The quantification of microfibers in the gastrointestinal tract of the *Loligo opalescens*

Edward Martinez, Alexandra De Leon, Andrea Huvard, PhD., Brian Swig, PhD.
Microfiber Research Team, California Lutheran University

California Lutheran UNIVERSITY

Background

Microfibers (MF) are largely composed of inorganic particles, considered microplastics, that present a great threat to the well-being of the global oceanic ecosystem which have received a substantial amount of attention from researchers in recent years as a result. They are typically generated by inorganic fabrics such as textiles. One of the major sources that contributes to the MF pollution in marine ecosystems is the process of washing items made of MF using laundry machines. It is estimated that 603.9 to 1,523.3 tons of microfibers are released in the global water supply using platen laundry machines and 705.1 to 2,408.1 tons of MF with pulsator laundry machines annually[8]. This estimate is based on the total global annual production of polyester fabric, polyamide fabric, and acetate fabric being 60.8 million tons in 2012[8].

As a result of the significant release of MF from polysynthetic items and inadequate waste water filtration, microfibers are threatening marine organisms. MF with low and high densities present in marine environments tend to float on the sea surface where they are ingested by a wide range of organisms passively or actively. Microplastics were found in 19.8% of the digestive tracts of 263 individuals of the 26 species of commercial fish they analysed off the coast of Portugal[7]. The presence of MF pollution in the ocean has a domino effect on oceanic food chains, initially being directly ingested directly by organisms of lower trophic levels and then indirectly ingested by their predators, such as the Loligo opalescens. 0 to 4 MF in roughly a third (N=10, 32%) of the digestive tracts of wild Atlantic mackerel (Scomber scombrus) that were being feed to a group of captive grey seals (Halichoerus grypus)[6]. Scat sample tests of the seals yielded a microfiber presence of 0 to 4 MF per sample in about half (N=15, 48%) of them[6]. The presence of MF in the captive seals that are isolated from free-floating ocean contaminants was indicative of their main contamination source is the wild Atlantic mackerel containing MF that were transferred via trophic transfer [figure 1].

Loligo opalescens is a common squid that lives in the waters off of the Southern California coast[1] [Figure 2]. Squid in this genus are predators and their prey are often entangled in their tentacles then bitten by the jaws and swallowed quickly. Unlike many other marine invertebrates, these squid are not filtering water rather they are carnivorous predators eating other animals. Organic material is digested in the midgut and non-digestible materials are passed out of the digestive system. It is interesting to determine if squid have non-digestible MF in their guts. If they do, we can assume that these fibers most likely came from their food.

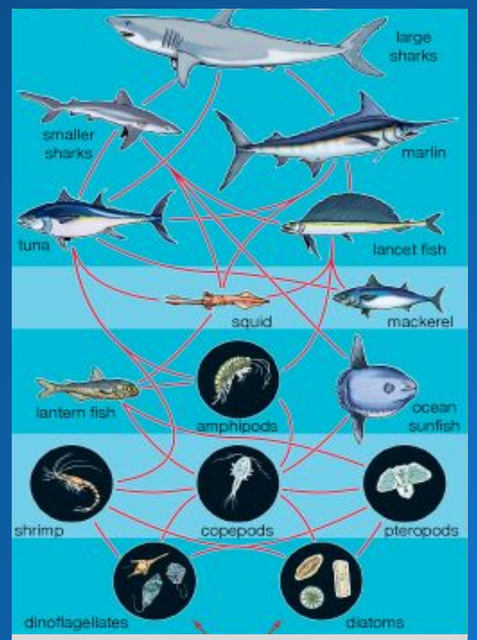

Figure 1. food web displaying a range of consumers from producers to quaternary consumers.[2]

Discussion

In recent decades, microfiber (MF) research has been done on other marine organisms, especially filter feeders and major commercial oceanic organisms like mussels and fish, but no records indicate that any work has been done on squid. It may not be the most widespread aquatic organism sold for human consumption, but it is the most commonly used as bait globally and as a food source for many of California's aquatic and land predators[9]. It is California's largest fishery, which yielded a revenue of $73.8 million in 2010. It's importance to the ecosystem of the North American west coast and to California's economy sparked my curiosity in understanding how it is being affected by MF pollution and what dangers it would be facing.

