A Prototype Citizen-Science Microplastics Sampling Protocol to Quantify the Magnitude of Pollution Along a Community Beachfront

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Introduction

In March 2019, a young Cuvier's beaked whale washed ashore in the Davao Gulf of the Philippines. This whale died from starvation, and after an autopsy opened its viscera, 88 pounds of tangled plastics and garbage were found (Borunda, 2019). Simultaneously, the Pacific Garbage Patch, a conglomeration of plastic bottles, trash, straws, and netting, double the size of France, continually and inexorably expands in mass. Despite the prevalence of plastics pollution in news media, the pollution is ignored and we continue with the glut of plastics consumption. As international efforts to curb plastic garbage remain stagnant, the plastics break down into smaller and smaller pieces, where they continue to infiltrate and poison the environment (National Geographic Society, 2012).

Microplastics are now the most prevalent form of debris in oceanic and freshwater bodies (Masura, Baker, Foster & Arthur, 2015). These particles, which range in size from a sesame seed (5mm) to smaller, enter the environment via runoff and break down into minute particles through mechanical, biological, and oxidative degradation. When a plastic particle enters the environment, the toxins it has absorbed diffuse into the soil and water (Lamizana, n.d.). Ingested by marine life, these particles have a high chance of traveling up the marine food chain (Masura, Baker, Foster & Arthur, 2015).

Generally speaking, there are three main categories of microplastics. Microbeads are a type of microplastic that are manufactured to be used mainly in wash-off cosmetics (acne face wash and face masks), personal care, and cleaning products. As they are not captured by most wastewater treatment systems, microbeads infiltrate bodies of water thereby harming the environment (Plastic microbeads, n.d.). Microfibers are a plastic particle found in all synthetic fiber clothing, most commonly polyester. At the molecular level, these fibers look like strands of hair. When washed, microfibers are released from the clothing and bypass filters in the average washing machine, and ultimately, end up in waterways (The Story of Microfibers, n.d.). All other types of plastics, ranging from car tire remnants to decomposed water bottles are categorized under the term microplastics.

A two-year study conducted by The National Oceanic and Atmospheric Administration (NOAA) Marine Debris Program and The National Park Service quantified microplastics throughout many national parks in the United States. Results from the study of parks in the Northeast (i.e. Fire Island National Seashore and Gateway National Recreation Area (Staten Island)) revealed that at the Fire Island site, an average of 106.3 microplastics particles per kilogram of sand were collected. Additionally, in Staten Island, an average of 88.8 microplastics particles per kilogram of sand were collected (Whitmire & Van Bloem, 2017). The conclusion that every single national park had some level of microplastics pollution abundant within its ecosystem means that they are ubiquitous in water throughout the world. The amount of microplastics was location dependent: the least amount of microplastics were found in national parks in Arkansas, and the most were found in The Great Lakes and The Apostle Islands National Lakeshore. Regardless of amount, all of these sites have a dangerous amount of plastics. The source of these microplastics is generally waste from urban areas, mainly wastewater discharge (Yu & Ladewig, 2017).

Research conducted by Magadini et al. (2018) on New York's faunal ecosystem found that microplastics pollution had infiltrated the food chain. Following oxidation and filtration of marine samples, the researchers used UV spectroscopy to categorize and count the number of plastics. FT-IR spectroscopy confirmed the types of microplastics present in the samples and results showed that both bottom feeders like clams and mussels, and mummichog fish had ingested hundreds of counts of plastic particles. Current research is still being conducted on the potential effects of microplastics on marine life. After ingestion by fish and shellfish, microplastics and the chemicals they contain travel the food chain where they may be ingested by humans raising concerns about potential human health impacts (Smith, Love, Rochman & Neff, 2018). Plastic particles can absorb harmful substances and act as a pathway for disease and environmental toxins (Lamizana, n.d.). Most plastics contain endocrine disrupting chemicals (most prevalently Bisphenol A) that, when digested, can have adverse effects on the individual (Chen, 2019). These disruptors can cause cancerous tumors, birth defects, and other developmental disorders (Lamizana, n.d.).

