AGRICULTURAL RESEARCH FOUNDATION
INTERIM REPORT
FUNDING CYCLE 2019 – 2021

TITLE: Kick-starting COZI – the Coastal Oregon Zooplankton Investigation – to understand microplastic and energetic loads at the base of our food web

RESEARCH LEADER: Dr. Leigh Torres, Marine Mammal Institute, Department of Fisheries and Wildlife, Oregon Sea Grant

MASTER'S STUDENT: Lisa Hildebrand, MSc degree obtained December 2020, Wildlife Science

COOPERATORS: Dr. Kim Bernard, College of Earth, Ocean, and Atmospheric Sciences
Dr. Susanne Brander, Environmental and Molecular Toxicology
Dr. Sarah K. Henkel, Integrative Biology

EXECUTIVE SUMMARY:
This project has established the Coastal Oregon Zooplankton Investigation (COZI). COZI is a cross-college collaborative effort led by four early-career female scientists that aims to improve our understanding of the quality of zooplankton in coastal Oregon by species, life history stage, location, and time. Funding from ARF for this COZI project supported the collection of zooplankton samples along the Oregon coast in 2019, and the analysis of zooplankton samples collected in 2017, 2018 and 2019. Analyses include species identification, community analysis, caloric content determination, and microplastic quantification and identification.

OBJECTIVES:
1. Determine how microplastic loads and caloric content vary by zooplankton species, life history stage (gravid or non-gravid), location and time of year in coastal Oregon.
2. Relate microplastic loads and caloric content of zooplankton species to determine how marine predators ingest microplastics relative to energetic gain.
3. Disseminate findings to the public, managers and scientific community to inform future research and management efforts aimed at reducing the impact microplastic in Oregon coastal ecosystems.

PROCEDURES:
<u>Sample collection</u>: Between 1 June and 15 October 2019 we collected 13 zooplankton samples from the nearshore area near Newport using a light trap. These samples were stored and frozen for further analysis along with samples collected in 2017 and 2018. The locations of sample collection in Newport, OR are illustrated in Figure 1.

Zooplankton samples were also collected in Port Orford, OR using a small tow net deployed from a kayak during July-August of 2016 and 2019 as part of a gray whale foraging ecology study. These Port Orford samples were collected from two sites, Tichenor Cove and Mill Rocks, and used for community analysis: n_{2016} = 21 samples; n_{2017} = 59 samples; n_{2018} = 243 samples; n_{2019} = 248 samples.

Sample processing: Zooplankton samples must be processed in a stepwise fashion to determine caloric content and microplastic loads by species and stage, and to avoid contamination during microplastic analysis. Therefore, all samples were first be sorted under a laminar flow hood by species and stage. Next, a portion of each sorted sample was processed to determine caloric value in a bomb calorimeter, and another portion of the same sample is being analyzed for microplastic load. Port Orford samples have been sorted to species and are ready for community analysis.

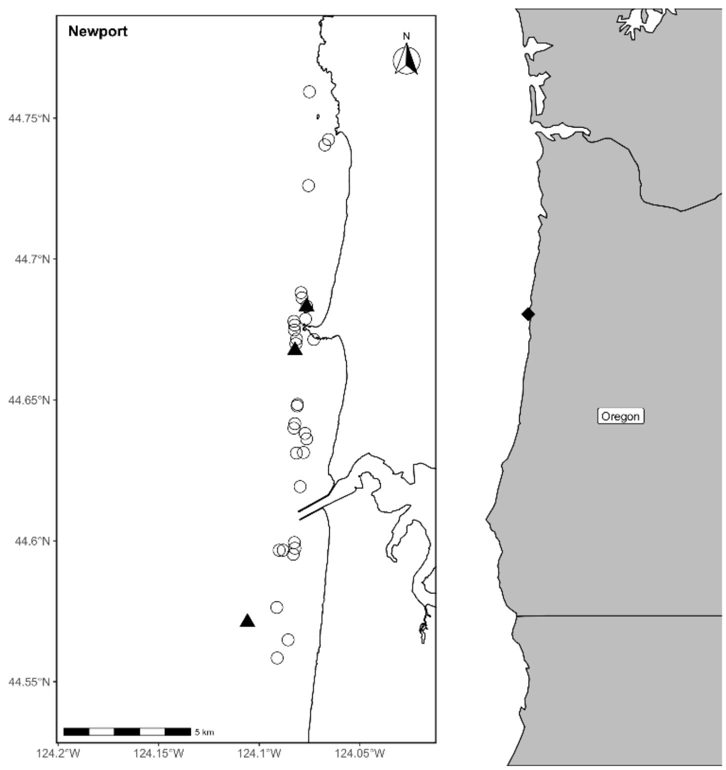

Figure 1. Locations of zooplankton samples collected with a light trap (open circles) or opportunistic collections of surface swarms of crab larvae (black triangles) in Newport, along the Oregon coast in the Pacific Northwest coast of the USA.

