

**AGRICULTURAL RESEARCH FOUNDATION
FINAL REPORT
FUNDING CYCLE 2018 – 2020**

TITLE: Spatial-temporal occurrence of microplastics in Black Rockfish (*Sebastes melanops*) off the coast of Oregon

RESEARCH LEADER: Susanne Brander, Ph.D.

COOPERATORS: National Oceanic and Atmospheric Administration, Marine Studies Initiative, SMURF Project, Oregon Department of Fish and Wildlife, National Science Foundation, Southern California Coastal Water Research Project, Hatfield Marine Science Center, Marine Studies Initiative

EXECUTIVE SUMMARY: Plastic production is increasing, which means the number of plastics entering the environment is also increasing. There is evidence that plastic is found in several marine organisms, but which and at what quantity needs to be determined. Microplastics are plastics <5mm in size and can be primary plastics, meaning they were made to be that size. These are known as nurdles and are used to form larger plastic goods by melting. Microplastics can also be a secondary plastic, which means they were degraded through different chemical, physical, or photooxidative means. Once this abundance is known, further toxicological studies can be completed to determine what impacts the plastics themselves or potential additives, fillers, and dyes have on the organisms consuming them. Black rockfish were collected from the Oregon Department of Fish and Wildlife (ODFW) and a charter company in Newport, OR. These fish were dissected, and the gastrointestinal tracts (esophagus, stomach, pyloric caeca, and intestine) were removed. The GI tracts, sans the GI tract contents, were dissected and digested through chemical degradation using potassium hydroxide (KOH) to create a liquid that could be sieved and vacuumed through pore sizes as small as 5µm. The contents found in the stomach organ were processed independently of the GI tract to see if potentially any of the prey contained microplastics.

A substantial proportion of the project thus far has been devoted to method development. Although more samples remain to be processed now that methods are finalized, the proportion of fish with suspected microplastics in Newport are 10.3 with a 95% confidence interval of 3.07% - 17.52%, while the percent of fish with suspected microplastics from the reserves are 26.7% with a 95% confidence interval of 11.03%-31.59%. 52% of all the fibers found were black, which is similar to results found in published papers. We will continue to verify the suspected plastics gathered through the project, work is expected to be completed in mid-2020.

OBJECTIVES:

1. To aid in filling data gaps on the ingestion of microplastics in the field with a commercially/recreationally important species in the Oregon Coast, black rockfish.
 - a. This research is being completed by using fish from two different populations. The first population is from off the coast of Newport, OR. The second population is from outside the marine reserves caught by the Oregon Department of Fish and Wildlife (ODFW). With this

2. Verify the suspected plastics/synthetics are truly anthropogenic.
 - a. In the literature, the most commonly found ingested morphology of microplastic is fibrous. A subset of all the suspected microplastics will be verified using Fournier Transform Infrared Spectroscopy (FTIR), which is an analytical technique accepted in the field to verify plastics. The FTIR produces an individual spectrum that is unique to each type of polymer. These spectrum can be compared to created libraries of spectra.
3. Outreach to the community.
 - a. Masters student Katherine Lasdin and PI Brander will be completing public engagements as requested by local organizations. PI Brander also brought a non-profit to OSU from the University of North Carolina, Wilmington, called Plastic Ocean Project. The goal was to convert local restaurants to become Ocean Friendly Establishments, which means they stopped using plastic straws or only gave them out upon request. Through this process, Katherine learned about using appropriate terminology to become more inclusive to all people. Some people need straws, and asking people to stop using them can be a problem for some people. This was in 2018-2019 and early 2020. Ocean Friendly Establishments also joined in with the OSU club, The Last Dam Straw, to reduce the plastic straw usage on campus, and several eateries joined in the movement.

