Microplastic ingestion by group and size of zooplankton in the Equatorial Pacific

Minako Cheney

University of Washington

School of Oceanography, Box 357940

Seattle, WA 98195-7940

Email: minache@uw.edu

3/8/2024

Abstract

Since the discovery of microplastics in the Sargasso Sea in 1972, more plastics have entered our oceans. These gradually degrade into what we know as microplastics. If they get small enough, they can fall into the size range that zooplankton feed on. If consumed by zooplankton it can affect the health of the zooplankton themselves, but also lead to possible health complications in humans. The study looks at what factors such as groups of zooplankton, bead size, and zooplankton size have an impact on ingestion of microplastics. The samples were collected on the *R/V Thompson G. Thompson* along a transect from American Samoa north to 5°N, 167°W from December 28th to January 9th. Samples were collected with a 1-meter 209 µm zooplankton net and a 333 µm Manta Net. The zooplankton used in the study mainly consisted of ostracods and amphipods, with a mixture of copepods. The zooplankton samples were injected with fluorescent polystyrene bead sizes of 7.3 µm, 10.6 µm, 15.8 µm, and 20.6 µm and put in either a solution of filtered or unfiltered seawater. They were then viewed under a fluorescent microscope to view possible ingestion of the beads. The findings were that zooplankton consume plastics even in the presence of plankton, which indicates possibility for consumption in a non-lab setting. Another finding was that size is a major contributor to zooplankton bead ingestion. Larger zooplankton consumed a wider range of bead sizes compared to smaller zooplankton.

Plain language summary

Zooplankton have been found to consume microplastics in previous studies. This study looked at the factors that may impact ingestion rates of microplastics. These factors include groups of organisms, bead size, and size of zooplankton. The findings include zooplankton in filtered seawater were more likely to consume plastic beads with the most common size consumed being 15.8 µm which is a size found within the range of food eaten by zooplankton. There was also a trend of increasing organism size correlating with higher ingestion rates of

beads. This finding matters because zooplankton are an important piece in the ocean food web as they get consumed by small fish and other organisms. Plastics carry harmful chemicals which are the main concern in trophic transfer from zooplankton to other organisms, as it implies that they may ultimately reach humans.

Introduction:

The global demand for plastic in 2011 was found to be 245 million tons (Andrady, 2011), but currently stands at 390.7 million tons. It was estimated in 2010 when the global demand was at 275 million metric tons, that around 4.8 - 12.7 million metric tons entered the ocean (Jambeck et al., 2015). Based on that, there is around 6.8 – 18 million metric tons entering the ocean today. Plastics predominantly enter the ocean through land-based sources such as mismanaged waste where the waste is not properly contained. Waterways, wastewater outflows, and winds or tides can transport these mismanaged sources of waste into the ocean (Jambeck 2015). Maritime sources contribute to a smaller but significant portion of the pollution through fishing which includes “Ghost nets” or abandoned fishing nets (Thushari et al., 2020).

Marine plastics were not a concern until relatively recently in 1972 when plastic particles were found in the Sargasso Sea (Carpenter and Smith, 1972). This initial finding led to others joining in the research of plastics in the ocean such as the 20-year-long study of plastics in the surface waters of the North Atlantic Subtropical Gyre from 1986 to 2008 (Law et al., 2010), and the discovery of the Great Pacific Garbage patch in the North Pacific Subtropical Gyre (Moore et. al., 2001). The discovery of the Great Pacific garbage patch has led to marine plastics becoming a high-priority area of research for ocean sciences due to their persistence and impact on the ocean itself, animals, and humans (Andrady, 2011).

When plastic is exposed to the environment, it degrades due to wave action, UV rays, temperature, and biodegradation. This creates what we know as Microplastics or plastic fragments smaller than 5mm in diameter (Andrady, 2011). Microplastics come in different forms with the primary microplastics composed of microbeads intentionally created for consumer products such as detergents, cosmetics, and synthetic particles manufactured for air blasting and boat antifouling. The secondary form of microplastics is plastic waste that has since degraded into smaller pieces and fibers (Lee et al., 2023, Galloway et al., 2017). It is estimated that in marine environments a plastic fragment with a diameter of 1mm could take approximately 300 years to degrade to a diameter of 1nm (Galloway et al., 2017).

