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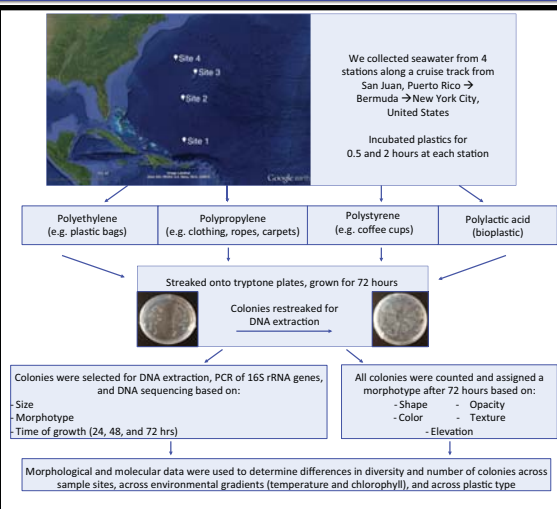
## Abstract

Plastic pollution, the primary form of marine debris, is an increasing environmental concern, including in the Sargasso Sea, located in the North Atlantic subtropical gyre, where plastics are known to accumulate. These plastics provide a previously unavailable habitat for microbial communities and successional colonizers. Plastic colonizers play a role in the fate and ecological impacts of plastics, which may transport invasive species, harmful algae, and potential pathogens. In order to understand how newly deposited plastics are colonized in the open ocean, we performed morphological and genetic analyses of the first colonizers of plastics, microbes. We studied the microbes that grew on different plastic types after incubation times of 0.5 and 2 hours at four sampling sites spanning different latitudes in the Sargasso Sea. We found differences in colonization across sites, as well as between plastic resin types. Our molecular results revealed a wide variety of biofilm forming genera such as *Marinobacter*, *Halomonas*, *Idiomarina*, *Alteromonas*, and *Pseudoalteromonas*. This study offers the first glimpse of the very early colonizers of newly deposited plastics in open ocean ecosystems.

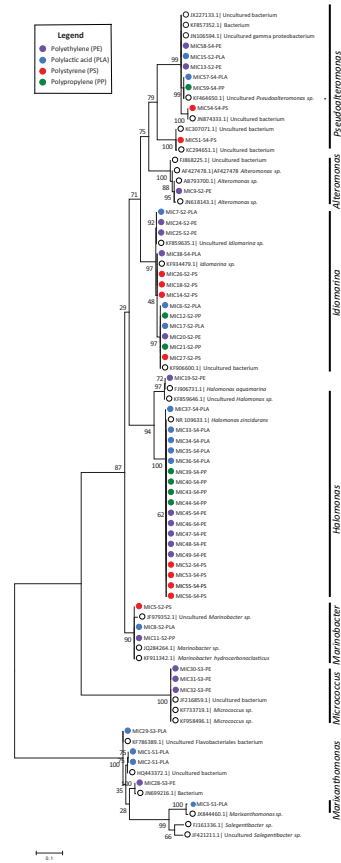
## Introduction

- Plastic pollution is a growing concern in ocean gyres, like the Sargasso Sea. Plastics, particularly microplastics (<5 mm) are known to accumulate there [1]
- These plastics provide new habitats in open ocean ecosystems for microbes and other colonizers. This could have significant effects on ecosystem health and function [2]
- Plastics can be directly deposited in the open ocean through fishing and shipping traffic, and by organisms that ingest and egest small pieces of plastic [1,3]
- Plastics could provide a means of dispersal for harmful algae, persistent organic pollutants (POPs), and invasive species [4-7] but little is known about the identity of the first colonizers of plastics in the open ocean
- Previous research has studied the effects of plastics, and the organisms that colonize them after 24 hours or more [8,9]
- We studied the early colonization (0.5 and 2 hours) of virgin plastic pellets, made of various resin types, in open ocean ecosystems to better understand the initial stage of succession in the communities of these novel ecosystems

## Methods

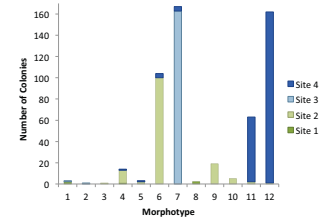


## Results & Discussion



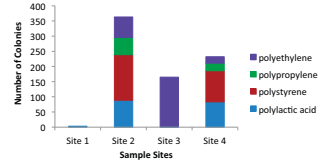
**Figure 1.** A phylogenetic tree based on partial 16S rRNA genes inferred using the Maximum Likelihood method. Taxonomic groups are shown to the right and resin types are color coded.

