

**Size Matters: The Variables Affecting Microplastic Ingestion Rates in  
Copepods**

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## **Abstract**

Copepods, the most abundant zooplankton, are known to consume microplastics. My study aims to see what factors, copepods size; bead size; location of captured copepod, played the biggest role in their ingestion rate. On the R/V Thompson, copepods were collected along the southern edge of the East Pacific Gyre and fed different microplastic sizes: 10.6  $\mu\text{m}$ , 10.9  $\mu\text{m}$ , and 15.8  $\mu\text{m}$ . The microplastics fed were fluorescent beads that glowed under a fluorescent light making ingestion visible through a microscope. At the University of Washington, the size of the copepods and the amount of beads ingestion were noted. I found that copepods larger than 300  $\mu\text{m}$  had the highest rate of ingestion for mainly the 10.9  $\mu\text{m}$  and 15.8  $\mu\text{m}$  bead sizes. Smaller copepods the 10.9  $\mu\text{m}$  bead sizes at high raters. My major finding was that the size of the copepods was the main factor in ingestion rates and size selectivity of the beads. Additionally, I found that there was an inverse relationship between ingestion rate and location; copepods consumed less if they were in an area of high plastic concentration. My findings agree with other studies that have proved microplastic ingestion in copepods and explains what can increase the amount of microplastics in a food web.

## **Plain Language Summary**

Anthropogenic pollution is defined as any pollution caused by humans. Right now, plastics are becoming a concerning issue of anthropogenic pollution. The reason is that since plastics take hundreds of years to degrade, they linger on our planet for hundreds of years. During that time, plastics break down into sizes as small as sand; these are called microplastics. There has been evidence of small organisms consuming these microplastics such as zooplankton.

Zooplankton are important to global food webs since they are regularly consumed by lower trophic level fishes and other smaller organisms. Copepods are the most abundant zooplankton in our oceans and have had evidence of microplastic ingestion in the field and in laboratory studies. In additional studies, evidence of these microplastics in copepods and other zooplankton have been found to go up in the food web to top predators such as seals, tuna, and even humans. This is concerning since plastics contain and carry cancer-causing pollutants.

Plastics themselves contain many toxins. What is also alarming is that other toxins stick to these plastics. What is found in plastics can be resin type toxins. Pollutants found on plastics are persistent organic pollutants (POPs), which are a form of organic compounds that cannot be broken down. Both resin type toxins and POPs are extremely harmful to humans. They are known to aid in mutations in humans that can lead to the formation cancer cells.

This study aimed to see if the size of the copepods, the size of the beads fed, and where the copepods came from impacted their ingestion rate of microplastics. What I found was that the size of the copepods played the biggest role in how much microplastic was. This is a logical conclusion since larger organisms eat more food. However, this also means more microplastics have a chance of being in food webs from these larger copepods.

## **Introduction**

Plastics are a form of anthropogenic pollution. The more plastic we use, the more plastics get into our oceans. Overtime, these plastics break down into microplastics and are distributed throughout the oceans (Barnes et al., 2009; Desforges et al., 2014). The Great Pacific Garbage Patch (GPGP) is one of the most well-known places of plastic pollution in the ocean. The currents in the Pacific gyre make it so that plastics discarded in the ocean are trapped within the

gyre. Because these plastics are trapped, they break down into microplastics that are also carried by the currents. Towards the center of the gyre microplastic abundance does decrease and is highest along the edges of the Pacific Gyre. (Desforges et al., 2014; Fernández de Puelles et al., 2019). Microplastics sizes are characterized as any piece of plastic under 500  $\mu\text{m}$ . At this small of a size, microplastics can easily be ingested by smaller organisms like zooplankton (Cole et al., 2013; Sun et al., 2016).

Copepods are the most abundant zooplankton in the oceans. On average, 82% of zooplankton in the oceans are copepods (Fernández de Puelles et al. 2019). They are a main source of food for many higher trophic levels in the Pacific Ocean. Studies show that copepods can eat a wide variety of microplastic sizes making this a concerning cause for organisms that regularly consume copepods (Cole et al. 2013).

