Analysis of Plastics in Bluefin Tuna (*Thunnus Thynnus*)

Teagan Cunningham [a], Nash Holley [b], and Mikayla Wallace [c]

[a] Bowdoin College, Earth and Oceanographic Sciences Major, Quahog Bay Conservancy Intern 2022
[b] Bates College, Environmental Studies Major, Quahog Bay Conservancy Intern 2022
[c] Maine Maritime Academy, Oceanography Major, Quahog Bay Conservancy Intern 2022

August 1st, 2022

Abstract

Plastic is very abundant on our planet. Over time, manufactured pieces of plastic disperse into small, secondary fragments called microplastics and nano-plastics which can be very harmful to the marine environment, marine organisms, and humans. When microplastics are ingested by marine organisms, their digestive tracts are blocked, causing the organism to starve. The objectives of this study were to discover how many microplastics (MP) and microfibers (MF) were in a *Thunnus thynnus*, also known as an Atlantic Bluefin Tuna, caught offshore near Saco, ME in early July 2022. To accomplish this objective, four samples were taken in total: one in the lining of the stomach and three in different sections of the intestine. These four samples were oxidized in sodium hydroxide, filtered to reduce organic matter, and later placed under a Zeiss Stemi 305 Microscope for counting. Both MP and MF were found in each of the four samples taken from the bluefin tuna digestive organs: combining all samples, there were a total of 462 MP and 345 MF. The end of the intestine had the most microplastics with a value of 232, while the beginning of the intestine had the most microfibers at 131. Plastic is becoming a major contributor to environmental degradation and harming all biotic organisms.

Introduction

Global annual plastic production is ~380 million tons, which pollutes our continents and oceans (Ritchie & Roser, 2018). Plastic debris is found almost everywhere on our planet. Eventually, it photodegrades into even more harmful fragments that are less than 5 mm long called microplastics (MP). Nano-plastics are even smaller, being any plastic fragment less than 1 μm (NP). There are ~5.25 trillion pieces of plastic currently in the ocean collecting in the five subtropical gyres (Mendoza & Jones, 2015). Only a couple hundred thousand of these pieces are in the surface waters; the majority of the debris is scattered throughout the deep ocean (National Geographic Society). Microplastics account for ~1.3 metric tons of oceanic pollution (The Pew Charitable Trusts). Microfibers (MF) are also harmful: they are primarily fragments from synthetic clothes released during every wash. Other sources of microfibers include threads from synthetic ropes, fishing gear, and plastic tarps. Unfortunately, microplastics and microfibers are found in almost all marine life. When microplastics are ingested by marine organisms they block their digestive tracts, causing the organism to believe they are full. Therefore, they do not hunt or eat, eventually starving themselves (The University of Nottingham). Once ingested, the toxins in the microplastics are absorbed by the organism's tissues (Savoca et al, 2021). This causes health issues such as tissue damage, oxidative stress, neurotoxicity, growth retardation, behavioral abnormalities, and differences in immune system gene expression (Bhuyan, 2022). Microplastics are most likely ingested by all organisms with 99% of fish having microplastics in

them (Savoca et al, 2021). Therefore, they move up the food chain and are eventually consumed by humans.

Atlantic bluefin tuna are one of the top predators in the marine food chain, therefore they help balance the ecosystem (World Wildlife Fund). Bluefin tuna range from 6 to 15 ft long and can weigh up to 2,000 lbs (Oceana, 2022). They have a lifespan of about 35 to 40 years. Since they are a migratory species, they travel all over the Atlantic Ocean at high speeds of up to 55 mph (Oceana, 2022). They consume many smaller fish and plastics as they swim a large portion of the ocean throughout their lifetime. Since bluefin tuna are near the top of the food chain, they should experience the repercussions from consuming microplastics and showcase an abundance of microplastics inside their digestive systems. Tuna have relatively simple digestive organs compared to humans (Figure 1A). First, the tuna eats a fish through its mouth. Then, the food travels to the stomach where it begins to be digested, and later goes through the intestine. The anterior intestine is the first half of the intestine which is directly after the stomach. The posterior intestine is the second half of the intestine which is connected to the ileorectal valve: this is where the digestion process and nutrient absorption are completed. The ileocecal valve connects the intestine to the anus of the tuna and allows the digested food to exit the fish. The objectives of this study were to discover how many microplastics and microfibers were in the 300 lb (live weight) Atlantic Bluefin Tuna caught offshore near Saco, ME, in early July 2022. Four samples were taken: three from the beginning, middle, and end of the intestine and one from the lining of the stomach. It was hypothesized that since Atlantic Bluefin Tuna are a migratory species and near the top of the food chain, they will contain a significant amount of microplastics. Additionally, the lining of the stomach is theorized to contain the most MFs and MPs compared to the other samples from the intestine because the stomach is first in the digestive process.