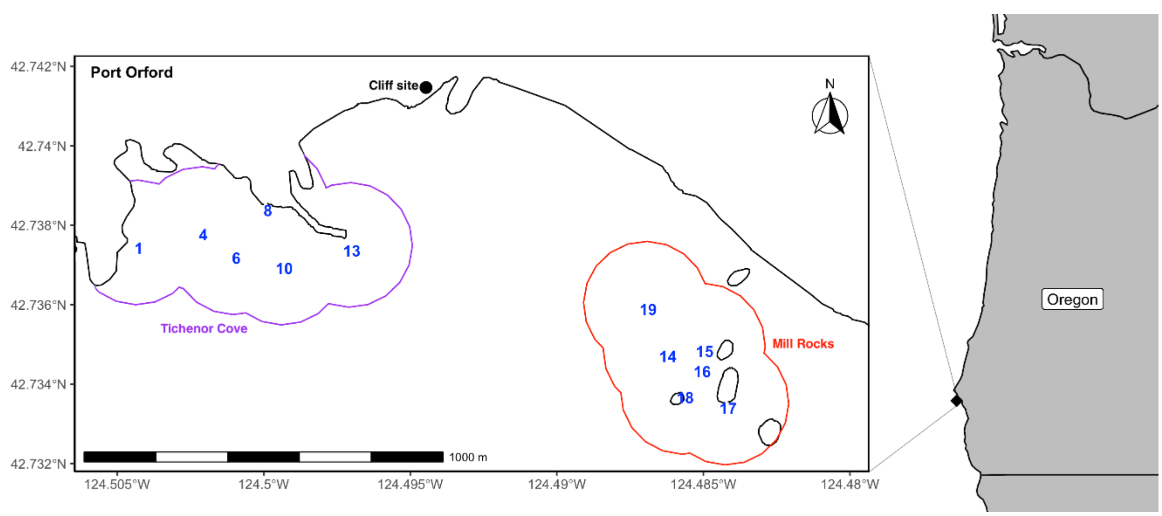

Figure 2. Locations of zooplankton sampling stations (blue numbers) collected during July-August in 2016-2019 with a tow net deployed from an outrigger on a research kayak off the coast of Port Orford, Oregon and used for community analysis. The two study sites are demarcated by the purple line for Tichenor Cove, and the red line for Mill Rocks.

SIGNIFICANT ACCOMPLISHMENTS TO DATE:

All zooplankton net samples collected in Port Orford and Newport have been sorted (Table 1). Bomb calorimetry of the sorted Newport samples was also completed in 2020. Microplastic analysis was interrupted due to the pandemic that limited lab access, but work was resumed with limited access in late 2020 and is currently in-progress with an anticipated completion date of June 2021.

Table 1. Progress on identification, bomb calorimetry and microplastic analysis of zooplankton samples collected in two different locations (Newport and Port Orford) with two different sampling methods (light trap and zooplankton tow net, respectively) over three summers (2017, 2018 & 2019). Dark green coloring indicates this step is complete, and dark red indicates incomplete work.

	Location, type				
Year	**Newport, light trap**	**Port Orford, zoop net**	**Sorted in hood?**	**Bomb calorimetry**	**Microplastics**
2017	10/10	59/59	4/10	10/10	0/4
2018	10/10	235/235	10/10	10/10	0/10
2019	11/11	248/248	11/11	11/11	0/11

Caloric content results:

In total, nine zooplankton species were identified in the Newport samples: the mysids *Alienacanthomysis macropsis*, *Exacanthomysis davisi*, *Holmesimysis sculpta*, and *Neomysis rayii*, the amphipods *Atylus tridens*, and *Polycheria osborni*, Dungeness crab megalopae and porcelain crab larvae, and an unknown species of *Caprellidae*. *A. macropsis*, *Caprellidae* sp., and *E. davisi* were rare, only being identified in 1, 1, and 4 light trap samples, respectively. These occurrences were represented by a single specimen, which prevented successful calorimetric determination for these three species.

