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Evaluation of microplastics in Japanese fish using visual and chemical dissections

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SUMMARY

Up to 12.7 million tonnes of plastic is estimated to be polluting oceans. Ranking as the fifth-highest plastic using country, Japan has an exceptionally high usage of single wrapped items. Additionally, as an island-nation, fish is vital to everyday life, making up approximately 40% of protein in Japanese diets. Based on these observations, I wondered how the overuse of plastic in Japan poses an ecological risk to marine species and their consumers local to Kanagawa Prefecture. To answer this question, I completed a plastic audit at a convenience store, took qualitative observations of plastic waste at three waterways, and dissected locally sourced fish to characterize ingested plastic. I found 83.4% of the convenience store's items within the recorded sections had plastic wrapping or pieces. Additionally, each waterway observed had both plastic and marine species present. Using visual and chemical dissection, all fish had microplastics present in their gastrointestinal tract, including two species that are typically eaten whole in Japan. Out of the fourteen microplastics found through the chemical digestion method, six were classified as plastic microfibers, four were likely thread plastic, three were see-through pieces of plastic film, and one was a foam pellet. Overall, these results are concerning as previous studies have found that microplastics can carry persistent organic pollutants. Both bioaccumulation and biomagnification result in large levels of contaminants building up at the top of the food chain. It is presumed that the increasing consumption of microplastics will have negative implications on organ systems such as the liver, gut, and hormones.

INTRODUCTION

Plastic contains organic polymers, which allows it to be durable and inexpensive, and results in a wide application of uses. While plastic has been used for hundreds of years, it was not until 1907 that the first completely synthetic plastic was created by Leo Baekeland. Now, over one-hundred years later, plastic is used around the world for thousands of purposes. According to Chemical & Engineering News' (C&EN) data, in 2014, Japan had the second-highest volume of "top 50" plastic producing companies after the United States (1). Approximately one-third of the plastic produced is used for packaging (2).

It is estimated that oceans are filled with up to 12.7 million tonnes of plastic litter, making it the largest area of marine pollution (3). Japan, an island nation, is ranked as the 5th highest plastic-using country, much of which is purely used for aesthetics reasons rather than functional ones (4). The average Japanese shopper uses 300 to 400 single-use plastic bags annually (5), 16 times more than in Britain, also an island-nation (6).

Once plastic is discarded, some of it ends up in recycling centers, but oftentimes a large percentage escapes and makes its way to waterways which leads to the ocean. According to Geyer *et al.*, 90.5% of worldwide plastic has never been recycled (7). Additionally, since Japan is on three fault lines, in the case of natural disasters, waste management would halt. This could lead to the release of plastic, as was seen during the March 2011 earthquake.

Marine plastic pollution has been documented as directly impacting 267 species worldwide, including fish, seabirds, and marine mammals (8). Plastic that recently entered the ocean will likely resemble the original design. These large pieces can confuse marine life as it might look like food. Once eaten, larger pieces of plastic can block the organism's gastrointestinal tract since they cannot break it down, eventually causing starvation (8).

At five millimeters or less in length, microplastics are tiny pieces of plastic that can be seemingly non-existent to the eye (9). Microplastics can either be primary or secondary; primary microplastics are originally a small particle size, whereas secondary microplastics are fragments of larger pieces (10). Once larger plastics are exposed to saltwater, UV light, and microbes, they are degraded into these smaller pieces (**Figure 1**).

There are multiple types of microplastics, including microfibers and thread plastic. Microfibers are small pieces of plastic that come off synthetic material, usually during a washing machine cycle. Because microfibers are so small, wastewater treatment centers are unable to filter them out, so the fibers enter the environment. Thread plastic, conversely, often come off nylon ropes or other fishing gear (11). Fishing gear makes up nearly 50% of the Great Pacific Garbage Patch trash, leading thread plastic to be a commonly found microplastic (12).