Evidence of microplastic ingestion in organisms has been found through numerous field and laboratory studies (Boerger et al., 2010; Cole et al., 2013; Desforges et al., 2015; Sun et al. 2016; Botterelle et al. 2019). In a laboratory study, it was observed that up to 50% of the copepods had consumed plastic particles fed to them (Cole et al., 2013) and in the northern South China Sea, it was found that copepods had consumed the most microplastics in comparison to other zooplankton groups (Sun et al., 2016). Other studies have shown that there are microplastics in higher trophic levels that consume zooplankton showing evidence of trophic transfer (Setälä et al., 2014; Ziccardi et al. 2016; Carber et al. 2018; Nelms et al. 2018; Miller et al. 2020). In a laboratory artificial food chain, evidence of microplastic trophic transfer in copepods to jellyfish was observed (Costa et al., 2020). Additionally, in a study by Nelms et al., 2018, evidence of microplastic trophic transfer from fish to top-predators, seals, was found. This

is alarming since microplastics contain harmful chemicals and can absorb carcinogenic pollutants in the ocean.

Microplastics can carry toxins that end up in the seafood we consume (Karbalaie et al. 2018; Smith et al., 2018). Examples of toxins found in microplastics are polypropoylene, polyethylene, polystyrene, and other resin types. Additionally, POPs existing in the ocean can adhere to microplastics. Some of these POPs include polychlorinated biphenyls, polycyclic aromatic hydrocarbons, and organochlorine pesticides (Smith et al., 2018). Other chemicals that can be absorbed into microplastics include phthalates, bisphenol A, flame retardants, PCBs, pesticides, fertilizers and heavy metals (Carbery et al., 2018). These pollutants are known to impact the human body by being an agent of genetic mutation leading to the development of cancerous cells within the body (Carbery el al., 2018; Karbalaie et al. 2018; Smith et al., 2018).

When looking at the study by Desforges et al. 2014, it was observed that many plastic particles were collected mainly towards the coast of Vancouver Island, with highest concentrations at the Queen Charlotte Sound and the Strait of Georgia. In another study by Desforges et al. 2015, a similar trend was observed in zooplankton in that the most ingested microplastic was from areas of high microplastic concentrations. In this 2015 study, it was discovered that copepods consumed more plastic that euphausiids and the most consumption was found in the Strait of Georgia.

My study aims to see if these trends of copepods consuming more microplastic where there are high concentrations of microplastics can be observed in other parts of the ocean. Additionally, I wanted to see what other factors play a role in microplastic ingestion in copepods, since they are the most abundant in the ocean and play a large part in many food webs. The factors I observed was the size of the plastic, the size of the copepods, and the location of where

the copepods were collected. I hypothesized that the size of the copepods and if they came from high plastic concentrations would be the main reason to copepod microplastic ingestion rates. Furthermore, the size of the beads would not play a major role in copepod feeding habits since all the bead sizes are under 20  $\mu\text{m}$  a size where >50% of copepods were seen to have ingestion (Cole et al., 2013).

## Methods

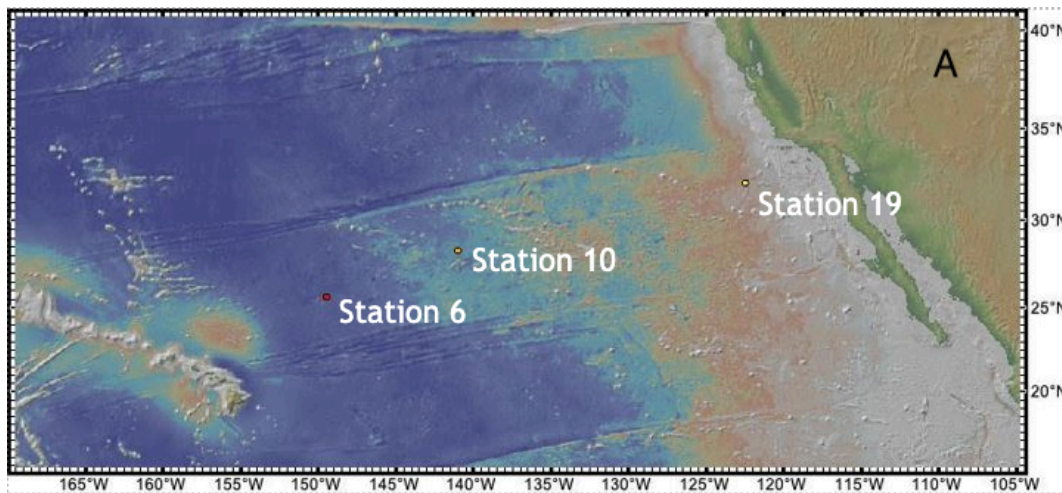


Figure 1. Map of sample sites. Station 6 and 19 were closest to shore. Station 10 was furthest from shore, but it was right along the edge of the East Pacific Gyre which is also the edge of the GPGP.

