al. (2015) for *C. lobatulus*. The tests leave a ring of residue on the substrate when pried loose, which is sometimes observed when picking, suggesting that once they die, calcareous benthic foraminifera would fall from the elevated substrates and onto the sediments as their attachment material degrades. A lack of appropriate examination of coarse substrates at cold-water coral sites may account for these abundance differences (e.g. Fentimen et al. 2020), implying that when it comes to estimates of the contribution of (especially calcareous) benthic foraminifera to global biogeochemical cycles (e.g. carbon cycle), best estimates may be much lower than the actual number and biomass of living calcifying specimens. The availability of hard substrates (including sediment grains) that were available to living foraminiferal populations in ancient oceans is likely to significantly influence the number of fossil epibenthics in micropaleontological samples from deep-sea cores.

4.2. Elevated vs. sedimentary populations

C. wuellerstorfi var. *lobatulus* (called *C. lobatulus* by Beaulieu 2001b) colonized glass sponge stalks, the most abundant elevated substrate in the region

with a density of ~1118 stalks ha^{-1} (Beaulieu 2001b; see Burkett et al. 2020 for details). Previous Station M studies documented the presence (although not abundances) and dominance of agglutinated foraminifera in the sediments (e.g. Drazen et al. 1998, Jeffreys et al. 2013). The results of our study demonstrate no similarity in dominant foraminiferal populations from SEA^{3}s and sediment cores, suggesting source populations for the colonization of the SEA^{3}s are not coming from the sediments, but from the surrounding biogenic substrates (e.g. glass sponges). If this is the case, it is tempting to consider epibenthic foraminiferal populations colonizing these substrates much like in island migration (e.g. Island Biogeography Theory). Within 6 mo to 1 yr in the deep Pacific Ocean, previously deployed SEA^{3}s have been colonized by hundreds of *C. wuellerstorfi* (Burkett et al. 2018, 2020), but this species is also commonly found living on/within surface sediments in many regions (e.g. Venturelli et al. 2018). Our results are consistent with previous findings correlating larger sediment grain sizes with larger numbers of *C. wuellerstorfi* (e.g. Venturelli et al. 2018), suggesting that attachment surfaces of grains at the SWI or other elevated hard substrates influence the number of epibenthic taxa in the habitat. These studies demonstrate that elevated substrates serve as the preferred habitats for *C. wuellerstorfi.* Distances from one elevated substrate, either those naturally occurring — such as the abundant glass sponges at Station M — or plastic debris could serve as source populations through reproductive material and propagules, which are likely capable of dispersing great distances and possibly even persisting until ideal conditions occur (e.g. Alve & Goldstein 2003, 2010, 2014). At Station M, glass sponge structures provide hard substrates for attachment, and likely function as biogenic habitat islands for *C. wuellerstorfi.* While our experimental design was not set up to test the idea of SEA^{3}s acting as biogeographic oases of attachment in a deep-sea habitat dominated by soft sediment, the results suggest a great deal about colonization and recruitment patterns, providing a glimpse of the ecological impact of introducing artificial microhabitat islands of plastic to the deep sea.

Future deployments of SEA^{3}s may be useful in determining sources of epibenthic foraminiferal populations and providing further insights into deep-sea propagule dispersion. At present, there is no material to document mechanisms of foraminifera on elevated substrate biogeography, but additional SEA^{3}s are currently being processed to test recruitment patterns. With currently available information, it seems highly unlikely that juvenile *C. wuellerstorfi* var. *lobatulus*, or their propagules, spend any part of their life in the sediments at Station M, as only a single *C. wuellerstorfi* var. *lobatulus* was observed in sediments there. It stands to reason that abundant adult populations of *C. wuellerstorfi* var. *lobatulus* on hard substrates would generate reproductive materials. Deep-sea foraminifera are thought to alternate from sexual to asexual reproduction generationally, which can be observed in the proloculus size of the test of the individual (Goldstein 1999). *C. wuellerstorfi* var. *lobatulus* produced via sexual reproduction have a small proloculus (microspheric), while those formed from asexual reproduction have a larger proloculus (megalospheric). Although this has yet to be definitively documented in deep-sea benthic foraminifera, releasing gametes into the water column would be an effective means of dispersal while facilitating the colonization of new elevated substrates by genetically varied offspring. Not only would the release of gametes facilitate genetic exchange between the plastic substrate populations, but it could drive the colonization of substrates, such as sessile epifaunal macrofauna or plastic debris, which may serve as hard substrate habitat islands. In contrast, asexual reproduction by adult founders might maintain the populations on newly colonized elevated substrates, given that genetically identical offspring are likely to be successful in the same environment as their parent. Potential evidence of asexual reproduction has been observed on SEA^3 substrates, where microspheric *C. wuellerstorfi* adults have been observed with apparent megalospheric juveniles (Burkett et al. 2015, 2018, 2020), and further work is ongoing to document numbers of megalospheric and microspheric individuals on SEA^3 materials at various lengths of deployments.