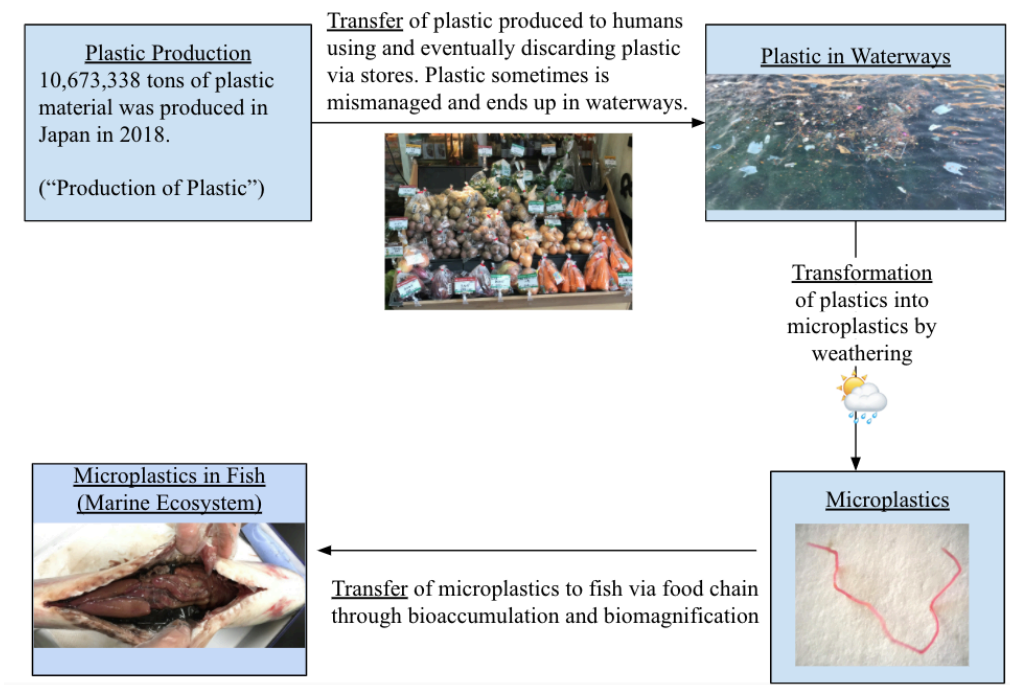

Figure 1. Systems diagram of plastic's movement from production to entrance into marine ecosystems.

Microplastics especially pose a large issue since they can bind to persistent organic pollutants such as pesticides. This binding can increase as microplastics move due to ocean currents (13). If ingested, marine organisms absorb the pollutants which can have a negative impact on their liver, hormones, and gut (11). Both the plastic and pollutants begin bioaccumulating within organisms over time which eventually biomagnifies as it moves up trophic levels. This results in the largest levels of contaminants being present at the top of the food chain.

Ingestion of microplastics often occurs because they resemble plankton, or small organisms that float in the ocean (9). Zooplankton, a primary consumer, were found to accidentally consume microplastics as they are similar in size to phytoplankton. Because zooplankton are near the bottom of the food chain, microplastics accumulate throughout trophic levels until they reach the top of the food chain, where carnivores can be found. This study estimated that humpback whales, which consume 1.5% of their body weight in krill and plankton daily, are ingesting 300,000 microplastic particles every day (14).

Despite humans being estimated at the trophic level 2.2 (out of 5.5) due to an omnivorous diet; we are still predicted to be consuming nearly 2000 microplastic particles weekly. The health implications of this are still unclear (15). However, scientists have found that a certain level of ingested microplastics can cause mild inflammation in the respiratory tract (16).

Fish are a major part of Japanese culinary culture, making up 40% of the protein in their diet. Japanese people eat on average 69.1 kg of fish annually (17). This means the total population (126.8 million people in 2017) consumes about 8.8 million tons of fish annually.

Previous research by Tanaka and Takada (2016) found microplastics present in anchovies from North Tokyo Bay (18). Because the bay is only 55 km away from the center of the Kanagawa Prefecture and connected to the Pacific Ocean, I wondered whether Kanagawa Prefecture residents are at risk of eating contaminated fish. From my background research and Tanaka and Takada's publication, I came up with the research question: how does the overuse of plastic in Japan pose an ecological risk to marine species and their consumers in the Kanagawa Prefecture?