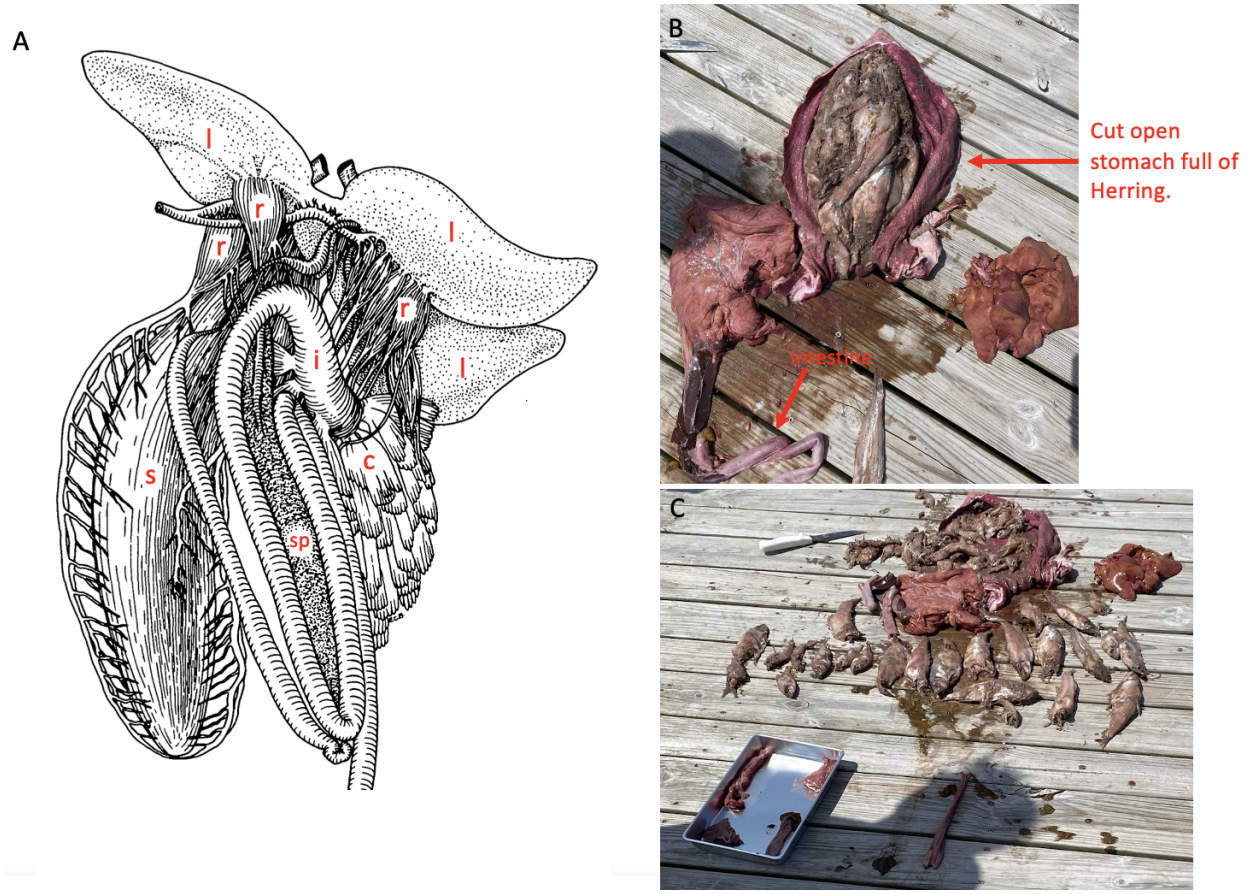

Figure 1: Digestive organs of the bluefin tuna. The large retia (r) behind the liver (l) which supplies blood to the stomach (s), intestine (i), spleen (sp) and pyloric caeca (c) is labeled in the drawn image taken from Carey, Kanwisher, & Stevens, 1983 (A). The cut open, sampled tuna stomach and intestine (B). Laid out Herring's from inside the stomach with the samples displayed in the steel dissection tray (C).

