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Initial Microbial Colonizers of Microplastics in the North Atlantic Ocean

Presented to the Faculty of the Biological Sciences Department

in Partial Fulfillment of the Requirements for a Bachelor of Arts

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Project Advisors:

Dr. Linda Amaral-Zettler, Dr. Erik Zettler, and Dr. Marianne Moore

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Abstract

The negative impacts of plastics on marine life such as fish, seabirds, marine mammals, and sea turtles that ingest plastic and become entangled in plastic are well documented. Less is known, however, about the potential for plastics to transport organisms, such as invasive species, potential pathogens, and harmful algae. Rafting organisms that become attached to plastics in the marine environment comprise a new community, known as the Plastisphere. Our results revealed that initial colonizers of the Plastisphere in coastal and pelagic regions of the North Atlantic Ocean include a variety of heterotrophic bacteria (e.g., *Pseudoalteromonas, Alteromonas, Marinobacter hydrocarbonoclasticus*) that are known to form biofilms and metabolize hydrocarbons. We also found that all eleven genera of our initial colonizers (e.g. *Pseudoalteromonas*, *Alteromonas,* and *Vibrio*) and even some specific initial colonizer oligotypes within these genera are present in much later stages of succession in the Plastisphere. Therefore, these initial colonizers remain a part of the community long after introduction into the environment, and may therefore continue to play a role in the ecological impacts and ultimate fate of plastic in the marine environment. This study offers the first glimpse of the early microbial colonizers of the Plastisphere in coastal and pelagic ecosystems.

Introduction

Plastic products have revolutionized the way we live our lives, from single-use products used in medicine and food service, to durable structures used in the construction of our cars and houses. However, at the end of the useful life of these products, many are not disposed of properly and are discarded into the environment, both on land and into the oceans. An estimated 4.8 to 12.7 million metric tons of plastic waste enter the ocean each year (Jambeck et al. 2015),

equivalent in weight to adding two Empire State Buildings worth of plastic to the ocean every week. In fact, plastic pollution has become the primary form of marine debris (Derraik 2002). With plastic production increasing at an average rate of 9% since 1950 (GESAMP 2015), the amount of plastic marine debris (PMD) will probably continue to increase unless waste management methods are improved.

Once PMD enters the marine environment, it can be broken down into microplastics, which are fragments less than five millimeters in length (Derraik 2002; GESAMP, 2015; Arthur et al. 2009; Fig. 1). This can occur by a variety of environmental mechanisms, including mechanical breakdown, photodegradation,[1] and thermooxidative degradation[2] in the marine environment (Andrady, 2011). The fate of PMD in the ocean also varies depending on the type of polymer from which a plastic, also known as a resin, is made.

Figure 1. Microplastics found in a neuston sampling net from a Sargasso Sea surface sample collected from a half hour tow, hundreds of kilometers from shore, in April 2015.

[1] Photodegradation: the breakdown of plastics by UV radiation, often through oxidation reactions.
[2] Thermooxidative degradation: the slow breakdown of plastics at moderate temperatures through oxidation.

The three most frequently occurring resins found floating in the ocean, and examined in this study, are polyethylene, polypropylene, and expanded polystyrene (Hidalgo-Ruz et al. 2012). These petroleum based plastics have been produced for over 100 years, and can take hundreds of years to degrade (Oberbeckmann et al. 2014). An alternative to petroleum-based plastics is biobased plastics, such as polylactic acid (PLA) and polyhydroxyalkanoate (PHA). By definition, bioplastics or biobased materials are organic materials that use new carbon sources, such as corn or sugar cane, as opposed to old fossil carbon (GESAMP, 2010, Narayan 2005). Organic materials are defined here according to International Union of Pure and Applied Chemistry (IUPAC), and are materials containing carbon-based compounds in which the carbons are attached to other carbons, oxygen, hydrogen, or other elements in a chain, ring, or other three dimensional structure (Narayan 2005). Therefore, in order to categorize a plastic as biobased, the material must be organic and contain recently fixed new carbon from biological sources.

Some of the advantages of using biobased over petroleum-based plastics include that they are made from renewable materials, and they may be more readily biodegradable (Lörcks 1998; Verlinden et al. 2007). Although bioplastics might biodegrade in commercial composting environments, these high nutrient, high temperature conditions are very different from the marine environment (GESAMP, 2010). In fact, in tests lasting the duration of several weeks to two months, bioplastics like PLA did not biodegrade at all in seawater, and PHA only partially disintegrated in seawater (CIWMB, 2007). Therefore, though biobased plastics may be a better alternative, both petroleum and biobased plastics raise environmental pollution concerns due to their persistence if improperly disposed.

Plastic pollution is a concern throughout the world's ocean due to their persistence and slow degradation, but also because ocean currents transport plastics readily to distant locations.

Though discharge of plastics from ships at sea is now prohibited (International Maritime Organization, 2011), some loss of plastics at sea still occurs (Jambeck et al. 2015). The vast majority of PMD, an estimated 80%, originates from land-based sources (Jambeck et al. 2015), which can then be transported by currents. Plastic pollution has now been documented in a wide variety of marine environments: along the shorelines of even the most remote islands, floating in the open ocean, in the seabed of continental shelves, in the deep sea, and in Arctic sea ice (Barnes et al. 2002; Woodall et al. 2014; Obbard et al. 2014).

In the open ocean, particularly high concentrations of microplastics accumulate in mid-ocean gyres (Cole et al. 2011). Since mid-ocean gyres, such as the Sargasso Sea included in this study, are substrate-poor pelagic ecosystems, marine microplastics provide a previously unavailable habitat for surface-dwelling or attached microorganisms and subsequent colonizers. The accumulation of nutrients on plastic surfaces (Zobell and Anderson 1936; Zobell 1943) facilitates the colonization of plastics, as does the hydrophobicity of plastics. One hypothesis behind this is that since bacteria and microplastics are both hydrophobic and nonpolar, they have a tendency to aggregate in water (Rosenberg and Kjelleberg 1986; refer to Appendix Fig. A. for more detail). Increasingly prevalent PMD in the ocean could therefore alter the distribution of marine microorganisms such as microbes, because solid surfaces are believed to play a significant role in the spatial distribution of bacteria in the ocean (ZoBell and Anderson 1936).

Impacts of PMD

The negative impacts of PMD on organisms such as marine mammals, sea turtles, seabirds, and fish that ingest plastic and become entangled in plastic are well documented (Carpenter et al. 1972; Derraik 2002; Laist 1987, 1997; Baird and Hooker, 2000; Denuncio et al.

2011). While many studies have focused on the negative impacts of PMD on organisms that can ingest and become entangled in plastic debris, less is known about other effects of plastic pollution (Derraik 2002).

One of these lesser-known effects of plastics is their ability to provide a new means of dispersal. For example, plastics have been found to transport persistent organic pollutants (POPs) and persistent bioaccumulative toxins such as polychlorobiphenyls and *polycyclic aromatic hydrocarbons* (GESAMP, 2010; Rochman et al. 2013). Since plastic substrates accumulate nutrients (ZoBell and Anderson 1936; Zobell 1943) and provide a substrate in an otherwise substrate-poor, open ocean environment, plastics can also act as a fomite (an object capable of carrying pathogens and transferring them from one individual to another) for a variety of organisms. The community that survives on plastics includes bacteria, protists, and animals (Carpenter et al. 1972; Reisser et al. 2014; Masó et al. 2007; Minchin 1996; Gregory 1978). Of particular concern is the finding that plastics can transport potential pathogens (Zettler et al. 2013), harmful algal species (Masó et al. 2007), and invasive species (Barnes 2002).

Organisms are able to attach to this artificial substrate and can be transported over long distances, since plastic is longer-lived in the environment than most natural substrates (Ilgenfritz 1975). Natural floating debris such as pumice and wood have always provided a means of transportation in the oceans for organisms that are able to hitch a ride (Barnes, 2002). However, the recent explosion of anthropogenic debris in the past couple decades, largely in the form of plastics, can more than double the rafting opportunities for organisms (Barnes, 2002). Relatively little is known about the impacts of this plastic-based biological community, known as the Plastisphere (Zettler et al. 2013).

The Microbial Community of the Plastisphere

The Plastisphere contains a diverse community of colonizers (Zettler et al. 2013; Oberbeckmann et al. 2014), with microbes being the earliest colonizers (Dang and Lovell 2000; Webb et al. 2009). Microbes are important in marine ecosystems in general because they drive primary production, nutrient cycling, and other ecosystem services. The microbes in the Plastisphere, however, are strikingly distinct from microbes found in ambient seawater (Zettler et al. 2013; Oberbeckmann et al. 2014). The microbial colonizers of plastics may have significant effects on marine ecosystem health and function, and the microbes that colonize may vary depending on the type of plastic, season, and location (Oberbeckmann et al. 2014, Amaral-Zettler et al. 2015). Understanding the composition and ecological significance of marine microbial communities on plastics is critical for determining how plastic pollution may alter marine and coastal environments.

The microbial community of the Plastisphere forms the initial biofilm, an organic, matrix-enclosed layer of microbial cells that coats plastics in the marine environment. The matrix of biofilms, consisting mainly of polysaccharides, can support a diverse community of microbes (Jones et al. 1969). Biofilms may offer protection to the microbial community from biocides or antibiotics, and may allow for interactions between members of the biofilm that promote survival and the maintenance of homeostasis (Fletcher 1991). Bacteria, which are key players in biofilm formation along with other microbes such as diatoms (Wahl 1989), often attach to solid surfaces using pili or exude a stalk-like holdfast during attachment, which may aid in the accumulation of nutrients near the attached bacteria (ZoBell 1943). Other bacteria use alternative methods, such as the extension of polymeric fibrils to the substrate surface (Marshall et al. 1971). In some cases, bacteria adhered to substrates have no attachment structures at all, and they are held in

place only by an extracellular, polysaccharide matrix layer (Jones et al. 1969).

Attachment of planktonic bacteria in seawater to the surface of PMD is the first step in biofilm formation (Dang and Lovell 2000). Then, primary colonizers increase in abundance on the surface and a biofilm begins to form as the bacteria grow, reproduce, modify the surface of the substrate, and produce a polymer matrix (Dang and Lovell 2000). Other planktonic microbes are then able to interact with the bacteria in the biofilm (Dang and Lovell 2000). The biofilm reaches a mature stage in succession as the community continues to interact competitively and synergistically, allowing for further colonizers to establish themselves in the community (Lee et al. 2008; Dang and Lovell 2002; Zobell and Anderson, 1936).

Biofilm formation is likely the beginning of life in the Plastisphere. The existence of a biofilm may also be a requirement for the attachment of larger organisms, such as diatoms and benthic invertebrates (Wood 1967; Wahl 1989; Cooksey and Wigglesworth-Cooksey 1995; Reisser et al. 2014; personal observation, Fig. 1). A variety of macrofauna, such barnacles (Fig. 1) and other suspension feeders, have been found to settle on floating marine substrates during later stages of succession (Wood 1967; Cooksey and Wigglesworth-Cooksey 1995; Thiel 2003). As previously discussed, we know that the Plastisphere can contain a diverse community of bacterial and eukaryotic organisms. Succession, however, the manner in which the species composition of this community changes over time, is largely unknown for this unique marine ecosystem, particularly in the earliest stages of colonization.

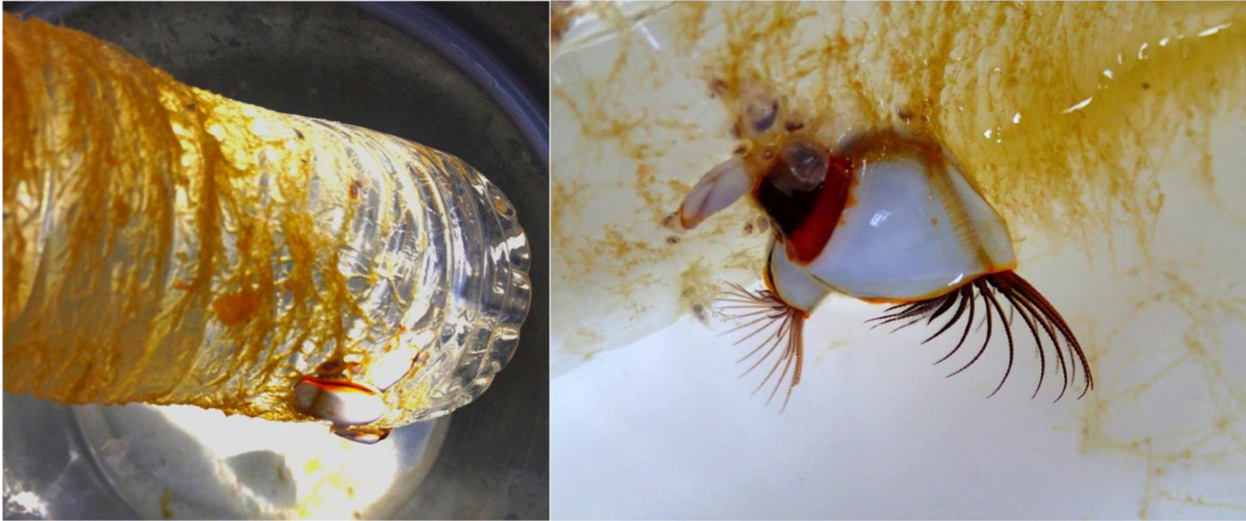


Figure 2. This plastic bottle (left), floating at the ocean surface in the North Atlantic Ocean and collected in a neuston sampling net in April 2015, had relatively large animals attached to it such as barnacles (right). The initial colonizers of plastics may lay the foundation for subsequent colonizers of the Plastisphere.

The earliest colonizers of any uninhabited environment are called pioneer or early successional species. Examples of uninhabited environments occurring after a disturbance include the slope of a volcano after an eruption, the side of a rock in the intertidal zone after wave action or ice has dislodged attached species, or the surfaces of clean plastics that have been deposited into the ocean. The pioneers of these inhospitable environments are typically able to withstand high physiological stress (Cain et al. 1956), and they are often capable of transforming their environment in a way that makes it more habitable for themselves and, subsequently, for other organisms (Cain et al. 1956). The concept of succession can also be applied to microbial communities. It is important to note, however, that later stages of succession in biofilm communities might not be driven by competition for resources or niches as is typical in macro-organismal communities. This is because later developing, more specialized microbial populations in biofilms are able to utilize mutually exclusive carbon sources, including carbohydrates such as glucose and cellulose, and derivatives of aromatic polymers such as

benzoate (Jackson et al. 2001). The pattern of succession in the earliest stages of microbial colonization appears to be somewhat random and difficult to predict precisely (Jackson et al. 2001). Our limited understanding of early colonization is more developed in a few specialized environments, such as in the formation of dental plaque (Kolenbrander 2000). In this environment, for example, colonization begins with a group of gram-positive organisms, primarily streptococci, and succession culminates with the colonization of gram-negative, anaerobic bacteria such as *Porphyromonas gingivalis* (Xie et al. 2000). We still know very little, however, about the role of the initial colonizers in the marine environment.