For the mysids, gravid females were identified through the presence of a brood pouch that contained any developmental stage of offspring (from eggs to larvae). Individuals that had a brood pouch without any contents were classed as empty brood pouch females and considered to have already released their young. For *A. tridens*, gravid females were identified by a clutch of eggs in their pereopods. Reproductive stages could not be discerned for *P. osborni* due to its small size.

Energetic value: A total of 284 energetic values were determined (Table 2). Caloric content did not vary significantly between years (Kruskal-Wallis χ^2 = 3.20, df = 2, p = 0.202). Therefore, samples were pooled across years for all subsequent analyses.

The energetic density between species (pooled across reproductive stage and month) varied significantly (Kruskal-Wallis χ^2 = 123.38, df = 5, p = <0.0001; Figure 3a). Dungeness crab megalopae (n=13) had the highest mean energetic density (4.21 $\pm$ 1.27 kJ g^{-1}) and the amphipod

P. osborni (*n*=11) had the lowest mean energetic density (0.83 ± 0.21 kJ g^{-1}). Caloric values of Dungeness crab megalopae and the mysid *N. rayii* (2.42 ± 1.06 kJ g^{-1}) were significantly higher than those of the mysid *H. sculpta* (1.60 ± 0.66 kJ g^{-1}), porcelain crab larvae (1.17 ± 0.14 kJ g^{-1}), and the amphipods *A. tridens* (1.25 ± 0.57 kJ g^{-1}) and *P. osborni* (Dunn's test, p <0.001). Dungeness crab megalopae and *N. rayii* caloric contents were also significantly different from one another, with the former having the higher caloric value (Dunn's test, p = 0.0006). The caloric contents of *H. sculpta* and *P. osborni* differed significantly (Dunn's test, p = 0.0006), with *H. sculpta* having a higher caloric content. All other pairwise comparisons were not significant (Dunn's test, p > 0.006). Comparison of the mean caloric values of the prey species tested in this study to the mean caloric value of Arctic amphipod *A. macrocephala* (2.02 kJ g^{-1}) showed that two Oregon zooplankton species have higher caloric contents, namely Dungeness crab megalopae and *N. rayii* (Table 2).

Table 2. Energetic values (wet weight) and sample sizes by zooplankton species and reproductive group collected from June-October in 2017-2019 in central Oregon.

Species	**Number of energetic values**	**Mean energetic value (kJ g^{-1})**	**Minimum energetic value (kJ g^{-1})**	**Maximum energetic value (kJ g^{-1})**	**Standard deviation**
Atylus tridens	28	1.25	0.47	2.93	0.57
Gravid	2	0.78	0.60	0.97	0.26
No brood pouch	25	1.31	0.47	2.93	0.58
Empty brood pouch	1	0.71	-	-	-
Dungeness crab megalopae	13	4.21	2.61	6.14	1.27
Holmesimysis sculpta	100	1.60	0.47	5.21	0.66
Gravid	33	1.74	0.83	3.04	0.58
No brood pouch	48	1.65	0.83	5.21	0.73
Empty brood pouch	19	1.23	0.47	2.36	0.49
Neomysis rayii	128	2.42	0.59	7.96	1.06
Gravid	37	2.50	1.27	7.96	1.04
No brood pouch	67	2.59	0.86	5.54	1.12
Empty brood pouch	24	1.86	0.59	3.47	0.75
Polycheria osborni	11	0.83	0.53	1.14	0.21
Porcelain crab larvae	4	1.17	1.00	1.31	0.14

Since significant differences between species were found, analyses to determine whether energetic values vary by reproductive stage and month were carried out within each species. Sufficient replicate samples for different reproductive stages and months were only obtained for the two mysid species (*N. rayii* and *H. sculpta*) and the amphipod *A. tridens*.

Reproductive stages had significantly different caloric values for *H. sculpta* (Kruskal-Wallis χ^2 = 10.34, df = 2, p = 0.005), however not for *N. rayii* (Kruskal-Wallis χ^2 = 9.49, p = 0.008) nor *A. tridens* (Kruskal-Wallis χ^2 = 3.35, p = 0.187) (Figure 3b). For *H. sculpta*, females with empty brood pouches had significantly lower caloric values (1.23 ± 0.49 kJ g^{-1}) than gravid females (1.74 ± 0.58 kJ g^{-1}; Dunn's test, p = 0.0007), but not than individuals with no brood pouch (1.65

$\pm$ 0.73 kJ g^{-1}; Dunn's test, $p = 0.008$). No significant differences were identified between *H. sculpta* gravid females and individuals without a brood pouch (Dunn's test, $p > 0.1$).

