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The plastic-associated microorganisms of the North Pacific Gyre

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ABSTRACT

Microorganisms likely mediate processes affecting the fate and impacts of marine plastic pollution, including degradation, chemical adsorption, and colonization or ingestion by macroorganisms. We investigated the relationship between plastic-associated microorganism communities and factors such as location, temperature, salinity, plankton abundance, plastic concentration, item size, surface roughness, and polymer type. Small plastic items from the surface of the North Pacific Gyre in 2011 were examined using scanning electron microscopy. Bacillus bacteria (mean 1664 ± 247 individuals mm^{-2}) and pennate diatoms (1097 ± 154 mm^{-2}) were most abundant, with coccoid bacteria, centric diatoms, dinoflagellates, coccolithophores, and radiolarians present. Bacterial abundance was patchy, but increased on foamed polystyrene. Diatom abundance increased on items with rough surfaces and at sites with high plastic concentrations. Morphotype richness increased slightly on larger fragments, and a biogeographic transition occurred between pennate diatom groups. Better characterizing this community will aid in understanding how it interacts with plastic pollution.

1. Introduction

Plastic pollution in the marine environment has many impacts on marine and coastal ecosystems including ingestion, entanglement, smothering, leaching of plasticizing chemicals, adsorption of persistent organic pollutants, and the transport of sessile or sedentary organisms to new environments via rafting (reviewed in Gregory, 2009). Small plastic fragments are the most common type of anthropogenic marine debris, and because of their small size they incorporate into and change the physical properties of marine and coastal sediments (Carson et al., 2011), and are ingested by a range of organisms from whales (Fossi et al., 2012) to mussels (von Moos et al., 2012).

The source of some small plastic debris is terrestrial, as evidenced by the abundance of pre-production pellets in global marine environments (Barnes et al., 2009). However, many of the fragments are the result of the degradation of larger items after they are introduced into the ocean (Andrady, 2011; Sudhakar et al., 2007). The rate that larger items degrade on the ocean surface has important implications for the distribution, impacts, and eventual fate of marine plastic pollution. Although the breakdown of plastics by ultra-violet radiation and other factors has been investigated in coastal experiments (e.g. Andrady, 1990; O'Brine and Thompson, 2010), the process is poorly understood in the open ocean.

The formation of biofilms on the plastic surface, in particular, is important to the plastic degradation process (Artham et al., 2009). For instance, organisms may protect the plastic from ultraviolet radiation and photo-catalysis either directly or via decreased buoyancy (Andrady, 2011), thereby increasing longevity. Alternately, the organisms themselves may actively accelerate the degradation process (Balasubramanian et al., 2010; Zettler et al., 2013). Fouling microorganisms likely mediate other processes of concern, such as interactions with persistent organic pollutants (Gouin et al., 2011), colonization by larger organisms and associated changes in buoyancy (Lobelle and Cunliffe, 2011), or inhibiting consumers' ability to distinguish plastic from live prey (Carson, in press). The transport via rafting of the microorganisms themselves is also of concern, because this community is distinct from the that of the surrounding seawater (Zettler et al., 2013), with potential impacts such as the introduction of disease vectors (Goldstein et al., in review) or harmful algae (Maso et al., 2003). Despite the recognition that microscopic organisms are key to understanding and solving many of the problems with plastic pollution (Harrison et al., 2011), the microfouling community on mid-ocean plastic fragments has not been adequately characterized.

Our goal is to relate the abundance and diversity of plastic-associated microorganisms in the North Pacific Gyre ecosystem to a variety of physical and biological factors. Specifically, we ask:

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1) What is the density of common organisms on the surface of open-ocean plastic fragments, as visually quantified by scanning-electron microscopy (SEM)?
2) How does the density or diversity of this community vary across the entire Eastern North Pacific Gyre, and in relationship to sea water temperature, salinity, plankton abundance, presence of larger organisms, and plastic concentration?
3) How does the density or diversity of this community vary in response to properties of the plastic fragments themselves, such as size, polymer type, and surface roughness?