Understanding the microbial communities that are the initial colonizers on microplastics is important for understanding the succession of life in the Plastisphere (Zettler et al. 2013). Previous studies have examined both microbial colonizers of plastics retrieved from the ocean and microbial colonizers of clean plastics exposed to marine environments for several weeks or months (Webb et al. 2009; Zettler et al. 2013; Oberbeckmann et al. 2014). Although some studies examined marine and estuarine microbial colonization of plastics during early stages of colonization, such as 1-3 weeks (Lobelle and Cunliffe 2011) or 24-72 hours (Dang and Lovell 2000), no studies have documented colonization during even shorter time periods in open ocean ecosystems.

To place our study on early colonization in the greater context of succession in the Plastisphere, we compared our results with theoretical models of ecological succession. The three general models of ecosystem succession, according to Connell and Slatyer's foundational 1977 paper, are the facilitation, tolerance, and inhibition models. In the facilitation model, early colonizers arrive to the uninhabited site and modify it in a way that make the environment less suitable for subsequent early colonizers to arrive, and help late succession organisms to enter the

community. In the tolerance model, the early colonizers still alter the environment to make it more difficult for other early colonizers to arrive, but their presence has little or no impact on colonization by late stage successors. In ecosystems that follow an inhibition model, early colonizers modify their environment in ways that inhibit subsequent colonization of both early and late stage colonizers, and only their elimination permits succession to continue. In all three models initial colonizers are eliminated as the ecosystem reaches its late succession stage (Connell and Slatyer 1977), due to active emigration or predation (Thiel 2003). Characterizing the initial colonizers and their presence or absence throughout later stages of succession in the Plastisphere will contribute to a better understanding of how this community develops over time.

Applications

In addition to contributing to our fundamental knowledge of the stages of succession in the Plastisphere, understanding the initial colonizers of virgin plastics is relevant for practical reasons. For example, these "plastiphilic" microbes may play an important role in the biodegradation[3] and ultimate fate of PMD on which they live (Ozaki et al. 2007; Lin et al. 2009; Reisser et al. 2014), since bacteria can attack plastic polymers biochemically (Zobell 1943; Mergaert et al. 1996; Shrivastav et al. 2011). Also, the microbial community that lives on plastics could affect fish and other marine organisms that ingest these plastics. Therefore, understanding the role of microbes in degrading plastics is essential to determining the fate of plastic in the ocean, for bioremediation projects, and for understanding the ecological impact of PMD. This topic is particularly relevant in the marine environment given that the oceans are the final destination of a significant portion of the world's hydrocarbon pollution (Atlas 1981). This

[3] Biodegradation: the chemical breakdown of organic molecules by microorganisms such as bacteria.

research is also useful to a variety of fields outside the realm of environmental science, such as in medicine, the military, and industry.[4]

Virgin plastics are plastic particles that do not yet have microorganisms attached and are essentially uninhabited. Plastisphere communities in the earliest stages of succession can develop on uncolonized PMD from fishing and shipping vessels (Cole et al. 2011), or from marine organisms that ingest and egest these virgin microplastics (Thompson et al. 2004; Cole et al. 2011), essentially "resetting" the microbial community (Amaral-Zettler et al. 2015). Plastic resin pellets, small granules of plastic <5 mm in diameter and generally disk or cylindrical in shape, are the raw material used for melting down in the manufacturing of many plastic products (Ogata et al. 2009). During both transportation and manufacturing, these resin pellets can be lost to the marine environment as virgin plastics (EPA, 1992; Ogata et al. 2009). Therefore, examining the colonization of virgin plastics in the marine environment is not only a useful tool for studying succession experimentally, but it also models an environment that is in fact found in the oceans due to plastic pollution.

Current Study Overview

Given our limited understanding of the initial colonizers and the microbial succession that takes place in the Plastisphere, the goal of this study was to characterize the earliest stages of

[4] Medicine: biofilms that form on devices such as catheters and heart implants with plastic components could contribute to opportunistic infections (Rosenberg and Kjelleberg, 1986; Chauhan et al. 2016; Gristina et al. 1988). Military: biofilms affect the fouling and hydrodynamics of vessel surfaces and other submerged equipment (Zobell 1943). Industry: if the microbial community adhering to the surfaces of ships, oilrigs, pipelines, or other submerged structures is found to biodegrade or negatively alter those surfaces, proactive measures might be taken to prevent the attachment of those microbes.

life in this community. In addition to describing some of the initial colonizers, which were previously unknown, we expected to find that the initial microbial colonizers would be distinct based on location, and to a lesser extent, based on the resin type of the substrate to which they attached. This is based on previous studies that have found that microbial communities differ based on location but not as strongly on resin type (Pommier et al. 2007; Carson et al. 2014, Zettler et al. 2013; Oberbeckmann et al. 2014; Amaral-Zettler et al. 2015).

We then investigated whether the initial colonizers persisted in later stages of succession in the Plastisphere. We did this by comparing the microbes found in our first colonizer experiments with the Plastisphere community found in more established Plastisphere communities characterized in previous studies. Though examining the model by which the Plastisphere community reaches late stage succession is beyond the scope of this study, each of the three major models of succession predicts that early colonizers drop out of the community in later stages (Connell and Slatyer 1977). Therefore, we expected that early colonizers would no longer be present in the Plastisphere after a few weeks, and that they would certainly be absent after several months of exposure to seawater. Though it is unlikely for real communities to follow any one model of succession perfectly, it is generally true that early successional species give way to later stage successors because as the environment changes over time, different species are favored due to the suitability of their life history traits in any given environment (McCook 1994).

In terms of life history traits, we expected that many of the first colonizers of the Plastisphere would have certain adaptations such as the ability to form biofilms, to metabolize hydrocarbons, and to be phototrophs. The ability to form biofilms would be useful to these early colonizers because biofilms offer protection from grazing predators, provide increased access to

nutrients, and facilitate antibiotic resistance through enhanced horizontal gene transfer (Chavez-Dozal et al. 2013; Fux et al. 2005; Donlan and Costerton, 2002; Rodríguez-Martínez and Pascual 2006). Bacteria that could metabolize hydrocarbons would be well suited to the early Plastisphere ecosystem because they could utilize the hydrocarbons of the resin as a source of carbon at a stage when organic material has not yet accumulated on the plastic surface. Alternatively, phototrophs that attach to microplastics floating near the sea surface would be able to utilize sunlight to create their own carbon sources from dissolved carbon dioxide. Later successional stage Plastisphere communities include autotrophs (e.g. cyanobacteria) and heterotrophs (Zettler et al. 2013), but phototrophs are particularly well adapted to be pioneers of newly settled communities as they generate new organic carbon in carbon-poor environments (Frey et al. 2013). We examined the presence or absence of these life history traits among pioneers of the Plastisphere once these initial colonizers were identified through our genetic analyses.

Methods

Seawater Sample Collection

We performed experiments in three different marine environments: the oligotrophic Sargasso Sea (SS),[5] the coastal waters of Woods Hole (WH), MA,[6] and in the semi-enclosed shelf waters of the Gulf of Maine (GOM)[7] (Fig. 3; Appendix, Table A). All glass and plasticware was cleaned by rinsing with 0.5-1 N hydrochloric acid, 6 rinses of deionized water (DI), 70% ethanol, and finally rinsed with 0.22 μm-filtered seawater to minimize contamination. We collected water samples from the ocean surface using a cleaned bucket. Approximately 1 L of

[5] Conducted by Amalia Alberini, Lena Goss, Caroline Graham, and Helena McMonagle
[6] Conducted by Linda-Amaral-Zettler, Erik Zettler, and Helena McMonagle
[7] Conducted by Erik Zettler

collected seawater was then transferred to cleaned polypropylene sealed containers that were 1.0 to 2.6 L in volume. We measured temperature and salinity at the top of the hour closest to our sampling time using a SBE-45 thermosalinograph (SeaBird Electronics, Bellevue, WA USA) for open ocean samples, and a YSI 85 hand-held instrument in Woods Hole and the Gulf of Maine. Chlorophyll and phosphate concentrations were determined at the closest surface station using standard methods (Parsons et al. 1984). Chlorophyll was measured by vacuum filtering 250-500 mL of seawater through a 0.45 μm membrane filter (GN-6 Metricel, Pall Corporation, USA), extracting in 96% acetone, and determining the concentration of chlorophyll-*a* using a calibrated fluorometer (Turner Designs 10-AU) with non-acidification optical filters. Phosphate concentrations were determined using the molybdenum blue chemistry and measuring the absorbance of the resulting solution at 885 nm using a OOI USB-2000 spectrometer to determine the phosphate concentration compared to known standards.

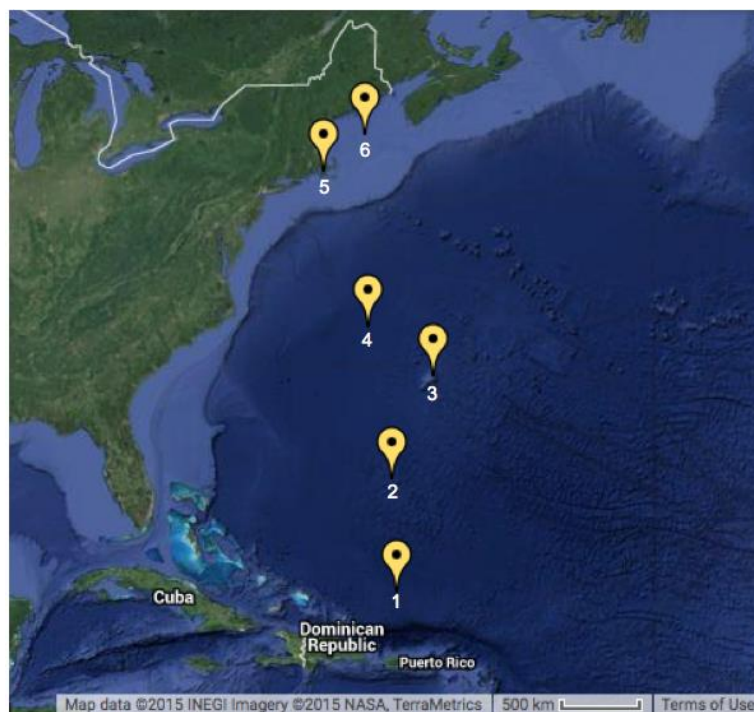


Figure 3. Map of seawater collection sites. Seawater for experiments was collected at six sites total. From south to north, sample sites 1, 2, 3, and 4 are in the Sargasso Sea (SS), site 5 is in Woods Hole (WH), and site 6 is in the Gulf of Maine (GOM).

Marine Microbial Colonizer Trials

For all experiments, we prepared 20-25 pellets of different resins types: polyethylene (Kristi Galus, Dow Chemical Company), polystyrene (purchased commercially-unknown manufacturer), polypropylene (purchased commercially-unknown manufacturer), polylactic acid (Ramani Narayan, Michigan State University), and polyhydroxyalkanoate (Scott Tuten, Meredian Holdings Group), into each vial. Polyhydroxyalkanoate was not used in the Sargasso Sea experiment (Sites 1-4). We then cleaned the pellets by rinsing them with 70% ethanol and 0.2 µm filtered seawater. Next, we deposited the pellets into each of the five separate containers of collected seawater. Incubation start times were staggered by 10-15 minutes for each resin so that all could be harvested after 2 hours. Pellets of each plastic resin were incubated in the polypropylene plastic containers containing seawater at room temperature (20-25°C) for two hours in the light with a gentle mixing after approximately 1.5 hours. For experiments at sea (SS and GOM), this additional mixing was not performed because the boats were in constant motion. For the SS experiment only, pellets were incubated and streaked[8] after time periods of both 30 minutes and two hours.

At the end of the incubation period, we gently removed six pellets from each incubation container using cleaned forceps (70% ethanol, air dried) and placed the pellets into 0.2 µm filtered seawater to remove any microbes that were not attached to the pellet surface. Pellets were then streaked onto agar. For the SS experiment, pellets were streaked onto seawater agar (SWA: 75% seawater, 25% DI-iH_2O, 20 g/L agar) and tryptone agar (Try: 75% seawater, 25% DI-H_2O, 1 g/L tryptone, 15 g/L agar). For the WH and GOM experiments, pellets were streaked onto tryptone and CHROMagar Vibrio (CaV) agar (CaV: CHROMagar™ Vibrio, VB910,

[8] Streaking is a technique used to isolate colonies from a culture, in this case by using sterile forceps to slide microplastics along agar surface to transfer microbes onto the agar.

Manufacturer, City, State). Samples were monitored for 72 hours and individual colonies were picked at 24, 48, or 72 hours and restreaked to confirm they were derived from a single cell and to increase biomass for DNA extraction and sequencing. Colonies were systematically selected for re-streaking[9] (and, ultimately, for DNA sequencing) based on four criteria. First, only colonies that were not touching or overgrown by a neighboring colony were re-streaked. Then, colonies were chosen based on the time period of growth before they became visible (24, 48, and 72 hours), assuming that colonies with different growth rates would likely differ genetically. Next, whenever possible, colonies with a morphology distinct from other selected colonies were chosen, again in an attempt to increase the diversity of the colonies sequenced. Finally, when there were still multiple colonies of the same morphotype eligible for re-streaking and DNA sequencing, the largest colonies were selected, as these would presumably produce sufficient biomass for DNA sequencing. All colonies selected for re-streaking and sequencing were also streaked onto multi-well seawater agar plates to archive for future work. We extracted DNA from individual cultures established for each plastic resin on each type of agar (TSW and CaV) using a Qiagen DNeasy Blood and Tissue kit (Valencia, CA). Following extraction, we measured the concentrations of DNA for each sample using a Nanodrop (SS experiment: ND-1000 UV/Vis Spectrophotometer, Thermo-Fisher/Life Technologies, Wilmington, DE; WH and GOM experiments: NanoDrop 2000/2000c, Thermo-Fisher/Life Technologies, Wilmington, DE) to ensure quality and quantity for amplification. We then amplified a portion of the 16S rRNA gene through a Polymerase Chain Reaction with forward primer at 10 μM 518F: 5'CCAGCAGCYGCGGTAAN- 3' and reverse primer at 10 μM 1046R: 5'CGACRRCCATGCANCACCT- 3'(Marteinsson et al. 2013) with an expected DNA amplicon

[9] Re-streaking was used after streaking microbes from original plastic pellets to transfer individual colonies onto separate culture plates to grow up their biomass and isolate them for DNA extraction.

size of 528 bp). For the SS experiment, we used gel electrophoresis (E-Gel precast agarose gel, 1.2% Agarose, Thermo-Fisher/Life Technologies, Wilmington, DE) and we compared the resulting band size to a molecular ladder (Quick-load 100bp DNA ladder, New England Biolabs Inc.) to ensure that the amplification of our target gene was successful. Following successful amplification, we performed a PCR product cleanup using a Qiagen QiaQuick Spin PCR Purification kit (Valencia, CA), used a Nanodrop to ensure quantity and quality of the cleaned DNA, and sequenced the product using Sanger capillary sequencing. The DNA products from the SS experiment were sent to Eurofins (Huntsville, AL) for sequencing. Products from the WH and GOM experiments were sequenced at the W. M. Keck Ecological and Evolutionary Genetics Facility in the Josephine Bay Paul Center for Comparative Molecular Biology and Evolution at the Marine Biological Laboratory in Woods Hole, MA on an ABI 3730XL (Applied Biosystems, Foster City, CA) capillary sequencer using the BigDye protocol with either 518F and 1046R or just 518F primers according to the manufacturer's instructions.