RESULTS

For the first part of my investigation, I recorded visual evidence of the presence of plastic in Japan. To do this, I conducted a convenience store plastic audit and took observations of three local waterways. This allowed me to see how much plastic was available to consumers and whether plastic pollution was present nearby. Next, I dissected locally purchased fish for microplastics. I used two methods of dissection: a visual dissection and a more in-depth chemical digestion. Data in this investigation found all fish dissected to have microplastics present. Additionally, these species of fish are typically eaten whole or raw in Japan, potentially exacerbating the impacts on human consumers.

Plastic items at Aeon, a convenience grocery store

Plastic was most often found in the bread, cereal, and snack section and the to-go food section. 99.1% of items in these sections were sold in plastic. Two snacks were boxed, the other 224 items were plastic-wrapped, primarily because they are not made freshly in the convenience store. All but

Table 1. Amount of plastic items at local Aeon convenience grocery store.

Section of Store	Items with Plastic	Items without Plastic	% of Total Items with Plastic
Frozen	261	3	98.9
Fruits & Vegetables	69	9	88.5
Fish, Poultry, & Meat	172	2	98.9
Drinks & Dairy	312	182	63.2
To-Go Food	116	1	99.1
Health	28	41	40.6
Breads, Cereals, & Snacks	224	2	99.1
Candies	60	10	85.7
TOTAL	**1242**	**250**	**83.2**

one to-go food item was served in plastic, as the purpose was to be convenient for shoppers. The most common type of plastic used for food items was plastic wrappers for snacks and bagging for frozen food. The least amount of plastic was found in the health section, where most of the medicine was in glass jars. However, vitamin squeeze pouches and pill packaging were plastic. 88.5% of fresh produce was wrapped in single-use plastic. Overall, 83.2% of this convenience grocery store's items within the recorded sections had plastic wrapping or pieces. Of the plastic-free items, 80% were alcoholic or health drinks (**Table 1**).

Recording observations on plastic pollution and marine species in waterways

Each waterway observed had both plastic pollution and marine species present (**Table 2**). In many instances, both were present at the same time (**Figure 2**).

Dissections

The purpose of my lab dissections was to look for the presence of plastic within fish. From the first part of investigation, I knew that there was plastic available to consumers in Japan, so I wanted to see if there was also plastic present in the fish people eat. I chose to purchase different species of fish from a variety of locations to allow for a wider application (**Table 3**).

Plastic Bags (among other plastic waste found in Motomachi Canal) | Jellyfish found in same location as plastic waste (different day)

Figure 2. Plastic bags or jellyfish? Two images taken of the same waterway on different days showing instances of plastic pollution and jellyfish.

Table 2. Plastic pollution and marine species found at three waterways.

Location	Examples of Plastic Pollution	Marine Species Example
Yokohama Bay	Plastic bags, plastic food wrappings, plastic bottles	Jellyfish
Motomachi Canal	Plastic bottles, Styrofoam containers, plastic wrappings	Seabird
Yuigahama Beach, Kamakura	Plastic bottles, plastic bags, plastic food wrappings	Various Fish

Table 3. Information on fish used for dissections

English Name (Japanese Romaji Name) *Scientific Name*	Number of Fish Dissected	Fish Origin	Relative Size	Store Type	Purchase Location
Flathead grey mullet (Bora) *Mugil cephalus*	1	Osaka Prefecture	Large	Local fish market	Chinatown, Yokohama
Pacific saury (Sanma) *Cololabis saira*	3	Miyagi Prefecture	Small / Medium	Large grocery and home store	Honmoku, Yokohama
Sweetfish (Ayu) *Plecoglossus altivelis*	1	Aquaculture	Medium	Fish market	Komachi-Dori, Kamakura
Sardines (Iwashi) *Sardinella zunasi* or *Sardinops sagax*	4	Chiba Prefecture	Very Small	Medium grocery	Minatomirai, Yokohama

Visual dissection following CLEAR

For my visual dissections, I followed CLEAR's method. It is aimed towards citizen scientists, meaning it is intended to be accessible to anyone. It had clear directions and illustrations that walked me through the methodology (19). Also, this method has been used in similar contexts, but not within Japan (to my knowledge).