Significant differences in energetic content between months were only detected for *H. sculpta* (Kruskal-Wallis $\chi^2 = 15.38$, df = 4, $p = 0.004$; Figure 3c), whereby September was significantly higher than all other months (June, July, August; Dunn's test, $p < 0.002$), except October (Dunn's test, $p > 0.4$). Linear regressions of caloric content by reproductive stage within each species over time (DOY) revealed that the energetic value of the gravid reproductive stage increased significantly throughout the season for both mysids, *H. sculpta* ($F_{1,31} = 15.71$, $p = 0.0004$, $R^2 = 0.32$) and *N. rayii* ($F_{1,35} = 6.138$, $p = 0.0182$, $R^2 = 0.12$) (Figure 4). All other linear regressions conducted were not significant ($p > 0.2$; Figure 4).

<u>Community analysis preliminary results:</u>

Community analysis of the samples collected in Port Orford was conducted using Non-metric multidimensional scaling (nMDS), which is gradient analysis approach that produces an ordination based on a distance or dissimilarity matrix. NMDS was conducted on the mysid and amphipod communities at all sampling stations in each year to compare community structure between the sampling sites of Tichenor Cove and Mill Rocks. All the other zooplankton species were removed from analysis due to low occurrence rates.

Within each year, the two sites were significantly not different from each other (Figure 5). However, the two sites became more similar to each other with each year, as indicated by the increased overlap of blue and green symbols from 2016 to 2019. This pattern may be an artifact of increased sample size in the latter years, yet the similar point distribution in 2018 and 2019 suggests some consistent community structuring between these years. The Port Orford habitat has undergone a significant change since 2016, with dramatic increases in purple sea urchins and subsequent decline in kelp. We have documented this change via our GoPro drops conducted at each kayak sampling station, as well as concurrent change in zooplankton availability and gray whale occurrence (Figure 6). We are currently integrating these results with the community analysis to determine drivers and correlations.

<u>Microplastic analysis</u>:

After sorting and identification of zooplankton, we are now analyzing 29 samples for microplastics. Each sample consists of ~2g of one zooplankton species from one collection effort. The digestion and sieving stages of this analysis process are complete, and the samples are now going through a vacuuming stage to aggregate plastics on a filter. Once this step is complete, all filters will be inspected underneath a microscope to identify and collect any potential piece of microplastic. Then, at least 25% of all of pieces will be selected and analyzed via FTIR (Fourier Transform Infrared Spectroscopy) to identify the material (e.g., plastic, cellulose, natural fiber, etc.).