DNA Extraction, PCR, and DNA Sequencing

For all experiments, we performed a phylogenetic analysis using sequenced DNA products to determine the identity and genetic relationships among colonies sampled. We searched for the closest relatives of our sequences using the *Basic Local Alignment Search Tool* (BLAST) algorithm (Altschul et al. 1990). Using the phylogenetic tree building software MEGA (Tamura et al. 2013), we then constructed maximum likelihood trees based on the Kimura 2-parameter model (Kimura 1980). This allowed us to examine the phylogeny among the initial colonizers that we found, and to visualize any grouping among initial colonizers by location or

by resin type (for example, to see whether a certain genus grew primarily on a certain resin). A bootstrap consensus tree was inferred from 100 replicates. Bootstrap values show the percent likelihood that the groups in the given phylogenetic tree are as shown given each of the 100 replicate calculations of the tree (Felsenstein 1985). Branches, corresponding to groups with less than 70% of bootstrap values, were collapsed. The Maximum Likelihood approach was used to estimate pairwise distances of a matrix. The Neighbor-Joining method was used to obtain the initial tree from this matrix. To model evolutionary rate differences among sites, a discrete Gamma distribution was used (5 categories, +G, parameter = 0.6064). A total of 96 initial colonizer nucleotide sequences were used to construct the phylogenetic tree. Identical sequences from the same resin type and collected from the same location were removed to simplify the phylogenetic tree. Positions containing gaps and missing data were excluded from the analysis. The final alignment used to built the tree consisted of 445 nucleotide sites.

Bioinformatics and Genetic Analyses

To compare identities of members of the initial microbial communities with those from environmentally collected and incubated microplastics, we used the Visual Analysis of Microbial Population Structures (VAMPS: vamps.mbl.edu) website and tools hosted at the Josephine Bay Paul Center at Marine Biological Laboratory (Huse et al. 2014). First, we ran our genetic sequences in a BLAST analysis. The BLAST identified the microbes isolated from the three initial colonizer experiments to the highest taxon possible. Then, we compared these microbes to those found on plastics that contained microbial communities in later stages of succession.

These later stage Plastisphere communities came from two different sets of samples. The first set of samples consisted of 43 samples from the Woods Hole Dock Time Series (WHDTS), a time series experiment conducted at the Marine Biological Laboratory dock in Woods Hole,

MA. Macroplastics (>5 mm) made of polyethylene, polypropylene, and polystyrene were exposed to coastal seawater for known time periods ranging from 2 weeks to 4 months (WHDTS incubated macroplastics; unpublished data). Sampling and sequencing of the Plastisphere community was conducted at weekly intervals for the first four weeks and monthly intervals thereafter by Linda Amaral-Zettler and Erik Zettler.

The second set consisted of samples of the community attached to 31 different microplastic (<5 mm) particles collected using a neuston net towed at the surface of the North Atlantic Ocean at least 15 km from shore. The microbial communities on these microplastics had been exposed to seawater for an undetermined time period (North Atlantic environmentally collected microplastics; Zettler et al. 2013 for methods). Microplastics were collected from May to June in 2012 and 2013. To our knowledge, there is no reliable way of determining the amount of time that environmentally-collected microplastics have been incubating in seawater. We estimate, however, that these plastics had been immersed in seawater for several weeks, if not several months or years.

From both the environmentally collected microplastics from the North Atlantic Ocean and the incubated plastics from the WHDTS, microbial communities were characterized using next generation sequencing, and these genetic data were compared in an oligotyping analysis with first colonizers. Oligotype analysis is a supervised computational method that can be used to discover the diversity of closely related but distinct bacteria in environmental samples, which we did using this case from the environmentally collected microplastics and WHDTS incubated plastics. Through our oligotype analysis, we determined whether initial colonizer genera occurred in any of the 31 environmentally collected samples, or the 43 WHDTS samples. Then, after determining whether initial colonizer genera were present in later stages of succession, as

represented through these two different sample sets, we used VAMPS to determine the four most abundant oligotypes per genus occurring among all 31 environmentally collected samples, and then among all 43 WHDTS samples. We used the variable regions, generated by VAMPS, that distinguish between these four most abundant oligotypes to search for these oligotypes among the sequences of our initial colonizers. This method allowed us to determine whether the specific initial colonizer oligotypes remained in later stage succession Plastisphere communities.

Results

Identification and Distribution Patterns of Initial Colonizers

We found eleven identifiable genera and various unknown initial colonizers of virgin microplastics within the first 2 hours of incubation in seawater. Initial colonizers, in decreasing order of abundance of sequenced colonies per genus, were as follows: *Pseudoalteromonas, Vibrio, Halomonas, Idiomarina, Alteromonas, Tenacibaculum, Marinobacter, Micrococcus, Enterovibrio, Marixanthomonas,* and *Polaribacter* (Fig. 4). Only one of these genera, *Pseudoalteromonas*, was found in all three locations (SS, GOM, and WHD), and seven genera were isolated in one location only (Table 1). For genera wherein we isolated more than one colony, almost all attached to more than one resin, with several genera attaching to four of the five resins used in our experiments (Table 2). There was more clustering by location (Fig. 5a) than by resin (Fig. 5b) among close relatives (bacteria that share common ancestry). A complete list of the colonies sequenced at each general location (GOM, WHD, and SS) and their identities as determined by the BLAST analysis can be found in the Appendix, Tables B-D).

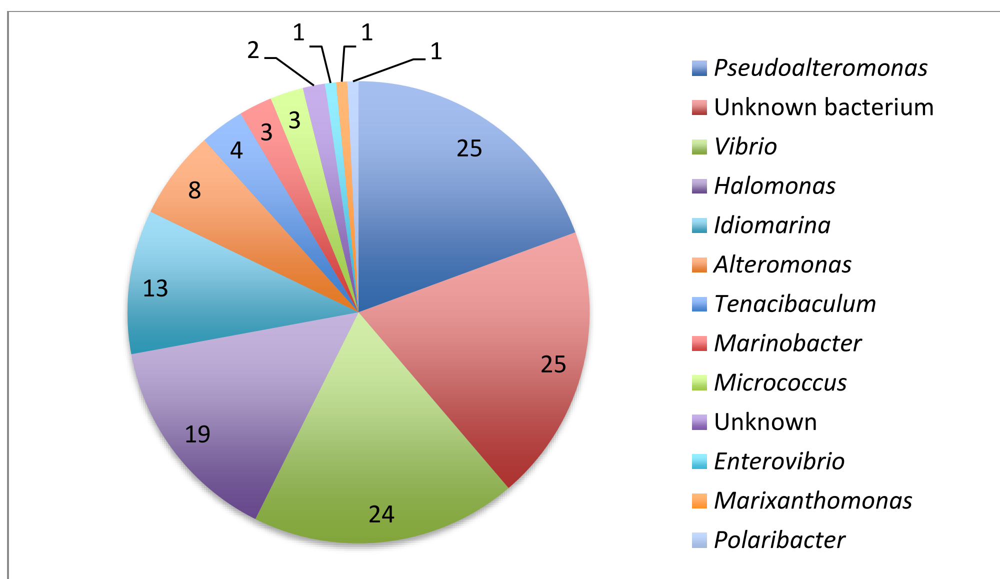


Figure 4. Identity of microbes initially colonizing virgin microplastics at all locations. A total of 129 colonies were sequenced and identified using a BLAST analysis. Numbers bordering the pie diagram represent the number of colonies identified per genus. Colonies that could not be identified were categorized as "Unknown bacterium" if known to be bacteria, or "Unknown" if the BLAST analysis could not determine their identity.

Table 1. Initial colonizers categorized by location in which they occurred on incubated microplastic pellets. Genera represented by only a single sequence are indicated with a (1).

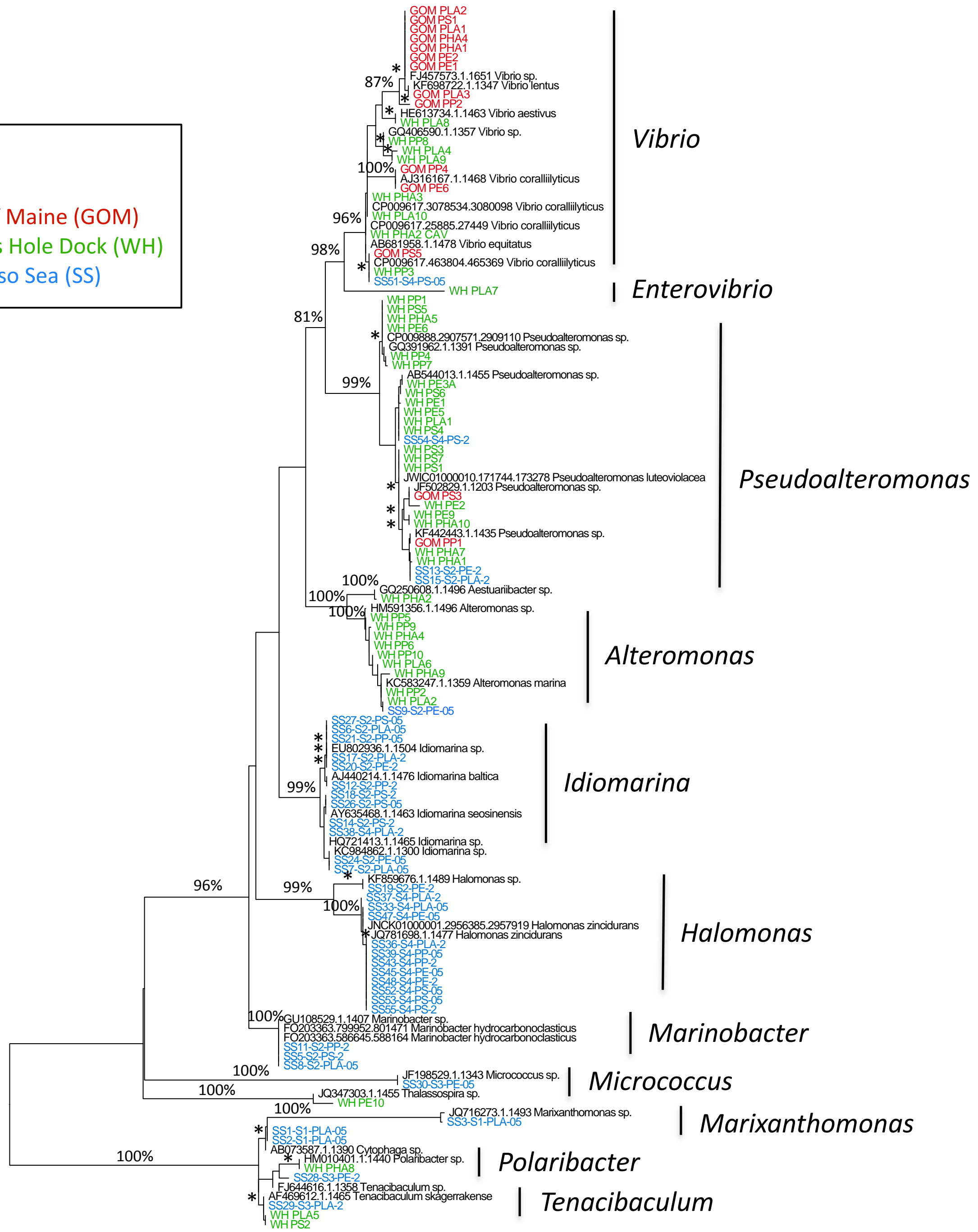
Genus	Number of locations in genus occurred	Woods Hole Dock (WHD)	Gulf of Maine (GOM)	Sargasso Sea (SS)
Pseudoalteromonas	3	X	X	X
Vibrio	2	X	X	
Alteromonas	2	X		X
Tenacibaculum	2	X		X
Polaribacter (1)	1	X		
Enterovibrio (1)	1	X		
Idiomarina	1			X
Halomonas	1			X
Marinobacter	1			X
Micrococcus	1			X
Marixanthomonas (1)	1			X

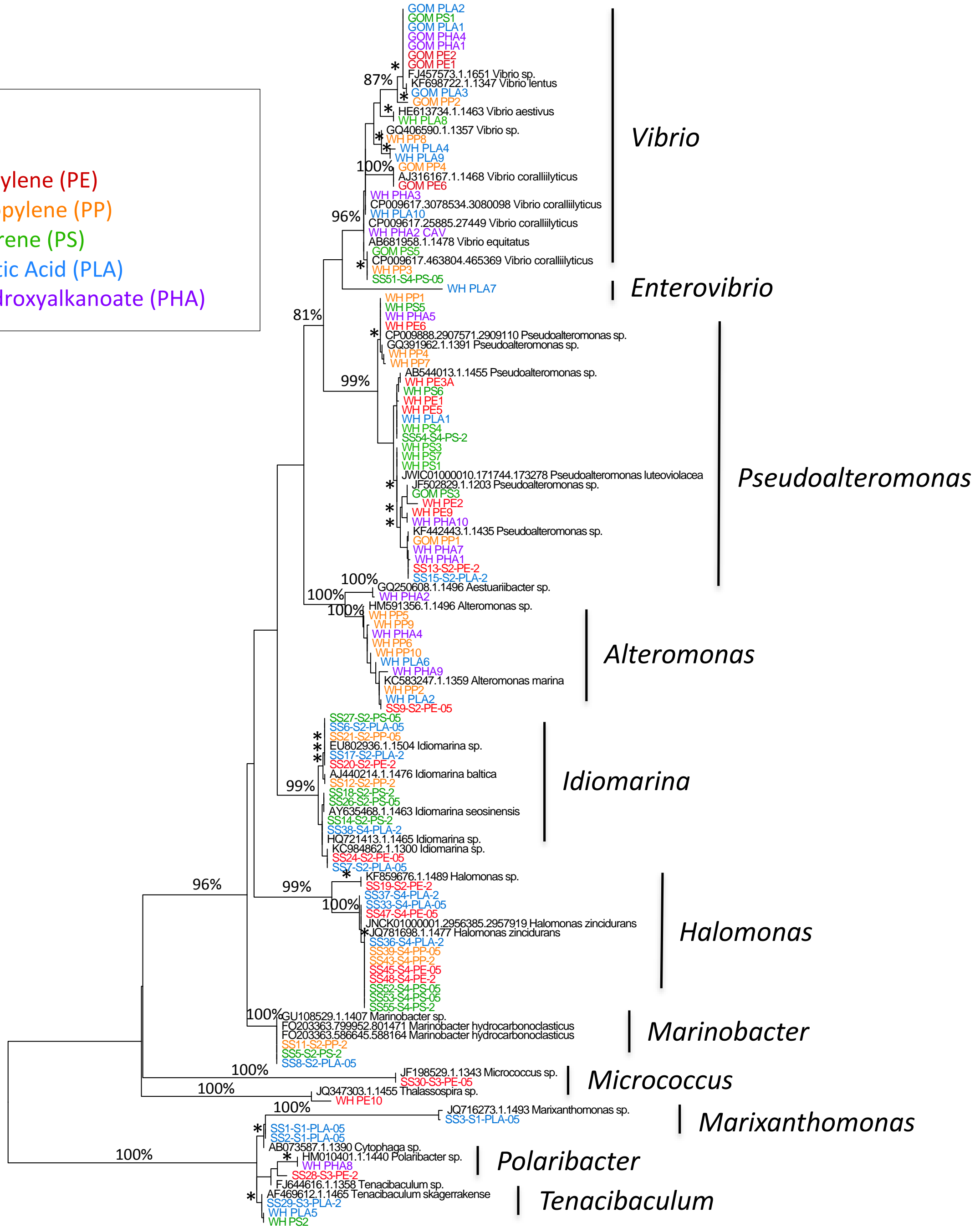
Table 2. Initial colonizers categorized by the resin where they were isolated. Genera that occurred among identified initial colonizers are marked with an "X". *PHA was used in GOM and WH experiments only. Genera represented by only a single sequence are indicated with a (1).