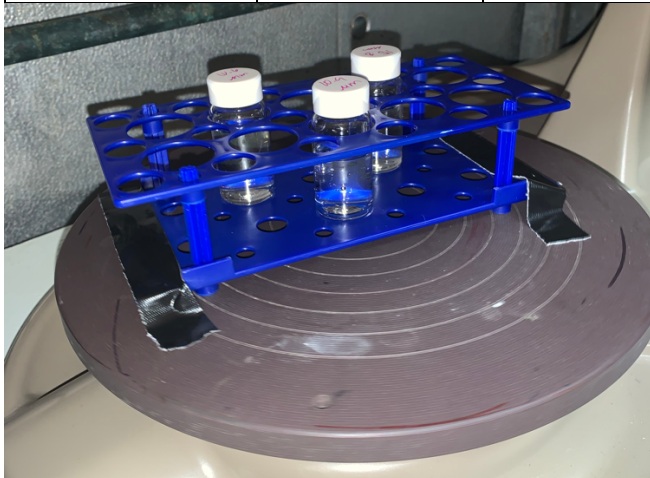
### *Sample Collection*

Collection was done on the R/V Thompson in 2021 on December 22, 23, and 28 (Table 1, Figure 1). A 1 m zooplankton closing net with a mesh size of 333  $\mu\text{m}$  was used to collect the zooplankton. Samples at each station were taken from a depth of 200 m between the hours of 2100 and 0300 when zooplankton are actively feeding (Table 1). The net was then hosed off with seawater to ensure all the zooplankton were off the net and in the collection bottle. The zooplankton were placed inside a 1 L jar with air stones attached to pumps, used to aerate the samples, inside a cooler. They sat for 24 to secrete any leftover organic matter or potential

microplastics left in their system. Samples were kept at ambient sea-surface temperatures to that of the sampling site which was determined by CTD casts. After the 24 hours, I collected live copepods from the 1 L jars and placed >10 living copepods in 10 mL vials each along with filtered seawater to make sure my samples are not ingesting additional plastic. 20  $\mu$ L of 20  $\mu$ m fluorescent polystyrene beads at sizes were pipetted into the of the vials. This was done for each of the bead sizes: 10.6  $\mu$ m, 10.9  $\mu$ m, and 15.8  $\mu$ m. The vials were rotated on a wheel for 24 hours at under 5 rpm to mimic the flow of the sea (Cole et al., 2013) (Figure 2).

*Table 1. Location, date, and time of sample collection.*

Station	Date	Latitude	Longitude	Local Time
6	12/22/2021	25.6595	-149.5	2:45
10	12/23/2021	28.3151	-141.026	21:50
19	12/28/2021	32.0978	-122.4999	0:46



*Figure 2. The set-up of the rotating wheel.*

### *Quantifying Ingestion*

The samples were placed in a solution of formalin to preserve until they got to the lab at the University of Washington in Seattle. There, the samples were rinsed 5 times with filtered seawater to wash off any microplastics stuck to them. A fluorescent microscope was used to

observe the ingestion of microplastics. Individual microplastic particles were counted and size of copepods were calculated. Those that had particles clumped together inside, were estimated by dividing the diameter of the particle mass from the diameter of 10 particles (Figure 3).

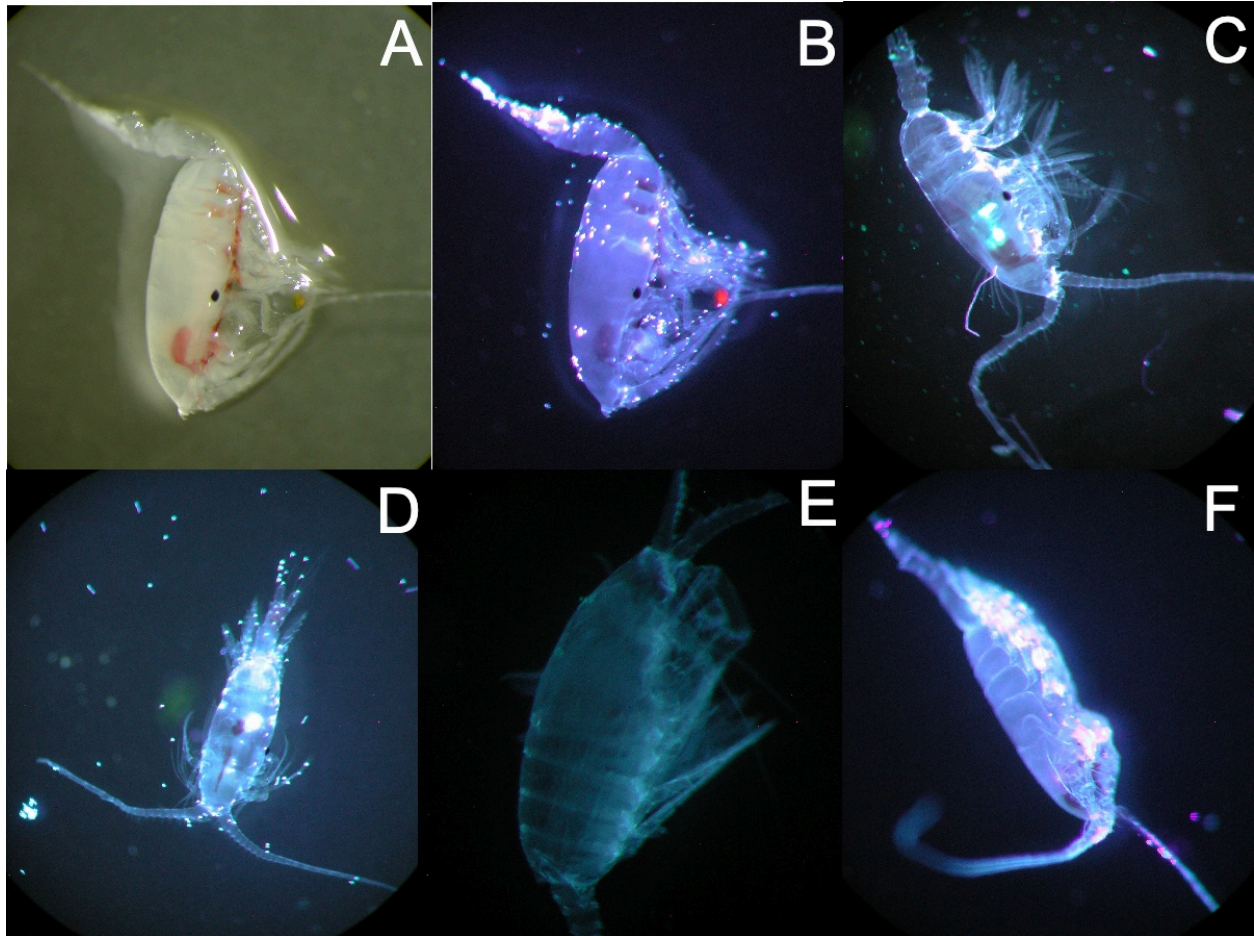


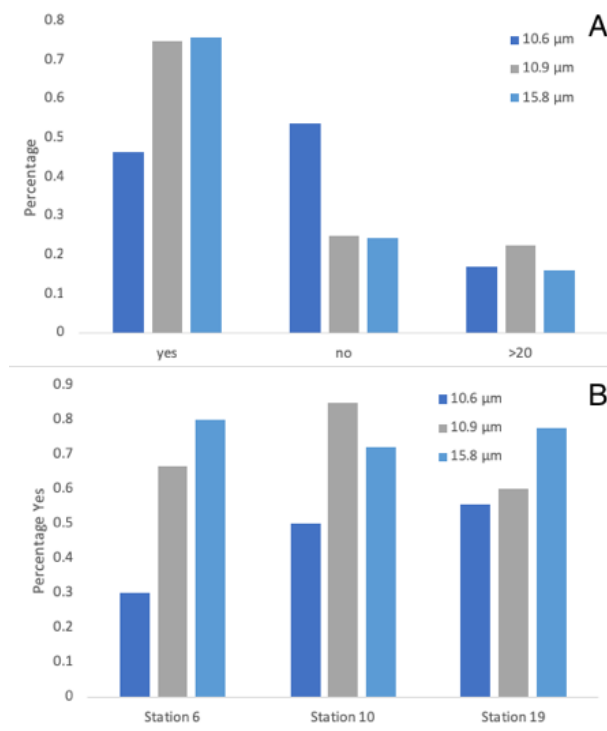
Figure 3. Pictures taken of copepods after ingestion. A) How the copepods were viewed without the fluorescent light. B) Fluorescent beads glowing under the light. C) Ingestion of the 10.6  $\mu\text{m}$  beads. The bright part in the copepod is a clump of the beads. D) Top view of a copepod where I could better see ingestion. If the beads looked more dull/blurry it indicated ingestion rather than the beads being on the copepod. E) No ingestion in copepods resembles this. F) In this view it looks like ingestion, but the beads were attached to their foot.

## Results

### *Bead Size*

When observing ingestion, I first wanted to see what percentage of copepods ingested the plastic beads. I found that with the 10.6  $\mu\text{m}$  bead size, less than 50% of the copepods ingested

the beads. Only the 10.9  $\mu\text{m}$  and the 15.8  $\mu\text{m}$  bead size had more than a 50% ingestion of the beads (Figure 4A). When looking at each station and the percentage of each of the beads that were consumed, I found that at station 6 and 19, around 80% of the copepods fed 15.8  $\mu\text{m}$  beads consumed them. At station 10 is where 85% of the copepods fed the 10.9  $\mu\text{m}$  beads ingested them. Consumption of the 10.6  $\mu\text{m}$  beads was the lowest at all, but at station 19 had the highest consumption of that size (Figure 4B). When looking at what size beads the copepods consumed more of, around 20% of the copepods had ingested more than 20 particles of the 10.9  $\mu\text{m}$  bead sizes. The 10.6  $\mu\text{m}$  and 15.8  $\mu\text{m}$  bead sizes had around the same percentage of copepods that consumed more than 20 particles (Figure 4A).



*Figure 4. Consumption of beads based on size and whether the copepods ingested the beads or not. A) Only 46% of the copepods had ingested the 10.6  $\mu\text{m}$  bead size. 75% of the copepods ingested the 10.9  $\mu\text{m}$  beads and 76% of the 15.8  $\mu\text{m}$  beads. 22.5% of the copepods had ingested more than 20 of the 10.9  $\mu\text{m}$  beads. B) 10.9  $\mu\text{m}$  and 15.8  $\mu\text{m}$  bead sizes had the highest percentage of copepods that consumed those sizes at each station. While station 19 had the highest consumption of the 10.6  $\mu\text{m}$ , it was only 56% of the copepods.*

### Location

Ingestion rate per copepods was calculated for each bead size fed at each location. At station 6 and 19, the highest rate of particle ingestion in each copepod was for the 10.9  $\mu\text{m}$  sized beads. Station 10 had the highest rate of ingestion for the 15.8  $\mu\text{m}$  beads and station 19 had the

highest rate of ingestion for the 10.6  $\mu\text{m}$  beads. Also at station 19, the lowest ingestion rate for the 15.8  $\mu\text{m}$  beads occurred. The 10.9  $\mu\text{m}$  dominated in ingestion rate the copepods at each station with the rate being no lower than about .7 particles/day (Figure 5A). However, if bead size was not a factor, the copepods at station 19 had the highest rate of bead ingestion at 2.32 particles/day per copepod. Station 6 had lowest total ingestion rate at 1.12 particles/day per copepod (Figure 5B)

I used data from Miller 2022 to observe the plastic pollution found at each station. Station 6 had the highest weight of plastic collected from the manta net tows. The weight of plastic captured decreased as we went further away from station 6 with station 19 having the lowest plastic weight (Figure 6).