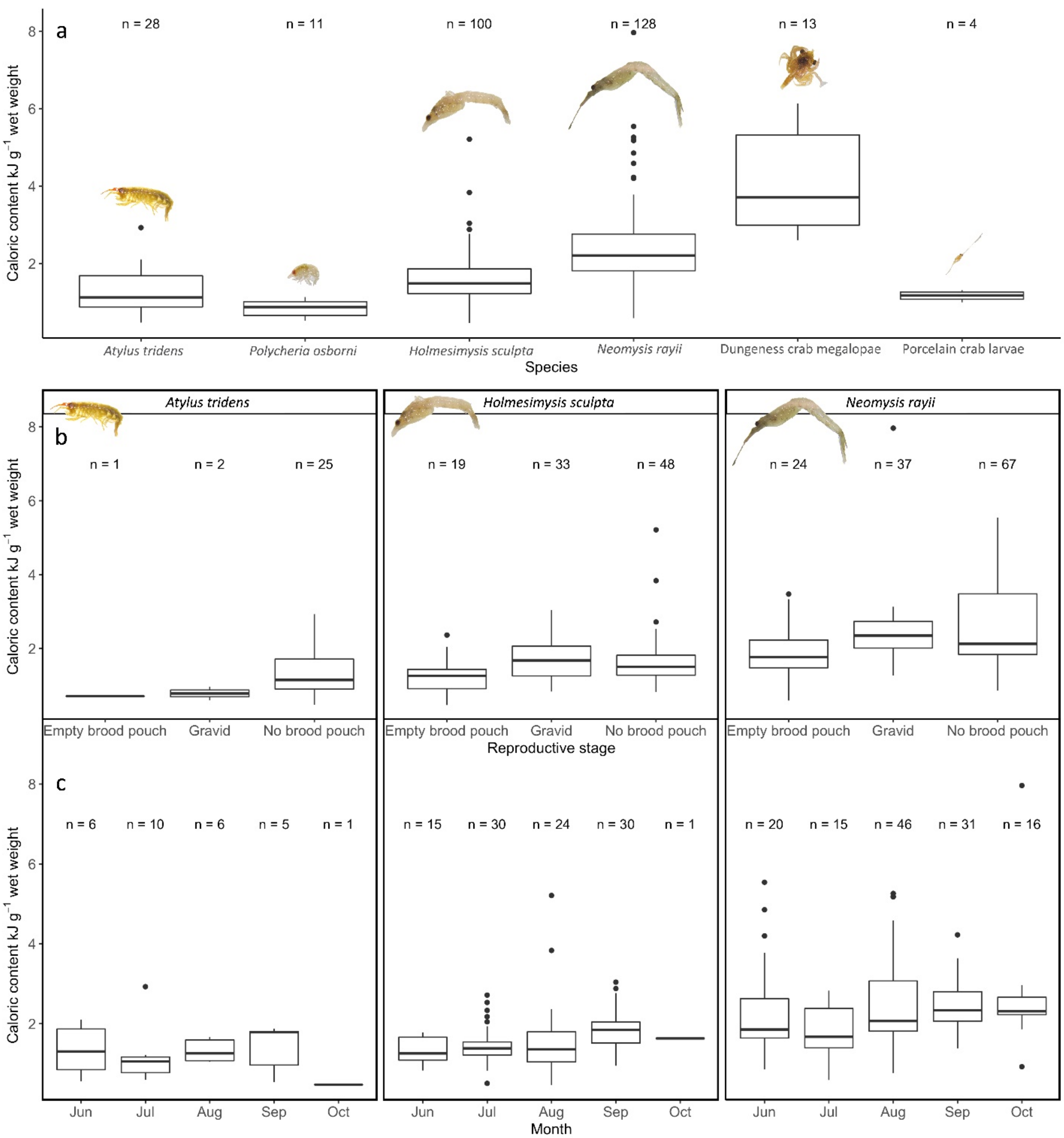

Figure 3. Median caloric content and interquartile ranges (kJ g-1 wet weight) by (a) species, (b) reproductive stage, and (c) month. Sizes of the zooplankton images are scaled at actual ratios relative to one another.