2. Methods

We collected pneustonic plastic from the Eastern North Pacific Gyre in July 2011 aboard the *R/VSea Dragon*. The voyage originated in Honolulu, Hawai'i, USA, and was to include a relatively uniform northeastern transit toward Vancouver, Canada with surface trawl samples collected at regular intervals. Unfavorable weather forced the vessel off the intended path and prevented the deployment of trawls during some days. The voyage resulted in 17 trawl samples spaced across the gyre (Fig. 1), all of which contained plastic (Table 1).

Samples were collected using a manta trawl with an opening measuring 61 cm wide by 16 cm high, trailing a 3 m net of 333-μm mesh with a removable cod end. The trawl was attached to the end of a spinnaker pole placed perpendicular to the long axis of the ship in order to tow the trawl outside the ship's wake. Trawls were deployed for exactly one hour, timed with a stopwatch, while maintaining an approximate speed over ground of 2 knots. A mechanical flowmeter was placed inside the trawl to calculate the exact trawl length given variation in boat speed or surface currents. Trawls were deployed in sea states of 4 (moderate) or less to avoid waves overtopping the trawl opening or the trawl skipping over waves, and to maximize the amount of debris present in the surface layer (Kukulka et al., 2012). Trawl contents were rinsed into a 333-μm sieve, transferred to sample jars, and preserved with buffered 5% formalin. Sea surface temperature and salinity were recorded before and after each trawl using a YSI Model 30 salinity, conductivity, and temperature probe.

In the laboratory, samples were rinsed in distilled water to remove the formalin, and then up to eight fragments or pre-production pellets in the 1–10 mm size class, depending on availability, were removed from each sample for analysis. This resulted in a total of 100 pieces from 15 of the 17 trawl samples, as two trawls did not contain plastic items of an appropriate size. The fragments or pellets were individually rinsed six times with increasing concentrations of ethanol (35–100%) to remove any water, then sputter-coated with gold using a Pelco SC-6. Each piece was then examined for the presence of attached microorganisms using a Hitachi S-3400N Scanning Electron Microscope (SEM). The extensive rinsing and preparation steps make it unlikely that any microorganisms in the trawl that were not attached to the plastic would be imaged during SEM analysis.

For each item, a degree of rotation was chosen using a random number table with integers from 1 to 360, and the viewing stage rotated accordingly. A portion of the piece was then surveyed in an edge-to-edge transect from top-to-bottom at 6000× magnification. All organisms were counted, either at 6000×, or higher magnification when necessary, and representative images saved. The total length of the transect was recorded for density calculations. Three transects at different, random orientations were completed for each piece, and the results pooled. Because the fragments could