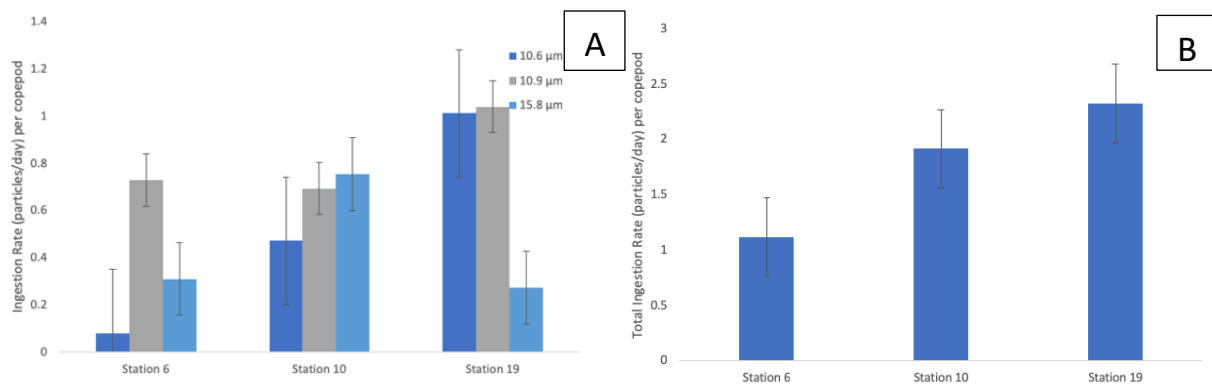


Figure 5. A) Ingestion rate per copepod. The highest rates for the 10.6  $\mu\text{m}$  and 10.9  $\mu\text{m}$  sizes both occurred at station 19 with a rate of 1.01 and 1.04 particles/day, respectively. The 15.8  $\mu\text{m}$  bead size had the highest ingestion rate at station 10 with each copepod consuming .75 particles/day. B) Total ingestion rate per copepod at each station.

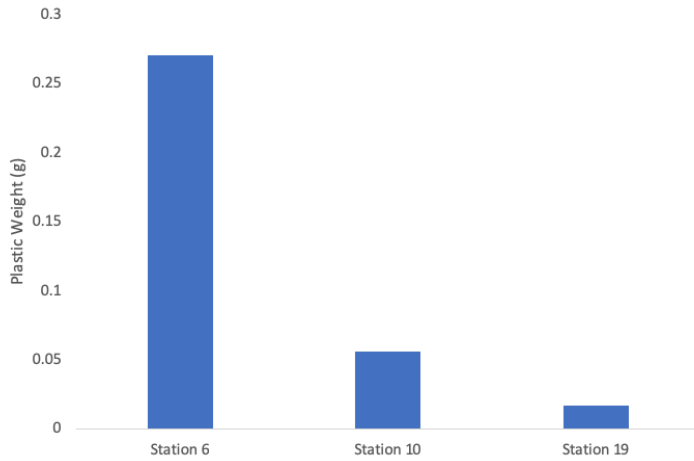


Figure 6. Weight of plastic captured in manta net tows. It is important to note that other stations along the GPGP (not in this graph) had high plastic concentrations. For example, Station 11, 13, and 14 had the highest plastic weight out of all the samples. Additionally, the distance between each station was about 2 knots.

### Copepod Size

Smaller and juvenile copepods were those with a size less than 300  $\mu\text{m}$  and larger copepods were those greater than 300  $\mu\text{m}$ . On average, the larger copepods had higher ingestion of all bead sizes. Many of these larger copepods had ingested more than 20 particles of all bead sizes while the smaller copepods had only 2 ingest more than 20 beads (Figure 7A). On average, my station 10 samples had larger copepods while station 19 had the smallest copepods in the samples (Figure 7B).