Microplastics are made of a variety of resin types including polyethylene, polypropylene, polystyrene, etc. These plastics are processed with additives during production such as flame retardants, plasticizers, and antimicrobial agents. Microplastics have also been found to have an accumulation of persistent organic pollutants (POPs) which bond more to plastics than to seawater. POPs persist for long periods, accumulate in organisms through trophic levels, and are known to be toxic to both humans and animals (Environment U.N., 2017). Some persistent organic pollutants include polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), and organochlorine pesticides (Smith et al., 2018). Multiple studies have found that human exposure to POPs can cause hormonal disruption, developmental effects, inflammatory effects, lipid metabolism disturbances, and cancer (Lee et al., 2023, Smith et al., 2018, Cole et al., 2013). One of many organisms that can be affected by these chemical additives and POPs are zooplankton, who have been known to consume microplastics.

Zooplankton are heterotrophic marine organisms consisting of Meroplankton which are larvae and Holoplankton which stay plankton their whole life. Zooplankton experience diurnal vertical migration which is where during the day they stay below the zone with visible light to avoid predation, and during night they swim up to feed on the phytoplankton (MarineBio Conservation Society, US EPA, 2013). They are important to the ecosystem as they are primary consumers and are a crucial source of food for the secondary consumers in the ocean such as fish, jellyfish, and even whales. The concern with microplastics and zooplankton is that since they are primary consumers, they have the ability to transfer microplastics across a larger trophic level (Fig. 1). The sizes of microplastics also overlap with the sizes of prey for zooplankton and the ingestion of microplastics by zooplankton has been reported (Cole 2013, Desforges 2015, Sun 2017). It is still unknown how much transfers between trophic levels, but it is known that many organisms expel microplastics at some point and retention is not long (Walkinshaw et al, 2020). Although microplastic retention is not long, the main concern comes with the chemical additives that make up properties of plastics. Ingestion of microplastics has been reported to cause effects on the behavior and growth and reproduction of zooplankton (Cole et al., 2013, Lee et al., 2023). Zooplankton feed in the surface ocean which has a high abundance of microplastics (Botterell et al., 2019. Their feeding method of filter feeding means they will consume microplastics along with plankton (Cole et al., 2013). These effects on behavior, growth, and reproduction are critical

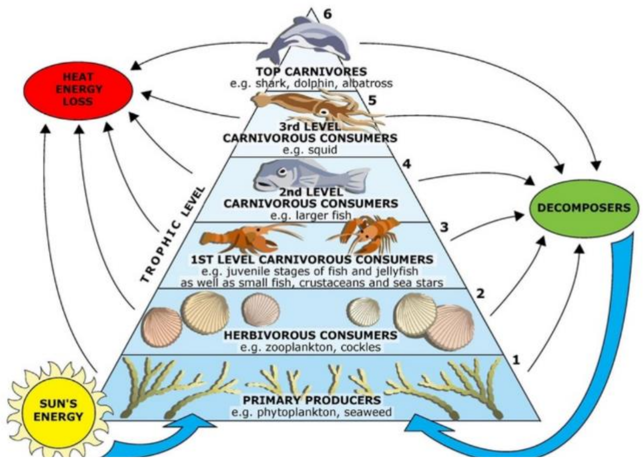

Figure 1. Diagram of the marine trophic pyramid (Marine food webs).

to understand because any decline in population of zooplankton can have a bottom-up effect on trophic levels eventually affecting the larger fish and marine mammals (NOAA Ocean Exploration).