Genus	Number of resins to which genus attached	Polyethylene (PE)	Poly-propylene (PP)	Polystyrene (PS)	Polylactic Acid (PLA)	Polyhydroxy-alkanoate* (PHA)
Pseudoalteromonas	5	X	X	X	X	X
Vibrio	5	X	X	X	X	X
Idiomarina	4	X	X	X	X	
Halomonas	4	X	X	X	X	
Alteromonas	4	X	X		X	X
Marinobacter	3		X	X	X	
Tenacibaculum	2			X	X	
Micrococcus	1	X				
Marixanthomonas (1)	1				X	
Enterovibrio (1)	1				X	
Polaribacter (1)	1					X

Most of the genera occurred in only one or two of the experimental locations (Table 1). Only one genus occurred in all three general locations (note that the samples found in the SS were collected in four different sites, Fig. 1), and seven genera occurred in only one general location (WHD or SS). The BLAST analysis results (Table 1) did not identify any bacteria of the genus *Vibrio*, one of the most common genera found, from the four SS sites (Table 1), although they are known to occur there attached to plastic debris (Zettler et al. 2013). Phylogenetic analysis, however, suggests that there may have been one isolated *Vibrio* colony at a SS site (Figure 5a). When we categorized the initial colonizers by resin type (Table 2), we found that most of the genera attached to at least two different resins. Of the four genera attaching to only one resin, three had only one colony isolated and identified belonging to that genus. The exception was *Micrococcus,* which was found only on PE though there were three colonies belonging to this genus.

Figure 5. Phylogenetic trees show clustering patterns by location (a) and by resin type (b). Neighbor-joining consensus trees for 100 bootstrap replicates were constructed from the sequenced 16S rRNA gene of each initial colonizer colony using the Maximum Likelihood method. Tree building was based on the Kimura 2-parameter model (Kimura 1980) and a discrete Gamma distribution to model evolutionary rate differences among sites (+G, parameter = 0.5696). The bootstrap values, indicating the percentage of trees in which the associated taxa clustered together, out of 100 randomized replicates, are shown next to the branches. Bootstrap values greater than 70% are shown either numerically or with an asterisk next to the corresponding branch. An asterisk represents a bootstrap value of 70%-100%. Both trees are drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 131 nucleotide sequences, which included both initial colonizers and at least one closest neighbor per genus. All positions containing gaps and missing data were eliminated. The alignment included a total of 445 nucleotide sites. Evolutionary analyses were conducted in MEGA6 (Tamura et al. 2013). Sample IDs include sample location (GOM, WH, or SS), resin type (PE, PP, PS, PLA, or PHA), site (for SS samples only, in reference to Fig. 1) and incubation time (for SS samples only, 0.5 or 2 hours).


a)
Legend
Gulf of Maine (GOM)
Woods Hole Dock (WH)
Sargasso Sea (SS)
GOM PLA2
GOM PS1
GOM PLA1
GOM PHA4
GOM PHA1
GOM PE2
GOM PE1
FJ457573.1.1651 Vibrio sp.
KF698722.1.1347 Vibrio lentus
GOM PLA3
GOM PP2
HE613734.1.1463 Vibrio aestivus
WH PLA8
GQ406590.1.1357 Vibrio sp.
WH PP8
WH PLA4
WH PLA9
GOM PP4
AJ316167.1.1468 Vibrio coralliilyticus
GOM PE6
WH PHA3
CP009617.3078534.3080098 Vibrio coralliilyticus
WH PLA10
CP009617.25885.27449 Vibrio coralliilyticus
WH PHA2 CAV
AB681958.1.1478 Vibrio equitatus
GOM PS5
CP009617.463804.465369 Vibrio coralliilyticus
WH PP3
SS51-S4-PS-05
WH PLA7
Vibrio
Enterovibrio
87%
100%
96%
98%
81%
WH PP1
WH PS5
WH PHA5
WH PE6
CP009888.2907571.2909110 Pseudoalteromonas sp.
GQ391962.1.1391 Pseudoalteromonas sp.
WH PP4
WH PP7
AB544013.1.1455 Pseudoalteromonas sp.
WH PE3A
WH PS6
WH PE1
WH PE5
WH PLA1
WH PS4
SS54-S4-PS-2
WH PS3
WH PS7
WH PS1
JWIC01000010.171744.173278 Pseudoalteromonas luteoviolacea
JF502829.1.1203 Pseudoalteromonas sp.
GOM PS3
WH PE2
WH PE9
WH PHA10
KF442443.1.1435 Pseudoalteromonas sp.
GOM PP1
WH PHA7
WH PHA1
SS13-S2-PE-2
SS15-S2-PLA-2
Pseudoalteromonas
99%
100%
GQ250608.1.1496 Aestuariibacter sp.
WH PHA2
100%
HM591356.1.1496 Alteromonas sp.
WH PP5
WH PP9
WH PHA4
WH PP6
WH PP10
WH PLA6
WH PHA9
KC583247.1.1359 Alteromonas marina
WH PP2
WH PLA2
SS9-S2-PE-05
Alteromonas
SS27-S2-PS-05
SS6-S2-PLA-05
SS21-S2-PP-05
EU802936.1.1504 Idiomarina sp.
SS17-S2-PLA-2
SS20-S2-PE-2
AJ440214.1.1476 Idiomarina baltica
SS12-S2-PP-2
SS18-S2-PS-2
SS26-S2-PS-05
AY635468.1.1463 Idiomarina seosinensis
SS14-S2-PS-2
SS38-S4-PLA-2
HQ721413.1.1465 Idiomarina sp.
KC984862.1.1300 Idiomarina sp.
SS24-S2-PE-05
SS7-S2-PLA-05
Idiomarina
99%
96%
99%
KF859676.1.1489 Halomonas sp.
SS19-S2-PE-2
SS37-S4-PLA-2
SS33-S4-PLA-05
SS47-S4-PE-05
JNCK01000001.2956385.2957919 Halomonas zincidurans
JQ781698.1.1477 Halomonas zincidurans
SS36-S4-PLA-2
SS39-S4-PP-05
SS43-S4-PP-2
SS45-S4-PE-05
SS48-S4-PE-2
SS52-S4-PS-05
SS53-S4-PS-05
SS55-S4-PS-2
Halomonas
100%
100%
GU108529.1.1407 Marinobacter sp.
FO203363.799952.801471 Marinobacter hydrocarbonoclasticus
FO203363.586645.588164 Marinobacter hydrocarbonoclasticus
SS11-S2-PP-2
SS5-S2-PS-2
SS8-S2-PLA-05
Marinobacter
100%
JF198529.1.1343 Micrococcus sp.
SS30-S3-PE-05
Micrococcus
100%
JQ347303.1.1455 Thalassospira sp.
WH PE10
100%
JQ716273.1.1493 Marixanthomonas sp.
SS3-S1-PLA-05
Marixanthomonas
100%
SS1-S1-PLA-05
SS2-S1-PLA-05
AB073587.1.1390 Cytophaga sp.
HM010401.1.1440 Polaribacter sp.
WH PHA8
SS28-S3-PE-2
Polaribacter
FJ644616.1.1358 Tenacibaculum sp.
AF469612.1.1465 Tenacibaculum skagerrakense
SS29-S3-PLA-2
WH PLA5
WH PS2
Tenacibaculum
0.07



b)
Legend
Polyethylene (PE)
Polypropylene (PP)
Polystyrene (PS)
Polylactic Acid (PLA)
Polyhydroxyalkanoate (PHA)
GOM PLA2
GOM PS1
GOM PLA1
GOM PHA4
GOM PHA1
GOM PE2
GOM PE1
FJ457573.1.1651 Vibrio sp.
KF698722.1.1347 Vibrio lentus
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GQ406590.1.1357 Vibrio sp.
WH PP8
WH PLA4
WH PLA9
GOM PP4
AJ316167.1.1468 Vibrio coralliilyticus
GOM PE6
WH PHA3
CP009617.3078534.3080098 Vibrio coralliilyticus
WH PLA10
CP009617.25885.27449 Vibrio coralliilyticus
WH PHA2 CAV
AB681958.1.1478 Vibrio equitatus
GOM PS5
CP009617.463804.465369 Vibrio coralliilyticus
WH PP3
SS51-S4-PS-05
WH PLA7
WH PP1
WH PS5
WH PHA5
WH PE6
CP009888.2907571.2909110 Pseudoalteromonas sp.
GQ391962.1.1391 Pseudoalteromonas sp.
WH PP4
WH PP7
AB544013.1.1455 Pseudoalteromonas sp.
WH PE3A
WH PS6
WH PE1
WH PE5
WH PLA1
WH PS4
SS54-S4-PS-2
WH PS3
WH PS7
WH PS1
JWIC01000010.171744.173278 Pseudoalteromonas luteoviolacea
JF502829.1.1203 Pseudoalteromonas sp.
GOM PS3
WH PE2
WH PE9
WH PHA10
KF442443.1.1435 Pseudoalteromonas sp.
GOM PP1
WH PHA7
WH PHA1
SS13-S2-PE-2
SS15-S2-PLA-2
GQ250608.1.1496 Aestuariibacter sp.
WH PHA2
HM591356.1.1496 Alteromonas sp.
WH PP5
WH PP9
WH PHA4
WH PP6
WH PP10
WH PLA6
WH PHA9
KC583247.1.1359 Alteromonas marina
WH PP2
WH PLA2
SS9-S2-PE-05
SS27-S2-PS-05
SS6-S2-PLA-05
SS21-S2-PP-05
EU802936.1.1504 Idiomarina sp.
SS17-S2-PLA-2
SS20-S2-PE-2
AJ440214.1.1476 Idiomarina baltica
SS12-S2-PP-2
SS18-S2-PS-2
SS26-S2-PS-05
AY635468.1.1463 Idiomarina seosinensis
SS14-S2-PS-2
SS38-S4-PLA-2
HQ721413.1.1465 Idiomarina sp.
KC984862.1.1300 Idiomarina sp.
SS24-S2-PE-05
SS7-S2-PLA-05
KF859676.1.1489 Halomonas sp.
SS19-S2-PE-2
SS37-S4-PLA-2
SS33-S4-PLA-05
SS47-S4-PE-05
JNCK01000001.2956385.2957919 Halomonas zincidurans
JQ781698.1.1477 Halomonas zincidurans
SS36-S4-PLA-2
SS39-S4-PP-05
SS43-S4-PP-2
SS45-S4-PE-05
SS48-S4-PE-2
SS52-S4-PS-05
SS53-S4-PS-05
SS55-S4-PS-2
GU108529.1.1407 Marinobacter sp.
FO203363.799952.801471 Marinobacter hydrocarbonoclasticus
FO203363.586645.588164 Marinobacter hydrocarbonoclasticus
SS11-S2-PP-2
SS5-S2-PS-2
SS8-S2-PLA-05
JF198529.1.1343 Micrococcus sp.
SS30-S3-PE-05
JQ347303.1.1455 Thalassospira sp.
WH PE10
JQ716273.1.1493 Marixanthomonas sp.
SS3-S1-PLA-05
SS1-S1-PLA-05
SS2-S1-PLA-05
AB073587.1.1390 Cytophaga sp.
HM010401.1.1440 Polaribacter sp.
WH PHA8
SS28-S3-PE-2
FJ644616.1.1358 Tenacibaculum sp.
AF469612.1.1465 Tenacibaculum skagerrakense
SS29-S3-PLA-2
WH PLA5
WH PS2
Vibrio
Enterovibrio
Pseudoalteromonas
Alteromonas
Idiomarina
Halomonas
Marinobacter
Micrococcus
Marixanthomonas
Polaribacter
Tenacibaculum
0.07

Initial Colonizers in Plastisphere Succession

We expected that initial colonizers might be replaced by other microbes as the Plastisphere community changed after a few weeks or months of exposure to seawater. We found, however, that all of the initial colonizer genera and even many of the specific oligotypes remained in much later stages of succession. Later stages of succession were represented by the Woods Hole Dock Time Series (WHDTS) macroplastic communities, collected in a separate incubation experiment characterizing Plastisphere communities over a period of four months, and the environmentally collected microplastic communities.

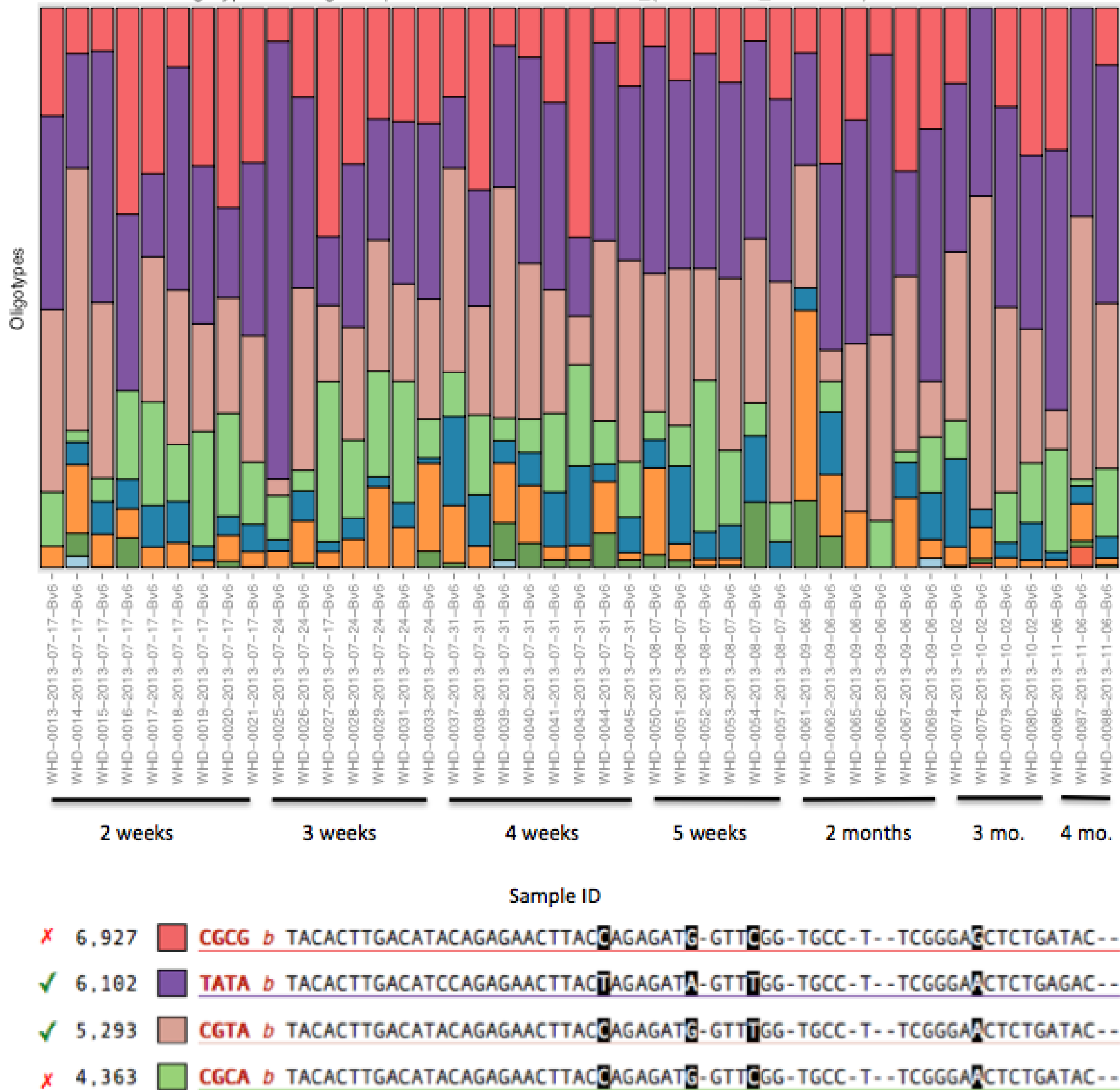
From our coastal site at WHD (Fig. 1., Site 5), initial colonizers belonged to six different genera (Table 1). Our oligotyping analysis found that all of these genera occurred in all 16 of the Woods Hole Dock Time Series (WHDTS) samples that were sequenced in the first two weeks of exposure to seawater. Furthermore, these genera occurred in almost all of the 43 WHDTS samples that were sequenced by the end of the four month-long time series. The only exceptions were *Enterovibrio* and *Tenacibaculum,* which occurred in 42 of the 43 samples. There were two samples that either did not contain *Enterovibrio* or did not contain *Tenacibaculum*, but both samples were sequenced less than two months into the time series, which does not suggest these genera dropped out in later stages of succession. Therefore, the initial colonizer genera at the coastal site in Woods Hole remained part of the Plastisphere community after two weeks and four months of incubation in seawater.

Using a more extensive oligotyping analysis of the 43 WHDTS samples, we compared the four most abundant oligotypes of each sample and found that although many of the same oligotypes persisted over the course of the WHDTS, there was variation in oligotypes and relative abundance of oligotypes over time (Fig. 6a-f). At least two of the four most abundant

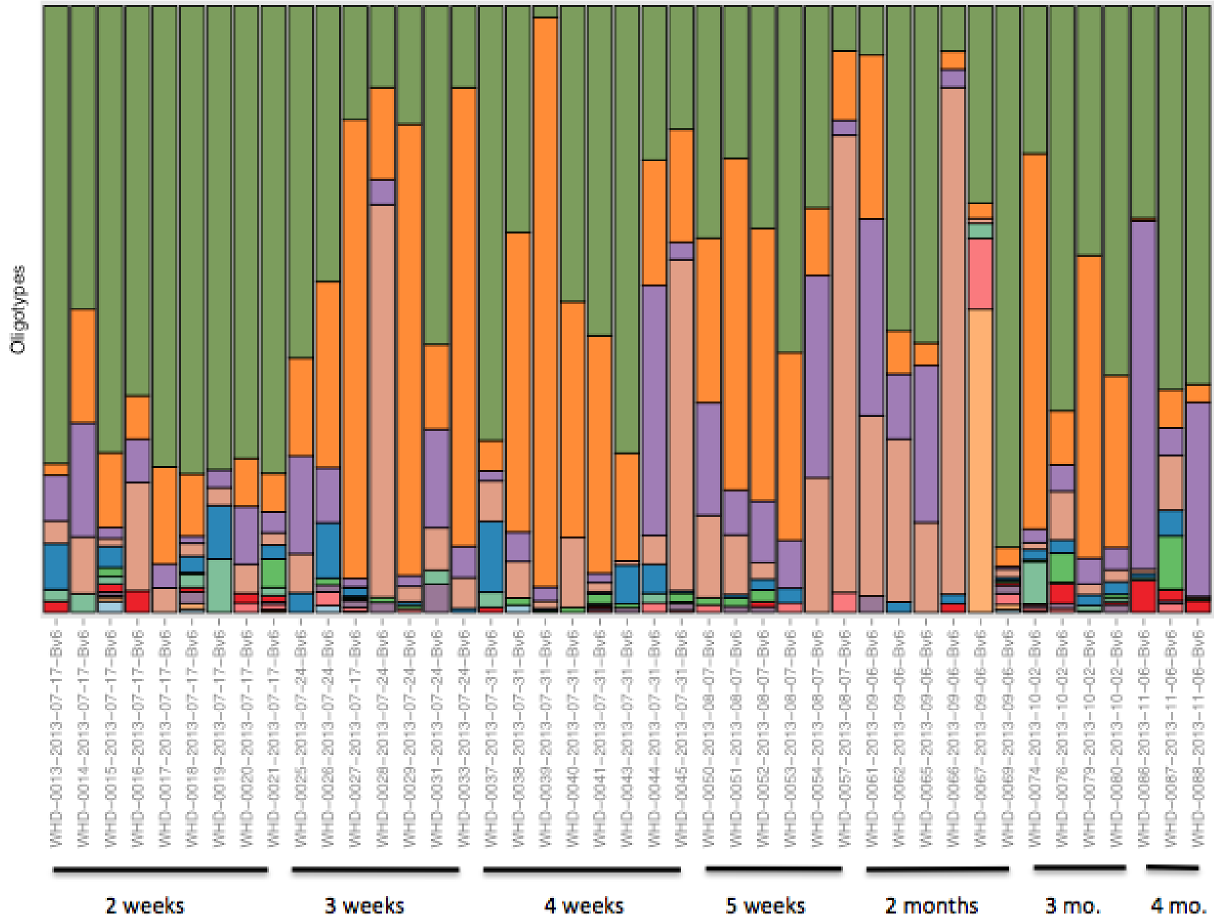
oligotypes of *Pseudoalteromonas* (Fig. 6a), *Vibrio* (Fig. 6b), and *Alteromonas* (Fig. 6c) matched the sequences of our WHD initial colonizer samples. For *Polaribacter* (Fig. 6d), *Tenacibaculum* (Fig. 6e), and *Enterovibrio* (Fig. 6f), none of the four most abundant oligotypes matched initial colonizer sequences from WHD. The abundance of the oligotypes present in the WHDTS samples varied considerably over the time series for all six WHD initial colonizer genera (Fig. 6a-f).

Figure 6. Stackbars show oligotype distributions among all 43 Woods Hole Dock Time Series (WHDTS) samples for each initial colonizer genus found at the Woods Hole Dock (WHD): *Pseudoalteromonas* (a), *Vibrio* (b), *Alteromonas* (c), *Polaribacter* (d), *Tenacibaculum* (e), and *Enterovibrio* (f). WHDTS samples consist of Plastisphere community sequences from polyethylene, polypropylene, and polystyrene plastics that were incubated in seawater for known time periods ranging from 2 weeks to 4 months. Sampling and sequencing of the Plastisphere community was conducted weekly for the first four weeks and monthly thereafter. Oligotype analysis was performed from 43 samples from the WHDTS for each initial colonizer genus with Oligotyping pipeline version 0.96 (available from http://oligotyping.org). The four most abundant oligotypes are color coded and listed below in stackbars. Sample IDs include location, sample numbers, and date of sampling from plastic surfaces. The numerical frequencies of each oligotype, indicating the total number of times the oligotype was found across all samples, is listed to the bottom left next to each oligotype sequence. Every oligotype listed is followed by a representative sequence of each distinct oligotype. A green checkmark indicates that the sequence was found among initial colonizer sequences from WHD, which includes colonizers of microplastics (<5mm) within the first two hours of incubation. Alternatively, a red cross means that the sequence was not found among any of the WHD initial colonizer sequences.

a)



b)



Sample ID

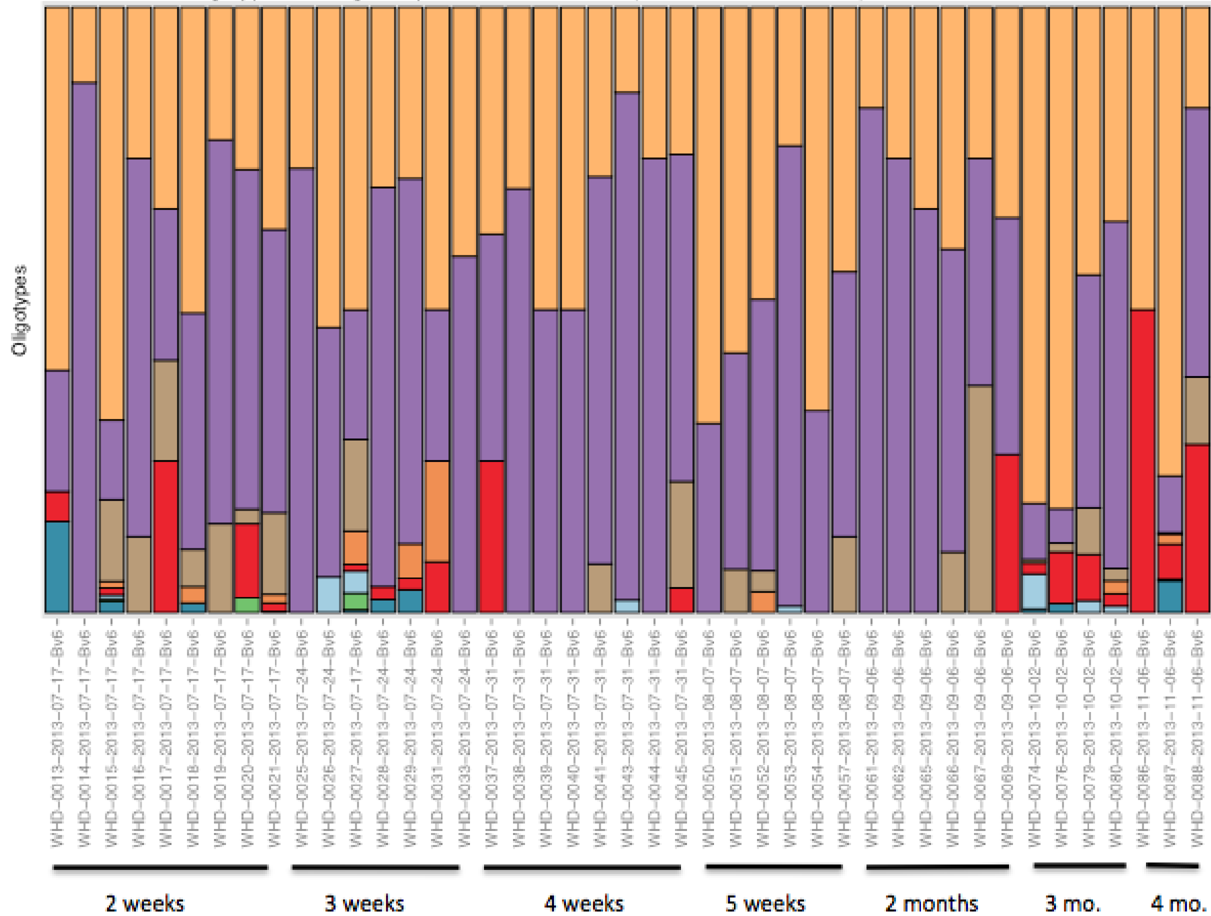
✓ 8,033 GGTC b TACTCTTGACATCCAGAGAAGCCAGCGGAGACGCAGGTGTGCC-T-TC-GGGAG-CT-CTGAGAC--

✓ 6,622 TTGA b TACTCTTGACATCCATAGAACTTTCCAGAGATGGATTGGTGCC-T-TC-GGGAA-CT-ATGAGAC--

✓ 901 CCTG b TACTCTTGACATCCTCAGAAGAGACTGGAGACAGTCTTGTGCC-T-TC-GGGAA-CT-GAGAGAC--

✗ 812 GGAC b TACTCTTGACATCCAGAGAATCTAGCGGAGACGCTGGAGTGCC-T-TC-GGGAG-CT-CTGAGAC--

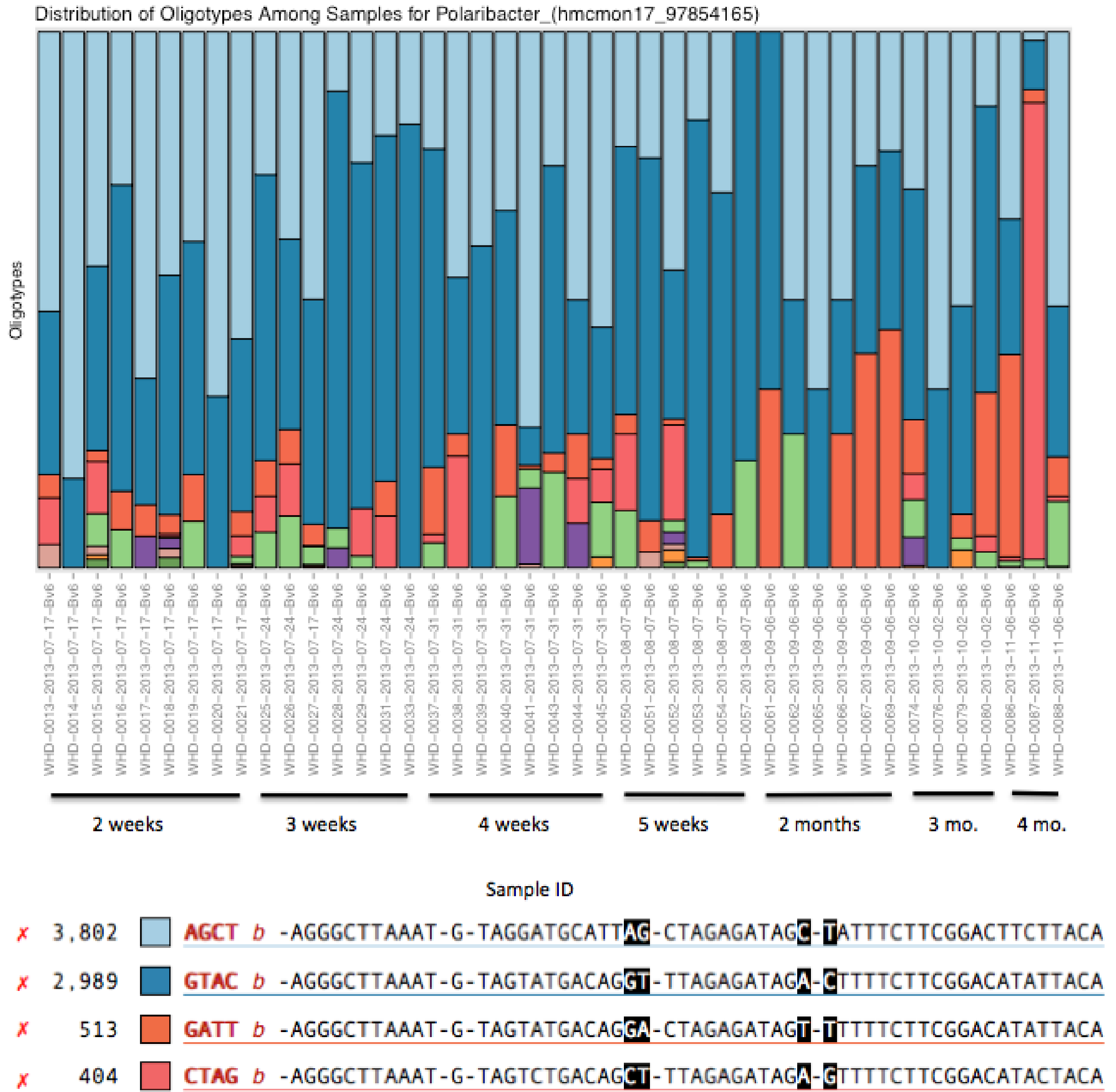
c)