The growing concerns about microplastics pollution has led to local legislative action. As recently as 2016, Suffolk County (New York) passed a law charging customers for plastic bags, following the rationale that only 2% of plastic is recycled with the remainder "clogging up waterways and roads" (Newsday, 2018). Despite customer dissatisfaction, since 2017, the use of plastic bags in bags was down 81.7 percent, about 1.1 billion bags (Parrish, 2019). No known laws exist for Nassau County nor from New York City or New Jersey, the closest areas to the Long Island South Shore in the tristate region. National efforts have banned plastic bag usage in six states (see appendix 1), in the Northeast and West Coast (Gibbens, 2019).

Research has demonstrated that microplastics are both abundant and ubiquitous to marine environments, with the NOAA study quantifying microplastics at several locations close to my home. As a concerned community member, my research goals for this experiment included a naturalistic survey assessing the magnitude of microplastics pollution in a marine ecosystem near my community and whether the measured quantities of microplastics pollution was associated with human population densities. I collected mole crabs (*Emerita analoga*) to quantify microplastics in their gastrointestinal tract.

Experimental Goals and Hypotheses

This experiment involved a naturalistic survey assessing whether microplastics pollution (particles < 5 mm) may be associated with the health of marine ecosystems. My study focused on quantifying the accumulation of microplastics in Emerita tapoida. I hypothesized the following:

1- As I sampled westward along Long Beach (noting its proximity to New York City, increasing population density, and more entry points for plastic waste) I would find larger numbers of microplastics inside the mole crab samples.

2- The number of microplastics would increase with the size and age of each mole crab, as older mole crabs would filter more content from the environment and thus, ingest more plastics pollution.

Methods

Mole Crabs (verified as *Emerita talpoida* through DNA barcoding) were selected as the target organism. Inhabiting the swash zones of sandy beaches, mole crabs burrow into the sand, awaiting the receding waves as an opportunity to secure their food (carcasses, smaller crabs, mollusks, worms, algae, plankton) (Deane, 2019). These marine invertebrates feed on organisms ranging from .004mm to 2mm in diameter and are themselves prey to fish and birds (The Pacific Mole Crab, n.d.).

Samples Collection. With written permission from the town council[1], we selected two areas: **Lido Beach** (T40°35'07.9"N, 73°37'08.0"W) and **Long Beach** (40°34’58”N, 73°41’02”W and 40°34’57”N, 73°41’29”W).

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[1] *The beach below the natural ordinary high water mark is public trust property and therefore anyone including you can be on the beach. It sounds like you will take just small samples and especially if you are going to replace them, the town should have no objection. If you could send me the results of your study, I would appreciate it as I am a bit of a science nerd with a special interest in Lake Michigan.*
Robert LeMay

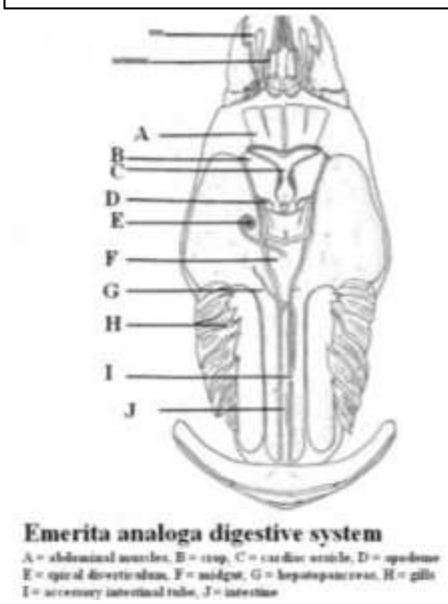
I constructed a core sample collector (see appendix 2) following the directions outlined by Tufts University and collected two cores six meters from the water line (University, T, n.d.). No invertebrates were found using this method and I transitioned to a 10” straining pan. Standing in the swash zone, I waited for the wave to recede and placed an edge of the strainer 2-3 inches deep in the sand. The receding wave washed through the mesh, depositing sand, shells and debris. I sifted through the sand and isolated the mole crabs which were stored in 99% ethyl alcohol and frozen.