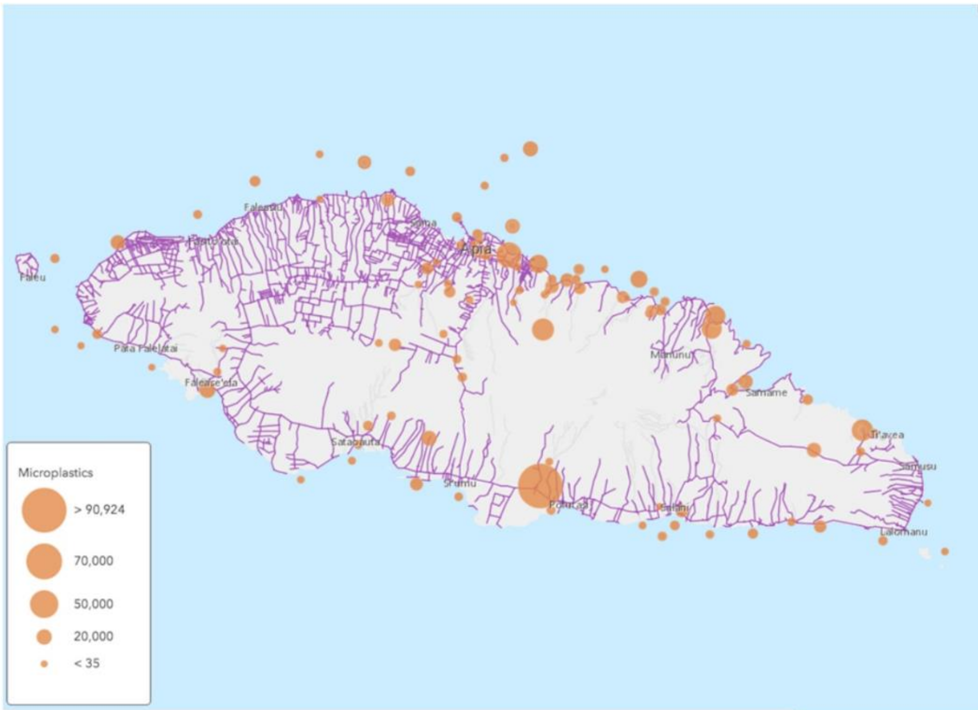

Figure 2. Shows distribution of microplastics around Samoa (Samoa Observer)

In 2020 there was a study which sampled freshwater, seawater, and mangrove swamps around the island of Samoa (Biosecurity of Upolu Fresh and Salt environment Water Resources). It revealed that in seawater there was from 35 pieces of microplastic per square micrometer to as many as 32, 709 with a median of 3,023 (Fig 2). Nearly all samples found in this study contained microplastics. Although this article does warn of the potential impacts of microplastics on humans, they put a stronger emphasis on larger plastics that kill larger marine animals and fish. They cited the need for stronger evidence to focus on microplastics, for which this study will aim to add evidence to the growing data on zooplankton microplastic ingestion.

Among the organisms found in the study, there was an overwhelming number of Myostracods. Ostracods are small organisms belonging to crustacea and grow between 0.5mm to 1.5 mm long on average (British Geological Survey). They will consume a wide variety of food which includes, but is not limited to algae, dead animals, and live snails and worms. They utilize antennae, mandibles, and maxillulae to feed. Ostracods can be non-swimmers that are swept upwards by currents and waves, but some are able swimmers. They are found in a wide variety of environments such as the ocean from coast to deep ocean, rivers, and lakes but overall, most live on the bottom near plants and sediments. Organisms living in the surface waters that are near the surface in marine environments tend to be planktonic. A previous study that looked at microplastic counts and zooplankton in the ocean found that ostracods do consume microplastics in forms such as fibers, fragments, and pellets. (Goswami et al., 2022, Lake Biwa Museum).

The goal of this study is to determine if the size of the organisms, size of beads, and type of groups will impact the ingestion rate of microplastics. The aim is to determine specific groups of zooplankton and their ability to consume different sizes of microplastics. Given past studies, I hypothesize that the zooplankton will ingest microplastics and that size of zooplankton, group of zooplankton, and bead size will be the main factors influencing microplastic ingestion.

Methods

Figure 3. Map of cruise transect. Shows the four stations at which samples were collected at 3°S, 0°, 3°N, and 5°N.