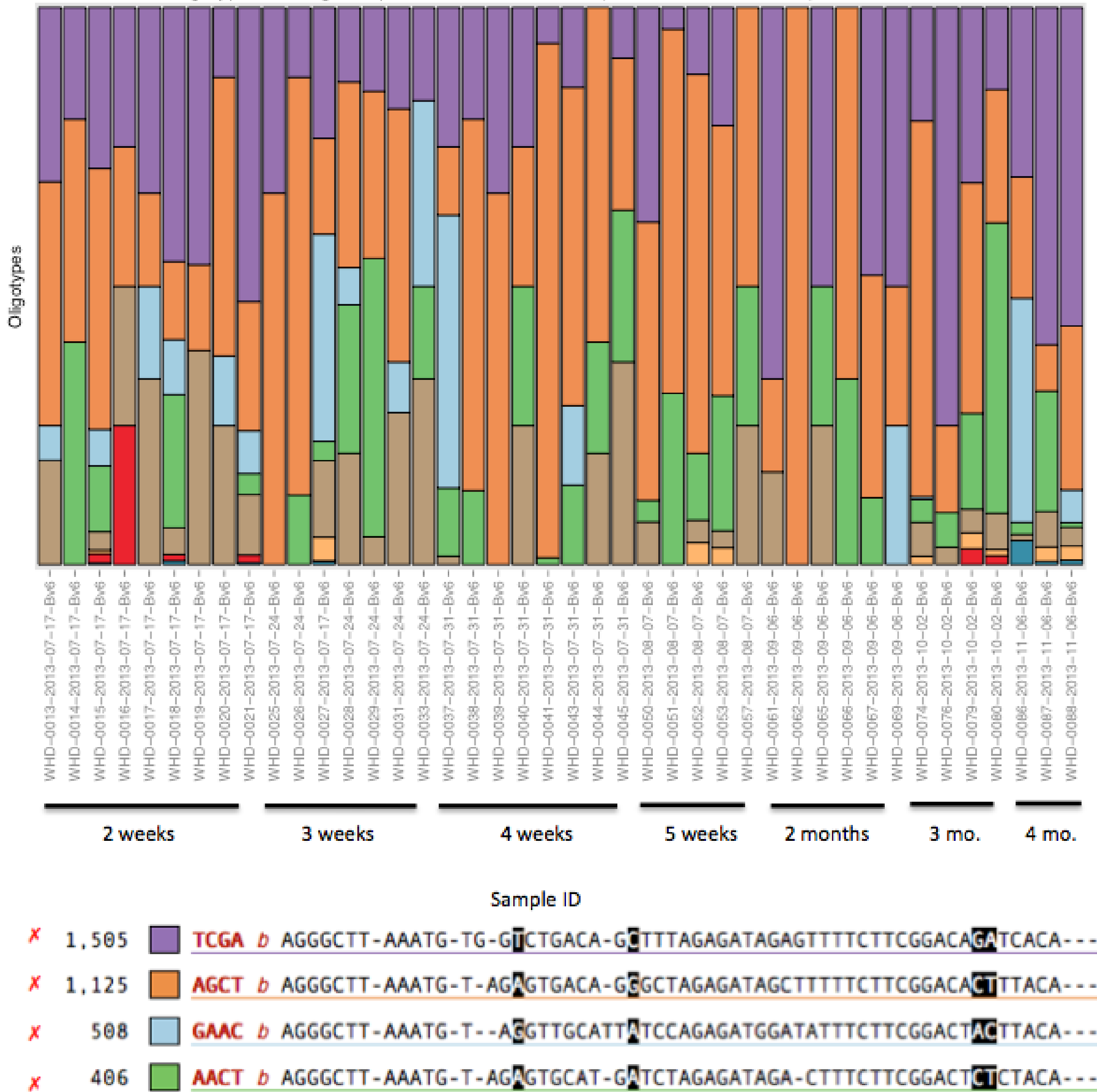
Sample ID

✓	2,956		ACGT	b	TACACTTGACATGCAGAGAACTTTCCAGAGATGGATTGGTGCCTTCGGGAACTCTGACAC-
✓	2,443		TGCA	b	TACACTTGACATGCTGAGAAGTTACTAGAGATAGTTTCGTGCCTTCGGGAACTCAGACAC-
✗	724		TCGA	b	TACACTTGACATGCTGAGAACTTTCCAGAGATGGATTGGTGCCTTCGGGAACTCAGACAC-
✗	156		AGTT	b	TACACTTGACATGCAGAGAAGAATTCAGAGATGGATTTGTGCCTTCGGGAACTCTGACAC-

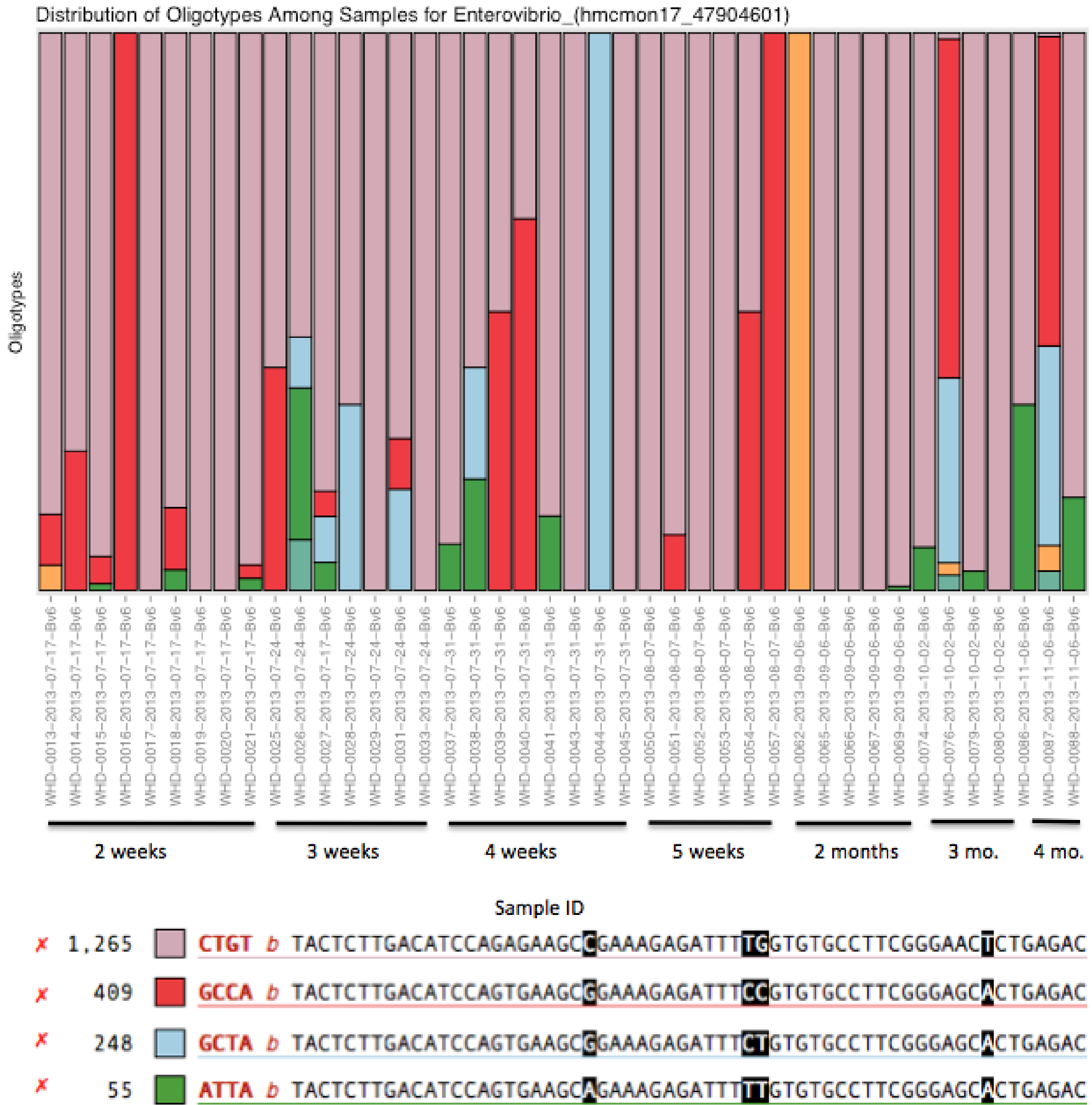
d)



e)



f)



From our pelagic sites in the GOM and SS, nine different genera (Table 1) constituted the initial colonizers. Our oligotyping analysis revealed that *Pseudoalteromonas* and *Alteromonas* occurred in all 31 of the Plastisphere communities that were sequenced from environmentally collected microplastics found in pelagic regions in the North Atlantic. The remaining seven

initial colonizer genera occurred in at least two-thirds of the 31 environmentally collected Plastisphere communities, with the ratio (number of environmentally collected samples containing this genus/number of environmentally collected samples total) given after each genus name: *Vibrio* (30/31)*, Halomonas* (30/31)*, Marinobacter* (30/31)*, Marixanthomonas* (28/31), *Tenacibaculum* (28/31), *Idiomarina* (27/31), and *Micrococcus* (21/31). Therefore, initial colonizer genera in the North Atlantic from the SS and GOM samples remained intact in later stages of the Plastisphere community that occurred on environmentally collected microplastics.

For eight of the nine pelagic genera (GOM and SS), with the exception of *Tenacibaculum*, a more detailed oligotyping analysis revealed that at least one of the four most abundant oligotypes from environmentally collected microplastics occurred among initial colonizers (data not shown). Therefore, not only did initial colonizer genera persist, but even some of the same oligotypes remained part of the community. We inferred this because some of the most abundant, later stage, pelagic Plastisphere oligotypes matched some of the initial colonizer oligotypes of each genera. The exception was the genus *Tenacibaculum,* for which none of the four most abundant oligotypes matched initial colonizer sequences.

Initial Colonizer Functional Traits

Functional trait analysis revealed several similarities among many of the initial colonizer genera. The majority of species in all eleven initial colonizer genera are heterotrophic (Table 3). Nine of these eleven genera included species that are known biofilm formers. Six of the genera contained species that metabolize hydrocarbons, according to previous studies on the metabolism of bacteria belonging to these genera (Table 3).

Table 3. The majority of initial colonizer genera were heterotrophs, and included species that are known to be biofilm formers and hydrocarbon metabolizers. Initial colonizer genera known to consist of species with the functional traits listed to the left (heterotrophic, biofilm forming, hydrocarbon metabolizing) are marked in blue. Boxes marked white indicate that it is unknown whether any species of the genus possess these functional traits. Sources for this information correspond with the numbers within each box: (1) Chang et al. 2016. (2) Sawabe et al. 2013. (3) Ivanova et al. 2000. (4) Mounier et al. 2014. (5) Anantharaman et al. 2016. (6) Cabaj and Kosakowska 2009. (7) Weiner et al. 1981. (8) Thompson et al. 2002. (9) Qurashi and Sabri 2012. (10) López et al. 2006. (11) Matsuura et al. 2013. (12) Kirchman, D. L. 2008. (13) Mai-Prochnow et al. 2004. (14) Certner and Vollmer 2015 (15) Yildiz and Visick 2009. (16) Jin et al. 2011. (17) Mnif et al. 2009. (18) Austin et al. 1977 (19) Chronopoulou et al. 2015. (20) Grimes et al. 2009.

	Alteromonas	*Enterovibrio*	*Halomonas*	*Idiomarina*	*Marinobacter*	*Marixanthomonas*	*Micrococcus*	*Polaribacter*	*Pseudoalteromonas*	*Tenacibaculum*	*Vibrio*
Genus is primarily heterotrophic	1	2	1	3	4	5	6	1	1	1	1
Genus includes biofilm-formers	7	8	9	10	4		11	12	13	14	15
Genus includes hydrocarbon metabolizers	16		17		4		18		19		20

Discussion

Identification and Distribution Patterns of Initial Colonizers

Genetic data and initial colonizer identification based on BLAST analysis revealed a diverse community of eleven genera of bacteria that attach to virgin microplastics within the first two hours of incubation in seawater (Fig.1). Of the 129 unique bacterial colonies that were isolated and identified at least to genus level, over half (63 colonies) belong to three genera—*Pseudoalteromonas, Vibrio,* and *Halomonas* (Fig. 1). The next most common genera were *Idiomarina* and *Alteromonas*. Past Plastisphere studies have found that *Vibrio*, *Alteromonas,* and *Pseudoalteromonas* are particularly abundant bacteria in environmentally collected microplastic communities (Zettler et al. 2013).

Phylogenetic analyses revealed greater clustering among close relatives by location than

by resin type (Fig. 5a and Fig. 5b), which supports our original hypothesis and the findings of Amaral-Zettler et al., 2015. Many closely related initial colonizers originated from the same location (Fig. 5a), while initial colonizers of the different resins were dispersed across the entire tree without as much clustering (Fig. 5b). Interestingly, *Vibrio*, one of the most common genera and a well-documented pathogen of humans and marine organisms, did not occur at any of the four SS sites. One SS colony, however, that appeared in our phylogenetic tree (Fig. 5a) seemed to be closely related to known *Vibrio* colonies, but its identity could not be confirmed from our BLAST analysis. This suggests that open ocean *Vibrio* may be later colonizers in this oligotrophic environment than shelf or coastal *Vibrio*, since *Vibrio* are a common Plastisphere colonizer in open ocean environments in the North Atlantic Ocean (Zettler et al. 2013).

Regarding the geographical distribution of marine microbes, it should also be noted that microbes of the same genus have been found in extremely different locations and conditions all over the world. For example, the initial colonizer genus *Marixanthomonas* has also been isolated from a deep-sea brittle star from the Fiji Sea (Romanenko et al. 2007), a location that shares little in common with our surface sampling in the Sargasso Sea. Nevertheless, our phylogenetic analysis shows some clustering among relatives by location even within genera (e.g. WH versus GOM clustering in the *Vibrio* clade). Some Plastisphere colonizers may be differentially distributed by location if they are specifically adapted to environmental variables that are characteristic to the region wherein they live (e.g. *Idiomarina* and *Halomonas,* Fig. 5a), while others might be more generalists and able to survive in a variety of environments (e.g. *Pseudoalteromonas*, Fig. 5a).