PROCEDURES:

Fish Collection and Preparation

- ~320 black rockfish collected by ODFW and charter companies during 2018 and 2019

Analysis for Microplastics

Digestive tract Dissection and Digestion

- Digestive tract removed, measured, weighed, and cut open
- Contents are separated from the GI tract membranes and placed into mason jars with potassium hydroxide for 48-72 hours at 50°C. The ratio of 10% KOH changed throughout the study from a 1:3 (w:v) to a 1:3 (w:v) of gut (or 100ml minimum) at 20% KOH and a 1:6 (w:v) of contents at 20% KOH.
- After digestion, all components of samples (guts and contents) are sieved on a 63µm sieve. After fatty residues that are remaining are then added to a mason jar with a 10% Alcojet solution to aid in the break-down of fat. Exoskeletons of all contents are removed from the sieve and examined under a microscope for the presence of microplastics. The sieves are rinsed 3X with Milli-Q/RO water. The remaining liquids from the contents are vacuumed using a Buchner funnel vacuum apparatus with a 5µm polycarbonate filter. The fat in Alcojet is allowed to sit for a minimum of 1 hour and then sieved through a 63µm and 1mm sieve. Same as before, all liquid is saved, and the sieves are appropriately cleaned. Once all the membrane liquid is together, the sample is vacuumed on a 5µm polycarbonate filter.
- Suspected plastics/synthetics will be kept for later verification in small acrylic jars in a small amount of 70% ethanol.

Control Procedure

- Researchers wore as much cotton as possible in the lab, and then wore 100% white cotton lab coats
- Dissections and sieving were completed under a hood with a HEPA filter

- Glassware is baked in an oven at 350°C for 12 hours after being washed, wiped with 70% ethanol, and rinsed 5 times with Milli-Q/RO water.
- Controls used: Damp filter paper/KOH/water to account for suspected plastics in air versus in sample

Analytical Chemistry

- Samples held in the acrylic jars with 70% ethanol are prepared for analysis via FTIR (Fournier Transform Infrared Spectroscopy)

SIGNIFICANT ACCOMPLISHMENTS: This project has been worked on by several people, including an undergraduate at the time from Oregon State University (Jordan Laundry) and REU student (Anika Agrawal). This project was taken over by Masters student Katherine Lasdin in fall 2018. Since starting the project, Lasdin has presented this research at several different local and national conferences, both as posters and oral presentations. The local conferences included the State of the Coast conference in October 2018, the Pacific Northwest Chapter of the Society of Ecotoxicology and Chemistry conference in April 2019, RAFWE, and the Pacific Northwest Chapter of the Society of Ecotoxicology and Chemistry conference in February 2020. National Conferences include the Western Society of Naturalists Conference in November 2018, the Coastal Estuarine Research Federation conference in November 2019. Lasdin has also presented information about plastics to the local college radio station in July 2019 and NW NEWS NETWORK with Tom Banse in September 2019. A collaboration was formed with Dr. Grorud-Colvert at OSU with the SMURF project. The SMURF (Standard Monitoring Units for the Recruitment of Fishes, <https://aquarium.org/smurf-aquarium-partners-with-researchers-to-monitor-fishes-in-marine-reserves/>) project has juvenile rockfish samples that will be used to complete the temporal aspect of the study. Lasdin gave a platform presentation at the 2020 AGU Ocean Sciences meeting in San Diego and both Brander and Lasdin presented results at the PNW Society of Environmental Toxicology and Chemistry meeting in Bremerton, WA in 2020.