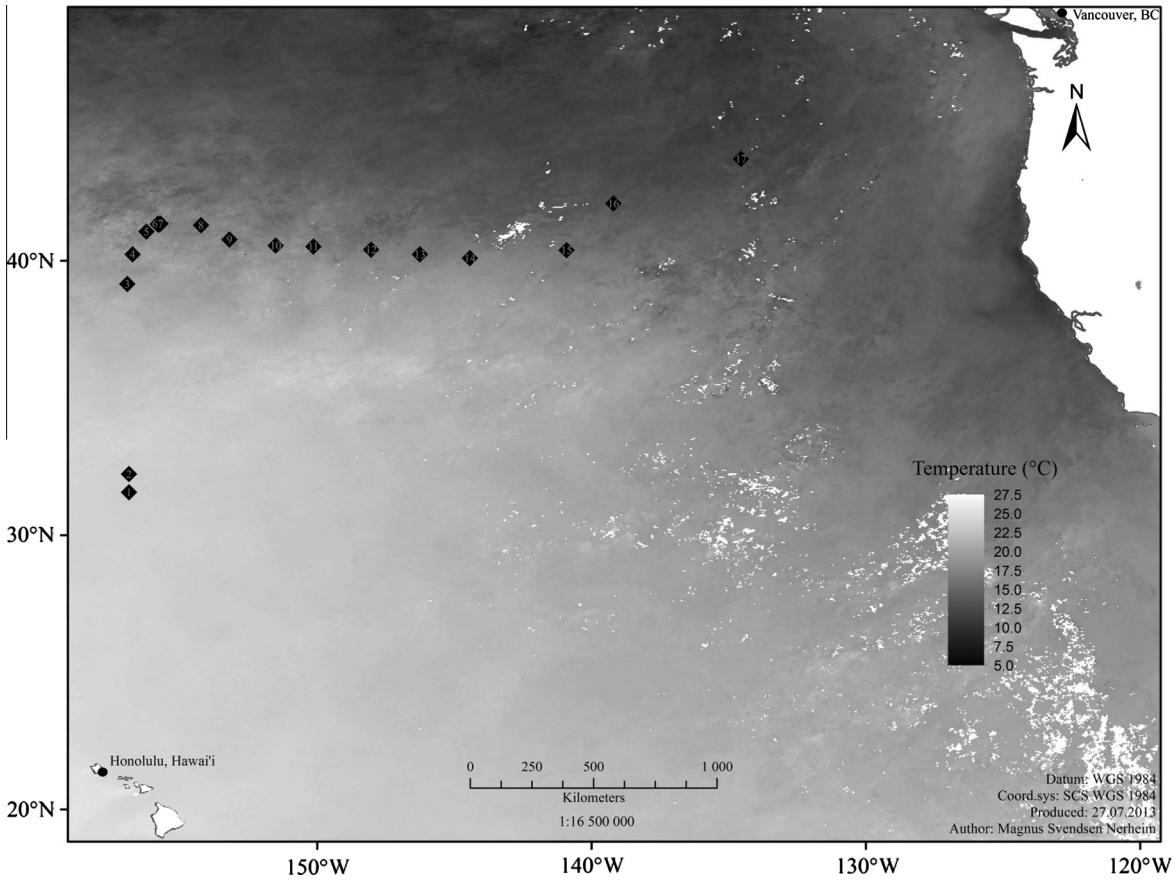

Fig. 1. Map of the 17 trawl locations in the Northeast Pacific Ocean and July 2011 average sea surface temperatures (Nerheim, 2013).

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Table 1
A description of sample collection stations in the Eastern North Pacific Gyre. *Manta trawls 13 and 17 did not contain fragments in the larger size categories as did the other trawls and were therefore excluded from SEM and FT-IR analysis. NA = total estimate of plastic abundance not available due to partial loss of sample.

Station	Date	Latitude (N)	Longitude (W)	Temperature (°C)	Salinity ($^0/_{00}$)	Plastic items	Plastic items (km^{-2})
1	7/11/2011	31.538	156.861	24.6	35.1	76	36,907
2	7/11/2011	32.203	156.833	24.7	35.1	82	33,558
3	7/14/2011	39.125	156.916	20.6	34.1	77	31,841
4	7/14/2011	40.201	156.707	20.2	33.8	150	45,921
5	7/15/2011	41.040	156.237	19.7	33.9	155	85,607
6	7/15/2011	41.321	155.707	19.5	33.9	859	219,585
7	7/15/2011	41.339	155.732	19.7	34.0	151	92,303
8	7/16/2011	41.284	154.255	18.4	32.2	489	217,432
9	7/16/2011	40.781	153.207	19.2	34.0	NA	NA
10	7/17/2011	40.546	151.539	19.1	34.1	43	14,447
11	7/17/2011	40.513	150.160	18.4	33.9	160	70,089
12	7/18/2011	40.404	148.053	18.1	33.7	210	94,353
13*	7/18/2011	40.237	146.270	18.2	33.8	177	43,924
14	7/19/2011	40.090	144.455	18.0	33.7	201	104,289
15	7/20/2011	40.367	140.932	16.9	33.5	141	63,158
16	7/21/2011	42.060	137.542	15.0	33.4	477	172,630
17*	7/22/2011	43.696	134.577	14.5	33.3	62	36,890

not be aged, and surface rugosity (a possible proxy for age) could not be quantified numerically, each piece was classified as either "smooth" or "rough" to represent the complexity of the surface qualitatively.

The microorganisms could not be identified to species as the preservation of samples in formalin precludes molecular techniques such as or next-generation genetic sequencing or community fingerprinting (see Zettler et al., 2013). They were instead classified visually using outward appearance. Diatoms were divided in three groups: group A, pennate diatoms with a raphe present (Bacillariophycidae); group B, pennate diatoms without a raphe (Fragilariophyceae); and group C, centric diatoms (Coscinodiscophyceae). Bacteria were divided into two categories: coccoid (spherical) and bacillus (rod-shaped). We use the term 'bacillus' here to describe the shape, which does not necessarily denote members of the genus *Bacillus*. Other, less abundant organisms that were encountered, such as dinoflagellates, coccolithophores, and radiolarians, were noted (Fig. 2).