Quantifying Microplastics

Isolating the GI Tract

Specimens were weighed and measured before dissection. The average weight of the mole crab was 1.16g and the average weight of the GI tract was 0.04g. Following the procedure of the UC Santa Cruz Physical and Biological Sciences guide for dissecting Emerita analoga, the specimen was placed on the ventral side, distal end oriented to the left of the dissection plate. Incisions were made from the dorsal to the ventral side, isolating the spiral diverticulum, the midgut, the accessory intestinal tube and the intestine (Dissection of Decapod Crustacean, n.d.).

Figure 1 and 2: I followed the diagram (left) in order to isolate the key structure of the mole crab (right)



Isolating, Staining, and Imaging Microplastics

For the first round of collections (24 samples), I adapted protocols published by NOAA (2015) and the University of Washington (Hydrogen Peroxide Procedure, n.d.). I placed the isolated GI tracts into plastic centrifuge tubes and added 20 µl of 30% hydrogen peroxide. Containers were incubated in a VWR WB02 water bath at 74ºC until no organic material was visible. The liquid was then filtered through GF/F 25mm filter paper and left to evaporate, leaving the plastics on the filter paper.

Nile Red is a lipophilic fluorescent dye, which when used for staining the microplastics, "absorbs onto plastic surfaces and renders them fluorescent when irradiated with blue light" (Maes, 2017). I placed the filters onto a glass well-plate and added 10mL of 0.01g nile red in acetone solution. When staining with Nile Red, "different types of plastic displayed different fluorescent colours" (Maes, 2017).

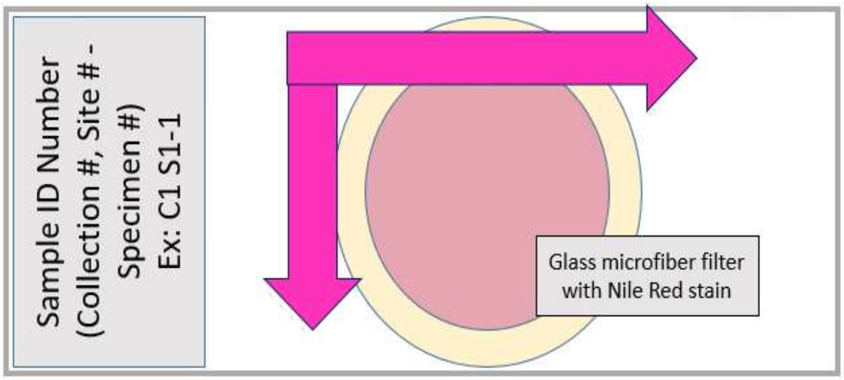
Under the UV microscope, I noticed that there was still a large amount of undigested tissue in the form of lipids which may have been a result of using hydrogen peroxide which is better at dissolving protein than lipids. Consequently, the samples were soaked in a solution of 15% sodium hypochlorite to allow for the digestion of any remaining lipid tissue.

As the samples were held and digested in plastic containers there may have been contamination of plastics from the container. As a result, I collected an additional nine samples with the same procedure, three from each the previous locations, with the following changes to minimize the chance of plastics contamination and to promote a more uniform digestion of samples:

- I used only glass containers to hold the crabs and contents
- During the digestion process, I used 15% sodium hypochlorite instead of the 30% hydrogen peroxide.

Quantifying Microplastics

I viewed each sample under a UV microscope and counted the number of microplastics by category: microbead, microfiber, microplastic.



Figures 3 and 4: I followed the same procedure for viewing slides under the UV microscope (left). Images of microbead, microplastic, and microfiber from left to right (below).