- Our genetic results revealed differences in taxa among sampling sites and plastic resins
- Certain genera had no preference for plastic type and grew on all four types (e.g. *Halomonas*), while other genera had a plastic type preference (e.g. *Marixanthomonas* (PLA))
- We found similar bacteria at sites 1 and 3, and sites 2 and 4 respectively. This might be due to similar environmental factors at sites, despite different latitudes

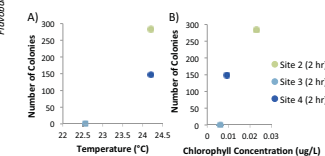


**Figure 2.** Total number of colonies of each morphotype on all plastic types across the four sampling sites.

- Our results revealed different dominant morphotypes at different sample sites
- No single colony morphotype occurred at more than two sites
- This could be because plastic-colonizing microbial communities differ based on location



- The total number of colonies found at each sampling site, grouped by plastic resin type.
- There were no statistically significant differences between the number of colonies or morphotypes on the different plastic types, but trends showed lower growth on polypropylene
- The number of colonies that grew at each sampling site varied substantially
- Morphotypes differed by plastic type, which could mean that plastic-colonizing microbes have preferences for certain resins



**Figure 4.** The total number of colonies found after 2 hour incubations at sites 2 through 4 (no 2 hr data available for site 1) compared across temperature (A) and chlorophyll (B) gradients.

- Regression analyses revealed positive trends between the number of colonies and temperature, and between the number of colonies and chlorophyll concentration if site 1 is excluded
- These environmental factors have been shown to correlate with greater productivity of microbial communities

## Policy Implications

- This research may inform future management related to:
  - Human health and environmental health, particularly concerning the ability of plastics in the ocean to transport invasive species, harmful algae, and pathogens
  - Responsible plastic waste management, which may include the removal of plastic colonizers to avoid the transport of invasive species
  - The selection and use of microbes for bioremediation, such as using bacteria that can metabolize hydrocarbons found in the environment



**Figure 5.** Microplastics were a typical finding in the plankton nets that we deployed throughout the Sargasso Sea. Photo: A. Siuda



**Figure 6.** The initial microbial colonizers of plastics may lay the foundation for macroorganisms to live in this floating ecosystem. Photo: H. McMonagle

## Conclusion

- Marine bacteria attached to various plastic types within 30 minutes of incubation
- Different genera dominated certain sample sites and plastic resin types
- Many early colonizers are part of genera that contribute to biofilm formation
- One of the early colonizers we found, *Marinobacter hydrocarbonoclasticus*, is a close relative of a bacterium that metabolizes hydrocarbons
- Species abundance may be related to chlorophyll and temperature
- This study contributes to a broader understanding of:
  - The succession of organisms that live on marine plastics by identifying some of the earliest colonizers
  - How open ocean microbial communities interact with solid substrates, such as human-introduced plastics

## Acknowledgements

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## References

- Cole, M., P. Lindeque, C. Halsband, and T. S. Galloway. 2011. Microplastics as contaminants in the marine environment: a review. *Marine Pollution Bulletin* 62: 2586-2597.
- Zettler, E. R., T. J. Minner, and L. A. Amaral-Zettler. 2013. Life in the "plastisphere": microbial communities on plastic marine debris. *Environmental Science & Technology* 47: 7137-7146.
- Wright, S. L., C. R. C. Thomson and T. S. Galloway. 2013. The physical impacts of microplastics on marine organisms: A review. *Environmental Pollution* 178: 483-492.
- Maso, M., E. Garce, F. Page, and J. Camp. 2007. Drifting plastic debris as a potential vector for dispersing Harmful Algal Bloom (HAB) species. *Scientia Marina* 67: 107-111.
- Andrady, A. L. 2011. Microplastics in the marine environment. *Marine Pollution Bulletin* 62: 1596-1605.
- Rochman, C. M., E. Hoh, T. Kurobe, S. J. Teh. 2013. Ingested plastic transfers hazardous chemicals to fish and induces hepatic stress. *Scientific Reports* 3.
- Barnes, D. K. A. 2002. Biodiversity: invasions by marine life on plastic debris. *Nature* 416: 808-809.
- Dang, H. and C. R. Lovell. 2000. Bacterial primary colonization and early succession on surfaces in marine waters as determined by amplified rRNA gene restriction analysis and sequence analysis of 16S rRNA genes. *Applied and Environmental Microbiology* 66: 467-475.
- Lobelle, D., and M. Cunliffe. 2010. Early microbial biofilm formation on marine plastic debris. *Marine Pollution Bulletin* 62: 197-200.