When possible ($n = 83$) plastic items were split to expose a fresh surface, and then analyzed for polymer type using a Thermo Nicolet Nexus 670 model Fourier Transform Infrared Spectrometer (FT-IR). Spectra measuring background absorbance were subtracted from the sample spectra, and the results were matched to the closest reference spectrum in the machine's Thermo Omnic software.

Remaining trawl samples were sorted and quantified under dissecting microscopes, after the methods of Lattin et al. (2004), to separate plastic from plankton. Plankton was dried overnight in an oven before being weighed. Plastic was dried and sieved into size categories, then separated by type (fragment, pre-production pellet, foam, line, or film), counted, and weighed.

We attempted to explain the resulting abundance and diversity of microorganisms using a series of analyses of variance (categorical independent variables) and linear regressions (continuous independent variables) that included environmental factors, other characteristics of the trawl site, and attributes of the examined fragments (Table 2). A map of the sample area along with remotely-detected sea surface temperature (Nerheim, 2013) was created using ESRI ArcGIS 10 (Fig. 1).

3. Results

All surface trawls collected in the Eastern North Pacific Gyre in the summer of 2011 contained plastic debris (Table 1). The trawls contained 219 pieces on average, composed of whole objects, fragments, pellets, line, foam, or films, weighing on average 5.4 g per trawl, and translating to approximately 85,184 pieces km^{-2} and 1.929 kg km^{-2} when the effective length of the trawls were taken into account. Although the trawls were of different effective lengths due to variable current flow and boat speed, the minimum amount sampled was 43 pieces (14,000 pieces km^{-2}) at site ten, and the maximum was 859 pieces (219,000 pieces km^{-2}) at site six.

One hundred plastic items between 1 and 10 mm maximum dimension were removed from 15 of the 17 trawls and examined for the presence of attached microorganisms using SEM (Table 3). Of the 83 items that could be identified using FT-IR, 59% were polyethylene, 33% were polypropylene, and 8% were polystyrene. All examined items had attached microorganisms, with bacillus bacteria (mean 1664 ± 243 individuals mm^{-2}) and pennate diatoms (1097 ± 154 mm^{-2}) the most abundant organisms by far. Coccoid bacteria (169 ± 39 mm^{-2}) and centric diatoms (9 ± 6 mm^{-2}) were also encountered in low densities, along with dinoflagellates, coccolithophores, and radiolarians at densities of less than one individual per piece.

The density of bacillus bacteria varied significantly with trawl site ($r^2 = 0.403$, $p < 0.001$) with particularly high densities at sites 8, 12, and 16 (Fig. 3). The density of these bacteria was also related to the type of plastic ($r^2 = 0.193$, $p = 0.002$) with polystyrene harboring significantly more than polyethylene or polypropylene (Fig. 4). The density of bacillus bacteria showed weak and negative correlations to the sea surface temperature ($r^2 = 0.056$, $p = 0.018$), salinity ($r^2 = 0.041$, $p = 0.045$), and longitude($r^2 = 0.054$, $p = 0.020$) of the trawl site. The density of coccoid bacteria was not significantly related to any factors except trawl site ($r^2 = 0.281$, $p = 0.008$), with relatively high densities at sites 7 and 14.

The highest densities of bacteria across the gyre were patchily distributed, compared to that of the diatoms, which generally peaked in the center of the sample transect (Fig. 3). Diatom density was significantly related to trawl site ($r^2 = 0.540$, $p = 0.005$), with high densities between sites 6 through 11, and again at 16. Densities were highest on fragments with rough surfaces ($r^2 = 0.420$, $p < 0.001$) and at trawl sites with higher concentrations of plastic ($r^2 = 0.301$, $p = 0.042$). As with the bacteria, diatoms had weak but significant, negative relationships with temperature ($r^2 = 0.050$, $p = 0.030$) and salinity ($r^2 = 0.050$, $p = 0.022$).