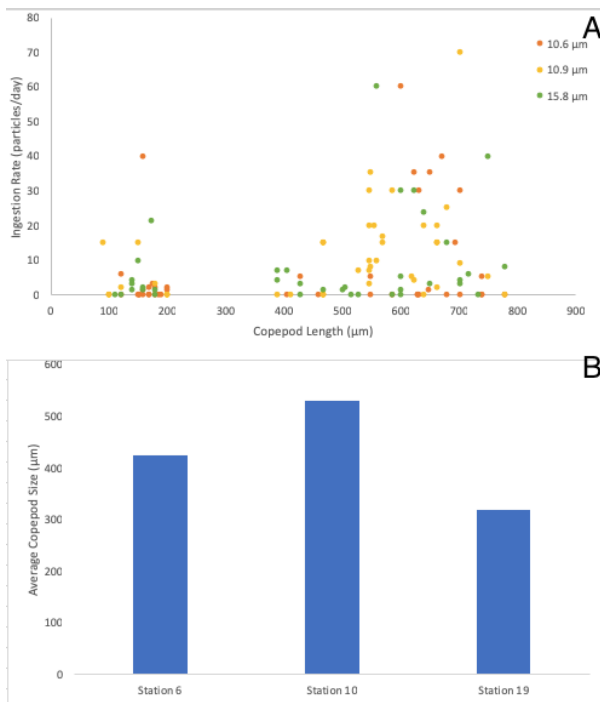


Figure 7. A) Copepod size and their ingestion rate. Copepods smaller than 300  $\mu\text{m}$  had highest ingestion rate of 40 beads/day. Larger copepods had a highest ingestion rate of 70 beads/day. In the smaller copepod group, the highest ingested bead size was 10.6  $\mu\text{m}$  while in the larger group it was more evenly distributed on what sizes were consumed. B) Average size of copepods caught at each station. The average size was 423.6  $\mu\text{m}$  at station 6, 531.3  $\mu\text{m}$  at station 10, and 318  $\mu\text{m}$  at station 19.

## Discussion

The 10.6  $\mu\text{m}$  were mostly not consumed in comparison to the 10.9  $\mu\text{m}$  and the 15.8  $\mu\text{m}$  sizes which is different than what was seen in Cole et al., 2013 (Figure 4). In their study, >50% of the copepods ingested beads sizes smaller than 20  $\mu\text{m}$ . What could explain this is the fact that even with such a small difference in bead diameter, especially in the 10.6  $\mu\text{m}$  and 10.9  $\mu\text{m}$  sizes, the volumes of these beads differ. The 10.6  $\mu\text{m}$  bead has a volume of 198.5  $\mu\text{m}^3$  and the 10.9  $\mu\text{m}$  bead with a volume of 215.8  $\mu\text{m}^3$  which is a notable difference in volume. This smaller volume explains why the individuals that did consume the 10.6  $\mu\text{m}$  had high ingestion rates (Figure 7A). A smaller volume leads to higher consumption of the bead and little left over for other copepods to consume.

The size of the copepods seemed to play the biggest roll bead consumption. Figure 7A showed that larger copepods had a greater ingestion rate in comparison to the smaller copepods. With the smaller copepods, the smallest bead size 10.6  $\mu\text{m}$  was mostly consumed. Station 19 had the smallest copepods and the highest consumption of the smallest bead sizes, 10.6  $\mu\text{m}$  and 10.9  $\mu\text{m}$ . Similarly, station 10 had the largest copepods and the highest consumption of the biggest bead size, 15.8  $\mu\text{m}$  (Figure 5A, Figure 7B).

What is interesting is that copepods that came from an area with more plastic caught seemed to consume less plastic. The most plastic caught from the manta net tows was at station 6. Station 6 also had the some of the lowest total ingestion rate of microplastics. Additionally, station 19 had the lowest plastics caught and highest ingestion rates in total (Figure 5B, Figure 6). The most probable explanation for this is that copepods did not expel much of the material in their digestion track during the 24 h period. This agrees with the findings from Devlyn 2022, where she observed that the zooplanktons at station 6 had the most microplastics already in them

and station 19 had the least amount. This also agrees with the study from Desforbes et al., 2015 in which the copepods in areas of high concentration of microplastics had consumed the most microplastics. This can serve as a proxy to the consumption of phytoplankton, or lack thereof, in areas of high microplastic consumption. This was seen in Cole et al., 2013 where they observed that zooplankton consumed less of the phytoplankton when more microplastics were introduced.

## **Conclusion**

Microplastics are a major concern since they contain carcinogenic toxins and are found throughout all trophic levels. I was expecting to see no favorability in bead sizes amongst all the copepods. What I found was that the copepods mainly consumed the 10.9  $\mu\text{m}$  and 15.8  $\mu\text{m}$  bead sizes. This can be explained by the fact that the 10.6  $\mu\text{m}$  has a smaller volume indicating that more is being consumed by certain individuals leaving less for the other copepods. The smaller copepods mainly ingest the 10.6  $\mu\text{m}$  beads. Larger copepods had the highest ingestion of all the bead sizes. With the copepods that came from areas of higher plastic concentration had more plastics already in them. Because of this, they ate less of the beads that were fed to them. This was seen with phytoplankton and microplastics in which copepods ate significantly less phytoplankton in the presence of microplastics (Cole et al., 2013). With this study, there is clear evidence of copepod consumption of microplastics, and their size is the main explanation for how much they can ingest and the sizes of plastics they prefer. Follow up studies on future predicted amounts of microplastics in our oceans would be helpful what could be the potential amount of plastic in a copepod.

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