Collection for samples was done on the *R/V Thompson G. Thompson* in 2023 from December 28th through January 10th (Fig 3). Samples were collected at 3°S, 0°, 3°N, and 5°N. Samples were collected using a 1 meter 209 µm and a 333 µm mesh Manta Net. The nets were rinsed off with seawater and the zooplankton were collected into two 4 liter jars. Due to time constraints, around 10 zooplankton were immediately pipetted into each of the eight 20 ml scintillation vials, 4 were filtered seawater to remove possible plastics and food for zooplankton, the other 4 being non-filtered seawater. 20 uL of 7.3 µm polystyrene beads were pipetted into one of the filtered and unfiltered seawater vials. This was then repeated for sizes 10.6 µm, 15.8 µm, and 20.6 µm. The vials were then rotated for 24 hours on a rotating wheel to simulate ocean wave movement. Samples after 24 hours were then put in formalin to preserve until arrival at University of Washington. They were then filtered and washed off with water to rinse off formalin and any beads stuck to the outside of organisms. They were then put under a fluorescent microscope to view possible ingestion of fluorescent beads (Fig. 4). Beads were counted, organism size was measured, and zooplankton were identified. If multiple

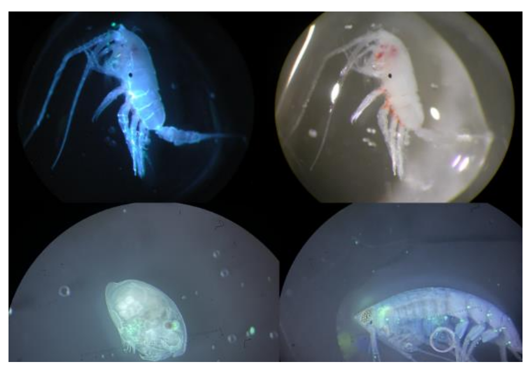

Figure 4. Top right is an organism with no fluorescence in normal lighting. The top left depicts same organism with no beads ingested under fluorescent lighting. Bottom left shows ostracod with beads ingested. Bottom left shows beads being ingested, but also provides a view of beads stuck to outside of the organism. Beads on the outside will appear brighter and less muted than those ingested.

beads were consumed, the diameter of the clump of beads was divided by the diameter of the bead.

Results

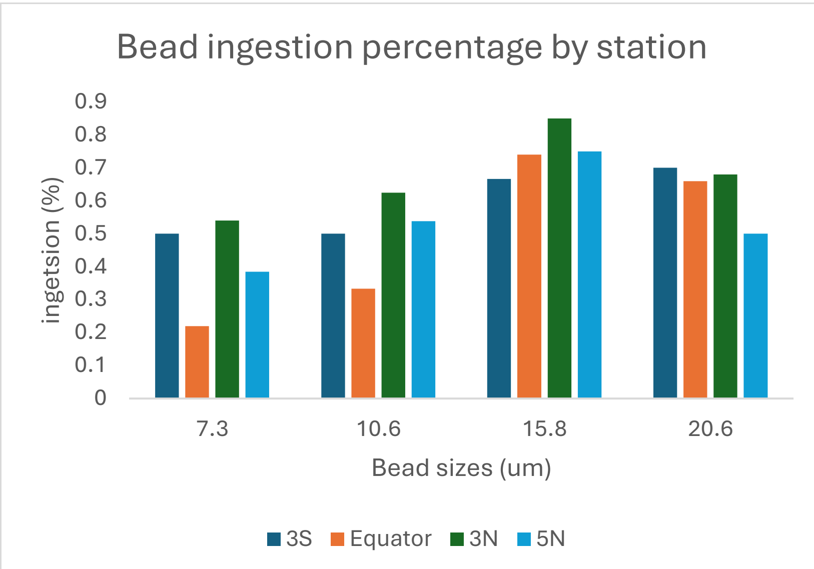

Figure 4 Bead ingestions shown for each station regardless of seawater solution. The highest consumed size on average was 15.8 µm with highest ingestion at 85% for 3°N. Lowest consumed bead was 7.3 µm with lowest ingestion at the equator.

Bead Size

When first analyzing the data, the percentage of ingestion of each bead size from the zooplankton examined. It was found that the most consumed bead size was 15.8 µm at an average consumption across all stations to be 75%, with the second highest being 20.6 µm at an average consumption of around 65%. The highest consumption of 15.8 µm was at 3°N with around 85% consumption and for 20.6 µm was at 3°N with around 68% ingestion. The lowest ingestion overall was for 7.3 µm and 10.6 µm which were both below the 50% mark. The highest ingestion at 5°N and 3°N and the equator was 15.8 µm, while station 3°S was highest for 20.6 µm.

Organism type and size

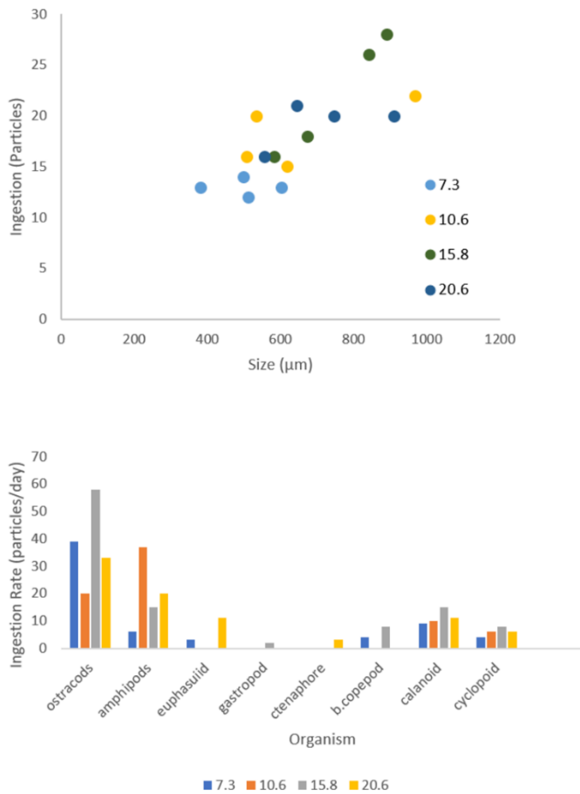

Figure 5 A. Positive correlation between Ingestion count and average organism size with an r squared value of 0.65 and p-value = 0.047 B. Ostracods had highest ingestion rates of 7.3 µm, 15.8 µm and 20.6 µm beads. The corresponding ingestion rates were 40, 57, and 33 beads per day. Amphipods ingested the highest for 10.6 µm beads at 35 beads per day.

Ostracods were the most abundant at this station and had the highest ingestion rate for 7.3 µm, 15.8 µm, and 20.6 µm polystyrene beads. The highest consumption for 10.6 µm was seen in amphipods at an ingestion rate of around 1.5 beads per day (Fig. 6). Higher ingestion of beads was seen to have a moderate positive correlation with increasing size with an r^2 value of 0.65. P value for Size to beads consumed was p = 0.047 (Fig. 6).

Water conditions

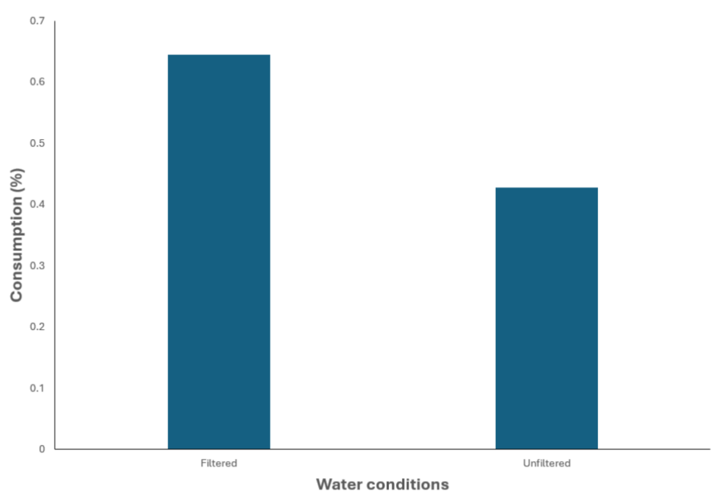

Figure 7 Displays percentage of consumption versus non consumption of all bead sizes by percentage. Around 65% of zooplankton sampled was found to consume beads.