The proportion of diatoms in group A (Bacillariophycidae) generally declined as the transect progressed north and east ($r^2 = 0.408$, $p = 0.010$), while the proportion in group B (Fragilariophyceae) increased concurrently (Fig. 5). Centric diatoms (group C) made up a small minority of all diatoms encountered, and did not show significant relationships to any of the variables in Table 2.

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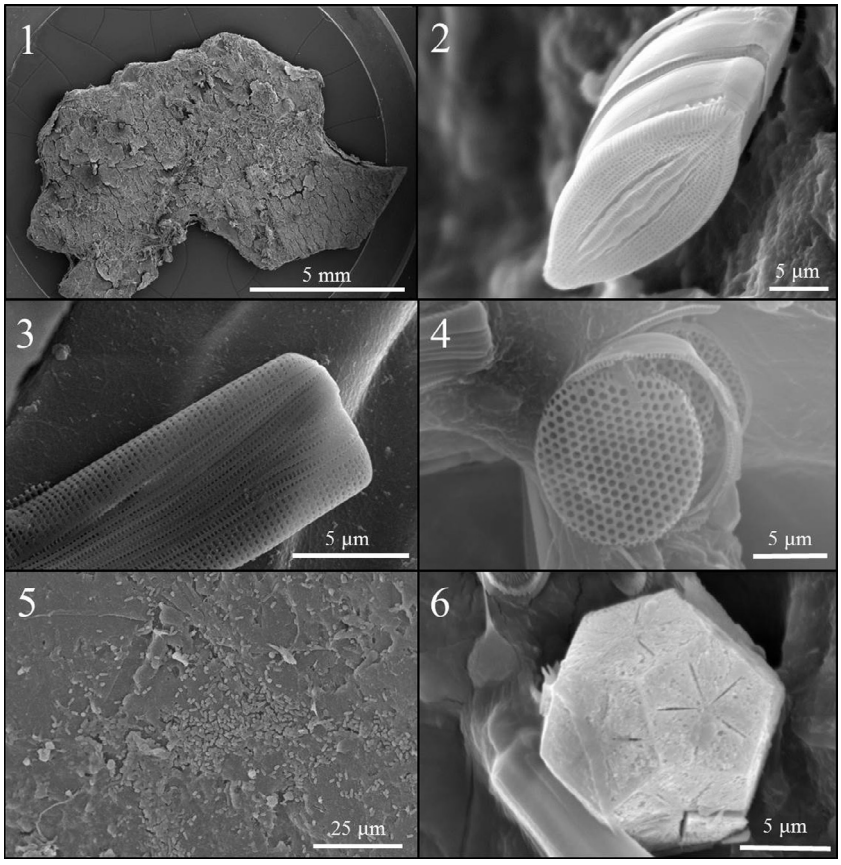

Fig. 2. Scanning-electron micrographs of 1: a typical plastic fragment collected from the surface of the North Pacific Gyre; 2: a common example of diatom group A (pennate diatom with raphe present) exhibiting a pointed oval shape; 3: a close-up of diatom group B (pennate diatom without raphe) exhibiting an elongate shape with squared ends; 4: the damaged frustule of diatom group C (centric diatom); 5: a fragment surface occupied by numerous bacillus bacteria; 6: a rare organism that was not a diatom or bacterium, in this case the radiolarian *Circorrhegma dodecahedra*.

Table 2
Variables used in analyses of variance and linear regressions to relate the abundance and diversity of plastic-associated microorganisms to various factors. Variables marked with an * are categorical.

Dependent variables (units)	Independent variables
Mean abundance, diatoms (#/mm^2)	Trawl site*
Mean abundance, bacteria (#/mm^2)	Latitude
Mean abundance, each subgroup (#/mm^2)	Longitude
Proportional abundance (%)	Temperature
Mean taxa richness (#)	Salinity
Mean taxa diversity (Shannon index)	Abundance of plastic
	Abundance of plankton
	Size of fragment
	Roughness of surface*
	Polymer type*
	Presence of macroorganisms*

Taxa richness, or the total number of different morphotypes on each piece, was positively related to the size of the piece ($r^2 = 0.083$, $p = 0.005$) and also varied with the site of collection ($r^2 = 0.442$, $p < 0.001$). Highest richness was found at sites 2, 7, 10, and 11 but did not uniformly change across the gyre. The Shannon diversity index of each piece, which takes the abundance of each group into account, was not significantly related to any factors tested.