The data collected suggest that the California market squid has been significantly impacted by MF pollution with an average MF content per squid of 28 [figure 3]. *Argonauta nuoryi* (*AN*), another member of the cephalopoda class and a tertiary consumer, had been analyzed in the Atlantic of southern Mexico to have an average MF count per sample of 67[2]. For context, *AN* has a length of about 80 cm[2], which is roughly four times larger than of the squid samples observed. Yet, the *Loligo* samples had an average that is roughly less than half of the average for the *AN*. It can be concluded, then, that *Loligo* has been significantly impacted by microfiber pollution present in the coastal waters of California, at least more so then *AN*.

The effect that MF pollution has had on species like the California Market squid [figure.2] may not isolated, it is an impact that could be carried onto its predators in a domino effect via trophic transfer. Research has suggested that MFs accumulate through the trophic levels of an ecosystem, meaning that predators will retain much of the microfibers present in their prey[4]. It was observed that the fish which had consumed *AN* had a significantly higher microfiber concentration then the fish that had not[2]. This research and my data suggests that the majority of the microfibers found in the California market squids obtained may have been transmitted through the consumption of its prey. However, further squid samples will need to be analyzed along with other data on MF in its prey in order to certify this conclusion. If this is the case, it could be an indication of how prevalent MFs may be in the aquatic ecosystem of Southern California given the squid's role as a keystone species [figure 1].

Moving Forward

Moving forward, it is my goal to continue to analyze more California Market squid samples from southern California to achieve a total of 200 processed samples this upcoming summer in order to certify the results. This would allow for a more comprehensive conclusion to be formed and allow the results to be published in a scientific journal.Along with this, I hope to explore the topic of trophic transfer in relation to the squid more profoundly in order to understand the extent of microfiber pollution in the ocean waters of Southern California and the dangers its ecosystem may be facing.

Figure 2. *Loligo Opalescens* (sample 18). Average squid length is ~21.96 cm (excluding the tentacles and arms).

Purpose

To analyze and record the quantity of microfibers present in the gastrointestinal tract of the california market squid.

Methods & Materials

1. Preparation of salt water solution in 600 mL Pyrex beaker
   a. 500 mL of distilled water mixed with 5 mL of sea salt
2. Dissection of squid digestive system
   a. Including the stomach, kidney, esophagus, and liver
3. Grinding of digestive system contents with a mortar and pestle into a paste
4. Suspend microfibers in 100 mL of salt water solution for one hour
5. Buchner vacuum filtration of microfiber solution
   [Repeat steps 4 & 5 a total of five times]
6. Place filter paper in petri dish
7. Utilize light microscope to count the number of synthetic fibers and their colors