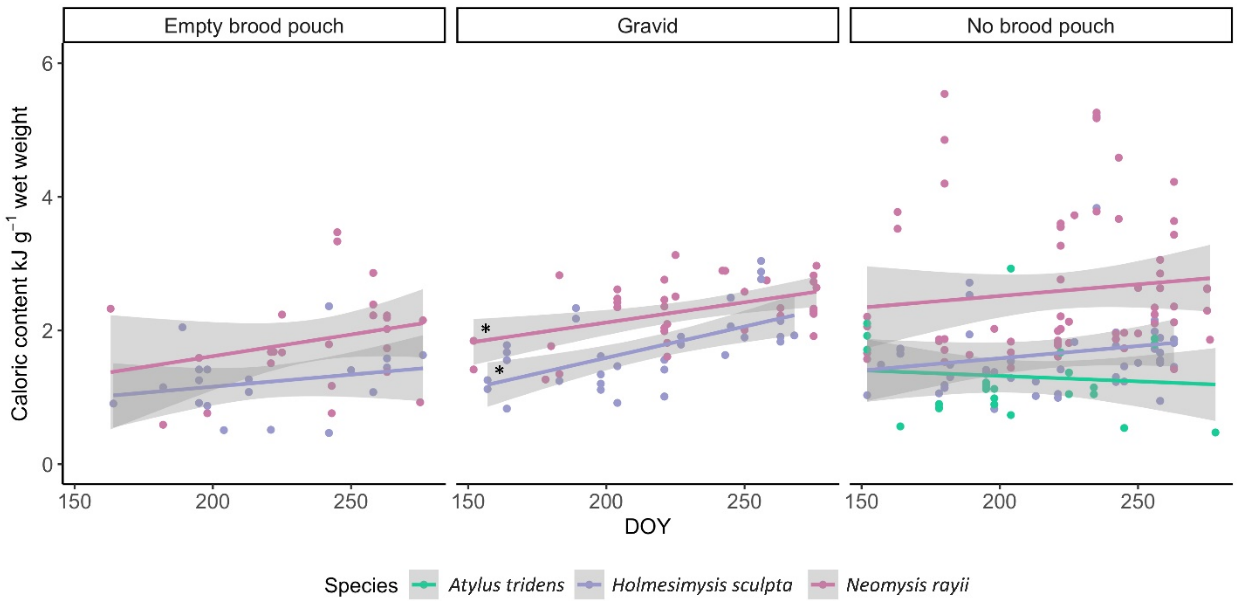

Figure 4. Caloric content (kJ g^{-1} wet weight) of different reproductive stages (empty brood pouch, gravid, and no brood pouch) as a function of day of year (DOY; ranging from June to October) for the mysids *Holmesimysis sculpta* and *Neomysis rayii*, and the amphipod *Atylus tridens*. *A. tridens* is only represented on one panel due to small sample size of this species for the empty brood pouch and gravid reproductive stages. Asterisks indicate significant regressions ($p < 0.02$).

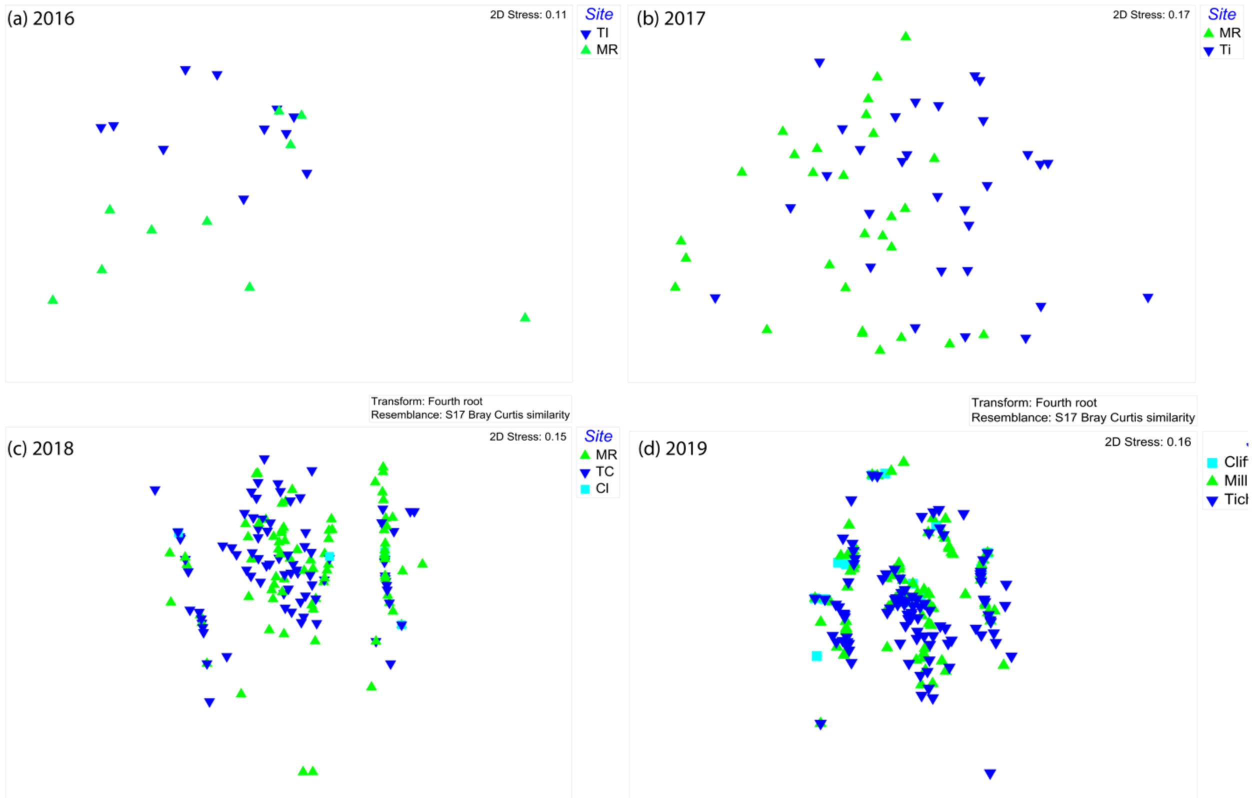

Figure 5. nMDS plot of the mysid and amphipod communities at Port Orford. In 2016, the samples were sub-sampled and 100 organisms counted, so panel (a) is based on relative abundances; subsequent years are based on actual abundances. TI and TC both = samples collected in Tichenor Cove; MR and Mill both = samples collected in Mill Rocks.