4. Discussion

The mean number of fragments encountered here (~85,000 km^{-2}) is considerably larger than averages found in the South Pacific Gyre in 2011 (~27,000; Eriksen et al., 2013) and North Atlantic Gyre over 22 years at 30°N (~20,000; Law et al., 2010). It is considerably smaller than a previous survey of the North Pacific Gyre (~330,000; Moore et al., 2001) although that study sampled from a much smaller area in the central gyre. The maximum number encountered (~220,000 km^{-2}), is smaller than the maximums reported in those three studies (NPG: ~970,000, SPG: ~400,000, NAG: ~580,000).

The concentration of plastic particles on the surface of the NPG is highly variable in space, similar to findings in other gyre ecosystems (e.g. Law et al., 2010). There was a general increase in plastic in the center portion of the transect near the 40th parallel (Table 1) indicative of an area of higher concentration as predicted by models (e.g. Maximenko et al., 2012). However, the central gyre holds an extremely heterogeneous mixture of debris, evidenced by the minimum concentration trawl that was also collected in the center of the transect, about 360 km from the maximum sample collected two days earlier.

Superimposed onto the patchy distribution of plastic particles is the patchy distribution of plastic-associated organisms. It has long been recognized that the plankton communities of the open ocean are patchily distributed, on the mesoscale (e.g. Garcon et al., 2001) to the microscale (Long and Azam, 2001), so it is not surprising that the plastic-associated organisms are as well. The distribution of bacteria on plastic surfaces was particularly patchy, with spikes in abundance at some sites that could not be well explained by environmental variables ($r^2 < 0.05$). The type of plastic did explain some of the variation in bacterial abundance, because polystyrene fragments harbored significantly more bacteria than did the more

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Table 3
Mean abundance of diatoms and bacteria by trawl site found on plastic fragments in the Eastern North Pacific Gyre. Diatom groups are A, pennate diatoms with a raphe present (Bacillariophycidae); B, pennate diatoms without a raphe (Fragilariophyceae); and C, centric diatoms without polarity (Coscinodiscophyceae). Plastic from trawls 13 and 17 were not examined.

Trawl	Pieces	Mean diatoms mm^{-2}				Mean bacteria mm^{-2}			Mean diversity (H′)	Max taxa richness (piece)
		Total	*A*	*B*	*C*	Total	Coccoid	Bacillus		
1	8	174	131	40	1	640	224	416	0.51	6
2	6	141	48	79	0	2216	146	2070	0.50	5
3	8	700	685	16	0	2349	0	2349	0.47	3
4	8	1044	612	417	0	2439	356	2083	0.72	5
5	8	1000	568	424	0	99	9	90	0.75	4
6	8	2770	502	2240	0	482	49	433	0.64	5
7	5	1175	588	531	1	990	827	164	0.85	7
8	8	2242	2129	80	33	3708	0	3708	0.62	4
9	5	1688	380	1194	0	597	7	591	0.82	5
10	4	2169	1017	961	0	962	153	809	0.96	6
11	6	2393	302	1584	104	708	230	478	1.02	6
12	8	344	77	179	9	3885	70	3814	0.61	6
14	5	305	39	217	0	520	518	1	0.40	6
15	8	648	63	404	1	1213	27	1186	0.55	6
16	6	1684	198	1187	0	5593	265	5328	0.54	5
	100	1188	502	595	9	1833	169	1664	0.64	5

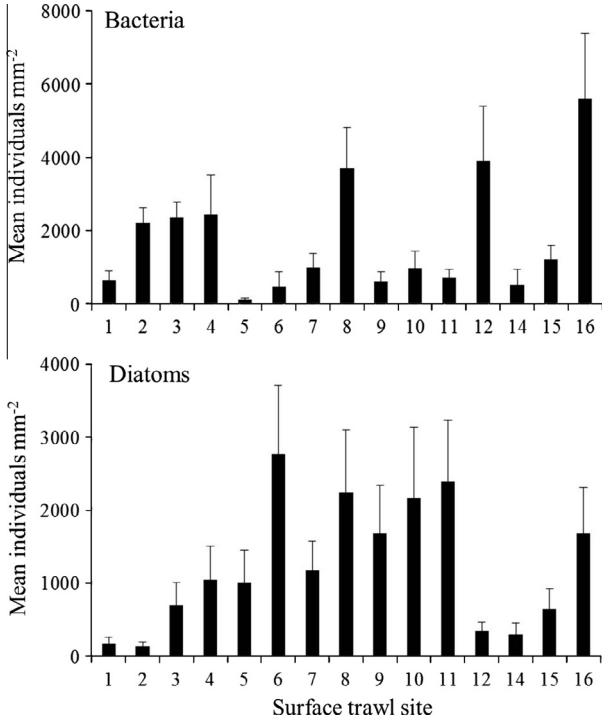

Fig. 3. Mean density of bacteria (top) and diatoms (bottom) found on small pieces of plastic trawled from the surface of the Eastern North Pacific Gyre in summer 2011. Error bars represent the standard error of the mean. No plastic pieces were examined for trawls 13 and 17.

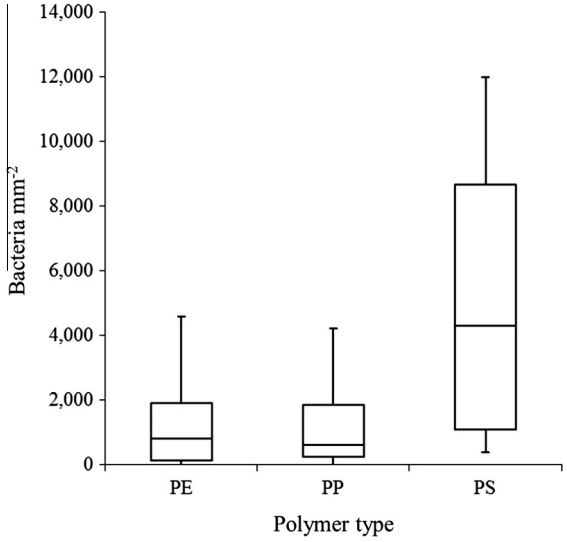

Fig. 4. Box plot of bacterial abundance by plastic polymer type. PE = polyethylene, PP = polypropylene, PS = polystyrene (foamed). Boxes extend from quartile 1 to 3, and whiskers extend 150% of the interquartile range above and below the first and third quartiles.

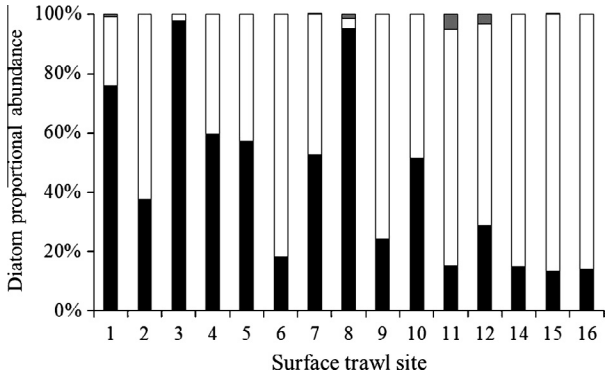

Fig. 5. Proportion of diatom groups by trawl site. Group A (pennate with raphe) are shown in black, group b (pennate without raphe) in white, and group C (centric) in grey.

common polyethylene or polypropylene (Fig. 4). All polystyrene found in trawls was foamed, as unfoamed polystyrene has a density greater than that of seawater. It is possible that the structure, or some other attribute, of foamed substrate is more favorable for marine bacterial growth than unfoamed polymers.

Although diatoms were also patchily distributed, their abundance did peak in the center of the transect, as did the concentration of plastic. The significant relationship between diatom abundance and plastic density could be because the same hydrodynamic forces which concentrate plastic along fronts and within eddies also concentrate drifting organisms (Pichel et al., 2007). The higher densities of plastic in these areas could make it easier for plastic-associated diatoms to colonize newly available surfaces. One might expect that total surface plankton biomass would also

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peak where plastic abundance is greatest if both are concentrated by small- and meso-scale eddies and fronts. However, as the time of day the trawls were deployed was not standardized, the vertical migrations of many organisms inflated the plankton abundance in trawls conducted in the early morning or late afternoon. Since most plastic remains at the surface in calm conditions regardless of the time of day, the two abundances were not related.

Qualitative surface "roughness" of particles was also positively related to diatom density, perhaps because it aids in attachment for the larger diatoms (Bravo et al., 2011), whereas it was not apparently important to bacteria. Diatom abundance and bacterial abundance were not significantly related to each other, despite evidence that bacterial colonization often precedes and facilitates colonization by benthic diatoms (reviewed in Wahl, 1989). If rough surfaces, often a result of plastic degradation, harbor more microorganisms, the degradation process may accelerate colonization leading to eventual sinking, or make the item more likely to be ingested, passing adsorbed persistent organic pollutants up the food chain (e.g. Tanaka et al., 2013). The central gyre, where plastic and some plastic-colonizing microorganisms are concentrated, may also see accelerated microbial effects compared to outlying areas due to the greater density of diatoms or other organisms.

Although no specific taxonomic identification of diatoms was possible, many appeared similar in outward appearance to certain genera. Diatom group A, pennate diatoms with raphe present, overwhelmingly had pointed oval or "American football" shapes similar to some members of the genus *Mastogloia*, a genus that is abundant in the North Pacific (Brzezinski et al., 1998) and is known to colonize plastic in the Atlantic Ocean (Carpenter and Smith, 1972). A minority had more elongate pointed oval shapes, similar to some members of the genera *Haslea* and *Frustulia*, or had bilobed or "peanut" shapes as do the genus *Diploneis*. The majority of diatom group B, pennate diatoms without a raphe, had very elongate, rectangular "rod" shapes, often with rounded-square ends, reminiscent of some members of the genera *Ardissonea*, *Protoraphis*, and *Fragilaria*. A small minority were colonial, in fan or star arrangements, similar to the members of the genus *Thalassionema*. Most centric diatoms (group C) were found in poor condition, making visual comparisons to known genera difficult, although all examples were circular and did not show polarity away from the central axis.

Goldstein et al. (in review) found that diversity of fouling organisms increased with the size of the plastic item in the North Pacific, in a new application of the theory of island biogeography. We found weak but significant evidence for a similar increase in microbial taxa richness, but not diversity, as the size of the plastic fragment increased. Neither richness nor diversity was significantly related to other environmental variables or attributes of the plastic particles. Although temperature and salinity were related to bacteria and diatom abundance, they accounted for less than 10% of the variation in those abundances. Therefore, our samples suggest that the plastic fouling community may be fairly uniform in composition, if patchily distributed, across the Eastern North Pacific Gyre. One significant biogeographic transition was noted – a shift from diatom group A to group B as the transect progressed northeasterly (Fig. 5). Of course, we have no information about the temporal stability of this community from these samples.

Diatoms dominate the microfouling community on the abundant plastic fragments in the Eastern North Pacific Gyre by mass. A surprisingly small number of general body shapes were encountered, although this may still represent considerable diversity at the species level, and may vary on larger debris than the particles examined here. If a relatively limited variety of diatoms or bacteria compose the majority of the microfouling community on open ocean plastic, it may simplify the study of the effects these organisms have on the degradation, buoyancy, colonization of larger organisms, and chemical adsorption properties of marine debris. However, the results of Zettler et al. (2013), published while this manuscript was in review, established considerable diversity on plastic fragments in the North Atlantic Gyre through molecular techniques. These techniques certainly would have uncovered greater diversity in our samples, particularly in bacteria. Species identification, as well as their relative abundances and distribution in space, are needed to assess the impacts of the microbial community on gyre ecosystems and the plastic within them. Species identification could also allow for more direct impacts of plastic microorganisms to be assessed, such as the transport of potential invaders, harmful algal species, or disease vectors.

Given the current rate of increase in global plastic production, it is likely that plastic debris will be present in significant quantities on the ocean surface for centuries. A major unstudied aspect of the issue is an estimate of the "end game" of surface pollution once the input of debris can be slowed. The rates or processes that control degradation and/or sinking in the open ocean are not well understood, but both are likely mediated by the formation of microbial biofilms (Harrison et al., 2011). Identifying the dominant organisms of this community, their distribution in the ocean, and their effects on the debris they inhabit, will be a crucial step in understanding the future of plastic pollution in the ocean.

Disclaimers

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