There were two conditions created for the zooplankton at each station where one had filtered water using a 0.2-micron filter, and the other with unfiltered surface seawater. It was found that around 60% of zooplankton across all stations consumed plastic beads in filtered water while around 40% consumed beads in unfiltered water. The highest consumption for filtered water occurred at 3°N, while lowest was at 3°S. The highest consumption for filtered water occurred at 3°N, while the lowest occurred at 3°S.

Discussion

The most important finding was that zooplankton still consumed beads even in the presence of phytoplankton which is representative of their normal environment. Although there was a higher percentage of consumption for filtered water. This just shows that when there is an absence of food, zooplankton will consume more microplastics and that it is not chance that this is higher than unfiltered water. The main point of the two conditions was that there is still consumption of beads for zooplankton even when they are in the presence of their normal food. This indicates that even in the open ocean, zooplankton can exhibit behaviors of microplastic consumption.

An important finding was that size was a main factor when considering zooplankton microplastic ingestion. There was found to be a moderate positive correlation between the average size of organism versus the count of beads ingested with the r^2 value being 0.65. Only looking at this we see that one of the main factors of consumption is the size of organism. If we include the p-value which was 0.047 we see that this occurrence is statistically significant and isn’t due to chance. Regardless, looking at the p-value tells us that size is one main factor influencing the ingestion of microplastics.

The organism groups that were most likely to consume microplastics were ostracods and amphipods. Ostracods had the highest ingestion rates for 3 out of 4 bead sizes, with amphipods having the highest for 10.6 µm. The most consumed bead sizes were on average 15.8 µm and 20.6 µm which given the average sizes of organisms being relatively high is logical as larger organisms would be able to consume more of the larger bead sizes compared to small organisms. Smaller bead sizes such as 7.3 µm and 10.6 µm were consumed by the smaller organisms found in the study (Fig 5).

Constraints for the project include the lack of a 24-hour rest period, which was cut from the original plan due to time limits between station and material limits. The study also included only 4 stations of which 5°N only had unfiltered water, and 2 stations had a smaller sample size due to lack of live zooplankton. This may be attributed to pulling the zooplankton net too fast up from 200-300 meters depth and due to spraying the cod end of the nets too harshly which may have led to more sensitive zooplankton to not surviving. The zooplankton groups included were also not indicative of the zooplankton diversity along the transect as they were chosen for the sampling due to being alive after the net tows.

Conclusion

Microplastics are a huge concern as they can contain toxins and chemicals that can bioaccumulate in the food chain and ultimately reach humans. These plastics also lead to disruptions in the behavior, growth, and reproduction of zooplankton. This study found that even in the presence of plankton, zooplankton exhibited behaviors of feeding on microplastics. This indicates that in the open ocean they have the ability to consume microplastics. Larger zooplankton were also found to consume larger bead sizes and overall consume more plastics. In zooplankton larger than 600 µm the most consumed bead sizes included 15.8 µm and 20.6 µm, while zooplankton smaller consumed more 7.3 and 10.6 um beads. Ostracods and amphipods were found to consume the most beads among the organisms sampled with ostracods consuming the most 7.3, 15.8, and 20.6 μm beads. This study shows that zooplankton can consume plastics even when they have food present and that size is one of the main factors when considering the size and amount of beads ingested. Future studies could look at the chlorophyll levels before and after the experiment to compare if there was ingestion of phytoplankton or algae and compare with bead consumption. More stations and replicates would be helpful in determining if zooplankton group is a major contributing factor to ingestion.

