What a Pain in the Gut: Comparative Methodologies for Identifying Microplastic Ingestion in Ecologically Relevant Organisms

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Introduction

- Increasing global plastic production and mismanagement of resulting waste has drastically impacted marine ecosystems[1].
- Chemicals used in plastic manufacturing, or those that adsorb onto the surface of plastics can act as endocrine disrupters[2].
- Microplastics, a category of plastic pollution, are an emerging contaminant in marine communities (Figure 1).
- Organisms may mistake them for prey items or accidentally ingest them during filter feeding. This exposes them to harmful contaminants that are contained within plastics or those that adsorb onto the surface of plastics[3].

Figure 1: Microplastics

- This study sought to examine two of the most common methodologies for plastic ingestion in two ecologically and/or economically important marine species.

Study Systems: Mole Crabs and Rockfish

Figure 2: Mole crab (top) and Rockfish (bottom)

- Mole crabs (*Emerita analoga*) are filter feeders that occupy the swash zones on sandy beaches (Figure 2 top).
- They are an important food source for shorebirds along the coast and are often used as bait.
- They feed using filter feeding antennae.
- As coastlines are likely to be more concentrated in plastic waste than the open ocean, it is possible that these crabs are being exposed to microplastics and are ingesting microplastics during feeding.
- Rockfish (*Sebastes* spp.) comprise a large fishery off of the U.S. West Coast, and catch in Oregon amounted to around 1 million USD in 2015[4] (Figure 2 bottom).
- Rockfish are are susceptible to microplastic ingestion as they are opportunistic feeders.
- Rockfish sampling was conducted at Oregon State University as a part of an REU project in 2018.
- Mole crab experiments were conducted at Bodega Marine Laboratory for a senior thesis project in the year 2019.

Methodology

KOH digestion and visualization

Rockfish were dissected, digestive tracts were extracted, and contents were analyzed.

Contents in tracts were digested at 50°C for 72 hours, 1:3 (w:v) in 10% potassium hydroxide.

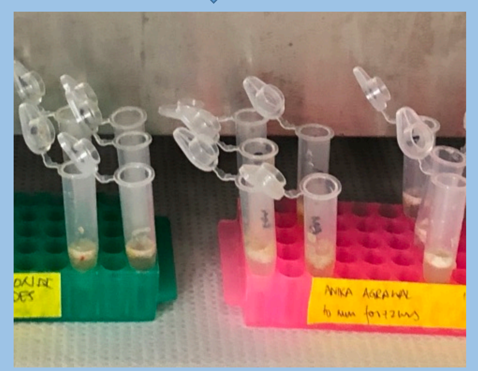
Digested contents were passed through a 1 mm and a 63 µm sieve and analyzed visually and through a microscope.

Suspected microplastics were photographed, measured and grouped by color and for further analysis via FTIR and Raman Spectroscopy.

Pros: Analyze large volumes, able to see fibers
Cons: Lots of room for human error as smaller plastics may not be visible to the naked eye

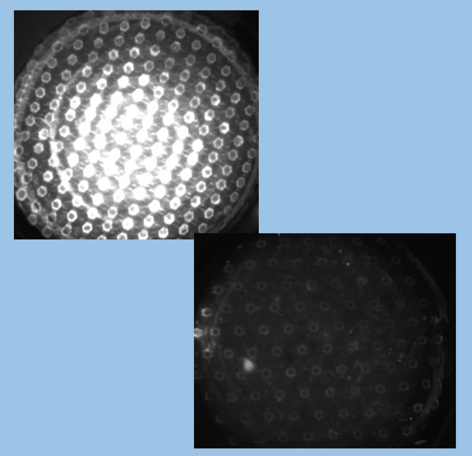
H_2O_2 digestion and Nile Red Staining

Mole crabs were dissected, viscera was removed and contents were analyzed.



Contents were placed in 5ml vials with 15% hydrogen peroxide at room temperature for 72 hours.

Digested contents were centrifuged down. The liquid was collected and that was passed through 0.22 µm filter and stained with Nile Red.



Stained filters were analyzed using fluorescence under a fluorescence scope. More fluorescence suggests the presence of microplastics.

Pros: Staining helps to compare on first glance based on intensity
Cons: Cannot see fibers and may be harder to quantify number of plastics

Results and Future Directions

- Microplastics were found in digestive tracts of both rockfish and mole crabs.
- Filter papers, which were used as controls for airborne contamination, contained blue and black fibers, that were also found in samples being analyzed, explaining the need for controls while analyzing microplastic ingestion via most methods (Figure 3).

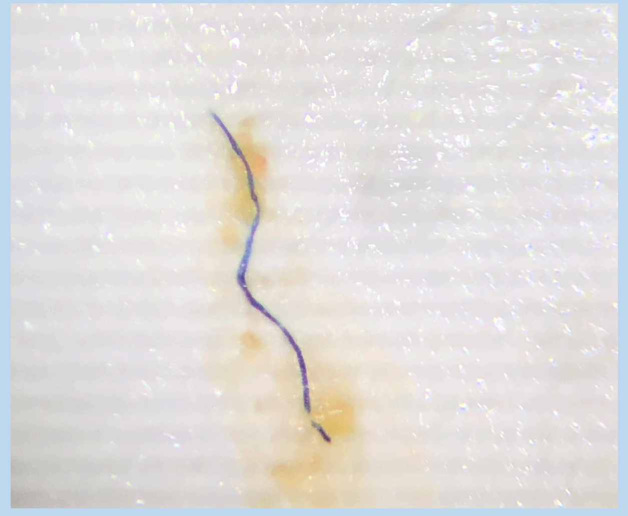


Figure 3: Example of a fiber found in both controls and organisms

- However, both methods are time consuming and it is necessary to create methods to quickly and efficiently analyze the presence of microplastics in organisms.
- Future studies could also look at the effects of microplastic ingestion on organisms.
- The presence of microplastics in organisms highlights the need to find alternatives to plastics to reduce marine plastic pollution (Figure 4).

Figure 4: Alternatives must be found to plastic to reduce marine and coastal plastic pollution

Acknowledgements

Part of this research project was conducted through the REU Program at the Hatfield Marine Science Center, Oregon during the Summer of 2018. Funding for this REU site is provided in by the National Science Foundation's Division of Ocean Science's located in Alexandria, VA. (NSF OCE-1758000).
The other part was conducted at Bodega Marine Laboratory, UC Davis and was funded by the Provost's Undergraduate Fellowship.
Special thanks to: Dr. Gary Cherr, Dr. Eric Sanford (UC Davis), and Dr. Susanne Brander (OSU)

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