Two species of fish were examined, both of which were found to contain microplastics. The first fish species dissected was the Pacific Saury, a common, inexpensive fish in Japan. Plastic microfibers were recorded in all three Pacific Sauries dissected. The second fish species was the Flathead Grey Mullet. One plastic microfiber was found in this fish.

Chemical digestion using KOH

CLEAR's method lacked in giving complete results since it relied on my eyes to differentiate between microplastics and fish biomass. However, since this method proved plastic being ingested was a relevant issue in the fish local to Kanagawa Prefecture consumers, it allowed me to move forward to a more advanced method. This provided a better representation of the microplastics in each fish (18).

Two different species of species were examined – Sardines and Sweetfish. Using the chemical digestion method, I found a total of 14 microplastics.

The mass of the fish varied from 8.47 to 1626 grams, included four different species, and were all bought from different locations. All the fish I examined contained plastic in some form regardless of size, type, or location purchased.

The most often found microplastic was microfibers (**Figure 3**), and the next most common was thread plastics. Out of the fourteen microplastics found through this chemical digestion method, six were classified as plastic microfibers, due to their thin, flexible structure (**Figure 4**). Four were likely thread plastic, which is stiffer and can evenly fray. Three were see-through pieces of plastic film, likely once a plastic wrapping or bag. The last microplastic found was a white circular plastic, most likely a foam pellet (**Table 4**).

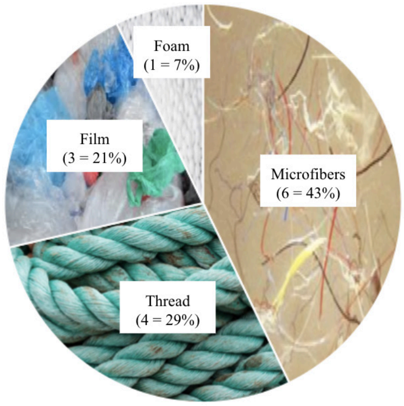

Figure 3. Types of microplastics (microfibers, thread, film, and foam) and their frequencies (measured as percentage of total counted) found in four kinds of dissected fish (flathead grey mullet, Pacific saury, sardines, and sweetfish).

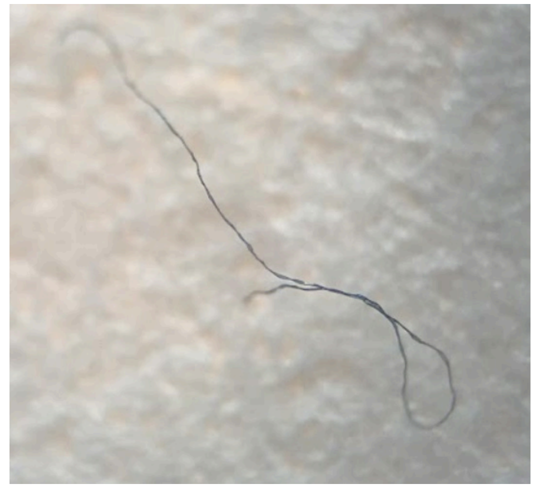

Figure 4. Blue/black plastic microfiber found in the sweetfish.

DISCUSSION

Every year it is estimated that up to 2.5 million tons of microplastics enter the ocean. With Japan being one of the top plastic users and one of the top fish-eating countries, it is not surprising that microplastics were found in all four Japanese-caught fish that I dissected.

It is problematic to humans that both Pacific Saury and Sardines were found to have microplastics because Japanese people tend to eat these fish whole. This means that the microplastics would be directly passed to humans and impact many systems (20).

The presence of microplastics in the Flathead Grey Mullet dissected is supported by a Hong Kong research paper. Cheung *et al.* found 60% of wild Flathead Grey Mullets had ingested microplastics, the most common type being plastic microfibers (21). In Japan, Flathead Grey Mullet is often served raw as sashimi or sushi. This is troublesome because microplastics can carry pollutants which absorb into the flesh of fish, meaning humans are also at risk for ingesting the pollutants.