While we could confirm only a single genus that occurred in all three locations, there were seven genera identified on all four or all five resins (Table 2). Polyhydroxyalkanoate (PHA)

was not deployed in the Sargasso Sea (SS) experiments, so occurrence on four resins was the maximum possible for SS colonizers such as *Halomonas* and *Idiomarina*, which we only isolated from SS sites (Table 1). Of the colonies we sequenced, only *Micrococcus*, *Marixanthomonas*, *Enterovibrio*, and *Polaribacter* were isolated exclusively from one resin, and we only sequenced one colony from the latter three genera so they could not have occurred on more than one resin in our samples. These results strongly support the hypothesis that colonizers were not differentially distributed by resin to as great a degree as by location.

Most of the initial colonizer genera were ubiquitous across all resin types, showing that there are some common microbes that colonize all types of plastic (Oberbeckmann et al. 2014). As evident when comparing genera containing the highest number of colonies (Fig. 4) with those genera found across each resin (Table 2), our findings suggest that when we selected a high number of colonies from a particular genus, we found those bacteria on all or most resins. Therefore, if more colonies had been sampled, it is possible that there would be even fewer resin-specific genera. According to the literature, characteristics of different plastic polymers play an essential role in the attachment, abundance, and types of bacteria colonizing the resins (Dang et al. 2000; Oberbeckmann et al. 2014). This may have important applications; for example, if certain pathogenic microbes are found to attach only to certain resin types, these plastics might be avoided for use in plastic-based aquaculture pens, water intake facilities for water desalinization, or other uses. One possible explanation why we did not see strong specificity by resin is that initial colonizers may be less resin specific than later stage colonizers of plastics examined previously. Further experiments testing the ability of various microbes to attach to different resins could confirm this. In addition, the surfaces of the microplastic pellets that we used may have had very similar surface characteristics at a microscopic scale (e.g. smoothness

and hydrophobicity), making the opportunities for attachment to these virgin microplastics similar across different resins.

Our evidence of differential distribution by location, but less so by resin, is consistent with previous research. Pommier et al. (2007) found that the composition of marine bacteria collected from surface waters world-wide were highly distinct between the different locations, showing that few marine bacteria are cosmopolitan and that many are endemic to specific locations. This challenges the common assumption that microbes are ubiquitous. Our findings also challenge this assumption, since closely related initial colonizers tended to cluster by location. Studies looking at other microbes such as diatoms attaching to plastic surfaces have also discovered a biogeographical transition of the types of abundant taxa at each location (Carson et al. 2013). Results from Zettler et al. (2013) show that certain types of plastic-colonizing microbes tend to be more abundant at different sampling locations and on different resins. In comparing the effect of these two factors, however, Amaral-Zettler et al. (2015) reported a greater difference among communities by location than by resin.

For several reasons, our experimental methods allowed us to detect the presence of initial colonizers on each resin type and at each location, but not their absence. First, some of the microbes that attached to the incubated microplastics may not have been streaked onto the agar. Secondly, any agar used for culturing is likely to select for certain microbes, and Jannasch and Jones (2003) found that cultural methods reveal significantly fewer bacteria than direct microscopic counts. Also, since we only picked colonies after a maximum of 72 hours of growth on the agar, extremely slow-growing colonies may not have been captured in our method. Therefore, our experiments allowed us to determine for the first time many of the initial colonizers present in extremely early Plastisphere communities originating from five different

resins and at six different locations, though we do not yet have a complete picture of the entire community at this early stage. Next generation sequencing of the entire initial colonizer community would not have been possible at this time, given the limitations of this method to sequence the DNA of extremely low biomass samples such as those of the earliest stages of colonization on the virgin microplastics.

Initial Colonizers in Plastisphere Succession

Though next generation sequencing was not used for initial colonizer sequencing, this technique was used to characterize the entire community in the WHDTS and environmentally collected microplastics, which were incubated for much greater time durations. Using those older communities that were sequencing using next generation techniques, we were able to compare our subset of the initial colonizer community with comprehensive community data of colonizers in later stages of life in the Plastisphere. We expected to see initial colonizers dropping out of the community in later stages of succession. According to the three models of succession presented by Connell and Slatyer (1977), a widely accepted, theoretical framework for ecological succession, early colonizers drop out of the community in later stages. This occurs because late colonizers are better adapted to late stage successional stage environments, so they replace initial colonizers (McCook 1994). This theoretical model was confirmed in the context of marine bacterial succession; for example, Dang and Lovell (2000) looked at early colonizers of various surfaces, including plastics, in salt marsh estuaries. According to this study, although bacteria within the genus *Alteromonas* were found after 24 hours of incubation, they were absent on any surfaces after 72 hours. Therefore, other microbes may have already replaced *Alteromonas* even at this relatively early stage in succession (Dang and Lovell 2000).

Contrary to our hypothesis, our results suggest that initial colonizers remain intact in much later stages of succession in both coastal and pelagic environments. All of the initial colonizer genera from the Woods Hole Dock (WHD) remained part of the community after not just two weeks but the full, four month long duration of the Woods Hole Dock Time Series (WHDTS), and even specific initial colonizer oligotypes occurred in the later stages of succession (Fig. 6). This confirms that initial colonizer oligotypes within these genera not only remain intact in later successional stage communities, but that they become some of the most abundant members of the community within their genera. Since we closely examined only the top four most abundant oligotypes of each genus from the WHDTS to determine whether they matched initial colonizer oligotypes, other initial colonizers oligotypes may have existed in WHDTS samples in lower abundances. Given that the WHDTS consisted of plastics with a known time of incubation in seawater, this dataset allowed us to confirm the presence of six initial colonizer genera, and initial colonizer oligotypes of three of those genera, on plastics incubated for up to four months. Finding initial colonizer oligotypes in the WHDTS samples also validated the methods we used to isolate initial colonizers since some of our initial colonizer oligotypes (collected in September, 2015) were the same as those in communities sequenced in different months and in a different year (July through November, 2013).

While the WHDTS provided samples with a known incubation time for comparison with initial colonizers collected from the exact same coastal location, it was even more surprising to find that of the initial colonizers occurred in the environmentally collected microplastic communities. These environmental microplastics were collected from various locations in pelagic regions (defined here as >15km from shore) in the North Atlantic from May-June in 2012 and 2013, yet the majority of the samples contained all of the initial colonizer genera and

all but one of those genera contained at least one initial colonizer oligotype. Although the precise age of these environmental microplastics is unknown, and dating these plastic particles is an area for future research, we predict that these plastics ranged in age from several weeks to several years old, given that some plastic objects float in the ocean for years (Ebbesmeyer and Ingraham 1992). It is possible that pieces of the original biofilm growing on these microplastics sloughed off and exposed bare polymer surfaces thus resembling again an earlier successional community. The least complicated interpretation of these results, however, is that these samples represent later successional stages of Plastisphere communities, and that initial colonizers remained present, in some cases as relatively abundant members of the community.

Although our results do not allow us to explicitly determine whether these Plastisphere communities are following a facilitation, tolerance, or inhibition model (Connell and Slatyer 1977), the community may be following some combination of the facilitation and tolerance models. Consistent with the facilitation model, many of the initial colonizers belong to genera that are known to alter their environment, as will be discussed shortly, which could be beneficial to other organisms that colonize in later stages. Simultaneously, the community may follow the tolerance model to some degree, since initial colonizers are able to tolerate later stage colonizers according to our results. Defining the specific model of succession for microbial communities is difficult because these communities, specifically those forming biofilms, are extremely dynamic, unpredictable, complex, and are often characterized by rapidly evolving interactions (Jackson et al. 2001; Faust and Raes 2012). To put these findings in context, a comprehensive review of fouling in the marine environment (Wahl, 1989) showed that fouling begins with the arrival of macromolecules on the newly deposited surface within the first few minutes of exposure, followed by the colonization of bacteria after one to several hours, diatoms and protozoa after

one to two days, and larvae and spores after one to several weeks. Although this review concluded that bacteria colonize after about one hour of submersion, we found some initial colonizers attached within as short an incubation time as 30 minutes (Appendix, Table A).

Microbial succession is well documented in other contexts as well. In the human gut microbiome, initial colonizers affect the time it takes for microbial communities to reach a stable, late successional equilibrium, but they may not drastically alter the composition of that late stage community (Trosvik et al. 2010). In dental plaque, early colonizers generally co-aggregate with certain other early colonizers, but not with late colonizers (Kolenbrander et al. 2002). Although these studies suggest that community structure of early microbial communities differs from that of late stage communities because initial colonizers disappear due to emigration or predation (Jackson et al. 2001), our findings show long-term prevalence of initial colonizers in later stage communities.

Future research might explore how early and late colonizers impact the settlement of larger protists, plants, or animals in the Plastisphere, potentially using methods similar to those used in studies of benthic settlement of coral larvae on coral reefs. Such experiments show that certain biofilm-forming bacteria, including our most prevalent genus *Pseudoalteromonas*, are sufficient to induce coral settlement, and more specifically that it is the compound tetrabromopyrrole that induces this ecological effect (Sneed et al. 2014). On floating, abiotic substrates in the ocean, filter feeders are one of the most common organisms to attach (Thiel 2003, e.g. Fig. 2). Further research might reveal whether bacteria or other microbial colonizers facilitate the settlement of filter feeders and other organisms on plastic marine debris (PMD). The discovery of any chemical cues that attract or repel organisms to PMD could also be useful,

particularly if those organisms are able to biodegrade plastic waste, or if they are undesirable colonizers of plastic devices or equipment exposed to the marine environment.

Initial Colonizer Functional Traits

Our functional trait analysis revealed several common traits among bacteria, which we expected given that certain bacteria are more adapted for colonization than others (Jackson et al. 2001). First, biofilm formation was a common functional trait exhibited by nine of the initial colonizer genera (Table 3). Since surfaces often accumulate higher concentrations of nutrients, the biofilm producers are likely to benefit from the existence of hard substrates (Zobell 1943), particularly in oligotrophic environments such as the Sargasso Sea. Biofilm formation, studied extensively in the biomedical field, provides fitness advantages for the bacteria embedded in the biofilm. The inter-cell matrix that composes the biofilm protects bacteria from host defenses in the context of the human body, offering protection from white blood cells and antibacterial peptides, as well as from antibiotics (Fux et al. 2005). Biofilms in the marine Plastisphere may provide similar defense from predators and biocides.

Second, all initial colonizer genera were heterotrophic (Table 3), contrary to our predictions that early colonizers, in an environment with little nutrient availability, would likely be phototrophs. While environmentally collected microplastics contained autotrophs, such as cyanobacteria (Zettler et al. 2013; Oberbeckmann et al. 2014), there were no known phototrophs among the initial colonizers we collected. This could be explained by methodological limitations. There may have been phototrophs present in our cultures that we simply did not select and sequence, because we did not sequence entire initial colonizer communities. Furthermore, phototrophs near the surface of both coastal and pelagic environments would naturally be

exposed to direct sunlight, whereas we cultured our initial colonizers in less intense artificial light (a 12:12 hour light/dark incubator for the GOM and WH samples, and a shelf with indirect natural sunlight exposure on the ship for the SS samples). Phototroph culturing was possible using our methods, but if any phototrophs were present, they may not have been readily cultured *ex situ*. This explanation is consistent with prior research findings that, in low light environments, heterotrophic bacteria may actually facilitate colonization by phototrophs in biofilms (Roeselers et al. 2007), as this would cause heterotrophs to colonize initially and before phototrophs.

Another possible explanation for why we did not find phototrophs is that many of the initial colonizers of the Plastisphere are adapted to obtain energy through alternative pathways, such as by metabolizing hydrocarbons of the resins to which they attach. Six of the eleven initial colonizer genera contain species known to utilize hydrocarbons as a source of energy (Table 3). It should be noted that bacteria of close relatedness and belonging to the same genus could have substantially different function, and given limitations of functional trait literature on these marine microbes, our analysis looked for the presence of each trait in any species of each genus. Therefore, we cannot confirm that all initial colonizers within these six genera metabolize hydrocarbons. With this caveat, we can speculate that the genes for hydrocarbon metabolism could have been conserved in many of the initial colonizers, which would allow them to attach in the extremely low nutrient environment of clean microplastics. In any case, some of the specific species within these genera that are confirmed hydrocarbon metabolizers were in fact the same species identified among our initial colonizers, such as *Marinobacter hydrocarbonoclasticus*. This bacterium has been isolated from Mediterranean seawater near a petroleum refinery

(Gauthier et al. 1992). It is able to use various hydrocarbons as the sole source of carbon and energy (Gauthier et al. 1992), and might therefore be well adapted to living in the Plastisphere.

Though one study looking for biodegraders of plastic substrates on polyethylene did not find any even after three weeks of incubation in seawater (Lobelle and Cunliffe 2011), our results confirm that bacteria that metabolize hydrocarbons are found on PMD within just two hours, and even thirty minutes, of incubation (e.g. *Marinobacter hydrocarbonoclasticus,* Appendix, Table A). These microbes could be useful in various applications, such as for waste management or bioremediation projects pertaining to the degradation of polymers in the environment. For example, *Micrococcus* is a prevalent marine bacterium capable of biodegrading petroleum (Austin et al. 1977), and *Pseudoalteromonas* may be useful in remediating oil spills (Chronopoulou et al. 2014). *Halomonas,* one of our most prevalent initial colonizers, can biodegrade PHA (Shrivastav et al. 2011). The latter example may also strengthen the argument for replacing plastics with bioplastics in production.

Microplastics are a relatively new but growing source of hard substrate in the marine environment, making it important to expand our understanding of the interaction between microplastics and the marine community. Our results offer the first picture of the very early colonizers of the Plastisphere on various plastics that occur in coastal and open ocean ecosystems, which contributes to our knowledge of the dynamics of the Plastisphere. Further research could examine the effects of microplastics and their associated Plastisphere on other organisms. For example, polystyrene microplastics ingested by oysters, negatively affects their energy uptake, fecundity, and offspring development (Sussarellu et al. 2016), but the direct impacts of attached microbes and potential pathogens to the oysters are still largely unknown. Additionally, this area of research may elucidate how the microbial community affects the

plastics themselves. Since microbes are likely to affect degradation and sinking of microplastics (Carson et al. 2013), the Plastisphere could play an enormous role in the ultimate fate of PMD in the oceans as well as affecting the organisms that interact with the Plastisphere.