Results

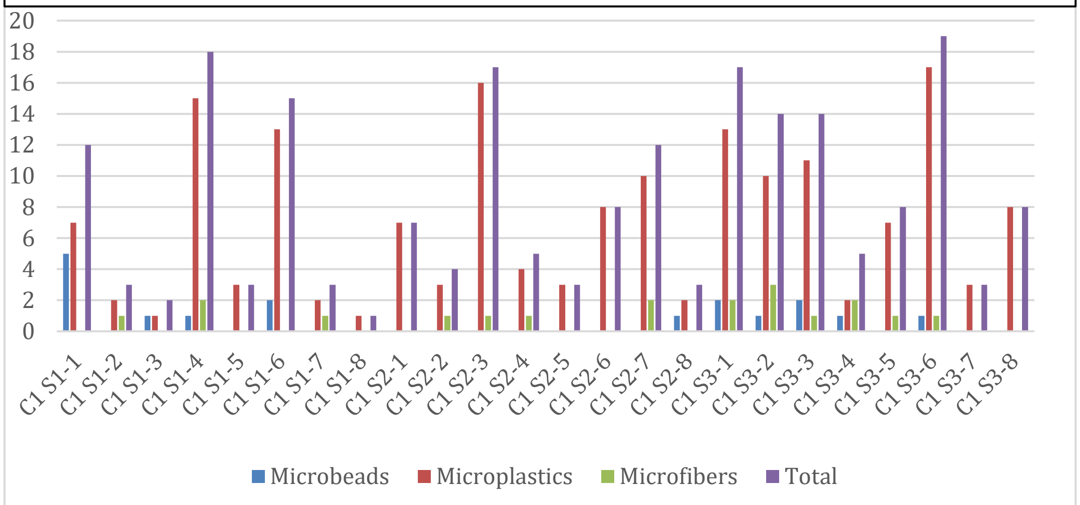
Bioinformatics using DNA Subway

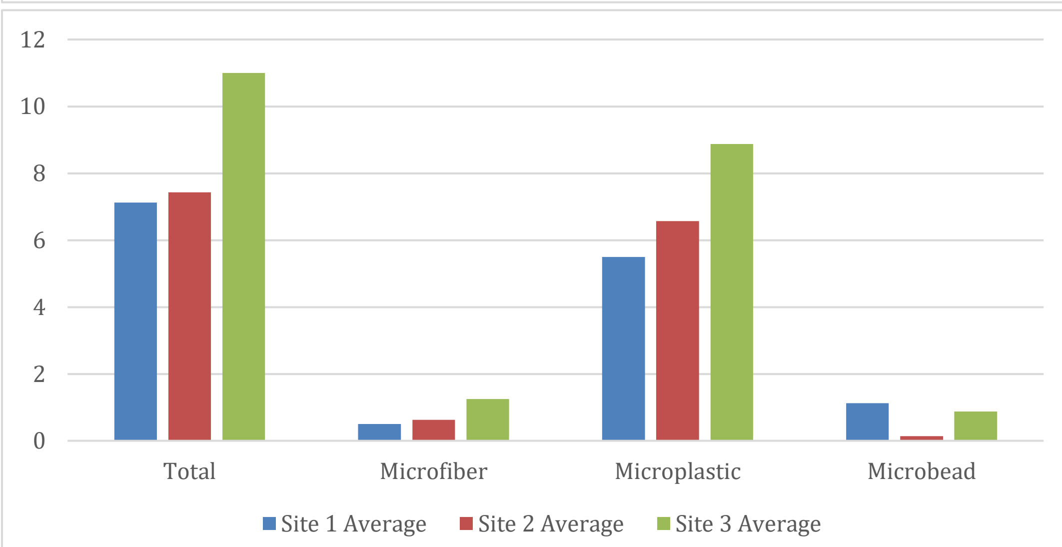
Results suggested that the closest match for the crabs was *Emerita talpoida*, the Atlantic Mole Crab (Emerita talpoida, 2018). Although close matches included *Emerita portoricensis* and *Emerita analoga*, these species of mole crab are non-native to the East Coast, instead inhabiting Puerto Rico (Puerto Rican sand crab, 2018) and the American West Coast, respectively (Emerita analoga, 2018).

Microplastics Counts

All three types of microplastics were found across the locations, non-bead/non-fiber microplastic particles were the most common form of pollution found in the samples across the sites. Microbeads were the second most form of pollution found.

Figures 5 and 6: The individual quantity of each crab's microbead, microplastic, microfiber, and total microplastic quantity organized by site and collection number.