When buying the Sweetfish, due to a language barrier, I did not know it was created by aquaculture. I chose to analyze it because I thought it would be interesting and applicable as aquaculture still encounters waterways. It was surprising

Table 4. Information on fish used for dissections

Fish Measurements				Plastic Observations	
Fish Type	Weight (g)	Length (cm)	Girth (cm)	Description	~ Length (mm)
Sardine 1	8.39	11.6	0.70	Red thread plastic - likely nylon rope (fishing gear)	5
				White foam plastic (soft pellet)	1
				Black thread plastic	3
Sardine 2	9.95	12.0	0.93	Black plastic microfiber - very thin, had loops	4
				Plastic clear sliver (flexible) - likely bag/wrapping	2
				Plastic clear sliver (flexible) - likely bag/wrapping	2
Sardine 3	8.47	11.9	0.82	Black plastic microfiber	8
				Black thread plastic	2
				Black thread plastic	4
				Plastic film, likely from plastic bag or wrapping	5
Sardine 4	11.0	12.6	1.18	Black plastic microfiber	4
				Black plastic microfiber	5
				Red plastic microfiber	3
Sweetfish	87.7	21.1		Blue/Black plastic microfiber	5

to find a microplastic in the Sweetfish, a fish created by aquaculture (fish farming), because aquaculture fish do not swim in the ocean, where most plastic is found. It shows that other water sources can also be contaminated with plastic. This is worrying as the Sweetfish, or Ayu in Japanese, makes up for around 14% of the total profit earned from freshwater aquaculture in Japan, thus highlighting its importance (22).

Since I do not have the ability to track microplastics' specific movements and origins, I cannot distinctly say a Kanagawa Prefecture residents' plastic is ending up in their local fish. However, while correlation does not equal causation, it is reasonable to believe that if there is more plastic being used there is a higher risk of encountering it in the food chain. Eventually, plastic will end up in a fish. It does not matter whether that fish is in Japan or not, as fish move through currents worldwide and plastic will pose the same threat despite location.

Plastic ingestion is not limited to fish, however. Once the pollution and wildlife share an ecosystem, ingestion is always possible. For example, the plastic bags found in Motomachi Canal resemble the jellyfish that inhabit the bay (**Figure 2**). This can lead to confusion for marine species, such as sea turtles, Brittle sea stars (23), and sea anemone (24) which were found to consume jellyfish in the waters surrounding Japan.

The ecological risk to marine organisms is drastic due to toxic pollutants clinging to the microplastics which can disrupt bodily functions related to hormones and the gut (25). Also, these pollutants can alter population dynamics, specifically trophic levels, as the extent of the problems can be more harmful to certain species, which then changes population levels. Furthermore, the issue only gets worse as you move throughout the trophic levels as both the plastic and toxins accumulate through each level, magnifying the issue at the top.

Despite being in trophic level 2.2, humans are still facing consequences of the bioaccumulation and magnification of plastics and toxins (15). The consequences for humans are still being researched but microplastics are hypothesized to, beyond a certain level of accumulation, inflame the respiratory tract. The persistent pollutants accompanied by the microplastics can also be toxic for humans if ingested (16).

An application that I would be interested to explore would be chemically analyzing microplastics for persistent organic pollutants (POPs). This, along with data collected on the organ health of fish, could highlight the impact of POPs on fish and predict the possible impacts for humans.

While most of the plastic I identified from my dissections was likely from synthetic materials or nylon ropes, Japan's abundance of plastic wrapping is still an issue. The plastic wrapping and packaging found in Japanese convenience stores can break down into microplastics. These types of microplastics were present in my investigation and have been found in fish around the world.

Possible identification methods that could aid further research include Fourier transform infrared (FTIR) and pyrolysis-gas chromatography-mass (Pyr-GC-MS). FTIR can show the specific chemical bonds and weathering of the plastic. Pyr-GC-MS can use thermal degradation to chemically identify microplastics and can be used for trace analysis. Together these methods can shed light on the specific types of plastic present and associated chemicals (12).

In conclusion, I was able to find both inputs and outputs of plastic within Japan's Kanagawa Prefecture (**Figure 1**). All fish dissected had microplastics present and the typical person in Japan consumes a high volume of fish weekly means that it is extremely likely humans are consuming these plastics. While the extent of the negative impacts on humans are currently unknown, it is still evident to a high extent that microplastics have a negative ecological impact overall.

Whether it is Japanese plastic ending up in these fish or not does not matter because the point is that microplastics are ending up in fish around the world. This research coupled with other studies done in North America, Europe, and elsewhere shows that microplastics are a global (and seemingly invisible to the human eye) issue. Because Japanese plastic has already been observed in local waterways, it can be predicted that this plastic will continue to break down into microplastics and be ingested by marine life at some point in the future.

MATERIALS AND METHODS

Counting plastic items in convenience store

People in Japan rely heavily on Convenience Stores for everyday shopping as they are "convenient" being close to train stations and people's homes. To see how much access people have to plastic items, I recorded the number of products that included plastic within the following categories: Frozen, Fruits & Vegetables, Fish & Poultry & Meat, Drinks & Dairy, To-Go Food, Health, Breads & Cereals & Snacks, and Candies. I also recorded qualitative observations to make note of specific trends, such as some items only having a plastic straw, while others had three layers of plastic. I chose to only focus on consumable items due to time constraints. For my results, I calculated the percentage abundance of plastic by dividing the number of products sold containing plastic by the total number of products sold.

Counting plastic pollution and marine species in waterways

I captured photos of both plastic and marine organisms in the following three waterways located in Kanagawa Prefecture (**Figure 5**). This was necessary to show the possibility of the plastic polluting the water being consumed by the marine organisms inhabiting the water. The photos were taken mid-day at the end of summer/beginning of fall.

Visual dissection

I followed Civic Laboratory for Environmental Action Research (CLEAR)'s fish dissection for marine plastic method

Figure 5. The three locations of waterways observed pinpointed on a map of Kanagawa Prefecture, Japan.

and recorded additional information about weight, length, and girth of the fish to highlight the variety of fish dissected (19). This method required me to first cut open the gastrointestinal tract of the fish. Then I placed the opened GI tract onto a coffee filter on a fine mesh strainer and poured distilled water over the contents. This allowed me to visually inspect for plastic. It was a good baseline method as it showed marine plastics were present in the fish. I used CLEAR's "Spotter's Guide" to identify the type of plastic present throughout my dissections.

Chemical digestion and analysis

Looking for a more advanced dissection method, I found Kühn *et al*'s research paper which highlighted the usage of potassium hydroxide (KOH) for isolating microplastics from the marine organisms (26). KOH dissolves organic material (GI) while leaving the plastic in the solution. After reading about KOH, I decided to seek inspiration from Tanaka and Takada's method (18). First, I dissected fish by cutting from anus to mouth, following CLEAR's method. Next, I prepared a 10% KOH solution in test tubes. I put the gastrointestinal tract into 10-20 mL (>3x the volume of the gut) of this solution to digest organic material. Then, I placed the test tubes with the solution and GI tracts in an incubator at 40°C for 10 days, stirring with a stirring rod every 3 days to break up non-digestible material. After 10 days, only non-digestible material was left, allowing me to identify microplastics.