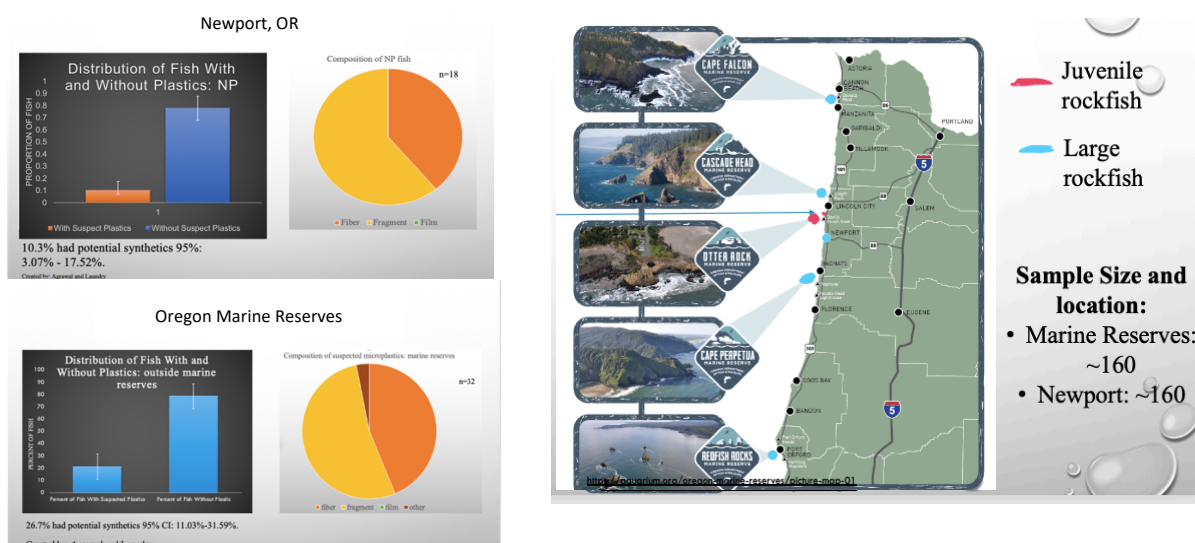


Figure 1. Map of study sites and sample sizes, and pooled results from Newport and reserves.

Data thus far collected from sites near reserves and new Newport OR (Fig 1) (from 2018 and the beginning of the 2019 field season) show that the percent of adult fish obtained near populated areas and associated with marine reserves that contained suspected synthetics or microplastics was 10.3% and 26.7%, respectively (Fig 1). Fish caught near marine reserves contained more suspected microplastics than those sampled near a medium-sized coastal town ($p\text{-value} = 0.016$). These data show that nearshore species may be impacted by waste management shortfalls and are consuming synthetic materials. Continued research is underway on already-collected samples to determine how much plastic is found in remaining samples caught during the 2019 field season which ended in fall 2019, we expect that this work will be completed by summer 2020.

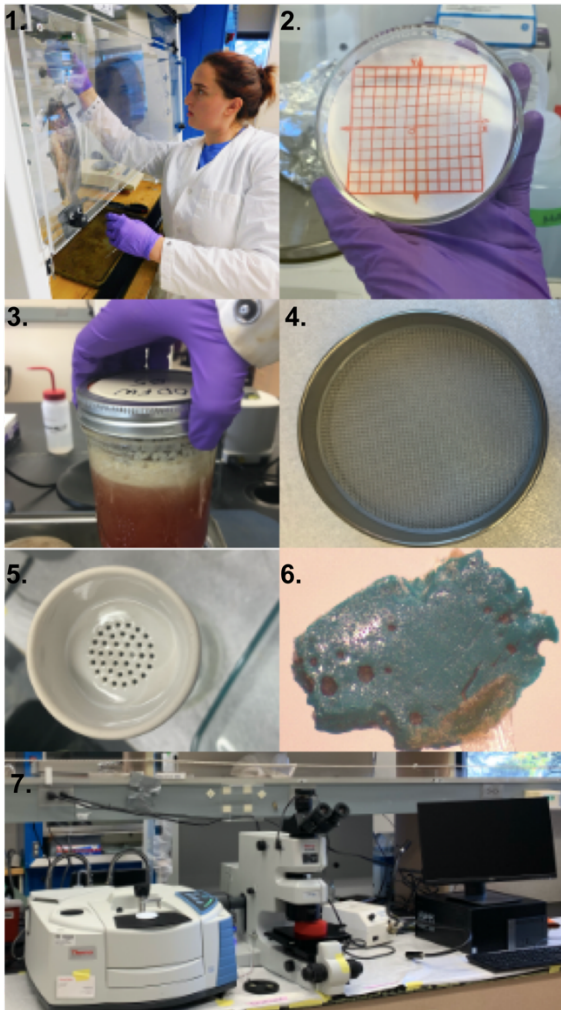
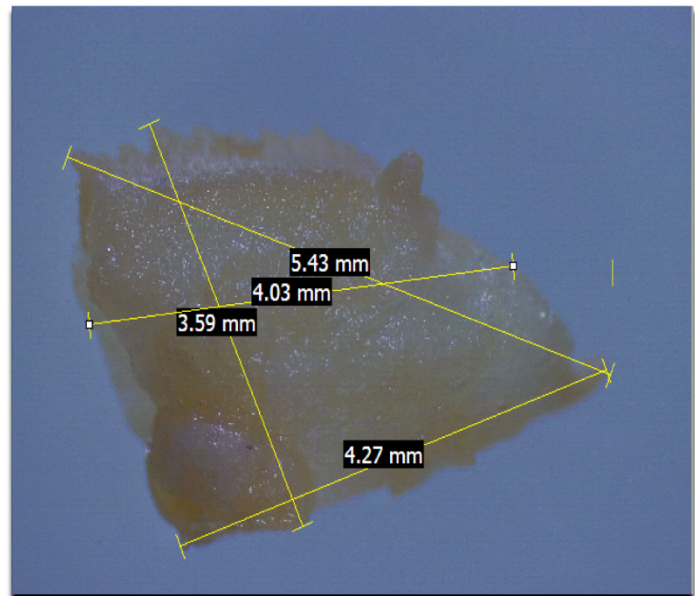


Fig 2. Method development and protocols in progress, facilitated by graduate student Katherine Lasdin, in the Brander laboratory at OSU. The plastic item pictured below is a piece of LDPE (low density polyethylene) obtained from a rockfish stomach.



We also are currently participating in a multi-lab project with the Southern California Coastal Water Research Project, University of Toronto, Thermo Fisher, and Horiba to aid in creating, standardizing, and seeing how protocols change in other microplastic labs. Through this study, PI Brander is the leader of an augmentation study to see if specific methods are better than others for certain tissues/organisms/mediums. PI Brander's Laboratory has also expanded the lab's research to other marine organisms looking for microplastics. Seven other studies have started/completed since the rockfish project started, including investigations into microplastics in

mussels, zooplankton, and otter scat. These studies have consisted of summer REU projects, undergraduate research projects, and masters theses.

BENEFITS & IMPACT: As listed above, this project is the pilot grant has allowed us to expand our microplastic studies at the university. Not only are we adding research to this “new” field with no standardized methods we are aiding in creating these new methods. The microplastic research completed at Oregon State University will aid in filling knowledge and data gaps regarding plastics in the Pacific Northwest through the newly created NSF-funded PNW Consortium on Plastics Research. The methodologies determined and used at OSU will enable us to share our data with other labs around the country for metadata analysis. For example, Brander taught a short course on microplastic methods and standardization at the Feb 2020 PNW SETAC meeting and is now viewed as an expert on this subject. Brander also was a co-editor of a recent special issue on microplastics published in the journal *Limnology and Oceanography Letters* in Feb 2020. She has authored four publications on microplastic pollution between 2019 and early 2020, and has been asked to serve as co-chair for an Ocean Protection Council committee on microplastic pollution.

ADDITIONAL FUNDING RECEIVED DURING PROJECT TERM:

Additional funding obtained in collaboration with Dr. Leigh Torres through the Agricultural Research Fund. The funding was titled, "Kick-starting COZI o the Coastal Oregon Zooplankton Investigation. This funding is enabling students to look at the presence of microplastics and available energy in food webs for grey whales. A student in PI Torres' lab is completing work in the hoods in the Brander lab. Funding via The Research Equipment Reserve Fund, Oregon State University, with letters of support from COMES, Hatfield Marine Science Center, MMI, MSI, and EMT, allowed for the purchase of a μ -FTIR. The μ FTIR enables us to verify the suspected synthetics/plastics in house and potentially form collaborations in the future.

Lasdin received a National Science Foundation Research Traineeship fellowship (NSF NRT) to support her second-year studies at OSU. This fellowship enables her to work on a transdisciplinary team at OSU that looks at how microplastics interact in the coupled natural-human system, accounting for risk and uncertainty and big data. Lasdin also received two travel funds to attend the Pacific Northwest Chapter of the Society of Ecotoxicology and Chemistry conference in 2019 and 2020 and a Oregon Marine Reserves scholarship. PI Brander also co-wrote a grant with PI Harper (OSU EMT) and received an NSF # GCR1935028 grant (3.3 million, 5 years) to continue studying micro and nanoplastics. Lastly, an OSU RERF grant for \$50,000 was obtained in mid-2019 to purchase a micro FTIR instrument that can identify polymers and other microscopic debris items, which is creating opportunities for additional internal and external collaborations.

FUTURE FUNDING POSSIBILITIES:

Brander plans to apply for an NSF Polar Programs grant with Portland State collaborator Dr. Elise Granek in 2020, along with applying for NIH funding for mammalian-focused studies with Dr. Stacey Harper of OSU EMT. She has also formed a collaboration with Dr. Chelsea Rochman of the University of Toronto to study the potential multigenerational impacts of plastics on dace in an experimental lake.