The lack of weight changes of calcite spar samples in SEA^{3}s after 2 yr on the seafloor confirms that calcite is not dissolving at a height of about 4 cm above the SWI at Station M (Table S2). Although the 4000 m water depth is near the average ocean CCD and below the average lysocline in the Pacific, this location is not corrosive to calcium carbonate in bottom waters slightly above the sediment. The presence of abundant, pristine planktonic foraminifera in the sediments is further evidence that this is the case for this location. Fresh surface material in the form of radiolaria and planktonic foraminifera in surface sediments is indicative of the connectivity of surface productivity to the seafloor, and undoubtedly influencing bottom water pH (Fig. S2). Interestingly, *C. mundulus* placed in pH <7.4 in the laboratory formed

organic and strongly agglutinated sediment cysts (Wollenburg et al. 2018). Foraminifera attached to SEA^{3}s commonly have soft cysts which can easily be removed with a paint brush and seem to be mostly composed of fine mud and organic material (Burkett et al. 2020). It is possible that organic coverings over foraminifera may prevent or decrease the number of tests falling from the elevated substrates and into the sediment, resulting in their absence in the sediments. One would expect that when a foraminiferan dies, the organic material covering the test as well as the material attaching it to the substrate would degrade and the test would eventually end up in the sediment, which was not observed in this study. It has been suggested that foraminiferal specimens which spend more time at the SWI are more likely to be physically and/or chemically destroyed, resulting in reduced preservation of epifaunal taxa compared to infaunal species (e.g. Loubere et al. 1993). The attached tests of foraminifera exposed above the SWI may be more susceptible to destruction by mobile macrofauna and/or organisms mining calcium carbonate. None of these proposed mechanisms have been documented at Station M, but all could account for the discrepancy between the elevated and infaunal foraminifera populations and the potential bias in the fossil record. This bias may be especially true for elevated epibenthic foraminifera, causing a significant underestimate of these populations in assessments based on fossil and living assemblages. Core-top assemblages have been commonly used to evaluate living populations, epifaunal/infaunal ratios of fossil assemblages are employed to assess paleoenvironmental conditions, and the distribution and ecological tolerances of taxa have been based on core-top abundances of species. An understanding of the taphonomic biases between fossil and living epibenthic taxa in the deep sea is critical for assessments of both modern and fossil populations and their habitats. The results of this study suggest that epibenthic deep-sea populations are not uncommon at 4000 m water depth in the Pacific Ocean where biogenic substrates are also present, and that these abundant calcareous foraminifera thrive on elevated substrates in an environment where they are not likely to be common in the sediment record. Future work is needed to investigate whether living epifaunal calcareous foraminifera are also abundant in other abyssal locations.

While the results of this study indicate a disconnect between elevated and infaunal populations, it leaves many uncertainties surrounding what environmental conditions are required for epibenthic species to occur in the abundances seen in some fossil records. Could these large populations be the result of proximity to elevated biogenic substrates that are not evident in the fossil record? The observation of higher foraminiferal diversities near areas where cold-water corals are common (e.g. Schönfeld et al. 2011, Fentimen et al. 2020, Stalder et al. 2021) also suggests that there is an influence of hard substrates on foraminiferal assemblages. If attached foraminifera are falling off elevated substrates, what percentage end up being preserved in the sediments? If they are not falling off the substrates, what happens to them? Uncertainties such as these could be addressed through additional SEA^3 experimentation.

5. CONCLUSION

A lack of significant differences in foraminiferal colonization of different plastics, except for the PlastiDip® covering of the cube and the black PET flagpole, suggest plastic types are not the driving force in colonization of plastics. The black plastic is likely to have low foraminiferal numbers as FTIR analyses suggest it contains polyethylene, which is avoided by bacteria. Plastic toxicity and/or the lack of bacterial food may result in lower numbers of other organisms as well. This has not been well documented in plastic debris in marine environments and should be studied further. More work is needed to identify differences and similarities between substrates that may influence colonization by foraminifera, including texture, composition, and bacterial populations. Results from this study confirm that many types of plastics serve as hard substrates for colonization by deep-sea foraminifera and that in providing suitable substrates for attachment, which may persist for extended periods of time, plastic debris in deep-sea environments dominated by soft sediments has the potential to alter the composition of local ecosystems. Vertical maxima at ~10 cm below the top of the flagpoles suggests that the impetus for colonization of the flagpoles is not driven by finding the highest point from the seafloor. It may be the result of adequate water flow for food acquisition. Additional observations of *Cibicidoides wuellerstorfi* var. *lobatulus* with tests reflecting the morphology of the substrate to which they are attached strongly suggest they sacrifice mobility for the sake of stability. Comparisons of sediment and attached SEA^3 populations at Station M demonstrate no significant overlap in species presence between the 2 environments, highlighting the potential fossil bias against

epifaunal species. Major ecological differences (e.g. absence of colonizing epibenthics in core materials) and reports of similar foraminiferal populations on glass sponges in the area (Beaulieu 2001a,b) suggest that Island Theory of Biogeography can serve as a framework to examine colonization patterns and genetic exchange between biogenic (glass sponges) and built material substrates (SEA^{3}s) at Station M. These results indicate that plastic pollution functions similarly to other elevated structures on the deep-sea floor.

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