Acknowledgments

I would like to thank all the crew members on the *R/V Thompson G. Thompson* who allowed us to sample and get our data for our thesis and for being very welcoming and supporting us throughout our time on the boat. I would also like to thank Professor Kathy Newell and the rest of the "Zoop Group" for being there for me throughout this entire process. Without the team, I don't think I could've completed my project. I also would like to thank my ocean senior class for reviewing my paper, listening to my endless questions, and providing amazing feedback. Lastly, I would like to thank my boyfriend Andy Yuen for being there for me when I was struggling with my project and needed support.

References

Andrady, A. L. (2011). Microplastics in the marine environment. *Marine Pollution Bulletin*, *62*(8), 1596–1605. https://doi.org/10.1016/j.marpolbul.2011.05.030

Athey, S. N., Albotra, S. D., Gordon, C. A., Monteleone, B., Seaton, P., Andrady, A. L., et al. (2020). Trophic transfer of microplastics in an estuarine food chain and the effects of a sorbed legacy pollutant. *Limnology and Oceanography Letters*, *5*(1), 154–162. https://doi.org/10.1002/lol2.10130

Avery-Gomm, S., O'Hara, P. D., Kleine, L., Bowes, V., Wilson, L. K., & Barry, K. L. (2012). Northern fulmars as biological monitors of trends of plastic pollution in the eastern North Pacific. *Marine Pollution Bulletin*, *64*(9), 1776–1781. https://doi.org/10.1016/j.marpolbul.2012.04.017

Boerger, C. M., Lattin, G. L., Moore, S. L., & Moore, C. J. (2010). Plastic ingestion by planktivorous fishes in the North Pacific Central Gyre. *Marine Pollution Bulletin*, *60*(12), 2275–2278. https://doi.org/10.1016/j.marpolbul.2010.08.007

Botterell, Z. L. R., Beaumont, N., Dorrington, T., Steinke, M., Thompson, R. C., & Lindeque, P. K. (2019). Bioavailability and effects of microplastics on marine zooplankton: A review. *Environmental Pollution*, *245*, 98–110. https://doi.org/10.1016/j.envpol.2018.10.065

Carpenter, E. J., & Smith, K. L. (1972b). Plastics on the Sargasso Sea Surface. *Science*, *175*(4027), 1240–1241. https://doi.org/10.1126/science.175.4027.1240

Cole, M., Lindeque, P., Fileman, E., Halsband, C., Goodhead, R., Moger, J., & Galloway, T. S. (2013). Microplastic Ingestion by Zooplankton. *Environmental Science & Technology*, *47*(12), 6646–6655. https://doi.org/10.1021/es400663f

Desforges, J.-P. W., Galbraith, M., & Ross, P. S. (2015). Ingestion of Microplastics by Zooplankton in the Northeast Pacific Ocean. *Archives of Environmental Contamination and Toxicology*, *69*(3), 320–330. https://doi.org/10.1007/s00244-015-0172-5

Environment, U. N. (2017, October 3). Why do persistent organic pollutants matter? Retrieved November 15, 2023, from http://www.unep.org/explore-topics/chemicals-waste/what-we-do/persistent-organic-pollutants/why-do-persistent-organic

Frias, J. P. G. L., Otero, V., & Sobral, P. (2014). Evidence of microplastics in samples of zooplankton from Portuguese coastal waters. *Marine Environmental Research*, *95*, 89–95. https://doi.org/10.1016/j.marenvres.2014.01.001

Galloway, T. S., Cole, M., & Lewis, C. (2017). Interactions of microplastic debris throughout the marine ecosystem. *Nature Ecology & Evolution*, *1*(5), 0116. https://doi.org/10.1038/s41559-017-0116

Goswami, P., Selvakumar, N., Verma, P., Saha, M., Suneel, V., Vinithkumar, N. V., et al. (2023). Microplastic intrusion into the zooplankton, the base of the marine food chain: Evidence from the Arabian Sea, Indian Ocean. *Science of The Total Environment*, *864*, 160876. https://doi.org/10.1016/j.scitotenv.2022.160876