	Total	Microfiber	Microplastic	Microbead
Site 1 Average (*SD*)	7.125 (6.749)	0.500 (0.756)	5.500 (5.606)	1.125 (1.727)
Site 2 Average (*SD*)	7.429 (4.926)	0.625 (0.744)	6.571 (4.719)	0.143 (0.354)
Site 3 Average (*SD*)	11.000 (5.806)	1.250 (1.035)	8.875 (4.998)	0.875 (0.835)

Figure 7: The averages are shown per site, as a total of all microplastics, microbeads, microplastics, and microbeads. Although statistically nonsignificant, Site 3 seemed to have more microplastics.

ANOVA analyses comparing total microplastics across each site were non-significant at the $p < .05$ level nor were any correlations found at the $p < .05$ level between total microplastics, the individual types of microplastics, and bodyweight and GI tract weight.

Discussion

Results indicated no significant differentiation between microplastics pollution across each site. When controlled for the size of the crabs, each site had approximately the same amount of plastics. Though statistically nonsignificant, I found that the greater the mass of the crab, the more plastics it contained. This may be a function of age as the older the crab, the more food and consequently, the more plastics it would ingest. Research by Watts (2014) has shown that microplastics are retained in the body of the crab for up to 14 days following ingestion. The main predators of sand crabs are fish, seabirds, and shore birds (Sand crab, n.d.) and therefore, further investigation is warranted into whether these animals also have plastics in them and if so, the quantity of those microplastics. As the microplastics move higher up the food chain, the prospect of these microplastics being consumed by humans raises concerns for and the need for future research on potential health risks.

Implications for Human Health

Studies show that every item intended for human consumption may contain microplastics particles in some form. People ingest about 70,000 microplastics annually from drinking water and eating food. People who drink solely out of plastic water bottles can potentially ingest an additional 90,000 microplastics per year (Safina, 2019).

Microplastics absorb toxins such as PCBs found in polluted waters (Christensen, 2017). These PCBs can lead to increased rates of melanoma, liver cancer, gallbladder cancer, biliary tract cancer, gastrointestinal tract cancer, and brain cancer, and may be linked to breast cancer (Clearwater, n.d.). Another pollutant found in these plastics is Bisphenol A (BPA). BPA is is a building block used in polycarbonate plastics. This chemical is an endocrine disruptor, possibly influencing multiple endocrine-related pathways (Rubin, 2011). When humans catch fish for consumption, they may unknowingly eat the plastics and the toxins these plastics carry. The average adult human consumes about 70,000 microplastics particles per year in their body (Science Daily, 2019). Research has found that microplastics can infiltrate organisms from the GI tract. When examining the microplastic consumption of mussels, Brown (2008) discovered that the plastics had entered the circulatory system. Further research needs to be conducted to determine whether these plastics can infiltrate the bloodstream of other organisms including humans.

Future Efforts and Research

The remainder of the jetties on the beach should be sampled, collecting the same specimens to document any long-term changes in biodiversity and microplastics pollution.

Researchers should sample at least three additional jetties, maintaining consistency with the equipment (use of straining pan) and procedures. I recommend that students collect a minimum of five (5) crabs of adequate size (around 3.175 cm in length) from each site. Future collections should consider spacing collection locations significantly far away from one another and collecting data in a longitudinal study from many locations over a series of months or years along the beach from end of Long Beach to the other. I also recommend that future researchers collect sand from each of the sampling locations so that microplastics can be isolated from the sand and counted in order to establish possible correlations between the microplastics in the sand and the organisms.

Researchers should use the dissection outline prescribed by PBSci, isolating and storing the GI tract of each sample and digesting them sequentially in 30% hydrogen peroxide followed by 15% sodium hypochlorite. When plastics quantification is conducted, researchers should locate any bright spot on the filter stained with nile red, which indicates a plastic. Microbeads appear as circular bright spots, microfibers look like bright red or green strings, and microplastics are any other shaped bright spot on the filter.

Future efforts must translate research into meaningful and immediate change that connects the scientific community, local governments, and citizens, and directs them toward reducing the vast quantity of plastic pollution in our environment. I propose the following research pipeline:

1. **Research must continue measuring the prevalence of microplastics in the environment.**

Future studies should promote a clearer understanding of the amount of microplastics in the environment so that the public can be informed of the full magnitude of microplastics pollution. The soil, groundwater, and oceans all need to be studied and closely monitored. This methodology should be applied to shoreline communities beyond Long Beach as beachgoers deserve to know their potential exposure to microplastics in the sand and water. Further research must examine the bioaccumulation of microplastics across Long Beach's marine and terrestrial food chains and its possible transmission to the residents of this community. Regardless of whether science rejects or affirms the danger of plastic pollution, the public needs to be informed of how individuals can contribute to limiting their role in adding microplastics to the environment.

2. **Research must identify vectors of entry for microplastics into the environment.**

The accumulation of microplastics in the environment is the consequence of years of ignorance and inaction toward limiting plastics use.

Plastic disposable ware. The constant cycle of producing and plastic cutlery is a main factor in environmental pollution. These single use forks, knives, spoons and straws end up in the environment after disposal. The constant cycle of producing and disposing is a main cause of ocean pollution.

Shipping Routes. Because organisms latch onto ships, the routes the ships travel should be examined to determine the impact these routes have on plastic pollution. Organisms that have previously ingested plastics carry these plastics to foreign destinations, once eaten by larger organisms, the plastics will continue up the food chain.

Clothing in the wash. More than ever, clothing is made up of plastic materials; polyester, nylon, and acrylic, in particular, make up about 60% of the material used in our clothing production. When washed, these microscopic fibers enter the sewage system-- there is no micro-filter in our washing machines-- and inevitably end up in our oceans. In one typical wash cycle, more than 700,000 fibers could be released (Resnick, B., 2019). A straightforward solution to this issue is to have people buy clothing made from natural-fibers, but this would be difficult to enact and enforce. Another solution would be to design a filter in a washing machine, similar to a lint trap in the dryer. This filter would need to filter plastics at a microscopic level, and allow water to pass through with ease.

Cosmetics. Within the beauty industry, extensive packaging is used to contain and to ship products. This realization was made in 2017, and since then many beauty companies have changed their ways to compensate for the pollution they created. Although further pollution has ceased, the pollution created prior to the packing changes has made a large impact. Some top natural brands are avoiding plastics altogether (Alternative Apparel, People Tree, Stella McCartney, and Matt & Nat), as all of these companies should also be avoiding plastic use (Munir, A., 2019).

3. **Research must identify and test plastics alternatives.**

Many biodegradable options exist that can limit the addition of plastics into the environment. For instance, the use of paper straws instead of plastics has been implemented in some restaurants, and I see no reason why the movement should not move to all restaurants. Small restaurants and fast food establishments can adopt biodegradable cutlery alternatives instead of the thousands of disposable plastic cutlery used each day. These alternatives already exist, and it is necessary for them to be popularized. Further research could follow a behavioral psychology approach designing effective ways to persuade and motivate the public to adopt plastics alternatives. From a materials engineering perspective, researchers can explore methods to address criticisms of plastics alternatives: designing biodegradable coatings that can maintain the integrity of paper straws during use, increasing the tensile strength of non-plastics utensils, and increasing the durability of biodegradable plates and bowls. Additional research must examine the carbon and resource footprint of plastics alternatives, ensuring that limiting the amounts of microplastics in the environment does not come at the cost of additional carbon emissions and chemical pollutants.

4. **Outreach must be made to corporate entities to reduce plastics use in their packaging.**

Meaningful change to the issue of plastic pollution can begin with changing the extensive use of plastics in my school, by designing an action plan so that all schools can switch from plastic straws and cutlery to paper and biodegradable products. After starting in my school, I will connect with local businesses and corporations. I aim to partner with local restaurants (fast food, family) and work with them to make them aware of how much they contribute to the plastics pollution crisis and how they can choose biodegradable alternatives to cups and straws. Integral to these efforts will be research examining best practices for engaging with administration and for-profit entities, and examining case studies (Silent Spring) where individual efforts brought about substantive actions. Consequently, research will help drive activism, increasing the likelihood of recreating historic successes that connect communities, businesses, and governments.

5. **Residents should be surveyed about their level of concern and behavioral science leveraged to design targeted outreach and information campaigns and to identify best practices of persuasion to change behavior.**

Surveys can evaluate the attitudes of individuals about the increase in plastic pollution in our current environment. Surveys would help researchers identify the priorities of any given community allowing education and outreach actions to be tailored to the needs, preferences, and cultures of those communities.

6. **Outreach to local government entities to promote and establish a precedent of change.**

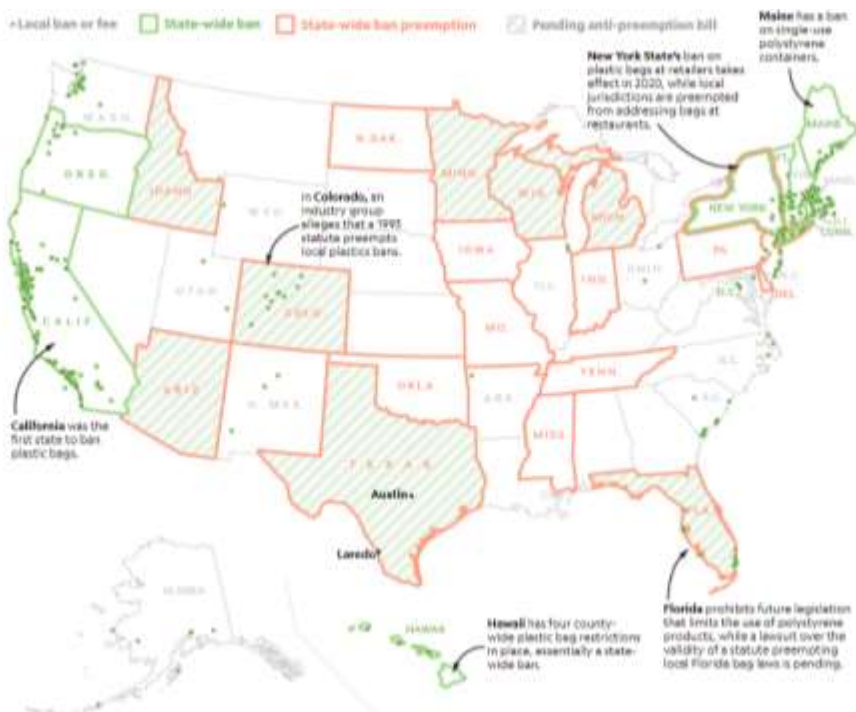
Finally, research must translate to community action which, in turn, will inform civic leaders of a determination for change on the part of their constituents. Efforts should be made to recruit local assemblymen to support legislation that limits plastics use. Progress has already been made in a neighboring county where plastic bags are currently taxed at five cents with each purchase increasing community use of reusable bags, thereby decreasing plastics consumption.

Conclusion

Humans are just beginning to grasp the full consequences of the unbridled exploitation of the environment: rising sea levels, raging wildfires, polluted oceans -- the Anthropocene presents two choices: environmental stewardship and remediation, or continued insults to the environment and our own, seemingly inevitable, self-destruction. My hope is that my efforts promote the realization that our looming destruction is not inevitable, that every person can make meaningful contributions through even minor lifestyle changes, and in this case, a shift in mindset becomes a powerful force for preventability an irreversible, plastic-polluted Earth. From the work of a 17-year old high school senior, and with politicians and the will of the community on the side of change, a decrease in plastics pollution is not only attainable but also inevitable.

Appendix Section

Appendix 1: (Gibbens, S. (2019, August 16). See the complicated landscape of plastic bans in the U.S. Retrieved from https://www.nationalgeographic.com/environment/2019/08/map-shows-the-complicated-landscape-of-plastic-bans/#close.**) – Bans on Plastics, and Bans on Bans**



Appendix 2: Core Sampler outlined by Tufts University

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