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REFERENCES

1. Tullo, Alexander. "C&EN's Global Top 50 Chemical Companies of 2014." *C&EN*, American Chemical Society, 2014, cen.acs.org/articles/93/i30/Global-Top-50.html. Accessed 15 Oct. 2019.
2. Helmenstine, Anne Marie. "Plastic Definition and Examples in Chemistry." *ThoughtCo*, DotDash, 8 July 2019, www.thoughtco.com/plastic-chemical-composition-608930. Accessed 15 Oct. 2019.
3. Jambeck, J. R., *et al*. "Plastic Waste Inputs from Land into the Ocean." *Science*, vol. 347, no. 6223, 12 Feb. 2015, pp. 768–771, science.sciencemag.org/content/347/6223/768, 10.1126/science.1260352. Accessed 8 Aug. 2019.
4. Dorger, Samanda. "These Countries Produce the Most Plastic Waste." *TheStreet*, TheStreet, 27 Feb. 2019, www.thestreet.com/world/countries-most-plastic-waste-14878534. Accessed 5 Sept. 2019.
5. "Time for Japan to Lose Its Plastic Dependency and Save the Oceans." *The Asahi Shimbun*, The Asahi Shimbun Company, 20 Oct. 2018, www.asahi.com/ajw/articles/AJ201810200027.html. Accessed 8 Aug. 2019.
6. Jowit, Juliette. "Drop in Plastic Bags Littering British Seas Linked to Introduction of 5p Charge." *The Guardian*, The Guardian, 5 Apr. 2018, www.theguardian.com/environment/2018/apr/05/drop-in-plastic-bags-littering-british-seas-linked-to-introduction-of-5p-charge. Accessed 8 Aug. 2019.
7. Geyer, Roland, *et al*. "Production, Use, and Fate of All Plastics Ever Made." *Science Advances*, vol. 3, no. 7, July 2017, p. E1700782, plasticoceans.org/wp-content/uploads/2018/05/Production_use_and_fate_of_all_plastics_ever_made.pdf, 10.1126/sciadv.1700782. Accessed 7 October 2019.
8. Laist, David W. "Impacts of Marine Debris: Entanglement of Marine Life in Marine Debris Including a Comprehensive List of Species with Entanglement and Ingestion Records". *Marine Debris Sources, Impacts, and Solutions,* edited by James M. Coe and Donald Rogers, Springer-Verlag New York Inc. New York, NY, 1997, pp. 99–139, www.researchgate.net/profile/David_Laist/publication/235768493_Impacts_of_Marine_Debris_Entanglement_of_Marine_Life_in_Marine_Debris_Including_a_Comprehensive_List_of_Species_with_Entanglement_and_IngestiOn_Records/links/02bfe5136037d75c73000000.pdf. Accessed 15 Oct.

2019.

9. Gewert, Berit, *et al*. “Pathways for Degradation of Plastic Polymers Floating in the Marine Environment.” *Environmental Science: Processes & Impacts*, vol. 17, no. 9, 2015, pp. 1513–1521, pubs.rsc.org/en/content/articlelanding/2015/EM/C5EM00207A#!divAbstract,10.1039/c5em00207a. Accessed 8 Sept. 2019.
10. Kataoka, Tomoya, *et al*. “Assessment of the Sources and Inflow Processes of Microplastics in the River Environments of Japan.” *Environmental Pollution*, vol. 244, 26 Oct. 2018, pp. 958–965. *ScienceDirect*, www.sciencedirect.com/science/article/pii/S0269749118338028, 10.1016/j.envpol.2018.10.111. Accessed 16 Oct. 2019.
11. Lebreton, L., *et al*. “Evidence That the Great Pacific Garbage Patch Is Rapidly Accumulating Plastic.” *Scientific Reports*, vol. 8, no. 1, 22 Mar. 2018. *Scientific Reports*, www.nature.com/articles/s41598-018-22939-w, 10.1038/s41598-018-22939-w. Accessed 9 Oct. 2019.
12. Wang, Wenfeng, and Jun Wang. “Investigation of Microplastics in Aquatic Environments: An Overview of the Methods Used, from Field Sampling to Laboratory Analysis.” *TrAC Trends in Analytical Chemistry*, vol. 108, 11 Sept. 2018, pp. 195–202, www.jlakes.org/uploadfile/news_images/hpkx/2018-11-09/1-s2.0-S0165993618303686-main.pdf, 10.1016/j.trac.2018.08.026. Accessed 12 Sept. 2019.
13. “The Problem of Marine Plastic Pollution.” *Clean Water Action*, Clean Water Action, 20 Dec. 2017, www.cleanwater.org/problem-marine-plastic-pollution. Accessed 8 Aug. 2019.
14. Valentine, Katie. “Zooplankton Are Eating Plastic, And That’s Bad News for Ocean Life.” *ThinkProgress*, 14 July 2015, thinkprogress.org/zooplankton-are-eating-plastic-and-thats-bad-news-for-ocean-life-dbe31be8ae2b/. Accessed 8 Sept. 2019.
15. Bonhommeau, Sylvain, *et al*. “Eating up the World’s Food Web and the Human Trophic Level.” *Proceedings of the National Academy of Sciences*, vol. 110, no. 51, 2 Dec. 2013, pp. 20617–20620. *Proceedings of the National Academy of Sciences of the United States of America*, www.pnas.org/content/110/51/20617, 10.1073/pnas.1305827110. Accessed 19 Oct. 2020.
16. de Wit, Wijnand, *et al*. “No Plastic in Nature: Assessing Plastic Ingestion from Nature to People.” World Wide Fund for Nature, June 2019. Accessed 7 October 2019.
17. Renton, Alex. “One in Ten Fish Is Eaten in Japan. So Why Don’t They Know There’s a Shortage?” *The Guardian*, The Guardian, 10 Apr. 2005, www.theguardian.com/world/2005/apr/10/japan.foodanddrink. Accessed 8 Aug. 2019.
18. Tanaka, Kosuke, and Hideshige Takada. “Microplastic Fragments and Microbeads in Digestive Tracts of Planktivorous Fish from Urban Coastal Waters.” *Scientific Reports*, vol. 6, no. 1, 30 Sept. 2016, www.nature.com/articles/srep34351, 10.1038/srep34351. Accessed 8 Aug. 2019.
19. Civic Laboratory for Environmental Action Research. “Marine Plastics in Fish: A Citizen Science Dissection & Analysis Protocol.” Accessed 7 October 2019.
20. Itoh, Makiko. “Autumn Is Peak Season for ‘Sanma,’ a Once-Common Fish That’s Playing Hard to Catch | The Japan Times.” *The Japan Times*, 14 Oct.2017, www.japantimes.co.jp/life/2017/10/14/food/autumn-peak-season-sanma-common-fish-become-hard-find/#.XW-xCJMzbBI. Accessed 4 Sept. 2019.
21. Cheung, Lewis, Ching Lui, and Lincoln Fok. “Microplastic Contamination of Wild and Captive Flathead Grey Mullet (Mugil Cephalus).” *International Journal of Environmental Research and Public Health* 15.4 (2018): 597. *Crossref*. Web. Accessed 7 October 2019.
22. Makino, M. “FAO Fisheries & Aquaculture National Aquaculture Sector Overview (NASO).” Food and Agriculture Organization of the United Nations, 11 July 2017, www.fao.org/fishery/countrysector/naso_japan/en. Accessed 11 Sept. 2019.
23. Miyake, Hiroshi, *et al*. “Scyphomedusa Aurelia Limbata (Brandt, 1838) Found in Deep Waters off Kushiro, Hokkaido, Northern Japan.” *Plankton Biology and Ecology*, vol. 49, no. 1, 2002, pp. 44–46. *The Plankton Society of Japan*, www.plankton.jp/PBE/issue/vol49_1/vol49_1_044.pdf. Accessed 15 Oct. 2019.
24. Yamamoto, Jun, *et al*. “Transportation of Organic Matter to the Sea Floor by Carrion Falls of the Giant Jellyfish Nemopilema Nomurai in the Sea of Japan.” *Marine Biology*, vol. 153, no. 3, 29 Sept. 2007, pp. 311–317. *ResearchGate*, www.researchgate.net/publication/225326725_Transportation_of_organic_matter_to_the_sea_floor_by_carrion_falls_of_the_giant_jellyfish_Nemopilema_nomurai_in_the_Sea_of_Japan, 10.1007/s00227-007-0807-9. Accessed 15 Oct. 2019.
25. Boucher, Julien, and Damien Friot. “Primary Microplastics in the Oceans: A Global Evaluation of Sources.” *IUCN*, 21 Feb. 2017, pp. 1–43, storyofstuff.org/wp-content/uploads/2017/02/IUCN-report-Primary-microplastics-in-the-oceans.pdf, 10.2305/iucn.ch.2017.01.en. Accessed 8 Sept. 2019.
26. Kühn, Susanne, *et al*. “The Use of Potassium Hydroxide (KOH) Solution as a Suitable Approach to Isolate Plastics Ingested by Marine Organisms.” *Marine Pollution Bulletin*, vol. 115, no. 1–2, Feb. 2017, pp. 86–90, www.sciencedirect.com/science/article/pii/S0025326X16309547, 10.1016/j.marpolbul.2016.11.034. Accessed 8 Aug. 2019.