Appendix

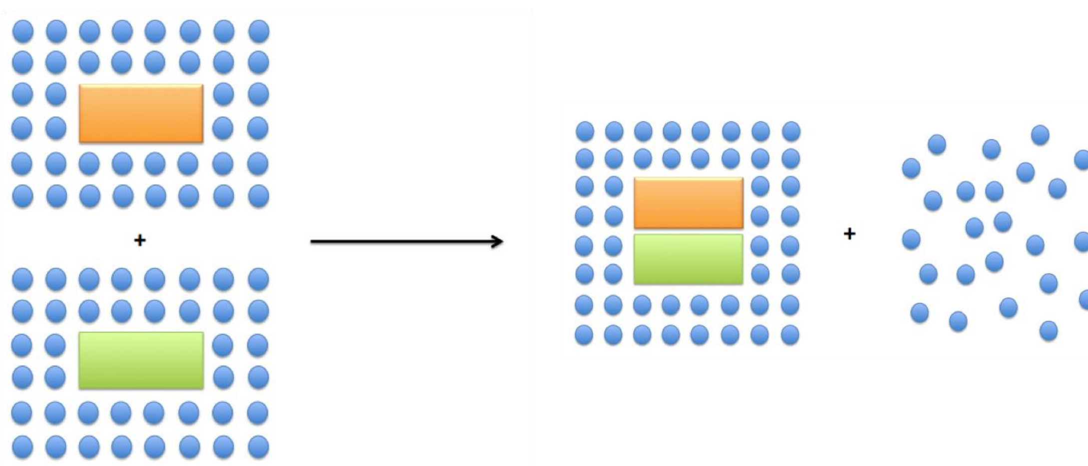


Figure A. When nonpolar bacteria (green rectangle), and microplastics (orange rectangles) adhere to each other, the release of water polar molecules (blue circles) previously separating them creates a favorable and spontaneous change in configuration. The previously ordered water molecules that separate the nonpolar solutes (i.e., bacteria and microplastics) move into the surrounding bulk of water molecules, leading to an increase in entropy (ΔS) and a smaller increased in enthalpy (ΔH). This makes the final configuration more favorable (Rosenberg and Kjelleberg, 1986).

Table A. Sampling location information. Site numbers correspond to map of seawater collection sites, Fig. 3.

Site #	1	2	3	4	5	6
Cruise	C-259	C-259	C-259	C-259	From shore in Woods Hole	Sorceror II
Date	24 Apr, 2015	28 Apr, 2015	10 May, 2015	13 May, 2015	6 Sept, 2015	10 Sept, 2015
Coordinates	21°52.8'N x 66°39.6'W	27°29.4'N x 66°52.6'W	32°30.0'N x 64°34.3'W	34°44.1'N x 68°12.4'W	41°52.5'N x 70°67.2'W	43°01.5'N x 068°23.3'W

Table B. BLAST Data with identities of first colonizers from the Sargasso Sea (cruise SS).

Sample Name	Sequence Aided ID
Cruise-Mic#-Site#-Plastic-Incubation Time in Hours-Agar-Colony	**Genus and species**
SS-MIC#1-Site1-PLA-.5-Try-A	Unknown bacterium
SS-MIC#2-Site1-PLA-.5-Try-B	Unknown bacterium
SS-MIC#3-Site1-PLA-.5-Try-C	*Marixanthomonas sp.*
SS-MIC#5-Site2-PS-2-Try-I	*Marinobacter sp.*
SS-MIC#6-Site2-PLA-.5-Try-B	*Idiomarina baltica*
SS-MIC#7-Site2-PLA-.5-Try-D	*Idiomarina zobellii*
SS-MIC#8-Site2-PLA-.5-Try-C	*Marinobacter hydrocarbonoclasticus*
SS-MIC#9-Site2-PE-.5-Try-H	*Alteromonas sp.*
SS-MIC#11-Site2-PP-2-Try-B	*Marinobacter hydrocarbonoclasticus*
SS-MIC#12-Site2-PP-2-Try-C	*Idiomarina baltica*
SS-MIC#13-Site2-PE-2-Try-A	*Pseudoalteromonas shioyasakiensis*
SS-MIC#14-Site2-PS-2-Try-K	*Idiomarina sp.*
SS-MIC#15-Site2-PLA-2-Try-B	*Pseudoalteromonas shioyasakiensis*
SS-MIC#17-Site2-PLA-2-Try-F	*Idiomarina baltica*
SS-MIC#18-SIte2-PS-.5-Try-A	*Idiomarina sp.*
SS-MIC#19-Site2-PE-2-Try-B	*Halomonas sp.*
SS-MIC#20-Site2-PE-2-Try-C	*Idiomarina baltica*
SS-MIC#21-Site2-PP-.5-Try-B	*Idiomarina baltica*
SS-MIC#24-Site2-PE-.5-Try-I	*Idiomarina zobellii*
SS-MIC#25-Site2-PE-.5-Try-J	*Idiomarina zobellii*
SS-MIC#26-Site2-PS-.5-Try-C	*Idiomarina sp.*
SS-MIC#27-Site2-PS-.5-Try-E	*Idiomarina baltica*
SS-MIC#28-Site3-PE-2-Try-A	Unknown bacterium
SS-MIC#29-Site3-PLA-2-Try-A	*Tenacibaculum sp.*
SS-MIC#30-Site3-PE-.5-Try-B	*Micrococcus sp.*
SS-MIC#31-Site3-PE-.5-Try-D	*Micrococcus sp.*
SS-MIC#32-Site3-PE-.5-Try-E	*Micrococcus sp.*
SS-MIC#33-Site4-PLA-.5-Try-A	*Halomonas zincidurans*
SS-MIC#34-Site4-PLA.5-Try-B	*Halomonas zincidurans*
SS-MIC#35-Site4-PLA-.5-Try-C	*Halomonas zincidurans*
SS-MIC#36-Site4-PLA-2-Try-A	*Halomonas zincidurans*
SS-MIC#37-Site4-PLA-2-Try-B	*Halomonas zincidurans*
SS-MIC#38-Site4-PLA-2-Try-C	*Idiomarina sp.*
SS-MIC#39-Site4-PP-.5-Try-A	*Halomonas zincidurans*
SS-MIC#40-Site4-PP-.5-Try-B	*Halomonas zincidurans*
SS-MIC#43-Site4-PP-2-Try-B	*Halomonas zincidurans*
SS-MIC#44-Site4-PP-2-Try-C	*Halomonas zincidurans*
SS-MIC#45-Site4-PE-.5-Try-A	*Halomonas zincidurans*
SS-MIC#46-Site4-PE-.5-Try-B	*Halomonas zincidurans*

SS-MIC#47-Site4-PE-.5-Try-C	*Halomonas zincidurans*
SS-MIC#48-Site4-PE-2-Try-A	*Halomonas zincidurans*
SS-MIC#49-Site4-PE-2-Try-B	*Halomonas zincidurans*
SS-MIC#51-Site4-PS-.5-Try-A	Unknown bacterium
SS-MIC#52-Site 4-PS-.5-Try-B	*Halomonas zincidurans strain B6*
SS-MIC#53-Site 4-PS-.5-Try-C	*Halomonas zincidurans strain B6*
SS-MIC#54-Site4-PS-2-Try-A	Unknown bacterium
SS-MIC#55-Site4-PS-2-Try-B	*Halomonas zincidurans*
SS-MIC#56-Site 4-PS-2-Try-C	*Halomonas zincidurans*
SS-MIC#57-Site4-PLA-NC-Try-A	*Pseudoalteromonas sp.*
SS-MIC#58-Site4-PE-NC-Try-A	Unknown bacterium
SS-MIC#59-Site4-PP-NC-Try-A	*Pseudoalteromonas sp.*

Table C. BLAST Data with identities of first colonizers from the Gulf of Maine (cruise GOM).

Sample Name	**Sequence Aided ID**
Cruise-Plastic-Agar-Date-Primer	**Genus and species**
GOM_PE1_TSW_10Sept15_517F.b.ab1	*Vibrio sp.*
GOM_PE2_TSW_10Sept15_517F.b.ab1	*Vibrio sp.*
GOM_PE3_TSW_10Sept15_517F.b.ab1	*Vibrio sp.*
GOM_PE6_TSW_10Sept15_517F.b.ab1	Uncultured bacterium
GOM_PHA1_TSW_10Sept15_517F.b.ab1	*Vibrio sp.*
GOM_PHA2_TSW_10Sept15_517F.b.ab1	*Vibrio sp.*
GOM_PHA3_TSW_10Sept15_517F.b.ab1	*Vibrio sp.*
GOM_PHA4_TSW_10Sept15_517F.b.ab1	Bacterium
GOM_PHA6_TSW_10Sept15_517F.b.ab1	Bacterium
GOM_PLA1_TSW_10Sept15_517F.b.ab1	Bacterium
GOM_PLA2_TSW_10Sept15_517F.b.ab1	*Vibrio sp.*
GOM_PLA3_TSW_10Sept15_517F.b.ab1	*Vibrio sp.*
GOM_PLA4_TSW_10Sept15_517F.b.ab1	Bacterium
GOM_PLA5_TSW_10Sept15_517F.b.ab1	*Vibrio sp.*
GOM_PLA6_TSW_10Sept15_517F.b.ab1	*Vibrio sp.*
GOM_PP1_TSW_10Sept15_517F.b.ab1	*Pseudoalteromonas atlantica*
GOM_PP2_TSW_10Sept15_517F.b.ab1	*Vibrio sp.*
GOM_PP3_TSW_10Sept15_517F.b.ab1	Metagenome
GOM_PP4_TSW_10Sept15_517F.b.ab1	Uncultured bacterium
GOM_PP5_TSW_10Sept15_517F.b.ab1	Uncultured bacterium
GOM_PP6_TSW_10Sept15_517F.b.ab1	Uncultured bacterium
GOM_PS1_TSW_10Sept15_517F.b.ab1	*Vibrio sp.*
GOM_PS2_TSW_10Sept15_517F.b.ab1	*Vibrio sp.*
GOM_PS3_TSW_10Sept15_517F.b.ab1	*Pseudoalteromonas sp.*
GOM_PS4_TSW_10Sept15_517F.b.ab1	Uncultured *Vibrio sp.*

GOM_PS5_TSW_10Sept15_517F.b.ab1	*Vibrio caribbeanicus* strain G7C_25m_08
GOM_PS6_TSW_10Sept15_517F.b.ab1	*Vibrio sp.*

Table D. BLAST Data with identities of first colonizers from the Woods Hole Dock (location WH, agar is TSW unless specified as CAV).

Sample Name	**Sequence Aided ID**
Cruise-Plastic-Agar-Date-Primer	**Genus and species**
WH_PE10_TSW_6Sept15_517F.b.ab1	Uncultured bacterium
WH_PE1_6Sept15_517F.b.ab1	Uncultured bacterium clone
WH_PE2_6Sept15_517F.b.ab1	*Pseudoalteromonas sp.*
WH_PE3A_6Sept15_517F.b.ab1	*Pseudoalteromonas sp.*
WH_PE3B_6Sept15_517F.b.ab1	*Pseudoalteromonas sp.*
WH_PE5_6Sept15_517F.b.ab1	Uncultured bacterium
WH_PE6_6Sept15_517F.b.ab1	*Pseudoalteromonas sp.*
WH_PE9_TSW_6Sept15_517F.b.ab1	*Pseudoalteromonas sp.*
WH_PHA10_TSW_6Sept15_517F.b.ab1	*Pseudoalteromonas sp.*
WH_PHA1_6Sept15_517F.b.ab1	*Pseudoalteromonas sp.*
WH_PHA2_6Sept15_517F.b.ab1	Uncultured bacterium
WH_PHA3_6Sept15_517F.b.ab1	*Vibrio parahaemolyticus* strain mer 9
WH_PHA4_6Sept15_517F.b.ab1	*Alteromonas sp.*
WH_PHA5_6Sept15_517F.b.ab1	*Pseudoalteromonas sp.*
WH_PHA6_6Sept15_517F.b.ab1	*Vibrio parahaemolyticus* strain mer 9
WH_PHA7_TSW_6Sept15_517F.b.ab1	*Pseudoalteromonas sp.*
WH_PHA8_TSW_6Sept15_517F.b.ab1	*Polaribacter sp.*
WH_PHA9_TSW_6Sept15_517F.b.ab1	*Alteromonas sp.*
WH_PHA_2_CAV_6Sept15_517F.b.ab1	*Vibrio harveyi* strain mer 2
WH_PLA10_TSW_6Sept15_517F.b.ab1	*Vibrio parahaemolyticus* strain mer 9
WH_PLA1_6Sept15_517F.b.ab1	Uncultured bacterium
WH_PLA2_6Sept15_517F.b.ab1	Marine metagenome
WH_PLA3_6Sept15_517F.b.ab1	Uncultured bacterium
WH_PLA4_6Sept15_517F.b.ab1	*Vibrio coralliilyticus* strain F75157
WH_PLA5_6Sept15_517F.b.ab1	*Tenacibaculum sp*.
WH_PLA6_6Sept15_517F.b.ab1	*Alteromonas marina* strain LAMA 972
WH_PLA7_TSW_6Sept15_517F.b.ab1	*Enterovibrio sp.*
WH_PLA8_TSW_6Sept15_517F.b.ab1	*Vibrio sp.*
WH_PLA9_TSW_6Sept15_517F.b.ab1	*Vibrio sp.*
WH_PP10_TSW_6Sept15_517F.b.ab1	*Alteromonas sp.*
WH_PP1_6Sept15_517F.b.ab1	*Pseudoalteromonas sp.*
WH_PP2_6Sept15_517F.b.ab1	*Alteromonas marina* strain LAMA 972
WH_PP3_6Sept15_517F.b.ab1	Uncultured bacterium

WH_PP4_6Sept15_517F.b.ab1	*Pseudoalteromonas sp*.
WH_PP5_6Sept15_517F.b.ab1	Uncultured gamma proteobacterium
WH_PP6_6Sept15_517F.b.ab1	*Alteromonas sp.*
WH_PP7_TSW_6Sept15_517F.b.ab1	*Pseudoalteromonas sp.*
WH_PP8_TSW_6Sept15_517F.b.ab1	*Vibrio sp.*
WH_PP9_TSW_6Sept15_517F.b.ab1	*Alteromonas sp.*
WH_PS10_TSW_6Sept15_517F.b.ab1	Uncultured bacterium
WH_PS12_TSW_6Sept15_517F.b.ab1	Uncultured bacterium
WH_PS1_6Sept15_517F.b.ab1	*Pseudoalteromonas sp.*
WH_PS2_6Sept15_517F.b.ab1	*Tenacibaculum sp.*
WH_PS3_6Sept15_517F.b.ab1	*Pseudoalteromonas sp.*
WH_PS4_6Sept15_517F.b.ab1	Uncultured bacterium
WH_PS5_6Sept15_517F.b.ab1	*Pseudoalteromonas sp.*
WH_PS6_6Sept15_517F.b.ab1	*Pseudoalteromonas sp.*
WH_PS7_TSW_6Sept15_517F.b.ab1	*Pseudoalteromonas sp.*
WH_PS8_TSW_6Sept15_517F.b.ab1	*Tenacibaculum sp.*
WH_PS9_TSW_6Sept15_517F.b.ab1	*Pseudoalteromonas sp.*

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Figure Sources

Figure 1: Helena McMonagle.

Figure 2: Helena McMonagle (left) and Brittany Mauer (right).

Figure 3: Sampling locations plotted at https://www.zeemaps.com/map?group=1712093#

Figure A: Helena McMonagle, after Rosenberg and Kjelleberg, 1986.