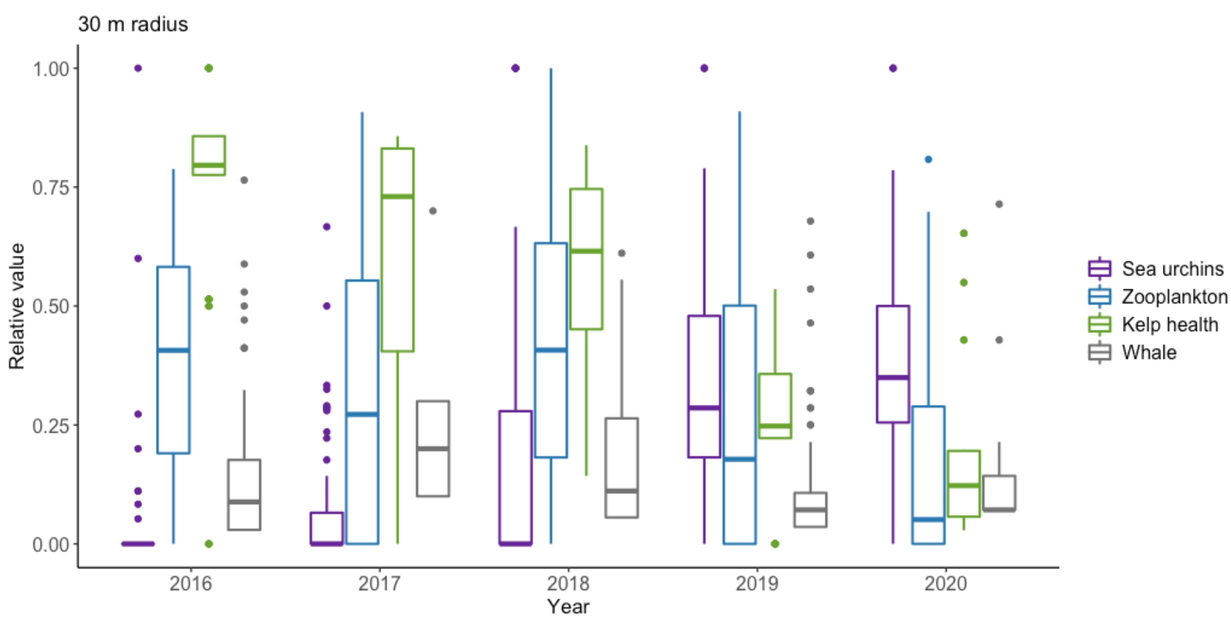

Figure 6. Boxplots indicating the change in relative value across five consecutive sampling years of sea urchins (purple), kelp health (green), zooplankton abundance (blue), and gray whale occurrence (gray) at all sampling stations in Port Orford. Preliminary results.

Engagement:
We have established a description of the COZI research project on PI Torres's GEMM Lab webpage to enhance communication about the project.

Master's student Lisa Hildebrand wrote a blog about microplastics in the marine environment.

PIs on this project (Torres, Brander) are also collaborating on the Pacific Northwest Consortium on Plastics that has recently been funded through the support of an NSF grant (2019-2024).

Multiple undergraduate students have been engaged in this research program including:

- Robyn Norman, Department of Integrative Biology, College of Science
- Haley Kent, Department of Integrative Biology, College of Science
- Elissa Bloom, Department of Chemical, Biological and Environmental Engineering, College of Engineering

ADDITIONAL FUNDING RECEIVED DURING PROJECT TERM:

PI Torres received a funding award from to the Office of Naval Research Marine Mammals and Biology Program to support research on gray whales off the Oregon coast, including prey collection and analysis.

Brander has also been funded as a co-PI on an Oregon Sea Grant SEED project (2021) to quantify plastics in Oregon seafood and to begin engaging with regulators at the state level (lead PI Granek, Portland State University).

FUTURE FUNDING POSSIBILITIES: