

Rory Mackintosh

HSC Depth Study

Microplastics in fish guts: How is the marine life affected by
Microplastics



Scientific Report

Aim: To investigate whether estuary fish species are more susceptible too coming into contact with or ingesting micro plastics compared to Oceanic fish species.

Background Knowledge:

- **What are Micro Plastics?**

Micro plastics are considered as small, barely visible pieces of plastics that are five millimeters or smaller in length, that are either broken down or produced to be this small (Smith, 2018). Micro Plastics are an environmental concern worldwide due to the fact they are polluting the worlds water ways and environments, entering our food sources, e.g. fish species. Micro Plastics also effect water mammals such as whales as they breath large amounts of water and krill which are full of these miniscule pieces of plastics. It is now also known that krill are being affected by these Micro Plastics which are eaten by various marine species. Because Krill are at the bottom of the food chain, most marine animals will be affected by Micro Plastics.

- **Where do Micro Plastics come from?**

Micro Plastics come from both primary and secondary sources. These sources are man-made and are the reason why micro plastics are so prominent in today's society.

Primary sources of Micro Plastics are those that come from industry or commercial places and are used as a product. These are extremely dangerous as they are normally made in very large numbers, meaning mass pollution. They are plastic pieces that are already 5mm or shorter without any degradation (Motivans, 2018). Some examples of primary Micro Plastic sources are:

Microbeads

Micro beads are tiny plastic pellets used in personal care, cosmetic and household cleaning products (e.g. facial and body scrubs, toothpastes and washing powders). They are very tiny pieces of manufactured polyethylene plastic. These microbeads have been found in oceanic species excrement and are a great example of what a Micro Plastic is. A couple of alarming statistics really show how microbeads are influencing the oceans habitat today. An estimated 8 billion microbeads alone, per day are emitted into aquatic habitats in the United states, which equals approximately 2.9 Trillion a year. (Gyres Institute, 2019)

Nurdles or Plastic Pellets

These are the size of a pea and are the raw plastic material for the production of most plastic products. They can be lost down factory drains or during transportation and end up on beaches and in the ocean. Because these are used in most plastic productions, they are extremely common in our waterways and beaches.

Secondary micro plastics are created from the degradation of larger plastic products once they enter the environment through natural weathering processes. Such sources of secondary micro plastics include water and soda bottles, fishing nets, and plastic bags. A common secondary micro plastic that is found are those of Hard Plastic and polystyrene balls, as they tend to float. (Motivans, 2018)

Both primary and secondary sources of Micro Plastics are threatening marine wildlife through ingestion, in particular microbeads, which are mistaken for fish eggs. Once in their stomach, the space for normal food is reduced, feeding behavior is altered, and energy levels drop. This often resulting in starvation from what we understand, they cannot actually break down the plastic and it stays in their digestive system, or it slowly passes through but clogs and slows the digestive system down, resulting in some of the stated issues. We have seen this happen recently in the media has shown the result of plastic pollution to whales. Similarly, they are mistaking plastic bags or ingesting plastic on accident, which is resulting in starvation, slowly killing the animals which are starting to turn up on beaches full of plastic.

Lord Howe Island off the Australian coastline also sees the issues between Plastic and the seabirds that nest there. They are seeing that the mother seabirds are hunting for food and often eating pieces of plastic. Unwillingly, these mother birds are feeding the chicks copious amounts of plastic, resulting in the starvation of both chicks and mother seabirds, ruining 2 generations of the species (Gill, 2018).

The Types of Micro Plastics

There are five main/common types of Micro plastics in the waterways today, which are:

- Fibers – micro pieces of clothing (fleece) and cigarette butts etc.

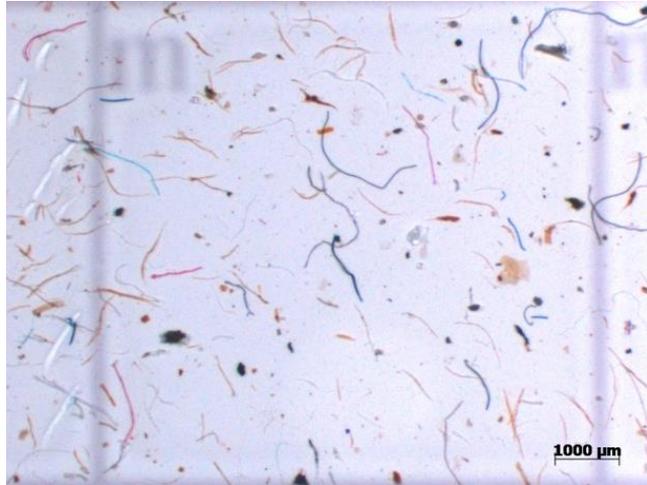


Figure 1: Microfiber microplastic. (1millionwomen, 2017, May 26)

- Fragments – Fragments are secondary source micro plastics that are small broken off pieces from larger plastics.



Figure 2: Microplastic Fragments. (Davis, 2017, June 6)

- Foam – e.g. Styrofoam



Figure 3: Microplastic foam. (Thorbecke, 2019, March 12)

- Microbeads



Figure 4: Microbead from Facewash product. (Whitebread, 2016, June 21)

- Nurdles



Figure 5: Microplastic Nurdle. (Entwistle, 2018, April 11)

These plastics are most commonly found under the 5mm size and can be very hard to see. (Flowers, 2016)

- **How to Identify Micro plastics**

There are multiple ways to identify micro plastics. Because there are various types of micro plastics, each individual piece must go through multiple tests to accurately be identified. The simpler ways to identify micro plastics are; Hot needle test to test fibers, using a light microscope to identify key features such as angular sides, colours and growth. Also using tweezers to see if the object stays the same shape under pressure or “bounces back”. (Liboiron, 2016)



Figure 6: spotters guide for micro plastic identification. (Liboiron, 2016)

The Hot Needle test is a good test to see if microplastics like fibers curl or shrivel to the hot needle. It is also good to see if objects like fragments melt and don't directly burn like organic pieces. It may also allow you to smell the melting plastic. (Liboiron, 2016)

- **How do Micro plastics make it to the water systems?**

Pollution Runoff

Runoff occurs when there is too much water on the land for it to absorb. For example, after a storm or heavy rains, when there is too much water. Excess water can flow off the land into drains that lead to storm water drains that lead both into the ocean and estuary systems, the water can directly flow into creeks, ponds or the environments in question. Run off can be due to natural sources or man-made causes.

Around the Central Coast and east coast of NSW areas the natural runoff processes that are most likely to affect us are:

- Soil erosion

Soil is eroded away and is carried into various bodies of water. These soils may not be supposed to be in the ocean and could create problems. (National Geographic, 2018)



Figure 7: Soil erosion into a river/estuary
(Plant Permaculture, 2015, April 10)

This soil can be contaminated with chemicals such as fertilizer, oils and even plastic pieces. This can be dangerous for both the animals of the ocean and the ocean itself. Soil runoff and degradation is common in heavy rains and floods. (National Geographic, 2018) The photo above is an example from a flood in England, where the soil runoff leads straight into a river (estuary system).

There are two classifications from the National Geographic's Definition. (National Geographic, 2018)

- Point sources (direct)
- Nonpoint sources (non-direct)

Both pollute in to the estuaries and oceans of the Central Coast.

Point source pollution is any form of runoff that runs directly to waterway. This might include a pipe from specific sewage treatment plant, factory, or even a home. Also, for people who live on the water, home storm water drains are also a form of point source runoff. For example, the sewerage pipe at Winnie Bay, a local area on the Central Coast that releases treated and sometimes raw sewerage directly into the oceans of the Central Coast. This creates an unsafe environment for fish, humans and any other animals that live in or are around the ocean.

Nonpoint Sources are the opposite in definition, any source of runoff that does not directly run into a waterway. Nonpoint sources of runoff can be large urban, suburban, or rural areas. In these areas, rainwater and irrigation wash chemicals into local waterways. Runoff from nonpoint sources includes lawn fertilizer, car exhaust, and even spilled gasoline from a car. Farms are a huge nonpoint source of runoff, as rainwater and irrigation drain fertilizers and pesticides into bodies of water (National Geographic, 2018). These are all sources that in a modern world are hard to minimize and even change. Unfortunately, not many people understand how much becomes runoff and ends up in our waterways.

Surfaces that don't allow water to pass through, such as roads, sidewalks and carparks increase runoff. Again, in a modernized world, these surfaces are hard to minimize or unchangeable. Materials as diverse as car-washing soaps, litter, and spilled gas from a gas station all become runoff.

Storm water drains in Brisbane water

A good example of storm water run-off into a system is the storm water drains on my property. Whenever it rains these drains directly wash into the Brisbane water estuary and come off most waterfront properties. They come from the drains in the gutter system and from people's properties.



Figure 8: Stormwater drains directly entering Brisbane Waters from suburban property

Illegal Dumping

As many people know, littering and illegal dumping of rubbish occurs daily around the world. From my first-hand experience, I have witnessed the masses of plastic and other pollution riddled throughout a small area.

Unfortunately, this also results in micro plastics being spread throughout our waterways. This is a common source of how secondary source micro plastics are being found, and only through education and action can we prevent any further problems arising.

- **Species of fish**

Yellowtail Scad (Yakka)

The Yellowtail Scad is a widespread species of the *Carangidae* family. It is also commonly referred to as the Yellowtail Mackerel, Yellowtail Horse Mackerel or the Yakka. (McGrouther, 2018)

How to Identify a Yellowtail Scad?

The Yellowtail scad is recognized by its silvery colour, with tinges of Brown and green dorsally. It has a strong forked yellowtail following from a thin caudal peduncle (McGrouther, 2018) (The narrow part of a fish's body to which the caudal or tail fin is attached.) Yellowtail are commonly found around the 20-30cm size, with a maximum length of 50cm (Busselton, 2019)

Where are they Found?

Yellowtail scads are found on both inshore reefs, offshore reefs and FADS and also schooling all throughout the ocean. They can be found from the ocean surface to any depth till 500m deep. Usually they are found in the southern waters of Australia, from southern Queensland around to southern Western Australia. (McGrouther, 2018)

They are also found in estuarine environments in areas with broken ground, such as rocky headlands, under bridges and places where there is current. Again, they are found from the surface to the bottom of the estuarine systems and are usually found in smaller numbers, throughout the Australian waters above.

Diet

Yellowtail scad are generally filter feeding animals that feed on microscopic zooplankton (Busselton, 2019). But regularly feed on 'burley' (consists of food scraps, fish scraps to attract various species, usually baitfish like Yakkas) and bait from fishermen. This would make them prone to being exposed to floating and in water micro plastics as, similarly to whales, filter water through their mouths catching the zooplankton, but unfortunately not having a control on whether or not they pick up the microplastics along with the zooplankton.



Figure 9: Yellowtail Scad (McGrouther, 2018, December 18)

- **The Estuary**

An estuary is a body of water that is a mix of both fresh and salt water (brackish) and are commonly found where a river or outlet meet the sea (NOAA, 2018). On the Central Coast for example, where Ettalong beach (Brisbane water estuary system) meets Box head is the estuary meeting the ocean.

Estuaries are considered as one of the most productive ecosystems worldwide. Many animals such as, Birds, fish, mammals and organisms, zooplankton, algae and weeds rely on estuary systems for homes, survival, food and migration stopovers. They are essential for wildlife and humans, with humans using them for jobs and food. They also make great foundations for human life, water, food, farming land and protection. (NOAA, 2018)

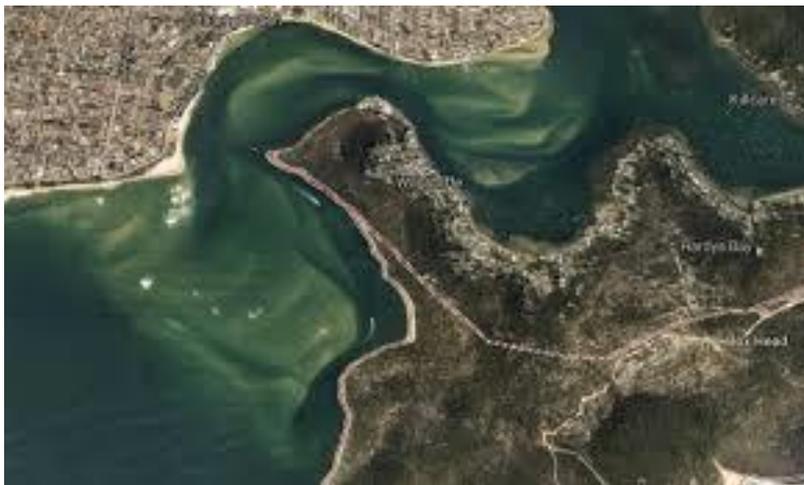


Figure 10: Where Brisbane water estuary meets the sea. (CCN, 2018, May 30)

- **The Ocean**

The Ocean covers approximately 70% of the earth's surface. Unlike the estuary, the ocean is a continuous body of water full of currents and mysterious animals and organisms. It is said we know more about space than what we do our own oceans.

The Ocean itself is divided into five major basins:

- The Pacific Ocean
- The Atlantic Ocean
- The Indian Ocean
- The Arctic Ocean
- The Southern Ocean

Currently the oceans are under a lot of investigations about climate change and its effects. It is saltwater and rich in minerals such as magnesium, calcium and chlorine. The oceans also regulate temperatures and create weather patterns. (Nunez, 2019)

Similar to an estuary, the oceans hold islands, reefs, currents and a vast amount of environments where animals thrive. These animals are fish, mammals, birds, crustaceans and jellyfish, with some of the strangest, alien-like creatures hidden in the depths. From microscopic plankton and Krill to 30m long Blue whales and bus-like sharks. The Ocean spans from 1 to beyond a 1000m where mysterious are hidden throughout. More than 80% of the oceans are unexplored and unmapped, meaning there could be cures, crazy animals and life-changing organisms to be found. (Nunez, 2019)

- **Life Cycle of a Microplastic
Krill**

Krill are macroscopic crustaceans found in the world's oceans and estuaries. They are essentially a fuel that runs the earth's marine world. Krill are filter feeders that eat microscopic phytoplankton and single-celled plants near the ocean's surface. Many fish like Yellowtail Scads and mammals like Humpback whales feed on krill (National Geographic, 2019). The krill are now unfortunately being exposed to microplastics and breaking them down further into "Nano plastics" (Warren, 2018). We do not know enough yet to see any real repercussions, but they are the beginning of the food chain, which eventually will directly affect humans.

Fish

Microplastics are now being seen as a frequent site in fish species. Filter-feeding fish like yellowtail scad that eat krill, would ingest both the krill with microplastics inside and microplastics along with those. We do know that fish are eating and ingesting

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microplastics, but unfortunately aren't certain if we are eating these microplastics (Hudson, 2018, June 8) , if the plastics are leaching toxic chemicals deeper into the fish. Are the fish we eat toxic? Unfortunately, we aren't sure, but if fish like figure 13 end up on a dinner plate, it wouldn't be very enticing.



Figure 11: Plastic filled fish. (Thompson, 2018, September 4)

- **Bioaccumulation**

Bioaccumulation is the process of breaking down or accumulation of substances such as plastics, pesticides and other chemicals inside the body or organism at a faster rate than it can be excreted. In relation to micro plastics, there is no data to show that the micro plastics ingested are bio accumulated into the flesh or body of the organism (fish).

Currently we do not know if the chemicals and plastics accumulated or ingested leach fatal or toxic chemicals into the fish's digestive system or further into the fish's bloodstream, flesh and interrupt any normal functions.

Hypothesis:

I believe the fish (Yellowtail Scad) will be more susceptible and ingest more micro plastics in the estuarine environment due to the high-density living areas, the storm water runoff and drains and the large amounts of human activity, compared to the ocean. Due to the ocean currents, the microplastics would be widely spread of the Central Coast beaches, whereas the estuarine tidal flow and current is predictable and stays in similar areas, where fish would come into new microplastics commonly. Also, the easily accessible estuaries are prone to illegal dumping.

Variables:

Independent: The Environment (location) in which the fish are caught, oceanic and estuarine

Dependent: The amount of micro plastics, measured by number per fish

Controlled:

- Equipment (all equipment used in the method)
 - Sieve
 - Filter paper
 - Tweezers
 - Needle
 - Knife
 - Microscope
 - Light
 - Sample container
 - Slides
 - Scissors
- Species of fish used (Yellowtail Scad)
- Method used (following steps 1-16 in method below)
- Amount of water used to wash guts, (1 Litre)

Materials:

- 1 x Sieve tower or sieve
- 15 x Filter Paper
- 1 x Light Microscope
- 3 x Microscope slides
- 1 x Scissors
- 1 x Scalpel or Fishing Knife
- 2 x Gloves
- 2 x Steel mesh gloves
- 1 x 30 m roll Baking paper
- 1 x Tweezers
- 3 x Sample container
- 1 x Water bottle
- 1 x Lighter
- 1 x Needle
- 1 x Light (for identification/finding of micro plastics)
- Micro plastic Identification Guide
- Dissection Diagram

Method:

1. Collect appropriate PPE and Equipment.
2. Set up equipment as shown in figure 10 and 16



Figure 12: equipment set up 1

3. Place fish on baking paper.
4. Put on your gloves and with a sharp knife/scalpel, make a cut from the fish's anus to the gill plate. (see dissection diagram)



Figure 13: Starting cut from anus.



Figure 14: End of cut at gill plate

5. Once you've cut the bottom of the fish open, as shown, cut the top of the guts out, at the base of the gills.

6. Once the gills and guts are outside the body of the fish, place the rest of the fish to the side.



Figure 15: The guts and gills removed from fish in 1 piece

7. Using the scissors or sharp scalpel/knife, make a cut from the top of the fish's guts towards the base, creating a large flap of flesh.
8. Wash the inside of the stomach thoroughly onto the filter paper with a washer bottle or tap.



Figure 16: washing the cut guts and gills

9. Wash the surrounding guts and gut cavity of the fish to cover all basis. As above, and let the water sit and drain out.
10. Once the stomach is washed thoroughly, examine the filter paper and sieve under a light. (Use tweezers or a needle to inspect the filter paper.) Check both sides of the filter paper, as some plastic and fibres may be on the underneath side.

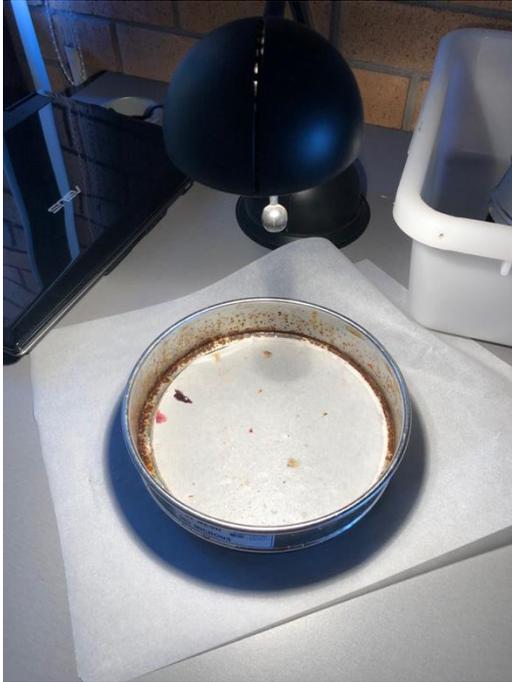


Figure 17: Gut content on filter paper



Figure 18: equipment set up 2

11. Pick up anything you think looks suspicious and place into sample jar or directly onto the microscope slide.



Figure 19: picking up microplastic with tweezers

12. Place each identified object on a microscope slide.

13. Place the slide onto the light microscope and look for micro plastic qualities. Refer to micro plastic identification photo.



Figure 20: Further inspecting object under light microscope

14. Depending on the micro plastic, apply a hot needle to the object and if it shrivels or melts, you can be certain it isn't an organic object and is in fact a micro plastic.
15. Record results.
16. Repeat for each fish, and record results in table as shown below.

Yellowtail Scad (Yakka) – Scientific Name, <i>Trachurus novaezelandiae</i>				AVG Amount of Micro plastics
Location:				
Fish Number	1	2	3	
Micro plastics (number per fish)				

Ethical Considerations:

As an investigation, some people may view this as unethical. For reasons such as harming and killing the Yellowtail Scad without 'consent' as such. But I have placed many different considerations to return the fish back into the ocean to keep the natural cycle of life going.

1. After catching the desired species of fish, to kill the fish as quickly as possible and in the most humane is to brain spike the fish. It dies quickly and without pain and does not suffer at all. Unfortunately, I cannot dissect a fish without putting it through suffering and possibly of it dying later.

2. After I have gutted and dissected the fish, the process to returning it to the ocean is simple. If a fish is sick and dies in the ocean, it is either eaten by a larger fish, crabs, star fish and many more species and creatures. As my home is based on the water, I have a healthy mud crab population outside my doorstep, in the Brisbane waters. I can either put the fish out the front for food for smaller fish species and the mud crabs to feed on. I can drop the fish in locations where they will be eaten and used in the continual cycle of life and death naturally.
3. Once I have found the micro plastics, due to their small size it's impossible to get rid of them. So instead of throwing them out again to continue the cycle. I have a small sample tube full of micro plastics, that way they don't continue the cycle and can stay in a controlled environment where they won't re-enter the waterways.

Risk Assessment:

Risk	Precaution
Sharp knife – cuts	Wear steel Mesh gloves when dissecting, to minimize the possibility
Fish Spines, Spikes – cause reactions (allergic) and cut or open skin. Venomous species. Infections	Wearing Steel Mesh Gloves to minimize possibilities. Research and understand fish spine/spike areas and whether they are venomous.
Fishing – e.g. boat problems, swell, rock fishing	Safety precautions and equipment – Read weather reports to stay safe, have all safety equipment (lifejacket, rock cleats, EPIRB and flares etc.) Understand Basic First Aid
Spearfishing – e.g. environment, weather, swell, equipment and drowning	Spearfishing environment can be very dangerous, boats, sharks and weather. Watch weather reports, understand shark behaviour and basic first aid. Boats be clear with a dive float, flag, whistle and mirror so you don't get run over. Be careful with equipment, unload guns and spears at all times but use, never point it at someone or yourself and make sure the safety works. Understand your abilities and don't push them too hard or you could 'blackout' and drown. NEVER DIVE ALONE
Burns – e.g. Hot needle test, light microscope light and lighter(matches)	Being careful around any hot equipment or flames. Wear appropriate PPE around hot equipment.

Fire – e.g. lighter catches something on fire, curtain, plastic, hair	Be aware of your surroundings. Get a trusted adult to use the lighter or matches for you. Have water and fire extinguisher close by.
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Results:

Oceanic Fish Species:

Test 1:

Yellowtail Scad (Yakka) – Scientific Name, <i>Trachurus novaezelandiae</i>				AVG Amount of Micro plastics
Location: THE TERRIGAL HAVEN – 3-5 meter's deep				
Fish Number	1	2	3	0.67
Micro plastics (number per fish)	1	1	0	

Test 2

Yellowtail Scad (Yakka) – Scientific Name, <i>Trachurus novaezelandiae</i>				AVG: Amount of Micro plastics
Location: THE TERRIGAL HAVEN – 3-5 meter's deep				
Fish Number	1	2	3	3.67
Micro plastics (number per fish)	2	6	3	

Estuary Fish Species:

Test 1:

Yellowtail Scad (Yakka) – Scientific Name, <i>Trachurus novaezelandiae</i>				AVG: Amount of Micro plastics
Location: SYDNEY HARBOUR – 3-10 meter's deep				
Fish Number	1	2	3	3.67
Micro plastics (number per fish)	3	3	5	

Test 2:

Yellowtail Scad (Yakka) – Scientific Name, <i>Trachurus novaezelandiae</i>				AVG: Amount of Micro plastics
Location: SYDNEY HARBOUR – 3-10 meter's deep				

Fish Number	1	2	3	4
Micro plastics (number per fish)	2	4	6	

Analysis:

Qualitative Data

Oceanic Yellowtail Scad

Fish 1:

1 x small black bio-fouled hard plastic which melted when came into contact with the hot needle during the hot needle test. curved and spiralled plastic piece. Looks like a plastic shaving. Had growth and melted which smelt like burnt plastic.

Fish 2:

1 x 3-5mm long black/green bio-fouled microplastic fibre/filament. Curved to the hot needle and then shrivelled when came into contact with the hot needle test.

Fish 3:

NIL

Fish 4:

1 x blue/green fibre. Looked like fishing braid with an unorthodox shape. Burnt but didn't shrivel to the hot needle.

1 x red and green fibre curled and shrivelled to the hot needle test.

Fish 5:

1 x piece of black/red fibre that shrivelled to the hot needle test. About 2mm long.

1 x hard angular black plastic. Burnt and smelt like plastic when exposed to the hot needle.

3 x black fibre's, varying between 2mm-5mm. All shrivelled to the hot needle test and had growth.

1 x 3-4mm clear plastic film. Unorthodox shape and burnt to the hot needle test.

Fish 6:

2 x white/clear fibre's, both shrivelled to the hot needle test. 3-5mm long each.

1 x hard clear fragment melted when exposed to needle which a strong burning plastic smell.

Estuarine Yellowtail Scad

Fish 1:

2 x clear/white fibre's, 2-4mm long that curled and shrivelled to the hot needle test.

1 x blue fibre, 5mm long and shrivelled to the hot needle test.

Fish 2:

- 1 x fragment/ film of blue, grey and clear piece. Resemblance to a plastic bag piece.
- 1 x purple and orange, fragment. Angular and distorted.
- 1 x blue, purple and clear fragment or film. Also resembled a plastic bag piece with a distorted shape.

Fish 3:

- 1 x Thick white fibre shrivelled to the hot needle test and melted with a burning plastic smell. 2-3 mm
- 1 x clear and black fragment. Had brown growth on it with distinct angular shapes
- 1 x small multi-coloured fragment. Blueish purple with red and orange. Very angular 1-2mm
- 1 x bright green fibre. Curled in contact with hot needle to shrivel completely. 3-4mm
- 1 x black fibre 2mm long, shrivelled and curled to the hot needle test

Fish 4:

- 1 x Bright blue microbead. Floated in salt solution. Spherical but very degraded under microscope.
- 1 x black and blue fibre. 2mm long and curled to the hot needle test

Fish 5:

- 2 x black fibre's, 1mm long. Both curled and shrivelled to the hot needle test.
- 1 x Purple fragment 1-2mm long. Looked blue from the eye but purple with light through it.
- 1 x pink fragment, very angular with lots of growth. Very bright

Fish 6:

- 2 x blue/purple fragments. Approximately 1-2mm long. Again, looked blue but wasn't under the light microscope. Very angular.
- 2 x 1-2mm long black fibres. Shrivelled and curled to the hot needle test.
- 1 x blue fibre. Burnt and smelt like burnt plastic and shrivelled to the hot needle test.
- 1 x long clear/white fibre. Shrivelled to the hot needle test and smelt like burnt plastic.

AVERAGE CALCULATIONS

Oceanic species

$$\text{AVG 3} = \frac{1 + 1 + 0 + 2 + 6 + 3}{6}$$

$$\text{AVG 3} = 2.167$$

$$\text{AVG 1} = \frac{1 + 1 + 0}{3}$$

$$\text{AVG 1} = 0.67$$

$$\text{AVG 2} = \frac{2 + 6 + 3}{3}$$

$$\text{AVG 2} = 3.67$$

Estuarine Species

$$\text{AVG 3} = \frac{3 + 3 + 5 + 2 + 4 + 6}{6}$$

$$\text{AVG 3} = 3.83$$

$$\text{AVG 1} = \frac{3 + 3 + 5}{3}$$

$$\text{AVG 1} = 3.67$$

$$\text{AVG 2} = \frac{2 + 4 + 6}{3}$$

$$\text{AVG 2} = 4$$

MICROPLASTIC IMAGES



Figure 21: Black Fibre on the underside of filter paper.

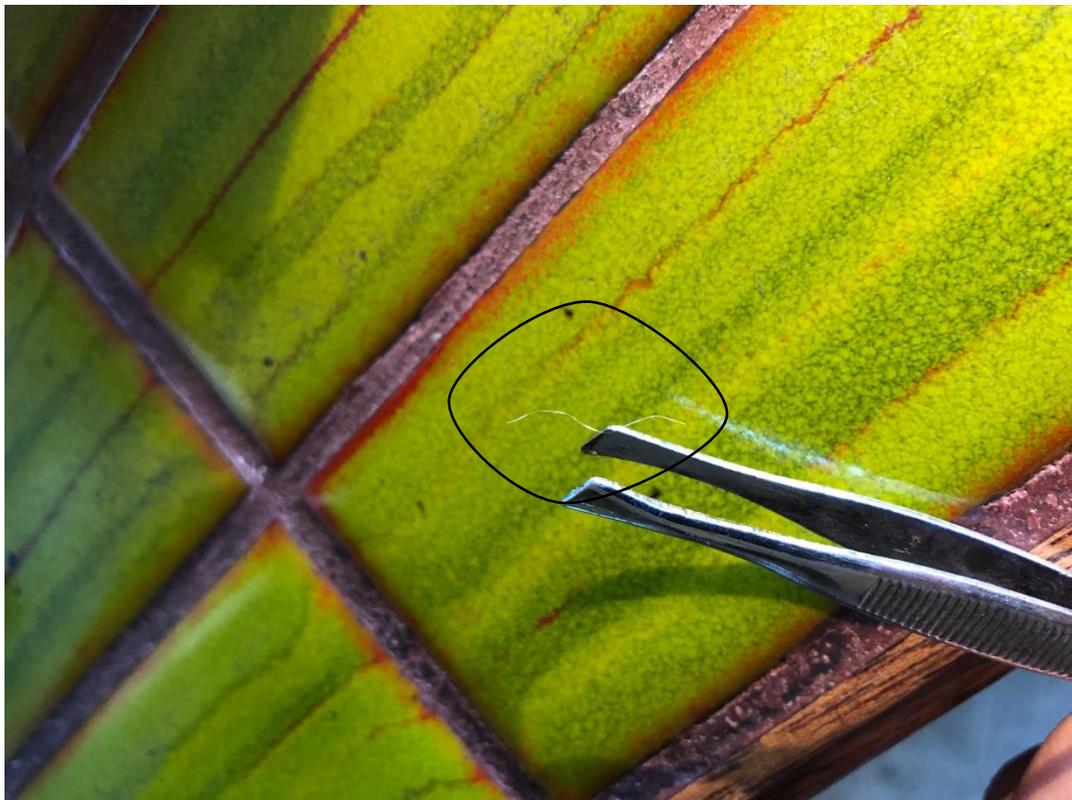


Figure 22: White fibre picked up by tweezers, ready for hot needle test.



Figure 23: red fibre after melting.



Figure 24: Pink unorthodox fragment.



Figure 25: hard clear and purple fragment

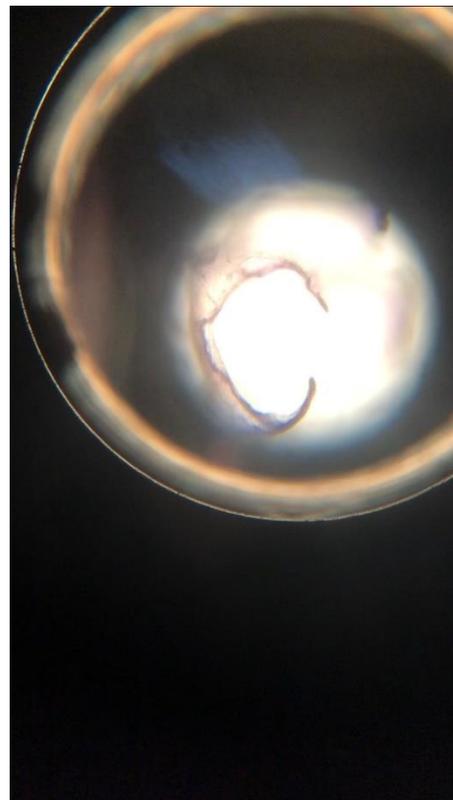


Figure 26: Fragments distinct angular shape

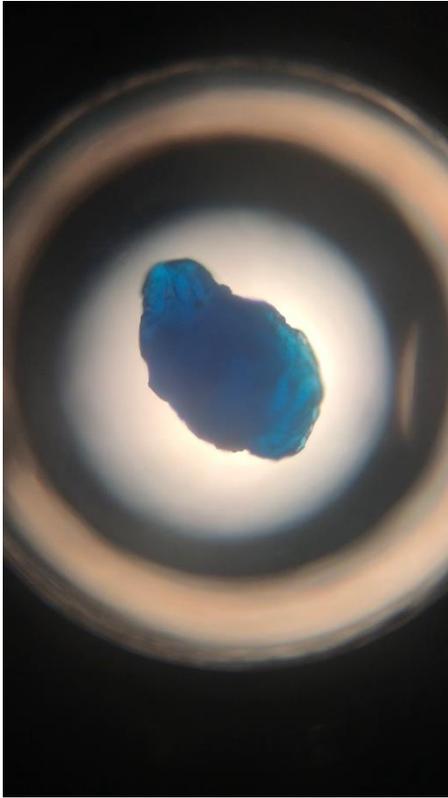


Figure 27: Degraded blue microbead

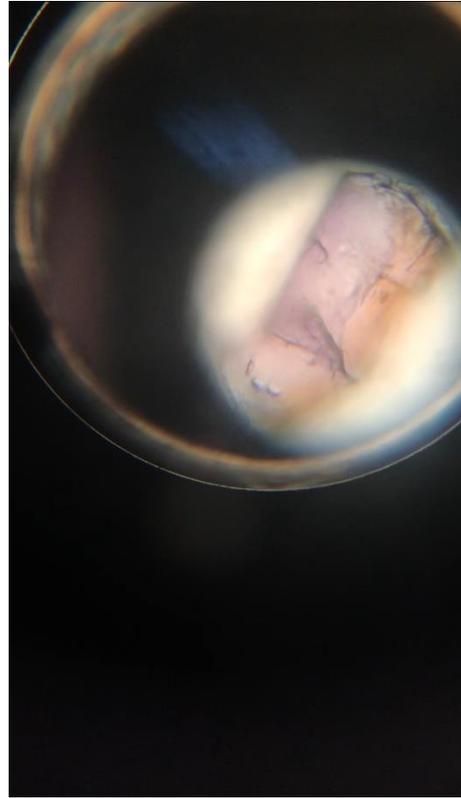


Figure 28: Angular, purple and orange fragment



Figure 29: unorthodox green fibre with growth.



Figure 30: Looked to be bag remains. Folded with growth and angular shapes

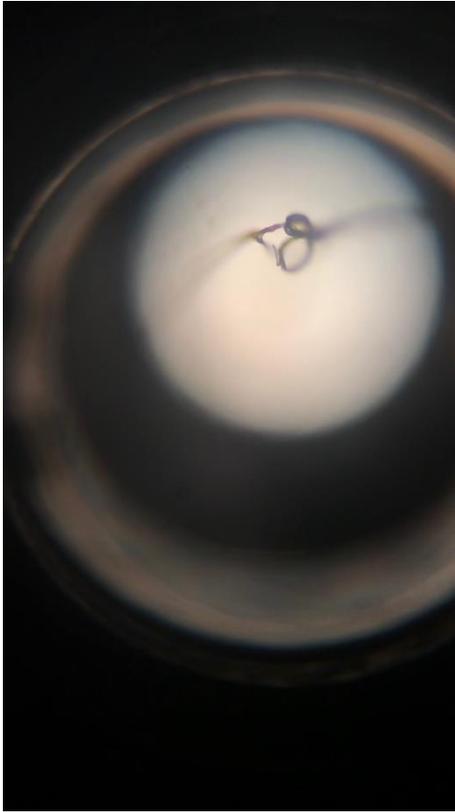


Figure 31: Twisted red and blue fibre.



Figure 32: clear red fibre.



Figure 33: Lower magnification of twisted green fibre. Clearly see growth



Figure 34: Clear and coloured fragment. Unorthodox shape with distinct angular sides

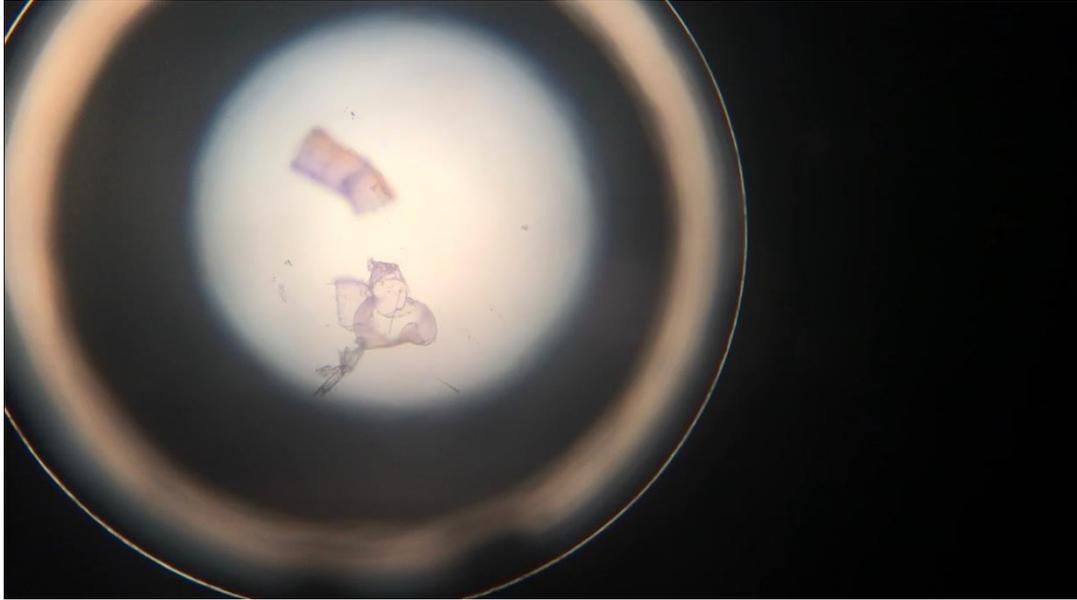
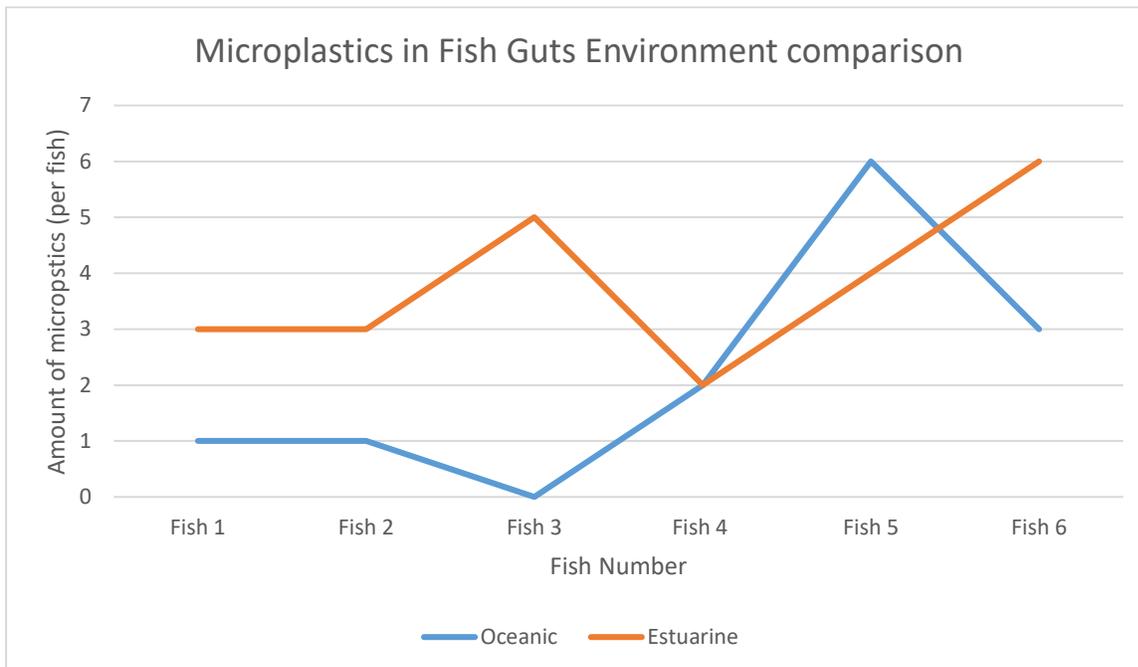


Figure 35: Wide shot of 2 fragments to compare the differences between microplastics

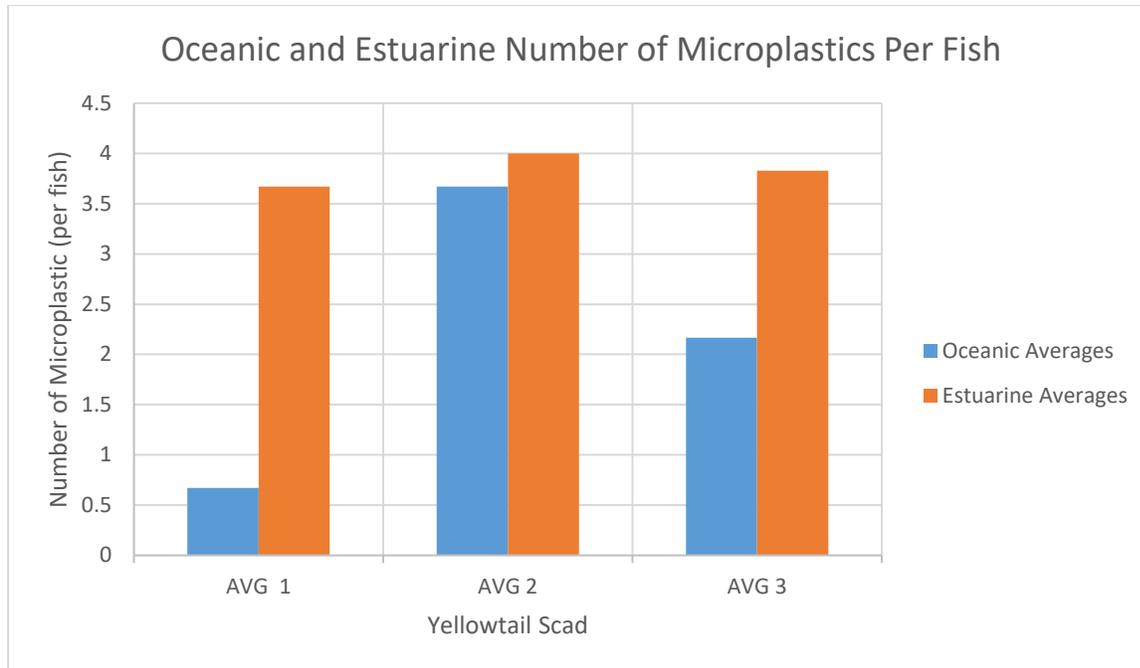


Figure 36: Blue/black microfiber on the top of the filter paper, caught in gut content

CHARTS



The Line graph above identifies the amount of microplastics in each fish's guts, from each environment. We can see that that estuarine Yellowtail Scad show a higher number of microplastics, however the numbers from the oceanic fish do overlap. The general trend shows that the estuarine Yellowtail scad had more microplastics compared to the Oceanic fish. Predicting for the future would be difficult due to the small sample size, but from this graph and following the trend, estuary caught fish would contain higher amounts of microplastics.



In the above chart you can clearly see that the estuarian species have higher averages for both tests 1 and 2. Also having a higher average over all the fish tested. The oceanic species do have lower averages, but it did seem to fluctuate from the information above. The estuary caught yellowtail had close and quite steady averages. This could have been due to the fact they were done after the oceanic species and the method was refined and investigator had a better understanding of the microplastics that seemed to be common.

Discussion:

During my investigation I did run into certain errors and problems that could have caused the results to be different. These are:

- Human error: When looking for the microplastics I well and truly could have missed pieces during each test. It is very difficult to find the microplastic pieces correctly identify them.
- Gross error: because I didn't have the most advanced equipment. I could have been making mistakes that made me miss plastics. Also not using the equipment properly such as the light microscope could've changed my perception on a certain object.
- Systematic Error: I re-used the sieve each test. I did wash the sieve but there could have been microscopic pieces left over from each test, meaning it could have affected the next test.
- Random Error: Due to the changing gut content, random errors may have occurred as each filtration was different to the next.
- The time spent on each differed as I stopped only 10 minutes after finding the last microplastic when there should have been a set time.

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- Minimal repetitions: Due to the massive population of Yellowtail Scad, 6 repetitions for each population is not very reliable. From the data we can pick trends and predict future or other results, but they aren't considered reliable.

Due to my human error in the earlier stages of the investigation. I believe as the investigation progressed it was easier to find microplastics due to the practice before hand. The earlier stages could have had more microplastics that I could not see as I wasn't sure on what to exactly look for, when in the latter stages of the investigation, I knew the fibre's, small fragments and colour's that seemed to be common in the yellowtails guts. To minimize this, before the investigation I trialled the method on other fish species such as tailors and bream to minimize the error when it came to the actual investigation.

The Gross error of investigator carelessness and not completely washing the sieve of each individual spec, may have affected the data, due to missing some microplastics in the sieve and when the next test comes around, I could accidentally pick up that microplastic or cause a gap between the sieve and filter paper where objects could escape both the filter paper and sieve. To resolve this error is simple, be careful and be intricate and precise when cleaning and using the equipment. Also, to get another trusted person to check or double check the work.

Due to the dynamic gut contents of the yellowtail, there were aspects of random error. Due to the yellowtail being in spawning season, majority of the fish were full of row and eggs full of stomach that caused problems as it made it very difficult to identify microplastics, but due to some being less full, the errors would've caused by chance. This also falls into category of my own human error as well, struggling to see the pieces due to higher amounts of debris.

The time spent on each test could have also directly affected my results. As it took a lot of time to go through each filtration of gut content, I decided to stop when I believed I couldn't see any more microplastics. I believe if I controlled the time, the results would have been closer. Also, I may have picked up more plastics if I spent a lot of time on each test. I found when I moved the light it would illuminate some pieces and found pieces a lot easier. If I was to do the investigation again, I would set a time limit of approximately half an hour, as I believe this would give me sufficient time to thoroughly inspect each piece.

The reliability of the investigation is limited due to the small amount of repetitions. This was due to the smaller amount of time. If I was to repeat this investigation, I would increase the amount of repetitions to a larger scaled experiment. This would allow me to make reliable and accurate predictions as I could remove outliers and conduct multiple repetitions covering all bases.

Because some of these errors, I believe they could have had an effect on the results. As the results were all reasonably close and only having a small sample size, I did not discount any of the results as outliers. The human error of possibly missing microplastics. These microplastics could then stick to the next filter paper and change the results of the next test. Because it was a

new concept for me and it took multiple tests to understand, change and get the hang of the method, this also could have affected my results.

Conclusion

In conclusion, I believe the investigation was completed well or to a satisfactory level. With more repetitions and practice I believe it could create an interesting set of concluding questions. I aimed to investigate how different or similar it was for estuary Yakka and Oceanic Yakka to ingest microplastics. Throughout the investigation to the concluding test, I believe I was providing relevant examples and sets of data that show what I aimed to do.

From the data, you can see that my hypothesis was supported, showing that the Estuary caught Yellowtail Scad were more susceptible to ingesting microplastics. This could be due to the higher density living, storm water and environmental runoff and smaller amounts of current compared to the Ocean caught Yellowtail Scad. It is clearly seen in Graph 1 and 2, with higher averages of 3.83 microplastics per fish (estuarine) compared to the 2.167 microplastics per fish (oceanic). The estuarine caught also had higher readings throughout the tests, however, the oceanic caught fish did fluctuate between low and high numbers. As above, with higher quality or more advanced equipment, with long periods of time and a higher number of sample size, I believe that the correlation between environments and susceptibility to ingesting microplastics will become clearer. But due to my small sample size and fairly close results, I can not conclude to say that one environment causes fish to ingest more microplastics compared to another.

Interestingly, the results were very similar for most fish species. Out of the total 36 microplastics found across the 12 yellowtail, 22 microplastics identified were fibers, which are synthetic materials like nylon. Because the yellowtail are generally filter feeders, these microfibrils enter the digestive tract where I believe the fibers stick to the stomach and tract walls, whereas the fragments are excreted easier compared to the fibers which seemed to be stickier and gets caught in the contents of the digestive system. Although there were other pieces, a degraded microbead, multiple fragments, clear film like pieces what looked to be a plastic bag remain.

Further questions drawn from this investigation that have been raised from my results are:

1. Do ingested microplastics leach toxic, poisonous or threatening chemicals into an organisms Body?
2. Are all or majority of Yellowtail Scad ingesting or coming into contact with Microplastics? Or is it just coastal fish?
3. What other species of fish and other animals are ingesting microplastics?
4. What are the direct repercussions of microplastics over certain periods of time?
5. How can we clean the waterways, or earth itself of microplastics? Where would we start?
6. Are we humans being affected by these fish eating microplastics accidentally?

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Evidently, I think this type of investigation will become part of a larger scaled scientific research issue. With further education and ongoing investigations into this subject, hopefully some of these questions could be answered and hopefully create a safer environment for not only fish and aquatic animals, but for the organisms and animals that eat these species. For a healthier and sustainable environment, tests similar to these would be extremely beneficial when it comes to the predictions of future repercussions of microplastics. As the report shows, humans could possibly already be affected by microplastics themselves, but unfortunately without further knowledge we can't see the damage they are doing to wildlife and possibly ourselves.

Bibliography

1millionwomen. (2017, May 26). Plastic Microfibres [Photograph]. Retrieved from <https://www.1millionwomen.com.au/blog/plastic-microfibres-what-we-know-so-far-and-how-help/>

Busselton, J. (2019). Yellowtail scad. Retrieved from <https://www.busseltonjetty.com.au/marine-research/fish-finder/bony-fish/yellowtail-scad/>

Davis, R. (2017, June 6). Microplastic pollution is the REAL threat to our oceans, warn scientists [Photograph]. Retrieved from <https://www.cleanwater.news/2017-06-16-microplastic-pollution-is-the-real-threat-to-our-oceans-warn-scientists.html>

Entwistle, A. (2018, April 11). Why the fuss about nurdles? [Photograph]. Retrieved from <https://phys.org/news/2018-04-fuss-nurdles.html>

Flowers, M. (2016). Zooming in on the five types of micro plastics. Retrieved from <http://www.waterkeeper.ca/blog/2016/11/15/zooming-in-on-the-five-types-of-microplastics>

Gill, V. (2018). Marine Plastic: Hundreds of fragments in dead seabirds. Retrieved from <https://www.bbc.com/news/science-environment-44579422>

Gyres Institute. (2019). Plastic microbeads. Retrieved from <https://www.5gyres.org/microbeads>

Hudson, M. (2018, June 8). You're eating micro plastics [Web Log/Article]. Retrieved from <https://theconversation.com/youre-eating-microplastics-in-ways-you-dont-even-realise-97649>

Liboiron, M. (2016). How to investigate fish guts for micro plastics. Retrieved from <https://civiclaboratory.nl/2017/12/30/how-to-investigate-fish-guts-for-marine-microplastics/>

McGrouther, M. (2018). Yellowtail Scad, *Trachurus novaezelandiae* Richardson, 1843. Retrieved from <https://australianmuseum.net.au/learn/animals/fishes/yellowtail-scad-trachurus-novaezelandiae/>.

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McGrouther, M. (2018, December 18). Yellowtail Scad [Photograph]. Retrieved from <https://australianmuseum.net.au/learn/animals/fishes/yellowtail-scad-trachurus-novaezelandiae/>

National Geographic. (2018). Runoff. Retrieved from <https://www.nationalgeographic.org/encyclopedia/runoff/>

National Geographic. (2019). Krill. Retrieved from <https://www.nationalgeographic.com/animals/invertebrates/group/krill/>

NOAA. (2018). What is an estuary? Retrieved from <https://oceanservice.noaa.gov/facts/estuary.html>

Nunez, C. (2019). Oceans. Retrieved from <https://www.nationalgeographic.com/environment/habitats/ocean/>

Plant Permaculture. (2015, April 10). Soil degradation [Photograph]. Retrieved from <https://planetpermaculture.wordpress.com/tag/run-off/>

Smith, B. (2018). How are microplastics created? Retrieved from <https://www.azocleantech.com/article.aspx?ArticleID=785>

Thorbecke, A. (2019, March 12). Plastic, Pollution, and Human Health [Photograph]. Retrieved from <https://blog.response.restoration.noaa.gov/plastic-pollution-and-human-health>

Thompson, A. (2018, September 4). From Fish to Humans, A Microplastic Invasion May Be Taking a Toll [Photograph]. Retrieved from <https://www.scientificamerican.com/article/from-fish-to-humans-a-microplastic-invasion-may-be-taking-a-toll/>

Whitebread, E. (2016, June 21). Microbeads [Photograph]. Retrieved from <https://www.greenpeace.org.uk/microbeads-story-so-far/>

Warren, M. (2018). Krill can break down micro plastics. Retrieved from <https://www.sciencemag.org/news/2018/03/krill-can-break-down-microplastics-may-not-be-good-thingBibliography>