Results

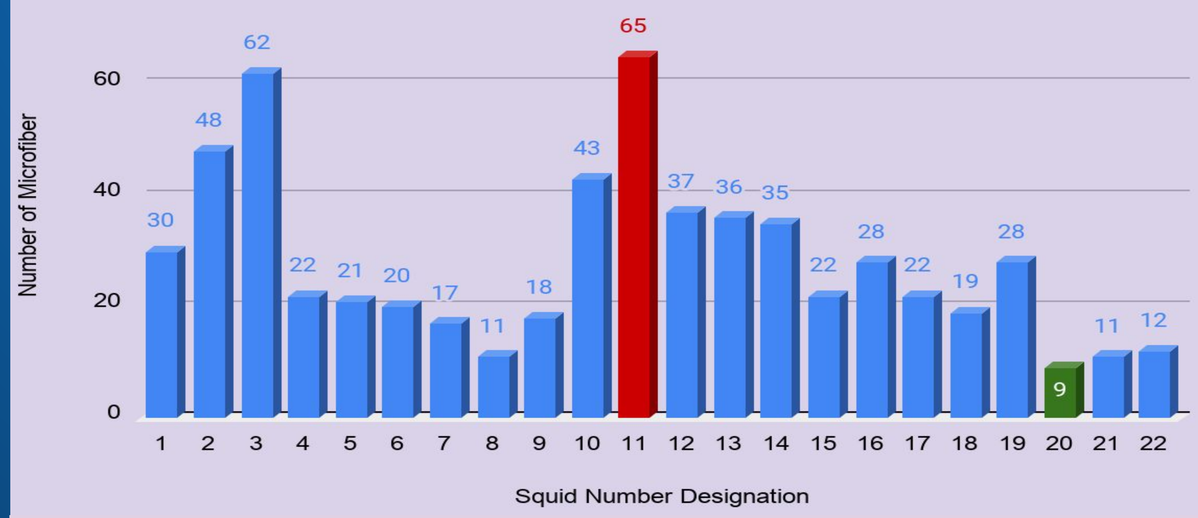

Figure 3. Bar graph comparing the total number of microfibers (MF) observed in each *Loligo Opalescens* sample (1-22). Maximum MF amount is 65 MF (red). The minimum MF amount is 9 MF (green). The average MF amount is 28 MF.

Acknowledgments

We would like to thank my mentors Dr. Huvard and Dr. Swig for their support and guidance. My partner, Alexandra De Leon, for her hard work and support. I would like to acknowledge ALLIES in STEM fellowship for funding my research. As well as OURCS, Dr. Knox, and California Lutheran University for the opportunity to conduct our research amidst this pandemic.

References

1. *California Market Squid.* (n.d.). NOAA Fisheries. Retrieved on June 4, 2020 from https://www.fisheries.noaa.gov/species/california-market-squid
2. Del, C. A.-P. M., Herrera-Galindo, E., & Cruz-González DG. (2019). Description of buoyant fibers adhering to argonauta nouryi (cephalopoda: argonautidae) collected from the stomach contents of three top predators in the mexican south pacific. *Marine Pollution Bulletin, 142,* pp. 504–509. https://doi.org/10.1016/j.marpolbul.2019.04.016
3. (n.d.). Food web [Photograph]. Encyclopedia Britannica. https://kids.britannica.com/students/assembly/view/90131.
4. Gouin, T. (2020). Toward an improved understanding of the ingestion and trophic transfer of microplastic particles: critical review and implications for future research. *Environmental Toxicology and Chemistry*, *39*(6), pp. 1119–1137. https://doi.org/10.1002/etc.4718
5. Ivar de Sul, J.A. & Costa M.F. (2013). The present and future of microplastic pollution in the marine environment. *Environmental Pollution, 185,* 352-364. https://doi.org/10.1016/j.envpol.2013.10.036
6. Nelms, S. E., Galloway, T. S., Godley, B. J., Jarvis, D. S., & Lindeque, P. K. (2018). Investigating microplastic trophic transfer in marine top predators. *Environmental Pollution* (Barking, Essex : 1987), *238*, 999–1007. https://doi.org/10.1016/j.envpol.2018.02.016
7. Neves, D., Sobral, P., Ferreira, J.L., & Pereira, T. (2015). Ingestion of microplastics by commercial fish off the Portuguese coast. *Marine Pollution Bulletin, 101*, 119-126. https://doi.org/10.1016/j.marpolbul.2015.11.008
8. Yang, L., Qiao, F., Lei, K., Li, H., Kang, Y., Cui, S., & An, L. (2019). Microfiber release from different fabrics during washing. *Environmental Pollution, 249*, 136-143. https://doi.org/10.1016/j.envpol.2019.03.011
9. Ziedberg, L.D. (2013). Chapter VI: Doryteuthis opalescens, opalescent Inshore Squid. In R. Rosa, R. O'Dor & G.J. Pierce (Eds.), *Advances in squid biology, ecology and fisheries: Part I - Myopsid Squids* (pp. 159-195). Nova.