Isobe, A., Iwasaki, S., Uchida, K., & Tokai, T. (2019). Abundance of non-conservative microplastics in the upper ocean from 1957 to 2066. *Nature Communications*, *10*(1), 417. https://doi.org/10.1038/s41467-019-08316-9

Jambeck, J. R., Geyer, R., Wilcox, C., Siegler, T. R., Perryman, M., Andrady, A., et al. (2015). Plastic waste inputs from land into the ocean. *Science*, *347*(6223), 768–771. https://doi.org/10.1126/science.1260352

Lake Biwa Museum. (n.d.). Retrieved February 5, 2024, from https://www.biwahaku.jp/smith/ostracod_food.html

Law, K. L., Morét-Ferguson, S., Maximenko, N. A., Proskurowski, G., Peacock, E. E., Hafner, J., & Reddy, C. M. (2010). Plastic Accumulation in the North Atlantic Subtropical Gyre. *Science*, *329*(5996), 1185–1188. https://doi.org/10.1126/science.1192321

Lee, Y., Cho, J., Sohn, J., & Kim, C. (2023). Health Effects of Microplastic Exposures: Current Issues and Perspectives in South Korea. *Yonsei Medical Journal*, *64*(5), 301–308. https://doi.org/10.3349/ymj.2023.0048

Manta Microplastics Sampling Trawl Net. (n.d.). Retrieved December 9, 2023, from https://www.nhbs.com/manta-trawl-net

Marine food webs. (n.d.). Retrieved December 8, 2023, from https://www.sciencelearn.org.nz/resources/143-marine-food-webs

Microplastics across Upolu's water measured. (n.d.). Retrieved March 8, 2024, from https://www.samoaobserver.ws/category/samoa/74191

Moore, C. J., Moore, S. L., Leecaster, M. K., & Weisberg, S. B. (2001). A Comparison of Plastic and Plankton in the North Pacific Central Gyre. *Marine Pollution Bulletin*, *42*(12), 1297–1300. https://doi.org/10.1016/S0025-326X(01)00114-X

Ostracods. (n.d.). Retrieved March 8, 2024, from https://www.bgs.ac.uk/discovering-geology/fossils-and-geological-time/ostracods/

Pasquier, G., Doyen, P., Kazour, M., Dehaut, A., Diop, M., Duflos, G., & Amara, R. (2022). Manta Net: The Golden Method for Sampling Surface Water Microplastics in Aquatic Environments. *Frontiers in Environmental Science*, *10*. Retrieved from https://www.frontiersin.org/articles/10.3389/fenvs.2022.811112

Savoca, M. S., McInturf, A. G., & Hazen, E. L. (2021). Plastic ingestion by marine fish is widespread and increasing. *Global Change Biology*, *27*(10), 2188–2199. https://doi.org/10.1111/gcb.15533

Small But Mighty: The Importance of Tiny Organisms in Marine Ecosystems: Coordinated Simultaneous Physical-Biological Sampling Using ADCP-Equipped Ocean Gliders: NOAA Ocean Exploration. (n.d.). Retrieved December 8, 2023, from https://oceanexplorer.noaa.gov/technology/development-partnerships/21adcp-gliders/features/zooplankton/zooplankton.html

Smith, M., Love, D. C., Rochman, C. M., & Neff, R. A. (2018). Microplastics in Seafood and the Implications for Human Health. *Current Environmental Health Reports*, *5*(3), 375–386. https://doi.org/10.1007/s40572-018-0206-z

Thushari, G. G. N., & Senevirathna, J. D. M. (2020). Plastic pollution in the marine environment. *Heliyon*, *6*(8), e04709. https://doi.org/10.1016/j.heliyon.2020.e04709

US EPA, O. (2013, November 21). Indicators: Zooplankton [Overviews and Factsheets]. Retrieved November 15, 2023, from https://www.epa.gov/national-aquatic-resource-surveys/indicators-zooplankton

Zooplankton ~ MarineBio Conservation Society. (n.d.). Retrieved November 15, 2023, from https://www.marinebio.org/creatures/zooplankton/