Methods

Instruments used

Four 250 mL beakers
Four safety goggles
Latex Gloves
INCU-Shaker Mini
Rocker 300 Vacuum Pump
1 liter vacuum trap
SAS Positive Pressure room (clean room)
Two Pyrex glass bowls
1000 mL flask
Metal clamp
53 µM Sieve
Vacuum filter reservoir
Vacuum filter funnel
Air Science Purair Ductless Negative Pressure Fume Hood (chemical hood)
Knife
1.0 M sodium hydroxide

Four Cytiva Whatman GF/F 47 mm filters
Zeiss Stemi 305 Microscope- brand
Petri dishes
Tin foil
Four 150 mm sterile Petri Dishes with covers
Stainless Steel dissection tray
Ohaus Adventurer Scale

Collection of Tuna Samples

The bluefin tuna was caught offshore near Saco, ME in early July 2022, and brought back to the dock on Bethel Point to be dissected with a knife (Figure 1B). Sample 1 was taken from the stomach lining of the tuna, two samples were taken from the intestine (Sample 2 from the anterior section, and Sample 3 from the posterior section), and Sample 4 was taken from the lining of the stomach. All samples were put on a small stainless steel dissection tray, brought to the lab, put in the SAS Positive Pressure Room, and then cleaned (Figure 1C).

Oxidation Process of Tuna Samples

Latex gloves were worn for all processes to reduce contamination of microplastics and fibers in the samples, as well as protect the analysts from sodium hydroxide. Before putting the samples into the 250 mL beakers, first the beakers were labeled 1-4. The empty weight of each beaker was measured using the Ohaus Adventurer Scale. Next, the tuna samples were placed into the beakers and weighed again to determine the sample wet weight in grams. The following equation was used to calculate sample weight: sample wet weight - empty beaker weight. Sample 1 weighed 11.950 g, sample 2 weighed 4.930 g, sample 3 weighed 9.570 g, and sample 4 weighed 11.825 g (Table 1). Samples were then brought into the Air Science Purair Ductless Fume Hood. 150 mL of 1.0 M sodium hydroxide was poured into all four of the 250 mL beakers that the samples were in. Samples were then put into the INCU-Shaker Mini at 60 ℃ and 125 rpm for 72 hours.

Sample Number	Sample wet weight (g)	Empty Beaker weight (g)	Sample weight (g)
1	119.650	107.70	11.950
2	114.330	109.40	4.930
3	110.930	101.36	9.570
4	122.855	111.03	11.825

Table 1: Measured wet weight of each sample and empty beaker weight to determine the calculated sample weight (g).

Filtration of Tuna Samples

All glassware and tools were rinsed with filtered deionized (DI) water prior to experiment. Once the samples were oxidized for 72 hours, each sample was taken out of the shaker and put into the clean room to prepare for filtration. Each sample was individually poured through a 53 µm sieve into a bowl underneath to filter out excess sodium hydroxide solution. The sieve was then turned over to be backwashed with DI water into a separate, clean bowl. Once the sieve

was completely backwashed and all material, each individual sample was carefully poured from the bowl into a new, clean 250 mL beaker. Samples were then poured into the vacuum filter reservoir, with a 47 mm Cytiva Whatman GF/F filter between the reservoir and the vacuum filter funnel which was connected by a metal clamp (Figure 2A). The sample then flowed from the funnel into a 1000 mL Erlenmeyer Flask (Figure 2B). The Rocker 300 vacuum pump was connected to a 1-liter vacuum trap by a tube which was used to collect all excess water. The vacuum pump was also connected to the flask itself through two tubes (Figure 2A). Once all samples were filtered, the filters were carefully placed into small tin holders with tweezers and put in labeled,150 mm sterile Petri Dishes with covers.

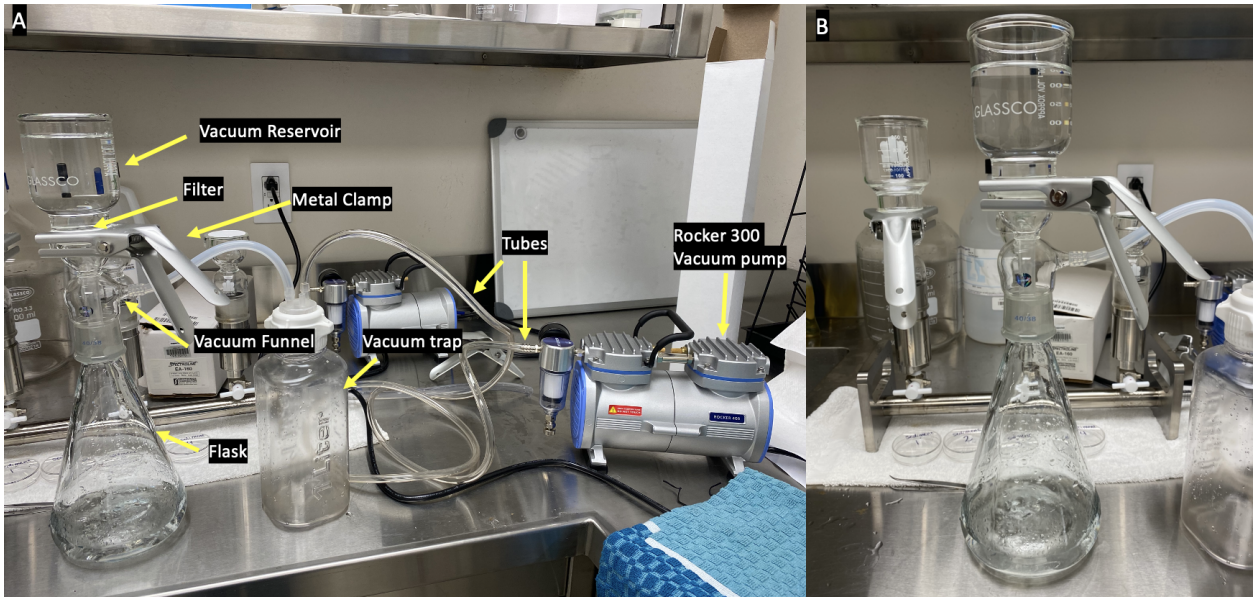

Figure 2: Images of the filtration system with vacuum reservoir, vacuum funnel, vacuum trap, filter, metal clamp, flask, tubes, and vacuum pump labeled (A). Close up of vacuum filtration from the first image (B).

Microscopic Counting of Microplastics and Fibers

To view microplastics and microfibers, each sample was individually taken out of its case and placed in a small, glass petri dish to be put under the Stemi 305 Microscope. 9 to 12 images were taken of each sample filter to locate all MP and MF (Table 2). All microscope images were analyzed on our laptops. To identify and count MF, all thin hair-like pieces were considered. For MP, they were considered to be any relatively dark or shiny reflective particle. It is important to note only the pieces that had dimensions to them were counted, not the nanometer sized dots. Additionally, the greenish-yellow lipid film was not counted as MP due to the inference of them being organic matter: it was deduced that they did not have enough time to oxidize in the sodium hydroxide. Additionally, some fish bones and algae were identified in the samples which were not counted. Once the MFs and MPs were counted, the following equation was used to calculate the number of each per gram in all four samples (Table 3): number of MP or MF / sample weight (g).

Part of Tuna	Sample Number	Number of Microfibers	Number of Microplastics	Average Microfiber Length (mm)	Average Microplastic Length (mm)
Beginning of intestine	1	131	113	7	3
Middle of intestine	2	70	42	5	3
End of intestine	3	89	232	7.5	3
Lining of stomach	4	55	75	8	12

Table 2: Number of microplastics and microfibers in each sample (1-4) with an estimated average length for each.

Part of Tuna	Sample Number	Microfibers per gram	Microplastics per gram
Beginning of intestine	1	10.96234310	9.456066946
Middle of intestine	2	14.19878296	8.519269777
End of intestine	3	9.299895507	24.24242424
Lining of stomach	4	4.651162791	6.342494715

Table 3: Calculated number of microfibers and microplastics per gram. The number of microplastics in each sample was divided by the sample weight in grams.

Results

The analysis of the bluefin tuna showed multiple plastic and fiber components. In the beginning of the intestine (sample 1), there were a total of 131 MF and 113 MP. The average length of MF in sample 1 was 7 mm, and for MP the average length was 3 mm. As for the middle of the intestine (sample 2), there were 70 MF, with an average length of 5 mm, and 42 MP with an average length of 3 mm. The end of the intestine (sample 3) had 89 MF, with an average length of 7.5 mm, and 232 MP with an average length of 3mm. Sample 4, being the lining of the stomach, contained 55 MF, with an average length of 8mm, and 75 MP with an average length of 12 mm. Sample 1 contained the most MF and sample 3 contained the most MP (Table 2).

The microfibers over all four samples ranged from 2 mm to 39 mm with an average of ~12 mm. The longest MF identified was in sample 1 with a length of 36 mm (Figure 3A). This MF is pink while most of the other MF found are black (Figure 3B-D). The pictured black MF was measured as 12 mm (Figure 3D). MFs are not always individually found: they can be seen in clusters like in Figure 3C where 15 MFs can be identified. The largest MPs were found in sample 4. This sample had the largest average length: 12 mm compared to the other samples having an average length of 3 mm. There were a total of 345 MFs and 462 MPs identified in the samples from the intestine and stomach of this bluefin tuna: combining all four samples.

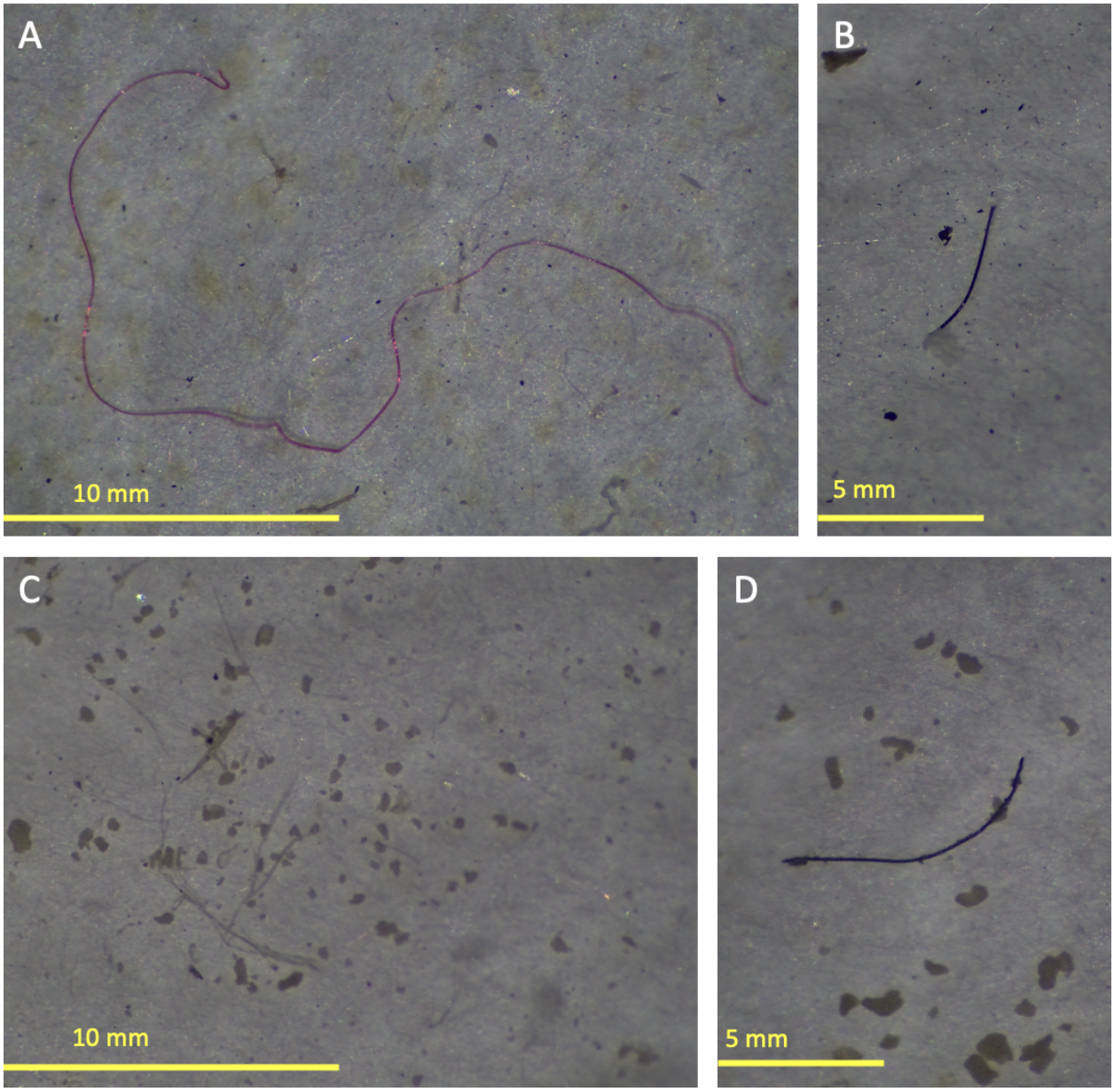

Figure 3: Images of microfibers from Zeiss Stemi 305 Microscope on 4.0 zoom from samples 1 (A), 2 (B & C), and 4 (D).

For each sample, the microfibers and microplastics per gram were calculated (Table 3). For the beginning of the intestine, there were 10.962 MFs per gram and 9.456 MPs per gram. The middle of the intestine had 14.199 MFs per gram and 8.519 MPs per gram. As for the end of the intestine, there were 9.300 MFs per gram and 24.242 MPs per gram. Finally in the lining of the stomach, there were 4.651 MFs per gram and 6.342 MPs per gram. Sample 2, the middle of the intestine, had the most MFs per gram while sample 3, the end of the intestine, had the most MPs per gram.

The plastic fragments ranged in size in this tuna from 3 mm to 20 mm with an average of 5.5 mm among all four samples. It should be noted that secondary plastic fragments above 5 mm in size or length exceed the definition of a microplastic. Despite this, it was decided to

include every piece of plastic that was observed in each sample, as to not inaccurately display the results. The black plastic fragment was measured as 7 mm in Sample 1 (Figure 4A). The black MP measures to be 3 mm while the colorful MP is 5 mm in sample 2 (Figure 4B &C).

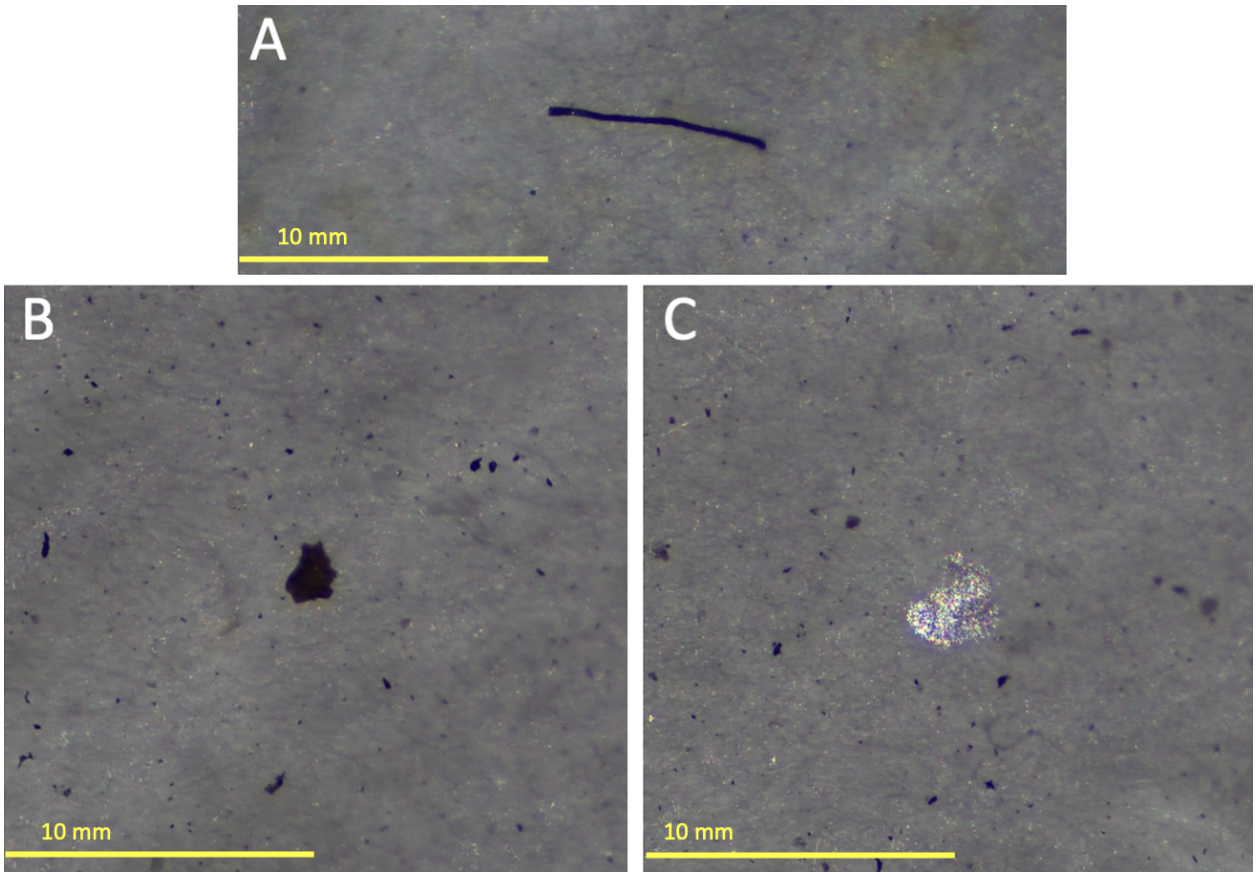

Figure 4: Microplastics images from Semi 305 Microscope on 4.0 zoom from samples 1 (A) and 2 (B & C).

Discussion

It is concluded that there is an abundance of microplastics and microfibers in the tuna caught offshore of Saco, ME. This aligns with the first hypothesis that since the Atlantic bluefin tuna are migratory and travel throughout the Atlantic Ocean, they amass an abundance of microplastics and microfibers. Additionally, since they are towards the top of the food chain and consume many smaller fish which also have MPs, tuna ingest MPs from their food and the water (Figure 1C). Therefore, it is theorized that larger fish are more toxic to consume relative to smaller organisms due to there being more MPs in their systems. Further research is needed to accurately analyze this.

In the sampled Atlantic bluefin tuna, the intestine has significantly more microfibers and microplastics per gram compared to the stomach. This is the opposite of our hypothesis. The end of the intestine, sample 3, had the most microplastics per g with 24 MP/g which is 9 g higher than the other samples. The beginning of the intestine, sample 1, had the most microfibers per gram at 14 MF/g which is 10 g more than the MF/g in the lining of the stomach. This is most likely because the microplastics are not dissolved in the stomach acid and travel

through the intestine. Although, they clog the end of the intestine which is relatively small causing the abundance of MP/g. It is unclear why the microfibers gather in the middle of the intestine. It is possible they get stuck to the walls of the intestine while other matter passes through.

After arriving at these results, it should be stated that there is a margin of error in these methods for identifying microplastics. Without current access to IR spectronomy equipment, any fiber or plastic identified cannot be completely confirmed to be inorganic. The makeup of each piece of material was studied under a microscope, and an educated guess was made on whether each piece was organic or inorganic. With a longer duration in the sodium hydroxide, further decomposition and oxidation would be expected. Sample 4, the stomach lining of the tuna, was the least dissolved at the end of the 72 hours. It also had the most solid form out of the samples before the sodium hydroxide was added. The other 3 samples were significantly more dissolved after the 72 hours, but small, less concentrated clods of organic material were present as well.

Prior microplastics studies have shown that microplastics found in the digestive system of this tuna are absorbed into the tissue: this meat is then consumed by humans (Bhuyan, 2022). Although, the tissue absorption rate from the intestine and stomach are unclear. Therefore, more research is needed to determine a quantitative and qualitative analysis of the impacts of microplastics in the seafood consumed by humans. This data allows us to make an estimate of how many microplastics are in our surface waters and predators, specifically Atlantic Bluefin Tuna. Therefore, microplastics continue to be a growing human health concern.

Conclusion

There are many things that are unknown about the impact of microplastics in our oceans. However, it is understood that they greatly threaten global pelagic biodiversity and have serious health effects to human populations that consume seafood. After plastics are broken up into secondary fragments or microfibers, they begin to travel up the food chain. First, they enter the stomachs of smaller sea animals, then enter the stomachs of their predators, and finally humans at the end of this chain. In this case, the tuna that was dissected most likely ingested microplastics through the consumption of smaller fish. After microplastics are ingested, they can have harmful effects on an animal's digestive system, by either blocking the path of food, or causing the animal to believe that they are full, due to the presence of the plastics in their stomach. Depending on the polymer of plastic, the toxins that some plastics release can be very harmful to animals and humans. The Atlantic bluefin tuna, as a migratory species that can travel through the ocean at speeds up to 55 miles per hour, travel a significant amount of the Atlantic Ocean. A considerable amount of microplastics have been found in the analysis of these tuna organs. Like this tuna, the tuna meat is consumed by seafood lovers all over the world which means humans are ingesting toxins that still have unknown consequences. It is confirmed that the intake of microplastics minimally causes human cell damage, inflammation, and immune reactions (Vethaak & Legler, 2021).

Future research could analyze microplastic concentrations in other marine organisms: oysters, clams, etc. It would also be beneficial to determine the type and potential origin of plastic within Atlantic Bluefin Tuna. Specifically, the polymers of plastics are most prominent in tuna and other marine life, as well as which of these plastics are the most harmful to the

organisms that ingest them. It is important to understand and continue research on how microplastics are affecting the health of marine life, our oceans, and humans.

Credit Authorship Contribution Statement

Teagan Cunningham: Methodology, Investigation, Writing- original draft, review, and editing.
Nash Holley: Methodology, Investigation, Writing - original draft.
Mikayla Wallace: Methodology, Investigation, Writing - original draft.

Acknowledgements

The authors thank Pat Scanlan and the Quahog Bay Conservancy (QBC) for their funding and guidance when conducting this research. We also thank Alec Bollinger for his support during sample collection.

References

"Atlantic Bluefin Tuna." *Oceana*, 14 July 2022, https://oceana.org/marine-life/atlantic-bluefin-tuna/.

Bhuyan, Md. Simul. "Effects of Microplastics on Fish and in Human Health." *Frontiers in Environmental Science*, vol. 10, 2022, https://doi.org/10.3389/fenvs.2022.827289.

"Microplastics Are a Big-and Growing-Part of Global Pollution." *The Pew Charitable Trusts*, https://www.pewtrusts.org/en/research-and-analysis/articles/2021/03/30/microplastics-are-a-big-and-growing-part-of-global-pollution.

"Ocean Trash: 5.25 Trillion Pieces and Counting, but Big Questions Remain." *National Geographic Society*, https://education.nationalgeographic.org/resource/ocean-trash-525-trillion-pieces-and-counting-big-questions-remain.

Ritchie, Hannah, and Max Roser. "Plastic Pollution." *Our World in Data*, 1 Sept. 2018, https://ourworldindata.org/plastic-pollution.

Savoca, Matthew S., et al. "Plastic Ingestion by Marine Fish Is Widespread and Increasing." *Global Change Biology*, vol. 27, no. 10, 2021, pp. 2188–2199., https://doi.org/10.1111/gcb.15533.

"The Big Problem of Microplastics." *The University of Nottingham*, https://www.nottingham.ac.uk/connectonline/research/2018/the-big-problem-of-microplastics.aspx.

"Tuna." *WWF*, World Wildlife Fund, https://www.worldwildlife.org/industries/tuna.

Vethaak, Dick A, and Juliette Legler. "Microplastics and Human Health: Knowledge Gaps Should Be Addressed to Ascertain the Health Risks of Microplastics." *Science*, vol. 371, no. 6530, 12 Feb. 2021, pp. 672–674., https://doi.org/10.1126/science.abe5041.